PULMONARY

May 17, 1973, 2:00 P.M.

Continental Ballroom 6

MODERATOR: Robert B. Mellins

1. 2:00 LUNG GROWTH AND FUNCTION FOLLOWING REPAIR OF CONGENITAL DIAPHRAGMATIC HERNIA. Mary Ellen B. Wohl, N. Thorne Griscom, Samuel R. Schuster, Robert G. Zwerdling and Denise Strieder. Harvard Med. Sch. Children's Hosp. Med. Ctr., Dept. of Cardiology, Radiology, Surgery and Medicine, Boston.

2. 2:15 HYPERSENSITIVITY LUNG DISEASE IN CHILDHOOD: AN EXPERIMENT OF NATURE. Donald N. Gillespie, Edward C. Rosenow III, and Edward J. O'Connell (Intr. by Gunnar B. Stickler). Mayo Clinic and Mayo Fndn., Rochester, Minnesota.

3. 2:30 ADENYLATE CYCLASE (AC) ACTIVITY IN FETAL RABBIT LUNGS AND ITS RESPONSE TO EPINEPHRINE (E), NaF, CORTISOL (C) AND GLUCAGON (G). Cynthis T. Barrett, Alex Sevanian and Solomon A. Kaplan, Department of Pediatrics, UCLA School of Med., Los Angeles.

4. 2:45 PULMONARY INACTIVATION OF PROSTAGLANDINS E_1 IN THE FETAL AND NEWBORN LAMB. Peter M. Olley, Flavio Coceani, and Geraldine Kent. Intr. by Dr. A. Sass-Kortsak. The Hospital for Sick Children and The Research Institute, Toronto.

5. 3:00 EVIDENCE OF LOWER AIRWAY OBSTRUCTION IN CHILDREN WITH HEART DISEASE. Etsuro K. Motoyama, Hiroshi Goto, Bernard Wu, Natalie de Leuchtenberg, (Intr. by C.D. Cook), Depts. Ped., Anesth. and Lung Research Ctr., Yale Sch. of Med., New Haven.

- 3:15 Intermission -

6. 3:35 LUNG ELASTIC RECOIL IN CYSTIC FIBROSIS. Anthony Mansell, A. Charles Bryan, and Henry Levison. Dept. of Peds., Research Inst., Hosp. for Sick Children, Univ. of Toronto.

7. 3:50 ALTERATIONS OF PULMONARY PRESSURE-VOLUME RELATIONSHIPS IN THE PERINATAL PERIOD: PHYSIOLOGIC EVIDENCE FOR RELEASE OF SURFACTANT AT BIRTH. W. Taeusch, Jr., I. Wyszogrodski, and M. E. Avery, McGill University-Montreal Children's Hospital Research Inst. & Dept. of Physiol., McGill University, Montreal.

8. 4:05 INDUCTION OF THE PULMONARY SURFACTANT IN THE FETAL PRIMATE BY THE INTRAUTERINE ADMINISTRATION OF CORTICOSTEROIDS. R. A. deLemos and G. W. McLaughlin. Dept. of Pediatrics, Wilford Hall USAF Medical Center and University of Texas Medical School, San Antonio, Intr. by M. J. Sweeney.

9. 4:20 THE EFFECTS OF POSITIVE END EXPIRATORY PRESSURE (PEEP) ON PULMONARY HEMODYNAMICA. J. R. Hessler, R. D. Garrison, D. V. Eitzman and S. Cassin, Depts. of Comp. Med., Pediatrics and Physiology, College of Medicine, Univ. of Fla., Gainesville.

10. 4:35 POSITIVE END-EXPIRATORY PRESSURE BREATHING IN INDUCED HYALINE MEMBRANE DISEASE. Lars Victorin, Dan Lindstrom, Hakan Sundell, Alex Tsiantos, A. B. Rill, Mildred Stahlman. Depts. Pediatrics and Radiology, Vanderbilt Univ. of Sch. Med. Nashville.

AMERICAN PEDIATRIC SOCIETY

First Plenary Session

APLASTIC ANEMIA (AA) FOLLOWING HEPATITIS--AN INDICATION FOR EARLY BONE MARROW TRANSPLANTATION. Bruce Camitta, Edwin Forman, Robertson Parkman, Joel Rappeport, Tessa Orellana and David Nathan. Harvard Medical School and Children's Hospital Medical Center, Boston.

Pancytopenia with aplastic bone marrow is a rare complication of viral hepatitis. Survival is < 15%. In contrast restoration of marrow function can be achieved in 50% of patients with severe AA using histocompatible bone marrow transplants. A 14-year-old boy developed AA during early recovery from viral hepatitis. Despite androgens and prednisone, progressive marrow failure with oral infection, bleeding and increased transfusion requirements ensued. Therefore 1.5×10^{10} marrow cells of a histocompatible male sibling were infused after "conditioning" by transfusion of donor blood followed by cytoxan (60 mg/kg/day x 4). Methotrexate was given post transplant to ameliorate potential graft vs host disease (GVH). Irradiated RBC, white cell and platelet transfusions were given as indicated. A "sterile" environment was maintained by asepsis and antisepsis. Early engraftment of hematopoietic elements was seen on day 10 and progressed rapidly thereafter. Mild GVH occurred but subsided. The patient is now in nearly complete hematologic remission. Immunologic reconstitution is occurring but somewhat more slowly. A similar case has recently been reported by Thomas, et al (Lancet 1:284, 1972). Bone marrow transplantation should be attempted early in the course of severe AA following hepatitis if an appropriate donor exists and should be seriously considered early in any patient with severe AA.

CONGENITAL ERYTHROPOIETIC PORPHYRIA (CEP) WITH A HITHERTO UNDESCRIBED PORPHYRIN PATTERN. Martin F. Seip and Leif Eriksen (Intr. by C.D. Cook), Depts. Ped. & Physiology, Univ. of Oslo, Norway.

A boy with the typical clinical picture of CEP showed an unusual porphyrin pattern. In addition to the expected very high urinary excretion of uroporphyrin I and high number of erythroblasts with nuclear fluorescence in the bone marrow, he showed large amounts of a 7 carboxylic porphyrin of the isomer III series in the urine. At 2 years of age the urine contained 11600 µg porphyrin per day, whereof 4330 µg uroporphyrin, 4330 µg 7 carboxylic porphyrin, 775 µg 6 carboxylic, 860 µg 5 carboxylic, 1270 µg coproporphyrin, and a small amount of 3 carboxylic porphyrin. The findings of increased amounts of protoporphyrin in plasma and feces are also at variance with the picture seen in classical CEP. These data indicate that we are dealing with a biochemically "new" type of CEP. Meticulous protection against light with wavelengths below 510 nm removed the skin lesions and brought about an almost complete compensation of the hemolytic anemia. Under light protection the ratio porphyrinogen/porphyrin in urine was 80/20, under incomplete light shielding 10/90. An important feature is thus the overproduction of porphyrinogen, which on light exposure in the skin is oxidized to porphyrin.

The data seem to exclude that impaired activity of the isomerase (uroporphyrinogen-III-cosynthetase), which has been claimed to be deficient in classical CEP, is the only or even the most important metabolic defect in this patient.

TRANSMISSION OF GROUP B STREPTOCOCCI AMONG PARTURIENT FEMALES AND THEIR NEONATES. Carol J. Baker and Fred F. Barrett (Intr. by Martha D. Yow). Baylor Col. of Med., Dept. of Ped., Houston.

Although Group B streptococcus has emerged as an important pathogen in perinatal infection, certain epidemiologic factors associated with these infections are ill-defined. The colonization rate of Group B streptococcus was studied in 205 third trimester females and their 206 neonates during a 4 month period. Maternal throat and cervicovaginal and infant throat and umbilicus or ear isolates were serotyped. In addition, blood and CSF isolates from 13 neonates with symptomatic Group B streptococcal infection during the study period were serotyped. Group B streptococci were recovered from 25% of mothers and 26% of neonates. Seventy-one percent of colonized and 12% of non-colonized mothers delivered infants who subsequently became colonized. Approximately 40% and 35% of isolates, respectively, from mothers and infants were types II and III. There were no statistically significant associations between neonatal colonization and race, sex, gestional age or site of colonization. However, neonates with proved Group B streptococcal infection were more frequently premature (69%),white (62%), and had type III isolates (58%) as compared to asymptomatic carriers. Type II strains were not as frequent among infected neonates. Neonatal colonization rate was 260/1000 live births as compared to an attack rate of 3/1000 for proved Group B streptococcal infection (mortality rate 1.1/1000). The gestional age and race differences among sick infants could not be related to colonization. Further studies seem indicated before prophylaxis of selected parturient carriers of Group B streptococcus could be justified.

POLIO IMMUNITY: Richard D. Krugman, Paul D. Parkman, Harry M. Meyer, Jr., FDA, Rockville, MD, George E. Hardy, Jr., Birmingham, Reuben D. Wende, Houston, Kenneth L. Herrmann and John J. Witte, CDC, Atlanta. Paralytic poliomyelitis in the U.S. has declined dramatically. However the 1972 Greenwich, CT epidemic shows that it remains a threat. Also, evidence has been found for increasing percentages of children lacking polio immunity (Horstmann, D.H., NEJM, 285:1432, 1971). Poor health care delivery, ineffective or unstable vaccines or lack of antibody persistence all might contribute to this problem. Studies were done to elucidate the relative importance of these factors. Administration of 3 doses of trivalent oral poliovirus vaccine (OPV) to 50 children in Guam, where polio antibody patterns are similar to those seen in the U.S., produced satisfactory responses despite high non-polio enterovirus isolation rates. Potency tests on OPV from 7 clinics in the U.S. indicated good stability. Antibody persistence was shown in 97-100% of 60 children immunized 5 years earlier in Birmingham, AL. These data indicate OPV to be potent, stable and effective and document antibody persistence. Thus poor health care delivery is implicated as the major factor. Increasing effectiveness of immunization programs by the simultaneous use of multiple vaccines is a possible solution. OPV and combined measles-mumps-rubella vaccine were used successfully in 400 children. Antibody responses were excellent and similar to those seen when the vaccines were given separately.

NEONATAL NARCOTIC ADDICTION-EXPOSURE TO HEROIN AND METHADONE, Carl Zelson and Sook Ja Lee, Dept. of Ped., New York Medical College-Metropolitan Hospital Center, New York.

Over the past 12 years, we have cared for more than 550 infants born to heroin addicted mothers. Approximately one-half of the infants required treatment. Recently, we have observed many gravidae on methadone alone or in combination with heroin. Their addicted infants appear to be more ill than those born to mothers on heroin. 12% of infants born of mothers on heroin required treatment, while 38% born to mothers on methadone were treated. Over a recent 13-month period, 58 infants were observed, 34 born to mothers who had used methadone alone or in combination with heroin, and 24 to mothers only on heroin. A comparison of the 2 groups showed the following: 1. the incidence of low-birth-weight infants was similar; 2. among infants born to mothers on methadone, weight and gestational age were more frequently concordant than for infants born to heroin addicted mothers; 3. methadone infants have higher average birth weights; 4. Apgar scores were lower in infants exposed to methadone; 5. the severity of withdrawal and the number of signs in each instance were greater in methadone infants; 6. seizures were also more frequent; 7. severe hyperbilirubinemia was more frequent in methadone infants; 8. hyaline membrane disease occurred in methadone infants, but has not been seen in heroin infants.

REDUCED DELIVERY RATES OF IMMATURE AND PREMATURE INFANTS FOLLOWING LIBERALIZATION OF NEW YORK STATE ABORTION LAW Jonathan T. Lanman, Schuyler G. Kohl, and James H. Bedell, The Population Council, New York, and Depts. of Ped. and of Obstetrics and Gynecology, Downstate Medical Ctr., New York.

Liberalization of the N.Y. state abortion law became effective on 1 July, 1970. In the following year, the delivery rate of immature (500-1000 gms.) and premature (1000-2500 gm) infants fell significantly at Kings County Hosp.-Downstate Med. Ctr. (KCH-DMC) in Brooklyn.

	deliveries	Birth rates/1000 deliveries	
		500-1000 gms.	1000-2500 gms.
1967 - June 1970	19,797	18	121
July 1970 - June 1971	6,706	8	98

KCH-DMC serves predominantly a black population occupying ward accomodations. Six other Brooklyn Hospitals, affiliates of DMC, serving predominantly a white population occupying private accomodations, experienced no corresponding change and had the lower rates in both periods. At KCH-DMC, newborns left for placement also declined significantly from 15/1000 deliveries to 7, but the change was not detected until 6 months after the change in the law. The lag presumably reflects the influence of those pregnancies already past 4-5 monthsthat could not be aborted. Similar reasoning would apply much less forcibly to pregnancies destined to produce immature or small premature infants, in which an early effect of the changed law was found.

AMERICAN PEDIATRIC SOCIETY

Second Plenary Session

INCREASED LH AND TESTOSTERONE RESPONSE TO LUTEINIZING HORMONE RELEASING FACTOR (LRF) AT PUBERTY. J.C. Roth, S.L. Kaplan, and M.M. Grumbach. Dept. Ped., Univ. Calif. San Francisco.

In order to explore the mechanisms initiating puberty we studied the pituitary responsiveness to LRF in 45 prepubertal, 11 pubertal and 14 adult subjects. Each subject received 2 μg/m^2 - 100 μg total dose of synthetic LRF by rapid intravenous infusion. Plasma LH and FSH and testosterone (T) levels (male subjects) were measured by specific radioimmunoassays twice before and for 8 hours following each infusion. Gonadotropin responses are expressed as mean maximal increment above control values in ng/ml ± SEM (LH (LER 960), FSH (LER 869)). We conclude that: (1) LRF releases small but comparable amounts of LH in prepubertal males (.76 ± .09) and females (.60 ± .14). This increases 6-fold ($p < .001$) in pubertal males (4.09 ± .45). (2) LRF releases more FSH ($p < .005$) in prepubertal females (2.19 ± .41) than in prepubertal males (1.03 ± .14), pubertal males (1.06 ± .20), or adult males (1.24 ± .23) whose responses are similar. (3) Increased sensitivity to LRF occurs prior to the attainment of secondary sexual characteristics but in association with concentrations of plasma FSH, estradiol, and T above prepubertal levels. (4) LRF raises plasma T levels (expressed as mean maximal increment above control values in ng/100 ml ± SEM) in adult males (170 ± 43) and in pubertal males (60 ± 18), but not in prepubertal males (2 ± 2). (5) The magnitude of LH and possibly FSH release induced by LRF appears to be affected by the degree of prior stimulation of the anterior hypophysis by LRF.

IMMUNOLOGIC STUDIES IN POSTPERICARDIOTOMY SYNDROME Mary Allen Engle, John McCabe, Brian Denham, Paul A. Ebert and John B. Zabriskie. The New York Hosp.-Cornell Univ. Medical Ctr., Depts. of Ped. and Surg., and The Rockefeller Univ., New York.

Etiology of the postpericardiotomy syndrome (PPS), which occurs in 25-30% of patients undergoing intrapericardial surgery, is unclear but may be due to an auto-immune reaction. A prospective study was undertaken in a consecutive series of 60 such children to determine whether heart-reactive antibody (HRA) developed and if so, whether it had any relation to the syndrome. Concurrent analyses were made on a double-blind basis at prescribed intervals in hospital and after discharge of HRA in serum, determined by immunofluorescent technique, and of clinical syndrome, each ranked on a scale of 0-4. When results were compared, a close correlation was found. The syndrome developed in 19 patients (31%), and antibody which bound to myocardium but not to pericardium appeared in all of these. All preoperative and 15 control sera from non-cardiac surgical subjects were negative. None of 13 patients with 0 antibody had PPS; 3 of 31 with intermediate HRA elevation had mild PPS and all 16 with HRA > 2+ had PPS, which was moderate or severe in 13. HRA appeared at the end of the first week, coincident with first clinical evidence of PPS, and lasted beyond clinical signs. Recurrence of PPS in 3 patients 4-8 months after surgery was associated with intermediate elevations of titer. We conclude that presence of HRA in high titer offers diagnostic confirmation of postpericardiotomy syndrome.

CELL RECEPTORS AND THE SELECTIVE TRANSFER OF PROTEINS FROM MOTHER TO YOUNG ACROSS TISSUE BARRIERS. Jonathan D. Gitlin and David Gitlin. Children's Hosp. of Pittsburgh.

Purified human IgG, IgE, albumin, insulin, growth hormone, and several heterologous IgG's were labeled with ^{131}I and ^{125}I. The proteins were added to homogenates of human placenta, and the mixtures were fractionated by differential centrifugation. Proteins were found to be selectively bound by tissue fractions containing cell membranes. IgG, actively transported from mother to fetus, was bound most avidly; albumin which traverses the placenta poorly is bound poorly. The placenta in vivo rapidly accumulates insulin but not growth hormone; in vitro there was strong binding of insulin to cell membranes in contrast to poor binding of growth hormone. Guinea pig IgG which readily traverses the maternofetal barrier in animals is also fixed to cell membranes; bovine IgG which passes less well is bound less well. Since the intestinal wall in suckling mice and rats is differentially permeable to IgG as is the placenta, labeled protein studies were carried out in these species as well; as observed by Waldmann, intestinal homogenates from sucklings proved preferential in protein binding. Thus, in those tissues across which proteins are transferred selectively, there is a related selective binding of proteins to cell membranes. Cell membranes from the placenta which bind such proteins are physically and selectively different from those of intestines, just as the kinetics and the specificity of protein transport across the two tissue barriers are different.

FOCAL SCLEROTIC LESIONS IN STEROID SENSITIVE NEPHROTIC SYNDROME. Norman J. Siegel, John P. Hayslett, Benjamin H. Spargo and Michael Kashgarian (Intr. by Charles D. Cook) Yale Univ. Sch. of Med., Depts. of Ped., Med. and Path., New Haven.

A prospective study (Lancet 1:1353, 1970) suggested that most children at onset of the nephrotic syndrome have either minimal change - steroid responsive or focal sclerotic - steroid resistant lesions by light microscopy. Also, age of onset <6 years and steroid responsiveness indicated with high probability a minimal change lesion. It was uncertain, however, whether minimal change and focal sclerosis were aspects of the same disease or were distinct entities.

In an analysis of 18 children with focal sclerosis on renal biopsy, 10 patients, age 1-6 at onset and biopsied after 3 to 17 years of polycyclic, steroid responsive or dependent nephrotic syndrome, had the lesion on both light and electron microscopy. In the remaining 8 patients the focal sclerosis was less extensive and could only be detected on electron microscopy. Of these 4 were steroid responsive and 4 steroid dependent.

These data imply: 1) in patients with a clinical course typical of minimal change lesion, focal sclerosis may be seen after many years of steroid sensitive nephrotic syndrome and therefore 2) minimal change and focal sclerosis may be aspects of the same entity; 3) electron microscopy may demonstrate early changes of focal sclerosis not visible on light microscopy.

RESPONSE TO GENETIC COUNSELING: A FOLLOW-UP SURVEY. Y. Edward Hsia and Ruth L. Silverberg. Depts. of Human Genetics & Ped., Yale Univ. Sch. of Med., New Haven. (Intr. by C. D. Cook).

A system of genetic counseling has been evolved which integrates psycho-social intake, non-directive team counseling by a physician and a social worker, and reinforcement by a written explanation to the counselees. A year later, 131 counselees were surveyed for: their recollection of genetic risks; their reproductive attitudes, including those toward prenatal diagnosis by amniocentesis; and their judgment of the value of the counseling. 100 responses were classified by: severity of disease (mild-24; serious-76) and recurrence risk (low <10%-75; high >10%-25). 73% had received a written account.

Their chance of having a healthy baby was remembered correctly by 77%; 10% were slightly optimistic and 10% slightly pessimistic; 3% had totally misunderstood. Of 11 counselees with high risk of a serious disorder, 4 wanted more children, 2 with use of amniocentesis. Of 44 with low risk of a serious disease, 24 wanted more children; of 17 who could be helped by amniocentesis, 12 intended to have it. There was a clear inverse relationship between the burden of the disease and their understanding of the genetic data. The importance of the written account was confirmed by: better retention and understanding of genetic data; better perception of the counselors' non-directiveness; better satisfaction with the counseling; and by the fact that only one family had discarded it.

Overall, 83% felt their questions had been answered satisfactorily, and 90% felt the counseling was worth the cost.

SURGICAL TREATMENT OF CHILDHOOD OBESITY. Paula M Botstein, Hans H Bode, John D Crawford, Paul S Russell, Harvard Med Sch, Mass Gen Hosp, Shriners Burns Inst, Depts of Ped & Surg, Boston.

Fear of metabolic complications has usually contraindicated surgery for intractable childhood obesity, but bypass may be justified and effective for children suffering hypothalamic hyperphagia. We have treated 4 adolescents (2 with Prader-Willi syndrome, 2 with bulimia after tumor removal) with jejunoileal bypass procedures leaving absorptive surfaces of 23-30 and 23-38 cm on either side of the excluded intestinal segment. Preoperatively, progressive obesity compromised cardiopulmonary function and caused social crippling despite intensive diet therapy. Prolonged hospitalization was required for weight loss sufficient (20 kg) to reduce operative risks in 1 boy; in another, a hyper-osmolar diet producing intestinal hurry achieved similar results. Operative complications (transient ileus, hyponatremia and hypercalcemia) occurred in 1 patient. Follow-up has ranged from 6 months to 9 years. Post-op losses were 12 to 29 kg depending on extent of residual functioning intestinal surface with ultimate weight stabilization despite continued over-eating. Cardiopulmonary function improved, and patients were socially rehabilitated. All have steatorrhea; 2 have had folate, B_{12}, or Fe deficiency. Post-prandial hypoglycemia, asymptomatic hypomagnesemia, and hyperoxaluria without renal stones each occurred once. Patients and parents considered operation worthwhile. We conclude that the conservative amount of bowel excluded prevented major weight loss and serious complications. (Children's Med. Res. Fund, HD T01 33, AM 04501).

PYRIDOXINE-RESPONSIVE HOMOCYSTINURIA: PROPERTIES OF NORMAL AND MUTANT CYSTATHIONINE SYNTHASE. Young Jin Kim and Leon E. Rosenberg, Depts. of Human Genetics and Ped., Yale Univ. Sch. of Med., New Haven. (Intr. by Charles D. Cook).

To define the biochemical basis for pyridoxine-responsive homocystinuria, we have extended our studies of cystathionine synthase (CS) in cultured fibroblasts from 2 patients with that disease. CS activity in crude fibroblast homogenates from these patients was 1-5% of that in control lines. Normal CS was purified nearly 7-fold by the sequential application of differential centrifugation, ammonium sulfate fractionation and calcium phosphate gel treatment: specific activity-9.5 nmoles/mg protein/60 min (u) in the crude homogenate; 62 u in the final preparation. This partially purified CS had the following properties: pH optimum 8.3; K_m for serine-1.5 mM; K_m for homocysteine-14 mM; K_m for pyridoxal-5'-phosphate (PP)-.014 mM. Partially purified CS from the two mutant lines demonstrated prominant chemical and physical differences from normal: its specific activity was much reduced (0.2-1.3 u); it was far more thermobile (heating at 55° for 10 minutes, which reduced normal CS activity by 40%, reduced mutant activity by >70%); and its affinity for PP was less than 1% of normal (K_m->5mM). Even at saturating concentrations of PP, CS activity in both mutant lines failed to increase to more than 2-3% of normal. *These findings suggest that the cells of patients with* pyridoxine-responsive homocystinuria contain a structurally altered, unstable CS protein whose activity is increased only slightly by its cofactor, pyridoxal-5'-phosphate.

THE PRENATAL DIAGNOSIS OF CYSTINOSIS. J.A. Schneider, F.M. Verroust, A.J. Garvin, E.O. Horger, III, and C. Jacobson, Univ. of California, San Diego, Sch. of Med., Dept. of Ped., La Jolla, Med. Univ. of South Carolina, Depts. of Path. and Ob-Gyn, Charleston, and George Washington Univ., Sch. of Med., Dept. of Ob-Gyn, Washington, D.C.

A 25 year old woman whose only child has nephropathic cystinosis requested prenatal study during her second pregnancy. Amniocentesis was done in the 18th post-menstrual week, and cultured amniotic fluid cells (AFC's) were studied with ^{35}S cystine (J. Ped. 77:468, 1970). The ratio of labelled cystine x 10^3/glutathione-N-ethylmaleimide (NEM) was 1800 in the AFC's at risk. This compared with 3900 and 1100 in two cystinotic fibroblast cultures, and is much greater than the values of 80 and 97 found in two heterozygous cultures, and 8 and 52 in two control AFC cultures. The ratio of labelled cystine/cysteine-NEM in the AFC's at risk was also in the cystinotic range.

The pregnancy was terminated by hysterotomy in the 23rd post-menstrual week. The free-cystine concentration of amniotic fluid was 0.059mM (normal- 0.03-0.09mM). No cystine crystals were seen during light microscopic study of alcohol-fixed tissue from all organs. The diagnosis of cystinosis was established by finding the free-cystine content of the liver, placenta, kidney, thymus, spleen, and thyroid to be 50-100 times greater than control values. Two weeks after the pregnancy was terminated, sufficient AFC's from the fetus at risk were available for direct measurement of their free-cystine content, which was 3.9nmole 1/2cystine/mg protein (normal- < 0.2).

SOCIETY FOR PEDIATRIC RESEARCH

Plenary Session

VAGAL INFLUENCE ON RESPIRATORY CONTROL IN NEONATES. Anthony Olinsky, M. Heather Bryan, A. Charles Bryan. Dept. of Paediatrics, Univ. of Toronto and the Research Inst., The Hosp. for Sick Children, Toronto, Canada (Intr. by P.R. Swyer).

We have studied the influence of vagal stretch receptor information on respiratory control in neonates. The method was described by Hering and Breuer, but has significant differences from their classic lung inflation reflex. If the airway is occluded at end expiration the next inspiratory effort produces no volume change and hence no stretch receptor activity. If control depends on this information, in its absence, the inspiratory effort will be prolonged. If control does not require stretch receptor information the duration of the inspiratory effort will be normal. Fifteen premature infants, gestation 27-33 weeks were studied initially at 14 hrs. to 14 wks. Ten full term infants were evaluated at 52 hrs. to 7 days after birth. Prolongation of inspiratory time following occlusion was expressed as percentage decrease in frequency. Premature infants showed marked slowing (54%±11) whereas full term infants showed little slowing (26%±17); the difference was highly significant. In 5/7 prematures restudied significant slowing (52%±8) was still evident at 3-7½ mths. after birth. Therefore unlike adults and term infants we have shown that control of respiration in the premature infant is dependent upon vagal stretch receptor information and that this dependence persists for many months after birth. These findings may be of significance in periodic breathing and the Sudden Infant Death Syndrome.

EFFECT OF EXCHANGE TRANSFUSION ON ALTERING MORTALITY IN: (1) *INFANTS WEIGHING LESS THAN 1250 GRAMS AT BIRTH AND* (2) INFANTS WITH SEVERE RESPIRATORY DISTRESS (RDS). M. Delivoria-Papadopoulos, L. D. Miller, P. A. Branca, R. E. Forster and F. A. Oski. University of Pennsylvania, School of Medicine, Philadelphia, and the Upstate Medical Center, Syracuse, New York.

Previous studies of newborn infants have shown that exchange transfusion with adult blood produces an increase in the P_{50} (P_{O2} for 50% HbO_2 saturation, pH 7.4). The purpose of this communication is to report the therapeutic benefits achieved employing exchange transfusion with blood less than 72 hours old anticoagulated with citrate-phosphate-dextrose (CPD). Two treatment groups were studied. The first consisted of 43 infants weighing less than 1250g at birth. Exchange transfusion was performed within the first 8 hours of life. Of 21 infants receiving exchange, 19 survived (90%); 11 of 22 (50%) of the controls survived ($p < .01$). Within this group 4 of 5 infants less than 1000g survived while 3 of 8 controls survived. The second group consisted of 33 infants with severe RDS; 16 received an exchange transfusion (mean weight 1510g) and 13 survived (81%). Of the 17 non-exchanged controls (mean weight 1950g), there were 8 (47%) survivors ($p < .01$). These results indicate that exchange transfusion, presumably by facilitating tissue oxygenation, is of value in the management of both infants with low birth weight and those with severe RDS.

THE EFFECT OF WEIGHT REDUCTION ON THE CELLULARITY AND METABOLISM OF ADIPOSE TISSUE FROM OBESE CHILDREN. Fredda Ginsberg-Fellner and Jerome L. Knittle. Mount Sinai School of Medicine, Department of Pediatrics, New York, New York 10029

Obesity in both adolescents and adults is characterized by an excess of total adipose cells. Weight reduction in these individuals is accomplished solely by a decrease in cell size and long-term maintenance is usually unsuccessful. An extension of such studies to younger children is therefore of great importance. 18 children, ages 2 to 11½, were investigated. 11 were hospitalized for a large part of their weight reduction and 5 were studied as outpatients. Prior to reduction total weight ranged from 22.8kg. to 83.6kg. and % ideal weight was 189±9%. Weight reduction resulted in a 41±4% decrease in ideal weight concomitant with adipose cell size reductions in all cases. However, as in adults, cell number did not change appreciably. In vitro metabolic studies revealed depression of epinephrine stimulated lipolysis (27±10%↑) and a 151±11% increase in production of $14CO_2$ from glucose 1-C14 in the presence of 1000uU/ml insulin. The latter results differ from the marked in vitro unresponsiveness to insulin reported in obese adults. To date, 11 subjects with initial cell number less than the maximum for non-obese adults ($40x10^9$) and who were significantly reduced, have maintained their decreased % ideal weights from 6 months to 2½ years. However, subjects with greater cell numbers have regained at least to their previous percentile. These results support the hypothesis that, to be effective, dietary intervention in obesity should be instituted early.

THE RESPONSE TO SYNTHETIC LUTEINIZING HORMONE RELEASING FACTOR (LRF) IN PATIENTS WITH SUSPECTED HYPOGONADOTROPIC HYPOGONADISM D.C.L. Savage, J. Roth, M.M. Grumbach, and S.L. Kaplan. Dept. Ped., Univ. Calif. San Francisco, San Francisco, California.

The plasma LH and FSH response to an IV injection of 10 - 100 μg LRF has been assessed in 36 patients with idiopathic hypothalamic hypophysiotropic hormone deficiencies or hypogonadotropic hypogonadism. Thirteen of 16 patients with presumed isolated growth hormone deficiency but only 3 of 15 with multiple pituitary hormone deficiencies had a normal prepubertal response. Two patients in this group with arrested puberty also had a prepubertal response. Thirteen patients had neither a rise in plasma FSH or LH. Of 5 patients with isolated hypogonadotropin hypogonadism (4 with anosmia), 1 failed to respond to LRF, 3 had a prepubertal, and 1 a pubertal response. Seven of the 16 non-responders received intermittent subcutaneous dose of LRF but only 2 of these had an increased responsiveness to IV LRF following this therapy. These results, in the light of the different response to LRF in prepubertal and pubertal children, suggest that the site of the defect in hypogonadotropic hypogonadism is the hypothalamus. The defect may be partial or complete. The failure to respond acutely to exogenous LRF may be the consequence of a chronic deficiency of endogenous LRF secretion. In contrast to the effect of TRF deficiency, chronic deficiency of endogenous LRF appears to alter the pituitary responsiveness to acute administration of LRF.

ORGAN CULTURE OF FETAL LIVER: A NEW MODEL SYSTEM. R. deBelle, A. Brown, N.R. Blacklow, R.M. Donaldson, and R. Lester, Dept. Med., Boston Univ. Med. Sch., Boston, (Intr. by M.E. Avery).

The study of structure and function in the developing liver has been made possible through the development of a fetal hepatic organ culture system which can be maintained in vitro for 3 weeks. Morphology, protein synthesis, bile acid synthesis and conjugation were studied in human and rat fetal liver, 15 weeks and 20 days gestation, respectively.

Using rat fetal liver, tissue losses were encountered, but morphologic integrity of suviving tissue was maintained for 3 weeks. ^{14}C-leucine incorporation into tissue protein was maximal during the first incubation day, remained constant thereafter, and could be 99% inhibited in the presence of 3.6×10^{-4}M cycloheximide. ^{14}C-cholesterol was incorporated into ^{14}C-cholate, and glycine and taurine conjugates of ^{14}C-cholate were formed throughout the period of study. Taurine conjugation equalled 62% of total synthesized conjugate on day one, but only 26% on day ten. Relative taurine conjugation increased more than 3-fold with 8×10^{-4}M taurine added to the medium. Similar results were obtained with human tissue.

Conclusions: (1) Fetal hepatic tissue can be maintained morphologically and metabolically in organ culture for prolonged periods; (2) Specific hepatocytic functions--bile acid synthesis and conjugation--are demonstrable; (3) Taurine depletion results in reversal of the G/T ratio, and the ratio can be restored by taurine repletion; (4) The system will permit study of the effects of pharmacologic and viral agents.

CHARACTERIZATION OF THE SECRETORY IMMUNE SYSTEM OF MIDDLE EAR: IMPLICATIONS IN OTITIS MEDIA. P.L. Ogra, J.M. Bernstein*, and T.B. Tomasi, Jr.*. Depts. of Peds., ENT, and Med., Sch. of Med., State Univ. of N.Y. at Buffalo.

Employing the techniques of immunoelectrophoresis, hemagglutination-inhibition, radioimmunodiffusion and direct fluorescent-antibody (FA) staining, the middle ear washings and biopsy materials of middle ear mucosa from 20 patients with secretory otitis media were examined for the distribution of major classes of immunoglobulin; presence and nature of antibody activity against mumps, measles, rubella and poliovirus; and immunohistologic localization of immunocompetent lymphoid cells. Appreciable amounts of Secretory Component, and γG, 7SγA and 11S secretory γA immunoglobulin were regularly found in the ear fluids. However, no γM or γE was detected. The ratio of γG:γA immunoglobulin in the serum and middle ear fluids was approximately 10:1 and 10:4 respectively. Specific antibody activity in the middle ear fluid against the viruses listed above was essentially limited to γA immunoglobulin, although such activity in the serum was predominantly associated with γG immunoglobulin. FA studies of mucosal tissues of middle ear demonstrated characteristic staining for Secretory Component in the surface epithelium, and the presence of γG and γA containing plasma cells in the submucosa. These data suggest the existence of a distinct secretory immune system in the middle ear. The implications of these observations may be applicable to the mechanism of protection, or pathogenesis of microbial infections in the middle ear.

ORIGIN OF MURAL GAS IN NECROTIZING ENTEROCOLITIS, Rolf R. Engel, Norman L. Virnig, Carl E. Hunt and Michael D. Levitt, (Intr. by William Krivit), Univ. of Minn., Minneapolis, Minn. 55455.

Necrotizing enterocolitis (NEC) is a catastrophic complication among ill neonates. During 1971-72 there were 31 deaths among 55 newborns with NEC in our neonatal ICU. While intramural gas is the radiologic hallmark of NEC, origin of the gas is unknown. Mural and luminal gas were sampled at surgery. H_2 accounted for more than 30% of the gas from both locations. N_2, CO_2 and traces of O_2, but not CH_4, comprised the remainder. Since ambient air and human metabolism do not provide H_2, other sources must account for the large quantities of H_2. Bacteria isolated from the blood of 9 babies with NEC were studied for their ability to form H_2. When incubated for 18 hours with 1.8 ml of blood, the organisms proliferated, but little or no H_2 (< 0.1 μm) was formed. However, copious H_2 (> 40 μ moles aerobically and > 80 μ moles anaerobically) was produced by each of the 9 isolates after either 0.2 ml of milk or 50% glucose was added to the blood. Similarly, enteric H_2 production also requires exogenous substrate since 12 normal infants did not exhale H_2 until feedings were started. Furthermore, all 55 cases of NEC developed symptoms of the disease only after beginning oral or gavage feedings. New avenues for investigating the cause and prevention of NEC are provided by the observation that H_2 is a major component of the mural gas in NEC, that the associated bacteria make H_2, and that neither microbial H_2 production nor the disease occur in the absence of exogenous substrate.

SKIN TESTS IN ACUTE LYMPHATIC LEUKEMIA. D.H. Char, R.B. Herberman, A. LePourhiet and B.G. Leventhal, National Cancer Institute, Bethesda, Md.

With evidence for tumor associated antigens in leukemia, and the advent of immunotherapy for ALL, there has been much interest in assaying the immunologic status of these patients. We have serially tested ALL patients with membrane extracts from autologous blast, autologous remission, and allogenic blast cells; and with standard skin test antigens (mumps, SKSD, etc.) in an attempt to correlate these measures of cellular immunity with status of disease, prognosis and as a guide for immunotherapy. Tests with standard skin test antigens showed no difference between normals and patients in remission or relapse. There was an impressive difference in skin test responses to autologous blast material. 45% of patients in remission were positive versus less than 7% in relapse. Changes in clinical condition correlated with a change in responsiveness to those antigens. Tests with allogenic blast material also gave a statistically significant difference between patients in remission and relapse, and correlated with response to autologous material. There was a slight increase in remission length for patients who were positive to autologous material. Patients on different treatment protocols appeared to have some differences in immunologic responsiveness.

SUCCESSFUL TREATMENT OF THE CONGENITAL NEPHROTIC SYNDROME BY RENAL TRANSPLANTATION. John R. Hoyer, M.D., S. Michael Mauer, M.D., Carl M. Kjellstrand, M.D., Theodore J. Buselmeier, M.D., Richard L. Simmons, M.D., Alfred F. Michael, M.D., John S. Najarian, M.D. and Robert L. Vernier, M.D. University of Minnesota, Departments of Ped. and Surg., Minneapolis, Minn.

The congenital nephrotic syndrome (CNS) is a uniformly fatal disease unresponsive to all forms of medical therapy. It is characterized by massive proteinuria, severe growth failure, malnutrition, increased susceptibility to infection, and progressive renal insufficiency. Renal transplantation offers a new therapeutic approach in this disease. We have now successfully transplanted kidneys to 3 children with CNS and mild to severe uremia, including one of Finnish extraction. These 3 children, ages 2½, 2½ and 4½ years at transplantation, now have normal serum creatinine levels at 1, 5 and 14 months after renal transplantation and have not had recurrence of nephrotic syndrome or significant proteinuria. The child followed for 14 months has grown 4 inches since transplantation.

This experience contrasts sharply with our reported experience with steroid resistant idiopathic nephrotic syndrome of older children and adults. Three of 4 such patients had recurrence of the nephrotic syndrome within the first few weeks after renal transplantation. (Lancet ii, 343, 1972)

These observations suggest that the basic defect leading to massive proteinuria in the CNS resides in the kidney and that cure of this disease is possible through renal transplantation.

BEHAVIORAL SCIENCE

BEHAVIOR OF INFANTS BORN TO NARCOTIC-ADDICTED MOTHERS Reuben E. Kron, Mitchell Litt, and Loretta P. Finnegan, Phila. Gen. Hosp. and the Depts. of Ped. and Psychiat., Univ. of Penna. Sch. of Med. (Intr. by Maria Delivoria-Papadopoulos)

This report describes abnormalities in the nutritive sucking performance of congenitally addicted infants undergoing narcotic withdrawal.

A series of 50 infants born to mothers addicted either to heroin or to methadone were studied by an objective method for measuring sucking behavior. Sucking rates as well as average pressures and amounts of nutrient consumed during sucking were significantly reduced for the addicted infants relative to a control group born to normal mothers and a second control group born to toxemic mothers. The subgroup of infants born to methadone-addicted mothers was significantly more depressed with regard to sucking behavior than the infants of heroin addicted mothers. Furthermore infants treated with paregoric (an opiate) for symptoms of the narcotic withdrawal syndrome showed significantly less depression of the sucking response than those treated with sedatives such as phenobarbital. These results raise questions about a number of a priori assumptions regarding the safety and efficacy of current treatment methods for maternal and neonatal addiction.

POTENTIAL DEVELOPMENTAL CONSEQUENCES OF SLEEP APNEA IN INFANCY. Lois B. Henning and Alfred Steinschneider (Intro.by Frank A. Oski) State University of New York, Upstate Medical Center, Department of Pediatrics, Syracuse, N.Y.

Studies of the sudden infant death syndrome have led to the hypothesis that recurrent prolonged apneic episodes during early infancy may result in sudden death or produce sufficient cerebral hypoxia to cause developmental defects. It has also been noted that infants with frequent brief apneic episodes during the rapid eye movement (REM) phase of a standardized sleep test are likely to have prolonged periods of apnea. The present study obtained data on the possible relationship between sleep apnea in the newborn period and subsequent development. Thirty-six normal term infants were observed during a single complete nap in the first week of life, during which a polygraphic recording was made of respiration and eye movements. The % of REM epochs during which at least one apneic episode occurred of two sec. or more (% Apnea-REM) was determined. Later, at a median age of 8 mo., each child's mental (MDI) and psychomotor (PDI) development was determined using the Bayley Scales of Infant Development. The average MDI and PDI scores were 112.6 and 110.9 respectively. A Chi-square analysis revealed that subjects with % Apnea-REM of greater than 18 had significantly ($p<.01$) lower MDI scores whereas PDI scores were unaffected. These results support the hypothesis relating sleep apnea and subsequent development. However this relationship applies to mental development with essential sparing of psychomotor functions.

THE LAY THERAPIST AND THE TREATMENT OF THE BATTERED CHILD. C. Henry Kempe, Helen Alexander and Ruth S. Kempe. Univ. of Colo. Sch. of Med., Dept. of Ped., Denver, Colo.

For the past four years, we have utilized the services of 24 lay therapists in the treatment of 65 sets of parents involved in serious child abuse. A group of 4 or 5 therapists consult once weekly with a social worker. 10% of parents were suffering from major psychiatric illness or a very serious aggressive personality disorder and were considered unsuitable for this form of treatment. Treatment is based on the concept that most abusive parents were themselves seriously deprived in infancy and childhood and require a warm "mothering" friendship with someone who is available 24 hours a day in times of crisis and with whom the parents can develop a feeling of trust so that they can subsequently learn to have substantive human relationships with others. The time invested by the lay therapist varies from an average of 20 hours during the first week of treatment to 6 hrs./wk. after discharge from the hospital. The lay therapist is only assigned to a single family during the acute stage of treatment. Improvement is generally noted within 4 months and is marked within 9 months. 80% of 72 abused children who needed to be separated from their parents could be returned home safely within 8 months of the beginning of treatment; 10% required up to 20 months of separation. No re-injuries occurred in this series. Lay therapists are paid $2 an hour and are less expensive and at least as effective as traditional health professionals in the therapy of parents of abused children.

RECOVERY OF INTELLECTUAL DEVELOPMENT OF MALNOURISHED INFANTS IN BOSTON. John D. Lloyd-Still, Irving Hurwitz, Peter H. Wolff, Harry Shwachman, Harvard Med. Sch., Dept. Ped. & Psych. The Children's Hosp. Med. Ctr., Boston

Intellectual performance, sensory motor abilities and social adaptation were studied in 41 subjects (2-21 years) who had severe malnutrition in infancy. Severe malnutrition was defined by a weight below the 3rd percentile for at least 4 of the first 6 months. Thirty-four subjects had Cystic Fibrosis, 4 had congenital lesions of the intestine, and 3 had protracted diarrhea. Mean birth weight was 2.9 kg. (range of 1.9-3.75 kg.). Mean weights during the first 6 months corresponded to 70th percentile malnutrition (58% had 3rd degree malnutrition). The control group consisted of 41 siblings. Mean I.Q. of 31 parents was 108 (S.D. ± 11.3). The Hollingshead 2 factor index of social position of 28 fathers averaged 3. Socio-economic deprivation was not present. The results of the Merrill-Palmer Intelligence test for the malnourished group and the controls revealed significant differences in favor of the controls. No differences were found in the older populations for whom the WISC and WAIS were used. The Lincoln-Oseretsky test of motor development and the Vineland Scale of social maturity showed no significant differences. These results are consistent with the hypothesis that malnutrition in infancy can affect intellectual development in the first 5 years of life. Beyond this age given adequate socio-economic support, no significant differences were observed.

MEMORY/ATTENTION VERSUS VISUAL/PERCEPTUAL CORRELATES OF LEARNING DISORDERS IN A PEDIATRIC POPULATION. J. T. Heriot, Intr. by S. B. Friedman, Univ. of Roch. Sch. of Med. Strong Mem. Hosp., Dept. of Ped., Rochester, N. Y.

The results of two studies conducted at the Psychodiagnostic Lab. cast doubt on the long standing and intense interest on the part of pediatricians, psychologists, optometrists and educators in the visual/perceptual processes. There has been an implicit assumption that the visual/perceptual processes are thought to have etiological significance for learning disorders and it is widely held that visual perceptual remediation offers hope for the alleviation of these disorders. The results of these studies suggest that it is the memory/attention dimension which is most significant. In a study of 341 children referred for learning difficulties, 52% had difficulties in reading or arithmetic with respect to their pro-rated IQ's. Of the children who had difficulties in both reading and arithmetic, 8.7% had difficulties in spatial relationships (SR) - a task with an important visual perceptual component, 47% had difficulties in visual attention (VA), 53% had difficulties in auditory rote memory (digits forward-ARM), and 78% in auditory rote memory (digits backward-ARMB). Of the 48% who did not have difficulties in reading or arithmetic, 10.5% had deficits in SR, 22% had deficits in VA, 7.4% had deficits in ARM and 17.3% had deficits in ARMB. Results were essentially the same in a non-referred, randomly selected population (grades K-12). SR versus VA, ARM and ARMB differences were significant for the underachieving group ($P<.001$).

THE CONTROLLING BEHAVIOR OF MOTHERS OF CHILDREN WITH PHENYLKETONURIA. Steisel, I. M., Katz, Kathy S. and Harris, Sandra L. St. Christopher's Hospital for Children, Temple University School of Medicine, Philadelphia; and Psychology Department, Rutgers University, New Brunswick.

The imposition of a strict diet upon a child with phenylketonuria requires fairly constant surveillance and control by his mother. Do mothers of children with PKU also attempt to control their children's behavior in other situations? Eighteen children with PKU on restricted diet, and their mothers, were observed while engaged in a dyadic problem-solving task. Verbal (and some non-verbal) interactions were rated independently by two examiners on ten variables for the adult and four for the child. Eighteen normal children of comparable IQ, age, sex distribution and social class served as controls. The mothers of PKU children evidenced less verbal output ($p<.025$) and more non-verbal intrusion ($p<.005$) than the other mothers. Total score for controlling behavior was in the predicted direction ($p<.001$). The scores of the two groups of children did not differ. Physicians who prescribe strict diets should be aware of the probability of parental control of children's behavior in non-eating situations.

MINOR PHYSICAL ANOMALIES AND ACADEMIC PERFORMANCE IN FIRST GRADE CHILDREN. Rosenberg, John B. and Weller, George M. St. Christopher's Hospital for Children, Temple University School of Medicine, Philadelphia and Montefiore Hospital & Medical Center, Speech & Hearing Clinic, New York.

This study employed a list of minor physical anomalies developed by Goldfarb and Botstein and later modified by NIMH investigators to assess the association with later cognitive functioning of congenital characteristics which are unnoticeable to the untrained eye. The number of anomalies found among 99 first grade public school children was not significantly related to measures of performance IQ, motor inhibition or classroom behaviors (as rated by teachers), but they were inversely related to verbal IQ ($p<.05$). Moreover, a strong statistical relationship was found between high number of anomalies and academic performance: children with many stigmata were far more likely to repeat the first grade ($p<.001$). These data indicate that congenital factors influence later cognitive functioning, and suggest that prenatal agents responsible for the appearance of physical stigmata may also be responsible for defective academic achievement.

BEHAVIORAL SCIENCE

Read by Title

THE EFFECT OF IRON DEFICIENCY ANEMIA ON SCHOLASTIC ACHIEVEMENT, BEHAVIORAL STABILITY AND PERCEPTUAL SENSITIVITY OF ADOLESCENTS. Thomas E. Webb and Frank A. Oski, Department of Psychology, University of Pennsylvania, Philadelphia, Pennsylvania, and Department of Pediatrics, State University of New York, Upstate Medical Center, Syracuse, New York.

In an attempt to document the effects of iron deficiency on cerebral function, students, 12 to 14 years of age, were compared in their scores of the Iowa Test of Basic Skills, teacher ratings of personality disturbance employing the Peterson-Quay Behavior Problem Checklist, and their performance on a standard visual after-image task. The composite score of the Iowa Test was found to be significantly lower among 92 anemic students than among 101, age and sex matched, controls. Teacher evaluations revealed that the anemic males displayed significantly more conduct problems such as distractibility, over-activity, disruptiveness, and negativism than did the non-anemic males. The iron deficient group demonstrated a significantly longer latency (4.08 sec) than did the non-anemics (1.81 sec) in reporting visualization of an after-image. These findings suggest that iron deficiency does alter cerebral metabolism by producing disturbances in perception and attentiveness which ultimately result in impaired scholastic performance.

HANSEL AND GRETEL PHENOMENON OF ELDERLY MOTHERS, N. Bingol, S. Iosub, M. Fuchs E. Wasserman Ped.N.Y.M. College, N.Y. 10029

One hundred two infants, born to 100 mothers (68% Puerto Rican, 32% Black) who were 36 or older at conception were compared to 101 infants born to 100 control mothers, of the same ethnic and socio-economic background, who were between 18 and 35 years. The infants were observed from birth to 18 months of age. The mean age of the elderly mothers was 39.8 and of the controls 24.7. The data were analyzed at birth and again at 18 months of age by gestational period, sex, weight, length and head circumference. There was a significant number of children whose weight was over the 90th percentile at 18 months in the offspring of elderly mothers (21.4%), as compared to the control group. (5.07%). $p < 0.05$. There was no significant difference in the mean birth weight of the babies born to elderly mothers and the controls (3050 gram and 300 gram respectively). The number of malformations was increased in the study group but this was not statistically significant. The higher percentage of overweight infants may indicate an overconcern of elderly mothers in respect to feeding their offspring considering possibly that an "obese child is a healthy child."

TREATMENT OF ASTHMA THROUGH BIOFEEDBACK TRAINING. Aman U. Khan (Intr. by J. L Schulman). Children's Memorial Hosp., Chicago.

The present study is designed to assess the effectiveness of "conditioned bronchial dilation" as a treatment for asthmatic attacks. The treatment involves the instigation of bronchial constriction followed by "biofeedback training" in bronchial dilation. The bronchial constriction is induced through suggestion, voluntary hyperventilation, exercise, or with the help of medication. The biofeedback training is carried out in 2 phases. During the first phase (5 sessions), a preliminary training is given, in the absence of bronchial constriction, to allow for formation of an "initial link" between the rewarded response (bronchodilation) and whatever internal or visceral cues are available to the child for self-initiation of bronchodilation. The child breathes into a biofeedback apparatus and tries to increase his expiratory peak flow rate. He is rewarded for increasing his peak flow rate by a signal light and by verbal praise. During the second phase of the training (10 sessions), the "conditioned link" is utilized to help the child overcome the bronchial constriction, which is induced experimentally by one of the above mentioned means. A refresher training period of 5 sessions is provided after each of the 1st, 2nd, 3rd, and 6th month, to reinforce the conditioned link. The results indicate that the experimental group (15 asthmatic children between the ages of 8 to 15) had significantly less number of asthmatic attacks, emergency room visits, hospitalization days and amount of medication as compared with a comparable control group over a period of 6 months.

CHILD ABUSE--A COMMUNITY HOSPITAL TEAM APPROACH Robert S. Chabon, Geoffrey B. Barnes, Leonard J. Hertzberg (Intr. by E. Kaplan). Sinai Hospital of Baltimore.

A battered child health team has been established in a Baltimore community teaching hospital to provide coordinated care for abused children and their parents. The program is federally grant supported and operates in close liaison with the local Child Protective agencies. The team consists of a pediatrician, psychiatrist, social worker, public health nurse, and community aide. It has provided intensive support to 22 families in its first operational year. Utilizing consultants and tools in collaboration with the University of Maryland School of Social Work the program has undertaken an investigation into the structure and dynamics of abusing families in order to establish criteria for predicting child abuse. Preliminary results of this study suggest that a statistical approach might prove feasible for such criteria, and that certain provocative family lifestyles are identifiable as high risk for child abuse.

ANOREXIA NERVOSA: SUCCESSFUL APPLICATION OF A FAMILY THERAPY APPROACH. Salvador Minuchin, Lester Baker, Ronald Liebman, Leroy Milman, Bernice Rosman and Thomas Todd. Child. Guid. Clin. and Child. Hosp. of Phila. (Intr. by Robert Kaye)

Anorexia nervosa is a disease with significant mortality (10 to 15%) and morbidity (long hospitalizations, frequent relapses). Many therapeutic approaches have been advocated with poor success.

Our group has put forward a conceptual model of the "psychosomatogenic family", characterized by the qualities of overprotectiveness, enmeshment, rigidity and lack of conflict resolution. The involvement of the child in family conflict is also conceived of as a critical variable in the evolution of a psychosomatic symptom.

In the past 3½ years, structural family therapy based upon this model has been used to treat successfully 20 cases of anorexia nervosa. These patients were seriously ill, as evidenced by a mean weight loss of 25% (range 20 to 38%). Hospitalization has been reduced to a mean of 17 days (range 10 to 25 days). The mean duration of family therapy has been 6 months (range 4 to 9 months). Followup for 6 to 40 months in 16 completed cases indicates there have been no relapses or development of pathology in other members of the family.

These results suggest that a family therapy approach may be a significant breakthrough in the treatment of anorexia nervosa.

A DEVELOPMENTAL PROGRESS QUESTIONNAIRE (DPQ). William K. Frankenburg and Bonnie W. Camp. Univ. of Colo. Sch. of Med., Dept. of Ped., Denver, Colorado.

Though extensive use is made of parents' reports pertaining to the development of their children, there is a paucity of data concerning the validity and reliability of these reports. As a first step in developing a valid developmental questionnaire 70 developmental questions (based upon 70 Denver Developmental Screening Test [DDST] items) were composed and arranged into age categories from 3 months to 6 years. Initially, 102 mothers were asked to answer questions pertaining to the developmental status of their children. Parent reports were then validated by administering the DDST to the same children. Agreement between parent report and DDST findings varied from 62% to 100% (mean 89%) for each of the items. Subsequently, questions with low agreement were revised. The DPQ was then administered to 720 mothers (360 low socio-economic class and 360 middle to high socio-economic class) and the DDST was administered to their children to determine the validity of parents' reports. No significant differences were found between the two examiners or the socio-economic classes for agreement between the DPQ and the DDST. The mean agreement for all of the items was 95%. Contrary to expectation, mothers significantly underestimated (3.2%) rather than overestimated (1.9%) the development of their children ($p < .01$). These findings demonstrate that with carefully worded questions it is possible to achieve valid parental reports of the current developmental status of their children.

THE RELATIONSHIP OF DRUG USE TO NUTRITIONAL, CYCLICAL AND ROUTINE HEALTH PRACTICES AMONG ADOLESCENT BOYS. C. Jack Friedman, Maarten S. Sibinga and Alfred S. Friedman, Phila. Psychiatric Center and Dept. of Ped., Temple Univ. Med. Sch. at St. Christopher's Hosp. for Child., Phila., Pa.

The possible relationship between use of drugs and nutritional factors, cyclical patterns of sleeping and wakefulness and related health practices among adolescent boys was studied.

The 498 subjects came from correctional institutions, a vocational program and intact advisory groups in a high school.

Method: Drug use was assessed by a questionnaire; use and extent of use of 7 classes of drugs were determined. Health practices were evaluated by answers to 21 statements of regularity or irregularity of eating and sleeping, as well as height and weight.

Results: Drug users were significantly lower in weight percentile scores than non-drug users. Their eating habits were more irregular, they ate lunches at school less often and their own evaluations of their health were poor. The greater the drug use the more sleeping-waking disturbances they had.

The findings demonstrated a strong association between the use of drugs and disturbances in eating, sleeping and related health practices. It is conceivable but by no means proven that these practices predated drug use and that boys whose activity-rest patterns were irregular had a greater inclination to use drugs.

Health practices and eating and sleeping-waking habits can be assessed to provide an indirect measure of drug use.

A COMPARISON OF THE PEDIATRIC HISTORY AND PEDIATRIC NEUROLOGICAL EXAMINATION IN THE DIFFERENTIAL DIAGNOSIS OF LEARNING DISABILITIES. PATRICIA L. HARTLAGE AND LAWRENCE C. HARTLAGE. DEPTS. OF PED. AND NEUROLOGY, MED. COL. OF GEORGIA, AUGUSTA, (INTR. BY ALEX F. ROBERTSON, III). The purpose of the study was to determine and compare the absolute and relative contributions of the pediatric history and pediatric neurology examination in the differential diagnosis of common types of learning disabilities. One hundred children who had been comprehensively diagnosed were studied, including 43 with minimal cerebral dysfunction (MCD), 20 with dyslexia, 11 with dull normal intelligence, and 17 with emotional disturbances. In the MCD children 70% had abnormalities on the history and 88% on the neurological examination. Among dyslexics, histories were non-contributory and although there were soft signs in nearly 60% of the cases, it should be noted that these signs almost always involved right-left confusion rather than motor system abnormalities. Children of dull normal intelligence were free of neurological findings, with developmental history significant in almost all cases. Neither medical history nor neurological examination were contributory in emotional disturbance. False negatives on the basis of both history and examination were most likely to occur in dyslexics and emotionally disturbed children. False positive interpretations of neurological findings need to be guarded against in children of lower intellectual abilities, since neurological maturation needs to be compared to the child's general intellectual maturity rather than his chronological age.

A FIVE YEAR FOLLOW-UP OF CHILDREN PREVIOUSLY IDENTIFIED AS CASES OF "SUSPECTED ABUSE". Stanford B. Friedman and Carol W. Morse, Depts. Ped. & Psychiat. Univ. Rochester Sch. Med. & Dent., Rochester, N.Y.

156 children under 6 years of age seen for injuries in an Emergency Department were previously studied and their injuries judged by the investigators to represent unreported "suspected abuse", "gross neglect", or an "accident" (Ped., 42:128, 1968). 5 years later, all cases of "suspected abuse" and "neglect", and a random sample of "accidents", for a total of 54 children, were included in a study involving interviews of parents and survey of medical facilities for subsequent contact with these children. 41 (76%) of these families were located and available for study, including 15/17 and 7/10 of the abuse and neglect cases, respectively. Subsequent injuries requiring medical attention occurred in 87% of the previously diagnosed "suspected abuse", in 71% of the "neglect", and in 58% of the "accident" cases. A similar distribution will be reported for the total number (77) of injuries. 4 children were again judged to have been victims of abuse, and all were in children previously judged to be in the "suspected abuse" category. 75% of the siblings of "abuse" and "neglect" cases, and 52% of the siblings of "accident" cases, also sustained injuries over the 5 year period.

PLANNING & EVALUATING A PROGRAM TO PREVENT INFANTILE IRON MALNUTRITION Ray Hepner, Norma Maiden, Margaret Breitenstein, Alice Rose, George Lentz, Dept.Ped,Sch.Med,Univ.Md,Baltimore

This report presents methods of and use of iron nutrition surveillance in C&Y Project 606A. Use of surveillance data for measuring dietary iron requirements of children 0-8 yrs of age was presented to APS in 1972. This paper describes how a Model Cities program was planned by us to be carried out by them.

Computerized data on wt,hem,hct at 4,6,9,12mos give prevalence of anemia due to Fe^{++}deficit & Fe^{++}deficit in mgms/child & mgms/Kg. In'67-'69 Fe^{++}deficit was 100mgms/child & 8mgms/Kg by 1 yr (955 pts).

Fe^{++} Deficiency by Age before Program (955 pts)

	4/12	6/12	9/12	1 yr
Mgms/child	25	40	70	100
Mgms/Kg	4	6	7	8

From the above surveillance data, Fe^{++} enriched formula was sent to all newborns to age 9 mos to prevent 70mgms deficit/child at 9 mos, & to reduce deficit from 8 to 1mgm/Kg at 1 yr.

The program in '70-'72 reduced prevalence of hem $<$10gms% with MCHC $<$30 from 55% to 2.5% & Fe^{++}deficit from 100 to 26 mgms/child & 8 to 1.2Kg at 1 yr (848 pts).

	N	Hemoglobin (gms%) Mean	% $<$10	Fe^{++} Deficiency at 1 yr. Mgms/Kg	Total Body
Before	(955)	10.1±1.2	55	8	100
After	(848)	11.6±0.6	2.5	1.2	26

Conclusions: Age specific nutrition surveillance provides objective data for accurate program planning & for evaluating impact of programs implemented by others.

THE LONG TERM PROGNOSIS OF CHILDREN BORN WITH LINEAR SKULL FRACTURES, Marvin Green, Herbert Rich and Myron Gordon, Depts. of Ped., Obs. and Gyn., New York Medical College - Metropolitan Hospital Center, New York.

Although the immediate effect of simple linear skull fractures in neonates is considered to be benign, the long-term outcome is uncertain. Among 31,500 children in the Collaborative Study, 16 were diagnosed with skull fractures at birth. These infants were followed for periods up to 4 years. Neurological examinations were made in the neonatal period and at 1 year and psychological tests at 8 months and 4 years. This report deals with the associated obstetrical events as well as the immediate and long-term outcome. Comparisons were made with the core population by means of appropriate statistical tests of significance. There was a higher proportion of primigravidae (13/16, $p < .01$), and of mid-forceps applications (11/16, $p < .01$), while the incidence of OA presentations was lower (9/16, $p < .05$) than in the core. Apgar scores less than 4 were not encountered, and no newborn infant was neurologically abnormal. Only 1 child weighed more than 4000 grams. Mental and motor scores at 8 months were not significantly different from core, and none of the children examined were neurologically abnormal at 1 year. Moreover, the distribution of I.Q. scores at 4 years also failed to show significant differences. These findings suggest that linear skull fractures in the neonate are not associated with impairment of CNS function later in life.

NUTRITION SURVEILLANCE AS A TOOL FOR PROGRAM PLANNING & EVALUATION Ray Hepner, Norma Maiden, Priscilla Guild, Margaret Breitenstein, Viviana Amzel, George Lentz, Dept.Ped.,Sch.Med., Univ. Md., Baltimore. (Intr. by Felix Heald)

This paper presents nutrition surveillance in school health programs by C&Y Project 606A for 5,284 Baltimore inner city children from 1967-71, & use of these data for program planning & evaluation. Annual measurements at ages 5,8 & 11 yrs included hemoglobin, serum albumin & vitamin A, weight,height & humeral skinfold(HSF). All ages had low means & excess abnormal values.

Public support (government & private) for free school meals was gained by use of these data, & meals rose from 127 to $>$3,700/day for our 5,284 elementary school children.

Meals began in '69 for grades K,1,2,in '70 for 3,4,& in'71 for 5,6 so that "not yet fed" ages serve as controls for"fed" ages while surveillance continued. "Not yet fed" remained the same, while "fed" mean values rose($P = <.01$) & excess abnormal values fell ($P = <.01$) 1 yr after meals began for each group.

Age	5yrs		8yrs		11yrs	
Meals Status	Before	After	Before	After	Before	After
Number Subjects	165	259	181	196	205	197
	% Abnormal Values (all significant, $P = <.01$)					
Hem $<$ 11.0gms%	25	6	7	1	6	2
Alb $<$ 3.6gms%	47	0	22	4	26	2
Vit A $<$ 20mcgm%	24	8	8	1	7	1
HSF $<$ 3rd%ile	68	28	36	8	24	4

Conclusions are: nutrition surveillance provides objective data for proving need, gaining support, designing programs & evaluating program impact.

NESTING IN THE HUMAN MOTHER AFTER MOTHER-INFANT SEPARATION. John Kennell, David Chesler, Harriet Wolfe, and Marshall Klaus. Case Western Reserve University School of Medicine, Department of Pediatrics, Cleveland, Ohio.

Even in nurseries where mothers are permitted early handling of their premature infants there is still a distressing incidence of mothering disorders. Successful adoption in animal mothers requires close contact and isolation with young and may take many days rather than the usual few hours. To enhance the attachment of a mother to her premature infant we developed a procedure somewhat similar to arrangements made for cross-adoption in animals: 15 mothers whose infants ranged in weight from 1005-2150 g at birth were provided with a special 3-day period of close physical contact with privacy (nesting) just before discharge, during which the mother gave complete care to her infant with support readily available. During the nesting 13/15 mothers were unable to sleep the first night, 6/15 rearranged the furniture, and 8/15 changed the feeding schedules. These mothers also showed and expressed increased confidence in caretaking. One month after discharge their feeding performance was compared in detail by time-lapse film analysis with a matched group who had only early contact. There were no significant differences in measures of attachment or caretaking skills. *These observations suggest the need to study alternative strategies such as earlier nesting or increased physical contact to enhance maternal attachment and optimize mothering behavior.*

MEDICAL STUDENT SELF-EVALUATION IN A FIRST-YEAR CLINICAL SCIENCE PROGRAM. John Kennell, Caroline Tempio, and Marcia Wile. CWRU Sch. of Med., Dept. of Ped., and Sch. of Applied Social Science, Cleveland, Ohio.

Self-evaluation as an appraisal technique for first-year medical students was initiated in 1968 at CWRU. Previously the major responsibility for evaluating student performance and progress resided with the faculty. On the basis that ongoing self-assessment can contribute to the self-knowledge and discipline vital for professional functioning, students were given responsibility for evaluation which emphasized student appraisal of progress confirmed and supplemented by preceptor observations. Each student was to assess his performance, abilities and growth in such areas as interpersonal relationships, group participation and sense of responsibility. At 2 one-hour student-preceptor conferences the student had the opportunity to discuss his self-appraisals. In 1971 the total preceptor group membership from the classes of 1972 and 1973 was individually interviewed by the third author. The purpose was to obtain feedback on the effect of the self-evaluation experiences on the students' professional development. Most of the students viewed the 2 self-evaluation experiences as worthwhile and contributing to professional growth. Changes in behavior and attitudes toward patients, peers, and professional associates were cited, as well as in perceptions of self.

DO MOTHERS' ANSWERS TO A QUESTIONNAIRE ADEQUATELY EVALUATE THE DEVELOPMENT OF INFANTS? Hilda Knobloch, Susan Gross, Robert Holsapple, Hugh Lafave, Frances Stevens, Judith Tate. Albany Medical College & N.Y. State Dept. of Mental Hygiene, Albany.

To answer this and detect deviant infants early, questions from a Developmental Screening Inventory of behavior for ages 20-32 weeks were mailed for all 603 infants born in a year when they were 28 weeks old. Information about hearing, convulsions and parental concerns also was requested. A home screening exam was done for all suspects and half of the normals. All infants with suspected dysfunction or seizures and a subsample of the normals had a full Gesell Developmental and Neurologic Examination. Comparisons could be made between the M.D. diagnosis and the M.D. evaluation of mothers' answers for 82 of 87 seen. The total incidence of dysfunction was low, 6%. The mothers identified the 5 with major deficits and the 7 otherwise normal infants with seizures. For the 22 with minor neuromotor problems, one mother said yes for all of the behavior items. Two infants had unilateral disabilities but could achieve and could not have been detected by the questionnaire; one of these was screened because of seizures.

Less than 10% of infants in need of early supervision are not identified by the 80% of mothers who respond to a mail questionnaire or staff visit. About the same percentage of normal infants are overreferred. In office or clinic practice the questionnaire is eminently satisfactory. More difficult logistic problems are posed if one wishes to achieve 100% coverage of a given population.

EDUCATIONAL OBJECTIVES FOR HOUSE STAFF IN THE PEDIATRIC INTENSIVE CARE UNIT (ICU) Richard E.Kravath,Albert Einstein Col. of Med.,Montefiore Hosp. & Med. Ctr.,Dept. Pediatrics, The Bronx, New York. (Intr. by Laurence Finberg)

*Explicit statement of objectives has importance to an educational program.*Educational objectives for a pediatric ICU were written, based on the work of Gronlund, stressing what is learned rather than what is taught and listed in steps of increasingly complex cognitive and affective behavior. Examples of cognitive behavioral objectives follow: (1) Knows indications,contraindications,actions,side effects and toxicity of commonly used drugs. (2) Evaluates information properly: recognizes assumptions,resolves conflicting opinions of consultants. (3) Applies knowledge of basic sciences to patient care. Examples of affective behavioral goals follow: (1) Aware of social factors in illness. (2) Accepts importance of his role in dealing with death and critical illness;ability to recognize and manage his own emotions; supports family of dying patient. (3) Accepts his own limitations but is independent to the limit of his ability. This formulation has forced a critical look at the methods and content of the educational program. A result is that less time is spent on trivia,and attempts to achieve minor goals less often frustrate the achievement of major ones. Affective goals and higher levels of cognitive functioning are easily overlooked in the traditional medical setting,and it is *sometimes better to ask a question than to give an answer, or* an order. This scrutiny of goals was helpful in the ICU and should be useful in other areas of medical education.

APPARENT NON-RELATIONSHIP OF HEMOGLOBIN LEVELS WITH BLOOD LEAD LEVELS IN AN INNER CITY PRESCHOOL POPULATION. A. H. Lubin, G. M. Owen and P. J. Garry, Ohio State Univ. College of Med., Dept. of Ped., Children's Hospital, Columbus, Ohio.

Elevated blood lead levels are believed to cause anemia. A recent survey of 417 inner city children (88% black) showed an inverse or non-relationship of hemoglobin levels and percent transferrin saturation with blood lead levels.

Sex	Age	Lead Level (μg%)	Hemoglobin (mg%)	Transferrin % Saturation	N
Females	1-6 yr.	< 40	12.1±1.1	21.0± 9.7	137
		≥ 40	12.3±1.0	20.6±10.2	39
Males	1-6 yr.	< 40	12.2±1.2	19.5± 9.3	130
		≥ 40	12.4±0.9	18.3± 8.8	61

Although anemia was prevalent (13% of children ≤ 11 gm. Hb; 7.5% ≤10.5 gm. Hb.) as were elevated blood lead levels (23% of children with blood lead levels ≥ 40 μg%), the anemia was primarily iron deficiency in nature. For children 12-23 months old(e.g.) holding transferrin saturation constant, hemoglobins tend to be higher in the children with elevated lead levels.

	Hemoglobin by %Saturation Transferrin (N)			
Lead Level(μg%)	< 10	10-14	15-19	> 20
< 40	10.4(15)	11.6(8)	12.0(7)	11.8(13)
≥ 40	11.7(13)	11.7(4)	11.8(3)	13.1(7)
	p=< 0.01	NS	NS	p=< 0.025

These data suggest that adverse affects of lead on heme integrity and synthesis are less common than generally believed and are seen primarily in children with lead levels ≥ 60 μg%.

THE EFFECTS OF NON-AVAILABLE DATA ON PREDICTING THE RISK OF PARENTAL CHILD NEGLECT IN A GENERAL CLINIC. Edwin J.Mikkelsen and Paul J. Nelson. (Intr. by Gerry Van Leeuwen), University of Nebraska Medical Center, Department of Pediatrics, Omaha.

The decade since Kempe's delineation of the Battered Child Syndrome has seen scores of articles published on all aspects of child abuse, and yet there have been few projects designed to explore the ill-defined area of child neglect. Believing that the neglecting parent can be equally damaging to a child's development and that these parents have a latent capacity for abuse, we have set out to develop a screening device for child neglect.

A questionnaire was developed by a survey of our Departmental Staff as to factors they believed were indicators of child neglect. The resultant check list was completed on every 5th of 4,350 outpatients seen over a 4 month period in our General Clinic. At the end of each questionnaire, the clinic physician assessed the patient's child neglect risk on a scale of 0 to 4. An extensive chart audit utilizing all University and Douglas County Welfare records was then performed by the authors on every 10th family of the 870 randomly selected in the first evaluation. In this subsample, we tabulated the factors picked up by the clinic staff and ourselves, and we also determined correlation coefficients between the presence of each factor and the assessed risk. The results aptly show that the evaluations made by the clinic staff were deficient primarily in the area of social history.

THE UNIVERSITY MEDICAL CENTER AND SCHOOL HEALTH
P.R. Nader, Int. by R.J. Haggerty, Depts. of Peds. and Psychiatry, University of Rochester School of Medicine, Rochester, New York

Research results after 5 years experience with one of the first Medical Ctr. School Health training programs indicate: 1) new roles for health personnel in 2 (urban and suburban) demonstration school health projects have influenced teachers and effected the nature and rate of referrals when compared to similar schools without such projects. 2) three separate 1% county-wide samples of children's (5-17) school functioning from 1967-71 document about 1/4 to have experienced school problems. Proportionally more exist among boys, blacks, and ghetto dwellers. 3) a large (7414) survey of high school students' drug use shows student perception of drug use "among friends" to exceed their own admitted use. 4) other studies describe elementary school children's visits to the nurse, and attitudes and knowledge regarding health, drug, and sex education.

Community-school programs are also effective (a) in carrying out preventive health roles for pediatricians; and (b) providing labs in child development and sites for multidisciplinary training of medical/nursing students, pediatric residents, fellows, practitioners, graduate students in education, psychology and school personnel.

THE DECREASING UTILIZATION OF CHILD HEALTH SERVICES
Klaus J. Roghmann and Robert J. Haggerty. Univ. of Rochester, Sch. of Med. and Dentistry, Dept. of Ped. Rochester, New York

A drop in the utilization rate of child health services has been observed nationwide. This trend, together with the dropping birthrate and the stepped up training of nurse practitioners may significantly reduce the future need for more pediatricians. Data from community surveys in 1967, 1969 and 1971 are analysed to examine this trend and the concurrent shifts in the source of care.

Over the four year period the utilization rate dropped 15%, from 6.6 visits per year to 5.5. The reduction was greater in the private sector (-16%) than in the public sector (-9%). The reduced utilization of the emergency room (-12%) was partly compensated by increased services at clinics and health centers. Some of the reduction was due to the changing age structure: the proportion of infants and preschoolers in the total child population has been shrinking. Some changes were due to legislative action. Changes in New York Medicaid regulations led to a shift from the private to the public sector. The liberalized abortion law reduced the number of high risk pregnancies. These factors will continue to operate, but a leveling off in the decline can be expected.

PHYSICAL IMPAIRMENT IN CHILDREN WITH LEARNING DISORDERS (L.D.) A PILOT PROJECT
Philomena Newberry, David L. Sackett, William Feldman (Intr. by Alvin Zipursky) McMaster University Medical School, Dept. of Pediatrics and Clinical Epidemiology, Hamilton, Ont. Canada

In order to assess the pediatrician's role in the management of learning disorders, 25 children (mean age 11 yrs. 11 mos.) in 3 L.D. classes had complete physical examinations including neurological assessments, full-scale audiometry, visual acuity and the Werry-Weiss test for hyperactivity. Significant results revealed visual problems (acuity < 20/40) in 28%, visual handicap (<20/70) in 4%, hearing impairment (>35 d.b. hearing loss) in 40%, "soft" neurological signs in 64%, and hyperactivity in 30%. Although 52% were discovered to be failing in Kindergarten or grade 1, medical assessment prior to our study had been done in only 28%, in spite of the fact that all patients had private physicians.

Our study suggests that pediatricians trained in assessing children with L.D. be affiliated with school boards in order to assess all Kindergarten children whose performance is inadequate.

ALLERGY TO FOODS: A COMMON CAUSE OF GROWTH FAILURE.
Douglas H. Sandberg, Patricia W. Conly, Charles W. Bernstein, and William W. Cleveland. Dept. of Ped., Univ. of Miami Sch. of Med., Miami, Florida.

A group of 100 children with growth retardation for which no demonstrable cause had been found have been found to have a history of respiratory allergy in 72% of first-degree relatives. Forty-two percent of the patients had a positive history of allergic respiratory disease and 49% of the patients had a history of food intolerance as infants. Typically, linear growth rate curves showed a period of normal growth followed by a decrease in rate beginning at 6-18 months of age with eventual resumption of a normal rate in most instances. Of 15 patients carefully studied, 10 had abnormal concentrations of serum immunoglobulins. Eight of the 15 children have shown an increased rate of growth on diets excluding certain foods (e.g., milk, wheat, corn). In several children, food hypersensitivity was tested by oral challenge with suspected foods with measurement of alteration of C3 complement by crossed immunoelectrophoresis. Six children with chronic disease such as glygocen storage disease, diabetes, cirrhosis, etc., have increased their rate of growth on similar diets; 2 mg/Kg of zinc daily has not increased the rate of growth in these children. Cyproheptadine temporarily increased appetite, weight gain and linear growth rate in a few children who presented with poor appetite.

ANTHROPOMETRY OF PRESCHOOL CHILDREN: DIFFERENCES BETWEEN BLACK AND WHITE CHILDREN by George M. Owen and A. Harold Lubin. Ohio State Univ., Col. of Med., Children's Hosp., Dept. of Ped., Columbus, Ohio.

Both on an absolute basis and when examined with socioeconomic status held constant, black preschool children in comparison with white preschool children are taller, heavier and have less subcutaneous fat. The magnitude and direction of mean differences are summarized for 621 children, 271 black and 350 white, of comparable socioeconomic status.

Age Interval (yrs)	Black-White Differences Height(cm)	Weight(kg)	Skinfold(mm)
	Boys		
1.50-2.49	+1.29	+0.37	-0.09
2.50-3.49	+1.86*	+0.70*	-0.29
3.50-4.49	+1.27	+0.48	-0.37
4.50-5.49	+2.20*	+1.10*	-0.45
	Girls		
1.50-2.49	+1.21	+0.64*	-0.28
2.50-3.49	+1.52	+0.83*	-0.24
3.50-4.49	+1.74	+0.51	-0.34
4.50-5.49	+0.65	-0.26	-0.22

*($p < .05$)

These findings, based on a national probability sample, suggest the need for different standards when assessing nutritional status of childhood populations.
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AGE SPECIFIC HOSPITALIZATION RATES BY AGE OF INITIATION OF COMPREHENSIVE CARE. Fred Seligman and Marta Carvajal, (Intr. by William W. Cleveland), University of Miami School of Medicine, Department of Pediatrics, Miami, Florida.

During a 4 1/2 year period 1083 hospitalizations of patients occurred after the patients were accepted for comprehensive health services. Annualized rates of hospitalization were computed by age of registration into the program. These data indicate that age specific hospitalization rates tend to increase the older the child is when accepted into comprehensive care. For example, at age 9-12 months rates of hospitalization are 41.8, 53.7, 139.1, and 461.5 for children who entered comprehensive services at ages 0-3 mo., 3-6 mo., 6-9 mo., and 9-12 mo. respectively ($p < .01$). Regardless of age that care is initiated, rates lower than the national pediatric average (62.6) are achieved after a child is in care for about 1 year; e.g., for patients registered at 6-9 mo., age specific rates are 254.0, 139.1, 65.6, 57.1, and 38.8 at 6-9 mo., 9-12 mo., 12-18 mo., 18-24 mo., and 2-3 years, respectively. This data supports the notion that comprehensive services in regards to hospitalization rates have the greatest impact the younger the patient is when care is first initiated.

PHYSICIANS AS CHILD BATTERERS. Maarten S. Sibinga and William B. Carey (intr. by Victor C. Vaughan, III), Depts. of Ped., Temple Univ. Med. Sch. at St. Christopher's Hosp. for Child. and Univ. of Pa. Med. Sch. at The Child. Hosp. of Phila., Pa.

PROBLEM: Physicians are generally alert to child battering by parents but child battering by physicians has not been adequately acknowledged and dealt with. As with parental child abuse, the frequency and severity of this form of battering is probably seriously underestimated and largely ignored.

DEFINITION: Any unnecessary traumatic procedure to a child whether injury results or not, even though this is usually cloaked in the guise of orthodox medical treatment.

PATHOGENESIS: May stem from the physician's personality, education or experience; or pressure from parents may make the physician resort to physical manipulations which reflect poor preparation for insightful management of family interactions or doctor-patient relationship.

CLINICAL EXAMPLES: Tonsillectomy, adenoidectomy; urethral dilatation or cystoscopy for enuresis; Denis-Browne splint for tibial torsion; "shot of penicillin" for common cold, etc.

MANAGEMENT: Parent can comply, complain, withdraw. Physician can resist pressure, reform self, intervene in parents' pathology. Other physicians usually react with a conspiracy of silence. Prognosis of this phenomenon is related to reform of the medical system and to public education. Prevention remains primarily the responsibility of the medical profession. Removal of financial incentive may be necessary. How long can we stand idly by while this form of battering persists?

THE SCHOOL NURSE PRACTITIONER: Assessment and Implications. Henry K. Silver, Judith Bellaire, Norman Hilmar, John Lampe, Patricia McAtee, Nancy Nelson, Aria Rosner. Univ. of Colo. School of Med., Dept. of Peds., Denver.

The school nurse practitioner, a new category of health professional, can provide extensive care for the well school-aged child and assess the factors in others that may operate to produce learning disorders, psychoeducational problems, perceptive-cognitive difficulties, and behavior problems, as well as those causing physical disease.

Evaluation of school nurse practitioners and comparison of their performance with that of "regular" school nurses shows that school nurse practitioners spend twice as much time in furnishing health care to students; provide more thorough and extensive assessment of health problems; spend less time with administrative activities; and have more contacts with students, school personnel, and parents. We have also found that school nurse practitioners exclude only half as many patients from school; manage a significantly greater proportion of patients themselves; give parents more meaningful recommendations for specific management of their children's health problems; and are only half as likely to refer pupils to physicians or others for consultation or care.

School nurse practitioners can carry out a number of activities that most general practitioners and many pediatricians are not prepared to perform. The effective utilization of school nurse practitioners would significantly improve the availability of health care for children and have favorable economic implications for school systems and parents.

PRIVATE PEDIATRIC PRACTICE: PERFORMANCE AND PROBLEMS. Barbara H. Starfield, Henry M. Seidel, Gertrude S. Carter, William F. Garvin, Johanna M. Seddon. The Johns Hopkins University Medical Institutions, Baltimore, Maryland.

All 105 pediatric practitioners in the Baltimore area were invited to join in a study assessing the quality of health management. The 15 who volunteered helped to set the criteria which were modifications of the standards set by The American Academy of Pediatrics. Medical records of 5-year olds were audited for recording of preventive procedures, throat cultures for suspected phyaryngitis, and urine cultures for suspected urinary tract infection. Some procedures were uniformly well performed; the performance of others varied widely. None of the practices had high rates of recording for all procedures. Existing records proved inadequate to assess the sequence of performance of activities and the relationships between the problems, diagnostic procedures, responses, and follow-up. Data from questionnaires sent to all 105 physicians indicated that the performance of the 15 participants was at least as high as that which would be found if all the practitioners in the area had participated.

This study has implications for the way in which peer review activities are organized and carried out.

DEVELOPMENTAL PATTERN OF BLACK INFANT IN LOW SOCIO-ECONOMIC ENVIRONMENT. Rosalind Y. Ting. (Intr. by David Cornfeld). The Children's Hospital of Philadelphia, Department of Pediatrics, University of Pennsylvania.

Using Gesell developmental schedule a comprehensive examination was done on 50 Black healthy infants ranging from 6 months to 36 months of age living in a low socio-economic section in Philadelphia. Comparable numbers of Black infants from higher standard neighborhood and infants from other ethnic background of the same age range were also evaluated with the same technique. Data analysed included motor, adaptive, language and personal-social behaviors. Each area in each child is calculated by DA/CA into + or - % rate. The results show one striking feature, 2/3 of the Black infants from low socio-economic environment have a lower score in language behavior of ≥ - 15%, primarily in their expressive language. The lack of spontaneous or responsive vocalization is more noticeable among infants than among toddlers. However the quality of speech is inferior among toddlers, as poor articulation and limited vocabularies occur in much higher incidence, compared with other groups. Background social history is also being compared and analysed. Further comparison is being made between the infants who are enrolled in a Day Care Center and those who are reared in home environment only.

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LEAD POISONING IN A SCHOOL FOR CHILDREN WITH MENTAL RETARDATION. L. Weiner, B. F. Oski, H. L. Weinberger and F. A. Oski. Department of Pediatrics, State University of New York, Upstate Medical Center, Syracuse, New York.

Surveys for the detection of lead poisoning have concentrated primarily in non-institutionalized children under the age of 6 years. In order to determine the scope of this problem in an older age group as well as assess its prevalence among children with moderate to severe mental retardation, a survey was conducted in a daytime school for patients with mental retardation. Blood lead and red cell delta-aminolevulinic acid dehydrase (ALAD) activity was measured in 89 children of whom 62% were over age 6 years. Study revealed that 17% of this population had significantly reduced red cell ALAD activity, suggesting the presence of an increased body lead burden. Blood lead values of greater than 40 micrograms percent were recorded in 8% of the students. A random survey of children under age 6 years of age tested in the pediatric ambulatory facility revealed that 32% of the population had blood lead values in excess of 40 micrograms%. This study indicates that lead poisoning is a problem in the older child as well as the toddler. It remains to be determined if it represents a significant etiologic factor in the mental retardation of 62% of the school population in whom no specific cause of the mental retardation is presently recognized.

CARDIOLOGY

First Session

"SLUGGISH" SINUS NODE SYNDROME IN CHILDREN AFTER OPEN HEART SURGERY FOR ACYANOTIC CONGENITAL HEART DISEASE

Nugent, E.W., Varghese, P.J., and Rowe, R.D.
Johns Hopkins Hospital, Department of Pediatrics, Baltimore

Sinus node function (SNF) was assessed in 5 patients who developed atrial dysrhythmia following repair of acyanotic congenital heart defects. SNF was evaluated by: (1) submaximal exercise, (2) intravenous atropine 0.01 mg/kg, and (3) overdrive atrial pacing (OAP) at a rate of 120-150/min. With exercise the rate did not increase in 2 patients while in the others the rate increased transiently and the P vector in the frontal plane shifted from +30° to +75° suggesting a transient shift of pacemaker to the sinus node (SN). Response to atropine was similar to exercise. In all patients escape intervals measured after OAP were markedly prolonged. Most patients escaped to a high right atrial focus although in one the His bundle was the focus. In all patients 1:1 conduction to the ventricle occurred at varying paced rates excluding any A-V nodal dysfunction. The prolonged escape interval in these patients indicates SN dysfunction (SND) as the etiology of their atrial dysrhythmia. In 3 patients "sluggish" response of the SN was seen as a transient increase in atrial rate with exercise. The appearance of SND in the postoperative period suggests that the SN might have been damaged at the time of surgery. The prognosis in these patients is guarded because they may develop fatal bradytachyarrhythmias.

THE Q-oTc INTERVAL AS AN INDEX OF HYPOCALCEMIA: CORRELATION WITH IONIZED CALCIUM IN PREMATURE AND FULL TERM NEWBORNS. Richard Colletti, Mary Pan, Edward Smith and Myron Genel. (Intr. by Norman S. Talner). Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

Clinical tradition asserts that the ECG reflects hypocalcemia as prolongation of the Q-T interval. The usefulness of the ECG as a rapid and reliable screen for neonatal hypocalcemia was assessed by correlating the limb lead ECG Q-T interval corrected for heart rate (Q-Tc) with simultaneous total calcium (TCa) and ionized calcium (ICa) in 26 full term (FT) and 30 premature (<38 wks gestation) newborns. Hypocalcemia was arbitrarily defined as TCa<7.5mg% and ICa<3.5mg%.

In FT infants the Q-Tc correlated moderately with TCa ($r=-.47, p=.02$) and improved with ICa ($r=-.67, p<.001$). In contrast, in premature infants the Q-Tc did not correlate with either TCa or ICa. However, use of the corrected Q-oT interval (Q-oTc), taken as the interval from the origin of the Q-wave to the origin of the T-wave, resulted in significant correlations in the premature group (TCa $r=-.36, p<.04$; ICa $r=-.49, p<.01$), and improved the correlation for both TCa ($r=-.66, p=.001$) and ICa ($r=-.71, p<.001$) in the FT group.

Prolongation of the Q-oTc was defined as ≥.190 in FT infants and ≥.200 in premature infants; application of these criteria separated hypocalcemic from normocalcemic infants with little overlap (χ^2 $p<.001$ for both groups). Thus the ECG is a useful index of neonatal hypocalcemia if the Q-oTc interval is used, rather than the Q-Tc, particularly in prematures.

CLINICAL VALIDATION OF AN IMPROVED SPHYGMOMANOMETER CUFF FOR INDIRECT BLOOD PRESSURE MEASUREMENT. Leonard Steinfeld, Ivan Dimich, Mt. Sinai Sch. of Med., N.Y.C.; Harold Alexander, Myron Cohen, Stevens Inst. of Tech., Hoboken, N.J. (Intr. by Dr. H. L. Hodes.)

The accuracy and reliability of the auscultatory technique of indirect blood pressure measurement depend primarily on two variables, sphygmomanometer cuff (SC) design and detection of Korotkoff sounds (KS). Laboratory model and clinical studies indicate that the inflatable portion of the (SC) must completely encircle the extremity and must be sufficiently wide so that intracuff pressure is accurately transmitted to the underlying artery without interference by intervening tissues. An encircling cuff, extending from the base of the deltoid to a level just above the antecubital crease appears to meet these requirements, as determined from 55 comparisons of simultaneous direct and indirect pressures obtained at the time of arterial catheterization, in subjects whose weights varied from 4 to 120 pounds. A new inexpensive (?disposable) integral cuff-bladder assembly has been designed, made of a polyolefin material with a cloth backing which fits easily and snuggly the extremity. The cuff is available in a variety of widths and lengths so as to adapt properly to any size extremity. The arterial wall oscillations responsible for (KS) can be recognized earlier and more precisely with an ultrasonic (US) Doppler shift transducer than with a microphone or stethoscope. Thus, the (US) transducer is preferable for marking the earliest peak systolic event. Recognizable markers in the (US) signal at end diastole have been identified and will serve to indicate end diastolic pressure. Clinical trials utilizing the improved cuff coupled with the (US) transducer have yielded results, especially in infants, which are more accurate and reliable than those obtained with other indirect techniques.

THE RESPONSE OF THE ATRIOVENTRICULAR SPECIALIZED CONDUCTION SYSTEM (AVSCS) TO RAPID ATRIAL STIMULATION (RAS) IN CHILDREN. Ehud Krongrad, Carl N. Steeg, Albert L. Waldo, and Welton M. Gersony. Col. of P&S, Columbia Univ., Babies Hosp., Dept. of Ped., N.Y.

The response of the AVSCS to RAS was studied during cardiac catheterization in 24 patients, (ages 2 mo. to 18y.), with various congenital heart defects. Atrial pacing via a catheter placed at the high right atrium was initiated at 100-150 stimuli/min., (S.P.M.), and gradually increased to rates as high as 600 S.P.M. Simultaneous His bundle electrograms were obtained in most patients. The effective heart rate/min., (E.H.R.), plotted against the S.P.M. was characterized by an M shaped curve. The initial ascending limb was produced during 1:1 conduction, (to rates as high as 270/min.), the first descending limb resulted from Wenkebach periodicity which occurred at 160 to 270 S.P.M., the second ascending limb was the result of 2:1 AV block beginning at rates of 200 to 300 S.P.M., and the final descending limb occurred as a result of varied conduction, which was initially noted at rates of 220 to 435 S.P.M. The S.P.M. required to induce Wenkebach or 2:1 AV block in this group of children were higher than reported in adults. With increasing S.P.M., the E.H.R. was not observed to exceed the maximum E.H.R. induced during 1:1 conduction. The site of AV block was shown to be in the AV node. The data may also suggest that the rate of increase of the P-R interval during 1:1 conduction at increasing S.P.M. may be a predictor of the S.P.M. at which AV block will occur. The definition of the response of the AVSCS during RAS is helpful in the understanding and treatment of various arrhythmias in children.

VENTRICULAR VOLUME IN TRANSPOSITION OF THE GREAT VESSELS WITH INTACT VENTRICULAR SEPTUM (TGV). Otto G. Thilenius and Rene A. Arcilla. Univ. of Chicago, Wyler Children's Hosp., Dept. Pediatrics, Chicago.

Right (RV) and left (LV) ventricular enddiastolic (EDV), endsystolic (ESV) and stroke volume (SV) were estimated in 7 infants with TGV, using the biplane angiographic parallelepiped method. These data were compared to normal infants, using age, height, weight and heart rate as variables. The cineangiograms were obtained before (age 2-14 days) and 6-30 months after a Rashkind procedure. Prior to balloon septostomy EDV, ESV, SV and ejection fraction (EF) of RV and LV were normal; RV and LV output was identical. After septostomy, LV data (per M^2 BSA) showed no significant change; however, RV-EDV and ESV were 50% larger than predicted, and RV output exceeded LV by 18%. RV and LV EF remained normal in spite of the reversed ventricular pressure relationship. -The normal ventricular dimensions in the newborn with TGV suggest uncompromised hemodynamics in utero. The well matched blood flow in the 2 circulations are evidence for the balanced net shunts across the atrial septal defect and bronchial circulation.

in ml	RV: EDV	ESV	EF	Q	LV: EDV	ESV	EF	Q
Before Rashkind	17.7	7.4	.58	1.31	12.0	2.3	.80	1.28
After Rashkind	42.0	19.4	.54	2.44	25.6	5.8	.77	2.06

POST-EXERCISE ARRHYTHMIA AFTER TOTAL CORRECTION OF TETRALOGY OF FALLOT

Frederick W. James, Samuel Kaplan, and Te-Chuan Chou, College of Medicine, Univ. of Cincinnati, Children's Hospital, Dept. of Pediatrics, Cincinnati, Ohio

Sudden unexpected death occurred in 4 patients (pts) many years after adequate anatomic correction of tetralogy of Fallot (TOF). These pts had unifascicular block and 2 had occasional premature ventricular extrasystoles (PVE) at rest. Since the deaths of these pts appeared to be related to arrhythmia 12 randomly selected pts with corrected TOF (1-14 years postoperative) were subjected to strenuous exercise. The purpose was to determine whether ventricular arrhythmia could be induced by the stress of exercise. Occasional PVE were observed in 4 pts before exercise. Unifascicular block (10), bifascicular block (1) and normal QRS duration (1) were present in the selected pts. Heart rates reached 140 to 220 beats/min with pulse pressures of 50 to 112 mm Hg. During exercise 1 pt developed atrial extrasystoles and after exercise 6 pts developed unifocal and multifocal PVE. One pt who developed multifocal PVE and had a history of syncope was treated with quinidine which abolished the arrhythmia. When quinidine was withdrawn the multifocal PVE returned after exercise. The remaining 5 pts are asymptomatic without treatment. Post-exercise arrhythmia was not noted in the control pts. From this pilot study we concluded that strenuous exercise induced or aggravated ventricular arrhythmia in pts who had "excellent" correction of TOF.

MYOCARDIAL CONDUCTION DEFECTS & FETAL BRADYCARDIA. M. Yeh, H.O. Morishima & L.S. James, Division of Perinatology, College of Physicians & Surgeons, Columbia University, N.Y., N.Y.

Increased use of fetal heart rate monitoring during labor has drawn attention to the occurrence of premature ventricular contractions particularly during variable deceleration (cord occlusion). Experiments have been conducted in 12 pregnant baboons to explore this phenomenon & to determine its mechanism. Arterial & venous catheters & EKG electrodes were inserted into fetal vessels & a device placed round the base of the umbilical cord for occlusion of the vessels. After surgery, with the fetus intact in utero, the umbilical cord was occluded intermittently for periods ranging from 30 secs. to 12 mins; fetal B.P. & EKG were recorded continuously & fetal pH & PO_2 monitored serially. In 3 fetuses, the cord was occluded before & after administering atropine. During cord occlusion, all fetuses became hypoxic & acidotic; onset of bradycardia occurred in 12±4 secs. Sinus bradycardia was seen in all instances, appearing 15-20 secs. after cord occlusion. Elevation of S-T segment was seen in 40% of occlusions, appearing in 30-60 secs; A-V conduction defects were seen in 53% & complete heart block in 65%, appearing after 1-2 mins. of occlusion. Upon releasing the occluder, recovery of both HR & BP was prompt; EKG & conduction defects reverted to normal in 10-20 secs. & PO_2 rose immediately. Recovery of pH was slow. EKG, & conduction abnormalities were not seen with cord occlusion after atropine administration, suggesting mediation via the vagus nerve. These experiments demonstrate a previously unrecognized hazard from short periods of cord occlusion.

COMPARISON OF THE HEMODYNAMIC EFFECTS OF EXERCISE AND OF ISOPROTERENOL INFUSION IN PATIENTS WITH PULMONARY VALVULAR STENOSIS. William A. Neal, Russell V. Lucas, Jr., and James H. Moller, Dept. of Ped., Univ. of Minn., Mpls, Minn.

In children with congenital cardiac anomalies, isoproterenol (Is.) has been used to simulate exercise (ex.). To determine if these states are comparable, 28 children with pulmonary valvular stenosis were studied by cardiac catheterization at rest, during supine submaximal exercise, and during Is. infusion. Similar heart rates were obtained on ex. (142/min) and Is. (139/min). Cardiac index (CI), and right ventricular systolic pressure (RVSP) were measured during each physiologic state. The change in pulmonary valve area from rest was calculated (ΔPVA cm^2/M^2).

The mean hemodynamic parameters are summarized:

	VO_2 $cc/min/M^2$	A-VO_2 Diff. Vol.%	CI $L/min/M^2$	Stroke Index $cc/beat/M^2$	RVSP mm.Hg	Δ PVA
Ex.	691	9.0	7.6	53.5	111	-0.06
Is.	225*	3.4*	6.2*	45.7*	118	-0.22*

*$p < 0.01$

At similar heart rates CI was significantly greater with ex. than Is. Since RVSP was similar in the two states, the pulmonary valve area was significantly smaller with the pharmacologically induced stress. The study indicates that the hemodynamic effects of Is. are not comparable to those produced by exercise in patients with pulmonary stenosis.

CARDIOVASCULAR EFFECTS OF PROPRANOLOL AND PRACTOLOL IN PUPPIES. Nestor J. Truccone, Henry M. Spotnitz, and Welton M. Gersony. Col. of Physicians and Surgeons. Columbia Univ., Dept. of Ped., N.Y.

Previous studies of beta blocking agents, propranolol (PROP) and practolol (PRAC), in adult subjects suggest that PRAC does not have the marked negative inotropic effect of PROP at higher dosage. The immature circulation has recently been shown to display even greater sensitivity to PROP, whereas the effects of PRAC are unknown. In order to investigate the effect of PROP and PRAC in immature subjects, dose response curves were studied in 11 (PROP) and 10 (PRAC) intact puppies, (1.4-1.8kg) anesthetized with chloralose and morphine, and maintained on a volume respirator. Beyond beta blocking levels, (0.3mg/kg), defined by the absence of response to isoproterenol, PROP continued to depress heart rate, (H.R.), cardiac index, (C.I.), LV dp/dt, and dp/dt/p. At 2.1mg/kg, C.I. averaged 66% of C.I. at blocking levels (90ml/kg/min vs 135ml/kg/cm) and LVEDP increased, indicating a profound negative chronotropic and inotropic effect of PROP in large doses. Beta blockade with PRAC was achieved at a dose level of 1.2mg/kg. In contrast to PROP, larger doses of PRAC (to 8.4 mg/kg) did not cause further depression of LV function. Starling ventricular function curves (4 PRAC, 4 PROP) with H.R. paced at 180/min., confirmed the above observation. After 2.1mg/kg of PROP (7x blocking dose), C.I. averaged 53±13.0 cc/kg/min. at an EDP of 14.5mmHg, while after 8.4mg/kg of PRAC (7x blocking dose), C.I. averaged 238.5±31.3cc/kg/min. at an EDP of 14.0mmHg ($P<0.01$). These results suggest that PRAC is a safer agent for induction of beta blockade in infants and children.

EFFECTS OF CYCLIC AMP ON ISOLATED DUCTUS ARTERIOSUS Erni Kreil, Warren Zapol, Geoffrey Sharp, John Ayromlooi, Saliem Abrahams, Richard Kitz (Intr. by Daniel Shannon) Harvard Med. Sch., Mass. Gen. Hosp., Depts. Anesthesia & Med., Boston.

Flow impedance of the isolated late gestation perfused fetal guinea pig ductus arteriosus (DA) was determined at low and high oxygen tensions. Drugs influencing the adenyl cyclase system were tested at low and high oxygen tensions.

AGENT	No. TRIALS	CONC.	O_2	% MEAN INHIBITION OF PRODUCED CONSTRICTION
Cyclic AMP	8	3-15	mM	-42
Dibutyryl cAMP	7	2-5	mM	-90
Monobutyryl cAMP	2	1-3	mM	-91
5' AMP	6	3-5	mM	-37
Glucagon	6	1-70	µM	-43
Theophylline	5	3-9	mM	-100
Imidazole	7	5-40	mM	+43 (% activation)

Cyclic AMP, its lipid soluble derivatives and 5' AMP produced significant DA relaxation. Glucagon, an activator of adenyl cyclase, produced profound relaxation. Imidazole, which activates phosphodiesterase (PDE), caused rapid constriction of nitrogen-relaxed DA, whereas theophylline, which inhibits PDE, causes relaxation. The results suggest adenyl cyclase is present in DA smooth muscle and may regulate the state of constriction.

CARDIOLOGY

Second Session

CREATION OF COUNTERCLOCKWISE SUPERIORLY ORIENTED FRONTAL PLANE LOOPS (CCW-SOFPL) - IN ISOLATED BLOOD PERFUSED CANINE HEARTS. Ehud Krongrad, Michael R. Rosen, Charles Merker, Brian F. Hoffman, (intr. by Welton M. Gersony). Col. of Physicians and Surgeons, Columbia Univ., Babies Hospital, Department of Pediatrics and Pharmacology, New York, N.Y. 10032.

The surgical creation of CCW-SOFPL ("left anterior hemiblock") is reportedly associated with a high incidence of late development of complete heart block (CHB). Experimental creation of CCW-SOFPL has been previously produced in the canine heart only following extensive damage to the left bundle branch system approached from within the left ventricle. To further investigate ethiologic mechanisms leading to the development of CCW-SOFPL isolated blood perfused canine hearts were suspended in a specially designed Lucite chamber. Six simultaneous frontal "surface" ECG recordings were then obtained from leads attached to the chamber wall. These showed a normal (0-90°) frontal plane axis. Using a bipolar electrode probe-syringe combination, the His bundle (HB) was identified by recording HB electrograms in the right atrial and right ventricular septa. Lidocaine injections (0.05ml, 2%) or discrete cautery lesions were then made at various HB recording sites. ECG pattern of CCW-SOFPL (range -80° to -165°) resulted always from injury to the branching portion of the HB. Our results indicate that 1) CCW-SOFPL may not always represent damage to the left bundle but may be due to injury to the HB. 2) CCW-SOFPL can be produced by small lesions to the HB entirely from within the right ventricle. We conclude that similar lesions might occur during surgical correction of congenital heart diseases leading subsequently to the development of CHB.

ECHOGRAPHIC DETERMINATION OF LEFT VENTRICULAR VOLUMES Richard A. Meyer, James Stockert, and Samuel Kaplan, College of Medicine, Univ. of Cincinnati, Children's Hospital, Dept. of Pediatrics, Cincinnati, Ohio 45229

The purpose of this study was to validate the determination of left ventricular (LV) volumes by echocardiography in infants and children. Twenty-five patients between the age of 2 months to 15 years with a variety of congenital cardiac diseases underwent left ventriculography (Biplane AP and lateral cine). There was a significant correlation in the measurement of the minor dimension of the LV in end diastole from the lateral view of the cine with that measured by ultrasound ($r=+0.84$, $dF=23$, $p\ 0.01$). End-systolic volumes in the lateral cine were determined by echocardiography. Average ratios of the cine AP major and minor dimensions to the cine lateral minor dimension were 1.55 and 1.12 respectively. Ventricular volumes using the ellipsoidal model ($V=\pi Ab^2/6$) were calculated for end diastole (Ved) and end systole (Ves) from the following equations: $Ved=\pi(1.55Bd)(1.12Bd)(Bd)/6$ and $Ves=\pi(1.55Bs)(1.12Bs)(Bs)(.94)/6$ where Bd + Bs represent the echodimensions in diastole and systole and (.94) represents the percent shortening of the major axis during systole. The Ved calculated from the above equation correlated significantly with the end-diastolic volume determined by angiography. ($r=0.876$, $dF=23$, $p<0.01$). We concluded that echocardiography is useful for estimation of LV volumes and valuable since it can be repeated with changing hemodynamic states.

HYPOXIC PULMONARY VASOCONSTRICTION - FACT OR FANCY? B.S.Langford Kidd, Asrar B. Malik and Peter M. Olley, Hosp. for Sick Children, Dept. of Ped. and Univ. of Toronto, Dept. of Physiol., Toronto, Ontario, Canada.

The factors modifying the pulmonary vascular response to airway hypoxia have been studied in experiments on intact dogs anesthetized with Pentobarbital 30 mg/kg. Pressures were measured through cardiac catheters in the pulmonary artery (PAP), pulmonary artery wedge (PwP), left atrium (LAP) and aorta, and flow ($\dot{Q}$) was estimated using indocyanine green. In spontaneously breathing dogs, hypoxia (PaO_2 >40 torr) produced an initial increase in pulmonary vascular resistance (PVR) (PAP-LAP/$\dot{Q}$) followed by a decline to control levels by 10 minutes. PwP-LAP did not change significantly. The late decline in PVR was abolished when $PaCO_2$ and pH were controlled. In experiments where $PaCO_2$ and pH were varied independently, a decrease in $[H^+]$ reduced the hypoxic rise in PVR while an increase in $PaCO_2$ increased it. Increased $[H^+]$ and reduced $PaCO_2$ had no effect. When pH and $PaCO_2$ were controlled, neither α- nor β-adrenergic blockade with Phenoxybenzamine 2.5 mg/kg or with Propranolol 2.0 mg/kg altered the hypoxia induced increase in PVR. These experiments support the thesis that the pulmonary vascular response to airway hypoxia is an increase in PVR, that it is mediated locally, and that its magnitude is affected by both pH and $PaCO_2$.

Supported by the Medical Research Council (Canada) and the Ontario Heart Foundation

ECHOCARDIOGRAPHIC ASSESSMENT OF NORMAL NEONATAL CARDIAC FUNCTION

David J. Sahn, William J. Deely, Arthur D. Hagan, William F. Friedman, Div. of Ped. Cardiol., Univ. of Calif. San Diego, Sch. of Med. La Jolla, CA. The angiographic determination of the mean rate of circumferential fiber shortening (VCF) is of proven sensitivity in detecting altered left ventricular function in the adult. Accordingly, a simple ultrasound method was developed for deriving VCF in neonates. VCF was determined in 72 full term neonates (ages 5 to 150 hours, weight range, 6-10 pounds) during sleep. For the group, VCF averaged 1.51± 0.04 (SE) circumferences/sec. with a range of 0.92 to 2.2 circ/sec. Sub-groups values were: <12 hrs. (N=13) 1.48 + 0.08 circ/sec; 12-24 hrs. (N=16) 1.46 + 0.05 circ/sec.; 24-48 hrs. (N=26) 1.44 + 0.06 circ/sec.; 48-72 hrs. (N=19) 1.57 ± 0.06 circ/sec.; 72-150 hrs. (N=8) 1.61 ± 0.11 circ/sec. The differences between age groups were not significant statistically. In contrast, VCF was noted to be depressed significantly in the first hour of life when normal infants were evaluated serially. An excellent correlation existed between angiographic and echo determinations of VCF ($r = +0.97$, $p<0.001$). These studies validate ultrasound determinations of internal shortening velocity in the neonatal period and attest to the reproducibility of the method. The technique will be especially valuable for the serial, non-invasive assessment of LV performance in infants with congenital heart disease, respiratory distress syndrome, sepsis, metabolic disturbances, and for evaluating the consequences of drug therapy, respiratory interventions, and cardiac surgery.

AN EXPERIMENTAL MODEL FOR THE STUDY OF RED CELL CHANGES WITH CHRONIC SHUNT HYPOXEMIA.

Amnon Rosenthal, S. Bert Litwin, Myron B. Laver, and Lawrence N. Button, Harvard Med. Sch., Children's Hosp. Med. Ctr. and Mass. Gen. Hosp., Depts. of Cardiology and Anesthesia, Boston, Mass.

Shunt hypoxemia was created in seven dogs by an inferior vena cava (IVC) to left atrium (LA) anastomosis and ligation of the proximal IVC. Three dogs with a sham operation (Op) served as controls. The carotid artery was exteriorized and fixed subcutaneously to facilitate collection of arterial blood. Serial determinations of PaO_2, $PaCO_2$, blood pH, hemoglobin (Hb), 2,3-Diphosphoglycerate (DPG), P50 (O_2 tension at 50% Hb saturation), inorganic phosphate and blood volume were performed prior to and at regular intervals after Op. Sham dogs were studied up to 60 days post-Op and IVC-LA dogs up to 425 days. The IVC-LA dogs maintained a mean PaO_2 of 45±7 (SD) mmHg and developed mild respiratory alkalosis (mean $PaCO_2$ 24±3 mmHg; pH 7.44±.05). The immediate post-Op period (4 hrs.-13 days) in all the IVC-LA dogs was characterized by an increase in mean P50 from 27.0±0.7 to 32.5±1.9 mmHg (peak value at 2 to 7 days) and then maintained at 30.7±1.2 mmHg ($p<.01$). Changes in P50 were associated with a rise in pH, DPG and serum inorganic phosphate. A rise in Hb concentration occurred 10-21 days post-Op from an initial mean of 14.1±1.7 to 19.2±1.8 gm% ($p<.01$). Mean red cell volume increased from 32±7 to 59±6 ml/kg ($p<.01$) and plasma volume remained constant. We conclude that the IVC-LA shunt model is suitable for the study of adaptive mechanisms in red cell metabolism and hemoglobin oxygen affinity.

ACUTE EFFECTS OF RED CELL VOLUME (RCV) REDUCTION ON PULMONARY BLOOD FLOW (PBF) IN POLYCYTHEMIA OF CONGENITAL HEART DISEASE.

Amnon Rosenthal and Donald C. Fyler. Harvard Med.Sch., The Children's Hosp. Med. Ctr., Dept. of Cardiology, Boston.

The acute effects of isovolumic RCV reduction on PBF and pulmonary vascular resistance (PVR) were measured at catheterization in 16 patients (pts) with severe secondary polycythemia. Reduction in mean hematocrit from 75% (range 65-84%) to 64% (range 53-76%) resulted in an increase in systemic blood flow (SBF) in all pts (paired t test: $p<.01$). In those with D-transposition of the great arteries (D-TGA) and no significant pulmonary stenosis (PS) (n=5) there was an increase in mean PBF from 8.5 to 12.5 L/min/m^2 ($p=.03$), a fall in mean PVR from 4 to 2.7 ($p=.06$), and an increase in mean effective pulmonary flow (EPF) from 1.4 to 1.9 L/min/m^2 ($p<.01$). Systemic oxygen saturation (SaO_2) decreased slightly. By contrast RCV reduction in pts with severe pulmonary stenosis or atresia, ventricular septal defect (VSD) and normally or abnormally related arteries (n=11) was associated with a decrease in mean PBF from 3.7 to 3 L/min/m^2 ($p<.01$), a decrease in the mean left-to-right shunt from 2.2 to 1.5 L/min/m^2 ($p<.01$) and an increase in mean right-to-left shunt from 1.6 to 2.7 L/min/m^2 ($p<.01$). These changes induced a fall in mean SaO_2 from 78 to 72% ($p<.01$). The data suggest that when PBF is largely derived from the systemic circuit (severe PS or atresia with VSD) RCV reduction will diminish PBF and SaO_2. However, when PBF is independent of the systemic circuit (D-TGA) an increase in PBF and mixing (EPF) may be expected.

COMPARISON OF INDICES OF CARDIAC CONTRACTILITY IN REFLECTING EFFECTS OF POSITIVE AND NEGATIVE INOTROPIC INFLUENCES

George Benzing, III, James E. Stockert, Ed Nave and Samuel Kaplan, College of Medicine, Univ. of Cincinnati, Children's Hospital, Dept. of Pediatrics, Cincinnati, Ohio

Control cardiac performance of 8 dogs was assessed by quantification of mean left ventricular hydraulic output power (LVP) and maximum contractile element velocity (Vmax) at a left ventricular end-diastolic pressure of 9.0 mm Hg. Systemic vascular resistance was quantitated for each. During bypass coronary blood flow was interrupted for 45 minutes. Myocardial function was evaluated again at the same ventricular end-diastolic pressure and systemic vascular resistance. During infusion of epinephrine 0.5 microgram/kilogram/minute studies were repeated.

After myocardial ischemia the average of the percentages of the control left ventricular hydraulic output power was 49 percent ($p<.01$). In contrast an average of 95 percent of the control (N.S.) Vmax values was observed. During epinephrine infusion the averages of the percentages of the LVP and Vmax of that observed after ischemia were 171 and 123 respectively. The change in Vmax obtained during an epinephrine infusion from that obtained following ischemia did not correlate significantly ($r=0.520$, $df=6$) with the change in LVP.

These observations suggest that Vmax does not reflect appropriately the decrease in cardiac performance, quantitated by LVP, following myocardial ischemia, or the increase in LVP during an infusion of epinephrine.

MYOCARDIAL METABOLISM AND FUNCTION DURING CHOLINERGIC STIMULATION IN THE NEWBORN LAMB.

S. Evans Downing, John C. Lee and James F.N. Taylor. Yale Univ. Sch. of Med., Dept. of Pathology, New Haven, Connecticut

This study was designed to assess the influences of cholinergic stimulation on coronary flow (CF), oxygen metabolism (MVO_2) and contractility (MC) of the left ventricle in the newborn. Fifteen lambs 1-7 days of age were anaesthetized with pentobarbital (20 mg/kg) and prepared for continuous measurement of CF, heart rate (HR), aortic (AP) and left ventricular end-diastolic (LVEDP) pressures, dP/dt max, and cardiac output (CO). HR, AP and CO could be controlled as desired. Simultaneous arterial and sinus blood samples were analyzed for O_2 content, hematocrit, pH, PO_2 and PCO_2. LV MVO_2 and % O_2 extraction (Ext.) were calculated. Acetylcholine (ACh) infusion (10-20 µg/min/kg) caused a bradycardia (40/min), increase of LVEDP (5 cm H_2O) and fall of dP/dt max (600 mm Hg/sec) with constant AP and CO. CF increased and Ext. decreased, but MVO_2/beat was unchanged. Similar changes were observed with constant HR (pace). Supramaximal efferent stimulation of right or left vagus nerves produced bradycardia (80/min) but no significant changes in contractility, CF, Ext. or MVO_2. It is concluded that while cholinergic sensitivity can be demonstrated, vagal innervation of the myocardium is insufficient to importantly influence MC, CF or MVO_2 in the lamb.

CONTRAST DILUTION ANALYSIS: A NON-INVASIVE TOOL FOR EVALUATION OF CONGENITAL HEART DISEASE.

Farzin Davachi, Joseph D. Cohn, Gordon Hass, Walter R. Stankewick and Louis R. M. Del Guercio. (Intr. by W. Yakovac). Dept. of Peds. and Surgery, Saint Barnabas Medical Center, Livingston, New Jersey 07039.

A method has been devised to obtain multiple contrast dilution curves across the cardiopulmonary silhouette. In infants and dogs with congenital heart disease, one ml. meglumine diatrizoate is injected into the external jugular vein or central circulation. Solid state x-ray detectors are externally positioned across the heart and contrast dilution curves are recorded during transport of the contrast material. The topological relationship of anatomical segments and flow distributions through the heart and lung define the shape of the recorded curves. Recordings were performed in twenty dogs including animals with congenital heart disease. On the basis of curve analysis formulated upon curve variance and skewness, a function of the second and third moments about the mean, shunt phenomena could be predicted within the animal subjects. Left to right shunt phenomena were readily detected in infants with congenital heart disease by the appearance of a prolonged downslope on the indicator curve. Right to left shunts produce a premature hump on the left heart curves. The injection of a minute amount of non-radioactive, radiopaque contrast material allows inscription of multiple, externally recorded contrast dilution curves without the need for cardiac catheterization or arterial cannulation. Analysis of these curves provides assessment of cardiac hemodynamics and evaluation of shunt phenomena in animal and human subjects.

RELATIONSHIPS AMONG POTASSIUM, ACETYLCHOLINE AND OXYGEN INDUCED CONTRACTION OF THE DUCTUS ARTERIOSUS. S. Noel and S. Cassin, Dept. of Physiol., College of Medicine, Univ. of Fla., Gainesville, Fla. 32601.

Treatment of the guinea pig ductus arteriosus with 10 ug/ml acetylcholine prior to exposure to oxygen (Po_2 = 144 mmHg) augmented contraction of the ductus arteriosus in response to oxygen (n = 6). Oxygen-induced (Po_2 = 144 mmHg) contraction (n = 44), acetylcholine-induced (10 ug/ml) contraction (n = 6) as well as oxygen (Po_2 = 144 mmHg) plus acetylcholine-induced (10 ug/ml) contraction (n = 6) of the ductus arteriosus were inhibited by 40 mM magnesium. In contrast, excess potassium (46 mM)-induced contraction (n = 6) of the ductus arteriosus was not inhibited. Manganese (3 mM) which inhibits influx of calcium across the smooth muscle cell membrane prevented contraction of the ductus arteriosus to oxygen (n = 7) but not to excess potassium (n = 6). From these studies we suggest that oxygen-induced contraction of the guinea pig ductus arteriosus is dependent on the influx of extracellular calcium, related to acetylcholine-induced contraction but unrelated to excess potassium-induced contraction (n = 6). (Supported in part by NIH Grant #HE 05979-01.)

CARDIOLOGY

Read by Title

DETERMINATION OF CARDIAC OUTPUT AND CARDIAC DISTRIBUTION OF THE ISOLATED FETAL LAMB PREPARATION. Errol R. Alden, Thomas A. Standaert and W. Alan Hodson. Dept. Ped., Univ. Wash., Seattle, WA.

The isolated fetal lamb preparation using a membrane oxygenator and the umbilical vessels was assessed to determine its validity as a model for physiological studies on the fetus. The blood gases and umbilical blood flow of 25 fetuses approximated in utero values for an average of 4 hrs. Mean arterial blood O_2 and CO_2 tensions were 25±9mm and 45±11mm respectively. The umbilical flow was 144±38ml/Kg.min. Metabolic acidosis and rising lactate levels commonly occurred. Oxygen consumption was calculated to be 5.9ml/Kg.min. The cardiac output (C.O.) and its distribution was determined 10 times on 7 fetuses using radioactive microspheres (50μ). At the time of injection, the PO_2 was 26±10mm, PCO_2 32±27mm, pH 7.32±0.06. The C.O. output was 312±76ml/Kg.min., 65% of the expected in utero value. The distribution of C.O. was comparable to available chronic in utero data with the exception of the gastrointestional tract (50% above expected values).

The metabolic acidosis was not related to maldistribution, but the low C.O. may have been brought about by umbilical flow that was 80% of normal. A reduced C.O. coupled with a reduced blood oxygen capacity (hemodilution with maternal prime blood) then led to hypoperfusion of the tissues and subsequent metabolic acidosis. The technical factors limiting the umbilical flow have been resolved and the oxygen capacity of the blood has been increased.

LIVER TEST BEFORE AND AFTER ANGIOCARDIOGRAPHY IN PEDIATRIC AGE GROUP. Agustin W. Castellanos and Heriberto Mercado. Univ. of Miami Sch. of Med. Variety Children's Hosp. Cardiology Dept. Miami, Fla.

The purpose of this investigation is to know if the contrast media injected during performance of angiocardiography may alter the hepatic functions in patient with congenital heart disease. Group A: 30 normal neonates. Group B: 11 neonates with congenital heart lesions. Group C: 100 cases of congenital cardiopathies from 1 month to 12 years of age. Liver tests carried out: bilirubin, cephaline-flocculation, thymol turbidity, Lactic dehydrogenase, alkaline phosphatase, serum glutamic transaminase, Bromsulphatein. In many cases creatine-phosphokinase and prothrombin time. One or several liver tests were found abnormal in 13% of the group A, 91% of the group B and 63% of the group C. The Fisher Exact Test was done. The younger the patient, the more severe the disease and the greater the amount of dye, the more severe the abnormalities of the liver tests. In one case the patient's death could be related to a severe cardiac damage due to the dye. Liver tests should be done in sick patient when angiocardiography is indicated.

MITRAL VALVE ANNULOPLASTY FOR MITRAL REGURGITATION (MR) FOLLOWING SUB-ACUTE BACTERIAL ENDOCARDITIS (SBE) D. Danilowicz, A.L. DeGuzman and G. Reed. (Intr. by E.F. Doyle). Depts.Ped. and Surg.; N.Y.U. Medical Center, New York, N.Y.

Two girls, ages 9 and 17 years, were admitted for surgery following an episode of SBE which resulted in severe MR and congestive failure. The 9 year old had a secundum atrial defect(ASD) and the 17 year old had no known pre-existing heart disease.

At operation, the 9 year old had two ASD's and a flail posterior half of the aortic leaflet of the mitral valve due to ruptured chordae. Annuloplasty was performed through the ASD and the defects were sutured. The child is now 3 years post-op with no symptoms or murmur.

At operation, the 17 year old had a flail anterior leaflet with ruptured chordae and a tear in the leaflet; this was corrected by annuloplasty. The girl is now 1 year post-op with no symptoms. There is no murmur at rest; a grade 1/6 rumble appears after exercise. Cardiac catheterization 9 months after operation was normal at rest; a 7 mm. Hg early diastolic gradient occurred after exercise. There was no regurgitation on left ventricular angiogram and valve movement was only minimally limited.

These two patients demonstrate the successful use of conservative annuloplasty in the treatment of MR due to ruptured chordae following SBE.

BACTERIAL ENDOCARDITIS FOLLOWING PROSTHETIC PATCH REPAIR OF VENTRICULAR SEPTAL DEFECT. A.L. deGuzman, E.F. Doyle, and M. Finegold. Depts. of Ped. and Path., N.Y.U. Med. Center, N.Y.N.Y.

Bacterial endocarditis (BE) after prosthetic patch repair of ventricular septal defect (VSD) has usually ended fatally in reported cases. Three of our cases from a total of 165 who survived patch closure of VSD developed BE over a 14 year period. Prophylactic antibiotics were used in all cases. In 2 the infection was detected early in the postoperative period while the 3rd case was diagnosed 6 months after surgery. The causative organisms were hemolytic staph aureus, staphylococcus albus and serratia marcescens. The latter followed contamination from a malfunctioning air conditioning system in the operating room.

Clinical subsidence of the infection was effected with antibiotic therapy in each case. However, progressive fatal heart failure resulted from development of a large left to right shunt which resulted from separation of the patch. Each case at autopsy showed persistent infection on the patch; in 1 case this was of mild degree.

This experience demonstrates the failure of vigorous medical therapy and the need for removal and replacement of the infected patch as soon as the infection is reasonably controlled especially if heart failure has ensued.

THE NATURAL HISTORY OF THE SIMPLE VENTRICULAR SEPTAL DEFECT
Doris A. Evans, M.D., Jerome Liebman, M.D., F.A.A.C., Jay L. Ankeney, M.D. and Victor Whitman, M.D., Rainbow Babies and Children's Hospital, Cleveland, Ohio

The natural history of 456 patients with a simple ventricular septal defect (VSD) is reported. Cardiac catheterization was done in 122. The average period of follow-up for all patients was 32 months. Sixty-seven of the catheterized patients presented in the first two years of life and 46 of this group had congestive heart failure (CHF). Twenty-seven of the patients had a pulmonary artery pressure (PAP) at least 3/4 systemic, of whom 14 had elevated calculated pulmonary vascular resistance. Twenty patients had more than one catheterization and the calculated pulmonary vascular resistance stayed the same in 12, increased in 4, and decreased in 4. None of the patients developed an exclusive right to left shunt. All patients with a PAP greater than 3/4 systemic had right or combined ventricular hypertrophy (CVH) on their electrocardiograms. Two of the catheterized patients had complete spontaneous closure of their defect. Of 7 catheterized patients who died, 5 were medical deaths related to the VSD and 2 followed surgical intervention; none had more than grade 3 changes in the pulmonary arterioles at autopsy. There was no mortality or significant morbidity due to the VSD per se in the non-catheterized patients. Thus, significant morbidity occurs in only twelve percent of patients with simple VSD. These high risk patients can be identified in early infancy. All have RVH on their electrocardiograms and the vast majority have CHF.

BRAIN ABSCESS IN CONGENITAL HEART DISEASE. Charles Fischbein, Amnon Rosenthal, Alexander S. Nadas, Keasley Welch, and Edwin G. Fischer. Harvard Med. Sch., The Children's Hosp. Med. Ctr. Depts. of Cardiology and Neurosurgery, Boston.

Brain abscess (BA) remains a serious complication of congenital heart disease (CHD). To determine the risk factors for BA in patients with CHD we reviewed 26 consecutive cases admitted to our institution between 1960 and 1973. Data were compared to an appropriately matched control group of 58 patients with cyanotic CHD. The incidence of BA in the cyanotic CHD population was 2.0%. Tetralogy of Fallot and D-transposition of the great arteries accounted for 81% (21/26) of the cases. Median age for the BA group was 9 4/12 yrs. and the youngest patient was 3 3/12 yrs. The mean arterial oxygen saturation (SaO_2) for the BA patients was 75% compared to 86% for the control group ($p<0.01$). Within the BA group mean Sa O_2 for the survivors (16/26) was 81% compared to 64% for the deceased ($p=0.01$). Mean hematocrit for the BA group was 56.4% and for the control 52.2% ($p=0.1$). Alpha strep was the commonest organism isolated (5/20). Hemophilus aphrophilus, the second most common (3/20) organism, has also been isolated from dogs and its growth is enhanced by low pO_2 and high pCO_2. The overall mortality for the group was 38% (10/26). Complete recovery of survivors occurred in 54% (8/15) and residual neurologic impairment in 46% (7/15). We conclude that the morbidity and mortality of BA is inversely related to Sa O_2. Corrective cardiac surgery(in contrast to palliative procedures)prior to two years of age would practically eliminate the hazard of BA from the CHD population.

ALTERED ELECTRICAL AND MECHANICAL PROPERTIES OF RIGHT VENTRICULAR MYOCARDIUM IN CATS WITH EXPERIMENTALLY INDUCED RIGHT VENTRICULAR HYPERTROPHY. Henry Gelband* and Arthur Bassett*. University of Miami School of Medicine, Miami, Florida. (Intro. by W.W. Cleveland)

Previous published data have reported that right ventricular (RV) myocardial contractility decreases 2 days after the development of RV hypertrophy in cats. To determine whether there are associated cellular electrical changes we recorded simultaneously, isometric force (Po) and transmembrane potentials from RV papillary muscles (PM) in cats after partial surgical banding of the main pulmonary artery. Sham and banded operated cats were sacrificed 1,3,7,10,21 and >90 days following surgery. PM from sham and 1 day operated cats had normal electrical-mechanical properties. RV systolic hypertension was present in all animals. In addition, RV water content was increased but did not fully account for the increased weight. RVPM from 3 day banded hearts had decreased Po, and maximum dP/dt, associated with a shift of transmembrane potential plateau to a more negative value. The mean shift in origin of the plateau was 24.7 mV for 15 RVPM from 3 day banded hearts. Although Po remained decreased, alterations in transmembrane potential was less obvious in RVPM 7 days after surgery and was normal 21 days after surgery. The altered transmembrane potential plateau during the development of early myocardial hypertrophy may result from changes in the ionic environment, principally Ca^{++}, which may be related to the depression of Po.

COMPLEX PRESSURE AND FLOW RESPONSES TO COMBINATIONS OF AFFERENT INPUTS IN THE NEONATE. Norman Gootman, Margo Schleman, Phyllis M. Gootman and Nancy M. Buckley. Long Island Jewish-Hillside Medical Center, New Hyde Park, N.Y. (Intr. by Philip Lanzkowsky)

Studies of responses to different patterns of sciatic nerve stimulation (SNS) and baroreceptor inhibition or stimulation were carried out in 17 piglets (1 to 14 days of age) anesthetized with halothane (0.25 to 0.5%) in a mixture of N_2O-O_2. The sciatic nerve was stimulated (SNS) before and during bilateral common carotid occlusion (BCCO) or carotid sinus infusion (CSI). Simultaneous recordings included aortic pressure (P), ECG, and carotid, renal and femoral flows (F) by calibrated electromagnetic probes. Blood gas composition and body temperature were monitored and controlled. High frequency (SNS) pressor responses were augmented during BCCO; low frequency SNS depressor responses were not altered when BCCO was applied after SNS had begun, but were converted to pressor responses if SNS was carried out after BCCO was applied. Femoral flow responses to SNS plus BCCO or BCCO plus SNS were smaller than to SNS alone. Patterns of changes in flow in the other beds were not as obvious. Flow responses to low or high frequency SNS were smaller when combined with CSI before or during stimulation. Our results show that the cardiovascular controlling system in the neonate is capable of occlusive or summated responses. Supported by Rozenberg-Toner Heart Fund.

BACTERIAL ENDOCARDITIS (BE) IN INFANCY. David H. Johnson, Amnon Rosenthal, and Alexander S. Nadas, Harvard Med. Sch., Children's Hosp. Med. Ctr., Dept. of Cardiology, Boston, Mass.

The risk of BE in children under two years of age with sepsis was evaluated by reviewing the clinical files (1945 - 1972) and autopsy records (1930-1969) at our institution. Autopsy review disclosed 847 infants with bacterial sepsis, 12 of whom had BE (1.2%), documented by culture and pathologic examination; only one case of BE was noted in the clinical survey. Sepsis was caused by gram positive organisms in 71% of the cases in the first two decades of the study while gram negative organisms accounted for 70% in the ensuing twenty years. Of the 12 BE cases, six had congenital heart disease (CHD). Three cases of BE without CHD had underlying conditions which may have predisposed them to BE; infected epidermolysis bullosa, extensive burns, and intracardiac foreign body in one case each. Endocardial involvement in patients without CHD was confined to the atrio-ventricular valves. Of the 61 cases of bacterial sepsis associated with CHD, six had BE (10%) while in 786 cases of sepsis without CHD six had BE (0.8%) ($p<0.001$). Four of the five cases of BE occurring after the introduction of antimicrobial agents died; the single survivor, now aged one year, has severe aortic regurgitation. This study indicates that BE in infancy has a high mortality and that infants with CHD and sepsis are at a greater risk for developing BE.

PROGNOSTIC IMPLICATIONS OF LEFT VENTRICULAR FUNCTION IN ENDOMYOCARDIAL DISEASE. By L. Jerome Krovetz*, and Barry J. Maron, Johns Hopkins University School of Medicine, Departments of Pediatrics and Biomedical Engineering, Baltimore.

Since 1965, 42 children with endomyocardial disease have been catheterized; presumptive diagnosis was endocardial fibroelastosis (EFE) in 24, cardiomyopathy (CAM) in 11, and anomalous left coronary artery (ALC) in 7. Findings in these children were compared to catheterization data in 60 children without significant hemodynamic abnormality. Left ventricular end-systolic and left ventricular end-diastolic volumes (LVEDV) were measured using single plane cineangiography and ejection fraction (EjF) calculated. Left ventricular end-diastolic pressure was elevated in 24% of the survivors and in 71% of the fatal cases. LVEDV was increased in 40% of survivors and 60% of the fatal cases. Thirteen of 16 patients with EFE, 3 of 9 patients with CAM, and none of 2 with ALC, had EjF less than normal. One-half of the survivors and all 5 patients who died had low EjFs, ranging from .06 to .52 (normal .57-.90). In 8 patients, LV pressures were measured during the course of cineangiocardiography and pressure-volume loops drawn. Three types of pressure-volume relationships were seen: 1) high LVEDV and low EjF, 2) high LVEDV and normal EjF, and 3) normal LVEDV and normal EjF. The significance of these in relation to prognosis will be discussed and illustrative examples shown. Although prognosis could not be determined by any single variable, only 8 of 22 survivors had more than 1 abnormality and 4 of these are still symptomatic.

INTRAVASCULAR HEMOLYSIS IN CONGENITAL HEART DISEASE. W. Pennock Laird, Ashby Taylor and Bertram Lubin Children's Hospital of Philadelphia, Philadelphia

In the absence of hepatocellular disease, low serum haptoglobin levels are a sensitive index of intravascular hemolysis. The incidence of intravascular hemolysis in congenital heart disease is not known although it has been reported in individual cases and following cardiac catherization. Therefore haptoglobin levels have been measured in 25 children (age 10 mo-14 yr) both before and after cardiac catherization. In 9 children haptoglobin levels were 20mg% or less before cardiac catherization (mean=7.4 ± 2.4mg%). In the remaining patients, the mean haptoglobin level was 86.8 ± 10.7mg% (normal lab range 40-120mg%). Low haptoglobin levels did not correlate with hemoglobin levels, red cell indices, presence of cyanosis, or hemodynamic state. In 14 of 16 children with normal precatherization haptoglobin levels, a 25% decrease occured following cardiac catherization. Renografin 76 Dye, 1-4cc/kg total dose, was injected in all cases. In none of these was hemoglobinuria observed although this complication has been noted. These studies indicate a significant incidence of intravascular hemolysis in congenital heart disease. This process may play a role in the development of thrombotic complications and anemia seen in these children.

THE ANATOMY OF THE FORAMEN OVALE IN RELATION TO BALLOON ATRIAL SEPTOSTOMY. C.C. Laura Meng, C. Robert Wells, Marie Valdes-Dapena, James B. Arey, Iain F.S. Black and Anna C. O'Riordan. St. Christopher's Hospital for Children and the Dept. of Pediatrics, Temple University School of Medicine, Philadelphia Penna. (Introduced by Angelo M. DiGeorge).

Herein reported are the results of 97 balloon atrial septostomies (BAS) in infants with complete transposition of the great arteries and other types of cyanotic congenital heart disease. Complications of the procedure are described, as well as ways in which these may be avoided. Necropsy examination has revealed two types of foramen ovale. Patients with Type A foramen ovale (72%), in whom the valve of the foramen is a thin-walled structure, are more likely to benefit from BAS than those with Type B, (28%), in whom the valve is thicker. The proper equipment for and technique of pullback of the inflated balloon catheter from the left to the right atrium is very important, but the nature of the foramen ovale plays a major role in determining the success or failure of the BAS. In the Type A foramen ovale a large atrial septal defect can be relatively easily created with satisfactory long-term palliation. Gross descriptions of the valve of the foramen support this thesis.

FLUORESCENT ANTI-HEART IgM AND ELEVATED LEVELS OF SERUM IgM IN NEWBORNS WITH CONGENITAL HEART DISEASES. James J. Nora, M.D. and E. Joy Weishuhn, B.S., Univ. Colorado Med. Center, Denver, Co. 80220, and Barbara J. Bourland, M.D. and Shirley C. Watson, B.A., Baylor Col. of Med., Houston, Tx. 77025.

Thirty-five percent (13 of 37) of newborns, studied at less than two weeks of age, with congenital heart lesions had both abnormally elevated serum levels ($>$ 20 mg%) of IgM and anti-heart IgM demonstrable by a fluorescent technique. An association but not a causal relation between the elevated serum IgM and the congenital heart defects may be deduced. Alternative interpretations of the presence of anti-heart IgM in the sera of these newborns include: modification of the antigenicity of the developing heart with teratogenic effect produced by the invading organism, but not the anti-heart IgM; and direct teratogenic influence of the anti-heart antibody of fetal origin raised against both the invading organism and shared (or modified) antigens in the host.

ATHEROSCLEROTIC CHANGES OF THE ILIAC ARTERIES IN CHILDREN WITH A SINGLE UMBILICAL ARTERY - THE EARLIEST FORM OF ATHEROSCLEROSIS IN HUMANS. Wladimir W. Meyer and John Lind, Karolinska Inst., Dept. of Pediatrics, Stockholm, Sweden, and University of Mainz, Inst. of Pathology, Mainz, West-Germany.

The absence of one umbilical artery is associated with an asymmetrical development of the iliac arterial tree. At the side of the single umbilical artery the common and the internal iliac arteries - the only connection between the abdominal aorta and the placental circuit - are large and thick-walled in comparison with the same arteries of the other side of the body which do not participate in the placental circuit. A striking pathological lesion of the enlarged arteries at the side of the single umbilical artery is the early and regular development of marked intimal lipidosis and atherosclerotic plaques. Since these changes could be found as early as at the age of thirteen months, they represent the earliest atherosclerotic lesions observed in humans so far. The evaluation of local structural and hemodynamic factors, which are mainly responsible for the development of these early lesions, may be important for the better understanding of the pathogenesis of human atherosclerosis especially of its early stages in childhood.

ISOPROTERENOL AND RIGHT VENTRICULAR OUTFLOW OBSTRUCTION. P. Syamasundar Rao, Leonard M. Linde and Shoichi Awa, Med. Col. of Georgia and UCLA Sch. of Med., Depts. of Ped., Augusta and Los Angeles.

The right ventricular outflow pressure gradients produced by isoproterenol (IP) are due to the differences in the expression of the total fluid energy. The side pressure, the end pressure and the flow velocity in the pulmonary artery (PA) and the right ventricle (RV) pressure were measured in 11 dogs before and after IP. The RV and end PA pressures were equal ($p = >0.05$) and were slightly higher than the side PA pressure in the control state but increased to markedly higher levels ($p = <0.01$) after IP. IP increased the velocity of the flow in the PA ($p = <0.01$). The RV and side PA peak systolic pressure difference (mean = 8.82 mm Hg) increased to a mean of 19.09 mm Hg ($p = <0.01$). This change in pulmonary valvar pressure gradient is proportional to the increase in PA flow velocity induced by IP. The side pressure measures only potential energy and the end pressure measures the potential and kinetic energies. In the RV, only potential energy is recorded where kinetic energy is practically nonexistent. The gradient between the RV and side PA pressure in this study and the increase in gradient across the RV outflow tract after IP, documented by other workers, is due to partial transformation of the fluid energy into kinetic energy in the PA.

DIAGNOSIS AND MANAGEMENT OF SYMPTOMATIC PATENT DUCTUS ARTERIOSUS (PDA) IN RESPIRATORY DISTRESS SYNDROME (RDS). William A. Neal, Russell V. Lucas, Jr., F. Blanton Bessinger, Carl E. Hunt, Dept. Ped., Univ. of Minnesota Hosp., Minneapolis.

A clinical diagnosis of PDA was made in 76 (19%) of 396 RDS admissions to our unit in 1970-72. Twenty-eight patients had a continuous murmur, the remainder a systolic murmur in the left subclavicular area plus a wide pulse pressure. Congestive heart failure (CHF), present in 15 patients (20%), was defined as tachypnea, tachycardia and hepatomegaly which appeared or worsened concurrently with appearance of the PDA. A continuous murmur was present in 14 of 15 patients with CHF and 14 of 61 patients without CHF. Left ventricular hypertrophy (ECG) was present in 14 of 15 patients with CHF and in only 2 patients without CHF. A left ventricular strain pattern was present in 9 of 15 patients with CHF and absent in patients without CHF. Initially, the patients with CHF were managed medically. Nine improved, 1 died and 5 were unimproved. The 5 unimproved patients were operated, but the initial 3 patients did not improve. Two subsequent patients (below) improved after operation. Because of the initial operative failures, left-to-right shunts and ventilation-perfusion ($\dot{V}A/\dot{Q}$) abnormalities were measured in the next 4 patients considered for operation. One patient had no net shunt, was not operated, and ultimately died of RDS. A second had a 50% left-to-right shunt, was not operated, and improved. Two patients had $>$60% left-to-right shunts, were operated and improved immediately, and had significantly improved $\dot{V}A/\dot{Q}$ abnormalities.

FUNCTIONAL CLOSURE OF VENTRICULAR SEPTAL DEFECTS IN TRICUSPID ATRESIA. P. Syamasundar Rao and Leonard M. Linde, Med. Col. of Georgia and UCLA Sch. of Med., Depts. of Ped., Augusta and Los Angeles.

Anatomic closure of ventricular septal defects (VSD) in tricuspid atresia is well documented. This report documents functional closure of such VSDs. Two infants with proven diagnosis of tricuspid atresia and VSD (by angiography) presented with "cyanotic spells" and disappearance of holosystolic murmur. The "spells" were temporarily relieved by administration of morphine and oxygen. Both patients had aorto-pulmonary shunts but died. At autopsy, the diagnoses were confirmed and the VSDs were small but patent and surrounded by muscle. There was no valvular or infundibular pulmonic stenosis.

Clinical and autopsy findings suggest that there was intermittent functional closure of the VSD during life. The VSD in tricuspid atresia is in the muscular septum entirely surrounded by muscle, so that contraction could occlude the VSD completely. The clinical presentation of the "spells" is very similar to that seen in tetralogy of Fallot. The mechanism of onset of "spells" in tetralogy itself is not clear. Infundibular spasm precipitated by acute increase in circulating catecholamines has been advanced as a mechanism. It is postulated that the ventricular septal muscle contraction is precipitated in a manner similar to infundibular spasm in Fallot's tetralogy.

SUDDEN INFANT DEATH SYNDROME AND CARDIAC ARRHYTHMIAS. P.S. Rao, W. B. Strong and O. B. Kwon, (Intr. by A. F. Robertson), Med. Col. of Georgia, Dept. of Ped., Augusta.

The cause of sudden infant death syndrome (SIDS) is not known. The major hypotheses include infection, airway obstruction, prolonged apnea and failure to interrupt apnea. Our recent observations in a 3 month old girl support cardiac arrhythmias as another possible etiologic factor. The infant had several episodes of paroxysmal supraventricular tachycardia (PST) and three episodes of ventricular fibrillation (VF). Evidence for Wolff-Parkinson-White syndrome (WPW) was also present (predisposing to recurrences of PST). On two occasions, the infant appeared ill and was taken to the hospital where VF was documented. If she had VF at home, it is unlikely that she could have survived until hospitalization. Therefore, it is postulated that she had PST at home and developed VF at the time of hospital admission. A third episode of VF occured in the hospital. The infant was promptly defibrillated on all three occasions. Recurrence of the arrhythmia was prevented by administration of digitalis and procaineamide. If the episodes of arrhythmia were not initially symptomatic, the infant would have died. Supporting cardiac arrhythmias as one of the mechanisms of SIDS are: 1) the findings in this infant, 2) the observations by James of the immaturity and electrical instability of the conduction system in infancy, 3) increased incidence of cardiac arrhythmias in premature infants, and 4) peak incidence of PST in this age group. The sequence of events appears to be: WPW $\longrightarrow$ PST $\longrightarrow$ VF $\longrightarrow$ Death.

HYPOPLASTIC LEFT HEART SYNDROME. J. Razook, W. M. Thompson, Jr., R. Elkins (Intr. by H. D. Riley, Jr.) Child. Mem. Hosp., and V. A. Hospital, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma.

The hypoplastic left heart syndrome, characterized by mitral and/or aortic valve atresia, continues to be a lesion which is resistant to corrective and/or palliative surgery. Twelve patients with hypoplastic left heart syndrome have been seen at Children's Memorial Hospital. Of these, the last three underwent surgery following catheterization. The basic problems being attacked are: to insure adequate left to right shunting via an atrial septal defect, to create a permanent means of maintaining systemic flow via an artificial ductus, to prevent pulmonary hypertension and hypervascularity by pulmonary artery banding. An adequate atrial septal defect is created by either atrial septostomy at catheterization or an atrial septectomy at surgery. Systemic flow is insured via an artificial ductus from the main pulmonary artery to the aorta, just distal to the atretic segment. Finally the pulmonary vascular bed is protected by banding of the right and left pulmonary artery decreasing flow to the lungs and maintaining adequate perfusion pressure of the systemic circulation.

Of three patients operated, the following results have been obtained: The first patient survived twelve hours, the second three days, and the third two and one-half months with an acceptable growth pattern. We have been gratified by the course of our last patient and feel that this approach represents sound operative intervention for at least palliation of a fatal lesion.

NORMAL ATRIOVENTRICULAR CONDUCTION IN CHILDREN. Nigel K. Roberts and John D. Keith. University of Toronto, Hospital for Sick Children, Department of Paediatrics and Research Institute, Toronto, Canada.

A new study on normal atrioventricular conduction in children has been undertaken. Previous reports have indicated that the P-R interval varies both with age and heart rate. We have with the aid of His bundle electrograms been able to define normality for the component parts of the P-R interval-namely the P-A interval (conduction from the sino-atrial node to the atrioventricular node) 4-38 m.secs.,the A-H interval (atrioventricular nodal delay) 54-118 m.secs. and the H-V interval (from the A-V node through the His bundle to the Purkinje System) 20-42 m.secs.

124 children who had His bundle electrograms performed were divided into seven age groups (under 1 yr.,1-2,3-4,5-6, 7-8,9-12,13-17 yrs). A significant increase in only the H-V interval was found with increasing age ($p < 0.01$). This change was shown statistically to be unrelated to the heart rate. By way of confirmation electrocardiograms were taken at rapid paper speed and the P-R intervals and heart rate tabulated. A mean change in rate of 43% was obtained with sinus arrhythmias in 21 children (age 3-14) and of 34% with exercise in 33 children (age 2-18). There was no significant change in the P-R interval with the changes in heart rate obtained in these 54 children.

DETECTION OF MYOCARDIAL DAMAGE DURING CARDIAC SURGERY BY ISOENZYME ANALYSIS OF SERUM CREATINE PHOSPHOKINASE. C. R. Roe, H. N. Oldham, Jr., G. W. Young and W. C. Sealy. Duke University Medical Center, Departments of Pediatrics and Surgery, Durham, North Carolina.

Electrophoretic detection and quantitation of the MB isoenzyme of creatine phosphokinase (CPK-MB) in serum has proved to be a highly specific index of acute myocardial damage in the medical and surgical patient. Techniques for demonstration of this isoenzyme have been successfully applied to the recognition of myocardial damage following major cardiac operative procedures. To determine the intra-operative (IO) onset of damage reflected by appearance of CPK-MB, serial analyses have been performed on patients (during and immediately following) major non-cardiac and cardiac surgery. IO analyses on 3 patients undergoing thoracotomy failed to reveal CPK-MB in serum. Elevated CPK activity was due to release of CPK-MM from skeletal muscle trauma. IO isoenzyme analysis of 3 of 12 aorto-coronary by-pass grafts, 3 of 6 aortic valve replacements and 3 of 4 atrial septal defect repairs indicates that these procedures may also be performed without significant damage. The rapid IO appearance of serum CPK-MB in the remaining cases revealed onset of damage 1. during induction of anesthesia, 2. during cardiopulmonary by-pass and, 3. postoperatively. Serial isoenzyme analysis during the perioperative period will provide a more critical correlation between intra-operative events and myocardial damage.

CONGENITAL HEART DISEASE IN INFANTS OF DIABETIC MOTHERS. Thomas W. Rowland, John P. Hubbell, and Alexander S. Nadas. Dept. of Ped., Harvard Med. Sch.; Dept. of Cardiology, The Children's Hosp. Med. Ctr., Boston.

A review of 470 infants of diabetic mothers revealed 19 cases of proven congenital heart disease (4%), five times the incidence in the general population. Transposition of the great arteries, ventricular septal defect, and coarctation of the aorta together comprised over one-half of the cases, which were verified by catheterization, autopsy, or surgery.

No relationship between age of onset, severity, or duration of maternal diabetes and incidence of heart defects was observed.

Cardiomegaly on chest x-ray, heart murmur, and respiratory distress were common and gave little assistance in differentiating heart disease from other neonatal difficulties typical of these babies. In those infants with serious cardiac anomalies necessitating diagnosis in the newborn period, however, the abnormal electrocardiogram, presence of cyanosis without x-ray evidence of pulmonary disease, or loud or diastolic murmur served as important clues to the identification of babies with congenital heart disease.

ACID-BASE ALTERATIONS ASSOCIATED WITH CONGESTIVE HEART FAILURE IN CHILDREN BEYOND INFANCY: PATHOGENESIS. Shyamal K. Sanyal, S.K. Jain, M.K. Thapar, D.B. Sarkar and S. Madhavan (Intr. by Donald Pinkel)., Dept. of Pediatrics, Safdar-Jung Hospital, New Delhi, Patel Chest Institute, New Delhi, India, and St. Jude Children's Research Hosp., Memphis, Tenn.

Acid-base alterations associated with congestive heart failure have been *scantily documented in children beyond infancy. A prospective study was* designed to determine (1) acid-base profile and (2) pathogenesis of acid-base changes in children with heart failure. These patients were above 5 years of age. Arterial blood samples were obtained before and 1 week after start of decongestive therapy and analyzed for pH, pO_2, pCO_2, bicarbonate, lactic and pyruvic acid. Detailed pulmonary function studies were done in 12 children.

In 75 children with heart failure, the mean values for pH, pCO_2 and bicarbonate were 7.30 ± 0.110, 53.3 ± 16.51 and 26.1 ± 6.32 as compared to 7.42 ± 0.044, 33.2 ± 3.91 and 22.1 ± 3.61 ($p < 0.001$). Acidosis was observed in 56 (respiratory in 7, mixed in 49), and alkalosis in 7. Lactic and pyruvic acid levels were elevated in 14 of 20 children. Vital capacity, FEV_1, total lung capacity and maximum voluntary ventilation were significantly reduced and functional residual capacity increased ($p < 0.01$) in patients with heart failure. Air velocity index was less than 1 in 6 of 10 patients. Five of the 6 patients with abnormal air-velocity index showed elevated pCO_2. Seven patients expired. In each fatal case the pH was less than 7.1 and pCO_2 above 60 mmHg.

We conclude that congestive heart failure produces significant acid-base alterations in children beyond infancy. Compromise of pulmonary function, restrictive and/or obstructive in nature, and high blood levels of lactic and pyruvic acids play an important role in the pathogenesis of these changes.

LIGATION OF PATENT DUCTUS ARTERIOSUS IN PREMATURE INFANTS REQUIRING ASSISTED VENTILATION. Bijan Siassi, Luis Cabal, Carlos E. Blanco and Arnold G. Coran. (Intr. by Paul F. Wehrle.) Los Angeles County-USC Medical Center.

Surgical ligation of patent ductus arteriosus (PDA) was performed in 25 premature infants in respiratory failure requiring assisted ventilation (IPPB):

	Gestation (Wks)	Birth Wt. (g)	Wt. at Surgery (g)	Age at Surgery (Days)
Mean	30	1336	1386	23
Range	24-34	800-2520	750-2190	8-49

The magnitude of L → R shunt was measured in all cases by dye dilution and oximetry averaging 74 ± 12%. In addition, clinical diagnosis of PDA was confirmed in 9 infants by cardiac catheterization and cineangiography. Nineteen infants had severe RDS requiring IPPB within the first 2 days after birth. Heart failure in these instances was characterized by persistent tachycardia (>160 BPM), low diastolic arterial pressure (<25 mmHg), increasing respirator pressure and requirement for oxygen. Thirteen infants in this group survived. All 6 infants who first required IPPB after 8 days of age because of apnea or congestive heart failure survived.

The data suggest that surgical ligation of PDA in premature neonates with minimal lung disease has negligible surgical risk. With evidence of pulmonary oxygen toxicity (>60% oxygen for >150 hours) the prognosis was worsened. Infants with L → R shunt <65% did not appear to benefit significantly from this procedure.

VALVE REPLACEMENT DURING ACTIVE RHEUMATIC FEVER. Arnold W. Strauss, David Goldring, Antonio Hernandez, Alexis F. Hartmann, Jr., John Kissane, Clarence S. Weldon and Robert McKnight. Washington University Medical School, Departments of Pediatrics, Pathology, Surgery and Radiology, St. Louis, Missouri.

A study of pediatric patients requiring valve replacement for mitral insufficiency (MI) is presented to determine differences in response in patients with inactive rheumatic fever (2), those with clinically or pathologically active rheumatic fever (4), and those with isolated congenital MI (2). The case histories, laboratory and hemodynamic data, surgical pathology, surgical results and post-operative course were compared. Data from the few previously reported experiences by others were analyzed. The results showed that congestive heart failure in both active and inactive rheumatics was largely due to mechanical factors, not active myocarditis; that all survived and were dramatically improved; that there were no differences in the clinical, hemodynamic or post-operative findings; that there was no evidence of reactivation of rheumatic fever post-operatively or after cardiac catheterization and that, contrary to the traditional concept, valvular surgery is not only feasible, but mandatory, in some cases of rheumatic fever whether the disease is active or not.

Effect of Birth Asphyxia on Blood Volume and Cardiovascular Hemodynamics of Newborn Lambs.A.C.Yao,T.Lu, R.Castellanos, and B. Matanic S.U.N.Y.Downstate Med.Ctr.Dept.of Ped.Ob-Gyn. and Lab.Animal Sci.Brooklyn,N.Y.(Intr. by Q.H. Qazi).

Blood volume and cardiovascular hemodynamics were studied in five asphyxiated newborn lambs with immediate cord clamping(A)following caesarean section of ewes with acutely induced hypoxia.Eight non-asphyxiated lambs served as control:four of these had immediate cord clamping(B)and the other four had delayed clamping(C)at caesarean section.Gestational age ranged from 138-148 days and weight from 3.25-4.9 kg.Blood volume was measured by double label(RIHSA^{125} and Cr^{51})dilution technique: (Values: Mean ± S.E.)

Groups	RCV (ml/kg)	BV ml/kg)	HCT (%)
A	43.2± 1.7	103.9± 5.6	42.6± 1.2
B	33.0± 2.1	99.5± 8.2	33.0± 1.1
C	49.3± 2.6	103.3± 7.6	50.3± 2.2

Blood pH,pO2, pCO2 were monitored.Heart rate,arterial, atrial,right ventricular or pulmonary arterial pressures were measured by simultaneous catheterizations via the umbilical artery and vein and cardiac output determined by indocyanine green dilution.The hemodynamic measurements in the asphyxiated lambs(A)were significantly higher than group B,but similar to group C,except for slightly lower cardiac output.

It is suggested that lambs asphyxiated in utero, despite immediate cord clamping, start out with greater blood volume and circulatory overload and thus are like lambs with delayed cord clamping.

DEVELOPMENTAL BIOLOGY

First Session

SERUM GONADOTROPIN AND SEX STEROID CONCENTRATIONS IN THE HUMAN FETUS. Jeremy S.D. Winter, Richard Boroditsky, Charles Faiman and Francisco I. Reyes. University of Manitoba, Depts. of Paediatrics, Obstetrics-Gynaecology and Physiology, Winnipeg, Manitoba.

Individual serum concentrations of FSH, HCG, testosterone (T) and estradiol (E) were measured by radioimmunoassay in 23 male and 24 female human fetuses delivered by hysterotomy. Results were correlated with crown-rump (CR) length (5-24 cm), fertilization age (10-24 weeks) and maternal serum concentrations of HCG and E. In females, serum T was less than 100 ng%. In males, T levels were significantly higher, with values up to 300 ng%. These showed a pattern similar to that we have reported in testicular T concentration, with highest values at 7-14 cm. CR (11-16 weeks). Both fetal and maternal HCG levels declined with fetal age, with no apparent sex difference. Maternal/fetal HCG ratio in paired samples was 33/1. Fetal serum E levels (30-900 ng%) were similar to maternal values and showed no sex difference. Fetal male FSH concentrations were negligible; but high FSH levels (10-240 µg LER-907/100 ml) were seen in female fetuses. These data provide further evidence for HCG-mediated T production by the fetal testis at the time of genital differentiation. The finding of elevated FSH levels in female fetuses in the face of high serum E concentrations is unexplained.

ANTI-HEART ANTIBODY PRODUCTION OF CARDIOVASCULAR MALFORMATIONS IN THE MOUSE. James J. Nora, M.D., Vincent N. Miles, M.D., Jill H. Morriss, M.D. and E. Joy Weishuhn, B.S., Univ. of Colorado Med. Center, Denver, Co. 80220, and Michael R. Nihill, Texas Children's Hosp., Houston, Tx. 77025.

Cardiovascular malformations have been produced in 22% of C57BL/6 mice by injecting on 4 consecutive days of gestation (days 8-11 or days 12-15) anti-heart antibody (RAMHS) which has been raised in rabbits and adsorbed against mouse kidney and liver to yield an antiserum with high anti-heart activity and low activity against other organs, demonstrably kidney. Cardiovascular maldevelopment occurred in 9% of mice injected with anti-kidney antibody (RAMKS), 7% receiving normal rabbit serum and 1% receiving normal saline. A highly significant ($p < .001$) difference between the RAMHS and normal saline groups demonstrates that anti-heart antibody is teratogenic to the developing heart; and a significant difference ($p < .05$) between RAMHS and RAMKS antibody in the frequency of induction of cardiovascular anomalies suggests that there is organ-specific activity to anti-heart antibody. The type of cardiovascular anomalies caused in the mice studied were the same (VSD in C57 and ASD in A/Jax) as those which occur spontaneously or are produced by other teratogens such as dextroamphetamine. Anti-heart antibody would thus not appear to produce a specific type of maldevelopment but rather make manifest the malformation to which there is an hereditary predisposition (genetic-environmental interaction).

COLLAGEN IN EARLY HEART DEVELOPMENT. Johnson, Randall C., Francis J. Manasek, Walter C. Vinson, and Jerome M. Seyer (Introduced by Alexander S. Nadas) The Children's Hospital, Depts. of Cardiology and Orthopedics and Harvard Medical School, Dept. of Anatomy.

A regulative function has been ascribed to extracellular matrix (ECM), especially collagen, for development of many embryonic organs. It is possible that abnormal ECM production results in abnormal parenchymal development. We examined chick cardiac ECM at the time of early heart formation (6-13 somites). The electron microscope revealed the presence of crossbanded unit collagen as well as accumulations of basal lamina-like material in the cardiac jelly. Carboxymethyl cellulose chromatography of collagen extracted from hearts of lathyritic embryos incubated with ^{3}H-proline showed that collagen was being synthesized at the time of organ formation and differentiation. Both α 1 and α 2 chains were synthesized in a ratio of 2:1. This is the earliest that collagen has been found in the developing heart. Since there are no connective tissue cells present at these times the collagen must be a parenchymal product. We propose that this collagen, possibly in conjunction with matrix glycosaminoglycans, may play a crucial role in organ development.

(Supported by HL 10436, HL 13831, and AM 15671. F.J.M. is a recipient of a Career Development Award.)

DIURNAL PATTERNS OF DNA SYNTHESIS: MODIFICATION BY DIET AND FEEDING SCHEDULE. Peter R. Dallman and Robert A. Spirito, Dept. of Pediatrics, University of California-San Francisco.

Rates of cell division and DNA synthesis in man and other mammals have a marked diurnal fluctuation which may be modified both by the nature of the diet and the schedule of feeding. Rats of the Sprague Dawley strain were studied at 32 days of age when they are growing rapidly and liver DNA synthesis is still active. The diurnal pattern of DNA synthesis in rat liver was investigated at 3 - 4h intervals after 10d of adaptation to a 12h light - 12h dark cycle. The initial group was given a normal diet ad-libitum. The incorporation of thymidine-^{3}H into nuclear DNA varied over a 5-fold range with a peak at the end of the dark period as previously shown. Incorporation of thymidine-^{3}H into mitochondrial DNA remained relatively constant. Next, administration of a protein deficient diet (3.5% protein) for 4d was found to obscure the diurnal peaks of incorporation of thymidine-^{3}H into liver DNA and of thymidine kinase activity normally observed in animals fed a diet containing 26% protein. The effect of feeding schedule was then studied by restricting exposure to the stock diet to either the first 8 hours of dark (close to normal feeding behavior) or to the first 8 hours of light. The 12h shift in the feeding period resulted in a corresponding shift in the timing of peak incorporation. The results indicate that the prominence of the diurnal peak of nuclear DNA synthesis in the liver is dependent on the protein content of the diet and on the timing of food intake. (Supported by USPHS grant AM13897).

POLYAMINE BIOSYNTHESIS IN HUMAN FETAL LIVER AND BRAIN. John A. Sturman & Gerald E. Gaull, Dept. Ped. Res., N.Y. State Inst. Res. Mental Retard., Staten Island, N.Y., & Dept. Ped., Div. Med. Genet. & Clin. Genet. Center, Mt. Sinai Sch. Med., CUNY, New York, N.Y.

Our previous studies with human fetal tissues indicated that the transsulfuration pathway is inoperative and that remethylation favoring DNA synthesis was increased. We now have studied the S-adenosylmethionine (SAM) and ornithine decarboxylases in fetal (2nd trimester) and mature human tissue. These enzymes are involved in the biosynthesis of putrescine and the polyamines spermidine and spermine, compounds which are thought to be regulators of the synthesis of RNA and proteins.

The ability of fetal liver extracts to decarboxylate SAM was 20-fold greater than extracts of mature liver, and the concentration of the product, spermidine, was 4-fold higher in the fetal liver. Extracts of fetal brain had only one-third of the activity of extracts of mature brain when assayed in the presence of exogenous putrescine, but similar activity when assayed in the absence of putrescine. The concentration of putrescine in fetal brain was 8-fold that in adult brain, but the concentrations of spermidine and spermine were similar. Ornithine decarboxylase activity was low in all tissues.

These results suggest that at this stage of development fetal liver is adapted for rapid RNA and protein synthesis in that it has high polyamine concentrations and increased capability of polyamine synthesis. Fetal brain tissue appears to have the capability for rapid polyamine biosynthesis, but this is not manifested by increased concentrations.

DNA SYNTHESIS IN CATCH-UP GROWTH. Hector G. Jasper & Jo Anne Brasel. College Physicians & Surgeons, Inst. Human Nutrition & Dept. Pediatrics, New York.

Since the biochemical events associated with catch-up growth are not well documented, we have studied liver growth after nutritional rehabilitation in rats. Radiothymidine uptake into DNA and DNA polymerase activity were related to cell growth parameters. Undernutrition from 0-11 days of age reduced cell number(DNA 55% normal)without changes in cell size (Protein/DNA 96%). Within 48 hrs of refeeding DNA increases to 68% normal and to 80% by 7 days with no significant changes in cell size. Therefore proliferative cell growth was the major event in the first days of catch-up. Mean thymidine uptake was 38% normal in malnourished rat liver; with refeeding it reached normal by 48 hrs, peaked at twice normal at 72 and returned to normal at 96 hrs. DNA polymerase activity/mg DNA(denatured primer) rose from malnourished levels of 54% normal to 108% at 12 hrs refeeding and to 180% normal at 24 & 48 hrs. Therefore the very prompt cellular proliferation in these severely stunted rats, shown by significant increases in DNA content and thymidine uptake, is preceded by elevations in DNA polymerase activity. Thus it may be of interest to investigate levels of polymerase activity in retarded and accelerated human growth.

EVIDENCE FOR FETAL GENE EXPRESSION IN 21-TRISOMIC FIBROBLASTS TRANSFORMED BY SV_{40} VIRUS. Oliver W. Jones, Pamela N. Porter and David L. Bull. (Intr. by Jerry A. Schneider). Univ. of Calif., San Diego, Dept. of Med., La Jolla, Calif. 92037.

It has been postulated that genes for certain tumor viruses, which later in life may act as determinants of cancer, may be important also as gene determinants during normal embryogenesis. The viral information manifest through fetal gene expression should be detectable during a certain period of normal fetal development. De-repression of fetal gene activity should occur in tumor cells and in cells transformed with oncogenic viruses.

We have established that normal human fetal tissue contains a form of thymidine kinase which is unique and can easily be distinguished from adult thymidine kinase. The fetal enzyme is exclusively cytoplasmic and is absent in normal adult tissue. Human cancer cells contain fetal thymidine kinase almost exclusively.

Fibroblasts derived from an individual with Down's syndrome contain primarily adult thymidine kinase. 21-Trisomic fibroblasts transformed by SV_{40} virus reverts to synthesis of fetal thymidine kinase and the adult form disappears. By all criteria, gel electrophoresis, sucrose gradient sedimentation, heat lability and sensitivity to inhibitors, thymidine kinase in transformed 21-Trisomic fibroblasts is identical to normal human fetal enzyme and is not an SV_{40} virus-coded protein. It is possible that the human fetal gene for thymidine kinase may fulfill criteria of a virus expression, vertically transmitted as part of the inherent genetic information in human cells.

MATERNAL NUTRITION AND FETAL GROWTH. Richard L. Naeye, William A. Blanc, Depts. of Pathology, Pennsylvania State University College of Medicine, Hershey, Pa. and Columbia University College of Physicians & Surgeons, New York City.

A study of 521 gestations found fetal growth to be influenced by maternal nutrition. Maternal stature, one reflection of preadolescent and adolescent nutrition had little correlation with fetal growth but mother's pregravid body weight as well as both diet and weight gain during pregnancy did have such a correlation. Neonates undernourished before the third trimester had growth retardation of almost all fetal body organs including the brain. If liver and adrenal findings are representative, this growth retardation was due to a smaller number of cells in the various organs. By contrast, late gestational undernutrition had less effect on bones, brain, heart, lungs and kidneys. Both reduced cell size and number contributed to small organ size in this latter period. Fetal growth improved with successive pregnancies except in poorly nourished mothers whose successive offspring became more growth retarded. Neonates of best nourished mothers had significantly larger brains than infants from other groups. Fetal growth retardation in offspring of poor and black mothers was due to poorer maternal nutritional status including more iatrogenic low calorie diets during pregnancy. The growth retardation associated with toxemia was partially related to prescribed low calorie diets.

INDEPENDENT BIOSYNTHETIC CONTROL OF α AND β CHAINS OF HEMOGLOBIN. George R. Honig, Jeffrey L. Wolf, and R. George Mason, Univ. of Illinois College of Medicine, Univ. of Ill. Hospital, Department of Pediatrics, Chicago, Illinois.

Nonpathologic erythroid cells normally synthesize α and β hemoglobin chains at very nearly equal rates. An experimental model was used to investigate the proposed possibility that β chain synthesis might depend in part on the concomitant synthesis of α chains. Erythroid cells were obtained from rabbits having a variant hemoglobin in which the α chains contain 3 isoleucine (ileu) residues while no ileu is present in the β chains. Cells were incubated in media containing a radioactive amino acid and L-O-methylthreonine, an inhibitor of protein synthesis which is a specific antagonist of L-ileu. With bone marrow cells, which have no detectable uncombined α chain pool, incubation with the ileu antagonist produced an apparent inhibition of both α chain and β chain synthesis. In order to eliminate interference from other proteins synthesized by bone marrow cells the following steps were performed: 1) DEAE-Sephadex chromatography, 2) hemoglobin gel filtration (G-75), 3) globin gel filtration, and 4) CMC chromatography. The purified globins demonstrated no significant effect on β chain synthesis accompanying inhibition of α chain synthesis. These findings suggest that α and β chains are synthesized under independent control. Globin synthesis studies with bone marrow cells may require extensive purification of biosynthetic products for meaningful interpretation of experimental results.

DRUG-BILIRUBIN INTERACTION: AN ALTERNATE HYPOTHESIS FOR KERNICTERUS by Joseph Krasner, Ph.D., Michael Thaler, M.D. and Sumner J. Yaffe, M.D., Department of Pediatrics, State University of New York, Children's Hospital, Buffalo, N.Y. and Univ. of California, San Francisco, Calif.

The administration of drugs to the newborn infant has been implicated in the production of kernicterus by direct displacement of bilirubin from its binding to serum albumin. An in vitro experiment in which whole rat brain was submersed in an aqueous bilirubin-bovine albumin solution was used to examine an alternate hypothesis for drug-induced bilirubin neurotoxicity. C^{14} bilirubin was utilized to determine loss of bilirubin from the solution and uptake by the brain. In the presence of 7.4 mg/100ml of salicylate, bilirubin concentration in the solution decreased 13.5 %/mg of brain tissue present when compared to the same system in which no drug was added. A 38 mg/100ml sulfisoxazole solution produced a 2 fold decrease in bilirubin concentration of the incubation mixture. Histological sections of the brain tissue showed marked yellow coloration when drug was present with no discernible color in the absence of drug. These results suggest that high concentrations of bilirubin alone are not sufficient to produce yellow staining of the brain. The presence of either drug is necessary. They may exert their effects by either acting as a transport carrier for the bilirubin or by producing an alteration in brain membrane permeability.

DEVELOPMENTAL BIOLOGY

Second Session

CORTISOL AND LUNG CHOLINE PHOSPHOTRANSFERASE (CPT) ACTIVITY IN THE FETAL RAT AFTER *in utero* DECAPITATION. Philip M. Farrell and Will R. Blackburn, (Intr. by Paul A. di Sant'Agnese), NIH, Bethesda, Md., and Penn. State Univ., Col. Med., Hershey, Pa.

Fetal rabbits injected with corticosteroids show increased pulmonary lecithin synthesis through the choline incorporation pathway as mediated by CPT (Science 179:297, 1973). In contrast, reduced lecithin synthesis is present in the lungs of hypophysectomized fetal rats (decapitated at 16 days gestation). In this work, the latter animals have been employed to further study the role of cortisol in regulating lung surfactant synthesis.

Measurements of plasma cortisol revealed that fetal rats show a rise beginning on day 19 and reaching a peak of 7.5 µg/100 ml on day 20. Lecithin synthesis as measured by ^{14}C-choline incorporation with lung slices rises in parallel fashion beginning within 24 hours after the change in cortisol. In decapitated fetuses, however, cortisol was found to remain at trace levels throughout gestation. Lung CPT activity was significantly reduced in decapitated fetuses at 22 days gestation from 7.7±0.4 (control mean) to 5.1±0.4 picomoles lecithin/min/mg lung ($p<.001$). Early attempts to raise the enzyme level by injecting the decapitated animals with dexamethasone have been partially successful, a group of 5 showing a mean CPT of 7.4. Nuclear dexamethasone receptors were found to be quite adequate in the decapitated fetus, peaking on day 19 along with the controls. In conclusion, these findings support the hypothesis that in the fetus cortisol plays a key role in controlling lung CPT and regulating lecithin synthesis.

HUMAN FETAL LUNG CELLS IN MONOLAYER CULTURE: GROWTH ENHANCEMENT WITH CORTISOL. Barry T. Smith, John S. Torday, and C.J.P. Giroud (Intr. by M.E. Avery). Dept. Exp. Med., McGill Univ. and The McGill Univ.-Montreal Children's Hosp. Res. Inst., Montreal, Canada.

Human fetal lung cells were obtained by trypsin dispersion of lungs removed from six 10-20 week fetuses at hysterotomy. The cells were grown in Ham's F10 medium in 95% air/5% CO_2. The addition of 2 µg/ml (.55 µmole%) of cortisol (F) to the feeding medium resulted in marked enhancement of growth within 24 hours of implantation. F-treated cells reached confluence in an average of 10 days, as opposed to 16 days for control cells.

At intervals up to 14 days after implantation, cultures were incubated with equimolar amounts (1.8 nmole) of ^{3}H-cortisone (E) and ^{14}C-F. In all cultures there was a significant conversion of E to F but this was almost doubled in F-pretreated cultures.

The specificity of these observations was shown: 1) by comparing the effect of F with that of 6 other corticosteroids, each differing from F at one molecular position (E; corticosterone; 11-deoxycortisol; 4-pregnen-11β,17 α,20 α,21-tetrol-3-one; 5-pregnen-3β,11β,17 α,21-tetrol-20-one; and 21-deoxycortisol); 2) by demonstrating growth inhibition of fetal skin fibroblasts and a lack of effect on fetal kidney parenchymal cell growth with F; and 3) by demonstrating the inability of these other cell types to convert E to F.

HUMAN LUNG: A TARGET TISSUE FOR GLUCOCORTICOIDS? Philip L. Ballard* and Roberta A. Ballard* (Intr. by W.H. Tooley) Cardiovasc. Res. Inst., NHLI Sp. Ctr. of Res. - Pulm. and Dept. of Ped., University of California, San Francisco, Ca.

Glucocorticoids accelerate pulmonary development and induce early appearance of alveolar surfactant in fetal lamb and rabbit. The lungs of these animals contain cytoplasmic glucocorticoid receptors, specific proteins which mediate the earliest steps of hormone action in target tissues. To evaluate the potential responsiveness of human lung to glucocorticoids, we examined cytosol fractions of human fetal lung for specific binding of ^{3}H-dexamethasone. All specimens (n=16) from fetuses of gestational age 12 to 26 weeks obtained at hysterotomy or postmortem contained binding activity with a mean value of 0.20 pmoles receptor sites/mg protein (range 0.11-0.34). The concentration in lung was consistently greater than in corresponding liver specimens (mean 0.08 pmole/mg protein, range 0.03-0.10). The binding reaction requires 12-24 hrs at 0° to reach equilibrium, and dissociation of dexamethasone from receptor has a $t_{\frac{1}{2}}$ of 30-50 hrs. The mean equilibrium dissociation constant was 11.5 nM (range 4-21). *Other steroids with glucocorticoid activity compete for binding, while inactive steroids do not. The receptor-steroid complex which is formed in the cytoplasm transfers to nuclear sites at 20° or 37°, but not at 0°, in both cell-free and intact cell preparations. We conclude that human fetal lung contains receptors essential for the initial events in glucocorticoid action. This suggests that human lung is a direct target tissue for corticosteroids and is responsive to these hormones during fetal life.*

FATTY ACID SYNTHESIS BY THE DEVELOPING LUNG. Ian Gross and Joseph B. Warshaw, Harvard Med. Sch., Mass. Gen. Hosp., Shriners Burns Inst., Boston Hosp. for Women, Boston, Mass.

Long chain fatty acids (FA) are important components of pulmonary surfactant and of lung membranes. The lung can incorporate endogenously synthesized and exogenously supplied FA into surfactant. We have investigated developmental profiles of de novo synthesis of FA and microsomal and mitochondrial FA elongation in lung and liver of the rabbit. After 23 days gestation de novo FA synthesis by FA synthetase in the supernatant fraction of lung homogenates was similar to adult controls (20 n moles/mg/hr). In contrast, FA synthetase activity of fetal liver was greater than in adults (60 as compared with 15 n moles/mg/hr). Hepatic FA synthetase activity decreased at birth but increased again after weaning. Acetyl-CoA carboxylase, the initial step in de novo FA synthesis, followed a similar developmental pattern. Pulmonary mitochondrial and microsomal FA elongation activity was low during fetal development but increased 3 to 4 fold after birth. Hepatic microsomal and mitochondrial FA elongation showed a similar developmental profile. These findings indicate that the developing rabbit has active de novo FA synthesis in both lung and liver. However, in contrast to liver, FA synthetase activity of lung did not decrease after birth in response to the high fat diet provided by milk. Pulmonary mitochondrial and microsomal FA elongation *do not appear to be important until after birth.* It is likely that FA synthesized in the lung as well as those derived from the liver are important for both pulmonary membrane growth and the production of pulmonary surfactant.

DEVELOPMENTAL CHANGES IN SOME CYTOPLASMIC ENZYMES OF ADIPOSE TISSUE IN HUMAN NEWBORN INFANTS. Milan Novak, Peter Hahn, Duna Penn, Ellen F. Monkus and Lawrence T. Kirby, Dept. of Ped. Univ. of Miami, Sch. of Med., Miami, Fl., and Dept. of Ped. & Obst. & Gynec., Univ. of Brit. Columbia, Sch. of Med., Vancouver, Can.

The human newborn obtains a large amount of energy from fat, fetal glycogen being rapidly utilized after birth. In adipose tissue the rate of lipolysis depends on glycogen content and utilization. In the fetal period the major part of fatty acids are synthesized. After birth increased lipolysis, and later ingestion of milk, supplies the organism with fatty acids so that suppression of fatty acid synthesis would be expected. We studied the activities of citrate cleavage enzyme (CCE), malic enzyme (ME), phosphoenolpyruvate carboxykinase (PEPK), glycerophosphate dehydrogenase (GDH) and glucose-6-P dehydrogenase (G-6-PDH) in cytosol of subcutaneous adipose tissue. ME activity was highest during the first day of life and then decreased. CCE had the same tendency, but only the difference between newborns and adults was significant. Both G-6-PDH and GDH activities were higher in the neonate but no developmental changes were noted. CCE catalyses citric acid breakdown which provides acetylCoA and ME and G-6-PDH furnish "reduction potential" for fatty acid synthesis. GDH and PEPK may be involved in glycerol production.

Our results imply that the capability of the adipose tissue to synthesize fatty acids decreases rapidly in the first days of life so that early exogenous fatty acid requirements should be considered in the management of the neonate.

CEREBRAL OXIDATION OF GLUCOSE AND D-BOH-BUTYRATE BY THE ISOLATED PERFUSED HUMAN FETAL HEAD. Peter A.J. Adam, Neils Räihä, Eeva-Liisa Rahiala, and Martti Kekomäki. Case Western Reserve Univ.,Cleve.,and the Univ. of Helsinki,Depts. of Pediatrics.

Even though arterial:jugular venous gradients for D-B-OH-butyrate(BOHB)have been demonstrated in starved adult man and the young infant, its complete cerebral oxidation to CO_2 has not been demonstrated in a physiological system. In order to determine whether BOHB can replace glucose(G), 8 human fetal heads,obtained by abdominal hysterotomy at 12-17 weeks gestation,were perfused through the internal carotid arteries with 2.8mM glucose-6-C^{14}($G6C^{14}$) or 3.8mM B-OH-butyrate-3-C^{14} ($BOHB3C^{14}$).Oxidative metabolism was quantified by serial collection of gaseous $C^{14}O_2$ from the closed perfusion system,and from the recirculating medium. G and BOHB were utilized at physiological rates, as tabulated (mean ± SD):

Substrate	(n)	μmole/min. g brain Utilization	CO_2 Production
Glucose	(4)	0.13 ± 0.03	0.05 ± 0.04
BOHB	(4)	0.26 ± 0.11	0.06 ± 0.02

If these rates are compared to fetal weight and steady state metabolic data in newborn infants or mammalian fetuses, cerebral G consumption accounted for about 50% of total fetal G uptake.Even though BOHB was taken up at twice the rate of G, unlabeled G did not interfere with the rates of BOHB utilization;and CO_2 production correlated closely with BOHB uptake. Apparently BOHB may replace glucose as the "essential" substrate for cerebral oxidative metabolism early in fetal life.

THE INFLUENCE OF HIFAT DIET ON BRAIN GLYCOLYSIS. P.T. Ozand, J. Stevenson, J.T. Tildon & M. Cornblath, Univ. of Maryland Sch. of Med., Dept. of Ped., Baltimore, Md. 21201 & Rosewood State Hosp., Owings Mills, Md. 21117.

After 24 hours of starvation, newborn pups of dams fed a HiFat (HF) diet have a significant reduction of brain glycogen as compared to control pups (C)(Ped.Res., 6:395, 1972). To determine whether this difference was associated with an alteration in brain glycolysis, glucose-6-phosphate (GP), Pyruvate (P), and Lactate (L) were measured in the brain of HF pups on 1,2,3,5,7,14 and 21 days of life, and compared to levels in C pups. Brain GP in the normal rat falls progressively during the first 5 days of life from 4.82 ± 0.45 to 2.74 ± 0.30 μmoles/gm protein. In HF pups the GP levels were higher at birth (6.13) and fell much more rapidly (2.10 by the 2nd day of life). Starvation for 24 hrs decreased these values about two fold in HF pups but had no effect on C pups. In C pups the ratios of L/P was relatively constant (12.8, 15.8, 16.9, 9.7 on days 1,2,3 and 5 respectively), but on identical days the ratio in HF pups were significantly higher (43.2, 76.9, 72.3 and 79.6). Cross fostering experiments confirmed that these effects were inductive changes in response to hyperlipemia and hyperketonemia. The results suggest a shift in the regulation of brain glycolysis during development which is accelerated in HF pups and appears to be associated with the reduction of pyruvate to lactate. (Supported in part by NIH grants HD-03595-04 and HD-06291-01 and the John A. Hartford Foundation.)

FETAL GROWTH RETARDATION AND CELL METABOLISM Uwe Stave, Fels Research Inst., Yellow Springs, Ohio

Is fetal growth retardation accompanied by deviations in metabolic fuel utilization in different organs? We have analyzed 5 key enzymes in liver, heart muscle, brown adipose tissue (BAT) and 3 different brain areas of 24-h-old hypotrophic (ave. 35.8 g) and healthy control rabbits (ave. 59.7 g). The 5 key enzyme activities represent glycolysis (phosphofructokinase), Krebs cycle (citrate synthase), fatty acid oxidation (3-hydroxyacyl CoA dehydrogenase), fatty acid synthesis from glucose (ATP citrate lyase, ATP-CL), and ketone body utilization (3-ketoacid CoA transferase). Glycogen was also measured. Liver of hypotrophic animals had a 50% reduced ATP-CL while the ketone body production was markedly increased. The myocardium showed a trend for low key enzyme activities. In BAT the glycolytic capacity was reduced. In brain the potential for fatty acid oxidation was increased by 40%. The glycogen content was low in most tissues. The hypotrophic newborn is disadvantaged by starting extrauterine life with abnormally low carbohydrate reserves. Enzymatic adjustments for a more efficient fatty acid utilization take place primarily in brain stem but, also in midbrain and cortex. Krebs cycle activity is always within normal range, thus oxidative phosphorylation is not hampered. One practical conclusion is to provide nutrients soon after birth.

PHASES OF FETAL METABOLIC ADJUSTMENT TO MATERNAL STARVATION. Michael A. Simmons, Giacomo Meschia, Edgar L. Makowski, and Frederick C. Battaglia. Division of Perinatal Medicine, University of Colorado Medical Center, Denver.

Protein catabolism accounts for ~25% of fetal sheep O_2 consumption in the fed steady state. The present study describes the role of protein catabolism in the fetal metabolic response to maternal starvation. Six ewes had catheters placed in a maternal artery (A) and a fetal artery (a). After a 5-day recovery period, while the ewes were on a usual or an enriched protein diet, A and a glucose and urea concentrations were measured for 6 to 12 days, then total starvation begun. Urea a-A differences were unaffected by maternal diet despite significant differences in absolute urea concentrations, implying no increased protein catabolism in the fetus despite a higher maternal protein intake. Starvation led to increased urea a-A differences, with a peak at 4 days of starvation and a return to fed-state levels by 7 days of starvation. Glucose (a, A, A-a) fell sharply to a plateau by 48 hours. At the time of peak urea production, ~ 80% of the fetal O_2 consumption can be accounted for by protein catabolism. In starvation, the small A-a glucose difference implies a fetal glucose uptake of << 2 mg/min/kg but is still compatible with a surviving fetus. These data describe two well-demarcated phases of fetal metabolic adjustment to maternal starvation. A stable fetal glucose concentration results from an initial phase of increased protein catabolism and a second phase whose substrates are unknown.

GLUCONEOGENIC CAPACITY OF NEONATAL PIG LIVER. Thomas A. Helmrath and Loran L. Bieber (Intr. by William B. Weil), Michigan State Univ., Col. of Human Med., Dept. Human Develop. and Biochem., E. Lansing.

Failure to initiate hepatic gluconeogenesis may be a factor in producing neonatal hypoglycemia. Newborn piglets appear to be a model in which to study this problem, since they are known to become hypoglycemic when starved from birth. Activities of the gluconeogenic enzymes have been reported to be both substantial and low in piglets. We have investigated the activity of soluble and mitochondrial phosphoenolpyruvate carboxykinase (PEPCK) and mitochondrial pyruvate carboxylase (PC), and conversion of lactate-^{14}C to glucose-^{14}C in liver slices in fed and fasted 1 day old and fasted 24 day old

	Fed (1 day)	Fasted (1 day)	24 day (fasted)
P.C.	53.6±11.0(9)	82.2±16.1(7)	178±23.7(7)
PEPCK Soluble	37.8±3.9(9)	47.2±2.8(7)	52.0±5.9(7)
Mitochondrial	150.2±19.8(9)	171.6±14.7(7)	285.0±16.7(7)
Glucose-1 hr. umoles/g dry wt.	10.1±0.90(6)	6.97±0.67(7)	26.5±3.4(7)

piglets. 24 day old pigs had significantly ($P<.01$) higher PC and mitochondrial PEPCK specific activity than the 1 day old. Liver slices from 1 day old fasted piglets converted lactate to glucose at a greater rate than the 1 day fed, though the total glucose formed by either was 1/2 to 1/4 that of slices from 24 day old pigs. This suggests that glucose consumption exceeds the limited glucose generation in the piglets and is a cause of the hypoglycemia. (Supp. NIH - HD - 05821-03S1).

DEVELOPMENTAL BIOLOGY

Read by Title

PLACENTAL VILLITIS OF UNKNOWN ETIOLOGY: HARBINGER OF SERIOUS DISEASE? Geoffrey P. Altshuler. (Intr. by Irwin J. Light). Univ. of Cincinnati Med. Ctr. Fels Div. of Ped. Research. Dept. of Ped. Cincinnati.

Although light microscopy of human placental tissue reveals diagnostic features of cytomegalic inclusion disease and toxoplasmosis, and characteristic features of herpes, rubella and syphilis, villitis of unknown etiology has been described (Amer.J.Obstet.Gynec. 113:616, 1972). These lesions are present in 1% of all placentas. Recently 5 serious examples occurred in our newborn nurseries, four of which were fatal. Complete autopsies were performed. In the first, a 36 weeks gestation boy, autopsy disclosed severe hydrocephalus and bilateral cataracts. A 36 weeks gestation dysmature girl died with hyaline membrane disease and intra-ventricular hemorrhage. She also had bilateral cataracts. Another dysmature boy of 36 weeks gestation expired with extensive acute infarcts in multiple organs including the placenta. A fourth male neonate of 30 weeks gestation died with multi-cystic dysplastic kidneys. A fifth infant is alive at 8 months age, but has a head circumference greater than the 98th percentile. Because of hepatosplenomegaly a liver biopsy had been performed at 6 weeks age. This was consistent with viral hepatitis. Clinical and autopsy studies have excluded known neonatal infectious agents. Findings indicate an infectious cause but no etiologic agent has been identified. Nevertheless these placental lesions may be associated with brain and liver damage, congenital anomalies, dysmaturity, and prematurity.

EFFECT OF CYPROHEPTADINE ON GROWTH IN SHORT, UNDERWEIGHT CHILDREN, Alia Antoon, Hans H. Bode, John D. Crawford, Harvard Med. Sch., Shriners Burns Inst., Massachusetts Gen. Hosp., Dept. of Ped., Boston.

In a double blind study we have investigated the effect of cyproheptadine 6-12 mg/m^2 daily on growth velocity and weight gain in 24 short, underweight children. Except for two with growth hormone deficiency, none had primary endocrine, skeletal or metabolic disorders. There was close correlation between the administration of the drug, increased appetite and rate of weight gain in 15 of the patients. Favorable changes in staturał growth velocity were observed only after malnutrition was alleviated, suggesting that caloric deficiency was contributing to the stunted growth. With the cessation of therapy appetite, weight gain, and growth velocity returned to pretreatment levels. The most frequent side effect was mild sedation. A similar number of short, undernourished children have received cyproheptadine apart from the controlled study with demonstrable improvements in growth. Diagnoses in the latter group have ranged from idiopathic hypercalcemia to Silver's syndrome, acyanotic heart disease and hyperkinesis due to minimal brain dysfunction. It is concluded that a 3 month trial of cyproheptadine is indicated in children with short stature in whom suboptimal caloric balance may be a contributing factor. Continuing treatment is indicated in those showing growth acceleration. Aided by HD-T01-00033 and The Children's Medical Research Fund.

MALIGNANT TRANSFORMATION PRODUCED BY CYTOSINE ARABINOSIDE. William F. Benedict, Myron Karon, and Richard Kouri. Division of Hematology, Children's Hospital of Los Angeles, USC School of Medicine, Los Angeles, California, and Microbiological Associates, Bethesda, Maryland.

1-B-D-arabinofuranosylcytosine (ara-C) is an effective chemotherapeutic agent for the treatment of acute leukemia. The nucleoside is an inhibitor of DNA synthesis. As part of a major program to investigate the nature of chemical carcinogenesis, we have observed that as little as 10^{-7}M ara-C will produce transformation in fetal hamster cells, either in mass culture or with a quantitative transformation within 24 hours. This dosage is in the range obtained clinically. The transformed cells produce fibrosarcomas within one month when injected into newborn hamsters.

The fact that a water soluble nucleoside antimetabolite can induce malignant transformation in the hamster system similar to that produced by carcinogenic hydrocarbons and alkylating agents has at least two important implications: (1) antitumor agents used to treat acute leukemia might have a paradoxical effect and be involved in the induction of late recurrent disease seen after long disease free intervals; (2) patients who receive ara-C for the treatment of infectious disease in an effort to preserve life and/or prevent brain damage, need careful follow-up to determine the relevance of the animal carcinogenetic model to the human situation.

POSTNATAL Hb A AND Hb F SYNTHESIS IN INFANTS WITH SEVERE INTRAUTERINE GROWTH RETARDATION (IUGR). Harry Bard (Intr. by C. Roy) Univ. de Montréal, St-Justine's Hosp., Dept. of Ped., Montréal, Canada.

A previous study showed that term small for gestational age newborn infants (TSGA) synthesize significantly more Hb F than term newborn infants who are appropriate in weight for gestational age (TAGA) Ped. 45:766, 1970. To determine if the delay in switching to Hb A synthesis persists after birth in TSGA infants, Hb A and Hb F synthesis was measured at 2 week intervals in 9 TSGA infants weighing less than 2000gm born to toxemic mothers. For comparison 8 preterm infants appropriate in weight for gestational age (PRAGA) but within the same birth weight range as the TSGA infants and 9 TAGA infants were studied at the same intervals. Hemoglobin synthesis was determined by incubating reticulocytes with ^{14}C leucine, separating the Hb A and Hb F by ion exchange chromatography and finally liquid scintillation counting of the separated A and F fractions. Hb F synthesis was significantly elevated at birth in TSGA infants (81.4%), compared to TAGA infants (59.7%), $P<.005$, but was less than in PRAGA infants (87.2%) $P<.05$. At 4 weeks of age Hb F synthesis in TSGA infants (50.8%) was significantly less than in PRAGA infants (79.2%) $P<.005$ but no different than in TAGA infants (54.6%) $P<0.4$. IUGR secondary to toxemia favours Hb F synthesis inutero. After birth these TSGA infants revert to the proportions of Hb A and Hb F synthesis that correspond to their post-conceptional age.

STUDIES ON THE DEVELOPMENT OF FATTY ACID OXIDATION BY NEONATAL PIG LIVER. Loran L. Bieber, Thomas A. Helmrath, and M. A.K. Markwell (Intr. by. William B. Weil), Michigan State Univ., Col. of Human Med., Dept. Biochem. and Human Develop., E. Lansing.

Fatty acid oxidation stimulates hepatic gluconeogenesis in the rat. Since neonatal piglets have limited hepatic gluconeogenesis, we studied the capacity of piglet liver to oxidize fatty acids. The development of carnitine palmitoyltransferase (CPTase) and mitochondrial fatty acid oxidation and were investigated in 1 day, 24 day, and FFA infused piglets. Hepatic CPTase is low at birth and doubles by 24 hours of age, but develops slower in fasted than fed piglets.

	CPTase	Palmitoyl CoA Carnitine Oxidation	Pyruvate Oxidation
1 day Fasted	25.5±2.5(10)	31±4(10)	96±12(7)
Fed	27.4±4.4(9)	21±2.8(9)	66±11.6(9)
FFA Infused	29.0±2.3(18)	35±4.8(6)	53±10.3(10)
24 day Fasted	28.0±3.6(7)	65±4.2(7)	36±3.0(7)

Carnitine stimulated palmitoyl CoA oxidation is significantly ($P<.01$) higher at 24 days vs. 1 day and infusion of FFA had no additional effect. Pyruvate oxidase activity was significantly ($P<.01$) lower at 24 days. Although CPTase activity was maximal the capacity to oxidize fatty acids in one day old animals was one half that of 24 day old animals. This suggests that the limited capacity to oxidize fatty acids may contribute to the reduced hepatic gluconeogenesis in neonatal piglets. (Supp. - NIH - HD - 05821-03S1).

PHYSICAL GROWTH OF HEALTHY CHILDREN FROM BIRTH TO 7 YEARS, Glen S. Bartlett (Intro. by I.A. Schafer), Dept. of Ped., Case Western Reserve University at Cleveland Metropolitan General Hospital, Cleveland, Ohio

Weight, length, and head circumference data on over 35,000 apparently healthy children followed from birth to 7 years in the Collaborative Study on Cerebral Palsy have been used to generate distance, increment, and bivariate tables and graphs and to make sex and ethno-racial comparisons of physical growth. Based on these 15,821 white, 17,259 Negro, and 2281 Puerto Rican children of varied socioeconomic background: Whites are heaviest and have largest head circumferences at all ages and are longest through 3 years; Negroes are lightest through 3 years, shortest through 1 year and tallest at 4 years and beyond, and have intermediate head circumferences; Puerto Ricans are generally intermediate in weight and length through 1-3 years and smallest thereafter, and have the smallest head circumferences. Males are heavier, longer, and have larger head circumferences than females. Weight, length, and head circumference increase at each age with increasing socioeconomic status, partly explaining the ethno-racial differences: Higher status Negroes and Puerto Ricans are heavier, taller, and have larger head circumferences than lower status whites. Growth of all 3 ethno-racial groups differs appreciably from that indicated by current standards based on small numbers of children from similar socioeconomic backgrounds.

BIOCHEMICAL, ENDOCRINE AND STRUCTURAL CORRELATIONS IN DEVELOPING FETAL LUNG. Will Blackburn, G. Giannopoulos and M.A. Lopata (Intr. by N. M. Nelson). Pa. State Univ.Col. Med., Hershey, Pa.

Studies of fetal rat lung development revealed rapid cell proliferation during days 17-18 followed by a progressive slowing of this process until birth. Lung analyses indicated major alterations during day 19 in utero: (Values in picograms/cell)

Age (Days)	16	17	18	19	20	21	22
Cells x 10^6	6.5	18.6	48.7	113.3	155.5	183.2	198.5
Water	315.3	210.3	274.2	222.0	241.7	283.5	316.5
Protein	37.1	24.7	32.3	26.1	28.4	33.4	37.2
RNA	14.2	12.2	11.8	3.4	6.5	9.7	8.5
Glycogen	----	7.5	9.2	12.8	16.5	8.9	5.9
Lipid	16.0	14.8	13.4	9.3	13.6	15.3	17.8
P-Lipid	6.7	6.6	6.2	5.3	7.0	9.7	10.5
Lecithin	3.6	4.7	4.9	3.0	4.4	6.1	5.0

Type II cells first appeared in EM analyses on day 19, representing 2.3% of lung cells. Their concentration rose to a peak (17.5%) on day 21. Surface tension studies correlated with the appearance of type II cells and rising lecithin levels during days 20-22. Nuclear dexamethasone receptors also reached a peak level between days 19-20 and were associated with rising plasma levels of corticosterone. Collectively these data suggest day 19 as the initiation for type II cell differentiation and the onset of surfactant synthesis. The appearance of receptor protein, cortisol, and enzymes fundamental to lecithin synthesis are closely coupled events which precede the appearance of effective surface activity in fetal lung.

EFFECT OF THYROIDECTOMY ON BRAIN LIPIDS AND CEREBROSIDE FATTY ACIDS IN THE OVINE FETUS. Allen Erenberg, John H. Menkes, William Oh and Delbert A. Fisher. Harbor General Hospital, Torrance, and UCLA School of Medicine, Depts. of Pediatrics.

We have found that third trimester thyroidectomy (Tx) of the ovine fetus impairs carcass and lung growth and bone maturation, but does not affect growth of other organs (liver, heart, thymus, kidney, spleen, brain). We studied the effect of thyroid ablation on the composition of fetal brain. Five ovine fetuses were Tx at 90 to 100 days gestation and sacrificed 19 to 43 days post-Tx. Cerebral (Cb) and cerebellar (Cl) tissues were analysed for DNA, RNA, protein, total lipids (TL), phospholipids (P), Cholesterol (Ch), cerebrosides (C), and sulfatides (S), and for 18 carbon and 24 carbon cerebroside fatty acids (CFA 18, CFA 24) composition in the Cl. Differences between control and Tx are summarized in the table:

DNA		RNA		Prot		TL		CFA 18	CFA 24
Cb	Cl	Cb	Cl	Cb	Cl	Cb	Cl	Cl	Cl
NS	NS	NS	NS	↓	NS	↓	↓	↑	↓

The data indicate that third trimester fetal Tx in the sheep does not impair cell replication in the brain, but does reduce protein synthesis in the Cb and myelination in the Cl.

CHOLINE PHOSPHOTRANSFERASE (CPT) AND LECITHIN SYNTHESIS IN NEWBORN RABBITS TREATED WITH CORTICOSTEROIDS. Philip M. Farrell (Intr. by Paul A. di Sant'Agnese), NIH, Bethesda, Md.

Fetal rabbits injected with corticosteroids show elevated *lung CPT activity and enhanced lecithin synthesis through the* choline incorporation pathway (Science 179:297, 1973).

To determine if newborns could respond in similar fashion, 28 day gestation rabbits were delivered by C-section and treated with I.M. hydrocortisone (ca. 50 µg/g body wgt. q 6 hours) or saline. All animals had moderate respiratory distress but maintained a normal blood pH. The steroid treated group differed in no way from the controls in terms of clinical condition and lung appearance. At various intervals from 3-12 hours after birth, the animals were sacrificed and lecithin synthesis measured *in vitro* by incubating lung slices with ^{14}C-choline. Incorporation of isotope into lecithin was the same for 12 control and 12 steroid treated rabbits. Likewise, there was no difference in lung CPT activity.

An improved assay for CPT has been developed in this work and various kinetic features of the enzyme examined. The apparent K_m of rabbit lung CPT for CDP-choline was found to be 3×10^{-5}M. Although a K_m for the diglyceride substrate could not be determined, the enzyme was established as fully saturated in the presence of 1 mM dipalmitin. The absolute CPT activity at saturating substrate concentrations in newborn rabbit lung was 127 picomoles lecithin/min/mg protein. This relatively slow velocity is consistent with a possible role of CPT as the rate limiting enzyme in the choline incorporation pathway.

PANCREATIC ALPHA & BETA CELL RESPONSIVENESS IN FETAL & NEWBORN LAMBS. Robert H. Fiser, Dale L. Phelps, Allen Erenberg, Mark A. Sperling, William Oh & Delbert A. Fisher, Dept. of Ped., Harbor Gen. Hospital, UCLA Sch. of Med., Torrance, CA.

Hormonal control of energy and growth substrate flow in the developing fetus and newborn are not well defined. In an attempt to clarify the role of the pancreas, we have studied the in vivo response of the pancreatic alpha and beta cells in 10 fetal and 9 newborn lambs. For the fetal studies chronically catheterized preparations were used. Plasma glucose, immunoreactive insulin (IRI), and glucagon (GLU) levels were monitored serially after the following stimuli: glucose (6 mg/Kg/min), alanine (1 mM/Kg), and 2-deoxyglucose (50 mg/Kg); the latter to produce selective intracellular glucopenia.

Control plasma IRI levels were similar in the fetus and newborn and glucose/IRI ratios were relatively increased in the newborn vs. the fetal animals ($P < .001$). Plasma IRI was increased after glucose infusions in both the fetus and newborn ($P < .001$) and the response increased with gestational age. GLU levels were unchanged. Alanine did not alter plasma glucose, IRI or GLU in either the fetus or newborn. Deoxyglucose infusions decreased IRI ($P < .05$) in the neonatal period but had no effect in the fetus.

These data indicate 1) that the effects of IRI and GLU in control of intermediary metabolism probably are of minimal importance in the fetus and 2) that maturation of beta cell responsiveness to stimuli precedes that of the alpha cell.

LATER GROWTH AND CARBOHYDRATE METABOLISM IN INFANTS OF DIABETIC MOTHERS (IDM). Pamela Fitzhardinge, Charles Bauer, David Schiff, Donald Fields, Eleanor Colle. McGill Univ., Montreal Children's Hospital, Research Inst., Montreal, Quebec.

At birth, infants of diabetic mothers are hyperinsulinemic and large for gestational age. Fourteen IDM underwent serial studies of growth and carbohydrate metabolism in the first year. Growth rates were compared with 24 normal infants of the same age. The IDM grew more rapidly in length and less rapidly in weight than the controls. ($p < .02$). Twenty-one other IDM were studied at ages 4 to 9 years (mean 6.6 yrs.) and tended to be slightly short for their age ($p < .10$) with a wide variation in weight (mean weight normal). Glucose disappearance rates at 6 and 12 months were normal. Insulin response was increased. There was no correlation between insulin response and rate of growth. Oral glucose tolerance tests were done on 13 of the older IDM. Ten were normal. Hyperglycemia and hyperinsulinism were present in 3 children, two of whom were obese.

In summary, linear growth is rapid the first year but seems to decelerate before 4 years. Insulin production continues to be excessive at least for the first year but does not produce hypoglycemia. By 4 years the insulin levels are normal except when associated with obesity.

EFFECT OF ACUTE GRADED REDUCTION IN AMBIENT pO_2 ON OXYGEN CONSUMPTION IN THE 18-19 DAY CHICK EMBRYO. Ira H. Gessner Univ. of Fla., Col. of Med., Dept. Ped., Gainesville, Fla.

Normal, intact 18-19 day chick embryos were placed in a constant pressure, variable volume Warburg respirometer, immersed in a 38° C. waterbath. After a stabilization period O_2 consumption was measured directly at 10 minute intervals for a period of 60 minutes. Constant O_2 consumption in room air was obtained at an average of 0.31 cc./min. for 120 embryos. Varying air-nitrogen mixtures were then introduced. After a 5-minute equilibration period, O_2 consumption was measured at consecutive 10 minute intervals for 30-60 minutes, and found to be stable at the following levels:

Number of embryos	pO_2	% of control
120	160 (room air)	100
21	150	96
28	140	92
24	130	86
27	120	75
20	100	54

Measurement of normal 18-19 day chick embryo blood gases in this laboratory has shown arterial pO_2 = 22 mm. Hg. $\pm$3, and allantoic (respiratory) vein pO_2 = 45 mm. Hg. $\pm$5. Thus, the normal chick embryo is exquisitely sensitive to small decreases in ambient pO_2 reacting immediately by reducing O_2 consumption despite the fact that the embryo's arterial pO_2 is at much lower levels. This suggests that the critical oxygen environment for the chick embryo is room air.

NON-GLOBIN PROTEIN SYNTHESIS BY EARLY HUMAN ERYTHROID CELL PRECURSORS. Frances M. Gill, Shlomo Friedman and Elias Schwartz, Children's Hosp. of Philadelphia, Philadelphia, Pa.

The relative production of globin and non-globin proteins by early human red blood cells (RBC) has not been ascertained. We have studied protein production in RBC of differing maturity in a girl with sickle cell disease during an aplastic crisis. The first bone marrow (BM) showed 10% pronormoblasts, myeloid precursors and lymphocytes. After incubation of BM cells with ^{14}C-leucine, hemolysate was prepared and proteins were separated by Sephadex G-100 chromatography. Proteins of molecular weight greater than that of hemoglobin (void volume peak) contained 53% of the total radioactivity. Proteins eluting in regions corresponding to molecular weights of 64,000 and 16-32,000 were separated by carboxymethyl cellulose (CMC) chromatography in 8M urea. In neither sample was radioactivity associated with β^S or α globin peaks. A second BM taken 6 days later showed enormous hyperplasia of late normoblasts. Studies similar to the above showed only 5.5% of the radioactivity in the void volume peak. The hemoglobin radioactivity peak on G-100 contained radioactivity associated with β^S and α globin peaks recovered by CMC chromatography. The β^S/α ratio was 1.03. Peripheral blood taken 2 days after the second BM and two months later had findings on G-100 and CMC similar to those of the second BM.

These results indicate that human pronormoblasts produce primarily non-globin proteins, while more mature RBC produce mainly globin.

ALTERED CARBOHYDRATE UTILIZATION IN SICK PREMATURE INFANTS. Ronald L. Gutberlet, Arturo Q. Santos, Sylvia De Coello, (Intr. by Marvin Cornblath.) Univ. of Maryland Sch. of Med., Dept. of Ped., Baltimore.

Sick newborns may have abnormal glucose utilization manifest by glycosuria, which may be related to an altered renal threshold. To evaluate this, blood glucoses (BS) taken before and after a 6 hr. urine collection were compared with urine glucose. 56 blood and 44 urine measurements were made over 36-hr. periods on 8 infants (GA 28-34 wks.; BW 737-1730 gm.; age 3-4 days) receiving continuous I.V. glucose as part of clinical management. Constant rates were maintained for 12-hr. periods. Blood (40-201 mg.%) and urine (7-2212 mg.%) glucose show a continuing relationship (r= +.78; SE .15).

Blood Glucose (mg.%)	40-60	61-80	81-120	121-201
Excretion (mg./6 hr.)	5.8	8.3	13.7	57.2

Three sickest infants; 1 RDS, 2 apnea, were compared to the 5 "well" infants for CHO tolerance. % of I.V. glucose excreted was 10X greater in ill infants (1.82% vs. 0.15%) and was related to higher BS levels. BS rose with increased infusion rates in 3/3 of ill and 1/5 of well infants. After increase in infusion rates, glucosuria rose during the 1st 6 hrs. of the 12 hr. steady state but fell during the 2nd 6 hrs., an average of 38% in 10/12 samples. Lactosuria and galactosuria were present in all patients. Lactose paralleled glucose excretion while galactose did not. Five infants fed a lactose formula, had greater excretion than those NPO (17.3 vs. 3.1 mg./kg./6 hr.). Serum insulin reflected BS values and clinical illness (9-31 μU for BS > 100 mg.% and 0-19 μU for BS < 100 mg.%). HGH levels were high but not related to BS or Insulin. In conclusion; glycosuria reflects BS levels; sick infants have decreased utilization in the presence of high circulating insulin; lactosuria may indicate intact absorption and intolerance.

DEVELOPMENT OF THE VENTRICULAR SEPTUM IN THE CHICK. Jung Y. Harh, Glenn C. Rosenquist and Milton H. Paul. Johns Hopkins University School of Medicine, Department of Pediatrics, Baltimore, Maryland and Northwestern University Medical School, Department of Pediatrics, Chicago, Illinois.

Nylon fibers 12 μ in diameter and 200 μ in length were used as markers to study the development of the ventricles in the chick embryo heart from Hamburger-Hamilton stage 22 to 31 (4-7 days of incubation). When two fibers were placed in the myocardium of the ventricle, one on each side of the future site of the ventricular septum and within 200-250 μ of it, the distance between the fibers gradually shortened until both fibers were found within the myocardium of the septum. The tips of the fibers were always directed toward the interventricular foramen. When fibers were implanted in the ventricle at a distance greater than 250 μ from the prospective site of the septum, the distance between the two fibers increased and neither fiber became embedded in the septum. The portion of the ventricular wall at stage 22 that will later contribute to the ventricular septum is a zone about 400-500 μ wide that extends from the conoventricular sulcus along the ventral wall of the ventricle, around the apex, to the atrioventricular junction dorsally. The septum grows centrifugally from the interventricular foramen. The crest and smooth portion of the septum are formed by fusion of small trabeculae that were most centrally located within this belt-like zone, and the trabeculated portion from material that was nearer the borders of the prospective ventricular septal zone.

INTERSCAPULAR TEMPERATURE AND PLASMA GLYCEROLS. Carol B. Hersh, Paul H. Perlstein, Charles J. Glueck and James M. Sutherland, Univ. of Cinti. Col. of Med., Dept. of Pediatrics, Cincinnati.

A significant rise in plasma glycerol was measured in cold stressed newborn infants. This reflection of lipolysis did not correlate with a persistence in interscapular skin temperature. 24 full-term infants under 3 days of age were studied. Mean rectal (RT), interscapular (IT), and deltoid (DT) temperatures before and after a 20-minute exposure in a 22-23° C room are tabulated below.

	RT ± SE	IT ± SE	DT ± SE
Precooling	36.80 ± .07	35.40 ± .12	33.86 ± .21
Postcooling	36.48 ± .09	34.00 ± .11	32.32 ± .16
Change in Temp	-.32 ± .51	-1.40 ± .10	-1.54 ± .14

The changes in RT are significantly different (p<.01) from the IT and DT changes. The latter two changes are not statistically different from each other. As a measure of lypolysis, plasma glycerols were determined in 16 of these babies. The mean precooling glycerol of 0.305 ± (SE) .006 mM/L was different (p<.001) from the postcooling glycerol of 0.332 ± (SE) .007 mM/L. Since the rise in plasma glycerol did not coincide with a persistence in interscapular temperature, the use of interscapular temperature as a measure of brown fat activity is not supported by this study.

PERINATAL VITAMIN D METABOLISM, Laura S. Hillman and John G. Haddad (Intr. by P. R. Dodge) Washington Univ. Sch. Med., St. Louis Children's Hospital, Dept. of Ped. and Jewish Hospital of St. Louis, Dept. of Med., St. Louis, Missouri.

To evaluate placental transfer of 25-hydroxy Vitamin D (25-OHD) and infant utilization of dietary Vit D, 25-OHD levels were measured in maternal blood near delivery, cord blood, and serial heelsticks during the first 6 weeks of life. Random term births, premature births (28 to 38 weeks) plus 13 sets of twins (32-40 weeks) were studied. Cord blood 25-OHD correlated directly with maternal blood levels at all gestational ages and was similar in both twins despite dizygosity and weight difference. Cord blood 25-OHD did not correlate with gestation age, birth wt., number of infants, or apparent maternal vitamin intake. Blood levels in black mothers and cord levels in their twin or single infants were lower than white counterparts of similar gestation and size. Term infants on standard Vit D enriched formula maintained or increased their 25-OHD levels. Premature infant levels initially fell or remained low despite enriched formula and standard Vit D supplements. However, in all infants by 4-6 weeks falling levels had stabilized or 25-OHD was increasing. 25-OHD levels remained similar within twin pairs and changed with time like single infants of the same gestational age. Thus premature birth does not appear to alter placental transfer of 25-OHD but neonatal Vit D metabolism may be delayed.

REDUCED BRAIN GLUCOSE WITH ELEVATED PLASMA GLUCOSE DURING ANOXIA: INCREASED ANOXIC SURVIVAL AFTER PRE-TREATMENT WITH GLUCOSE. Jean Holowach-Thurston, Richard E. Hauhart, and Elizabeth M. Jones, Wash. Univ. Sch. of Med., St. Louis, Mo.

During 6 min. of anoxia in newborn mice, brain glucose fell 72% despite a doubling of plasma glucose levels. This is a study of the mechanism of this paradox and the effects of glucose administration. The increase in brain lactate was compatible with an uptake of glucose from the blood at a rate 5 times that calculated for animals with an adequate O_2 supply. Apparently brain glucose falls because the increase in the rate of anaerobic glycolysis exceeds that of glucose transport. The effect of glucose on increasing anoxic survival was dramatic. Dosage: glucose or mannitol 30 mmoles/kg, NaCl 15 mmoles/kg (s.c.). Pre-anoxic interval <1-140 min. In 8-12 day-old mice survival after 4.9 ± 0.2 min. anoxia at R.T. in 39 NaCl controls was 5% vs 82% in 39 glucose-treated littermates. In newborns at 34° only 1 out of 11 NaCl controls survived 25 min. anoxia compared to survival of all 11 glucose-treated littermates. Even after decapitation the head of glucose-treated animals exhibited a delay in the onset of terminal gasping and time of the last gasp ($p<0.001$). No life-sparing effect was seen with mannitol; zero % survival in 11 mannitol-treated 9-11 day-old mice and in 10 NaCl controls vs 55% survival in 11 glucose-treated littermates. In glucose-treated mice, brain glucose was 3 times the control value ($p<0.001$). This increase, coupled with the findings in the isolated head after glucose treatment, suggests that glucose plays an important role in survival of the brain during anoxia.

THE HUMAN SIMIAN CREASE (SC) AND ITS' VARIANTS: A MINOR BIRTH DEFECT PROVIDING A MODEL FOR INVESTIGATION OF SERIOUS CONGENITAL MALFORMATION. Ernest B. Hook, Birth Defects Inst., N.Y.S. Dept. of Health and Albany Med. Col.

Investigation of approximately 5000 apparently normal newborn infants and their mothers revealed a prevalence of the SC or its' variants in 7.5% of males, 4.5% in female, and 3% in mothers. Approximately a two fold increase was found in older individuals with ventricular septal defect or idiopathic mental retardation than in apparently normal individuals in the same age range. These data, in conjunction with those of others, suggest that the simian crease in an otherwise apparently normal infant is a prognosticator of diminished fitness, although the magnitude of this effect is still to be determined. In the newborn study, factors associated with an increased frequency of simian creases were, among others, lower birth weight, lower socioeconomic status, presence of SC in mothers, and heavy maternal tobacco smoking. Stratification of the data by birth weight suggested that the heavy maternal smoking association with SC is not mediated purely by impaired fetal growth. Data from these and other studies have led to the hypothesis that the SC is strongly affected by an incompletely dominant allele whose penetrance is influenced by environmental factors. (Supported by grants from N.I.C.H.H.D., N.I.H. and the A.M.A.- E.R.F.)

CHANGES IN THE STRUCTURE OF HUMAN PLACENTAL DERMATAN SULFATE DURING DEVELOPMENT

Alexander M. Jamieson, Ting-Yang Lee and Irwin A. Schafer, Department of Pediatrics, Cleveland Metropolitan General Hospital and Division of Macromolecular Science, Case Western Reserve University.

Optical mixing spectroscopy was used to measure the molecular weights of dermatan sulfate (DS) isolated from human placenta at 12-18 weeks gestation and at 40 weeks term. The effect of hyaluronidase digestion on molecular weight was then investigated as a probe for glucuronic acid substitutions in this polymer. Molecular weights before hyaluronidase digestion: 23,000 ± 1500 for term placenta vs. 21,700 ± 1500 for young placenta. After hyaluronidase digestion, molecular weights were 15,700 ± 1500 term vs. 10,000 ± 1500 for young placenta. These data indicate clear differences in the macromolecular structure of dermatan sulfate during development and suggest two possibilities: (1) fetal DS has more glucuronic acid substitutions than molecules of term DS or (2) each polymer has the same number of substitution but in fetal DS they are nearer the center of the polymer chain. Since molecular weight of DS parallels its solution viscosity, the structure of this polymer may regulate the viscosity of the ground substance during development, influencing both cellular migration and molecular transport.

CONTROLLED FOLLOW-UP STUDY OF LOW BIRTH WEIGHT INFANTS AT 4-6 YEARS OF AGE TREATED WITH PHOTOTHERAPY.

Jerold F. Lucey, Jean R. Hewitt, E. Stanley Emery, Steven Goldstein and Susan Collins. Dept. of Ped., Univ. of Vt. Col. of Med., Med. Ctr. Hosp. of Vermont, Burlington.

The purpose of this study was to examine the possible relationships between neonatal phototherapy in low birth weight infants with physiologic jaundice and its long-term effect in growth (height, weight, head circumference), intelligence, speech, hearing and neurologic function. A total of 99 infants (55 light treated and 44 controls) who were part of a study (Ped. 41:1047, 1968) were studied at 4-6 years of age.

Results to date (1/20/73) have failed to reveal any significant difference attributable to phototherapy between these two groups, (Table I) when the multiple factors influencing head growth (S.F.D., temp., caloric intake) are considered. An answer to the question will require a larger more carefully controlled study in which control of the light environment of the "normals" is precisely defined (see Lucey, et al., A FLUX DAY--Abstract).

	No. of Infants	Ave. Wgt.	Head Cir. Nl.	Head Cir. Sm.	Psychomet. Nl.	Psychomet. Abnl.	Hearing Nl.	Hearing Abnl.	Neurolog. Nl.	Neurolog. Abnl.
CONTROL	44	2134gms	43	1	30	10	40	8	34	2
LIGHT	55	2072gms	48	5	22	9	3]	6	46	3

Study supported by NIH Grant PHS R01 05561, United Cerebral Palsy Grant and Easter Seal Foundation Grant.

METHYLATION OF TRANSFER RNA (tRNA) AND RIBOSOMAL RNA (rRNA) *in vivo* AND *in vitro* BY FIBROBLASTS FROM NORMAL HUMANS AND PATIENTS WITH CYSTIC FIBROSIS (CF).

Michael Klagsbrun and Philip M. Farrell (Intr. by Paul A. di Sant'Agnese), NIH, Bethesda, Md.

It has been reported that fibroblasts in tissue culture from patients with CF show decreased methylation of total RNA (Rennert et al., Pediatrics 50:485, 1972). Such a change could result from a deficiency of one or more of the tRNA methylases.

In this study, control and CF fibroblasts were cultured in the presence of L-(methyl-^{14}C) methionine and (5-^{3}H) uridine. RNA was then extracted and fractionated by sodium dodecyl sulfate sucrose density gradient centrifugation. The ratios of ^{14}C/^{3}H incorporation revealed that tRNA, 18S rRNA, and 28S rRNA of CF cells were methylated to at least as great an extent as in the control cells. In addition, the methylated base composition of human fibroblast tRNA and rRNA was identified for the first time by two dimensional chromatography. The observed bases were the same for CF and control cells and identical to the pattern previously found for RNA from yeast, various animal tissues, and HeLa cells.

Methylation rates were also measured *in vitro* by incubating fibroblast extracts with *E. coli* tRNA and S-adenosyl (methyl-^{3}H) methionine. It was found that the activities of CF extracts were equal to or greater than those of control fibroblasts and the patterns of methylated bases were again identical. In conclusion, CF fibroblasts show normal methylation of tRNA and rRNA *in vivo* and no deficiency in tRNA methylases.

EFFECTS OF NEPHROTIC SYNDROME ON THE OUTCOME OF PREGNANCY IN RATS

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Heymann's model of autoimmune nephrosis (AN) in rats, which morphologically closely resembles membranous glomerulonephropathy in humans, was used to study the interrelations of pregnancy and nephrotic syndrome without azotemia. 12 AN rats (22 pregnancies) were compared with 10 normal control rats (10 pregnancies). AN rats showed an increase in proteinuria ($P < 0.001$) during the middle and last weeks of gestation. Among AN rats spontaneous complete abortions were more frequent (54.5 vs 0%) and the liter size was smaller (6.3 vs. 12.3). The gestational increase in proteinuria was more marked in AN rats who aborted than those delivering liveborns ($P < 0.01$). No significant difference in birth weight was noted between AN and control rats and no congenital malformations were seen in either group. Survival rate of offspring was better in controls than in AN rats at 3 weeks of age (37.16 vs 11.1%). Nephrotic syndrome seems to affect the ability of pregnancy to reach term, liter size and survival of the offspring.

CAN METABOLIC DATA PREDICT LINEAR GROWTH POTENTIAL.

Ingeborg Krieger and Charles F. Whitten. Wayne State University and Children's Hospital of Michigan, Detroit.

Rapid weight gain occurs in malnourished infants (MN) after realimentation. Forced feeding of anorexic infants with congenital heart disease (CHD) also causes rapid weight gain. The extent to which this response is predictive of linear growth recovery is not clear. N-balance and calorie efficiency were therefore studied in infants with known poor growth potential (9PD) who were force-fed. Data are compared with those of 4 new and 16 previously studied controls (MN) who showed linear catch-up growth. N-balance was measured continuously for an average of 14 days. The BMR was calculated from oxygen consumption. Calorie efficiency was measured by relating residual calories to weight gain (residual calories = total calorie intake minus BMR minus a fixed amount for activity). The ratio (7.8) was significantly higher than in controls (4.8) $p < 0.02$. The difference was due to a relatively low BMR. Calorie inefficiency was thus not evident when relating the total calorie intake of 134 cal/kg/day (controls 148) to weight gain, 6.4 gm/kg/day (controls 8.2). Although N-absorption tended to be lower, N-retention per body weight was similar to controls. N-retention per weight increase was in the majority higher, but equally high N-retentions were seen in MN after an initially rapid weight gain slowed down. Although these findings agree generally with reported data on the progeny of underfed mother rats they were not sufficiently consistent to be predictive of the potential for linear catch-up growth.

ALPHA$_1$-FETOPROTEIN (AFP) AND FETAL ABNORMALITY.

Aubrey Milunsky and Elliot Alpert. (Intr. John W. Littlefield) Harvard Medical School, Massachusetts Gen. Hosp., Dept. of Ped. and Med., Boston.

Elevated amniotic fluid (AF) AFP levels have been observed in pregnancies with anencephalic fetuses. In association with fetal distress/death, AFP levels apparently rise in maternal serum. Hence the possibility arose that pregnancies with defective fetuses may show AFP in AF and maternal sera. We have thus far examined AFP levels by electroimmunodiffusion in 65 amniotic fluid samples obtained at 14-24 weeks (2 at 32 weeks) in pregnancies with a known outcome. The outcome was normal in 52 (group A), spontaneous abortion/fetal death in 4 (B), and therapeutic abortion for defective fetuses was provided in 9 (C). AFP levels > 2 mg % were found in 4 cases in A, 2 in B and 4 in C. Striking elevations occurred in amniotic fluids from 2 anencephalic fetuses and another fetus that died on the day of/or after amniocentesis. Elevated levels were also noted in 1 of 2 fetuses (AF) with Down's syndrome, in 1 of 2 with Tay-Sachs disease, in Klinefelter's syndrome (1), and prior to spontaneous abortion (1). The cause of markedly elevated AFP in AF from defective or dying fetuses remains obscure. Preliminary studies suggest that at least in anencephaly the AFP does not rise concurrently in AF and maternal serum. Nevertheless, measurement of maternal serum AFP or other fetal specific proteins may ultimately provide an important screening method to select high risk patients for amniocentesis and further prenatal studies.

UMBILICAL GLUCOSE/O_2 QUOTIENT OF THE HUMAN FETUS. Frank H. Morriss, Jr., Giacomo Meschia, Edgar L. Makowski, and Frederick C. Battaglia. Division of Perinatal Medicine, University of Colorado Medical Center, Denver.

To determine the role of glucose as a metabolic substrate in the term human fetus, blood samples for umbilical venoarterial molar differences of glucose (ΔG) and O_2 (ΔO_2) were obtained from umbilical artery (a) and vein from 24 fetuses at elective cesarean section. Maternal venous (V) glucose concentration was sampled simultaneously. Glucose/O_2 quotients (G/O_2) were calculated according to the equation: $G/O_2 = 6\ \Delta G/\ \Delta O_2$.

Results: For 8 appropriately-grown fetuses delivered from fasting mothers with stable blood pressures, $\bar{x}$ $[glucose]_V = 82.2$ mg%, $\bar{x}$ $[glucose]_a = 55.9$ mg%, $\bar{x}$ $[glucose]_{V-a} = 26.3$ mg%, $\bar{x}$ $G/O_2 = 0.81$, and $G/O_2 = -0.19 + 0.038[glucose]_{V-a}$, ($p < 0.01$). Hypotension which occurred in 12 mothers was significantly associated with conduction anesthesia, with fetal hypoxemia, and with an increased G/O_2. For all pregnancies, umbilical arterial glucose concentration was correlated with maternal glucose concentration: $[glucose]_a = 5.5 + 0.60\ [glucose]_V$, ($p < 0.001$).

Thus, glucose can supply approximately 80% of substrate for aerobic glycolysis in well-oxygenated term human fetuses of fasting mothers.

IN VITRO EFFECT OF DECREASED OXIDATIVE PHOSPHORYLATION ON LIPOLYSIS IN HUMAN NEWBORN ADIPOSE TISSUE. Duna Penn, Milan Novak and Ellen F. Monkus, Dept. of Ped., Univ. of Miami, Sch. of Med., Miami, Florida

The substantial amounts of carbohydrates are believed to be exhausted shortly after birth and fat is then used to meet metabolic and nutritional requirements. Energy yielding substrates such as glucose and pyruvate enhance lipolysis more than norepinephrine in neonatal adipose tissue. Adult tissue responds well to norepinephrine but is insensitive to added glucose. Lipolysis seems to be controlled by hormone sensitive lipase, which in turn is dependent on the adenyl cyclase-cyclic AMP system. Hence the activation of lipolysis requires energy (ATP) production.

In vitro glycerol release measured in intact fragments of neonatal adipose tissue suspended in glucose containing medium (100 mg%) was significantly lower ($p < 0.0025$) in medium equilibrated with nitrogen than in incubations equilibrated in room air. Glucose stimulated lipolysis in suspensions of neonatal adipose cells also decreased below the basal level in the presence of oligomycin. The concentration of oligomycin used (5 µg/ml) was sufficient to block 60% of norepinephrine stimulated oxygen consumption in adipocytes.

Thus anaerobic glycolysis is insufficient to maintain glucose stimulated lipolysis. These findings are in agreement with the reduction of blood glycerol and free fatty acids in acute hypoxia. They offer additional clarification of the profound disturbances in metabolism in neonatal asphyxia.

HEMOPEXIN (Hx) IS SYNTHESIZED ONLY BY THE LIVER IN THE HUMAN FETUS. Ursula Muller-Eberhard, Scripps Clinic and Research Foundation, Dept. Biochemistry, La Jolla, Ca.

The site of uptake of ^{3}H-heme and ^{125}I-Hx, injected i.v., is the hepatocyte (J. Lab. Clin. Med. 76:426, 1970), which appeared also to be the site of synthesis of Hx (Gitlin and Biasucci, J. Clin. Invest. 48:1433, 1969). The relative amounts of Hx, albumin (Alb), and transferrin (Tr) were determined in their immunoprecipitates (IP). For 13 fetuses, 12-25 weeks of gestation, culture fluids of liver, placenta, and colon were analyzed. Cell suspensions of these organs had been incubated with ^{14}C-lysine and -isoleucine by Dr. P.F. Kohler according to J. Clin. Invest. (in press, March, 1973).

Human serum (50 µl) was added to aliquots of culture fluids as a source of carrier protein. Then, 20 µg bovine IgG (BGG) and anti-BGG at equivalence were added to precipitate most of the unspecific radioactivity. In the resultant supernatants, the three proteins were precipitated by their respective monospecific antisera. The radioactivity of each IP was calculated in percent of the total radioactivity residing in protein precipitated by 98% ethanol (average 73% of total cpm). Placenta and colon cultures incorporated no ^{14}C into the IP of Hx, Alb, and Tr. For liver cultures, the IP of Alb contained 23 to 59%, that of Hx 3 to 5%, and that of Tr < 1%. Each IP had 120 (twice background radioactivity) to 3,000 cpm per 5 µl culture fluid. The ratio of Hx:Alb was approximately 1:10, which indicates a very active fetal metabolism of Hx. Supported by USPHS Grants #HD-04445 and HL-08660.

THE CELL CYCLE IN PATIENTS WITH CHROMOSOMAL ANOMALIES. Ian H. Porter and Betty Paul, Birth Defects Institute, N.Y.S. Dept. of Health and Dept. of Peds., Albany Med. Col. of Union Univ.

Retarded growth and the other clinical signs which the autosomal trisomy syndromes have in common may be caused by a decreased rate of cell proliferation. We measured the length of cell cycles in patients with abnormal chromosomal constitutions by pulse labeling cultured fibroblasts and counting labeled metaphases. The patients with the trisomy 18 and Down's syndromes all demonstrated an increase in the total length of the cell cycle and particularly in the length of G1. Cells from a patient with mosaicism (66% 46,XY; 28% 47,XY+21) had a normal cell cycle. Thus, it appears to be the increase in the length of the cycle in these syndromes which slows the rate of cell proliferation causing growth retardation and perhaps affecting differentiation at specific stages in development. The finding of an alteration in the G1 stage in neoplasms may be significant in view of the fact that patients with abnormal chromosome constitutions have an increased susceptibility to malignant transformation.

CARBONIC ANHYDRASE (C.A.) ACTIVITY IN BRAIN OF MALNOURISHED RATS. Santiago J. Muzzo, Jorge A. Bassi & Pedro Rosso (Intr. by Myron Winick) Coll. Physicians & Surgeons, Inst. Human Nutrition, New York.

Since carbonic anhydrase is felt by some to be located almost solely in neuroglial cells, it was used to study the question of which cell type is decreased in postnatal malnutrition, produced in rats during lactation by increasing litter size to 15 and feeding the dam a 12% casein diet. The cell number deficit in the malnourished rats at 21 days was only partially recovered by 3 weeks of rehabilitation. Total brain C.A. activity minus red cell activity (net C.A. activity) was also decreased by malnutrition($1.04\pm0.15 \times 10^6$ vs $0.77\pm0.09 \times 10^6$, $p<0.001$) and remained less than control levels after refeeding ($1.83\pm0.26 \times 10^6$ vs $1.63\pm0.18 \times 10^6$, $p<0.02$). However net C.A. activity/mg DNA was similar in both groups. A parallel decrease of 20-25% in the number of cells and in total net C.A. activity was found in the malnourished group and a subsequent parallel increase of 10-11% was noted in both after refeeding. Based on evidence that carbonic anhydrase is almost exclusively found in neuroglia, the present data suggest that the reduction in cell number produced by postnatal malnutrition in rats is primarily due to a reduction in the glial cell fraction.

Osmotic composition of human fetal kidney in relationship to its function in utero. W.J. Rahill and J. Koo, Dept. of Ped. State Univ. of N.Y. at Buffalo.

The human fetal kidney functions in utero but what purpose it serves is unknown. From human fetuses 6-16 cm in crown-rump length (gestation age 75-122 days) kidneys were quick frozen (10-30 min.). Inner (medullary) and outer (cortical) specimens were taken from larger kidneys. In 8 fetuses mean plasma Na was 169 (157-184) K 11 (9.0-12.1) meq./L. Tissue concentrations of Na, K, Cl and urea were also high and fell as fetal size increased (Na 189, n=13, CR 6.0-9.3 to 144 meq/L water n=16, CR 6-16, K 101 to 79, urea 23 to 13 mm/L water). Urine, however, was dilute (sp. gr. 1.002-1.004, n=8). Ratios of Na and urea in inner and outer sections (Na 1.10, urea 1.05 n=16) showed no gradient. These results suggest that the fetal kidney at this stage actively promotes growth by conserving solutes, with water retention occurring secondarily. The absence of corticomedullary gradients for Na or urea indicates that the postnatal mechanism of water conservation via the countercurrent multiplier and exchanger functions of the renal medulla are absent. These data suggest a major function for the human fetal kidney in early gestation. (Supported by the Kidney Foundation of Western New York and the Kidney Disease Institute of New York)

OXYGEN (O_2) TOXICITY ON FETAL KIDNEY AND LUNG IN ORGAN CULTURE. Jack S. Resnick, David M. Brown & Robert L. Vernier. Univ. of Minnesota, Dept. of Ped., Minneapolis, Minnesota

Optimal O_2 concentrations have not been evaluated for developing organs, despite evidence of deleterious effects of high O_2 concentrations on various immature organs (lung and retina). We have investigated the effects of varying O_2 concentrations on developing fetal mouse kidney and lung in an organ culture system. We also investigated the effects of hypokalemia on these developing organs.

The fetal kidney developed severe toxic cell changes in pO_2=707 mm Hg while dichotomous branching and nephrogenesis were inhibited when compared to kidneys in pO_2=140 mm Hg. Intermediate oxygen concentrations had similar, but less severe effects. The fetal lung showed similar toxic cell changes and inhibition of normal bronchiogenesis in pO_2=707 mm Hg compared with lungs grown in pO_2=140 mm Hg. In both kidney and lung, there was a suggestion of an age related toxicity; younger fetal organs (11-11½ days) were more severely affected than older organs (12-13+ days). No adverse effects of hypokalemia on organogenesis were noted. These findings show that O_2 in high concentrations is toxic to developing fetal mouse lung and kidney in organ culture. Supported by USPHS grants AM-15146, HD-50477.

	Oxygen Conc.	No. successful cultures / total no. cultures (%) Kidney	Lung
I.	95% O_2 (pO_2=707 mm Hg)	0/374(0%)	0/110(0%)
II.	Intermediate O_2 (pO_2=485 mm Hg)	13/53(24.5%)	-----
III.	Room Air (pO_2=140 mm Hg)	143/148(96.6%)	93/96(96.6%)

EFFECTS OF ADRENOCORTICOSTEROIDS ON DEVELOPMENT OF THE KIDNEY AND LUNG IN ORGAN CULTURE. Jack S. Resnick, Robert L. Vernier, & David M. Brown. Dept. of Ped., Univ. of Minn., Mpls., Minn.

Experimental polycystic kidney disease has been produced in newborn rabbits by the administration of corticosteroid compounds to the pregnant female, and may be related to the development of hypokalemia. We have studied effects of corticosteroids upon the development of fetal mouse kidneys and lungs in an organ culture system using direct photomicroscopy and histology. Deoxycorticosterone was toxic and inhibited normal epithelial branching and nephrogenesis/bronchiogenesis at 2.5 x 10^{-4}M but had no effect on kidney or lung at 10^{-4}M. No differences were noted between high or low potassium media. However, an age dependent effect was present, where older kidneys were less effected. No abnormalities were noted with hydrocortisone (10^{-6} to 2.5 x 10^{-4}M) or 9-α fluorohydrocortisone (9-α Fl.) (10^{-4} to 10^{-3}M) in either high (7.3-12.1 mEq/L) or low (1.8-6.0 mEq/L) potassium media. However, measurements of surface area of kidneys suggests that 9-α Fl. (5 x 10^{-4}M) inhibits overall growth. 11½ day control kidneys had a mean surface area of 5.66 units while steroid treated kidneys had a mean surface area of 4.05 units ($p < .001$). The potassium concentration of the media was not an independent variable upon the surface area. These studies demonstrate a toxic or inhibitory effect of some corticosteroids compounds on normal development of fetal mouse kidney and lung. Careful observations are necessary in studies evaluating the use of corticosteroids in pregnant mothers to prevent hyaline membrane disease. Supported by USPHS grants AM-15146, HD-50477.

Porphyrin synthesis by cultured human fetal cells. Arleen Rifkind and Soja Bennett. Cornell Univ., N.Y., N.Y. 10021. Intro. by Maria New.

Metabolism of foreign compounds and steroids requires heme enzymes. The ability to regulate porphyrin and heme synthesis could affect fetal risks from exposure to environmental chemicals. Delta aminolevulinic acid synthetase (ALAS), the rate limiting enzyme in porphyrin synthesis can be induced by drugs and hormones in adult liver and kidney. While embryonic avian liver is responsive to ALAS induction, livers of fetal and neonatal rats, rabbits and guinea pigs are relatively refractory. In contrast, many tissues can synthesize porphyrins when supplied with the precursor delta aminolevulinic acid (ALA). Liver and kidney cells from 11 and 18 week human fetuses, were grown in primary culture. Porphyrins were formed when ALA was added to the culture medium. Maximum liver cell conversion of ALA to porphyrins occurred in cells grown for 3 days. By 5 days porphyrin synthesizing activity had decreased by 90%. Whereas chick embryo liver cells form mostly coproporphyrin, human liver cells formed more protoporphyrin. Human fetal cells did not synthesize porphyrins when drugs and hormones were added to the culture media. In summary:Human fetal liver and kidney cells capable of metabolic activity were grown in culture. They were able to synthesize porphyrins from precursors but not in response to drugs and hormones. The findings suggest that in humans as in other mammalian species, ALAS is not inducible during early fetal development, and that the fetus may not be able to synthesize heme as readily as the adult after exposure to chemicals.

Stimulation of Tumor Cells Exposed to Antibody. W. T. Shearer, G. W. Philpott, C. Stewart, and C. W. Pa. *Barnes Hospital, Washington University School of Medicine*, Department of Medicine, St. Louis, Mo. 63110

It is known that antibodies to surface antigens on lymphocytes stimulate blast transformation and increased ^3H thymidine uptake. We confirm a similar role for antitumor antibody in immunologic enhancement of tumor cell growth. We have studied the ability of anti-trinitrophenyl (TNP) antibody to augment ^{125}iododeoxyuridine (^{125}IUdR) uptake in TNP-substituted HEp-2 cells in tissue culture. After 30 minute exposure of TNP-HEp-2 cells to anti-TNP antibody a 1.5-fold increase in cell uptake of ^{125}IUdR ($P<0.01$ triplicate determinations) and a 1.5-fold increase in the number of cells as measured by counting cell nuclei ($P<0.05$ triplicate determinations) was observed as compared to control cells after 72 hours incubation. 98% of the radioactivity remained in the cell pellets after 2 extractions with 6% trichloroacetic acid and 85% of the radioactivity was in the isolated cell nuclei.

These findings may explain the phenomenon of immunologic enhancement of tumor growth. Supported by Grants from the National Institutes of Health.

RELATIONSHIP OF CALCIUM, PHOSPHATE, URINARY CYCLIC AMP, AND ACID EXCRETION IN THE NEWBORN. S. R. Siegel, J. C. M. Chan, R. Fiser, and W. Oh, UCLA Sch. of Med., Harbor Gen. Hosp., Dept. of Ped., Torrance, Ca., and USC Sch. of Med., Children's Hosp. of Los Angeles

Calcium (Ca) and phosphate (PO_4) balance, urinary 5'cyclic adenosine monophosphate (CAMP), and acid excretion were studied in 46 newborn infants, (gestation: 28-41 wks.) at 24-48 hrs. of age. Sera was obtained at the end of the timed urine collection period (2-14 hrs.). The 28-33 wk. gestation infants were fed by continuous intravenous 10% dextrose in water; 34-41 wks. with oral commercial formula. Serum Ca and PO_4 increase with gestational age (G.A.) ($r = 0.75$ and 0.38 respectively). Urinary Ca excretion is unrelated to both serum Ca and G.A. Urinary PO_4 excretion was higher in 28-33 wk. infants (19.1 ± 4.8 μM/kg/hr, M±S.E.M.), than term babies (0.98 ± 0.45) and unrelated to both CAMP and titratable acid (T.A.). Percent fractional urinary PO_4 excretion of filtered PO_4 is inversely related to G.A. ($r = -0.67$). CAMP/Ccr, average 56.7 ± 5.3 pM/ml Ccr, was unrelated to G.A. Ammonium, T.A. and net acid excretion increase with G.A. ($r = 0.43$, 0.48 and 0.52 respectively), and statistically similar in 10 small for date when compared with term infants. Thus, the reduction in PO_4 reabsorption in the 28-33 wk. infants may be due to decreased TM_{PO_4}, resulting from the competitive inhibition of glucose reabsorption and perhaps tubular immaturity. Limitation in T.A. in low G.A. infants is not related to reduced PO_4 excretion.

IRON DEFICIENCY: PROTECTIVE ADAPTATION IN LIVER DNA SYNTHESIS. Martti A. Siimes and Peter R. Dallman, Dept. of Pediatrics, University of California, San Francisco.

During the development of iron deficiency in the rat, certain abnormalities in liver DNA synthesis were found to accompany progressive anemia without apparently being secondary to it. Rats were given a semi-synthetic diet with or without added iron, from age 10 days. As anemia progressed (Hct 68% of controls), total liver weight and RNA content started to decline whereas DNA remained normal. Only as the anemia became more severe (Hct 40% of controls) were diminished liver DNA and retardation of body growth detected. Incorporation of ^{32}P into DNA, which should be indicative of total DNA synthesis, became decreased in the iron deficient groups. In contrast, incorporation of ^3H-thymidine into DNA and the activity of thymidine kinase remained normal or increased even in severe iron deficiency (Hct 30% of controls). The latter two parameters reflect the activity of salvage pathway by which thymidine from DNA catabolism is reutilized. The fact that protein calorie malnutrition decreases both types of DNA synthesis suggests that the maintenance of the synthesis from salvage pathway is characteristic of iron deficiency rather than a non-specific result of decreased intake of the diet. Next, the response to treatment with IM iron dextran indicated that changes in liver nucleic acid metabolism were not secondary to anemia. There was a marked increase in RNA and DNA synthesis, as well as in total liver weight, within two days, prior to any substantial response in Hct. (Supported by USPHS grant AM13897.)

TRANSMISSION OF LIGHT THROUGH LIVING TISSUE. Thomas R.C. Sisson, and Matthew Wickler, Dept. Pediatrics, Temple Univ. School of Medicine, Philadelphia (intr. by Angelo M. Di George).

The transmission of 3 types of fluorescent light (broad-spectrum daylight - F20T12/D, broad-spectrum blue - F20T12/B, and narrow-spectrum blue - F20T12/BB) was measured through various thicknesses of abdominal wall in 18 Wistar rats. The lamps had the following flux 19.2 cm. from the light source: F20T12/D = 1.45 mw/cm^2, F20T12/B = 0.90 mw/cm^2, and F20T12/BB = 1.30 mw/cm^2.

Transmission by F20T12/D lamps through full-thickness walls (0.20 cm.), dermis-epidermis (0.086 cm.) and muscle-fat-subcutaneous tissue (0.108 cm.) was 14.4%, 141%, and 20.2% respectively; by F20T12/b it was 3.5%, 5.5%, and 11.0% respectively; by F20T12/BB it was 6.5%, 8.0%, and 12.2% respectively. There was a reduction of transmission as distance from a light source increased.

These data indicate that broad-spectrum (white) light has greater tissue penetrance than narrow-spectrum blue light or broad-spectrum blue, which has in the latter case a smaller flux in the regions above 500 nm. (green, yellow, red) than white light. However, the transmission of violet and blue visible light is of a degree that will permit photochemical reactions to take place in tissue below the dermis, including the vascular bed of the body integument. This has implications not only in the use of phototherapy but also in the choice of environmental lighting in newborn nurseries.

REGULATION OF PROLINE BIOSYNTHESIS IN CULTURED HUMAN FIBROBLASTS: FEEDBACK INHIBITION OF PYRROLINE-5-CARBOXYLATE REDUCTASE. David L. Valle, Sylvia J. Downing and James M. Phang, (Intr. by Paul A. di Sant'Agnese). Metabolism Branch, National Cancer Institute, NIH, Bethesda, Md.

In mammalian cells, the synthesis of proline from ornithine requires two reactions. The first, catalyzed by ornithine-δ-transaminase, converts ornithine to Δ^1pyrroline-5-carboxylate (PCA). PCA is then converted to proline by pyrroline-5-carboxylate reductase. We have developed sensitive radioisotopic assays which enable us to measure both these enzymes in extracts from as few as 10^5 cultured cells. Using these techniques, we studied the PCA reductase of a normal human male fibroblast line (D550). Enzyme activity was constant throughout all phases of culture growth at 600 $\pm$ 100 nmoles proline produced/hour/mg protein. The Km for PCA was 4 x 10^{-4} M. Unlike a previously described rat liver PCA reductase, we found the fibroblast enzyme to be exquisitely sensitive to feedback inhibition by proline. Kinetic analysis revealed a competitive type inhibition. Under the conditions of our assay, there was 50% inhibition at 2 x 10^{-4} M proline and greater than 90% inhibition at 1 x 10^{-3} M proline. Hydroxyproline and numerous analogues of proline were at least an order of magnitude less effective as inhibitors. This specific, sensitive negative feedback inhibition by proline may play an important role in regulation of proline biosynthesis in peripheral mammalian cells.

DEVELOPMENT OF METHIONINE-ACTIVATING ENZYME (MAE) IN FETAL AND NEONATAL LUNG OF HUMAN, MONKEY AND RABBIT. Hans J. Sternowsky, Niels C.R. Raiha, Gerald E. Gaull. Dept. Ped. Res., NYS Inst. Basic Res. Ment. Retard., Staten Island; Dept. Ped., Clin. Genet. Cent., Mt. Sinai Sch. Med., City Univ. N.Y.; Dept. Ped. & Med. Chem., Univ. Helsinki.

Methionine as S-adenosylmethionine is the major donor of methyl groups such as those used for the synthesis of the surfactant lecithin by the "methylation pathway".

MAE spec. act. is lower (1.1 μmoles/mg prot/h) in fetal rabbit lung at term than in mid-pregnancy (5.1). Rhesus monkeys show an increase near term from 1.2 to 8.9 and gradually reach mean adult spec. act. (1.0) within 5 months. Human fetal lungs (N=19) show an increase in spec. act. (1.2 to 7.3; r=0.87; $p<0.01$) with increasing crown-rump length (3.5 to 25 cm). After birth, MAE spec. act. decreases within 5 days to 2.8.

Binding capacity of MAE for methionine is high ($S_{0.5}$=1.8mM) compared to that for ATP ($S_{0.5}$=6.4 mM). The pH optima in human fetal liver (7.0) and human fetal lung (6.4) suggest a special adaptation to low intracellular pH in lung.

Rabbit fetuses cannot survive premature birth prior to two days before term, whereas monkey and human fetuses can. It is possible that the survival of prematurely-born primates is facilitated by the increased activity of lung MAE, providing the necessary methyl groups for lecithin biosynthesis. The inability of the rabbit to survive may be because of its decreased ability to activate methionine in lung.

SERUM ALPHA-FETOPROTEIN AND MALIGNANT TERATOMA IN CHILDREN. Jaw J. Wang, Edward Sarcione, Mary Bohne, and Lucius F. Sinks. Roswell Park Mem. Inst., Buffalo, New York and The State Univ. of New York at Buffalo, Buffalo, New York.

Alpha-fetoprotein (AFP) is a specific circulating embryonal and fetal alpha$_1$-globulin with a molecular weight in humans of about 70,000. The protein is not detectable in human serum after the first several weeks of life, but it re-appears with the development of a primary liver cancer or embryonal tumors of the ovary and testis. Seven children with gonadal or sacrococcygeal malignant teratoma, and 20 with a variety of other malignant tumors, e.g. neuroblastoma, lymphosarcoma, rhabdomyosarcoma etc. were tested qualitatively and quantitatively for serum AFP. AFP was detected by Ouchterlony's double diffusion technique, using monospecific rabbit antiserum to human alpha$_1$-fetoglobulin. The quantitation was made by the radial immunodiffusion technique of Mancini. Elevated levels of AFP in serum (25.7 - 7.8 mg%) were observed in 5 of 7 children with malignant teratoma. Of the 2 negative sera, both came from patients who had been free of active disease for more than 2 years. Four patients with active disease had either no response or partial response to therapy; serial study of AFP showed continued elevation. One patient achieved complete tumor regression following chemotherapy, and his AFP returned to normal. On surgical re-exploration, no tumor could be found. In the control group of 20 children with other tumors, none had a detectable level of AFP. AFP is thus useful in the diagnosis and assessment of the efficacy of therapy in children with malignant teratoma.

SUBCELLULAR DISTRIBUTION OF GH; EFFECT OF CORTISOL AND ESTROGEN. Joseph Thomas, Vaddanahally T. Maddaiah, Platon J. Collipp, Viswanathan Balachandar and Shang Y. Chen. Nassau County Med. Ctr., Dept. of Ped., East Meadow, N. Y.

It has been shown that mitochondria from hypox rat liver accumulate growth hormone significantly within 5 minutes of intravenous injection. HGH has been shown to stimulate mitochondrial protein and RNA synthesis, and cortisol and estradiol blocked the stimulatory effect of HGH. The liver subcellular distribution of ^{3}H-HGH 10 minutes after intravenous injection into cortisol and estradiol treated (5 days) hypox rats is shown below (homogenate = 100%). Cortisol treatment reduced mitochondrial uptake of labeled hormone significantly ($p<.05$). There was no significant difference in the other fractions, or in subcellular distribution in the estrogen treated rats.

Treatment	Nuclear	Mitochondrial	Microsomal	Cytosol
Saline	20 ± 2.6	32.7 ± 4.1*	22.7 ± 4.6	24.3 ± .86
Cortisone	20.2 ± 1.6	25.6 ± 2.6*	26.7 ± 3	27.1 ± 4.1
Estrogen	16.2 ± 2.5	34.3 ± 1.7	27.7 ± 2	21.5 ± 1.5

Hypox rat liver by electron microscopy had increased numbers of mitochondria and decreased mitochondrial matrix density. GH treatment reduced some of these changes. When GH was injected along with cortisol or estrogen, intensity of growth hormone effect was decreased. These results may help explain the observed blocking effect of cortisone on the stimulatory effect of HGH on mitochondrial protein synthesis and perhaps on linear growth in children.

THE STUDY OF CELLULAR MECHANISMS FOR THE INCREASED LEVELS OF FREE CYSTINE AND THE MOLECULAR UTILIZATION OF THE CYSTEINE IN CYSTINOTIC CELLS. John R. Waterson, William P. Winter, and Roy D. Schmickel (Intr. by William J. Oliver). Univ. of Michigan Medical Center, Univ. Hosp., Dept. of Ped., Ann Arbor, Mich

The most striking finding in cystinosis is the high level of free, intracellular cystine, yet it has not been determined how this pool is formed or how it is utilized. Since the pool of free intracellular cystine can be derived from the dietary extracellular cystine or from intracellular synthesis, we tested both sources a cause of the high intracellular levels. In cystinotic fibroblasts extracellular ^{35}S-cystine rapidly appeared in the high intracellular pool, in the peptide glutathione, and in protein. In contrast, ^{35}S-methionine did not appear in the high cystine pool, but was present as ^{35}S-cysteine in glutathione and protein. It appears that extracellular but not synthesized cysteine contributes to the high intracellular pool. The utilization of cysteine was also studied since a low affinity (K_m) of cysteine for cysteinyl-tRNA synthetase would be a cause of increased intracellular cysteine. The K_m for cysteine using cysteinyl-tRNA synthetase from normal and cystinotic cells was 2.5 x 10^{-6} M and 1.7 x 10^{-6} M respectively. The specific activity of cysteinyl-tRNA synthetase was 5.8 pmoles/μg protein/10 min for normal cells and 6.1 pmoles/μg protein/10 min for cystinotic cells. In addition the acceptor activity of $tRNA_{Cys}$ in cystinotic and normal cells was comparable.

PARENT-SPECIFIC HEIGHT STANDARDS FOR PREADOLESCENT CHILDREN OF THREE RACIAL GROUPS, WITH METHOD FOR RAPID DETERMINATION. John Wingerd, Irene L. Solomon, Edgar J. Schoen. Kaiser-Permanente Medical Center, Department of Pediatrics, Oakland, California.

Since accurate appraisal of childhood growth depends upon consideration of family background, we have investigated the relation of children's heights to those of their parents in a large multiracial California population. 59,000 Height measurements of 11,233 children aged 1 to 9 years were recorded by the Child Health and Development Studies. For each child, midparent height was determined as the simple average of the heights of both parents. To determine centile points for children taking into account parents' heights, the data were treated as a whole and the necessary modifications for sex, race, and parents' height were derived by regression analysis. Parent-specific expected height tables for children of given age, sex, and racial group whose midparent height is between 156 and 184 cm have been prepared. The difference between the child's expected and actual height can be used to obtain the child's height centile rank corrected for parental height.

To make the data readily available for clinical use, we have also devised a "slide rule" for relating a child's height to that of his parents. It is hoped that the availability of the slide rule will sufficiently simplify the inclusion of parental height in the calculation of children's height centiles to make this corrected estimate a usual clinical practice.

HYPOTHYROIDISM AND HEPATIC MICROSOMAL DRUG METABOLISM DURING GROWTH. J.V. Aranda*, K.W. Renton*, G. Kunos*, G. Boyd*, N.R. Eade* and E. Colle. McGill Univ., Dept. of Pharmacology and Montreal Children's Hosp. Research Inst., Montreal, Canada.

Hypothyroid adult patients have diminished drug biotransforming capacity. In thyroidectomised adult rats, this is due to decreased activity of the hepatic microsomal mixed function oxidase(HMMFO). The effect of hypothyroidism on HMMFO during growth period has not been studied and to determine this, weanling rats were fed with 0.15% propylthiouracil(PTU) diet to induce hypothyroidism. The HMMFO components shown below, aminopyrine N-demethylation(AmpND), a type I substrate oxidation and aniline P-hydroxylation(AnOH), a type II substrate oxidation, were then measured. Compared to age-matched controls, the results indicate that PTU-induced hypothyroidism decreases electron flux and HMMFO activity, and preferentially inhibits a type II substrate oxidation during growth. This suggests that drugs which undergo hepatic biotransformation should be cautiously used in children with hypothyroidism.

Enzymes (M±SE)	Controls(6)	Hypothyroid(8)	P
NADPH oxidase*	7.90 ±0.26	4.94 ± 0.55	<0.001
cyt c reductase*	121.69 ±8.99	88.65 ±10.14	<0.05
cyt P450**	0.481±0.020	0.509± 0.020	N.S.
cyt P450 reductase*	2.03 ±0.28	2.15 ± 0.23	N.S.
AmpND*	2.59 ±0.30	2.73 ± 0.16	N.S.
AnOH*	0.270±0.009	0.103± 0.016	<0.001
T4 (µg %)	6.60 ±0.19	3.00 ± 0.21	<0.001

*nmoles/mg protein/min **nmoles/mg protein

SERUM α-FETOPROTEIN LEVELS IN AGA AND SGA NEWBORN INFANTS. Chap-Yung Yeung*, M. Adinolfi and John R. Hobbs (Intr. by John C. Sinclair), *McMaster University Medical Centre, Department of Pediatrics, Hamilton, Ontario, Canada.

The logarithms of the levels of serum α-fetoprotein were found to have a significant inverse relationship with both the gestational age and the birth weight of 140 normal appropriate-for-gestational age (AGA) newborn infants. 19 small for gestational age (SGA) infants had normal α-fetoprotein levels for their gestations but significantly lower levels for their birth weights. 5 other SGA infants who had demonstrable congenital anomalies were found to have significantly higher levels of the protein for their gestational ages but normal levels for their weights. They were cases of Down's syndrome, tracheo-esophageal fistula, agenesis of lung, congenital heart with polydactyly, and renal anomaly. It is suggested that estimation of the serum α-fetoprotein levels may provide an approach to the early detection of congenital anomalies.

THE EFFECT OF INTRAUTERINE MALNUTRITION UPON HEPATIC DRUG METABOLISM. Saroj Mehta, Margareta Eriksson, Charlotte S. Catz, Sumner J. Yaffe. Dept. of Ped., Sch. of Med., State University of New York at Buffalo, Buffalo, New York.

Two methods were utilized to produce intrauterine malnutrition in rats (1) dietary protein restriction with 8% casein (2) mechanical reduction of the circulation to one of the uterine horns on the 17th day of gestation. The hypotrophic rats produced may be compared to their normal littermates from the other uterine horn. In both types of malnutrition a decrease in body and liver weights was still present at weaning, although food intake was not restricted after birth. Oxidative pathways for drug metabolism were increased for aminopyrine and decreased for aniline in both experimental groups as determined in vitro. Reduction and conjugation were not altered in either group. The content of cytochromes P450 and b_5 did not change in the protein restriction group but was diminished in the hypotrophic rats. Sleeping time, following administration of hexobarbital, was prolonged in the protein restriction group but unaltered in the hypotrophic animals. This reflects the additional changes in receptor (brain) which are the consequences of prolonged intrauterine malnutrition.

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INHIBITION OF DRUG METABOLISM BY STEROIDS AND A VARIETY OF NATURALLY OCCURRING SUBSTANCES. Lester F. Soyka and Fred W. Deckert. Univ. of IL., Sch. of Basic Med. Sci., Dept. of Pharmacol., Chicago, IL. 60680

We have demonstrated that pregnanolone and related metabolites of progesterone inhibit hepatic microsomal drug metabolism (J Pharm Exp Ther 182: 320, 1972). Because such steroidal activity might explain the low rates of drug metabolism in the neonate, a survey was made to classify inhibitory substances. Of 75 steroids having a variety of structures and endocrine activities only 21-hydroxypregnane-3,20-dione was a more potent inhibitor of p-nitroanisole demethylation by hepatic microsomes than pregnanolone. Norethindrone, and 6 steroids having a structure closely related to pregnanolone, had equivalent inhibitory activity. Inhibition was greater when microsomes from female (virgin or pregnant) rather than male rats were used as the test system. Androgens had low activity; natural estrogens were inactive. Corticosterone was moderately inhibitory. Synthetic corticosteroids had little or no activity, as did cholesterol and related compounds, fatty acids and esters, ceramides, gangliosides, phosphatidyl inositide and other natural fatty acid conjugates, and a variety of indoles and lipids. Definition of the structural requirements for steroids to exhibit inhibitory activity and the exclusion of a variety of components of the endoplasmic reticulum strengthens our proposal that the potent inhibitory substances extracted from neonatal rat liver microsomes probably are steroids and possibly metabolites of progesterone.

DRUG PROTEIN BINDING IN THE NEWBORN INFANT by Joseph Krasner, Ph.D., George P. Giacoia, M.D. and Sumner J. Yaffe, M.D., State University of New York and Children's Hospital, Buffalo, N.Y.

The in vitro techniques of equilibrium dialysis and ultrafiltration were utilized to investigate the binding of diphenylhydantoin, salicylate, nafcillin and bilirubin to plasma proteins from newborn infants. At plasma concentrations of 16 µg/ml, the unbound fraction of diphenylhydantoin in cord plasma was 11%. The percentage of unbound diphenylhydantoin had a positive correlation with the serum concentration of bilirubin, suggesting a competitive binding site. The association of salicylate to cord blood albumin was $1.7 \times 10^5\ M^{-1}$ (95% bound at 5 mg %). Nafcillin had an association of $1.2 \times 10^4\ M^{-1}$ to proteins in cord blood serum (approx. 49% bound at 200 µg/ml). The binding of these drugs to newborn plasma proteins differs from that reported for adult plasma. The apparent association constant for bilirubin binding to purified albumin obtained from pooled cord blood ($5.2 \times 10^7\ M^{-1}$) exceeded the binding found with adult albumin ($2.4 \times 10^7\ M^{-1}$). A fluorometric method was used to study drug-bilirubin competition for protein binding sites in neonatal serum. Minimal displacement was found at usual therapeutic concentrations of salicylate, nafcillin, sulfisoxazole and diazepam.

INCREASED DIGITALIS REQUIREMENTS FOR ENHANCED MYOCARDIAL CONTRACTILITY IN NEWBORN RABBITS. Robert C. Boerth, Thomas P. Graham, Jr., and James B. White. Vanderbilt Univ.Sch. of Med., Division of Pediatric Cardiology, Nashville, Tennessee.

It is well known clinically that for therapeutic inotropic effects of digitalis the newborn and young child require 2 to 5 times more digitalis per unit body weight than do adults. However, it is not known whether this difference is due to an age-related change in myocardial sensitivity to this drug. In this study, dose-inotropic response relations of ouabain (O), a digitalis glycoside, were examined in isometrically contracting papillary muscle preparations from 8 newborn rabbits (NB) and 8 adult rabbits (A). NB ranged from 9 to 17 days of age (mean = 13). All data were divided by muscle cross-sectional area to correct for differences in muscle size. Prior to administration of O, developed stress (DS) was 0.29±0.08 g/mm^2 (mean ± SEM) in NB and 2.08±0.53 g/mm^2 in A, and dS/dt was 2.4±0.7 g/mm^2/sec in NB and 12.6±2.8 g/mm^2/sec in A. O produced linear dose-response curves of DS and dS/dt in papillary muscles from both NB and A, however the curves for NB were shifted significantly to the right of those for A. The dose of O at which DS was 50% of the maximal response (ED_{50}) was 2.92 X 10^{-7}M in NB compared to 1.72 X 10^{-7}M in A. It is concluded that newborn myocardium is less sensitive to the inotropic effects of digitalis than is adult myocardium, and that this could explain why relatively larger doses of digitalis are required in the newborn and young child for therapeutic effects.

TOBRAMYCIN: MATERNAL-FETAL PHARMACOLOGY. Betty Bernard, Salvador J. Garcia, Charles A. Ballard, Daniel Ivler, Lauri D. Thrupp, Allen W. Mathies and Paul F. Wehrle. Depts. of Peds. and Obstetrics, USC School of Medicine, Los Angeles, Calif.

To investigate the maternal-fetal transfer of tobramycin (TBM) and its distribution in the fetus, we administered a single 2 mg/Kg I.M. dose to 33 pregnant women (11-1st trimester, 22-2nd trimester) 2 to 22 hrs. prior to therapeutic abortion and sterilization by hysterectomy. TBM concentration was assayed microbiologically in maternal serum, myometrium, fetal tissues (placenta, brain, lung, liver, kidney) and fluids (amniotic, CSF, urine and serum). Maternal serum half-life of TBM was 2.0 hrs. while peak serum concentration at T_o was 5.4 ug/ml. Following maternal TBM administration, TBM half-lives were 5 hrs. in myometrium, cord blood and fetal urine. Mean TBM concentrations were determined for placenta (1.1 ug/gm), amniotic fluid (0.25 ug/ml), fetal kidney (2.1 ug/gm), fetal lung (0.4 ug/gm) and fetal liver (0.4 ug/gm) as in these samples, TBM was first detected at 2 hrs. and persisted to 22 hrs. TBM was not detected in fetal CSF after 16 wks. gestation while it was detected primarily in the 2nd trimester amniotic fluid; these differences are most likely due to maturation of the blood brain barrier and renal function.

Second trimester fetal kidney demonstrates active concentration of TBM as values of 1-3.8 ug/gm were found in 19 of 21 samples. None of the 20 simultaneous cord blood samples were above 1 ug/ml. Fetal urine levels were obtained only in the 2nd trimester and ranged from 0.1-3.0 ug/ml.

HEXACHLOROPHENE: PHARMACOKINETICS AND TOXICITY IN DEVELOPING MICE. Leland W.K. Chung, Margaret Warner, and Allen H. Neims. McGill Univ., Montreal Children's Hosp., Roche Developmental Pharmacology Unit, Dept. Ped. & Pharmacol., Montreal.

The role of hexachlorophene (HCP) in the newborn nursery rests in part on relationships between animal toxicity, human pathology, and blood levels. These considerations prompted us to study 1) pharmacokinetics; 2) hepatic uptake; 3) plasma protein-HCP-bilirubin interactions; 4) temporal aspects of CNS toxicity; and 5) relative sensitivity of developing, as against mature, brain. Adult female mice (C-57) were injected subcutaneously with ^{14}C-HCP (6 mg/kg). HCP distributes rapidly with highest levels in plasma and liver. Plasma HCP levels peaked at 2 hrs (8 ug/ml) and decreased in first-order fashion thereafter to 18 hrs with $t_{\frac{1}{2}}$, 4.5 hrs. Other tissues, except brain, revealed identical elimination half-times. In brain, HCP levels maintained a plateau for 6 hrs. No significant difference in distribution was observed between adult and 5-day old mice, but newborn animals displayed two-fold higher brain/liver ratios. Gel filtration patterns of cytosol from mouse liver suggest participation of ligandin in hepatic accumulation. With human serum, maximal levels of bilirubin and HCP seen in the nursery, do not alter appreciably each other's binding to protein. Within 48 hrs after a single injection of HCP, 80 mg/kg, status spongiosus is apparent in mice. Comparative studies with developing animals are incomplete, but do reveal increased sensitivity.

COMPARISON OF INTRAMUSCULAR AND INTRAVENOUS GENTAMICIN ADMINISTRATION IN NEWBORN INFANTS. John W. Paisley, Arnold L. Smith and David H. Smith (Intr. by William B. Berenberg) Children's Hospital Medical Center, Boston, Mass.

The intravenous administration of gentamicin to newborn infants has been reported to produce erratic blood concentrations, a factor detracting from its use in some clinical situations, e.g. small prematures and/or prolonged administration. An enzymatic assay was used to study the kinetics of serum gentamicin concentrations in 18 newborns following dosages of 0.7 - 2.4 mg/kg administered intramuscularly or in a 20 minute intravenous infusion. Peak serum concentrations were more reproducible following intravenous infusion, with a dose of 1.0 mg/kg producing a 2.2 µg/ml increment. The kinetics did not differ significantly in 11 infants in whom the intramuscular and intravenous routes were alternated. The mean $T1/_2$ in infants less than 1 week of age was 4.6 ± 2 hours, while in infants greater than 1 week, it was 3.0 ± 1 hours. Maximum concentration and $T1/_2$ was independent of gestational age, weight, hematocrit, serum bilirubin and total serum protein concentration. $T1/_2$ appeared to correlate with creatinine clearance. No accumulation in serum was observed, but urinary excretion could only account for 20-50% of an administered dose. The present study suggests that 2 mg/kg be given to infants every 8 hours to maintain peak concentrations in the therapeutic range. Ideal management should involve a quantitative assay on each patient because of wide individual variation.

PLACENTAL TRANSFER AND PHARMACOKINETICS OF DIGOXIN IN THE PREGNANT EWE. Sharanjeet Singh, Peter Fehr, Bernard L. Mirkin. Depts. of Ped., Obst., Phcl., Div. Clin. Phcl. Univ. of Minnesota, Mpls., Minn.

Digitalis glycosides have been used as an adjunct in the management of heart failure in utero. The disposition of digoxin in the maternal-feto-placental unit has been studied. Catheters were implanted in the maternal femoral vein (MFV) and artery and umbilical vein and artery of the pregnant ewe. Digoxin (0.007, 0.02 and 0.03 mg/kg) was administered via the MFV and sequential blood samples drawn from each catheter site. Amniotic fluid and tissue specimens were also obtained. Digoxin levels were determined by radioimmunossay (Smith 1969, Singh & Mirkin 1972). The maternal to fetal plasma digoxin ratio varied with the dose and time of sampling as follows:

Digoxin Dose	Maternal/Fetal Plasma Digoxin Ratio			
(mg/kg)	5 min.	60 min.	120 min.	180 min.
0.007	33	--	--	--
0.02	65	1.0	1.0	1.0
0.03	633	4.0	5.6	4.0

The conc. of digoxin in the amniotic fluid exceeded the fetal plasma conc. and approximated the conc. of the steady state maternal plasma conc. These data suggest that the amniotic fluid may act as a reservoir containing large amounts of digoxin. (Supported by USPHS Grants GM 01998 and GM 15477).

COMPLIANCE WITH DRUG THERAPY IN INSTITUTIONALIZED EPILEPTIC CHILDREN. J.T. Wilson and G.R. Wilkinson. Divisions of Pediatric & Clinical Pharmacology, Departments of Pediatrics & Pharmacology. Vanderbilt University, Nashville, Tenn. 37232.

A high index of compliance is expected in institutionalized patients since medical personnel supervise drug administration. This assumption was tested in 92 epileptic patients (74 children, 18 adults) residing at a state institution. Plasma levels of diphenylhydantoin(DPH), phenobarbital(PB), and primidone were used to assess compliance in a two phase study. In Phase I ambiguities in drug orders vs drug records were noted in 26% of the cases. Drugs given without authorization were found in 5%. Plasma levels paired by an interval of 2 months in Phase I varied by about 20%. Most patients (85%) fell outside a plasma level therapeutic window for DPH(10-20 µg/ml) with 8% having no detectable levels and an equal number > 20 µg/ml. PB levels in 35% were > 30 µg/ml. Results of Phase I were given to the medical staff at onset of Phase II. Two months later ambiguous medication records were found in 18% of a subset of 35 cases. Unauthorized drug in plasma was noted in 19%. Most patients (66%) given PB clustered within the 6-30 µg/ml range. Average plasma levels for both PB and DPH varied by > 10% between Phase I and II and marked intra-patient variation was found. Contrary to expectation, many institutionalized patients do not receive their anticonvulsants as prescribed. Concern about risks of inadequate treatment, drug toxicity, inappropriate dose and interpretation of drug trial data are raised by these findings. (Supported by GM 15431 & K4-HD42539.)

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Read by Title

MICROMETHOD FOR DETERMINATION OF LEVELS OF ANTICONVULSANT DRUGS IN WHOLE BLOOD. Henry W. Baird and Victor H. Auerbach, Dept. of Ped., Temple Univ. Sch. of Med. and St. Christopher's Hospital for Children, Phila., Pa.

Since reports both in this country and in Europe suggest that lack of compliance of the patient and erratic absorption may be major factors in poor control of seizure disorders in some children, it is useful in the management of the child with convulsions to know the levels of anticonvulsant drugs in whole blood or serum. Heretofore, analyses have required large samples of blood and been expensive. We have utilized a few drops of capillary blood collected on the filter paper routinely used for the PKU test of Guthrie. Samples corresponding to 0.1 ml of blood on the dried paper, 6 mm in diameter, are cut by paper punch for extraction and analysis by gas liquid chromatography (GLC). A Hewlett-Packard 7600 A, modified by a nitrogen detector containing a rubidium bromide crystal, can determine levels of several anticonvulsant drugs from a single run and perform 36 runs in triplicate automatically. The filter paper method using dried whole blood gives results similar to those of our usual procedure using serum, when assayed for phenobarbital, mephobarbital, diphenylhydantoin, primidone, carbamazepine, and mephenyltoin. The filter paper technique is more convenient than the traditional venipuncture; samples can be more easily mailed and stored. The sensitivity of the method is of the order of 100 ng of each drug per sample.

HEPATIC NECROSIS FOLLOWING ACCIDENTAL CHLORDANE EXPOSURE. William F. Balistreri, John C.Partin, and William K. Schubert, Children's Hospital Research Foundation, Cincinnati, Ohio.

Chlorinated hydrocarbon insecticides produce hepatocellular necrosis in experimental animals. Reports of human intoxication have emphasized CNS effects (convulsions, coma) without documentation of liver toxicity. An infant developed vomiting and seizures, jaundice, hepatomegaly, hypoprothrombinemia and elevated transaminases following acute, prolonged dermal exposure to liquid chlordane (octachloro-4.7-methanotetrahydroindane). Rapid clinical improvement followed exchange transfusion treatment. Changes of acute hepatocellular necrosis were documented by serial liver biopsy. Five days post exposure marked universal centrilobular necrosis, fatty infiltration and minimal inflammation were evident. Electron microscopy showed intact mitochondria with large dense bodies, abundant glycogen, dilated rough endoplasmic reticulum, active Golgi apparatus, and universal fat unlike Reye's Syndrome or viral hepatitis. Ten days post exposure histology improved, triglyceride and necrosis were diminished, and mitoses were frequent. After 4 months, hepatic architecture was intact; mild triglyceride accumulation persisted; serum transaminases were normal; no clinical residua were evident. Pesticide was not present in serum (5th day) or adipose tissue (10th day) (GLC). Hepatic and neurologic disease immediately following exposure implicate chlordane as the etiologic agent. Possible efficacy of exchange transfusion in suspected pesticide intoxication is emphasized.

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OROTIC ACID, A SERUM BILIRUBIN LOWERING COMPOUND: SUGGESTED MECHANISM OF ACTION. Michael I. Cohen and Helen McNamara. Albert Einstein Col. of Med.,Montefiore Hosp. & Med. Ctr., Dept. of Ped., The Bronx, New York.

Orotic acid is essential in the synthesis of uracil, cytosine, thymine and through a series of enzymatic steps to uridine diphosphate (UDP). A role for orotate in the metabolism of bilirubin has been shown, since uridine diphosphoglucuronic acid (UDPGA), the bilirubin acceptor compound and an orotic acid derivative, is essential for the conjugation of bilirubin. Orotic acid given to neonates decreases the serum bilirubin but no mechanism for this effect has been found. To clarify this problem we gave orotic acid to 18 rats. In assaying the liver tissue for glucuronyl transferase, exogenous UDPGA was given in varying concentrations. No enzymatic activity was noted in 6 which had no exogenous UDPGA and minimal activity noted if half optimal concentrations were added. This failure of orotic acid to stimulate endogenous rat UDPGA suggested that the serum bilirubin lowering effect of orotic acid in humans was not due to a stimulating action on the UDPGA synthesis. In the remaining 6 assays in which optimal UDPGA was added, enhanced glucuronyl transferase activity was noted. Subsequently 34 rats were offered orotic acid and contrasted to a control group of 42. Glucuronyl transferase activity was determined with optimal UDPGA added and found to be enhanced ($P<.001$) in the orotate treated group thus suggesting the mechanism of action of this compound is as a stimulant to glucuronyl transferase rather than to UDPGA.

EPIDEMIC HUMAN METHYLMERCURY POISONING- APPLICATION OF A MOUSE MODEL SYSTEM. Richard A. Doherty, Allen H. Gates (Intr. by Gilbert B. Forbes). Depts. Ped., Rad.Biol./Biophysics, Div.Genetics, Univ.Rochester Sch. Med., Rochester, N.Y.

The largest known epidemic of methylmercury poisoning recently occurred in Iraq. During a 2-month period 6,530 cases were hospitalized. There were 459 hospital deaths. The source was homemade bread prepared from seed wheat treated with a methylmercurial fungicide. Males and females of all ages were affected. Children of 1 to 9 years of age constituted 34% of admissions. 14 of 31 hospitalized pregnant women died. Followup studies of infants exposed *in utero* and by suckling are in progress.

We have used a mouse model to evaluate risk to the mammalian fetus and newborn. We have shown that 1)methylmercury passes freely from mother to fetus; 2)newborn mice ingest and absorb significant amounts of mercury when suckled by mercury-dosed mothers (cross-fostering technique); 3)suckling mice excrete mercury at rates less than 1% of adult excretion rates. These observations have been useful in designing and initiating human investigations and treatment. [Clinical data were obtained through joint efforts of investigative teams from the Univ. Baghdad and Univ. Rochester].

THE EFFECT OF POST-SUCKLING MALNUTRITION UPON HEPATIC DRUG METABOLISM. Margareta Eriksson, Charlotte S. Catz, Summer J. Yaffe, Department of Pediatrics, School of Medicine, State University of New York at Buffalo, Buffalo, New York.

Drug metabolism (in vitro) was studied in rats kept on a protein deficient diet (8% casein) for 3 and 6 weeks after weaning (21 days). Oxidative pathways using aminopyrine, hexobarbital and aniline as substrates were significantly decreased in the malnourished rats. A similar effect was measured for the reductive pathway with neoprontosil. Conjugation with p-nitrophenol was markedly increased. A decrease in cytochrome P450 and b_5 contents was significant. *In vivo* sleeping time, following intraperitoneal injection of hexobarbital was prolonged in the malnourished rats. A group of rats were refed on regular laboratory food for 3 weeks. The abnormal findings reverted to normal. Although post weanling malnutrition affected hepatic drug metabolism, it is important to note that in contrast to intrauterine or pre-weaning malnutrition, the changes were not permanent.

ABSTINENCE SCORE IN THE TREATMENT OF THE INFANT OF THE DRUG-DEPENDENT MOTHER. Loretta P. Finnegan, James F. Connaughton, John P. Emich. (Intr. by Maria Delivoria-Papadopoulos) Phila. Gen. Hosp.,Depts.of Ped. and Obs.,Univ.of Pa.,Sch.of Med., Phila., Pa.

Over the past 3yrs. 85% of 146 infants of drug-dependent mothers at P.G.H. manifested symptoms of abstinence. In the management of the first 121 infants careful observation for the onset and progression of symptoms preceded the use of drugs in the therapeutic regimen. Thus far the decision to use drugs or to increase dosage was influenced by arbitrarily applied clinical criteria providing inadequate basis for judgement in the treatment of this syndrome. A neonatal abstinence score has been devised to provide a more precise method of management in the last 25 infants of drug-dependent mothers. Twenty-one of the commonly seen symptoms are listed and each has been given a score of 1-5 according to its clinical significance (ie,convulsion=5, sweating=1). The infants are scored once every hour for the first 24 hrs. every 2 hrs. for the second 24 hrs. and every 4 hrs. for the duration of symptomatology. Infants whose score is 7 or less are not treated with drugs. Once a score of 8 or more is attained and sustained for 3 hrs. the infant is treated. Dosage schedules relating the score to particular dosages of detoxicant drugs are used. (the higher the score the greater the mg/Kg/day of drug). This abstinence score will be helpful in monitoring the symptomatology of the passively addicted infant and may provide more uniform criteria for assessment and treatment.

SALICYLATE DISTRIBUTION AND ELIMINATION IN CHILDREN WITH DOWN'S SYNDROME. George Giacoia, Charlotte S. Catz, Gerhard Levy, Somner J. Yaffe. Depts. of Ped. and Biopharmaceutics, Schs. of Med. and Pharmacy, SUNYAB, Buffalo, New York.

In 1970 Ebadi and Kugel reported lower binding of salicylate to plasma proteins and decreased formation of salicyluric acid the major metabolite of salicylic acid (SA) in children with Down's Syndrome (DS) as compared to normal controls (C). Because of the clinical implications of this finding this problem has recently been reinvestigated by us employing the techniques of pharmacokinetics. Four patients (6-12 years old) with DS confirmed by chromosomal analysis, and 4 C children of similar age received single oral doses of sodium salicylate, 20 mg/kg. Urine was collected at timed intervals fof 36 hours and blood was obtained to determine the binding of salicylate to plasma protein. There were no differences in protein binding of salicylate in the plasma of the patients and of their C (70-80% bound at a concentration of 20 mg%). The composition of urinary metabolites of SA and the kinetics of salicylate elimination by the children with DS was also no different from that of the C. Further there was no evidence of abnormal distribution of salicylate in the body, as reflected by its apparent volume of distribution.

DEPRESSION OF ERYTHROPOIESIS AND HAIR GROWTH DURING CHLORAMPHENICOL (CHLORO) TREATMENT IN A PATIENT WITH CYSTIC FIBROSIS (CF). Jean P. Kapp and Maarten S. Sibinga, Dept. of Ped., Temple Univ. Med. Sch. at St. Christopher's Hosp. for Child., Phila., Pa.

Optic neuritis and atrophy and peripheral neuritis are recognized probably dose-related complications of treatment with chloro. in CF. In other pts. treated with this drug, the risk of serious bone marrow toxicity has been estimated to range from 1 in 40,000 to 1 in 100,000. In CF, bone marrow depression has not often been reported.

We have observed a 3 1/2 year old girl with CF who became listless and anorexic after 8 days of chloro. treatment (60mg/kg/day). Tiredness and marked pallor later became apparent. After 6 weeks of chloro. treatment, erythroid bone marrow depression was manifested by a Hb. of 7.7 g%. WBC and platelets were normal. Blood counts preceding the use of this drug had been normal. There was no evidence of blood loss.

Three days after discontinuation of chloro., the patient's appetite and activity had returned to normal and 14 days after stopping the drug, the Hb. was 9.1 g% with a retic. count of 4.6%, a WBC of 5,500 and a platelet count of 274,000.

A few weeks later, it became apparent that her usually dark blonde hair had grown less fast, had become much lighter in color and was finer in texture during the period of drug administration than before or after the chloro. treatment. The mother also reported a great deal more loss of hair than usual which ceased when the drug was discontinued.

EFFECT OF HEROIN ON CORTISOL PRODUCTION IN PREGNANT ADDICTS AND THEIR FETUSES. Leonard Glass, B.K. Rajegowda, Trishit K. Mukherjee, Michael M. Roth and Hugh E. Evans. Columbia Univ. Dept. of Ped. and Obs., Harlem Hospital Center, New York, N.Y.

In order to assess the effects of heroin (which freely crosses the placenta) on fetal and maternal adrenocortical function, cord sera cortisol concentrations were determined by a fluorometric assay method (BMJ 2:310, 1972) on 18 non-asphyxiated infants of addicted mothers and 15 infants of nonaddicted mothers of similar birth weights and gestational ages. All infants were born by normal vaginal delivery. Cortisol levels were also determined on blood of 17 addicted and 12 nonaddicted mothers, drawn at the time of delivery.

While serum cortisol levels were comparable in both study (median 12.3 mg/100ml; range 8.3-20.6 mg/100ml) and control (median 13.0; range 4.8-21.0 mg/100ml) infants, addicted mothers had significantly lower concentrations (median 22.7 mg/100ml; range 12.0-71.0 mg/100ml) than nonaddicted mothers (median 38.5 mg/100ml; range 23.8-75.5 mg/100ml) ($p < 0.01$).

Heroin decreases cortisol production in adults by inhibiting secretion of ACTH. Decreased levels were found in pregnant addicts at the time of delivery, however, values were similar in infants of addicted and nonaddicted mothers. The reason for these differences has not been explained. The findings may reflect a decreased responsiveness of the fetal pituitary to heroin or a relative insensitivity of the fetal adrenal cortex to fluctuations in ACTH secretion.

CO-TRIMOXAZOLE: ABSORPTION, EXCRETION AND TOXICITY IN SIX CHILDREN. Edward B. Lewin, Jerome O. Klein and Maxwell Finland

Co-trimoxazole is an antimicrobial with activity against a wide variety of bacterial species of importance in infections of infants and children. Although the drug has been used extensively in children, there are no published pharmacokinetic data. Five infants, 7 to 17 months old, and an 11 year old child were treated with 185 mg trimethoprim (TMP) + 925 mg sulfamethoxazole (SMZ) (combined as co-trimoxazole pediatric suspension) per meter^2 daily in 2 equal doses. After the first dose, absorption was rapid. Peak values for TMP were reached between 1 and 4 hours and ranged between 0.5 and 2.0 ug/ml (geom. mean=1.2 ug/ml). Peak values for free SMZ were also reached between one and 4 hours and ranged from 16.0 to 27.5 ug/ml (geom. mean=18.7 ug/ml). Levels of both TMP and SMZ remained elevated through 12 hours without significant accumulation of either agent when measured one hour after subsequent doses on days two through four of therapy. Concentrations of TMP in urine ranged from 60-110 ug/ml during the interval 0-6 hours and from 32-105 ug/ml 7-12 hours after the first dose. In five of six patients, no untoward effects were apparent. In the sixth patient, granulocytopenia occurred after 25 days of treatment. This patient, a renal transplant recipient, received concomitant immunosuppressive therapy.

Harvard Medical School, Boston City Hospital, Depts. of Pediatrics and Medicine, Boston, Massachusetts.

DELAYED PRESENTATION OF NEONATAL METHADONE WITHDRAWAL Stephen R. Kandall and Lawrence M. Gartner, Dept. Pediatrics, Albert Einstein College of Medicine, Bronx, New York

Infants born of mothers with methadone addiction have been recognized recently to have more severe and prolonged symptoms of withdrawal than do infants born to mothers with heroin addiction. It has not been previously recognized, however, that methadone addicted infants may also have onset of initial symptoms of withdrawal late in the newborn period following an initial period of 2 to 4 weeks without symptoms. Of 71 infants at the Bronx Municipal Hospital Center in 1972 developing symptoms of narcotic withdrawal, 46 were born of mothers using methadone alone or in combination with heroin. Five of these 46 methadone addicted infants developed their initial symptoms between 2 and 4 weeks of age. Symptoms at presentation were similar to those observed in infants with early onset, but in one case the initial symptoms were seizures and in another the infant died at home following symptoms of increasing irritability and diarrhea.

The markedly increased usage of methadone and its severe and prolonged withdrawal symptoms in the newborn, coupled with the recognition that these symptoms may be silent for 2-4 weeks after birth, makes this a major public health problem. Further study of the developmental pharmacology of methadone in the neonate is needed, and increased surveillance of such potentially addicted infants is mandatory.

PHARMACOKINETIC STUDIES OF TRIMETHOPRIM-SULFAMETHOXAZOLE IN CHILDREN WITH GASTROENTERITIS. M.I. MARKS, M. KAZEMI, B. HALES, A.H. NEIMS. McGill Univ.-Montreal Children's Hosp. Research Inst., Montreal, P.Q.

Certain pharmacokinetic parameters of the combination, trimethoprim (TMP)-sulfamethoxazole (SMZ), were evaluated in 25 children, aged 10 months to 15 years, with gastroenteritis. Serum trimethoprim concentrations were measured by an agar-well diffusion microbiological technique while urine trimethoprim and all sulfamethoxazole concentrations were estimated by chemical methods. The drugs were well absorbed. Peak serum levels were achieved within one hour. At doses of 100 mg/kg/day SMZ and 20 mg/kg/day TMP divided q6h, the mean serum concentrations of SMZ (free) and TMP two hours after the ninth dose were 173.5 and 9.6 ug/ml, respectively. At the same time the urine contained 330 ug/ml TMP, 881 ug/ml "free" SMZ, and 2026 ug/ml SMZ. The mean SMZ free/TMP ratios for serum and urine were 23.8 and 2.9, respectively. Two patients were studied in detail. About 50% of the daily administered SMZ was recovered in the urine, with 55% of that fraction acetylated; 40% of TMP was recovered as such in the urine. The drugs were tolerated well (rash in 2 patients; vomiting in 3 patients) and lab. assessment of hepatic, hematopoietic, and renal function revealed no abnormalities during or after 7-days therapy. TMP-SMZ is absorbed and tolerated well by children with gastroenteritis. Serum and urine concentrations and ratios achieved should be adequate for activity in vivo against many common pathogenic bacteria.

HEROIN AND THE FETUS. Richard L. Naeye, William A. Blanc, Werner Leblanc, Depts. of Pathology, Pennsylvania State University College of Medicine, Hershey, Pa. and Columbia University College of Physicians & Surgeons, New York City.

Many neonates of mothers on heroin have small birth weights. Both fetal growth retardation and preterm delivery are at fault. Autopsy material was examined from: (a) 29 newborns whose mothers used heroin up to delivery (b) 10 neonates whose mothers used heroin only in early pregnancy (c) 3 infants with mothers on methadone (d) 7 neonates whose non-addicted mothers had hepatitis (e) 1044 newborn controls (f) placentas from 28 surviving infants of heroin addicts. The percent preterm (<38 wks) infants in the groups was: (a) 83% (b) 50% (c) 100% (d) 86% (e) 78%. The incidence of the amniotic fluid infection syndrome: (a) 57% (b) 70% (c) 0% (d) 27% (e) 54%. The incidence of hyaline membrane disease in the infants: (a) 40% (b) 0% (c) 67% (d) 57%. Body weights in % published normal values: (a) 86% (b) 99% (c) 114% (d) 103% (e) 106%. Using quantitative methods the subnormal size of organs in neonates of heroin addicts was due to a subnormal number of cells at all gestational ages. Near term infants had cells with a subnormal cytoplasmic mass. All the organ abnormalities can be explained by differing effects of maternal undernutrition at the various gestational ages. The amniotic infection syndrome appears responsible for many preterm deliveries.

METABOLISM OF DIAZOXIDE (3-methyl-7-chloro-1,2,4-benzothiadiazine-1,1-dioxide). Albert W. Pruitt, Bahjat A. Faraj, Zafar H. Israili and Peter G. Dayton. (Intr. by R.W. Blumberg), Emory University School of Medicine, Henrietta Egleston Hospital for Children, Dept. of Pediatrics & Medicine, Atlanta.

Each of two adult volunteers was given a single 300 mg dose of diazoxide-3-14C orally. The T ½ in plasma was 23 and 29 hours respectively. Urine was investigated for metabolites of diazoxide (D). Six days after dose, 87-90% of the administered radioactivity had been recovered in urine. 33-38% was unchanged drug; 21-29% was the 3-hydroxymethyl metabolite; 18-33% was the 3-carboxy metabolite; and in one subject studied, 4% was the sulfate conjugate of the 3-hydroxymethyl metabolite. Fecal excretion of radioactivity was 2% as unchanged D.

A male infant with onset of hypoglycemia at 3 days of age was started on D at 12 days of age because of poor control of blood sugar. After 6 days of D (7 mg/kg/day), severe hyperglycemia developed. D was discontinued and the plasma level of drug 48 hours later was 132 ug/ml. The plasma T ½ was 41 hours, which is significantly longer than T ½ 9.5-24 hours in children 6 months-6 years of age (Clin. Pharm. Ther., in press). Based on thin layer chromatography, both the unconjugated metabolites of D were present in the patient's urine collected at age 2 weeks. D was re-instituted at a lower dose at 5 weeks of age and the plasma T ½ was 40 hours, suggesting no increase in rate of metabolism of D.

ORAL CONTRACEPTIVES AND BIRTH DEFECTS: PRELIMINARY EVIDENCE FOR A POSSIBLE ASSOCIATION. James J. Nora, M.D. and Audrey Hart Nora, M.D. Univ. Colorado Med. Center, Denver, Co. 80220

A study of 10 patients with multiple congenital anomalies described by the acronym VACTEL (Vertebral, Anal, Cardiac, Tracheal, Esophageal, Limb) revealed exposure at the vulnerable period of embryogenesis to a progestogen-estrogen compound or a progestogen alone in 7 patients ($p < .001$). These hormones were taken either as a "pregnancy-test" or mistakenly without realizing that pregnancy already existed. Alternative hypotheses of single mutant gene etiology and chromosomal etiology of the VACTEL syndrome failed to support these modes. The multiple anomalies of skeletal, cardiovascular and gastrointestinal structures recapitulate the systems involved in the thalidomide syndrome but present a different pattern. A retrospective study of 224 patients with congenital heart disease disclosed that 20 patients received progestogen/estrogen at the vulnerable period of cardiogenesis compared with 4 of 262 controls ($p < .001$). The distribution of heart lesions was unusual in that truncoconal, great vessel malformations predominated. The deficiencies of retrospective studies are acknowledged, therefore, a prospective study, as well as a protocol for evaluation of teratogenic mechanisms of progestogen/estrogen in sub-human primates will be required for definitive investigation and are in progress.

DIPHENYLHYDANTOIN (DILANTIN) IN TREATMENT OF PATIENTS WITH GLYCOGEN STORAGE DISEASES, Marvin L. Rallison and William Jubiz (Intr. by M. E. Lahey), Univ. of Utah Col. of Med., Depts. of Ped. and Med., Salt Lake City.

Dilantin, known to have enzyme inducing activities, has been used in treatment of 4 subjects with glycogen storage diseases (GSD). The oldest patient, age 27, has Type I GSD, the youngest, age 5, Type VI, and the remaining 2 (siblings) have Type III GSD. Dilantin, in dosages of 3-5 mg/kg body weight daily, has been administered over a 2 year period. Reduction in liver size (by palpation and by liver scan), improvement in liver function tests, elevation of blood sugars, reduction in blood lactate levels and reduction in glycogen content* of liver cells was seen in response to Dilantin therapy. Response was most noticeable and persistent in the youngest patient and least demonstrable in the oldest. No consistent effect on growth of the younger children was noted. Enzyme activities* were not measurably changed in liver biopsy specimens obtained after 3 months of Dilantin therapy, despite clear evidence of clinical and chemical improvement. Only minimal gum hyperplasia has been noted in 1 subject with the Dilantin dosages used. Though enzyme induction by Dilantin cannot yet be demonstrated, clinical trials with Dilantin seem warranted in treatment of GSD.

*Performed by B.I. Brown, St. Louis, Missouri.

THE PLACENTAL CLEARANCE AND TISSUE DISTRIBUTION OF PHENOBARBITAL (PHB) IN THE BABOON. John B. Paton, David E. Fisher, Lester F. Soyka and Richard E. Behrman. Abraham Lincoln Sch., Univ. of Ill. Coll. of Med., Dept. of Ped., Chicago.

Five pregnant baboons at an estimated 160 days gestation (term = 184) were studied during steady state infusions into the fetus of antipyrine and PHB following pretreatment with PHB (200 mg/day x 3) to insure tissue saturation. Antipyrine diffusion clearance was 57 ± 13 ml/min/kg compared to a PHB diffusion clearance of 90 ± 34 ml/min/kg. Tissue levels of PHB were measured in two mother-fetus pairs. The fetal serum level (47 μg/ml) was 50% greater than maternal. In maternal tissues the highest concentrations were in liver, heart and kidney, whereas in the fetus the lowest concentration was present in the liver, while heart, kidney and lung had the highest concentrations. Brain stem levels in both mother and fetus were lower than serum, while cerebral and cerebellar concentrations were equivalent to serum in both. In the fetal liver, about 2% of radioactivity was the p-OH metabolite. Equivalent or even lesser amounts were found in maternal liver and kidney, and fetal kidney and muscle; none was detected in maternal skeletal muscle. Conclusions: (1) the placental clearance of PHB does not appear to be flow limited, (2) maternal liver/serum concentration ratio of 1.7 contrasts with 0.7 in the fetus, (3) the p-OH metabolite is either not formed by the fetal liver or is rapidly eliminated.

Supported in part by USPHS 05362 and PMA Foundation.

THE EFFECT OF CHRONIC MATERNAL MORPHINE ADMINISTRATION ON LUNG DEVELOPMENT AND GROWTH OF FETAL RABBITS. Dietrich W. Roloff and William F. Howatt. Univ. of Michigan Med. Ctr., Dept. of Ped., Ann Arbor.

The observation that premature infants of heroin-addicted mothers are less likely to have respiratory distress syndrome (Glass et al, Lancet 1971) stimulated us to study this effect in an animal model. Morphine (2.5 - 10mg/kg) was given to 13 pregnant rabbits subcutaneously every 6 hours from the end of the first week of pregnancy until delivery by hysterotomy on the 27th, 28th, or 29th day. Normal saline was given to 14 pregnant control animals. The fetal lungs were inflated and the amount of air entering the lungs (V_{max}) and the proportion of air remaining in the lungs at a deflation pressure of 10cm H_2O (V_{10}/V_{max}) were determined as reflecting fetal lung maturity. At 28 days, V_{max} and V_{10}/V_{max} values were lower in the fetuses from morphine-treated does than in the controls. No significant differences were found at 27 and at 29 days between the two groups. At all ages the weights of fetuses in the morphine group were lower than that of the controls. Taeusch et al (SPR 1972) found the same effect on fetal growth with maternal and direct fetal heroin injections given in late gestation but recorded no effect on lung maturation unless the drug was injected directly into the fetus. We conclude that maternal morphine addiction in rabbits interferes with fetal lung maturation as well as with fetal growth.

PARACHLOROPHENYLALANINE INDUCED CATARACT. Rowe, V.E.;Zigler, J.S.; Anderson, A.E.; Sidbury, J.B.; Guroff, G. From NICH, NIH, Bethesda, Md. and Duke Med. Ctr, Durham, N. C.

It had been previously shown that parachlorophenylalanine (PCP)-phenylalanine treatment of rats results in a high percentage of rats developing cataracts weeks to months after treatment. In our study we found that some rats developed bilateral cataracts, some unilateral and others none. The free aminoacids were determined by GLC in individual lens of control,cataractous, non-cataractous lens from unilaterally affected rats and lens from non-cataractous treated animals. The cataractous lens showed a significant decrease in all aminoacids except ornithine and aspartic acid. The unilateral normal appearing lens showed decreased glycine, arginine, serine and threonine compared with the normal. The PCP treated non-cataractous animal showed lowered valine and alanine concentrations. The soluble protein and glutathione was indistinguable among all the non-cataractous lens. No explanation for the delay in cataract formation following PCP treatment is apparent.

EXTRACTION OF INHIBITORS OF DRUG METABOLISM FROM LIVER MICROSOMES OF NEWBORN RATS. Ernest L. Sutton, Fred W. Deckert and Lester F. Soyka. Depts. of Pharmacol., and Peds., Univ. of IL., Chicago, Illinois 60680

A search has been made for inhibitory substance(s) which may be responsible for the impaired drug metabolizing activity of liver microsomes from immature rats. Washed microsomes from rats of 5, 10 and 90 days of age were extracted with various organic solvents, usually diethyl ether. The extracts were tested directly or following thin-layer chromatography (TLC). Demethylation of p-nitroanisole was chosen as a model drug metabolizing enzyme system. Extracts from each age group were inhibitory, with amounts from as little as 0.25 mg of microsomal protein. Equivalent inhibitory activity was present in extracts prepared from microsomes extracted at pH 3, 7 and 11. TLC plates were divided into five zones. Inhibitory activity was mainly present in the most rapidly migrating areas. Extracts from the 5 and 10 day olds were generally more potent than those from 90 day olds. A single UV absorbing spot (R_f 0.7 in $CHCl_3$: MeOH, 9:1) accounted for about half of the inhibitory activity of the most active band, but has not yet been definitively identified. The solubility and migratory properties of the inhibitor(s) coupled with our previous studies demonstrating that only steroids of a defined structure were inhibitory in our test system, suggest that the inhibitory substance(s) may be a metabolite of progesterone or a structurally related steroid. Supported in part by USPHS HD 053-62.

ORAL D-PENICILLAMINE THERAPY IN CHRONICALLY LEAD POISONED CHILDREN. Leonard F. Vitale, Amy Rosalinas-Bailon, Dave Folland, James F. Brennan, Beryl McCormick, Dept. of Peds., New Jersey Med. Sch., Newark, N.J.

(Intr. by Franklin C. Behrle)

Eight hospitalized, chronically lead poisoned black children (mean blood lead 72 ug%, mean Hb. 11.2 mg%, mean urinary ALA 28 mgs/L, range 14-72, and ALA-D 4.25 units, range 0-8) were treated with an oral dose of 29-40 mgs/kg/day of D-Penicillamine. During therapy, urinary lead excretion increased dramatically from a mean baseline of 23.7 ug/L (range 6-70 ug/L) to a mean level of 425 ug/L (range 132-1000). Urinary ALA dropped to 9 mgs/L (normal) in one week and to 5 mgs/L in 2 months when therapy was discontinued. ALA-D increased to 6 units in one week and to normal values (mean 125 units, range 112-144) in 2 months. Hemoglobin remained unchanged during the entire therapy. The blood lead dropped to 39 ug% in two months (range 24-43).

Since in all patients, urine lead excretion remains elevated during therapy, we believe, D-Penicillamine depletes lead storage sites in the body and is the therapy of choice for chronic lead poisoning.

THE SIMULTANEOUS MEASUREMENT OF RED CELL DELTA-AMINOLEVULINIC ACID DEHYDRASE (ALAD) AND THE FREE ERYTHROCYTE PROTOPORPHYRIN FOR THE DETECTION OF LEAD POISONING. L. Weiner, B. F. Oski, S. Piomelli and F. A. Oski. Department of Pediatrics, Upstate Medical Center, Syracuse, New York, and the New York University School of Medicine, New York, New York.

Both the measurement of ALAD and FEP have been adapted to micro methods and been advocated for mass screening for the detection of lead poisoning. In an attempt to evaluate the usefulness of these procedures, 105 subjects had simultaneous FEP, ALAD, blood lead, and hematologic indices performed. Analysis revealed a significant correlation between blood lead and ALAD, and ALAD and FEP. Although the FEP detected all subjects with blood lead above 50 micrograms %, it was also elevated in subjects with iron deficiency. The combined use of both tests (0.12 ml blood) provides a powerful screening tool for the detection of both iron deficiency and lead poisoning. The results can easily be interpreted as follows:

Test Result	Interpretation
↑ FEP; ↓ ALAD	Lead poisoning; or lead poisoning plus iron deficiency.
↑ FEP; ↔ ALAD	Iron deficiency.
↔ FEP; ↓ ALAD	Increased body lead burden with normal blood lead.
↔ FEP; ↔ ALAD	Normal subject.

DIGOXIN (D) TOXICITY IN INFANTS: CORRELATION WITH SERUM LEVELS. Ruth Yanagi, Richard Krasula, Alois Hastreiter and Lester F. Soyka. Univ. of Ill. Med. Ctr., Chicago, IL. 60680

Eleven infants (1 d-5 months) diagnosed as toxic by clinical and/or ECG findings were compared to non-toxic infants (17 d-10 months). Serum D concentrations 5-12 hrs following administration were determined by our RIA procedure (J Ped 81: 566, 1972). The digitalization period was considered to be the first two days of therapy:

	N	Dose (mg/kg/d)	Serum D (ng/ml)	
Toxic	6	.08	3.9 ± 0.6	
Non-toxic	7	.08	4.1 ± 0.7	
During maintenance therapy:				
Toxic	5	.018	3.2 ± 0.6	
Non-toxic	26	.017	1.8 ± 0.2	$p < 0.01$

One toxic pt with a value of 10 ng/ml was omitted from calculation of the mean. Only 2 of 26 values were > 3 ng/ml in the non-toxic maintenance group (21 pts), whereas 6 of 9 determinations in toxic infants were > 3, and the remaining 3 were > 2 ng/ml. Levels in older children (1-15 yrs., 53 pts., 85 determinations, 1.1 ± 0.1 ng/ml) were significantly lower than in infants ($p < 0.001$). Conclusions: D levels are: (1) not helpful in diagnosis during digitalization period, (2) correlated with clinical toxicity when > 3 in infants as in adults though some overlap occurs, (3) significantly lower in children than in infants. The high incidence of toxicity during digitalization suggests that the standard dose and schedule employed may be excessive for small, sick infants.

ENDOCRINOLOGY

First Session

FSH : ITS MEDIATING ROLE ON TESTOSTERONE SECRETION IN HYPOPITUITARISM. Raphaël Rappaport, Pierre C. Sizonenko, Nathalie Josso and Fernand Dray. (Intr. by Donough O'Brien). Descartes Univ. Med. Sch., Hôpital des Enfants Malades, Paris, France, and Univ. of Geneva Med. Sch., Dept. of Ped., Geneva, Switzerland.

22 hypopituitary males aged 8 to 28 years were given 1,500 IU of HCG, I.M., every other day for 12 days. Plasma radioimmunoassayable testosterone (T0), FSH and LH were measured before HCG. Testosterone response was evaluated on day 13 (T13). Among 15 patients with no sign of puberty, 5 had a normal T13 response (group I) and 10 were identified as low-responders (group II) when compared to 6 prepubertal (P1) controls. 7 hypopituitary subjects with signs of puberty (group III) were compared to 6 pubertal (P3) controls of similar bone age.

	T0(ng/100 ml)	T13(ng/100 ml)	FSH(mU/ml)×	LH(mU/ml)+
P1 controls	38 ± 10	490 ± 90	2.3 ± 0.2	5.5 ± 0.8
Group I	36 ± 11	714 ± 165	2.7 ± 0.6	10.4 ± 4.0
Group II	33 ± 9	168 ± 27***	1.2 ± 0.2***	9.1 ± 1.8
P3 controls	380 ± 30	1430 ± 120	4.2 ± 0.5	8.8 ± 2.1
Group III	323 ± 71	815 ± 147**	4.6 ± 0.9	11 ± 1.3

** $p < 0.01$, *** $p < 0.001$ × MRC 68/39, + MRC 68/40

There was a positive correlation between FSH and T13 in all patients ($r = 0.65$, $p < 0.001$), even in the prepubertal group ($r = 0.81$, $p < 0.001$). No such correlation was observed between LH and T13. It may be concluded that the testosterone response to HCG in hypopituitarism is dependent on the level of endogenous FSH.

AUTONOMOUS MATURATION OF THE BRAIN HYPOTHALAMIC PITUITARY GONADOTROPHIN SYSTEM. Jordan W. Finkelstein, Robert M. Boyar, Howard P. Roffwarg, Leon Hellman, Albert Einstein Col. of Med., Montefiore Hosp. & Med. Ctr., Inst. for Steroid Res., Dept. of Ped., Dept. of Oncology, Dept. of Psychiatry, Bronx, New York.

At the onset of puberty, follicle stimulating hormone (FSH) and luteinizing hormone (LH) are secreted in an episodic manner. Secretion during sleep is much greater than during wakefulness. This sleep augmentation phenomenon disappears when sexual maturation is complete. We have studied three girls with gonadal agenesis in order to determine if this maturational phenomenon is in any way mediated by normal gonads. 3 girls, one 12 year old with a small amount of pubic hair, one 17 year old with moderate pubic and axillary hair and one 26 year old with adult amounts of pubic and axillary hair were studied. None had normal estrogenic effects. Plasma was assayed for FSH and LH every 20 minutes for 24 hours. FSH and LH in all subjects were in the castrate range. The 12 year old demonstrated the sleep augmentation phenomenon typical of a normal girl in early puberty. The 17 year old showed some sleep augmentation but not as marked as in the first patient. This transitional pattern is seen in very late puberty as the adult pattern becomes established. No change was seen after bilateral oophorectomy. The 25 year old showed no evidence of sleep augmentation. These patients demonstrate that the maturational process which occurs in the brain-hypothalamic-pituitary gonadotrophin axis at the time of puberty is independent of normal gonadal function.

ENDOCRINE EFFECTS OF BRAIN SEROTONIN (5-HT) DEPLETION BY 5,6-DIHYDROXYTRYPTAMINE (5,6-DHT) IN PREPUBERAL MALE RATS. R. Collu, J. C. Jéquier, J. Letarte, G. Leboeuf, and J. R. Ducharme, Dept. of Ped., Ste-Justine Hosp., Univ. of Montreal, Montreal, Que.

Twenty-one day old male Sprague-Dawley rats received two successive intraventricular injections of either 5,6-DHT or vehicle. Controls were either pair-fed or fed ad libitum. The analysis of variance showed that brain 5-HT levels were significantly lower in experimentals vs pair-fed controls one (189 vs 407 ng/g, $p < 0.01$) and two (241 vs 384 ng/g, $p < 0.01$) weeks after treatment, body weights were significantly lower one week after treatment (41.5 vs 64.0 g, $p < 0.01$), tail lenghts were significantly smaller one (9.3 vs 9.8 cm, $p < 0.01$) and two (10.3 vs 11.2 cm, $p < 0.01$) weeks after treatment, and testes weights were significantly lower two 335 vs 582 mg, $p < 0.01$) and four (1570 vs 2027 mg, $p < 0.01$) weeks after treatment. Covariance analysis of body weight and pituitary growth hormone content showed a significant difference between experimentals and controls fed ad libitum two weeks after treatment (63.8 vs 133.7 mμg, $p < 0.01$). These data indicate that 5-HT plays a role in the control of growth and sexual maturation.

ONTOGENY OF POSITIVE FEEDBACK: LUTEINIZING HORMONE(LH) RELEASE IN PRE- AND POSTPUBERTAL GIRLS FOLLOWING EXOGENOUS ESTROGEN ADMINISTRATION. H.E.Kulin and E.O.Reiter, NIH, Bethesda, Md.

Sexual maturation is associated with changing sensitivity of negative feedback between gonadal steroids and the hypothalamic-pituitary axis. To examine the ontogeny of positive feedback(PF) pre- and postpubertal subjects were given intramuscular injections of 17β-estradiol($17\beta E_2$) such that sustained blood levels >100 pg/ml, measured by radioimmunoassay (RIA), were obtained. In each of 19 young adult women who received such a regimen during the first week of the menstrual cycle there was initial suppression of plasma and urinary LH(by RIA) followed by a 2-fold or greater increment in LH occurring while $17\beta E_2$ levels remained elevated (by definition PF). Follicle stimulating hormone changes were variable. Three normal prepubertal girls and an 11 year old girl with gonadal dysgenesis (GD) did not evidence PF, revealing only gonadotropin suppression. One early pubertal girl displayed a small increment in LH in the presence of elevated estrogen levels. One pre-menarchal, midpubertal girl with adult levels of gonadotropins and precocious puberty secondary to an hypothalamic cyst revealed a large LH rise during $17\beta E_2$ administration both before and after drainage of the cyst. A 14 year old girl with GD and adult castrate levels of gonadotropins also displayed PF. The data suggest PF between estrogens and the hypothalamic-pituitary axis in women is: 1) absent before puberty; 2) appears by midpuberty; 3) is present in the course of neurogenic precocious puberty; 4) matures in the absence of the ovary.

IMMUNOREACTIVE SERUM PARATHYROID HORMONE (IRPTH) ASSAY IN THE NEWBORN. David M. Brown & Arnold W. Lindall. Dept. Ped. & Lab. Med., Univ. of Minn., Minneapolis, Minnesota.

IRPTH was measured by radioimmunoassay in 33 newborn infants under 72 hrs. of age in an effort to relate neonatal hypocalcemia to parathyroid hormone (PTH) secretion. The mean IRPTH in newborns was 50% of the mean IRPTH of adults. It is noteworthy that Reiss et al, (J. Clin. Endocr. 34:767, 1972) have noted a progressive rise of IRPTH in mothers throughout the last 4 weeks of pregnancy. These observations would suggest a possible mechanism of calcium transport from the mother to fetus. IRPTH of newborns was inversely related to the serum inorganic phosphorus (P_i) (range 3.6-8.5 mg/100 ml) ($P < .001$). Above a Ca_s of 8.2 mg/100 ml, IRPTH and Ca_s were correlated positively. However, with Ca_s below 8.2 mg/100 ml, IRPTH rose as Ca_s decreased. In eight pairs of infants IRPTH and Ca_s was measured sequentially. Despite a progressive rise of IRPTH in some infants, Ca_s declined in the first 72 hrs. of life. No correlations were found between IRPTH and capillary pH or Ca_s and P_i. These studies suggest that neonatal hypocalcemia may be associated with either insufficient PTH or diminished end-organ response to PTH in the presence of increasing PTH secretion. Further studies of these relationships must include examination of PTH effects on P_i and calcium metabolism of bone, kidney and intestine in the newborn.

INTERFERENCE WITH THE ONSET OF PUBERTY AND REPRODUCTIVE FUNCTION IN FEMALE RAT BY PRE- AND/OR POST-NATAL CYANOKETONE (CTM), ANTI-BODIES TO LH:FSH, AND ACTINOMYCIN-D. Allen S. Goldman and Bernard H. Shapiro Childrens Hosp. of Philadelphia, Philadelphia.

Either CTM, a specific inhibitor of 3 -ol-dehydrogenase, antibodies to LH:FSH, or vehicle was injected in pregnant rats. Some pups also received CTM, at 1 day, or anti-LH:FSH on 1,2, and 4 days. Other pups were given CTM only on 1 and 4 days or actinomycin-D, only on 4,7,9, and 11 days. Pre and/or postnatal treatment with CTM significantly delays vaginal opening and the first estrus (43+1.2vs.35.9 +.3 days). Adult estrous cycles are changed from 4 to 5 days by an increase of diestrus from 2 to 3 days. Postnatal actinomycin-D also delays vaginal opening and first estrus (44+1.0vs.35.9+0.3 days). However, pre- and/or post-natal anti-LH:FSH induces earlier vaginal opening (32.2+1.1vs.35.9+1.3). Adult estrous cycles are changed to 5 days by an increase in estrus from 2 to 3 days. Although adult CTM or anti-LH:FSH-treated rats are fertile, weights of their fetuses and placentae are significantly reduced. Thus, the central mechanisms controlling onset of puberty and adult reproductive function may be damaged in fetal or neonatal life by inhibition of steroidogenesis, blockade of transcription, or by circulatory binding of endogenous gonadotropins.

FUNCTIONAL NEONATAL HYPOPARATHYROIDISM: ROLE OF GESTATIONAL AND POSTNATAL AGE: Reginald C. Tsang, I-wen Chen, Jean J. Steichen, Herbert Koffler, Lawrence J. Fenton, and Irwin J. Light, Univ. of Cinti. Col. of Med., Dept. of Ped., Cincinnati

Early neonatal hypocalcemia occurs in prematures in the first 2 days of life. Functional hypoparathyroidism is a possible pathogenetic factor. Parathyroid function was tested in neonates by serum parathormone (PTH) changes during citrate induced hypocalcemia. Sixteen "exchange" (citrated) blood transfusions were performed in jaundiced infants, gestation 32-42 wks, postnatal ages 6-86 hrs. Serum ionic Ca (Orion electrode) fell from pre-exchange levels of mean 2.97±SE 0.12 mg% to 1.20±0.10. PTH was assayed with a modified Arnaud method (Chen, Clin. Res. 20:440, '72) (detects PTH in 89% of normal adults, appears to recognize predominantly PTH-9500). In exchanges after 53 hrs postnatal age, maximum serum PTH correlated with gestation ($r=0.898$, $p < 0.025$). Before 52 hrs, maximum PTH correlated with gestation ($r=0.858$, $p < 0.01$). The maximum PTH change was significantly less in younger infants (68% of pre-exchange PTH) than in older infants (157%) (covariance $F=45.85$, $p < 0.01$). PTH changes corrected for dilution effects from low PTH in donor blood gave similar results: PTH in younger infants 131%, older 181% ($F=25.73$, $p < 0.01$). An additional 15 prematures with spontaneous decrease in serum Ca from birth to 24-48 hrs had no increase in serum PTH. In conclusion, functional hypoparathyroidism occurs in infants of shortened gestation and younger postnatal age, and could be a factor in neonatal hypocalcemia.

ENDOCRINOLOGY

Second Session

A CLASSIFICATION OF THE HYPOPARATHYROID SYNDROME BASED ON THE PTH RESPONSE TEST. H. Peter Kind, David K. Parkinson, Se Mo Suh, Donald Fraser, Sang Whay Kooh. Department of Paediatrics, University of Toronto, and Research Institute, The Hospital for Sick Children, Toronto, Canada.

Genetic implications make it necessary to differentiate the various hypoparathyroid syndromes. This is not possible by clinical examination alone and tests of renal phosphate handling are often equivocal. We found that urinary cyclic-AMP excretion (Uc-AMP) after I.V. PTH (8 u/kg) clearly divided 12 hypoparathyroid patients into 5 "Uc-AMP excretors" and 7 "non-excretors" and there was no overlap between the two groups. In general, the mean change in %TRP after PTH (Δ%TRP) was significantly greater in the "Uc-AMP excretors" than in "non-excretors", and this difference increased further on vitamin D therapy; however, there was overlap of individual values. In 1 additional patient, classed as a Uc-AMP "excretor" no phosphaturia occurred in response to I.V. PTH; calcemic response to repeated I.M. PTH was also defective. This patient represents a sub-group of "Uc-AMP excretors" with a defect in PTH responsiveness due, we suggest, to a block in the action of PTH beyond the c-AMP step. Only 3 of 7 patients classed as PTH non-responders (combined defects in Uc-AMP and Δ%TRP) had clinical stigmata of pseudohypoparathyroidism. Our evidence suggests that "pseudohypoparathyroidism" is a heterogeneous syndrome, and that a classification of hypoparathyroidism based upon the known actions of PTH is more appropriate.

INDUCTION OF TESTICULAR CYCLIC NUCLEOTIDE PHOSPHODIESTERASE BY ICSH AND FSH. Robert O. Christiansen and Marcia Desautel*. Stanford University School of Medicine, Dept. of Peds., Stanford, California.

Seven separate isozymes of cyclic nucleotide phosphodiesterase (PDE) are present in tissues of the rat. The mature rat testis contains isozymes c, e, and f. PDE-f is only found in the testis and appears concurrently with sexual maturation. Previous studies have shown that PDE-f is the low K_m (2.5×10^{-6}M) and PDE-c the high K_m (6.5×10^{-5}M) cyclic AMP PDE. PDE-f is located only in the seminiferous tubule and on subcellular fractionation is found in the nuclear fraction. Hypophysectomy in the sexually mature rat causes a disappearance of PDE-f in a matter of 17-21 days. When sexually immature rats are hypophysectomized at 28 days of age PDE-f does not appear. Experiments were begun 2 months after hypophysectomy using daily injections of purified ovine ICSH, ovine FSH and rat FSH, alone and in combination, for a period of 35 days. PDE-f was induced by rat FSH and the combinations of ovine ICSH and ovine FSH or testosterone and ovine FSH. Total PDE activity increased 3-5 fold in these groups, but did not significantly change in the various control groups. Seminal vesicle and prostate weights increased only in the ICSH and testosterone treated groups. Histologic examination showed mature spermatozoa in the rat FSH, ovine ICSH plus ovine FSH, and testosterone plus ovine FSH groups. PDE-f induction correlated with the appearance of mature spermatids. This represents the first demonstration that PDE is under hormonal control.

ENDOCRINE RESPONSES IN ANENCEPHALY. Joseph B. Warshaw and Alberto Hayek, Harvard Medical School, Massachusetts General Hospital, Shriners Burns Institute, Boston, Massachusetts.

We have investigated endocrine function in four anencephalic infants lacking hypothalamic tissue and having hypoplastic pituitaries. Intravenous glucose tolerance tests resulted in higher peak values for glucose and more rapid glucose disappearance rates than seen in normals. Basal levels of both thyrotropin and growth hormone were low as compared with values reported for normal newborns. The anencephalics showed a marked resistance to the hypoglycemic effects of insulin and there was no growth hormone increment in response to insulin induced hypoglycemia. However, administration of lysine vasopressin increased plasma growth hormone from less than 1 to greater than 10 ng/ml and plasma cortisol from 5 to 10 ng/100 ml. Serum thyrotropin increased from 5 to greater than 25 ng/ml after administration of synthetic thytropin releasing factor. Prolactin values obtained in three of the infants were in the normal or elevated range. The results suggest that anterior pituitary function mediated by the hypothalamus and its releasing factors is deficient in anencephaly. However, the anterior pituitary can release both growth hormone and thyrotropin when stimulated directly. The elevated prolactin values probably reflect the absence of hypothalamic prolactin inhibitory factor and may also be the basis for squamous metaplasia of the prostate observed in anencephalic males.

SOMATOMEDIN ACTIVITY IN PLASMA OF CHILDREN RECEIVING PREDNISONE. M.J.Elders, B.S.Wingfield*, L.M.McNatt*, and E.R.Hughes Depts. of Ped. UofA Med. Ctr. Little Rock, Ark. and U of W.Va. Morgantown, W.Va.

Growth retardation is a usual concomitant of prolonged high dose glucocorticoid therapy. The mechanism of this growth retardation has not been delineated. Seven children with nephrotic syndrome were treated with 1 mg prednisone/lb. body weight/day for 28 days. Somatomedin activity (Sm) was measured prior to treatment and at 4 hour intervals for 48 hours. After 8 hours on prednisone, the Sm had decreased and at 48 hours was less than 50% of the pre-treatment Sm and remained depressed as long as the patient was on continuous therapy. The patients were then placed on every other day therapy for 1-6 months. The Sm measured on "off" days approached pre-treatment levels. These findings were confirmed and extended to glucocorticoid treated animals. Young male normal or hypox rats were treated with saline or 2.5 mg of cortisol acetate q.a.d. for 10 days. Cortisol caused a 50% depression of $^{35}SO_4$ uptake into uronic acid in normal cartilage. The $^{35}SO_4$ uptake in hypox rats was 50% of the controls and was further depressed by cortisol. The addition of 1 mM cortisol to somatomedin stimulated embryonic chick cartilage in vitro depressed C^{14}-serine uptake 58%; the UDP sugars in the linkage region,16-38%; sulfate, 49%; and uronic acid content, 37%. These data suggest the cortisol induced growth retardation is primarily due to inhibition of somatomedin generation, but high concentrations of cortisol in vitro also directly affect biosynthetic activity of cartilage cells.

RELATIONSHIPS BETWEEN PLACENTAL CONCENTRATIONS OF CHORIONIC SOMATOMAMMOTROPIN AND TWIN GROWTH. Duncan R. MacMillan, Anne M. Brown, Adam P. Matheny, and Ronald S. Wilson. Univ. of Louisville Sch. of Med., Louisville, Ky. (Introduced by Jacqueline Noonan.)

Concentrations of chorionic somatomammotropin (HCS) in extracts of homogenized placentas from 125 viable twin pairs were determined by radioimmunoassay. The co-twin method was used to assess the relationships of placental HCS to parameters of fetal and postnatal growth. Twins with relatively lower placental HCS concentration as compared to their co-twins with relatively higher HCS had significantly lower placental weight ($p < .001$), lower birth weight ($p < .001$), and lower birth length ($p < .01$).

Somatic measurements at age 24 months were available for 27 of the pairs followed longitudinally. Twins with relatively lower placental HCS concentrations weighed less ($p < .02$) and were shorter ($p < .02$) at age 24 months than their co-twins with relatively higher placental HCS. In these 27 pairs, relative twin size at 24 months was more strongly related to placental HCS than to either placental weight or birth weight.

Placental concentration of HCS appears to be a sensitive index of placental function and its influence on fetal and postnatal growth. The data also suggest a somatotropic effect of HCS on the fetus not exerted through alteration of maternal metabolism, the influence of which is common to both twins.

RESTORATION OF GROWTH BY HUMAN GROWTH HORMONE (ROOS) IN HYPOPITUITARY DWARFS WITH ANTIBODIES INDUCED BY hGH (U.S.). Louis E. Underwood, Sandra J. Voina, Judson J. Van Wyk, Univ. of North Carolina Sch. of Med., Dept. of Ped., Chapel Hill.

Antibodies (Ab) to human growth hormone (hGH) have been detected in 12 of 37 hypopituitary dwarfs treated in our clinic with hGH supplied by the Nat. Pit. Agency (hGH-U.S.). In 9 of the children with Ab, the max. assoc. constant (k) was $\leq 10^5$ 1/mole and the Ab did not interfere appreciably with their responses to therapy.

Two severely hypopit. girls, shortly after the institution of hGH therapy, developed large quantities of anti-hGH Ab of relatively high binding affinity ($k > 10^6$ 1/mole) which blocked any response to continued therapy. Retarded disappearance from plasma of injected ^{131}I-hGH was observed in both pts. Plasma T 1/2's were 53.7 and 123.6 min, compared to a mean T 1/2 of 27.7 min in subjects without Ab or with low affinity Ab.

When hGH was discontinued in the pts. with high affinity Ab, the binding capacity of their plasma for GH fell to low levels within 3 months. Retreatment with hGH-U.S. was followed by a brisk, anamnestic reappearance of Ab. The administration of hGH prepared by the Roos method (AB Kabi, Stockholm) elicited no anamnestic Ab response and over the next 7 months, both pts exhibited dramatic catchup growth (14.2 and 7.9 cm/yr).

The finding that the antibody producing cells of these patients do not recognize Roos hGH as an immunogen suggests that the growth attenuating Ab formed in response to hGH-U.S. are directed toward a steric alteration of a portion of the hGH molecule rather than to authentic hGH.

THE MECHANISM OF FETAL TRIIODOTHYRONINE (T3) DEFICIENCY. Joseph Sack, Allen Erenberg, William Oh and Delbert A. Fisher, Harbor General Hosp., Dept. of Pediatrics, Torrance, Calif.

We have reported that serum T3 conc. are low and Thyroxine (T4)/T3 ratios high in fetal sheep and in the human fetus. Also, T3 turnover is low in fetal sheep indicating that the fetus is T3 deficient. This could be due to decreased T3 secretion, decreased conversion of T4 to T3 and/or decreased availability of T4 for deiodination. To resolve these possibilities, T4/T3 ratios were measured in homogenates of maternal and fetal thyroid glands of sheep and humans. In both species, the mean T4/T3 ratios were similar in maternal and fetal glands suggesting that fetal T3 secretion is not deficient. To study availability of T4, labeled T4 distribution was investigated in fetal sheep; the T4 was given IV by indwelling catheter. At the time of sacrifice (at 24 hrs.) 55% of the administered T4 was recovered in the fetus; most of the T4 radioactivity was in muscle (5.9%) and blood (20.5%). Only 1.3% was present in meconium as glucuronide, representing only 2.8% of the metabolized T4. Thus, most of the T4 presumably is available for deiodination. T4 to T3 conversion was studied in the thyroidectomized (Tx) sheep fetus by injecting 25 μg T4 and measuring serum T4 and T3 serially by radioimmunoassay. The serum T3 plateaued between 12 and 48 hours at a level of 0.23% of the T4 level. In Tx adult sheep this value is 0.57%. The results suggest that peripheral monodeiodination of T4 to T3 is deficient in the fetus at a time when total T4 deiodination probably is increased.

INSULIN AND PROINSULIN SECRETION IN CHEMICAL AND OVERT JUVENILE DIABETICS. George A. Burghen, James N. Etteldorf, Robert L. Trouy and Abbas E. Kitabchi. Col. of Med., Univ. of Tennessee, Depts. of Ped. and Med., Memphis, Tennessee.

Utilizing insulin specific protease and radio-immunoassay, total immunoreactive insulin (TIR) and proinsulin (IRP) were measured in plasma during an oral glucose tolerance test (GT) in 11 normal (N), 9 chemical diabetic (CD), and 9 nonketoacidotic overt untreated diabetic (OD) children. The results (mean values) in μU/ml are:

	Fasting		1/2 hr.		1 hr.		2 hr.		3 hr.		4 hr.	
	TIR	IRP	TIR	IRP	TIR	IRP	TIR	IRP	TIR	IRP	TIR	IRP
N	10.4	5.8	60.9	11.2	43.4	10.0	33.2	9.5	14.9	8.0	9.6	5.2
CD	16.8	8.9	91.3	26.8	77.4	25.8	97.4	32.3	50.1	18.0	25.8	12.6
OD	9.6	4.4	15.1	4.3	15.4	5.3	16.1	5.1	15.4	4.1	12.7	5.1

Fasting IRP in N, CD and OD consisted of 56%, 53% and 46% of TIR respectively. In CD although fasting TIR and IRP values were significantly elevated ($p<0.05$), during GT, IRP increased to more significant levels ($p<0.01$-$p<0.001$). Analysis of total response areas under the curves for TIR, IRP and glucose during 4 hour GT revealed a marked increase in TIR and IRP in CD with a disproportionate increase in IRP area; a marked decrease in TIR and IRP areas in OD in the presence of high glucose was noted, however, the IRP:TIR was the same as N. These studies demonstrate decreased TIR and IRP response to GT in OD. This is in contrast to increased TIR in CD during fasting state and in response to GT. The increase of TIR in CD was shown to be associated with a disproportionate increase in proinsulin.

INAPPROPRIATE TSH SECRETION IN A CHILD WITH T_3 THYROTOXICOSIS. Margaret H. MacGillivray, Delbert A. Fisher, George C. Schussler, John R. Warner. Depts. Ped. & Med., SUNY at Buffalo, N.Y. & UCLA, Torrance, Calif.

T3 thyrotoxicosis is a variant of hyperthyroidism in which the thyrotoxic patient has increased serum triiodothyronine (T3) and normal serum thyroxine (T_4) concentrations. The girl described in this report is unique because on numerous occasions, while receiving antithyroid medication, she exhibited elevated values of serum TSH and T3 in the presence of normal T_4 concentrations. At the initial examination, this 10 6/12-year-old patient was clinically and biochemically thyrotoxic [serum T_4 by column=13 ug% (normal 3-7); 24-hour I^{131} uptake=68%]. Throughout the next year, propylthiouracil (450 mg/d) and USP thyroid were administered, and her T_4 (4.2 ug%) was maintained in the normal range. However, the patient continued to appear mildly toxic; her enlarging thyroid gland remained excessively vascular (I^{131} uptake=67%). T3 thyrotoxicosis (440 ng/100 ml) and inappropriately elevated TSH (39 uU/ml) were documented in serum containing normal concentrations of T_4 by column (5.1 ug%). In the following 2 years, neither PTU (900 mg/d) nor Tapazole (45 mg/d) in combination with USP thyroid or Cytomel or Lugol's iodine successfully controlled the disease. Sella size and pituitary function other than the TSH defect were normal. Thyroid function was ablated with oral I^{131} (12.5 mc).

Conclusion: This patient appears to have an isolated defect in the regulation of TSH secretion associated with T3 thyrotoxicosis and LATS positive hyperthyroidism.

NATURAL HISTORY OF CHEMICAL DIABETES IN CHILDHOOD AND INTERVENTION WITH SULFONYLUREA THERAPY. Elsa P. Paulsen. Ped. Dept., Univ. of Virginia Med. Center, Charlottesville, Va.

Frequent measurements of plasma insulin after oral glucose in normal children and adolescents revealed two peaks: an early one and a later one of lesser intensity. Younger subjects (2-8 yr) and older ones (9-18 yr) differed in the height of the first peaks (47±2.6 vs 99±13 uU/ml, $P<.001$) but not in the second (35±14 vs 44±9 uU/ml, $P<.10$). Maximal responses to intravenous glucose or glucagon also differed between the two groups (46±3.3 vs 126±19 uU/ml, $P<.001$).

Chemically diabetic children under 9 yr. failed to have an early peak of insulin after oral glucose but had higher than normal levels late in the test. At 8-10 yr. early peaks of insulin appeared but insulin levels later in the test also rose, resulting in marked hyperinsulinemia which increased as pubescence advanced. Insulin responses after IV stimuli also increased with age and in puberty were far above normal.

Sulfonylurea therapy begun in 6 chemical diabetics 5-9yr. of age resulted in normal tolerance in 4-24 mo. More prolonged therapy (8-42 mo.) resulted in suppression of the initial peaks of insulin and glucose after oral glucose and abnormally high levels late in the test. After discontinuation of therapy tolerance passed thru a phase of normal glucose and insulin responses into a state of decompensated tolerance and marked hyperinsulinemia. The data contraindicate continuous use of sulfonylureas. The effectiveness of cyclic therapy to maintain normal glucose and insulin levels is under study.

Adenylate Cyclase (AC) Response to Glucagon of Fetal Liver Plasma Membranes. Solomon A. Kaplan and Barbara H. Lippe Dept. Ped., UCLA Sch Med., Los Angeles

Lack of response of liver enzymes, such as tyrosine amino transferase, to glucagon until just before birth in the rat fetus and lack of glycogenolysis until after birth suggested that fetal liver is unresponsive to glucagon until birth. Because glucagon exerts its effects by activation of AC, analysis of effects of glucagon on AC activity of livers from prenatal and postnatal rats was carried out. Units of enzyme activity are picomoles (PM) of cAMP produced per 10 min. incubation per mg. tissue. Average baseline activity of homogenates increased slightly from 22 in the 18 day fetus to 30 in newborns. Response to 32 ug. glucagon per tube was a 3-4 increase at this time from 30 to 135 PM and a further increase at 7 days of postnatal life. Similar responses were observed to 10 mM NaF. When plasma membranes were prepared and tested for AC response to glucagon, however, an adequate response was found in the prenatal animal which compares favorably with the postnatal response. In the 18 day old fetus baseline activity was 153 PM cAMP increasing to 465 with glucagon. In the newborn animal the values were 117 and 472. There was a slight increase in activity over the first week of life. Responses to NaF were similar to those observed with glucagon. It is concluded that lack of induction of certain enzymatic activity by glucagon in the fetal liver is not due to deficient glucagon receptors or AC activity but must represent a relative inability of cAMP to exert its effect on enzyme induction.

MALE PSEUDOHERMAPHRODITISM DUE TO 17αHYDROXYLASE DEFICIENCY IN TWO SIBLINGS. Marcos N. Alvarez, Mark D. Cloutier, and Alvin B. Hayles. Mayo Clinic and Foundation, Department of Pediatrics, Rochester, Minnesota.

Two genetically male siblings with female genitalia, lack of secondary sexual characteristics, amenorrhea, and hypertension who were the product of a consanguineous marriage are described.

Studies of steroid metabolism included the following: low urinary excretion of 17-ketosteroids, ketogenic steroids, cortisol F, pregnanetriol and aldosterone. Elevated levels of mineralocorticoids, desoxycorticosterone (DOC) and corticosterone (B) were demonstrated in urine and plasma. Low plasma aldosterone values were found and plasma renin levels measured in response to sodium depletion were suppressed. Plasma progesterone levels were increased.

This report confirms the association of male pseudohermaphroditism and 17αhydroxylase deficiency, and the genetic data is consistent with an autosomal recessive type of inheritance.

MULTIPLE MUCOSAL NEUROMATA (MMN) WITH MEDULLARY CARCINOMA OF THE THYROID. R.S. Brown, E. Colle, A.H. Tashjian, Jr., Montreal Children's Hosp. - McGill Univ. Research Inst., Montreal, and Dept. of Pharmacology, Harvard Sch. of Dental Med., and Harvard Med. Sch., Boston.

The demonstration of elevated plasma calcitonin (CT) levels has made possible the early diagnosis of medullary carcinoma of the thyroid in patients at particularly high risk. One such group are patients with the syndrome of MMN, medullary carcinoma and pheochromocytoma. Three new cases have been studied. Each exhibited the characteristic facies, and oral, lingual and conjunctival neuromata. One had a symptomatic thyroid carcinoma at age 15 years. Two others (aged 11 and 13 years) had elevated CT (0.85 ng/ml and 1.2 ng/ml) on routine screening. Operation confirmed the presence of medullary carcinoma. Venous drainage from the tumor at the time of surgery contained 67 and 320 ng/ml CT, respectively. Tumor tissue contained 92 and 650 MRC U/ml of CT, respectively and a neuroma from one contained detectable CT. Postoperatively, levels of CT were undetectable in one and 0.15 ng/ml in the other. Because the neuromata are present at birth, recognition of this syndrome by pediatricians is essential because of the early appearance of malignancies and its familial nature.

ENDOCRINOLOGY

Read by Title

SERUM ESTRONE (E_1) AND ESTRADIOL (E_2) IN NORMAL AND ABNORMAL CONDITIONS FROM INFANCY THROUGH ADOLESCENCE. K. Angsusingha, C. Richards, M. Brych, and F. Kenny, University of Pittsburgh School of Medicine, Department of Pediatrics, Pittsburgh, Pa.

Unconjugated E_1 and E_2 were separated by Sephadex LH-20 chromatography of ether extracts of serum and quantitated by radioimmunoassay. Prepubertal females had higher E_2 indicating activity of prepubertal ovary. E_2 rose in both sexes just prior to clinical evidence of secondary sexual development with further increases thereafter. Mean values in pg/ml serum:

AGE/STAGE	(N)	MALE E_1	E_2	FEMALE E_1	E_2
1-9 y/Tanner 1	(40)	13	< 2	15	4
< 12 y/Tanner 1	(12)	15	5	19	10
Tanner 2 + 3	(24)	30	8	58	60
Tanner 4 + 5	(24)	38	18	70	77

Sexually precocious females age 1-8 had elevated E_1 30 pg/ml; and E_2 38 pg/ml (N = 15). Medroxyprogesterone therapy (N = 3) decreased E_1 and E_2 to normal levels for age. The highest E_2 level, 214 pg/ml, was observed with granulosa cell tumor. E_1 and E_2 were not elevated in premature thelarche (N = 8), premature adrenarche (N = 8) nor adolescent male gynecomastia (N = 3). Turner's syndrome (N = 6) and anorchia (N = 5) resulted in normal E_1 and low E_2, consistent with predominantly adrenal origin of E_1 and ovarian or testicular origin of E_2.

RELIABILITY OF IV METYRAPONE AS A STIMULUS FOR HGH AND ACTH. George E. Bacon, Robert M. Larson, Martha L. Spencer, and Robert P. Kelch. (Intr. by William J. Oliver). University of Michigan, Department of Pediatrics, Ann Arbor.

Controversy exists concerning (a) the relationship between the releasing mechanism for ACTH and growth hormone (HGH), and (b) the reliability of metyrapone as a provocative test for HGH reserve. To investigate these questions, 16 children (10 short normal, 2 with partial HGH deficiency, and 1 each with precocious puberty, hypothyroidism, anorexia nervosa, and cerebral gigantism) received IV metyrapone, 70 mg/kg. over 4 hrs. Serum samples for Compound S were obtained at 0,4,6, and 8 hrs. An estrogen-primed arginine-insulin tolerance test (ATT-ITT) was performed 48 hrs. later in 14 subjects. HGH was measured by radio-immunoassay, and cortisol and Compound S by competitive-protein-binding assay. All subjects were found to have normal ACTH reserve; there was no discrepancy between the ITT and IV metyrapone result. Mean maximum level of Compound S following IV metyrapone was 18.1 ± 5.7 µg%. Peak concentrations occurred at 4 hrs. in 2 subjects, 6 hrs. in 6, and 8 hrs. in 8. Ten of 13 subjects with normal HGH release during ATT-ITT had concentrations greater than 7 ng/ml during metyrapone infusion. However, there was no correlation between the time of the maximum HGH and Compound S response.

In conclusion: (a) these data are not consistent with a common pathway for metyrapone-induced ACTH and HGH release; (b) the IV metyrapone test, in association with an estrogen-primed ATT, assesses ACTH and HGH reserve as reliably as the combined ATT-ITT.

INSULIN RESISTANCE, SKIN CHANGES AND VIRILISATION: A RECESSIVELY INHERITED SYNDROME PROBABLY DUE TO PINEAL DYSFUNCTION. Nicholas D. Barnes, Pasquale J. Palumbo and Alvin B. Hayles. Mayo Clinic and Foundation, Departments of Pediatrics and Medicine, Rochester, Minnesota.

*Two sisters among six siblings, the progeny of a first-*cousin marriage, developed generalized dark skin pigmentation, acanthosis nigricans, coarsening of the facial features and thickening of the skin of the extremities. Both also showed evidence of mild virilisation and somatic precocity but no abnormality of fat distribution or serum lipids. At the age of 12 years the older girl developed diabetes mellitus without ketosis. Her endogenous plasma immunoreactive insulin levels were very high and her hyperglycemia and glycosuria proved completely resistant to exogenous insulin in doses up to 700 U daily. Her condition has remained stable through 8 years without therapy. Her younger sister, at 8 years of age, maintained a normal fasting blood sugar but showed extremely high plasma insulin levels.

A condition closely resembling that described has been reported only once previously (Rabson SM and Mendenhall EN, Am.J.Clin.Path. 26:283, 1956). The three affected siblings died and at autopsy marked hypertrophy of the pineal glands was observed. Recent experimental evidence has established an important role for the pineal in the control of many aspects of neuroendocrine function and the features of this condition are consistent with a primary abnormality in pineal hormone synthesis.

THE OVARY AND THE UTEROTROPHIC PLACENTAL HORMONE. Francisco Beas, Cecilia Terán, Pablo Szendro and Walter Sierralta, University of Chile, School of Medicine, Pediatric Research Laboratory, Santiago, Chile.

In the present report the role of the ovary on uterotrophic action of UTPH is studied.

The uterine growth promoting activity of UTPH in normal and ovarectomized impuber mice was only observed when ovary was present.

In order to deep the knowledge of this relationship, water intake, weight increase, protein, DNA and RNA contents in uterus and ovary of UTPH treated, 17-B-oestradiol treated and sham injected mice were studied.

The C-14 formate incorporation in DNA and RNA of uterus and ovary of similar experimented groups were also studied at different pulses.

Significative differences were obtained between the above described experimental groups suggesting that, UTPH and 17-B-oestradiol, promote growth action throught different mechanisms at the level of the uterine and ovaric target cells. UTPH acts mainly at DNA synthesis and 17-B-oestradiol at RNA and protein synthesis, as has been described elsewhere.

ASSOCIATION OF DIABETES MELLITUS, OPTIC ATROPHY, NERVE DEAFNESS AND DIABETES INSIPIDUS. M.M. Belmonte, R. Bortolussi, E. Colle, and T. Gunn. Montreal Children's Hospital - McGill University Research Institute, Montreal.

Four children with diabetes mellitus (DM), optic atrophy (OA) and nerve deafness (ND) are reported. They are unrelated. Two are males and two females. Two are blind by legal criteria and two have symptomatic deafness. Two have partial pitressin sensitive diabetes insipidus. In two DM developed before OA. DM is insulin dependent in all cases and insulin levels prior to treatment in one case in whom it, was measured were low. Control of diabetes with insulin therapy did not retard the progress of the OA. In all cases ND and DI developed after the onset of DM. No other neurologic deficits are present. No abnormalities of growth hormone release are present. Amenorrhea is present in the two girls although secondary sexual development is normal. The siblings of the affected children show no abnormalities.

In addition two children with DM and fundal changes suggestive of optic atrophy and two children with optic atrophy having borderline abnormalities of glucose tolerance tests are being followed. It is suggested that children with primary optic atrophy of undetermined etiology should be screened for early signs of diabetes mellitus in an attempt to delineate the natural history of the disease in this group of individuals.

CONSTITUTIONAL SEXUAL PRECOCITY-PERSISTENT ALTERATIONS OF TESTICULAR HISTOLOGY AND CHROMOSOMES FOLLOWING CESSATION OF THERAPY WITH MEDROXYPROGESTERONE ACETATE (PROVERA®; MPA).
Alvro M. Camacho, Dorothy L. Williams, and J. Miguel Montalvo. Dept. of Ped. and Med., Univ. of Tenn., Memphis and Dept. of Ped., Univ. of Miss., Jackson.

We have reported on alterations of testicular histology and chromosomes in 4 patients with constitutional sexual precocity who were treated with MPA (J. Clin. Endocr. 34:279, 1972).

MPA was discontinued in one of these patients subsequent to testicular biopsy and he was re-biopsied 36 months later. He had received MPA for 71 months. In contrast to the biopsy while receiving MPA, histological sections of the second biopsy does not reveal an arrest in spermatogenesis and there is a regrowth of cells. However, defective spermiogenesis persists. Cellular degeneration previously noted at all stages of meiosis is limited primarily to spermatids. Leydig cells are now present in normal number and appearance. Electron microscope studies confirm the findings of defective spermiogenesis and reveal asynchrous maturation between nucleus and cytoplasm, abnormal membrane formation and other abnormalities. Meiotic chromosome preparations demonstrate less sticky degeneration with most chromosome figures showing normal morphology. However, aneuploid spreads remain prominent, with only an occasional polyploid cell.

These observations strongly suggest that permanent damage to testicular tissue may result from prolonged MPA treatment and that the use of this drug requires critical evaluation.

THYROTROPIN RELEASING HORMONE (TRH) INFUSION TEST:A USEFUL NEW TOOL.Marco Danon,Hans H.Bode,Farahe Maloof,(Introd. by John D. Crawford),Harvard Med.Sch.,Massachusetts Gen.Hosp.,Shriners Burns Inst.,Boston.

The characterization,synthesis and clinical availability of TRH has greatly enhanced understanding of the physiology of the hypothalamic-pituitary-thyroid axis.We have investigated the diagnostic usefulness of TRH infusion in 48 children.All controls showed TSH peaks within 30' after TRH (200 µg I.V.in 1'); TSH levels at 5' and 10' exceeded those at 2 and 3 hrs.In hypothalamic disease TSH release was delayed,the peak occurring after 30' and values at 2 and 3 hrs were higher than those at 5' and 10'.In primary pituitary disease TSH remained below 4 µU/ml.In hyperthyroidism TRH was ineffective in releasing TSH while in peripheral resistance to thyroid hormone the pattern of TSH release was similar to that of controls but the secondary thyroid response was enhanced.Untreated primary hypothyroidism was recognized by high baseline TSH levels;the further TSH rise after TRH was superfluous to diagnosis.In previously treated hypothyroidism a suppressed pituitary response to TRH was observed up to 3 weeks after the discontinuation of treatment.TSH measurements at 5',30' and 120' after TRH proved sufficient for diagnostic differentiation between hypothalamic and pituitary thyroid disorders.The reliability and economic advantages of the TRH infusion make it preferable to suppression tests in discriminating between eumetabolic Graves' disease, peripheral resistance to thyroid hormones and T_3thyrotoxicosis. Kinetic analysis of TSH response to TRH permits interpretation despite the differing sensitivities of various TSH assays.

MAXIMUM STIMULATION OF BETA-CELLS IN PREDIABETES.
Salvador Castells, Theodore W. AvRuskin, Chun Lu, Shiu-Ching Tang and Christina Juan. Downstate Med. Ctr. and The Brookdale Hosp. Med. Ctr., Depts. of Ped., Brooklyn, New York.

Ryan et al. found that oral glucose with IV tolbutamide and glucagon produces a greater release of insulin than obtained by any other stimuli. Ten children with a diabetic sibling, a diabetic parent and/or another family member of the non-diabetic parent were studied by the standard OGTT and the Ryan's test. Plasma insulin and growth hormone were determined by double antibody immunoassay. Five healthy children with no family history of diabetes served as controls. Both groups were 4-12 yrs. of age and of similar weight. Limits of normality at each time interval were defined as the mean±2SD. Four "genetically suspected prediabetics" had abnormally high plasma glucose during OGTT. Plasma insulin levels were not different from controls. Three of the 4 had abnormally low plasma insulin at 10 and 15 minutes during Ryan testing.

Decreased insulin release after maximum stimulation of the beta-cells with glucose-tolbutamide and glucagon in 3 children with suspected genetic diabetes may indicate an abnormality of insulin production in these children.
(Supported by NIH Grant RR-318).

PITUITARY AND THYROIDAL ADAPTATIONS TO CHANGES IN THYROXINE BINDING GLOBULIN.
Marco Danon, Hans H. Bode, Farahé Maloof, John D. Crawford, Harvard Med. Sch., Massachusetts Gen.Hosp., Shriners Burns Inst., Depts. of Ped. and Med., Boston.

Normally 75% of plasma T_4 is bound to TBG,providing a hormone reservoir large relative to tissue needs and permitting circadian rhythmicity of T_4secretion (Nicoloff,J.Clin.Invest. 49:1912,1970).The lesser T_4 reservoir in TBG deficiency should place an almost continual demand on the thyroid to replenish losses while in TBG excess the large T_4 pool should require infrequent TSH stimulation and T_4secretion.To test this hypothesis we studied pituitary and thyroidal responses to TSH releasing hormone (TRH) in 6 yr twin girls with TBG excess and 12 & 13 yr old brothers with TBG deficiency:

Mean Pituitary and Thyroidal Responses to TRH,200 µg I.V.

	T_4µg%	Δ%	FT_4ng%	Δ%	%FT_4	TSH µU/ml	peak
TBG↓	3.3→5.7	73	0.8→1.8	125	.023→.031	5.3→39.0	30'
TBG↑	25.0→27.0	8	2.3→2.9	26	.009→.011	2.1→25.0	5'
TBG N	7.0→8.0	14	1.1→1.4	27	.017→.018	2.1→20.4	30'

Constant activity to compensate for the increased T_4 clearance in TBG deficiency is suggested by the high normal baseline TSH levels whereas the rapidly dischargeable TSH in TBG excess indicates pituitary accumulation resulting from intermittent activity.Furthermore,the larger rise in TSH and greater T_4 discharge in TBG deficiency suggest heightened pituitary sensitivity to TRH and increased thyroidal responsiveness to TSH when the secretory systems are continually active.
HD TOl 33,AM 4501 and the Children's Medical Research Fund.

ENDOCRINE FUNCTION STUDIES AS AN AID TO GENDER ASSIGNMENT IN NEONATAL MALE PSEUDOHERMAPHRODITISM,John D.Crawford, W. Hardy Hendren,Samuel H.Kim,Harvard Med.Sch.,Massachusetts Gen.Hosp., Shriners Burns Inst.,Children's Service,Boston.

The neonate with ambiguous genitalia presents a social if not medical emergency as regards diagnosis,gender-role assignment and early completion of appropriate surgery. Chromatin negative,46 XY infants comprise the most difficult group,diagnostic possibilities ranging from congenital anorchia to incomplete testicular feminization (ITF).In addition to panendoscopic,radiographic and,as required,operative visualization of internal structures with gonad biopsy, basal and post HCG stimulation levels of pituitary gonadotropins and gonadal hormones improve diagnosis and prognosis. A patient with congenital anorchia showed LH 18,FSH 66 mU/ml and no rise in basal plasma testosterone from 70 ng% during 5 days HCG stimulation; a second with ITF had more modestly elevated gonadotropins (LH 14;FSH 9.5 mU/ml) and high basal testosterone (350 ng%) unresponsive to HCG.The normal genitalia of Kallmann's and anencephalic males bespeaks the importance in embryogenesis of chorionic gonadotropin as do the genital ambiguities seen in infants with placental insufficiency.Such neonates have shown hypoleydigism,but normal HCG responses and brisk rises in plasma gonadotropins following orchiectomy.HGH lack gives micropenis but not the hypospadias of "adrenal" defects of testosterone synthesis.These techniques help in etiologic and prognostic considerations and reinforce conviction that gender assignment must conform with reasonable functional expectations despite genetic,gonadal or hormonal inconsistencies.

SEXUAL PRECOCITY DUE TO THORACIC POLYEMBRYONIC EMBRYOMA.Marco Danon,Bruce Weintraub,John D.Crawford,Harvard Med. Sch.,Massachusetts Gen.Hosp.,Shriners Burns Inst.,Dept.of Ped.,Boston.

A 7 9/12 year old boy was referred because of a growth spurt,acne,pubic hair,penile enlargement without increase in testicular size,disruptive behavior at school and precocious sexual interests at home.Onset was precisely documented nine months earlier.Intravenous pyelograms,skull films and brain scan were unremarkable;the 17 ketosteroids were normal(4 mg/24 hours)but the plasma testosterone (0.4 mcg%),"LH" (25 mU/ml) but not FSH(3.0 mU/ml) and alpha$_1$fetoprotein (AFP,0.34mcg/ml) were elevated.Liver scan showed no evidence of hepatoblastoma. Specific assay proved the "LH" to be cross-reactive human chorionic gonadotropin (HCG,3.0 ng/ml).The source was a left, paravertebral polyembryonic embryoma containing both trophoblastic and gastrointestinal tissue.Assay of the tumor extract (10% wt/vol)following resection showed HCG 33 ng/ml.Plasma testosterone fell to the prepubertal range (0.06 mcg%) in 6 days;AFP disappeared in nine weeks;"LH" declined to 2.0 mU/ml in 12 weeks with specific HCG no longer detectable 16 weeks after surgery.No precocious behavioral changes were evident postoperatively;growth rate returned to normal and by 6 months pubic hair had disappeared.AFP is a common finding in patients with hepatoblastoma and HCG has been reported when the tumor is associated with precocity in males.AFP may also derive from teratomas because they frequently contain gastrointestinal tissue.This report appears to be the first of isolation of both AFP and HCG from a teratoma causing sex precocity.

RADIOIMMUNOASSAY (RIA) OF URINARY ESTRONE (E_1) AND ESTRADIOL (E_2) IN NORMAL AND ABNORMAL CONDITIONS FROM INFANCY THROUGH ADOLESCENCE. M. deLevie, C. Richards, M. Brych, and F. KENNY, Univ. of Pgh.,Sch. of Med., Dept. of Ped., Pittsburgh, Pa.

Because previous methods lacked sufficient sensitivity, we isolated individual estrogens by Sephadex LH-20 chromatography of ether extracts of urine, previously incubated with β glucuronidase for quantitation by RIA. E_1 and E_2, corrected for body surface area were higher in prepubertal females indicating activity of prepubertal ovary. Mean values in NG/24h/M^2 were:

Age Years	(N)	Tanner Stage	ESTRONE	ESTRADIOL
< 5	(14)	1	m 60 f 80	m 45 f 63
5-10	(25)	1	m 125 f 180	m 45 f 83

Adolescent values for E_1 and E_2 were higher in females; obesity was associated with increased values for both estrogens in both sexes. Endogenous ACTH (metyrapone) caused greater increases in E_1 than E_2. Estrogen excretion in males persisted despite adrenalectomy or anorchia; but was not detected in a female with autoimmune adrenal and ovarian destruction. Both E_1 and E_2 were elevated in girls with idiopathic sexual precocity and untreated AG syndrome (both sexes). Conventional cortisone therapy of AGS incompletely suppressed E_1 and E_2 excretion indicating persistent adrenal estrogen (+ precursor) production. No increase in estrogen excretion was detected in male adolescent gynecomastia nor infant females with premature thelarche. Hypopituitarism caused decreased E_1 and E_2 in both sexes.

SIGNIFICANCE OF ANTIBODIES TO HUMAN GROWTH HORMONE (HGH) DURING 18 MONTHS OF HGH THERAPY. S.D. Frasier, T. Aceto, Jr., A.B. Hayles and M.L. Parker. Depts. of Ped., USC Sch. of Med., Los Angeles; SUNY Sch. of Med., Buffalo; Mayo Sch. of Med., Rochester; Dept. of Med., Wash. Univ., St. Louis.

Thirty-one growth hormone deficient patients were treated with HGH for 18 months. Binding antibodies to HGH were measured in sera obtained at 6, 12 and 18 months and 6 months posttherapy. Binding antibodies were detected in 53.2%, 53.2% and 52% of all samples at 6, 12 and 18 months, respectively. During therapy, antibodies were intermittently present in 13 patients (42.0%) and present in all samples in 11 patients (35.5%). A 1:20 dilution of serum bound (mean ± SD) 41.1 ± 13.6%, 35.1 ± 20.7% and 31.3 ± 21.6% added $HGH^{131}I$ in patients with intermittent antibodies and 70.9 ± 14.0%, 75.6 ± 12.2% and 68.6 ± 23% added $HGH^{131}I$ in patients with persistent antibodies at 6, 12 and 18 months, respectively. Corresponding median antibody titers were 1:30, 1:60 and 1:30 in patients with intermittent antibodies and 1:60, 1:120 and 1:240 in patients with persistent antibodies. Growth rates (mean ± SD) while receiving HGH were 10.6 ± 3.7 cm/18 mos, 10.7 ± 3.5 cm/18 mos and 11.6 ± 4.6 cm/18 mos in patients with no antibodies, intermittent antibodies and persistent antibodies, respectively. Six months posttherapy, 22.2% of all samples were positive for antibodies. While long-term effects of binding antibodies to HGH are unknown, their presence does not significantly modify the response to 18 months of HGH administration.

EFFECT OF FREQUENCY OF EATING ON 24 HOUR PLASMA GLUCOSE PATTERN IN DIABETICS TREATED WITH LENTE INSULIN. Jordan W. Finkelstein, Robert M. Boyar, Howard P. Roffwarg, Jacob Kream, Leon Hellman, Albert Einstein Col.of Med.,Montefiore Hosp. & Med. Ctr.,Inst. for Steroid Res.,Depts. Pediatrics,Oncology, Psychiatry, Bronx, New York (Intr. By Laurence Finberg)

10 diabetic children had plasma glucose (PG) measured every 20 minutes for 24 hours in order to assess the degree of control of hyperglycemia following a single morning dose of lente insulin. Patients had free access to food at all times. Three groups of patterns of PG were obtained which were dependent on food intake. Group I patients ingested food at least once an hour and PG never fell below 400 mg% during waking hours.Group II patients ate less frequently between meals so that PG fell to 2-300 mg% at times. Group III patients ate only 3 meals daily and PG was in the normal range 4-5 hours after meals. In all groups PG fell to normal 4-5 hours after sleep onset. In all group I and some group II patients PG began to rise during the last hour of sleep and before breakfast. In all group III and some group II patients PG remained normal until breakfast. These data show that in the presence of lente insulin the PG pattern is dependent on the frequency of eating. The appropriate times to evaluate PG is 4-5 hours post-prandial the main daily meals, and after overnite fasting. A normal PG at these times indicates adequate control and correlates with 24 hour urinary excretion of glucose. A high fasting PG suggests inadequate insulin supply and high 4-5 hour post-prandial PG indicates excessive snacking.

STUDIES OF C_{19}-Δ^{16} STEROIDS AND CYCLIC AMP IN AN INFANT GIRL WITH VIRILIZING ADRENOCORTICAL CARCINOMA. Lytt I. Gardner, Matthew J. Barlow, Tania Gregory and D.B.Gower. Dept. of Ped. State Univ. of New York, Upstate Med. Ctr., Syracuse, NY and Biochemistry and Chemistry Dept., Guy's Hosp. Med. Sch., London, England.

An 18 month old girl with virilization was found to have an encapsulated right adrenal carcinoma (2x3 cm.) with great variation in nuclear size, frequent mitoses and ? blood vessel invasion. Preoperative urinary 17-KS, androsterone (ANDRO), dehydroepiandrosterone (DHA) and 3α-androstenol (3αA) were elevated; all were partially suppressed by 6 days of cortisol (F) therapy (100 mg/day). A month later the partial suppression was still manifest. During 3 days of ACTH (60 gm.gel/day) the excretion of these steroids tended to rise, but not markedly in the cases of ANDRO AND 3αA. Urinary pregnanetriol (P) and etiocholanolone (ETIO) were only slightly elevated preop; neither was influenced by F therapy. The excretion of P tripled during ACTH, but ETIO was unchanged. There was a trend to lower urinary C-AMP values postop. Urinary testosterone (TESTO) and androstadienol (3βDIENE) were also elevated preop; they showed no consistent response to F or ACTH. A week postop TESTO excretion was nearly normal; 3βDIENE was absent. The qualitative Allen's blue-color test for elevated urinary DHA remains a valuable diagnostic adjunct. Urinary TESTO and 3βDIENE may also have diagnostic value, since neither was suppressed by F in this study.

BODY COMPOSITION OF CHILDREN WITH OVERT DIABETES MELLITUS IN GOOD CONTROL. Margaret A. Flynn and D.Y.N. Murthy. (Intr. by R.L. Jackson) Depts. Nutrition and Pediatrics, University of Missouri Medical Center, Columbia, Missouri.

Body composition analysis by the ^{40}K technique offers a non-invasive technique to study the sequence and rate of repletion of the muscle mass and body fat in diabetic children after initiation of insulin therapy. Using this technique lean body mass of 462 normal children aged 3 to 18 years have been determined (Pediat. Res. 6:239, 1972). Body composition determinations were done by ^{40}K technique on 30 children with well controlled diabetes mellitus. All of the diabetic children had normal heights for age and their weights were within ±1 S.D. of their expected weight for their actual height. The total body K of 29 of the children were well within the normal range (84th and 16th percentile). Only one 10 year old boy who had been under treatment for 12 weeks after the onset of overt diabetes had a ^{40}K value at the 10th percentile. Serial observations are in progress to study the rate of change in body composition in children with recent onset of overt diabetes maintained in a high degree of control.

TESTOSTERONE 5 α-REDUCTASE ACTIVITY IN THE INCOMPLETE FORM OF TESTICULAR FEMINIZATION SYNDROME. Orville C. Green, M.D. Northwestern Univ. Medical School, The Children's Memorial Hospital, Department of Pediatrics, Chicago

Recent reports from several laboratories have suggested the failure of virilization that occurs in the syndrome of testicular feminization is due to a target organ enzymatic deficiency of testosterone 5 α-reductase. Those patients demonstrated total failure of virilization with absence of pubic hair and normal clitoral size. The clinical variant of this syndrome, in which pubic hair develops and clitoral enlargement is present, has been proposed as a partial defect of activity of this enzyme. Contradictory data has been presented by Perez-Palacios et al (Steroids 17:471, 1971) indicating that in both the complete and incomplete forms of the testicular feminization syndrome, 5 α-reductase activity may be present in target tissues. Enzymatic 5 α-reductase activity has been studied in slices of preputial clitoral skin and abdominal skin obtained during prophylactic gonadectomy from a 13 year old child with the incomplete form of the testicular feminization syndrome. 5 α-reductase activity in clitoral preputial skin was present and comparable to activity from foreskin of a normal 17 year old male. Gonadal tissue incubations with pregnenolone demonstrated testosterone production. The variant of the incomplete form of the testicular feminization syndrome cannot be explained by target organ deficiency of enzymatic 5 α-reductase activity or defective hormonal testicular synthesis of testosterone.

GLUCOCORTICOID, MINERALOCORTICOID, AND ESTROGEN METABOLISM IN A NEONATE WITH FAMILIAL CONGENITAL ABSENCE OF ADRENALS. F. Kenny, R. Depp, A. Allen, G. Klein, A. Vagnucci, and C. J. P. Giroud, Univ. of Pgh. and McGill Univ.Sch. of Medicine.

The propositus was the 4th affected male with 2 normal m. and 1 f. siblings, suggesting X-linked inheritance. Umbilical vein (UV) cortisol (F, 1.3 ug%), cortisone (E, 9.6), corticosterone (B, 0.3) and its sulfate (BS, 0.1) were low, as were umbilical artery (UA) values. The relatively larger amount of E suggests its origin from maternal F during placental transfer to fetus. Undetectable aldosterone (A1) in UV and UA indicate no fetal secretion nor materno-fetal transfer, since A1 is normally elevated in mother and fetus. Unexpectedly 11-deoxycorticosterone sulfate (DOC-S) was normal in cord and elevated in maternal serum. This could result from increased maternal secretion of DOC with transfer to fetal compartment and sulfurylation by fetal tissues. Low estrogen excretion by mother prior to term, and low serum estrone and estradiol in maternal and umbilical vessels were consistent with absent fetal adrenals. Normal maternal levels of F, E, B and BS show that the fetal adrenal has no role in their regulation in the mother. None of the cases had respiratory distress syndrome (RDS) suggesting that low levels of the steroids measured are not per se incriminated in pathogenesis of RDS. Three untreated siblings died at 14, 28, and 67 hrs of age. Propositus had poor circulation at 10 mins and hypotension at 30 mins with good short and long term response to gluco- and mineralocorticoid therapy.

DIFFERING SENSITIVITY OF HUMAN FETAL RECEPTOR SITES TO ARGININE-INDUCED INSULIN AND GROWTH HORMONE RELEASE. Katherine King, Robert Schwartz, Seppo Saarikoski, Kiyoko Yamaguchi, and Peter Adam. Case Western Reserve Univ., Cleveland Metro. Gen. Hosp., Dept.of Pediatrics;and Univ. of Helsinki, Dept. of Ob-Gyn.

Potentially,fetal hormonal secretion may be regulated when fetal plasma amino acid levels are elevated by raising the maternal levels.When 9 pregnant women at term were infused intravenously with arginine(arg),the fetal plasma insulin(IRI)and growth hormone(HGH)responses differed,as tabulated,even though fetal blood glucose concentration(G) was relatively constant:

Maternal Infusion	(n)	Fetal umbilical venous concentration(mean ± SD) G(mg%)	Arg(mM/L)	IRI(μU/ml)	HGH(ng/ml)
Saline	(25)	72 ± 17	0.1 ± 0.03	10.8 ± 5.2	31 ± 16
Arg(20g)	(9)	82 ± 9	1.1 ± 0.42	6.6 ± 3.8	57 ± 21

Fetal plasma IRI was relatively constant,whereas HGH rose.

In order to evaluate the early development of receptors for pharmacological doses of arginine(1.1 ± 0.6g/kg),it was injected directly into the carotid artery of 8 human fetuses, weighing 45 to 600 grams,while the placenta remained in utero. Surprisingly,fetal plasma HGH did not rise,whereas insulin rose in later midterm fetuses(>100g),and was related directly to fetal weight.Although changes of fetal plasma arginine at term potentially can regulate HGH,arginine in the physiological range will not control fetal insulin.Nevertheless, a pancreatic receptor for pharmacological doses of arginine apparently develops earlier than the hypothalamic or pituitary receptors for HGH regulation.

PERSISTENT SALT LOSS, POOR GROWTH, AND PREVALENCE OF URINARY INFECTIONS IN TREATED SALT-LOSING (SL) ADRENOGENITAL SYNDROME (AGS). F. Kenny, J. Kenny, H. Latorre, and K. Angsusingha, Univ. of Pgh. Sch. of Med., Dept. of Ped., Pittsburgh, Pa.

To test the hypothesis that maturation of 21-hydroxylase deficiency occurs in the SL form of AGS, longitudinal studies of 24 hr urinary Na and K were done in (A) 10 non-SL, (B) 4 SL receiving DOC pellets in infancy and ad lib salt thereafter (C) 16 SL receiving oral 9 α fluoro-cortisol (.05 - .1 mg/day) after dissolution of DOC pellets. Age range 1 - 17 years; total 85 patient-years.

mEq/24h/Kg Body Wt	Mean Urine Na(SD)	Mean Urine K(SD)
Group(A) Non SL	3.6 (1.7)	1.2 (.5)
Group(B) SL-DOC	6.4 (3.2)	1.1 (.3)
Group(C) SL-DOC→9 α F	6.4 (3.3)	1.4 (0.5)

Individual patients had relatively consistently low or high values. There was (1) no tendency for amelioration of Na loss when patients were grouped in 5 year age increments, and (2) 9 α fluorocortisol in conventional dosage fails to control salt loss or affect K excretion. (1) and (2) may contribute to the poor linear growth documented in treated AGS. Symptomatic urinary infection occurred in 20% of females. Prospective screening disclosed asymptomatic bacteruria in 16% of females, probably due to abnormal anatomy. This high incidence of UTI and bacteruria in AGS has not previously been appreciated. Persistence of "L" form bacteria in urine after antibotic therapy might be aggravated by high urinary salt content.

THYROID FUNCTION AND THE BMR IN GROWTH FAILURE OF PRENATAL ONSET (PD). Ingeborg Krieger. Children's Hosp. of Mich., Detroit

Small for date infants whose growth rate is consistently slow after birth may have a decreased growth potential (PD). Their BMR is low for size (height), a characteristic they share with dwarfing syndromes. Infants with growth failure due to malnutrition (MN) have a significantly higher BMR although thyroid function is decreased. If compared with normal on the basis of body weight the BMR is elevated in both groups. The extent to which changes in cell mass or thyroid function contribute to these findings is not clear. Total and free thyroxine (T4) were therefore measured together with the BMR before and during weight recovery in infants with PD (12), with growth failure due to chronic MN (9), and acute MN with weight loss (9). Height age was used as reference standard for the BMR. Both BMR and T4 were low in acute MN and rose to normal. BMR values in chronic MN were within normal range; increases with weight recovery were variable; T4 was low and increased. Infants with PD had a low BMR and a normal T4. Exceptions were one patient with superimposed acute weight loss and two with associated deprivation. Their BMR and T4 increased during spontaneous weight recovery; the final T4 was normal, but the BMR did not reach normal range. 5 patients with PD were force-fed. In contrast to MN, their BMR and T4 did not rise during rapid weight gain. The data show that the low BMR in PD is not due to decreased thyroid function, the low BMR in acute MN is. In the presence of a low T4 high BMR values in chronic MN may be due to an increase in metabolic mass.

17 α-HYDROXYLASE DEFICIENCY MIMICKING TESTICULAR FEMINIZATION. Ann K. Kershnar*, Danielle Borut*, and Maurice D. Kogut. Childrens Hosp. of Los Angeles, Dept. Ped., USC School of Med., Los Angeles, CA.

A 19-year-old phenotypic female (46,XY) developed hypokalemia (serum potassium (K) 2.8 mEq/L), alkalosis and hypertension (blood pressure 150/100). She had had bilateral gonadectomy (testes with normal male internal structures) at 12½ yrs. of age. 17-ketogenic steroids (17-KGS) were 3 mg, pregnanediol, 2.5 mg and tetrahydro-S <1.0 mg in a 24-hr. urine. Plasma progesterone was 400 ng/100 ml (normal value ≤80 ng/100 ml). Plasma renin activity was <30 ng/100 ml.

While receiving 180 mEq/K daily orally, serum K was 4.7 mEq/L and urinary K was 86 mEq/24 hrs. Following ACTH (25 units intravenously over 8 hrs.), serum K was 4.2 mEq/L and urinary K was 213 mEq/24 hrs.; urinary 17-KGS were 2 mg and pregnanediol 5.2 mg/24 hrs. Cortisol secretion rate was non-detectable before dexamethasone (D). Aldosterone secretion rates were 12.1 μg before D and 8.6 μg after suppression. Deoxycorticosterone (DOC) and corticosterone (B) secretion rates were measured. While receiving 15 mg hydrocortisone daily, the patient's blood pressure was 110/70, serum K, 5.0 mEq/L and urinary K, 21 mEq/24 hrs. on a normal diet.

17 α-hydroxylase deficiency in both the testes and adrenal accounts for the abnormalities. Hypertension and hypokalemia is probably caused by excess production of DOC and B; decreased aldosterone production may be due to suppressed renin-angiotensin system.

URINARY CAMP AND RENAL RESPONSIVENESS TO PARATHORMONE IN PREMATURE HYPOCALCEMIC INFANTS. Louie G. Linarelli, Caroline Bobik John Bobik, (Intro. by Frederic Kenny) Univ. of Pittsburgh Sch. Med.,Mercy Hosp. and Children's Hosp., Dept. of Ped.

The renal action of parathormone (PTH) is mediated via adenosine 3',5'-monophosphate (CAMP) and our previous studies in fullterm infants demonstrated a maturational proximal tubular response to PTH. Studies were performed to evaluate renal maturity in prematures and the role of CAMP in hypocalcemia. Normocalcemic infants excreted CAMP (N moles/mg creat) comparable to hypocalcemic infants days 1-10 of life. Five prematures 20 hrs to 8 days of life with birth weights of 1-1.9kg were infused for 1 hr with PTH 5U/kg/hr with serial blood and urines. CAMP responses were low with a peak delta response of 1.4 ± 0.4 N moles/mg creat. M ± range. Urinary CAMP response to PTH was equally reduced regardless of age, B.W. or hypocalcemia. Phosphate excretion and % TRP showed a more reliable response after the first 36 hrs of age. A serum calcemic response occurred with a rise of 1.1 ± 0.5 mg % M ± range 12 hrs post-infusion. Three premature infants demonstrated a 20 fold greater response to glucagon 30 ug/kg S.C. than to the PTH 5U/kg/hr I.V. with peak delta responses of 24.4 ± 3.9 vs 1.3 ± 0.4 N moles/mg creat., respectively. We find the opposite relationship in older children. In summary, a renal tubular immaturity to PTH with diminished CAMP metabolism is apparent. Bone CAMP response to PTH is probably normal. The CAMP immaturity is organ and hormone specific since the hepatic CAMP response to glucagon is more mature than the kidney is to PTH.

THE EFFECT OF GLUCAGON ON CAMP, GLUCOSE, INSULIN AND GROWTH HORMONE IN THE NEWBORN. L.G.Linarelli, H.M.Rubin, C.M.Bobik, J.R.Bobik, A.L.Drash, Univ. of Pittsburgh Sch. Med., Mercy Hosp. and Children's Hosp., Dept. of Ped.

Glucagon activates hepatic adenosine 3',5'-monophosphate (CAMP) leading to glucose release. Urinary CAMP excretion in response to glucagon was previously found to be of hepatic origin. Studies were performed to evaluate the maturity of the response to glucagon with hepatic CAMP and glucose release, pancreatic insulin (Ins) secretion and pituitary growth hormone (HGH) secretion in the neonate. Two groups of 6 fullterm newborns (2-4 days of age) were given either 30 μg/kg or 300 μg/kg S.C. and compared with children 7-12 years of age (30 μg/kg S.C.). Urinary CAMP and plasma glucose, Ins and HGH were measured. Glucagon stimulated a 10 fold rise in urinary CAMP excretion in all 3 groups. The peak glucose responses at 30-60 min. were comparable (153 ± 14.9, 144 ± 7.9, 136 ± 8 mg% M ± SEM) for 30 μg/kg infants, 300 μg/kg infants and 30 μg/kg children. Ins response was statistically less in the infant receiving 30 μg/kg, while HGH response over basal was the same. HGH secretion was greater at 300 μg/kg. The data are consistent with hepatic maturity with respect to glucagon mediated CAMP response and glucose release while pancreatic glucagon mediated insulin release is not yet maximal at low dose stimulation.

DIFFERENTIATION OF CHRONIC LYMPHOCYTIC THYROIDITIS AND SIMPLE GOITER IN PEDIATRICS. Monteleone, J. A., R. K. Danis, K. S.K. Tung, C. V. Ramos and V. H. Peden (Intr. by A. E. McElfresh). St. Louis Univ. Sch. of Med., Dept. of Ped. and Dept. of Path. St. Louis, Mo.

Eighty-one children with goiter were studied; thirty-five had open biopsies, 23 had one or more simultaneous needle biopsies, in the remainder, the diagnosis was made by clinical and laboratory findings. In 35 biopsied patients the histologic diagnosis was chronic lymphocytic thyroiditis (CLT) in 22, simple goiter (SG) in 13. Needle biopsy proved inadequate for diagnosis in 40 percent. In biopsied patients, thyroid antibody studies by the tanned red cell technique were positive in only a few cases of CLT,whereas an immunofluorescent technique revealed antibodies in 100 percent of cases of CLT and was negative in all cases of SG. A difference between PBI and T4 > 2.0 ug/ml. was found in almost as many cases of SG as in CLT. Clinical and laboratory findings are reported for the entire group of 81 patients.

Because others have reported a high incidence of juvenile diabetes mellitus in CLT, a group of euthyroid juvenile diabetics were studied for CLT. To date 20 have been studied, 6 were found to have goiter, but only 2 had CLT.

COMPARISON OF SLEEP INDUCED GROWTH HORMONE (GH) RELEASE WITH GH RESPONSES TO L'ARGININE AND INSULIN HYPOGLYCEMIC STIMULATION IN CHILDREN. Margaret H. MacGillivray, Michael E. Cohen, Cheryl Seifert, Kathleen Murray, Thomas Aceto, Jr. Children's Hosp., SUNY at Buffalo, N.Y.

This study compared physiologic secretion of GH during deep sleep, documented by the appearance of encephalographic delta waves, with that observed following stimulation with insulin-hypoglycemia and L'arginine. Peak GH concentrations (mean S.D.) in ng/ml are tabulated below:

	n	L'arginine	Insulin Hypoglycemia	Deep Sleep
Normal	15	18.4 ± 12.3	13.5 ± 6.9	21.3 ± 11.2
GH Deficiency	12	2.7 ± 1.8	1 ± 1	3.7 ± 4.5
Dwarfism With Normal GH	18	15.3 ± 10.3	8.4 ± 4.9	28.3 ± 15.0

Conclusions: In the normal children, there was excellent agreement between the release of GH during sleep with that observed following either L'arginine infusion or insulin hypoglycemia. Of the 12 hypopituitary patients studied, 2 patients had sleep induced GH concentrations ≥8 ng/ml but had deficient GH responses to both L'arginine and insulin hypoglycemia (peak GH <3.4 ng/ml). Measurement of plasma concentrations of GH during sleep is an additional diagnostic procedure for evaluating adequacy of GH responses in children with suspected pituitary dysfunction.

ALOPECIA TOTALIS WITH CHRONIC LYMPHOCYTIC THYROIDITIS - 2 CASES. Monteleone, J. A., K. S. K. Tung, C. V. Ramos and V. H. Peden (Intr. by A. E. McElfresh). St. Louis University School of Medicine, Department of Pediatrics and Department of Pathology, St. Louis, Missouri.

Alopecia totalis has been seen with lupus erythematosus and adrenocortical insufficiency. Two children with chronic lymphocytic thyroiditis were found to have alopecia totalis. Both patients had an enlarged thyroid though euthyroid. Both were found to have thyroid antibodies and antinuclear antibodies demonstrated by immunofluorescent technique. Open thyroid biopsy of one patient confirmed the diagnosis of chronic thyroiditis. Studies of scalp skin for the presence of antibody were negative.

Pituitary hormones (LH, FSH, TSH and HGH), adrenal hormones (cortisol, 17-keto- and 17-hydroxysteroids) and total estrogens were measured and found to be normal.

IATROGENIC CUSHING'S SYNDROME AND GLAUCOMA IN A BOY AFTER MISDIAGNOSIS OF VIRILIZING ADRENAL HYPERPLASIA. Hernan Mendilaharzu, Tania Gregory and Lytt I. Gardner, State Univ. of New York, Upstate Med. Ctr., Dept. of Ped., Syracuse, NY

A boy misdiagnosed as having the sodium-losing form of virilizing adrenal hyperplasia was treated with large doses of glucocorticoids (up to cortisol equiv. 80 mg/day) and mineralocorticoids (up to 2.0 mg/day desoxycorticosterone) from the newborn period until 4 2/12 years of age when his height-age was 8 months and bone age was 1 9/12 years; florid Cushing's syndrome and glaucoma were present. During steroid withdrawal, catch-up of linear growth took place transiently when the mineralocorticoid was discontinued and the glucocorticoid was being reduced. Catch-up of bone maturation was greatest after complete steroid withdrawal. At this time ACTH stimulation caused normal response of urinary corticosteroids; there were also normal responses of serum growth hormone and of plasma cortisol to insulin hypoglycemia. Cortisol production rate was normal. Plasma cortisol values were low-normal. Metyrapone responses were inadequate on two occasions, the last test being more than a year after corticosteroid therapy ceased. This suggests a selective interference with pituitary ACTH reserve and responsiveness. The glaucoma may also be associated with prolonged corticosteroid therapy, resulting in blindness in his right eye. Because of inadequate catch-up in linear growth, it seems likely that height will remain below the 3rd percentile, unless another catch-up response takes place.

ABNORMAL ANTERIOR PITUITARY FUNCTION TESTING IN ANOREXIA NERVOSA. Thomas Moshang, Jr., Vanitha Vaidya, John S. Parks and Alfred M. Bongiovanni. Dept. of Ped. Univ. of Pa. Sch. of Med. The Children's Hosp. of Phila. Phila. Pa.

Anterior pituitary function in 3 teenage females with anorexia nervosa was evaluated. All 3 patients had low triiodothyronine levels as determined by radioimmunoassay and low normal thyroxine levels. Two of the 3 patients had low normal basal TSH levels but responded promptly to TRH. The 3rd patient had a very low basal TSH and a blunted response to TRH. All 3 patients had a blunted GH response to propranolol-glucagon (P-G) provocative stimulation but normal GH sleep peaks. LH and FSH levels were determined over a 6 week period in 1 patient who was amenorrheic during that time, although previously regular Preliminary data indicate no mid-cycle gonadotrophin surge. Clomiphene was also given to this patient.

The data indicate that psychiatric abnormalities can alter pituitary function testing. It is of interest that GH peaks during sleep (a physiologic stimulus for GH release) was not altered although response to P-G was blunted. The normal response to TRH in anorexia nervosa (in two of the three patients) also described by Lunderg et al (Eur.JCI 2:150,1972), suggest that psychic alteration of pituitary function is mediated through the "final common pathway" of the hypothalamus-portal-pituitary system.

OXYGEN TRANSPORT IN JUVENILE DIABETIC KETOACIDOSIS. Paul Munk*, Melvin H. Freedman*, Henry Levison and Robert Ehrlich*. Dept. of Ped. Research Inst., Hosp. for Sick Children, Univ. of Toronto.

In six children with diabetic ketoacidosis the red cell 2,3-diphosphoglycerate (2,3-DPG) was decreased 2.91 ± 0.88 uM/ml RBC compared with a normal value of 4.42 ± 1.24; in contrast, in 23 children with controlled diabetes mellitus the red cell 2,3-DPG was increased 6.97 ± 1.49. Three patients had their acidosis corrected with insulin, intravenous fluids and sodium bicarbonate, and three with insulin and intravenous fluids alone. The red cell 2,3-DPG returned to normal values within 2-3 days of the beginning of therapy, as compared to the 4-5 day recovery period in adult patients with diabetic ketoacidosis. With respect to the oxygen dissociation curve, the measured P_{50} at pH 7.40 was low in all cases 22.7 ± 4.2 mmHg compared with a normal value of 27.21 ± 0.66 mmHg although all patients had an increase in the calculated in vivo P_{50} 31.6 ± 3.9 mmHg. The group of patients not receiving intravenous bicarbonate showed a decrease of the in vivo P_{50} as did the group receiving bicarbonate. This decrease was not significantly below normal so as to alter tissue oxygenation. We conclude from these results that intravenous bicarbonate as used in this study is not contraindicated in the management of children with diabetic ketoacidosis. Furthermore in terms of oxygen delivery, it is the P_{50} at the patients pH rather than at pH 7.4 which is the determining factor in tissue oxygenation.

GONADAL FUNCTION IN AGONADISM. Gary A. Parks, Kenneth W. Dumars, George A. Limbeck, W. Leslie Quinlivan, Lenore S. Levine and Maria I. New, Depts. of Ped., Univ. of Calif., Irvine and Cornell Univ. Med. Col., New York.

A 3½ y.o. "girl" with the diagnosis of "true agonadism" underwent endocrine evaluation which suggested the presence of Leydig cells. The child was a phenotypic female born with ambiguous genitalia (mild clitoromegaly and posterior labial fusion). At 1 year of age evaluation revealed a 46, XY karyotype and on exploratory laparotomy, absence of gonads, uterus, Fallopian tubes and Wolffian remnants was observed. Endocrine data at 3½ years revealed:

	pl.T(ng%)	DEA(ng%)	FSH(ng/ml)	LH(ng/ml)
Base line	63	43	0.72	1.9
Post HCG	159	34		

The rise in plasma testosterone (T) after human chorionic gonadotropin (HCG) is significant and suggests the presence of testosterone-secreting Leydig cells, responsive to HCG. The normal rather than high gonadotropin levels support this. The absence of Müllerian anlagen suggests that at some time during fetal development Müllerian inhibiting factor was present. The diagnosis of agonadism cannot rest on surgical exploration alone but must also include evaluation of the endocrine function of the gonad by an HCG stimulation test. Further, "true agonadism" is probably a misnomer and actually represents that form of dysgenetic male pseudohermaphroditism with a minimal amount of functioning testicular tissue present.

CREATION OF MUSCLE K DEPLETION BY DOCA AND NA DURING TREATMENT OF THE ADRENOGENITAL SYNDROME. Nichols, B. L., M. D., L. Librik, M. D., C. F. Hazlewood, Ph. D., and C. W. Clayton, M. D. *Depts. of Pediatrics and Physiology, Baylor* Col. of Medicine.

The reduced serum NA and increased serum K in the salt loosing form of the Adrenogenital Syndrome are well known. The response of muscle electrolyte composition was studied by percutaneous needle biopsy in 11 infants with this syndrome. Following treatment with DOCA pellets and increased sodium intake an increase in muscle NA and fall in K/dry weight was observed. This alteration in muscle composition reproduces in man the chemical pathology of chronic K depletion in experimental animals, however, in these children loss of muscle K was not associated with hypokalemia. Supported by NIH 00188, National Dairy Council, David Underwood Trust Fund.

FOLLICLE STIMULATING HORMONE (FSH) AND LUTEINIZING HORMONE-HUMAN CHORIONIC GONADOTROPIN (LH-HCG) CONCENTRATIONS IN PAIRED MATERNAL AND CORD SERA. Robert Penny, N. Olatunji Olambiwonnu, and S. Douglas Frasier. University of Southern California School of Medicine, Los Angeles County-USC Medical Center, Department of Pediatrics, Los Angeles.

FSH and LH-HCG concentrations were determined by radioimmunoassay in paired maternal and cord sera. The sera of 50 mothers and 51 infants, 25 female and 26 male (one set of twins), were assayed.

Mean (± SD) FSH concentration of mothers (3.3 ± 0.9 mIU/ml) giving birth to female infants was not different, $p > 0.1$, from that of mothers (3.7 ± 0.7 mIU/ml) giving birth to male infants. In contrast, mean (± SD) LH-HCG concentration of mothers (15.99 ± 3.1 IU/ml) giving birth to female infants was significantly, $p < 0.005$, greater than that of mothers 11.37 ± 5.0 IU/ml) giving birth to male infants.

Cord serum FSH mean (± SD) concentration was significantly, $p < 0.025$, greater in female infants (3.7 ± 0.5 mIU/ml) than male infants (2.4 ± 0.8 mIU/ml). However, mean (± SD) LH-HCG concentrations in female infants (0.122 ± 0.015 IU/ml) was significantly, $p < 0.005$, less than that of male infants (0.156 ± 0.040 IU/ml.

Poor correlation, on an individual basis and on a statistical basis, between maternal and cord serum FSH and LH-HCG concentrations was observed.

The data of this investigation are consistent with fetal pituitary gonadotropin secretion.

ANTITHYROGLOBULIN ANTIBODY TITERS (AAT) IN PAIRED MATERNAL AND CORD SERA. N. Ola. Olambiwonnu, Robert Penny, and S. Douglas Frasier. Univ. So. Calif. School of Med., Los Angeles County-USC Med. Ctr., Dept. of Ped., Los Angeles.

AAT and the concentration of thyroxine were determined in the sera of 100 normal mothers at parturition and in cord sera of their respective infants. Fifty mothers gave birth to male infants and 50 gave birth to female infants.

Significant AAT were demonstrated in 3 of the 100 mothers (1:64, 1:32, 1:32). One of the 100 cord sera, that of a male infant, was found to have a significant AAT (1:256). The infants of mothers with significant AAT did not have significant titers. Conversely, the mother of the infant with significant AAT did not have detectable AAT.

Mean (± SD) thyroxine concentrations (expressed as iodine) of mothers of male (6.9 ± 1.1 μg/100 ml) and mothers of female (6.7 ± 1.1 μg/100 ml) infants were not significantly different. Similarly, there was no significant difference between the mean (± SD) cord serum thyroxine concentrations of male (6.8 ± 1.1 μg/100 ml) and female (6.9 ± 1.5 μg/100 ml) infants.

On initial evaluation and evaluation after 6 months, all 4 infants theoretically at risk for thyroid disease were euthyroid.

Conclusions: 1. The incidence (3%) of a significant AAT in maternal serum is in agreement with prior observations. 2. The data raise the question of independent origin of maternal and cord antithyroglobulin antibody.

DIENCEPHALIC SYNDROME: EXCESSIVE GROWTH HORMONE AND FAT-INDUCED HYPERTRIGLYCERIDEMIA. Mihailo Petrovic and Edna H. Sobel. Albert Einstein College of Medicine, Bronx, N.Y.

A 7 month old girl with height age of 5 1/2 months and weight age of 1 month and clinical manifestation of diencephalic syndrome was studied. Tumor of the third ventricle was found. Fasting growth hormone was elevated (12mμg/ml) and concentrations increased after stimulation with arginine and glucagon to 20 mμg/ml.

Plasma triglycerides were 403mg% 1 1/2 hours and 675mg% 3 hours after feeding with corn oil supplemented formula. Recent review article (Archives of Disease in Childhood 47:338,1972) recognizes the possibility that elevated growth hormone might play a role in fat mobilization, but since there were no reports of serum lipid abnormalities, the authors consider pathophysiology of emaciation to be still open to question.

A number of lipolytic agents (ACTH, TSH, glucagon, norepinephrine and theophyline) regulate hormone sensitive lipase (HSL) and lipoprotein lipase (LPL) in a reciprocal manner when studied in vitro. On the basis of the laboratory investigations and findings in our patient, we can postulate that combination of both known stimulatory effect of growth hormone on HSL and presumed repressing effect on LPL provides the probable explanation for the failure to thrive in the patients with this syndrome.

TESTOSTERONE (T) METABOLISM BY CULTURED HUMAN SKIN FIBROBLASTS: FURTHER OBSERVATIONS. Leonard Pinsky, Morris Kaufman, Conrad St.-G. Hall. Depts. of Ped. and Biol., McGill Univ., and Cell Genetics Lab., Lady Davis Inst., Montreal.

It has been reported that very early passage fibroblasts from skin on the upper arm can be used to distinguish 2 year old children with testicular feminization (TF) from controls of the same age and either sex. When incubated with T for 48 hours, the TF cells performed oxidative metabolism primarily: *Their 17β-OH:17-keto ratio of T metabolites was <1. In equal*ly early passage fibroblasts carrier mothers had a ratio <1, while adult female controls were >1. We incubated fibroblast cultures from skin in the deltoid area of an adult male and female with T-4-^{14}C exactly as reported above. The 17β-OH: 17-keto metabolite ratio of both strains was reductive initially, but became oxidative within 5 population doublings. When the medium contained 6% fetal and 6% newborn calf serum, instead of 15% fetal, the pair of adult female and male deltoid strains as well as an adult female labium majus skin strain exhibited the same metabolic transition. A deltoid skin strain from a 7 month old male had a ratio <1 after 10 to 25 population doublings; labial strains of comparable generational age from 2 adults with TF had a ratio >1. We conclude that: (1) oxidative versus reductive metabolism of T by cultured skin fibroblasts is influenced by their generational age in vitro, and may preclude demonstration of clonal mosaicism in carriers; (2) a ratio <1 does not reflect the TF gene in labium majus skin strains of patients.

THE ROLE OF ESTRADIOL (E2) IN PUBERTAL DEVELOPMENT. R.L. Rosenfield, V.S. Fang, C. Dupon, M.H. Kim, and S. Refetoff. Univ. of Chicago Pritzker Sch. of Med., Dept. of Ped., Med., Obst. and Gynec., Chicago.

Studies have been undertaken to determine the relative sensitivity of various physiologic and developmental processes to E2 during puberty. Low-dose (1.5-2.0 mg) depot-E2 was administered i.m. to 6 teenagers with ovarian failure, in whom karyotype was XO in 4, XX_i in 1, XX in 1.

Peak plasma E2 levels of 72 ± 15 pg/ml (comparable to those at the time of menarche) occurred 3-7 days after one E2 injection. Peak estrogenization of vaginal mucosa appeared 1-2 weeks later. LH and FSH fell to 24 and 35% of control levels 3 weeks after E2 injection, by which time plasma E2 had returned nearly to control levels. The half-disappearance rate of 1 FSH pool=4.2 days. Estrogen binding globulin (TeBG) rose slightly, but not thyroxine binding globulin (TBG).

Monthly injection x 6 of depot-E2 in 1 completely suppressed LH and FSH. Menarche followed the 6th dose. Linear growth and bone age advanced proportionately at 1 yr. Available data after 5 mo. therapy indicates comparable results in 5 others.

These data indicate that 1) E2 effect on LH and FSH synthesis is persistent, 2) E2 suppresses FSH secretion in a different fashion than LH, 3) TeBG is more sensitive than TBG to E2, and 4) these E2 levels promote normal somatic and sexual pubertal growth. Furthermore, this type of E2 treatment appears to represent an optimal form of replacement therapy.

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Pituitary and serum levels of growth hormone (GH),prolactin (Pro) and luteinizing hormone (LH) in young male rats during "catch-up growth" following starvation. A.Root, G.Duckett and H.Kamali, Temple Univ. Sch.of Med., Albert Einstein Med.Ctr., Div. of Ped., Philadelphia, Pennsylvania.

Twenty-one day male rats, maintained at 73 C with free access to water in 14 hr-light:10 hr-dark cycles, were deprived of chow for 96 hrs and then permitted to refeed ad lib. Prior to and upon completion of starvation and periodically during refeeding these animals and control ad lib fed rats were sacrificed by decapitation without anesthesia. After 96 hrs of starvation tail length and body, pituitary, testicular and ventral prostate weights were significantly lower in deprived than in ad lib fed rats. These indices reached control values 55, 37, 13, 37 and 37 days after refeeding respectively. Pituitary GH content was significantly lower in starved than in fed animals upon termination of starvation, but increased to the control level within 24 hrs after refeeding. Pituitary Pro concentration was significantly higher in starved than in fed rats upon completion of starvation, declining to the control level 24 hrs later. Serum Pro concentrations were suggestively lower in starved than in fed rats until the sixth day of refeeding. Pituitary LH content remained significantly lower in starved than in fed animals until the thirteenth day of refeeding. It is concluded that serum and pituitary levels of the hormones measured do not bear a direct temporal relationship to the mechanisms of post-starvation "catch-up growth."

SIGNIFICANCE OF A NEWLY RECOGNISED FORM OF A MULTIPLE ENDOCRINE DEFICIENCY SYNDROME. R.L. Rosenfield M.D. and B.A. Porter, M.D., Univ. of Chicago Pritzker Sch. of Med., Chicago.

The rare combination of adrenal, parathyroid, and ovarian insufficiency occurring in one patient has never been adequately explained. The syndrome is usually thought to result from an autosomally inherited tendency toward autoimmune disease. Our studies indicate that these concepts are inade*quate to explain all forms of the syndrome.*

A 13 1/2 year old child has been studied in whom the diagnoses of primary hypoparathyroidism, primary hypoadrenalism, and primary ovarian failure were made sequentially. There was no clinical or laboratory evidence of impaired resistance to infection with Candida or other organisms. Thyroid, gastric parietal cell, and adrenal antibodies were undetectable (courtesy of Dr. Robert Blizzard).

The mother underwent premature menopause at 30 years.

This represents the first patient with this syndrome in whom a search for autoimmune and/or immunologic deficiency disease has been fruitless. In addition, there is seeming transmission of ovarian failure from mother to daughter in a manner compatible with dominant inheritance with variable penetrance.

These studies suggest that an autosomal locus is necessary for the functional integrity of the ovary. Furthermore, these findings best fit the hypothesis that the syndrome may result from failure of a single genetically determined control mechanism common to the affected endocrine organs.

Serum parathormone (PTH) concentrations in euparathyroid, hyperparathyroid and hypoparathyroid children. A.Root,R.Reben, A.Stopa,A.Gruskin,R.Goldstein,J.Baluarte and G.Duckett, Temple Univ.Sch.of Med., Albert Einstein Med.Ctr. and St.Christopher's Hosp., Philadelphia, Pennsylvania.

PTH concentrations were determined by non-equilibrium, double-antibody radioimmunoassay employing guinea pig antiserum to bovine PTH. Data are expressed in pg-equiv./ml of bovine PTH. Maternal PTH levels at parturition were higher than cord values (176 vs. 136 pg/ml). PTH levels were low and PTH was detectable in only 20% of sera from neonates less than 72 hrs of age. PTH concentrations ranged between <63-282 pg/ml and PTH was detectable in 82% of 38 sera from 25 normal males and 13 normal females (0.1-18 yrs). No relationships between PTH values and age, sex or stage of sexual maturation were discerned in this sample. In normal subjects PTH concentrations did not change following 1 mg glucagon i.v. (N=4), but increased 155 and 166% over control values after 2 mg glucagon i.m. (N=2). In 5 children with renal insufficiency PTH levels ranged between 705-3750 pg/ml. In 3 patients with hyperparathyroidism secondary to cystinosis, congenital renal hypoplasia and pseudohypoparathyroidism PTH values declined respectively to 37,26 and 13% of control levels during calcium infusion. PTH levels fell slightly during long-term administration of alumina gel to a child with renal insufficiency as serum calcium and phosphate levels normalized. In 2 males with idiopathic hypoparathyroidism PTH values were <63 and 130 pg/ml respectively.

TESTICULAR 3β-HYDROXYSTEROID DEHYDROGENASE (3βHSD) DEFICIENCY IN 3βHSD FORM OF CONGENITAL ADRENAL HYPERPLASIA (CAH). George Schneider, Myron Genel, Robert L. Rosenfield, Allen S. Goldman and Alfred M. Bongiovanni. Depts. of Ped. and Med., Yale Univ. Sch. of Med., New Haven, and VA Hosp., East Orange, New Jersey; Univ. of Chicago Pritzker Sch. of Med., Chicago, and Univ. of Pa. Child. Hosp. of Philadelphia, Philadelphia.

The 3βHSD form of CAH usually leads to death in infancy. To date only one pubertal subject has been described and it was suggested that testicular 3βHSD had normalized. We recently studied testicular function in a 14 year old boy with this syndrome. Off therapy, urinary pregnenetriol was 7.4 mg/day, pregnanetriol 8.4 mg/day, plasma dehydroepiandrosterone (DHA) 1416 ng% and Δ^5-androstenediol (Adiol) 470 ng%, all ↑. Plasma LH and urinary gonadotropins were elevated. After 3 days dexamethasone, all Δ^5 steroids fell but Adiol was twice normal-285 ng%-whereas plasma testosterone (T) was low normal-280ng%. Testicular biopsy revealed spermatogenic arrest, few Leydig cells and absent 3βHSD activity histochemically. HCG stimulation led to a disproportionately greater rise in Adiol than in T. Testicular incubations using pregnenolone and DHA as substrates confirmed the 3βHSD defect with only 10-30% as much Δ^4 products formed as in a control. Oral 17α-OH pregnenolone was almost completely excreted as pregnanetriol, suggesting normal hepatic 3βHSD activity. These studies indicate that both gonadal and adrenal 3βHSD activity remain impaired during puberty in this syndrome.

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A RAPID RADIOIMMUNOASSAY PROCEDURE FOR SERUM TRIIODOTHYRONINE. Christine B. Sekadde, W. Roy Slaunwhite, Jr. and Thomas Aceto, Jr., Sch. of Med., State Univ. of New York at Buffalo, Children's Hosp. of Buffalo, Depts. of Biochem. and Ped., Buffalo.

A rapid, simple radioimmunoassay for the measurement of serum triiodothyronine (T_3) suitable for use in a clinical laboratory has been developed using a T_3 binding antisera produced in rabbits by immunization with T_3 conjugated to human serum albumin. The influence of time, temperature and pH on the assay was investigated. Incubation at 37°C and pH 8.0 for 30 min. as compared to the customary incubation at 4°C for 72 hrs. did not affect reproducibility or accuracy. Separation of bound from unbound T_3 was achieved in 10 min. by the addition of polyethylene glycol (Carbowax 6000) at 0-4°C. T_3 was prevented from binding to thyroxine binding globulin by the addition of 8-anilino-1-napthalene sulfonic acid. To minimize the differences in protein concentration between standards and unknown, hypothyroid serum was added to all standards. The T_3 values obtained by this modification were essentially identical with those using the longer method. The interassay coefficient of variation is 10.4% (n = 6), and the accuracy is 96%. One person can process 50 samples in duplicate in one working day. T_3 (in ng/ml) in euthyroid subjects is 1.46 ± 0.25 (SD) (n = 50), in one myxedematous subject, 0.50; in hyperthyroid subjects, 3.67 ± 0.99 (5) and in cord blood, 0.71 ± 0.24 (130) with five additional values in the range 1.5 - 3.0.

AUTONOMOUS HYPERPARATHYROIDISM IN AN ADOLESCENT FOLLOWING PROLONGED THERAPY FOR HYPOPHOSPHATEMIC RICKETS (H.R.) Yeshawant B. Talwalkar, James E. Musgrave, Neil R.M. Buist, Robert A. Campbell, John R. Campbell (Intr. by Richard W. Olmsted). Dept. Peds., Univ.of Oregon Med.Sch., Portland.

A 14 year old girl was noted to have hypophosphatemic rickets at 22 months of age. Urinary amino acids and glucose were normal. Family history suggested H.R. Treatment with Vit. D and oral phosphate supplement improved growth and ameliorated rickets. Transient episodes of hypercalcemia, during 10 years of therapy, were associated with gradual onset of hypertension (140/105), impaired concentrating ability (669 mOsm/L) and prednisone-resistant hypercalcemia. Calcium infusion failed to suppress paratharmone (PTH). Preoperatively, serum PTH/calcium levels (μlEq/ml/mg%) were 24/11.8, 32/12.5, 20/14, 77/11. At surgery hyperplastic parathyroid glands were removed. Histology revealed adenomas in 2 parathyroid glands. Postoperative PTH/calcium were 3.5/9.9, 1/9.5. Pre and Postoperative serum calcium (CA.), serum phosphorus (P.), %tubular reabsorption of phosphate (TRP) and phosphate excretion index (PEI) were:

	Ca.	P.	TRP.	PEI	PTH
Preop	11-14	1.2-3.3	59-76	0.20-0.60	20-77
Postop	8-9.7	1.4-2.9	50-78	0.26-0.72	3.5-6

Differences between pre and postoperative P., TRP, and PEI were not noted, suggesting the absence of a PTH sensitive component of phosphate transport in H.R.

MATERNAL AND FETAL ARGININE VASOPRESSIN (AVP) KINETICS DURING PREGNANCY IN THE SHEEP. Ronald Skowsky & Delbert A. Fisher. Dept. of Med. Long Beach VA Hosp. Long Beach, CA. & Harbor Gen. Hosp. Torrance, CA.

We have employed a radioimmunoassay for AVP and a serum extraction procedure to prevent the degradation of AVP by vasopressinase to quantify AVP secretion in maternal and fetal sheep during pregnancy. Six ewes (113-114 d gestation) had catheters placed in the femoral artery and vein of the fetus and the jugular vein of the mother. Five to 7 days postoperatively, tracer doses of 125-I-AVP and 131-AVP were injected into the fetus and mother. Mean (and SEM) basal AVP values were 49.1 ± 8.7 μU/ml in the fetus and 1.3 ± 0.3 μU/ml in the mother. Mean (and SEM) fetal blood AVP production rate (PR) was 46.5± 8.1 mU/kg/hr, more than 100-fold greater than in the mother (0.30 ± 0.09 mU/kg/hr). The PR in 2 newborn lambs (2-3 d) was 16.2 ± 1.1 mU/kg/hr and basal AVP values 17.5 ± 2.2 μU/ml. The PR in nonpregnant ewes was 0.23 ± 0.02 mU/kg/hr and basal AVP values 1.17 ± 0.14 μU/ml. These data indicate that the posterior pituitary, like the anterior pituitary, is hyperfunctioning during the last trimester and that this hypersecretion decreases rapidly after birth. Also AVP-PR is not different in pregnant and nonpregnant adult animals.

THE HALF-HOUR PITRESSIN TEST OF PITUITARY-ADRENAL FUNCTION IN CHILDREN. Chandra M. Tiwary and Arlan L. Rosenbloom, Univ. Fla. Col. Med., Dept. Ped., Gainesville, Florida.

The study was designed to assess and standardize plasma cortisol (C) response to IM aqueous pitressin (ADH) thought to function as a corticotropin releasing factor without directly affecting adrenocortical function. Thirty children and adolescents (5-18 years) either without suspicion of adrenal disease or with proven normal primary adrenal function received .5 IU ADH per year of age. Blood specimens were taken before and 30, 60 and 90 min. after injection and analyzed for C, growth hormone (GH), insulin and glucose. Mean basal C was 14 μg% (S.D. 6.5, range 2-33), mean 30 min. level 25 μg% (S.D. 5.9, range 15-39), mean 60 min. level 26 μg% (S.D. 8.8, range 2-44). Mean rise was 15 μg% (S.D. 7.6, range 4-34) and mean peak level 29 μg% (S.D. 8.8, range 15-48). Criteria comparable to those applied to similar tests in adults were developed which utilize only the baseline and half-hour specimens. All 30 subjects met these criteria which include at least 2 of the following: (1) baseline level of >5 μg%, (2) rise above baseline of >10 μg% at 30 min. and (3) 30 min. value of >15 μg%. One patient with panhypopituitarism and dubious metyrapone response met none of the criteria. One of the normal subjects also met none of the criteria when retested while taking chlorpromazine. No changes occurred in glucose or insulin concentration; half had a diagnostic rise in GH (>5 μg/ml). There were no significant changes in pulse or blood pressure monitored every 15 min.

ARGININE VASOPRESSIN METABOLISM IN THE SHEEP AND RHESUS MONKEY FETUS. Fred G. Smith, Jr., Ronald Skowsky, Richard A. Bashore, Robert Bauer and Delbert Fisher. UCLA Sch. Med., Harbor Gen. Hosp., Dept. of Ped., Los Angeles, Calif. and Torrance, Calif.

Arginine vasopressin (AVP) secretion has been studied in the Rhesus monkey and intrauterine non-stressed sheep fetus, using a sensitive and specific radioimmunoassay (Clin. Res. 20:180, 1972). Indwelling catheters were surgically positioned in the femoral artery and vein and urachus of 3 near-term monkey fetuses and 3 intrauterine sheep fetuses at 114, 125, and 127 days gestation (term 145 days). Fetal and maternal concentrations of AVP were measured during hypertonic saline infusion, dextran volume expansion, and water loading. 3% NaCl (12 ml/kg) was infused over a 10 min. period. The initial AVP levels in the monkey and sheep fetuses were 7.7 and 13 μIU/ml and rose to 21.9 and 81.2 μIU, 30 min. after infusion. The maternal AVP concentrations did not change significantly. 10% dextran in saline (10 ml/kg) was infused over 10 min. in 2 fetuses and 5% dextrose and water (10 ml/kg) in 2 fetuses. The AVP concentrations following dextran rose to a mean of 31.0 μIU/ml at 30 min., then decreased to 8.8 μIU/ml, 45-60 min. after infusion. Fetal AVP levels following water loading decreased from 25.5 to 11.3 μIU/ml, 45 min. after water loading. These studies indicate that fetal AVP concentrations are high in the fetus and suggest that the fetal hypothalamic posterior pituitary axis is functioning and may be stimulated and suppressed.

MULTI-GLANDULAR DISORDERS IN ASSOCIATION WITH McCUNE-ALBRIGHT SYNDROME IN A MALE. VIRGINIA V. WELDON, ROBERT LANG, LAURENCE S. JACOBS, LOUIS V. AVIOLI. (Intr. Ralph Feigin). Washington Univ. Sch. of Med., St. Louis Children's Hosp., Depts. of Ped. and Med., St. Louis.

A 12 yr old white male presented with pathologic fractures in infancy, developed thyrotoxicosis at age 3, subsequently underwent early puberty, and later developed progressive osteopenia despite normal Vitamin D intake. Findings included proptosis, thyromegaly, congestive failure, cafe au lait spots, severe bony deformities and widespread polyostotic fibrous dysplasia and rickets radiographically. T_4 was 18.3 μg%, RAI uptake 57%, and TSH <1.5μU/ml. Baseline HGH levels were elevated (7.6-35.4 ng/ml) and remained so during sleep (8.7-44.7 ng/ml) with an irregular pattern; levels suppressed with glucose and rose excessively after L-Dopa, with an integrated 60 minute response of 1186 ng-min (normal 446±75 ng-min). Baseline prolactin levels were normal, and remained fairly stable during sleep (4.2-9.6 ng/ml). These data suggest abnormal hypothalamic regulation of HGH secretion; however the undetectable TSH rules out the possibility of hyperthyrotropic thyrotoxicosis in this patient. Serum Ca was normal, PO_4 low (2.0-3.0 mg%), alkaline phosphatase progressively rose to 4000 I.U., and excretion of hydroxyproline was 193/mg/24 hrs. Hydrated 2 hr TRP was inappropriately low (65-77%) when the serum PO_4 was low. Oral PO_4 was not absorbed normally as reflected by low serum levels and diminished excretion. Serum 25-OH Vitamin D level was normal. PTH levels are pending. This case thus represents poly-glandular disturbances which may be interrelated.

GASTROENTEROLOGY AND ENZYMOLOGY

EARLY-LABELED BILIRUBIN FORMATION (ELP) IN RATS DURING DEVELOPMENT, John D. Johnson and Dawn Channing, Stanford Univ. Sch. of Med., Dept. of Pediatrics(Intr. by Norman Kretchmer)

Bilirubin production is considerably elevated in the newborn period and ELP bilirubin formation may contribute to this increase. ELP bilirubin formation has been investigated in rats of various ages by determining ^{14}CO excretion after administration of glycine-2-^{14}C (gly) and δ-aminolevulinic acid-5-^{14}C (ALA). These determinations have been correlated with the activity of heme oxygenase in liver. ELP bilirubin formation from both gly and ALA is greater in suckling rats than in postweanling animals. During 30 hours after the administration of tracer quantities of gly, 0.015% $\pm$ 0.0004 is excreted as ^{14}CO in 4-day old rats, whereas only 0.008% $\pm$ 0.0007 is excreted as ^{14}CO in adult animals. Using ALA as the precursor of bilirubin, 1-9 day old rats excreted 5.8 to 6.8% of the administered isotope as ^{14}CO; adult animals excreted only 3.5%.

Peak activity of heme oxygenase in liver is present between 2-13 days after birth (0.33-0.36 nmoles/min/10 mg protein), but rapidly declines toward adult levels by the time of weaning (0.19 nmoles/min/10 mg protein). Developmental patterns of heme oxygenase activity in liver and ELP bilirubin production as measured by ^{14}CO excretion from gly and ALA are similar, and suggest that hepatic heme oxygenase activity may reflect rate of in vivo ELP bilirubin formation. Enhanced ELP bilirubin production in neonatal mammals may contribute to physiologic hyperbilirubinemia. (Supported by a grant from the Cerebral Palsy Res. & Ed. Found.)

pH AND BILIRUBIN TOXICITY. Thomas Nelson, Jorgen B. Jacobsen, and Richard P. Wennberg, (Intro.by W.Alan Hodson) Dept. Ped. Univ. of Washington, Seattle, WA.

We have evaluated the effects of pH on bilirubin-cell and bilirubin-albumin interaction to study the association between acidosis and kernicterus. The binding of bilirubin to albumin was examined using a new "peroxidase method" which quantitatively measures the unbound concentration. At each bilirubin:albumin ratio studied (0.3-1.4), the concentration of unbound bilirubin remained constant between pH 6.9-8.0. The effects of pH on bilirubin-cell interaction were studied two ways: (1) Viability. L929 cells were incubated overnight in MEM. The cells were then washed and incubated in $2X10^{-6}M$ bilirubin and $1X10^{-7}M$ albumin, either at pH 7.0 or 7.5. Control cells without bilirubin were also incubated at pH 7.0 & 7.5. After 20 min. exposure at 37°C, test media were replaced with MEM and the cells were grown 3 days, stained and counted. All cells exposed to bilirubin at pH 7.0 died. Cells exposed to bilirubin at pH 7.5 and control cells were equally viable. (2) Uptake of bilirubin was examined by rapidly separating L929 cells suspended in $2X10^{-5}M$ bilirubin and $1X10^{-6}M$ albumin at timed intervals. Bilirubin uptake at pH 7.0 was more rapid than at pH 7.5, and the total bilirubin removed from the solution after 20 min. incubation at pH 7.0 was almost double that removed at pH 7.5. These experiments suggest that the increased susceptibility to kernicterus at low pH is not due to a decrease in albumin binding, but may be related to changes in cellular affinity for bilirubin.

HEPATIC DYSFUNCTION ASSOCIATED WITH PARENTERAL ALIMENTATION: CLINICAL AND EXPERIMENTAL STUDIES. Michael I. Cohen, Iris F. Litt, S. Kenneth Schonberg, Fredric Daum, Ilya Spigland, Albert Einstein Col. of Med., Montefiore Hosp. & Med. Ctr., Dept. Ped., Dept. Path., The Bronx, New York.

The use of parenteral alimentation in children with varying nutritional problems has produced a myriad of technical and metabolic complications among which have been observations of abnormal liver function. 17 children without primary liver disease received total parenteral nutrition from 6 to 320 days. Abnormalities in serum transaminases, alkaline phosphatase and bilirubin occurred in 15 of these children and nausea, vomiting, RUQ pain and/or hepatomegaly were observed in 11. Only 2 patients had neither clinical nor chemical evidence of hepatic dysfunction. Liver biopsies from several patients demonstrated a triaditis, mild cholestasis, steatosis and occasionally moderate fibrosis. The appearance of these signs and symptoms after the initiation of intravenous nutrition and the presence of pathological confirmation raises concerns about the potential hepatotoxic effects of these fluids. An in vitro guinea pig liver explant system was developed to test this hypothesis. Hydrolysate solutions or the component amino acids were added to the explant and assayed for transaminase activity. Abnormalities were noted with the hydrolysate and with 4 single amino acids suggesting that these solutions may be responsible for the hepatic dysfunction noted.

IMPROVEMENT OF HEPATIC EXCRETORY FUNCTION BY PHENOBARBITAL THERAPY IN FAMILIAL INTRAHEPATIC CHOLESTASIS. Joseph R. Bloomer and Y. Edward Hsia, Depts. of Med., Human Genetics and Ped., Yale Univ. Sch. of Med., New Haven, Conn.

Phenobarbital (Pb) which stimulates bile flow experimentally, has had variable effectiveness in children with cholestatic syndromes. Pb therapy in two brothers with progressive familial intrahepatic cholestasis was dramatic. They both had severe pruritis, hepatomegaly, clubbing, jaundice, malabsorption, and stunted growth.

With Pb (6mg/Kg/d.) their itching disappeared, and their jaundice lessened. Their serum bilirubin, cholate, chenodeoxycholate and total bile acids decreased, but alkaline phosphatase and 5'-nucleotidase (5'-N.) increased. Pb also shortened plasma clearance of ^{131}I rose bengal. Stopping Pb for a month increased jaundice, but bile acid levels continued to fall.

Pb Treatment	Bilirubin Total	5'-N.	Bile Acid	I*Rose Bengal Plasma $t_{\frac{1}{2}}$
4 y.o. brother	mg/100 ml	u.	µg/ml	hours
Before Pb	15 - 21	24	109 - 170	58
During Pb	4.4	41	77	28
1 mon. off Pb	13	31	66	
2.y.o. brother				
Before Pb	30 - 33	23	115 - 131	88
During Pb	9.3	53	48	17
1 mon. off Pb	22	46	42	

Responsiveness to Pb appears to be a characteristic of this form of familial intrahepatic cholestasis.

EFFECTS OF PHOTOTHERAPY ON BILIRUBIN(B) METABOLISM AND SULFOBROMOPHTHALEIN(BSP) EXCRETION IN UNCONJUGATED HYPERBILIRUBINEMIA. M.Michael Thaler, Nancy H. Dawber, Joseph Krasner, Sumner J. Yaffe, and Louis Mosovich. Depts. of Pediatrics, Univ. California, San Francisco, and State Univ. of N.Y. at Buffalo

Kinetic studies of B metabolism were performed in a 3-year old with Crigler-Najjar syndrome treated with intense blue light. The child was injected with B-^{14}C (1mg, 18,000dpm/µg) and studied during consecutive 48-hr dark(D) and light(L) periods. The exponential decline in serum B specific activity permitted determination of:

	D	L
Total miscible pool:	248 mg	150 mg
Daily turnover:	68.7mg	62.4mg
Biological half-life ($T_{\frac{1}{2}}$):	60 hr	40 hr
Serum concentration:	17.5mg%	14.4mg%

Photodegradation products accumulated in blood during L, reaching 20% of circulating counts. During L, counts excreted increased 5 to 10-fold in bile and feces, and 2-fold in urine. Intact B excreted in bile accounted for 2.6% (0.7mg%), and 33.4% (5.0mg%) of total radioactivity in D and L periods, respectively. Similarly, biliary excretion of BSP increased 3 to 5-fold under lights. In both periods, there was no difference in specific activities of crystalline B recovered from corresponding serum and bile samples. Conclusions: 1. L reduces the total B pool and $T_{\frac{1}{2}}$; turnover is not affected. 2. Photodegradation products are excreted in bile and urine, but may be partially retained in blood. 3. Biliary excretion of B and BSP is greatly enhanced during L. 4. L has no effect on formation of B by the liver.

ANTIGEN ABSORPTION FROM THE SMALL INTESTINE: Mechanism of inhibition by class specific antibodies. W. Allan Walker, Kurt J. Bloch, and Kurt J. Isselbacher. Harvard Medical School, Mass. Gen. Hosp., GI Unit and Clinical Immunology Unit, Boston. (Intr. by J. Warshaw)

Adult small intestine can absorb antigens implicated in allergic and autoimmune diseases, and intestinal antibodies may protect against the ingress of these sensitizing macromolecules. Using a rat model, uptake of protein antigens (horseradish peroxidase (HRP) and bovine serum albumin (BSA)) by the small intestine was studied and effects of immunization (oral and parenteral) on absorption determined. Compared to controls, HRP and BSA absorption is inhibited after specific immunization without effecting the uptake of non-specific macromolecules under the same conditions. Rats immunized orally show more consistent and significant interference with antigen uptake than do parenterally immunized rats. Yet quantities of specific antibody in intestinal secretions and in mucosal extracts (measured by the Minden and Farr technique) is the same for both groups of animals. The type of local antibodies produced by these two methods of immunization is different, however. Using a radioactive immunodiffusion technique, only IgG1 antibodies are produced in orally immunized rats whereas both IgGa and IgG1 antibodies are produced in parenterally immunized animals. Therefore, absorption differences must relate to the type of antibody produced. IgGa is either less effective than IgG1 or IgGa may interfere with the effect of IgG1 on uptake of macromolecules.

SMALL INTESTINAL MORPHOLOGY AND DISACCHARIDASE (DD) ACTIVITIES IN INFANTS AND CHILDREN WITH CHRONIC DIARRHEA. Mira Bhatia, J. Rainer Poley, Donald J. Boon. Depts. of Ped. and Med., Univ. Oklahoma Health Sci. Ctr., and VA Hosp., Oklahoma City.

In 78 patients, 180 small intestinal biopsies were done. Seven of them had: celiac disease (4); giardiasis (1); sucrase-isomaltase deficiency (1); nodular lymphoid hyperplasia of the small intestine with dysgammaglobulinemia (1). Of the remaining 71 patients, 34 were aged 1 to 12 months (group A); 28 were 13 to 42 months old (group B); and there were 9 children aged 4 to 12 years (group C). Abnormal jejunal morphology was seen in 70 patients, showing one or more of the following: 1. Total or subtotal villous atrophy or villous dystrophy, sometimes patchy in distribution; 2. Infiltration of the lamina propria with mononuclear cells and/or eosinophils; 3. Vacuolization of epithelial cells at villus tips; 4. Edema of the lamina propria; 5. Epithelial atypia in crypts. In group A, a generalized DD deficiency (GDDD) was present in 24%, a partial DD deficiency (lactase and sucrase or lactase and isomaltase) in 12%. In group B, 4% had a GDDD, and 13% a partial deficiency. None had a GDDD in group C, but 15% had a partial deficiency. Patients with a GDDD had the most severe intestinal lesions. The etiology of the morphological changes is unclear, but could be a sequel to a local reaction to antigen(s) with or without the effect of bacterial endotoxins. Intolerance to dietary protein(s) other than gluten seemed the cause of the diarrhea in all 71 patients. Diarrhea was treated effectively by elimination of offending dietary protein(s).

SMALL BOWEL MUCOSAL DYSFUNCTION IN PATIENTS WITH CYSTIC FIBROSIS (CF). Claude L. Morin, Claude C. Roy, André Bonin and Roger Lasalle, Dept. of Ped., Ste. Justine Hospital, Univ. of Montreal, Montreal, Canada.

Jejunal biopsies were obtained in 21 children with CF, 14 with celiac disease (CD) and 14 controls and used at least for one of the following studies: 1^{o} disaccharidase activity (lactase, sucrase, maltase), 2^{o} hydrolase activity for L-alanyl-L-phenylalanine (L-Ala-L-Phe hydrolase), 3^{o} intestinal uptake of ^{14}C-L-Lysine and ^{14}C-L-phenylalanine expressed as a distribution ratio (DR). Results obtained showed a significant reduction in the three parameters of investigation in CD as compared to controls. Lactase activity was abnormally low in 4 out of 10 CF patients (sucrase/lactase = 19.2) as compared to 10 controls (sucrase/lactase = 2.9). Results (Mean±SE) of L-Ala-L-Phe hydrolase in 9 CF children (34.1±3.8) were similar to those of 5 patients with CD (33.1±5.7) and significantly lower ($p<.01$) than those of 10 controls (66.2±7.2). Uptake of lysine was normal in 12 CF patients but that of phenylalanine which is absorbed by a different intestinal transport system was significantly lower ($p<.01$) with a DR of 27.0±2.3 as compared to 38.7±1.8 in 7 controls. Out of 10 CF children in whom we were able to evaluate the 3 parameters of investigation, 6 were abnormal in at least 2 of these, while only one patient had normal results. This study suggests that there may be an intestinal component to the malabsorption of CF.

Supported by the Canadian Cystic Fibrosis Foundation.

EFFECTS OF TOTAL PARENTERAL NUTRITION (TPN) ON INTESTINAL MORPHOLOGY AND DISACCHARIDASE ACTIVITIES. H. L. Greene and G. B. Merenstein. (Intr. by David Karzon). U. S. Army Medical Research and Nutrition Laboratory, Denver, Colorado.

Three infants, ages 3-1/2, 6 and 18 months, were treated with TPN because of chronic diarrhea, weight loss and malnutrition. Intestinal biopsies were performed (1) before, (2) 14-16 days after initiation of TPN and (3) 4 to 6 days after beginning total oral alimentation. Blunted villi (v/c ratio < 1.5/1) were present initially in all patients. After TPN, the morphology was near normal in all patients and remained unchanged after initiation of oral feedings. Disaccharidase activities were uniformly low before TPN, increased during TPN but were highest after oral alimentation.

	Lactase	Sucrase	Maltase	Normal Range
(1)	2.1 ± 1.1*	8.9 ± 2.8	14.3 ± 6.3	L = 5-10
(2)	5.1 ± 2.0**	15.4 ± 4.7**	40.8 ± 6.7**	S = 9-19
(3)	7.3 ± 1.6	26.3 ± 5.1**	59.3 ± 8.0**	M = 27-60

*Activity µmoles/min/gm tissue. **Statistically different.

When the feedings were changed abruptly from intravenous to the oral route of administration, the infants developed diarrhea with significant intestinal fluid losses. Oral alimentation after TPN was better tolerated when the formulas were given as a 1:5 dilution and the concentration and volume increased gradually over 6-8 days. Smaller infants were able to tolerate continuous gastric drip feedings better than bolus feedings when oral alimentation was initiated.

SCANNING ELECTRON MICROSCOPY (SEM) OF THE MAMMALIAN INTESTINAL TRACT. Joseph A. Burke and Phillip Holland, Univ. of Kentucky Med. Sch., Dept. of Ped., Lexington, Kentucky.

SEM permits characterization of the topography and individual cell surface of the intestinal tract at the ultrastructural level. We have examined the entire intestinal tract of the normal rat and the gastric mucosal alterations induced by stress using SEM. The surface of the esophagus and forestomach is covered by keratinized squamous epithelium arranged in longitudinal folds. In the glandular stomach, mucous cells cover the surface and extend into the large (15-20µ) circular gastric pits. In the duodenum leaf-shaped villi are predominant with ridge-shaped villi interspersed. The crypts appear as circular pores between the villi. The villous epithelial cells are dome-shaped and covered with numerous microvilli producing a cobblestone appearance. Interspersed among these cells are goblet cell pores. The cecal surface is characterized by pits surrounded by raised concentric whorls of mucosa. In the colon and rectum the crypts are large (12-16µ) irregularly scattered pits which contain and are surrounded by numerous smaller goblet cell pores. The cell to cell junction of the polyhedral-shaped epithelial cells are clearly visible. Gastric surface alteration induced by stress is seen as numerous small craters limited to the superficial mucosa.

This is the first characterization to our knowledge of the entire normal mammalian intestinal tract surface using SEM. These observations indicate the usefulness of SEM for future investigation of a variety of intestinal tract diseases.

GASTROENTEROLOGY AND ENZYMOLOGY

Read by Title

SUCRASE-ISOMALTASE DEFICIENCY (SID)--AN UNDERDIAGNOSED CAUSE OF WATERY DIARRHEA? Marvin E. Ament, David R. Perera, Linda Esther. Dept. of Med., Univ. of Wash., Seattle. Introduced by E. Richard Stiehm.

SID, considered a rare cause of chronic watery diarrhea, has been described in less than 100 cases, 80% of which occurred outside the U.S. Our recent experience suggests that this condition may occur more frequently. During the past year we have found 7 children with SID in 4 families. 6 of these children had been incorrectly diagnosed from 6 months to 7-3/4 years, 3 as celiac sprue, 1 as irritable bowel, 1 as a complication of surgery for Hirschprung's disease and 1 as nonspecific gastroenteritis. 6 children were White and 1 Eskimo; 6 were male. All had either constant or intermittent watery diarrhea; 3 had crampy abdominal pain and distention. None exhibited growth failure. Only 2 of 4 mothers recognized the relationship between symptoms and sucrose-containing foods. Capillary sucrose tolerance tests were flat in all patients and produced acid diarrhea with ≤ 1/4% reducing substances. Sucrase-isomaltase was virtually absent in small bowel biopsies of the 6 SID patients assayed. A < 2% sucrose diet reversed symptoms within 1 day. All parents, all siblings, 4 grandparents, 2 uncles and 1 aunt showed no evidence of SID by sucrose tolerance test. 1 set of parents had 3 children with SID; these parents and 1 of their 2 other children were heterozygous for SID by small bowel enzyme assay. All patients with chronic unexplained diarrhea should be screened for SID by examining diarrheal stools for acidity and sugar.

DEVELOPMENT AND DISTRIBUTION OF ACTIVITIES OF INTESTINAL LYSOSOMAL ENZYMES AND DISACCHARIDASES IN THE HUMAN FETUS. Irena Antonowicz, Richard Grand, Harry Shwachman. Harvard Medical School, Child. Hosp. Med. Ctr.,Dept.of Ped.,Boston,Ms.

The developmental pattern and topographical distribution of seven acid lysosomal hydrolases and four disaccharidases were studied in 21 human fetuses, 5.6 cm in length (crown-rump) to 16 cm (approximately 8-20 weeks gestation). Small intestine was excised within 2 hours of delivery, stored on ice and immediately divided into three parts. The activity of all seven lysosomal enzymes; namely α- and β-glucosidase, β-glucuronidase, β-galactosidase, N-acetyl-β-glucosaminadase, aryl sulfatase and acid phosphatase showed values comparable to the activities found in normal infants and children. The distribution of these enzymes was not uniform along the whole intestine. β-glucosidase, acid phosphatase, N-acetyl-β-glucosaminadase and aryl sulfatase showed the highest activity in the ileum. No significant differences were found in the topography of α-glucosidase activity, while β-galactosidase and β-glucoronidase showed the highest activity in the jejunum. Lactase activity was readily detectable in all fetuses, even in the very young (5.6-6.8 cm), with an average value of 6.0 uM/gP/min. (Av. value for 1-3 month infants is 25.9). In contrast, sucrase, maltase and palatinase activities were the same as in normal infants and children. Our observations indicate that the lysosomal enzymes as well as sucrase,maltase and palatinase reach mature levels between 6-20 weeks gestation. The one exception is lactase, which does not reach the infant level during this gestation period.

ROLE OF POLYAMINES IN CYSTIC FIBROSIS (C/F). Santa N. Arvanitakis, John A. Mangos, Nona R. McSherry, Owen M. Rennert and David LaPointe, Univ. of Wisconsin Med. Sch., Dept. of Pediatrics, Madison, Wisc. and Univ. of Florida College of Med., Depts. of Pediatrics and Biochemistry, Gainesville, Fla.

Utilizing an in vitro system for the study of 3-O-^{14}C-methyl-D-glucose (MDG) uptake by rat jejunal epithelium, we have shown that addition of plasma from control children to the suspending medium resulted in moderate inhibition of MDG uptake while addition of the same amount of plasma from matching homozygotes and heterozygotes for the C/F gene caused significantly greater inhibition with Δ^{o} values of 17.4 ± 2.3% and 11.8 ± 3.2% respectively. Preincubation of C/F plasma with a preparation of "diamine oxidase" for 15 min at 37°C eliminated this increment in MDG uptake inhibition suggesting that polyamines may be involved in this effect. Serum and whole blood levels of spermidine (Sd) were found to be significantly higher in C/F homozygotes and heterozygotes than in matching controls. Addition of Sd, 10^{-3}M, to plasma of control subjects resulted in increase of the MDG uptake inhibition by 11.5 ± 3.7%. Preincubation of the "plasma + Sd" mixture with a preparation of "diamine oxidase" eliminated the Sd-induced increase in MDG uptake inhibition. It was concluded that Sd appears to be involved in this humoral effect of plasma from homozygotes and heterozygotes for the C/F gene raising the possibility that an abnormality in polyamine metabolism may play a role in the pathogenesis of C/F.

COMPARATIVE BINDING STUDIES OF BILIVERDIN TO BOVINE SERUM ALBUMIN WITH RESPECT TO BILIRUBIN. Marilyn L. Cowger and Jung J. Lee, Albany Med. Col., Dept. of Ped., and State Univ. of New York, Dept. of Chemistry, Albany.

Biliverdin (BV) is one product arising from photo-oxidizing bilirubin (BR). BV toxicity and its mode of albumin binding thus have clinical relevance. Highly purified BV has limited toxicity in mitochondrial systems as compared to BR. Optical rotatory dispersion, circular dichroism, photoelectric scanning ultracentrifugation, and Sephadex G-150 thin layer chromatography have been used to study the binding of each pigment to bovine serum albumin (BSA) as well as examining mixed systems of both pigments. At pH 4.5 with 5 mM salt BV binds to BSA in a 2:1 (BV:BSA) ratio. BR binds 1:1. Increasing the BSA 10-fold does not alter these ratios. In a mixed system with the protein limiting (pigments 1:1 and protein 0.5) the binding ratios are unchanged (2:1:1; BV:BR:BSA). At pH 7.4 with 0.2 M phosphate BV shows much free solubility. Half of the free BV migrates with an equivalent amount of BSA (1:1). BR has very limited free solubility and binds in a 1:1 ratio. In the mixed system the binding is 1:1:1. Combined data obtained using various methodologies all suggest that BV and BR are bound to different sites on the protein, and the presence of BV is not affecting the binding of BR. However, in mixed systems of the pigments (with and without protein) there is evidence to show that free BV decreases the solubility of free BR. This is not likely to be of clinical significance as it requires a fairly high concentration of BV. (Supported by NIH).

THE ROLE OF PARENTERAL ALIMENTATION IN THE PRIMARY MANAGEMENT OF REGIONAL ENTERITIS IN CHILDREN AND ADOLESCENTS. Michael I. Cohen, Scott J. Boley, Paul R. Winslow, Fredric Daum. Albert Einstein Col. of Med., Montefiore Hosp. & Med. Ctr., Dept. of Ped., Dept. of Surg., Bronx, New York. (Intr. by Laurence Finberg)

Parenteral alimentation has been used, albeit infrequently, in children with Crohn's Disease for improving nutritional status in preparation for operation and in anticipation of a prolonged postoperative period without oral intake. During the past 18 months we have utilized this modality in 2 teenagers for the above indications, and in an additional 7 patients with a new goal, that of primary management. In 6 children both the large and small intestine were involved, in 2 only the colon was diseased, and the last patient had miliary Crohn's Disease of the jejunum. Perianal involvement was noted in 4. Intravenous nutrition was maintained for 22-48 days when employed as primary therapy, when 5,000 calories per day were infused. None of the 7 patients received steroids, azathioprine or salicyloazosulfapyridine during parenteral alimentation. Remissions occurred in 4 of the 7 patients. In the 3 others partial improvement was noted in 2 and no change in the third. Remissions have persisted for 13, 12, 7 and 6 months respectively. Rapid healing of the perianal disease was noted in all 4 patients in whom it was present. This encouraging experience suggests that parenteral alimentation, while eliminating oral intake, has a primary role in the management of regional enteritis.

MALABSORPTION OF CRYSTALLINE VITAMIN B_{12} IN CYSTIC FIBROSIS. Julius J. Deren, Bimla Arora, Philip P. Toskes, John Hansell and Maarten S. Sibinga, Dept. of Med., Univ. of Pa. Med. Sch. and Dept. of Ped., Temple Univ. Med. Sch. at St. Christopher's Hosp. for Child., Phila., Pa. and Walter Reed Inst. of Army Research, Washington, D.C.

The malabsorption of crystalline vitamin B_{12} has been documented both in pancreatic injury associated with alcohol abuse in the adult and following partial pancreatic extirpation in the rat. It was of interest, therefore, to study vitamin B_{12} absorption in cystic fibrosis (CF) since severe insufficiency of the exocrine pancreas is generally observed in this disease

A dose of 0.25μc radioactive ^{57}Co-B_{12} was utilized and pancreatic supplements were withheld for 24 hours prior to and during performance of the urinary excretion tests.

All subjects studied had a severe depression in vitamin B_{12} absorption which was corrected by administering pancreatic supplements concomitantly with the labeled vitamin B_{12}. In spite of the defect in vitamin B_{12} absorption, no evidence for vitamin B_{12} depletion was found. This may be related to the normalization of vitamin B_{12} absorption by the administration of pancreatic supplements but we were also able to demonstrate that the ingestion of food restored vitamin B_{12} absorption to some degree in CF. The current data suggest that in spite of this observed defect in the absorption of crystalline vitamin B_{12} in cystic fibrosis, vitamin B_{12} supplementation is not required in this disease.

HUMAN LIVER, GLUCOSE-6-PHOSPHATASE (G). Platon J. Collipp, Shang Y. Chen, Vaddanahally T. Maddaiah, Arlan Carsten, Joseph Thomas and Viswanathan Balachandar. Nassau County Med. Ctr., Dept. of Ped., East Meadow, N.Y. and Brookhaven Nat. Lab., Upton, N. Y.

Human liver obtained at autopsy was found to contain active G. Both crude liver homogenates and Al_2O_3 solubilized microsomal preparations were found to be inhibited by KCl, and using classic techniques the inhibition was found to be competitive. Several divalent cations were shown to inhibit irreversibly. At physiologic concentrations (0.12M) the activity of G was reduced by approximately 10% and inhibition increased to 70% at 1.5M KCl. NaCl was found to inhibit only slightly at these concentrations. Potassium depletion as seen in starvation, uremia and hyperaldosteronism has been reported by others to be associated with hyperglycemia and impaired insulin release, and restoration of body potassium reduces the blood glucose to normal.

The molecular weight of human and rat G was found to be approximately 70,000 when determined by radiation inactivation and by acrylamide gel electrophoresis. This was true in normal as well as fasted and also diabetic rats, conditions associated with marked changes in enzymatic activity.

JEJUNAL DISACCHARIDASE ACTIVITY IN PANCREATIC INSUFFICIENCY Z. Myron Falchuk, Clementine Sessoms, and Lynn M. Taussig (Intr. by Paul A. di Sant'Agnese) NIH, Bethesda, Maryland

Lactase deficiency is said to occur more frequently in patients with cystic fibrosis (CF) than in the normal population. In this study jejunal biopsies were done on 8 patients with cystic fibrosis (age 13-41 years) and 9 patients with hereditary pancreatitis (HP) (14-44 years) to assess the relation between disaccharidase activities and pancreatic function. Lactase, trehalase and sucrase activities were measured. 23 controls (21-35 years) were also biopsied.

Pancreatic enzymes were absent and stool fat excretion was greater than 5% in all patients with CF and in 5 of 9 patients with HP.

Jejunal lactase activity was significantly lower in the CF group than in the normal group ($p<.01$). As a group the HP patients did not differ from controls. However, while the 4 HP patients who had normal fat excretion and measurable pancreatic enzymes had normal lactase levels, the 5 HP patients with marked pancreatic insufficiency had very low lactase levels. Trehalase activity was also decreased ($p<.02$) in the CF group. Trehalase activity in the HP patients paralleled pancreatic function in a manner similar to the lactase activity. Sucrase activity was normal in all patients. These observations suggest that lactase deficiency in patients with steatorrhea is common. The deficiency may not be a primary genetic defect but rather related to the pancreatic insufficiency in those patients.

MEASUREMENT OF Na^+ EFFLUX FROM ISOLATED INTESTINAL EPITHELIAL CELLS IN EXPERIMENTAL DIARRHEA. D. Grant Gall, Dan G. Butler, Mary H. Kelly, J. Richard Hamilton, Research Inst., Hosp. for Sick Children, Dept. of Ped., Univ. of Toronto, Toronto, Canada.

To study the relationship between sodium transport and the intraluminal accumulation of Na in diarrhea, we measured the rate of appearance of ^{22}Na in medium with and without ouabain ($10^{-3}M$) from loaded jejunal epithelial cells. Optimal conditions were determined for isolation, ^{22}Na loading, ^{22}Na efflux, and ouabain inhibition of efflux in rat and pig cells. In suspension, cells maintained intact membranes (80% excluded trypan blue) and constant Na transport characteristics over 3 hours. Na efflux rate constants were significantly higher in normal rat cells (20.5 ± 0.9/hr, with ouabain 3.4 ± 0.2/hr) than in normal pig cells (6.7 ± 0.2/hr, with ouabain 2.8 ± 0.4/hr). For rat cells, removal or replacement of glucose with equimolar sucrose in incubation medium reduced total Na efflux rate constants (10.7 ± 0.4/hr, ouabain 3.5 ± 0.3/hr). We produced a net intraluminal accumulation of Na in jejunal segments of piglets by perfusion with hypertonic mannitol (450 mOsm/kg) and by a specific viral enteritis (TGE). Cells isolated from both these groups showed significant increases in total Na^+ efflux rate constants (mannitol, 12.3 ± 4.6/hr, TGE, 13.8 ± 1.7/hr). However only TGE produced a significant increase with ouabain (4.4 ± 1.2/hr). We have succeeded in consistently measuring Na efflux from intestinal cells in vitro and have identified abnormalities in 2 distinct types of diarrhea.

TRACE ELEMENTS AND VITAMINS (V) IN TOTAL PARENTERAL NUTRITION (TPN). H. L. Greene, G. Merenstein, M. Hambidge, H. E. Sauberlich and Y. F. Herman. (Intr. by David Karzon). U. S. Army Medical and Nutrition Laboratory, Denver, Colorado.

Urinary excretion of vitamins B_1, B_2 and B_6 in two adults receiving TPN indicated that this method was not an appropriate means of determining the V requirements. Red cell transketolase and glutathione reductase activities were measured serially in two infants as an indication of vitamins B_1 and B_2 adequacy. Without vitamins B_1 and B_2 for 14 days activities indicated V depletion. Transketolase activities returned to control values within 24 hrs. after 2 mg/1000 Cal/d of B_1, and glutathione reductase activity returned to control values within 72 hrs. after 0.08 mg/kg/d of B_2. Cu and Zn concentrations were found to be low in the TPN solution and the same infants had plasma Zn and Cu determinations with and without treatment with intravenous Cu and Zn. The following changes in plasma levels occurred:

Day	0	30	40	44	Normal Range
Zn	64,82	45,40	58,64	114,84	75 ± 11 µg/100 ml
Cu	49,80	23,38	54, -	63,88	87 ± 9 µg/100 ml
		(1)		(2)	

Treatment (1) Zn, 20 µg/kg/d; Cu, 15 µg/kg/d.
(2) Zn, 40 µg/kg/d; Cu, 30 µg/kg/d.

Because V, Cu and Zn depletion may occur rapidly during TPN, the intravenous requirements of these micronutrients must be determined and proper additions made.

A RECOMMENDED DIETARY ALLOWANCE OF VITAMIN K FOR INFANTS. Herbert I. Goldman. (Intr. by Philip Lanzkowsky). Long Island Jewish-Hillside Medical Center, Department of Pediatrics, New Hyde Park, New York.

At present, there is no official Recommended Dietary Allowance of vitamin K. Nevertheless, we have collected 165 recent case reports of vitamin K deficiency in infants after the newborn period, with 28 deaths and 48 instances of intracranial hemorrhage. Dietary vitamin K deficiency was almost universal in these infants, demonstrating that such deficiencies are an important problem of which the reported cases undoubtedly represent but a small fraction. To prevent such deficiencies, a Recommended Dietary Allowance for infants of 3 mcg/kg/day of phylloquinone, or its equivalent, is suggested. This figure is based upon 3 different lines of evidence, all of which yield approximately equivalent values: 1) the dietary histories of 165 reported cases, 2) several studies of the effect of dietary vitamin K intake on deficiency in normal newborn infants, and 3) a study of the effect of dietary vitamin K intake on deficiency in older infants with diarrhea. Recent assays of cow's milk for vitamin K content, using phylloquinone as a reference standard, were used in the computation of the suggested Recommended Dietary Allowance.

DIFFERENTIAL DIAGNOSIS OF NEONATAL HEPATITIS(NH) AND BILIARY ATRESIA(BA) BY THE MEASUREMENT OF SERUM AND INTRALUMINAL DUODENAL BILE ACID CONCENTRATIONS. Manouchehr Karjoo, Philip G. Holtzapple, and Bertram H. Lubin. Univ Pa, Childrens Hosp Phila., Philadelphia

In 8 patients with prolonged neonatal jaundice, serum and intraluminal duodenal bile acid concentrations (DBA) were determined before and after gall bladder stimulation. The clinical course and routine laboratory studies did not allow differentiation between NH and BA. The initial peroxide hemolysis test (PHT) ranged between 75-95% in all patients. The ultimate diagnosis was established by open liver biopsy and operative cholangiogram in 7 of the 8 patients. In 3 infants with NH the unstimulated DBA was found to be lower (0,315, 716ug/ml) than in the unstimulated controls (range 921-1842ug/ml). Restudy in the patient with the undetectable level 5 weeks later revealed normal DBA concentrations. In 5 infants with BA the initial DBA were lower (range 0-52 ug/ml) and there was no increase after stimulation. In 2 infants studied 5 weeks later there was no change in the DBA concentration. Serum bile acids were elevated in all patients but were higher in the patients with hepatitis. These changes are consistent with the results of the PHT. The combination of the hemolysis test and the levels of duodenal and serum bile acids may be helpful in the evaluation of prolonged neonatal jaundice.

INFLUENCE OF PHOTOTHERAPY(PT) ON LIVER STRUCTURE. Joseph R. Goodman and M. Michael Thaler. Univ. California, Dept. of Pediatrics and VA Hospital, San Francisco.

Previous reports raise the possibility that PT may damage the liver in jaundiced infants and animals. The effects of PT on liver ultrastructure were studied in jaundiced Gunn rats(J) and normal littermates(N). Shaved 4 week old rats were exposed to 450 ft candles of continuous fluorescent light(F), or to 5,000+ ft candles of intermittent monochromatic light(M) (range 430-520nm). "Dark" controls were maintained in ambient light. Food and water were freely available. Hematocrit, serum bilirubin and liver biopsies were obtained at specific intervals. PT with F and M had no effect on hematocrit. Exposure to F (96hr) or M (24hr) decreased the serum bilirubin by 50% and 70% of initial values, respectively. Hepatic lobular patterns, glycogen and fat were unaffected by PT. No inflammatory, necrotic or cholestatic changes were present. Thin sections (1µ) disclosed dark cytoplasmic deposits and large vacuoles in J and N rats after 96 hr PT with F or after 24 hr PT with M. Electron microscopy confirmed the normal appearance of canaliculi, nuclei and cytoplasmic structures in all rats. After 96 hr PT with F, or 24 hr with M, marked sinusoidal congestion was noted, and large membrane-limited vacuoles containing granular material appeared in J rats. The light-induced changes disappeared in 1-2 weeks after discontinuing PT. These results indicate that intense illumination, which lowers bilirubin strikingly, has no effect on hematocrit and induces relatively minor, reversible structural changes in liver of normal and jaundiced rats.

ENTEROKINASE ACTIVITY IN THE HUMAN INTESTINE. Emanuel Lebenthal, Irena Antonowicz and Harry Shwachman. Harvard Medical School, Child. Hosp. Med. Ctr., Dept. of Ped., Boston, Mass.

Enterokinase initiates pancreatic digestion of protein by conversion of trypsinogen into trypsin, which in turn activates the other proteolytic proenzymes from the pancreas, chymotrypsinogen, procarboxy-peptidases and proelastases. Thus enterokinase is a key enzyme for the utilization of dietary protein. Enterokinase activity was measured in 40 small intestinal mucosal biopsies performed on patients between 2 months to 16 years of age suffering from a variety of intestinal disorders. In addition, these specimens were analyzed for lactase, sucrase maltase, palatinase and protein content. In celiac disease (12 patients) there appears to be no difference in activity in treated vs. untreated and the values are slightly higher than in 9 controls (2.14 units ± 1.28 vs. 1.55 ± 1.14). Differences in lactase activity occur in the untreated celiac patients vs. controls. The determination of enterokinase activity in the intestinal mucosa obtained at post mortem reveals the highest activity in the duodenum, with 17-40% of activity in the jejunum and 8-25% in the ileum. A study of 18 aborted fetuses between 10 to 20 weeks of gestation showed no enterokinase activity in the small intestine while intestinal lactase and α-glucucosidases were already present. These data show no correlation between enterokinase and brush border enzymes. In addition, a significant difference is observed in the developmental pattern of enterokinase and disaccharidases.

TRANSPORT OF BILIRUBIN IN PLASMA BY FREE FATTY ACIDS (FFA). Kwang Lee and Lawrence M. Gartner, Dept. of Pediatrics, Albert Einstein College of Medicine, Bronx, N.Y.

In the course of development of a method for measurement of reserve albumin (alb) binding capacity for unconjugated bilirubin (UB) using the fluorescent dye, Direct Yellow-7 (DY-7) a unique observation regarding the role of FFA was noted. Linear enhanced fluorescence (ΔF) of DY-7 was noted with addition of increasing concentrations of alb. Addition of up to 2 moles of UB/M of alb resulted in linear inhibition of ΔF with two distinct regression lines corresponding to each mole of UB added/M of alb. Addition of from 3 to 16 moles of Na oleate/M alb in the absence of UB resulted in no change in F. However, addition of equal concentrations of Na oleate in the presence of UB resulted in return of ΔF toward the ΔF observed in the absence of UB suggesting that FFA was capable of shifting UB from its primary alb binding sites to a state of solubilization in FFA rather than displacing UB from its primary sites.

This observation suggests that UB exists in two states in plasma: 1) either bound to primary alb binding sites or 2) in solution in FFA which is itself bound to alb. Increasing concentrations of FFA or decrease in affinity of UB to alb binding sites results in a shift of UB from primary alb binding sites to FFA. Thus, FFA may function as a transport vehicle for UB and may favor the transfer of bilirubin into cells, including those of the CNS. Since FFA uptake by brain cells is enhanced in the normal newborn and is further enhanced as a result of hypoxia, the presence of UB in FFA may cause kernicterus.

THE ROLE OF COW'S MILK PROTEIN IN IRON ABSORPTION. George L. McElroy and Calvin W. Woodruff. Univ. of Missouri Sch. of Med., Dept. of Ped., Columbia.

Human infants fed a prepared formula containing 2.4% protein had a higher incidence of iron deficiency than those fed 1.5% protein (Gross, S., J. Pediat. 73: 521, 1968). The addition of iron, 8 mg/L, prevented iron deficiency. The hypothesis that the higher concentration of protein interfered with iron absorption was tested in weanling rats. Four groups of 15 Sprague-Dawley rats were fed prepared formulas identical except for protein and iron concentrations; 1.5 or 2.4% protein and trace or 12 mg/L iron. Growth after 24 days was greater in the 2.4% protein group ($p < .01$) but iron intake did not influence growth under these conditions. Significant iron deficiency as measured by low serum iron saturation occured only in the rapidly growing high protein, low iron animals ($p < .01$). Iron absorption was measured with ^{59}Fe in a whole body counter. The per cent uptake after 5 days was 25.9 and 29.3 in the iron supplemented groups ($p > 0.1$). The low protein, low iron rats took up 45.2% ($p < .01$). The high protein, low iron group which was also deficient in iron took up 62.9%, significantly more than the low protein, low iron group ($p < .01$). These observations indicate that the higher protein concentration does not interfere with iron absorption in the rat.

CHARACTERIZATION OF FETAL PEPSINOGENS IN HUMAN AMNIOTIC FLUID. W.M. Liebman and I.M. Samloff (Intr. by J.W. St. Geme, Jr.), Depts. Peds and Med., UCLA Sch. Med. and Harbor General Hospital, Torrance, California.

There are two immunochemically distinguishable groups of pepsinogens, group I (Pg I), consisting of 5 electrophoretically faster migrating fractions (Pg 1-5), and group II (Pg II), consisting of 2 fractions (Pg 6,7). The first appearance of zymogen granules or of detectable pepsinogen has been previously investigated by histochemical or electron microscopic techniques and by conventional assays of proteolytic activity, respectively. In this study qualitative electrophoretic analysis was performed on 43 samples of human amniotic fluid, 13 to 40 weeks of gestation. Pg I was present in all of the samples, while Pg II was present only in those samples of gestational age, 32 weeks or older. No definite differential pattern of the fractions was noted, although the first faction of Pg I was not present in any of the samples. The metachronous appearance of Pg I and Pg II suggests that they are of fetal origin, and that the synthesis of Pg I by fetal gastric mucosa may precede that of Pg II.

ROLE OF SECRETIN IN TRANSDUCTAL FLUXES OF Cl AND HCO_3 IN THE RAT PANCREAS. John A. Mangos, Nona R. McSherry and Santa N. Arvanitakis. Univ. of Wisconsin Med. School, Dept. of Pediatrics, Madison, Wisc.

The transductal fluxes of Cl and HCO_3 were studied in the rat pancreas using micropuncture and stationary microperfusion techniques. In the unstimulated pancreas or during secretory stimulation with pilocarpine (P) or pancreozymin (CCK-PZ), the fluxes of Cl, HCO_3 and H_2O were zero and the pancreatic juice had the composition of the primary secretory fluid (PSF). During stimulation with secretin (S), the PSF was modified through transductal fluxes of Cl and HCO_3 against concentration gradients (Cl net efflux: 261 + S.D. 37, HCO_3 net influx: 238 + S.D. 57 nEq/min·gm wgt). During stationary microperfusion of interlobular ducts with PSF-like pancreatic juice, it was demonstrated directly that no net fluxes of anions occurred in the unstimulated or in the P or CCK-PZ-stimulated pancreas; in the ducts of the S-stimulated pancreas, the Cl of the injected fluid decreased from 113.2 $\pm$ 4.0 mEq/L to 101.6, 89.5 and 81.2 mEq/L at 3, 9 and 21 minutes without demonstrable water fluxes; S did not induce anionic fluxes when added to the injected fluid. It was concluded that secretin, which is the secretory stimulant of the rat pancreas, plays also an important role in the initiation and maintenance of the pancreatic transductal fluxes of anions.

EFFECT ON NITROGEN RETENTION OF UNEVEN DISTRIBUTION OF PROTEIN RELATIVE TO CALORIES. William C. MacLean, Jr., George G. Graham. Johns Hopkins Univ Sch Med (Ped), Hygiene & Pub Hlth (Internat. Hlth) Baltimore

Nutrition supplementation programs are based on the untested tenet that protein is most effectively utilized when consumed in parallel to calories. Metabolic balance studies were performed on 8 Peruvian males, age 4-26 months, weight 6-8 kg. Diets contained 2 g protein and 125 Kcal/kg/day, non-protein calories evenly distributed between fat and carbohydrate. Each child served as his own control. During control periods (6-12 days) protein was evenly distributed in 5 meals. During study periods (6-15 days), ½ daily protein was given in a single meal to 3 children, all the protein being given in a single meal to the remaining 5 children. Apparent N retentions, changes in serum albumin and in weight were similar on both diets. The % of daily urine N excreted in the 4 hours following the protein load was the same as that excreted during the same 4 hour period on the control diet. The infant appears to be able to digest, absorb, and utilize protein when given as a bolus unrelated to calories. Long term trials of complete single meal protein supplementation are being undertaken. This may have important implications for current nutrition supplementation programs. (USPHS Grant AM-09980)

HEURISTIC MODELING FOR DIAGNOSTIC AID: NEONATAL HEPATITIS AND BILIARY ATRESIA. Charles E. Mize, Wm. M. Lively, and Stephen A. Szygenda. Univ. Tex. Health Sci. Ctr. (Southwestern) and Southern Methodist Univ., Dallas.

Heuristic liver modeling and learning program techniques have been integrated to seek a method for presurgically differentiating anatomic biliary tree obstruction and hepatitis in neonates. A dynamic liver model has been developed, and then programmed for computer utilization of clinical data input. The resultant model flow leads to 1 of 4 categories: 1) hepatitis, 2) atresia, 3) normal, 4) no diagnosis possible (NDP). Data is integrated in the learning section of the program to use patients' cumulative data to improve the model. That is, program learning uniquely provides revised model solutions, as data from additional cases become available to modify the program. The diagnostic section uses that improved model to predict a diagnosis based on next patient data.

Using a grouped set of data (serum SGOT and direct bilirubin, disease duration, and Rose Bengal fecal excretion), an initial model solution was constructed from data of 26 patients with proven diagnoses. With this model, 18 other patients were tested separately, with a resulting high degree of diagnostic reliability (one error, 3 NDP). Subsequent program learning, employing all 44 patients, revised the model to provide uniformly correct prediction in each case, as determined from independent medical/surgical diagnoses. The technique promises to aid in specific diagnosis of these syndromes and offers possibility of more general application.

DOES HEATING REALLY ENHANCE THE DIGESTIBILITY OF MILK OR ALTER ITS ANTIGENICITY? James E. Nagel, D. Lee Miller, Gilbert A. Friday and David Gitlin. Children's Hosp. of Pittsburgh.

The assumption that heating enhances the digestibility of milk as well as reduces its antigenicity is a commonly held belief among pediatricians and allergists. This assumption was reinvestigated in the present study. Whole cow's milk, casein, β-lactoglobulin and α-lactoalbumin were heated to 100° C, and the in vitro digestibility of the heat-treated proteins with proteolytic enzymes was compared to that of the non-heated proteins. No differences were noted in the rate or completeness of digestion of casein or whole milk. A slight increase in the rate of digestion of β-lactoglobulin and α-lactoalbumin was noted, but this difference was relatively insignificant. The in vivo digestibility of ^{131}I radiolabeled whole milk and BLG was also not increased by heating. The effects of heating and proteolytic digestion upon the antigenicity of these proteins was investigated in allergic individuals who manifested positive immediate skin tests to the native antigens. Heating was found to be ineffective in significantly reducing the size of the wheal and flare. Heating plus in vitro digestion of these proteins did in some instances reduce the size of the skin reaction, but this was neither significant nor consistent. These data do not support the belief that heating of milk improves its digestibility, nor does heating and proteolysis appear to remove its antigenicity.

ABNORMAL JEJUNAL BIOPSY IN CHILDHOOD DIABETICS: LIPID ACCUMULATION IN THE JEJUNAL MUCOSA. John C. Partin, Robert Bobo and Wm.K.Schubert. Children's Hosp.Research Fndn., Cincinnati.

Most studies have claimed that the intestinal mucosa is normal in diabetics, including most of those suffering from diabetic diarrhea, with or without steatorrhea (Vinnik, I.E. et.al. Gastro.43:507,1962; Whalen, G.E.,et.al. Gastro. 56: 1021-1032,1969; Trier, J.S., Fed.Proc. 26:1391,1967). Four children ages 8, 12, 12 and 16 years presented with abdominal pain and diabetes; none suffered keto-acidosis but all were receiving suboptimal insulin doses. Three of the four had Type IV hyperlipoproteinemia (Fredrickson) and moderate hepatomegaly. None had measurable steatorrhea, but one, age 16, had definite relief of pain on a low fat, medium chain triglyceride substituted diet. Fasting jejunal biopsies examined by serial paraffin sections and serial frozen sections stained for lipids demonstrated expansion of the villus core from muscularis mucosae to villus tip by large amounts of triglyceride The lacteals were dilated but the epithelium appeared normal. One child, age 8, had numerous cuffs of macrophages about the veinules at crypt and midvillus level; these macrophage nests contained much birefringent material, probably cholesterol.

Triglyceride accumulation could result from endogenous epithelial very low density lipoprotein synthesis or defective chylomicron clearance, perhaps an early expression of diabetic neuropathy. The small intestinal mucosa of diabetics demand further investigation using lipid histochemistry and electron microscopy. Support:NIH/C.R.C.#RR-123

BRUTON'S AGAMMAGLOBULINEMIA (BD): A CASE WITH HYPOPARATHYROIDISM SECONDARY TO MALABSORPTION AND HYPOMAGNESEMIA. M. Norman, P. Holtzapple and J. Parks, U. of Pa., Sch. of Med., Dept. of Peds., Phila., Pa.

A 9 year old male with BD has recurrent infections, severe failure to thrive, and chronic diarrhea since 3 years of age. Four years after the onset of diarrhea, persistent hypocalcemia developed, and was unresponsive to oral Ca, Vitamin D supplements and a gluten free diet. Serum Ca ranged between 6 and 8 mg%. Features of malabsorption included an abnormal UGI xray, low serum iron, Vitamins B12, E and folate, low prothrombin, excessive fecal fat and chronic inflammatory changes on small bowel biopsy. Serum Mg ranged between 0.8 and 1.3 mg%. Serum immunoreactive parathyroid hormone (IPTH) was undetectable despite hypocalcemia.

Following parenteral Mg therapy, serum Ca and Mg rose to normal and IPTH to twice normal levels within 24 hours. Tetany, serum Ca, Mg and IPTH are well controlled on twice weekly maintenance MgSO4 injections. Diarrhea has abated, weight gain is significant, but steatorrhea persists. Documented malabsorption is uncommon in BD, and serum Ca levels are usually normal. Functional hypoparathyroidism secondary to hypomagnesemia is related to severe malabsorption occurring in this boy with BD.

MICROVILLUS DENSE BODIES (MVDB): VITAMIN D RELATED SMALL INTESTINAL EPITHELIAL STRUCTURES. John C. Partin, Jacqueline S. Partin and Wm. K. Schubert. Children's Hosp. Research Fndn., Cincinnati, Ohio 45229.

Microvillus Dense Bodies are electron scattering dense plaques measuring 250-500 Å which are closely applied to the inner lamina of the small intestinal microvillus plasma membrane. MVDB require glutaraldehyde, osmium and 0.19mM $CaCl_2$ for ultrastructural preservation (Sampson et. al. Cal.Tiss. Rev. 5:305, 1970; Oschman and Wall, J. Cell Biol. 55:58,1972). MVDB were first clearly recognized in the small intestinal mucosa of a hypoparathyroid boy treated with 500,000 I.U. of Vitamin D_2. We now show that the number of MVDB is related to the Vitamin D state of the rat. Quantitative electron microscopy was performed on duodenal mucosa of normal diet (ND), rachitogenic diet (RD), and rachitogenic diet + Vitamin D_2 (RD + D_2) weanling Charles River rats. The ratio MVDB/microvillus sections (MVS) was determined for each group by counting MVDB/MVS in 90,000 MVS. The ratio MVDB/MVS was ND=.0275, RD = .0044, RD + D_2 = .0583. Assuming 10,000 microvilli per cell, the number of MVDB per cell brush border would be ND = 4120, RD = 660, RD + D_2 = 12,330. The number of MVDB differs significantly ($p < .01$) between ND and RD + D_2 and RD and RD + D_2. Thus Vitamin D_2 treatment increases the number of MVDB in rachetic and normal rats. MVDB may be associated with Vitamin D activated calcium transport in the small intestine.

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PATHOLOGICAL EVIDENCE OF CYSTIC FIBROSIS PATIENTS WITH MECONIUM ILEUS. Ella H. Oppenheimer and John R. Esterly. The Johns Hopkins University, Baltimore, Maryland and The University of Chicago, Chicago, Illinois.

The morphologic findings in 37 infants with a history of meconium ileus showed a wide diversity in the sites and severity of changes indicative of cystic fibrosis. No pancreatic lesions were present in 10 patients, 9 of whom were less than one month of age. Anatomic lesions compatible with a diagnosis of cystic fibrosis were found in 34 of the 37 cases, and the changes in the remaining 3 cases were suggestive or compatible with that disorder. There was no significant difference in the frequencies of extra-pancreatic organ involvement between the group with pancreatic changes and those with a histologically normal pancreas. It is suggested that meconium ileus is always a manifestation of cystic fibrosis albeit with variation in organ involvement.

ELECTRON MICROSCOPY (EM) OF LIVER IN A-BETALIPOPROTEINEMIA: EVOLUTION OF SEVERE MICRONODULAR CIRRHOSIS. John C. Partin, Jacqueline S. Partin and Wm. K. Schubert. Children's Hosp. Research Foundation, Cincinnati, Ohio 45229.

An infant diagnosed as a-betalipoproteinemic (serum cholesterol 32mgm%, no immunoprecipitable beta-lipoprotein, acanthocytosis, typical intestinal biopsy and mother, aunt and sib with hypo-betalipoproteinemia) received liver biopsies at 10, 13, 25 and 30 months of age. Serum transaminases were elevated (SGOT 142-283) throughout the period. At initial biopsy the liver was white and contained large fat droplets in all liver cells; there was no inflammation or macrophage accumulation, but by electron microscopy, there was evidence of early cirrhosis. When placed on medium chain triglyceride diet after the first biopsy, fat droplets were reduced and the patient's clinical status improved. EM changes were, however, progressive: I.Progression of septal fibrosis to severe micronodular cirrhosis; II.The appearance of large patches of collagen-like fibrils, 150Å x 2000Å, within hepatocytes; III.Progressive increase in size of mitochondrial dense bodies, many measuring 1000Å at last biopsy; IV.Probable reduction in total hepatocyte population. The hypobetalipoproteinemic sibling (serum cholesterol 82mgm%, SGOT 32, acanthocytosis) was biopsied at 5 and 10 months of age. Frozen sections and EM showed excessive fat but no cirrhosis. Peribiliary lamellated bodies and fat increased between biopsies. The serious hepatic lesion in this patient emphasizes the need for liver biopsy in all childhood a-betalipoproteinemics. Support:NIH #RR-123

HUMAN GROWTH HORMONE (hGH) AND INTRALUMINAL FAT DIGESTION. J. Rainer Poley, J. Darrel Smith, John B. Thompson, University of Oklahoma Health Sciences Center, Departments of Pediatrics and Medicine, and VA Hospital, Oklahoma City; Peter D. Klein, Patricia A. Szczepanik, Argonne National Laboratories, AEC, Argonne, Illinois.

Micellar dispersion of lipolytic products by bile acids above the critical micellar concentration (CMC) is most important for normal fat digestion. Since no information is available on fat digestion in growth hormone (GH) deficiency, we have studied the influence of hGH in 6 GH deficient patients prior to treatment, after 10 days of 2 U/day and 4 and 12 months of 3 U/week. Jejunal micellar bile acids (JMBA) and lipids (JML) were isolated after ultracentrifugation of jejunal contents after two liquid test meals. Values were corrected for PEG. Four patients responding with good growth acceleration to exogenous hGH, increased JMBA continuously during treatment from levels at or below the CMC to normal adult levels after 12 months. JML increased to 100 to 250% over pretreatment values. In 2 patients not growing after exogenous hGH, no increase in JMBA and JML occurred. An increase in JMBA was apparently due to an expansion of the bile acid pool. A moderate increase in synthesis, pool sizes and turnover rates of primary bile acids was documented in 2 growing patients with kinetic studies using deuterated bile acids. Aside from this hepatic effect of hGH, an intestinal effect on bile acid metabolism must also be present, which permits a more effective enterohepatic circulation of the bile acid pool.

AMYLASE ISOENZYMES IN PANCREATIC INSUFFICIENCY IN HUMANS. Lynn M.Taussig, Robert O. Wolf, and Richard J. Deckelbaum (Intr. by Mary Ellen Avery) The Montreal Children's Hospital, Montreal and NIH, Bethesda, Maryland.

Separation of serum and urine amylase into its pancreatic and salivary isoenzymes has been shown to be of diagnostic benefit in certain situations. To assess the clinical value of amylase isoenzymes in states of pancreatic insufficiency, the isoamylase patterns of duodenal fluid, serum, and urine were studied in 11 patients (age 3-24 years) with cystic fibrosis. Other pancreatic enzymes and stool fat were also evaluated. The isoamylases were separated by disc polyacrylamide gel electrophoresis, squeezing the gels against starch agar slides, and developing the slides in an iodine solution.

All patients had evidence of marked pancreatic insufficiency. Total serum amylase concentrations were normal.

Pancreatic isoamylase activity was not present in any of the duodenal fluid samples. Serum and urine revealed normal salivary isoamylases but markedly decreased or absent pancreatic isoamylases (usually pancreas contributes more amylase than salivary glands to serum and urine). These results indicate that: 1) Study of serum or urine isoamylase patterns may be beneficial in evaluation of pancreatic function and diagnosis of pancreatic disease and insufficiency, thereby often avoiding the need for duodenal intubation; 2) Absence of pancreatic amylase in duodenal fluid does not always imply total destruction of pancreatic acinar tissue; 3) In humans, salivary glands and pancreas are the major sources of serum amylase.

FECAL BILE ACIDS AS AN INDICATOR OF BACTERIAL COLONIZATION OF SMALL BOWEL IN CHRONIC DIARRHEA. J. T. Rodriguez,M.D., T. L. Huang, Ph.D., J. Alvarado,M.D., J. V. Ordonez,M.D. and B.L. Nichols,M.D. Section of Nutrition and Gastroenterology, Dept. of Pediatrics, Baylor Col. of Med., Houston, Texas and Roosevelt Hospital and School of Med. Univ. of San Carlos, Guatemala City, Guatemala.

In children with chronic diarrhea, without steatorrhea and with no evidence of malnutrition, bile acid metabolism was found to be disordered. Subjects with increased growth of anaerobes in duodenum have significantly higher wet weight of feces per day than the controls. As a result of intensive bacterial deconjugation, total conjugated bile acids especially glycine conjugates, conjugated cholic, deoxycholic, ursodeoxycholic and isodeoxycholic acid were all significantly lower in the feces of patients with heavy colonization of duodenum by anaerobes. There was no apparent correlation between aerobic flora and bile acid pattern in the feces. The patients neither have enteropathogens nor Tropical Sprue. This would suggest, that chronic non-specific diarrhea could be related with changes in bacterial flora and secondary alteration of bile acids.

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STANDARDIZATION OF THE MANGOS FACTOR ASSAY AND STUDIES OF ITS SALIVARY GLAND DISTRIBUTION: Anne Taylor, LeRoy W. Matthews, Thomas F. Boat. Dept. of Pediatrics, CWRU, Cleveland, Ohio.

Mangos (Ped. Res. 1:436, '67) found that the rate of Na reabsorption (RNa) is inhibited in rat parotid ducts after exposure to mixed cystic fibrosis (CF) saliva. Confirmation of these findings has proved difficult indicating a need for standardization of the retrograde perfusion technique. Using a ratio of perfusate (ml) to dry gland weight (gm) between 1.8 and 2.8, we have reproduced these results and investigated the effects on RNa of stimulated secretions from individual glands of CF and normal subjects. Secretions collected separately from submaxillary, sublingual and parotid glands were perfused into 1 of the rat's 2 parotid glands. After pilocarpine stimulation, serial collections were made from both glands and Na concentrations determined. Percent inhibition of RNa in the perfused gland compared to the control gland at similar flow rates is tabulated. These results confirm the presence of a Na reabsorption inhibitory factor in mixed saliva of CF patients and indicate that not all CF salivary glands produce this factor. Secretion of the inhibitory factor only by glands which contain significant numbers of mucus cells suggests that these cells play a role in its production.

Secretion	Normal			CF			
	n	% inhibition	SD	n	% inhibition	SD	p
Mixed saliva	11	17.1	±8.05	12	46.2	±25.3	<.01
Sublingual	5	14.8	±9.99	6	36.1	±8.74	<.01
Submaxillary	6	17.0	±11.72	8	39.1	±16.93	<.02
Parotid	5	11.1	±5.36	5	10.9	±8.64	ns

BICARBONATE TRANSPORT BY SMALL INTESTINE IN PROXIMAL RENAL TUBULAR ACIDOSIS (PRTA). Morris Schoeneman*, Fima Lifshitz, Cornell Univ.Med.Col., N.Y.C., North Shore Hosp, Manhasset, N.Y., Univ. of Md. Sch.of Med.& Rosewood State Hosp., Baltimore, Md.

Transintestinal intubation with a double lumen tubing was done on an infant with PRTA, and in a comparative control patient with congenital hydrocephalus. The first part of the jejunum was perfused at a rate of 2.5 ml/min with Krebs-Hanseleit isotonic buffer containing 1 mM tyrosine and 20 mM D-glucose, with or without 30 mEq/l HCO_3^-. A non-absorbable marker, polyethylene glycol (PEG) was added. All perfusate samples were measured for pH, PCO_2, HCO_3^-, osmolality, glucose, Na, K, Cl, tyrosine and PEG. In the PRTA patient, HCO_3^- lumen to blood fluxes were 0.9-1.42 uEq/min/cm. of intestine as compared to a mean of 1.6 uEq/min/cm in the control. In contrast the PRTA patient had a blood to lumen flux of HCO_3^- of 0.4 uEq/min/cm compared to 0.059 uEq/min/cm in the control patient. This difference in HCO_3^- excretion occurred while the PRTA patient's serum bicarbonate level was 11.8-12.5 mEq/L and that of the control was normal. Moreover, when the blood levels of the PRTA patient were increased to 19-20 mEq/L, intestinal excretion of HCO_3^- increased to 2.4 uEq/min/cm, which could account for a total HCO_3^- loss of 5,000-10,000 uEq/min/cm. The massive intestinal excretion of HCO_3^- in PRTA was associated with normal reduction in pCO_2 level of the perfusate but with a defective Cl^- excretory mechanism. These data suggest a normal H^+ secretory mechanism but a defective HCO_3^-/Cl^- pump in PRTA.

EFFECTS OF ANTACIDS ON GASTRIC EMPTYING IN CHILDREN T. S. Vats, A. Hurwitz, R. G. Robinson, and W. Herrin. (Intr. by J. T. Lowman). Kansas Univ. Med. Ctr., Dept. of Ped., Med., and Diag. Rad., Kansas City, Kansas.

Variations in drug absorption and therapeutic efficacy are recognized clinically but have not been studied extensively in patients. In animals aluminum-containing antacids delay gastric emptying and depress drug absorption. This is the first report of antacid-induced delay in gastric emptying in patients. Leukemic children were studied since they receive antacids as prophylaxis against ulcers. Water, as a control, aluminum hydroxide gel and a magnesium-aluminum antacid were studied in random sequence in each of six children at a dose of 4ml/10 lbs. three times at hourly intervals. After the third dose of antacid or water each child drank 200 µCi of ^{51}Cr sodium chromate in saline and was then placed supine under a gamma camera interfaced with a PDP-15 computer. Disappearance of radioactivity from the stomach was plotted.

In each patient the 50% emptying time after water served as his control. 83.6 ± 11.2% of the radioactivity was retained following aluminum hydroxide ingestion at corresponding times ($p<0.05$). The mean maximal emptying rate after water was 5.1 ± 0.8 %/minute and after aluminum hydroxide was 1.8 ± .7 %/minute ($p<0.05$). As expected, magnesium aluminum antacid delayed gastric emptying to a lesser extent than aluminum hydroxide gel. These data clearly demonstrate that aluminum hydroxide gel delays gastric emptying in man. Further studies of antacid interaction with other drugs are in progress.

PHENYLKETONURIA: INTESTINAL TRANSPORT OF AROMATIC AMINO ACIDS, GLUCOSE AND ELECTROLYTES WITH HIGH OR MODERATE HYPERPHENYLALANINEMIA. Raul A. Wapnir* and Fima Lifshitz. Univ.of Md.Sch.of Med. & Rosewood State Hosp,Baltimore, Md.,Cornell Univ. Med.Col.,N.Y. C.,North Shore Hospital, Manhasset,N.Y.

An institutionalized adult phenylketonuric (PKU) femal was perfused through an intestinal double lumen tube when her plasma phenylalanine (Phe) level was 30.8 mg/100 ml (1.867 mM) and at the end of one month on a low-Phe diet, when her plasma Phe was 13.8 mg/100 ml (0.835 mM). Healthy volunteers served as controls. Their plasma Phe was below 2.65 mg/100 ml (0.16 mM). The perfusates were Krebs-Henseleit bicarbonate buffers with 1,2, or 5 mM L-Phe, 1 mM L-tryptophan (Trp), 1 mM L-tyrosine (Tyr) or one which was amino acid-free including 40 mM D-glucose (Glu) and polyethylene glycol as a non-absorbable marker. The intestinal absorption of the untreated PKU was significantly lower than that of controls for Phe (-4.3 to -18.1%), Trp (-8.6%), Glu (-10.3%) and Na (-40.9%). However, it was normal for Tyr and K. In contrast, when circulating Phe in the PKU was reduced by dietary treatment, the intestinal transport of Phe,Trp and Na was similar to that of normal individuals. Glu absorption remained at lower levels. These findings contribute to explain the appearance of unusual Phe and Trp metabolites in the urine and feces of untreated PKU as a result of impaired absorption and substantiate earlier findings that a reduction of plasma Phe below 15 mg/100 ml suffices to offset some of the biochemical imbalances associated with the primary enzymatic defect in PKU. (Supported in part by NIH grant HD-03959-04 and the John A. Hartford Fndn.).

BILE SALT METABOLISM IN INFANCY: EFFECT OF BACTERIAL OVERGROWTH. J.B.Watkins, P.Szczepanick, D.L.Hachey, P.D.Klein, and R.Lester, Boston Univ. Med. Sch., Dept. Med. and Ped., Boston, and Argonne Nat.Lab., Argonne,Ill. (Intr. by R.Klein).

We have demonstrated deficiencies in bile salt pool size and synthesis rates in normal full term and premature infants. No information is available on bile salt kinetics in infants with abnormal intestinal function. We now describe gross abnormalities of bile salt metabolism in a 3½ month old infant, operated at birth for ileal atresia who remained well for 3 months, developed a stricture, bacterial overgrowth and severe steatorrhea. Cholate pool size (determined by ^{14}C isotope dilution technique)equalled 12.7 mg/kg (2/3 the normal newborn value) while synthesis was 13.7 mg/kg/day. Keto derivatives (identified by TLC, GLC-mass spec) equalled 20% of the bile salt pool and were present in duodenal aspirate and stool. Only trace amounts of deoxycholate were found. Intraluminal bile salt concentrations averaged 1.25mM; 46% of this was deconjugated and in the conjugated fraction the glycine/taurine ratio was increased 4-fold. Conclusions: (1) Marked abnormalities of bile salt metabolism,i.e. increased losses, decreased pool size, intraluminal deconjugation and keto bile salt formation, result from bacterial overgrowth; (2) the infant responds with markedly increased synthesis, (3) a response inadequate to produce sufficient intraluminal bile salt concentrations; thus (4) bile salt deficiency compounded by bacterial overgrowth is a major contributory factor in the development of steatorrhea and malnutrition.

NUTRITIONAL STATUS OF CHILDREN IN PEOPLE'S REPUBLIC OF CHINA TODAY. Chi-Pang Wen, M.D. (Intr. by William B. Weil, Jr. M.D) Michigan State University, College of Human Medicine, East Lansing, Michigan

A staple food rationing system has practically eradicated overt starvation in People's Republic of China, even during periods of drought. The formula for rationing is extremely simple and intuitively designed. Each newborn baby is guaranteed a minimum of 3 Kg. of rice or wheat flour every month (averaging 400 Kcal/day from carbohydrate), with an increment of one additional Kg. for each additional gain in year of the child. Animal products are not rationed, except for pork in certain provinces. Periodic surveys of height and weight in school children in Peking revealed a consistent improvement over the last 18 years. The rapidly declining morbidity, mortality and case fatality rates are thought to be the combined results of a massive immunization campaign and improved nutritional status. Marasmus or kwashiorkor has never been encountered. Mild nutritional deficiencies, like cheilosis or underweight, are occasionally present, particularly in the rural areas. With extremely scarce resources, the Chinese rationing system, though offensive to American culture, worked effectively by realizing a close-to-perfect distribution and by minimizing the possible wastage of food to a population of 750 million.

RELATIONSHIP OF GASTROINTESTINAL GLUCOSE ABSORPTION WITH GESTATION AND FETAL GLUCOSE UPTAKE WITH FETAL GLUCOSE INFUSION. P. R. Williams, D. L. Phelps, and W. Oh, UCLA Sch. of Med., Harbor Gen. Hosp., Dept. of Ped., Torrance, Ca.

*To determine the relationship between gestation and gastro*intestinal (GI) absorption of glucose (G), studies were done in 5 early gestation (EG, 100-110 days) and 4 late gestation (LG, >130 d.) lamb fetuses, 2-5 days following hysterotomy for fetal carotid artery and orogastric catheter placement. G (3-5 gm/kg) was fed through the exteriorized orogastric catheter. In LG fetuses, blood glucose increased significantly at ½ to 2 hrs. following oral G feeding ($p < .05$). In EG fetuses, no significant rise in blood G was observed. Maternal G level remained unchanged. To determine the effect of fetal G infusion on the fetal glucose uptake (FGU), a separate study was done on 11 fetal lambs with chronic fetal catheter in place in the umbilical vein (UV), femoral vein (FV), and femoral artery (FA). Antipyrine method was used to determine placental blood flow (PBF) and FGU by the PBF and UV-FA G gradient. Fetal G infusion (6 mg/kg/min) resulted in a significant change in FGU (3.9 ± 2.2 vs. -3.0 ± 2.0 mg/kg/min, M ± S.E.M. [$p < .05$, n=7] at baseline and 2 hrs. post infusion respectively; minus sign of 2 hr. value indicates reversal of placental fetal G gradient). Saline infusion (n=4) did not show significant change in FGU. Thus, as the lamb fetuses mature, the GI G absorbability increases; a related study showed that exogenous G to the fetus could result in a net G uptake by the placenta rather than the fetus.

THE EFFECT OF EARLY MECONIUM EVACUATION ON THE SERUM BILIRUBIN LEVELS IN HEALTHY TERM INFANTS. Frederick H. Wirth, Jr., and Stephen E. Davis, USAF Med. Ctr., Dept. Ped., Scott Air Force Base, Ill. (Intr. by Arthur E. McElfresh)

The reabsorption of bilirubin from meconium is most pronounced in the first hours of life, and there is a significant relationship between a delay of the first meconium passage and the incidence of hyperbilirubinemia.

Initial observations showed that it took 67.1 hours for infants with a serum bilirubin greater than 12 mg.% to pass a yellow stool compared to 52.9 hours in healthy newborns ($P < .01$). Forty-one healthy term infants were randomly divided into 2 groups. The study group received one half of an infant glycerine suppository within 30 minutes after birth and every 4 hours just after feedings.

Daily serum and fecal bilirubin determinations were obtained. The fecal bilirubin procedure was developed for this study. It involves repeated chloroform extraction of a homoginate of meconium and direct spectrophotometric reading of the extracted bilirubin.

The treated infants were found to pass all their meconium at an average age of 27.6 hours as compared to 52.9 hours for the control group. Daily serum bilirubins were significantly lower in the treated group of infants on days 2, 3, and 4 of life. The amount of meconium and bilirubin excreted on day 1 was significantly greater in the study group.

SPECIFIC B_{12} MALABSORPTION IN A PATIENT WITH CYSTIC FIBROSIS Ravindranath Yaddanapudi*, Bhimsen R. Sirmokadam*, Ralph Carmel*, Nora Chang*, Wolf W. Zuelzer. From the Children's Hosp. of Mich. and Dept. of Ped. and Med. Wayne State Univ. Sch. of Med., Detroit, Mich.

A rare form of B_{12} responsive megaloblastic anemia, due to malabsorption of intrinsic factor (IF)--B_{12} complex in the intestine was observed in a patient with cystic fibrosis. The latter diagnosis was made in this W/F infant at 9 mo. on the basis of growth failure, intermittent diarrhea, foul smelling stools and marked elevation of sweat electrolytes. At 18 mo. severe anemia (Hb. 5.4 gms.%) was present with marked bone marrow megaloblastosis. Serum folate level was high normal and B_{12} level was less than 50 pg/ml. Schilling test with and without exogenous IF yielded no excretion of radioactive B_{12} though there was a dramatic response in Hb. levels and reticulocytes. IF activity in gastric juice was normal, IF antibodies were absent and transcobalamine levels were normal. Fecal fat excretion while patient was receiving pancreatic extract was normal. Urinary protein excretion was increased. These findings are consistent with the type of congenital B_{12} malabsorption described by Imurslund and Grasbeck.

The association of specific B_{12} malabsorption with cystic fibrosis has not been heretofore reported. Its occurrence in the present case despite pancreatic enzyme therapy is consistent with the presence of two unrelated absorption defects in the gastro-intestinal tract.

INTESTINAL GLUCOSE ABSORPTION IN GROWTH RETARDED (GR) RAT PUPS, M.K. Younoszai, Univ. of Iowa, Col. of Med., Dept. of Ped., Iowa City, Iowa

Rat pups raised in litters of 18 postnatally are GR. This may adversely affect intestinal function and perpetuate GR. We compared small intestinal absorption of 3-O-methyl-D-glucose (3OMG, a non-metabolisable hexose) and growth in normal control (C) pups, raised 7 to 9/litter to those in postnatally GR pups raised 18/litter. Absorption rate was measured in vivo under steady state conditions in the jejunum + ileum by a one pass perfusion technique at 7-8, 14-15 and 21-22 days after birth. The perfused solutions contained 3OMG, tracer ^{14}C- 3OMG, phenol red, 50 ug/ml (as non-absorbed indicator for volume changes) and sodium chloride in quantities sufficient to make the solution 297-304 mOsmoles/liter. The concentration of 3OMG in perfused solutions were 3 mg/ml for the 1 and 2 week and 15 mg/ml for the 3 week old pups. Absorption rate (mean±S.E.)of 3OMG, mg/g dry weight of perfused intestine were: at 1 week C 70±3, GR 53±9; at 2 weeks C 56±7, GR 79±7 ($P<0.05$); at 3 weeks C 275±18, GR 227±21. Intestinal growth in GR lagged behind those in C pups. Dry weight, mg (mean±S.E.) of perfused intestines were: at 1 week C 56±4, GR 52±5; at 2 weeks C 113±6, GR 69±6; at 3 weeks C 278±21, GR 125±13. Although absorption rate in GR was not significantly lower than in C pups, total amount of hexose absorbed was less in GR pups. The higher absorption rate in GR at 2 weeks suggest stimulation of intestinal transport during early phases of GR.

GENETICS

First Session

PRENATAL DIAGNOSIS OF METHYLMALONICACIDURIA. Maurice J. Mahoney, Leon E. Rosenberg, John Waldenström, Bengt Lindblad, and Rolf Zetterstrom. Yale Univ., Depts. of Human Genetics and Ped., New Haven; Univ. Gothenburg, Dept. Clinical Chem., Gothenburg; Karolinska Institutet, Dept. of Ped., Stockholm.

In two families with fetuses known to be at risk for methylmalonicaciduria (MMA), pregnancies were monitored by amniotic fluid (AF) methylmalonic acid determinations and by studies of cultured AF cells. The first pregnancy was studied at 12 menstrual weeks. A previous child had B_{12}-responsive MMA with a defect of intracellular B_{12} coenzyme accumulation. Methylmalonic acid was not detected in the AF and cultured AF cells metabolized propionate and methylmalonate normally and accumulated normal amounts of B_{12} coenzymes. Postnatal tests of urine and cells confirmed the absence of MMA. The second pregnancy was studied at 16 weeks. The type of MMA (B_{12}-responsiveness) had not been defined in a previously deceased child in this family. AF methylmalonic acid was elevated at 4.3 µg/ml. Cultured AF cells showed very deficient oxidation of propionate (2.7 nmoles/10^8 cells/3 hr; control 61) and methylmalonate (7.2; control 228). Methylmalonyl-CoA mutase activity was similarly diminished but B_{12} coenzyme accumulation was normal. MMA due to a defective methylmalonyl-CoA mutase apoenzyme was diagnosed and the pregnancy terminated. These studies, using both AF and cultured AF cells, have permitted the first prenatal diagnosis of MMA, plus indication of its biochemical basis, in time to elect abortion.

PEPTIDES OF ABNORMALLY LOW MOLECULAR WEIGHT IN ECTODERMAL DYSPLASIC HAIR. R.J.M.Gold & Z.Kachra (Intr. by C.Scriver) MRC Genetics Group, McGill Univ. Montreal, Quebec.

The S-carboxymethyl derivative of matrix protein was prepared from the hair of 4 normal subjects and from 4 ectodermal dysplasics of the autosomal dominant, hydrotic type. 50mg of the dry protein was dissolved in a 0.1M phosphate 8M urea buffer at pH 7, applied to a calibrated G-75 sephadex column, and eluted with the same buffer at 6ml an hour. The optical density of the effluent at 280 nanometres was monitored. The absorption profile from all normal subjects was characterized by a peak at 33,000 M.W. and virtually no absorption below, 15,000. This molecular weight range was not affected by wide variation of the preparative procedures. Selected fractions were rerun and appeared at their previous elution volume. The profile from severely affected ED subjects extended down to M.W. 3000. This was confirmed by applying ACTH and glucagon markers to the column. The profile of more mildly affected subjects from the same pedigree had intermediate characteristics. The findings are consistent with the hypothesis, previously advanced, that the gene whose mutation produces this disease codes for a constant subunit of several matrix proteins.

BIOCHEMICALLY MUTANT LYMPHOCYTES IN LONG-TERM CULTURE. Arthur D. Bloom, Elaine B. Spector, and Sandra A. Streeter. Univ. of Michigan Med. Sch., Depts. of Human Genetics and Ped.

We have established continuous cultures of human lymphocytes from patients with the following inborn metabolic errors: citrullinemia, the Lesch-Nyhan syndrome, Maple Syrup Urine Disease, galactosemia, and fructose-1, 6-diphosphatase deficiency. Each of these lines has maintained the karyotype of the donor, and, where tested, the cellular phenotype of the donor. Selective systems have thus far been applied to the UM-21 citrullinemic and the UM-10 Lesch-Nyhan cells. UM-10 cells were unable to grow in amethopterin-containing medium (HAT) and, unlike lymphocytes from normal donors, were able to grow in the presence of the purine analogue 6-thioguanine. The UM-21 cells were unable to incorporate ^{14}C-citrulline into TCA-precipitable cellular material, and continuous incubation of UM-21 in the presence of citrulline but without arginine allowed for establishment of a variant line, able to grow in citrulline. Maintenance of the mutant phenotype in the UM-21 parent line implies that these citrullinemic cells were deficient in argininosuccinic acid synthetase. Nine clonal sublines of UM-21 were homogeneous in their growth curves and labelling patterns in the presence of ^{14}C-citrulline, suggesting that the variant line is likely to have arisen by mutation in vitro. Our ability to select for or against the HGPRT(-) UM-10 cells will enable us to derive estimates of spontaneous and induced mutation rates at this locus in the human genome.

REPAIR OF ENZYME DEFICIENCY IN MURINE SOMATIC CELLS BY PROVISION OF REGULATORY ELEMENT OF CHICK ORIGIN. Bohdan Bakay, Carlo M. Croce, Hilary Koprowski and William L. Nyhan. Dept. of Pediatrics, UCSD School of Medicine, La Jolla, Ca. and The Wistar Institute, Philadelphia, Pa.

Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) is the site of the defect in the Lesch-Nyhan syndrome and an important enzyme in cell biology. In studies on the genetic control of this enzyme we have studied the expression of genes in progeny cells derived by hybridization of cells deficient in HGPRT with cells containing normal activity (HGPRT$^-$ and HGPRT$^+$). The HGPRT$^-$ 1R cell is a subline of the mouse L-cell selected in the presence of 8-azaguanine. 1R cells were fused with HGPRT$^+$ chick embryo fibroblasts and the progeny were grown in HAT media in which cells lacking HGPRT will not grow. Progeny cells in HAT contained the marker chromosome that distinguishes the 1R cell. HGPRT activity was present. Electrophoretic analysis showed clearly that this was not the chick enzyme but rather that it was mouse. These data indicate that genetic material of chick origin has determined the synthesis of a mouse enzyme. They are consistent with the operation of a regulator gene provided by the chick cells and responsible for the original defect in the mouse cell. Absence of backmutation of HGPRT$^+$ progeny cells to HGPRT$^-$ in media containing 8-azaguanine supports this conclusion.

GENETIC HETEROGENEITY IN I-CELL DISEASE (MUCOLIPIDOSIS II). Janet H. Glaser, William S. Sly, Chen-Kung Ho, and Elizabeth Neufeld, Washington Univ. Sch. Med. and NIAMD, NIH, St. Louis Children's Hosp., Dept. Ped. and Med., St. Louis, Mo.

Mucolipidosis II (I-Cell Disease) is characterized by severe skeletal dysplasia, severe psychomotor retardation, "Hurler-like" body configuration and normal urinary mucopolysaccharide excretion. We report here clinical and biochemical data on a mild form of this disorder in an 8 year old male with slowly progressive skeletal dysplasia and very mild psychomotor retardation (WISC performance IQ-103, verbal-81). Studies on cultured skin fibroblasts show 1)the typical partial deficiencies for multiple lysosomal hydrolases. 2)High levels of lysosomal hydrolases in the culture media. 3)Abnormal accumulation of ^{35}S-MPS, and 4)the previously unreported findings of stimulation of growth and MPS excretion by added platelet extracts.

Leukocyte enzyme levels are normal but serum levels are 20-100 fold elevated. The serum β-glucuronidase from the patient, which is 50 fold elevated, is not taken up normally by other fibroblasts. This finding is compatible with alteration or absence of the recognition portion of the hydrolase molecule, as recently proposed by Hickman and Neufeld for enzyme from I-cell fibroblasts.

Mild serum hydrolase elevations in parents of the patient suggest that the heterozygous carrier can be identified.

ANTENATAL DIAGNOSIS OF MUCOLIPIDOSIS II (I-CELL DIS.) Richard J. Warren[1], Colin J. Condron[1], David Hollister[6], Frans Huijing[2], Elizabeth F. Neufeld[4], Clara W. Hall[4], Allan G. W. McLeod[3], Andrew E. Lorincz[5]. Depts. Ped.[1], Bioch.[2], and Obst.[3], Univ. Miami Sch. Med.; NIAMDD,NIH, Bethesda, Maryland[4]; CDLD, Univ. of Alabama, Birmingham[5]; Dept. of Ped., UCLA, Los Angeles. Intr. by William W. Cleveland.

The antenatal diagnosis of mucolipidosis II (I-cell disease) has been predicted based on lysomal enzyme levels in cultured cells and extracellular fluids of patients with the disease. We report here the details of such a diagnosis. Significantly higher levels of lysomal enzymes were found in the amniotic fluid and the enzyme levels were decreased in the cells from the embryo. A microspectrofluorometric analysis revealed strikingly anomalous fluorescent spectra in both cultured and uncultured cells after staining with acridine orange. These data allow the conclusion that mucolipidosis II can be diagnosed in utero using direct assays of the fluid and cells thus obviating the necessity of tissue culture.

A POSSIBLE BIFUNCTIONAL ROLE FOR CYSTATHIONINE SYNTHASE. Oliver W. Jones and Mary A. Grishaver. Univ. of Calif., San Diego, Dept. of Med., La Jolla, Calif., (Intro. by Jerry A. Schneider).

Patients with homocystinuria due to cystathionine synthase deficiency fall into two major categories regarding biochemical responsiveness to pyridoxal phosphate: those who respond by reversal of methioninemia and homocystinuria and those who have no change in the specific aminoacidemia/aminoaciduria. We find an enzyme in human fetal tissue which catalyzes the reaction: H_2S + Serine $\xrightarrow[\text{sulfhydrase}]{\text{serine}}$ Cysteine, bypassing the reaction requiring cystathionine synthase.

Serine sulfhydrase is present in human fetal skin, liver and brain and has been purified 100-150 fold. There is an absolute requirement for serine and pyridoxal phosphate.

Serine sulfhydrase and cystathionine synthase have the following similarities: a. identical purification through six steps including elution at the same point by DEAE chromatography; b. similar temperature sensitivity; c. the same mobility on gel electrophoresis; and identical response to serine and sulfhydryl analogs.

Our results suggest that in human fetal tissue certain enzyme protein may have bifunctional catalytic properties. A point mutation on the enzyme polypeptide chain might render it incapable of catalyzing one reaction but capable of catalyzing another. It is possible that some homocystinuric patients might still have serine sulfhydrase activity and cysteine biosynthesis in response to pyridoxal phosphate, while the catalytic site for cystathionine synthase is rendered inactive by mutation.

NORMAL VALUES OF HEMOGLOBIN A SYNTHESIS IN THE DEVELOPING FETUS. Haig H. Kazazian, Jr., Andrea P. Woodhead, and Michael M. Kaback, Johns Hopkins Univ. Sch. of Med., Dept. of Ped., Baltimore.

The synthesis of hemoglobin A ($\alpha_2\beta_2$) by reticulocytes obtained from 42 fetuses of 5.7-20.0 cm crown-to-rump length was measured after ion exchange chromatography and accounted for 4.3-13% of the total hemoglobin synthesis. The percent of hemoglobin A synthesis varied directly with the crown-to-rump length of the fetuses ($R = +.46$, $p < .001$). The synthesis of hemoglobin A by reticulocytes of the four smallest fetuses of 5.7-8.0 cm crown-to-rump length was confirmed in the following manner. Column fractions containing the putative hemoglobin A were digested with trypsin and fingerprinted. After autoradiography of these peptide maps, radioactivity was found exclusively in the predicted α and β peptides. Therefore, the onset of adult hemoglobin synthesis in the human fetus occurs before the 68th gestational day. These results suggest that the antenatal detection of reduced levels of β-chain synthesis in the β-thalassemias is biologically feasible. However, our ability to diagnose the β-thalassemias antenatally still depends upon the development of a safe, reliable amnioscope to assist in the sampling of 5-10 μl of fetal blood under direct visualization.

COMPREHENSIVE TESTING FOR THALASSEMIA TRAIT. Howard A. Pearson, Richard T. O'Brien, Sue McIntosh and Gregg T. Aspnes, Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

Automated determination of mean corpuscular volume (MCV), using the Coulter counter Model S, is a valid screening test for thalassemia (New Eng. J. Med. Feb. 1973). This test permits relatively inexpensive screening of large numbers of individuals for these genetically determined conditions. Because diagnosis of thalassemia trait has clinical and genetic implications we have screened 500 persons from high risk populations. We have found a general lack of knowledge about thalassemia in both the public and profession. Many individuals in whom a diagnosis of thalassemia trait was made had been previously subjected to extensive and expensive blood tests and treated with hematinics including oral and parenteral iron, in spite of their Mediterranean background and lack of response to therapy. In Greek Americans the following frequencies of genetically determined blood conditions were established: β thalassemia 5.0%, α thalassemia 2.4%, Hgb S 1.0%, G_6PD (in males) 5.0%. Similar data are now being assembled for Italian Americans. Testing for thalassemia trait was well accepted when presented as part of a comprehensive program encompassing meaningful education, reliable testing and ethical individual genetic counselling.

ALPHA$_1$-ANTITRYPSIN DEFICIENCY: A VARIANT WITH NO DETECTABLE ALPHA$_1$-ANTITRYPSIN. Richard C. Talamo, Carol E. Langley, Charles E. Reed, Sohei Makino. Harvard Med. Sch., Children's Service, Mass. Gen. Hosp., Boston, and Univ. of Wisconsin Sch. of Med., Dept. of Med., Madison.

Severe hereditary serum alpha$_1$-antitrypsin (AAT) deficiency is usually characterized by levels of AAT of 10-35% of the normal amount (approx. 2.3 mg/ml) and the homozygous Pi (protease inhibitor) type ZZ. Pi^{ZZ} AAT deficiency is associated with early onset pulmonary emphysema, infantile cirrhosis or a combination of lung and liver disease.

No AAT could be detected in the serum of a 24 year old male with advanced emphysema by agarose electrophoresis, immunoelectrophoresis, double diffusion in agarose gel, quantitative radial immunodiffusion, nor by an electroimmunodiffusion method, capable of detecting as little as 0.7 mcg/ml of AAT. Pi typing analysis by acid starch gel electrophoresis and antigen-antibody crossed electrophoresis failed to demonstrate AAT in any electrophoretic region. A circulating AAT inactivator could not be demonstrated.

A family study revealed that both of his parents, both of his daughters, two of his 3 sisters and his maternal grandmother had Pi^M AAT (the commonest, "normal" AAT type), but had intermediate serum AAT levels, below the lowest AAT level usually found in healthy Pi^{MM} individuals.

The patient is thus homozygous for a new Pi allele, whose product cannot be detected by current methods. The other family members noted are heterozygous for this allele.

GENETICS

Second Session

NATURE OF X-AUTOSOME TRANSLOCATION AND CHOICE OF X-INACTIVATION. E.Sujansky, L.Y. Hsu, M. Lucas and K. Hirschhorn. Mt. Sinai Sch. of Med., N.Y.C., and Univ. College, London, Engl.

We have studied an X-autosome translocation in a young phenotypically normal female with primary amenorrhea. By both trypsin and fluorescent banding techniques, the abnormal karyotype was identified as 46,XXq-,-15,+t(X;15) (q13;p12). Autoradiographic studies showed that the normal X was late replicating in all cells. In reviewing 14 previously reported cases of X-autosome translocation in females, it appears that the nature of the translocation determines the pattern of X-inactivation. If a piece of autosome is translocated onto an almost complete X, this X is inactivated, with more or less spread of its late replication pattern to the attached autosomal piece. All of these patients are abnormal. If the X is broken into two major fragments in reciprocal translocation with an autosome, then the other, normal, X is inactivated. These patients may be normal, but like our case, may have gonadal dysgenesis. These patients with gonadal dysgenesis share with other such patients the fact that the same X is active in all cells (e.g., XO,X+morphologically abnormal inactive X), while normal females have different X's active in different cells and males have a Y. It is clear that females with primary amenorrhea require complete cytogenetic studies to identify X-autosome translocations, since they have normal X chromatin in buccal smears. Banding studies will reveal such translocations when the size of the exchanged pieces is about equal.

LOCALIZATION OF GENES ON THE X CHROMOSOME BY SOMATIC CELL HYBRIDIZATION. P. Gerald, G. Bruns, V. Monedjikova, Children's Hospital Medical Center, Boston and Wrentham State School, Wrentham, Mass.

A retarded female has been found to have the translocation t(19q+; Xq-), with breakpoint near the middle of the long arm of X. Autoradiographic studies demonstrate that the normal X chromosome in these cells is late-labelling. Leucocytes from this patient have been fused with mouse cells deficient in hypoxanthine-guanine phosphoribosyl transferase (HGPRT) and hybrid colonies have been selected by cultivation in HAT medium. Hybrids derived from normal cells express the X-linked genes, phosphoglycerate kinase (PGK) and glucose-6-phosphate dehydrogenase (G6PD) as well as HGPRT. Hybrid lines derived from the aforementioned patient's leucocytes possess human G6PD but lack human PGK. One of these lines has been chromosomally examined and contains the 19q+ element but lacks the Xq- element. These findings are consistent with localization of G6PD and HGPRT to the distal half of the long arm of the X. The loci for hemophilia A and color blindness are probably in the same region since both these loci are closely linked to G6PD. The data presented here, combined with the results of Ricciuti and Ruddle (in press), localizes PGK to the proximal half of the long arm of the X.

EPIDEMIOLOGY OF CHROMOSOMAL ANEUPLOIDY, Arthur Robinson, Walter Goad, Theodore T. Puck, Univ. of Colo. Med. Ctr., Denver and Los Alamos Scientific Lab., Los Alamos, N. Mex.

Between January 1, 1964 and December 31, 1972, 29,679 consecutive births have been screened at 2 Denver hospitals for aneuploidy involving the sex chromosomes and chromosome 21. The data confirm in a compelling manner previous conclusions of a seasonal distribution of these conditions. We can with 90% confidence now state that the total incidence of these states in newborns is at least 1.7 X greater during the period May-October than November to April. However, if one considers only those babies with Down's syndrome whose mothers were under 35 years of age at the time of conception, we can be 98% confident that the incidence of births May-October is at least twice that of November-April, and we can be 90% confident that it is at least 3 times the incidence. For sex chromosomal anomalies the May-October incidence was 0.21% and November-April was 0.09%. The probability that this is a random distribution is less than 2×10^{-3}. Since in Down's syndrome the maternal age effect counteracts the seasonal effect, it is not unexpected that among the sex chromosomal aneuploidies, the seasonal influence, although present, is less marked. The incidence of sex chromosomal abnormalities is $\geq 0.15\%$. Twelve per cent of these have died in the first 2 weeks of life, a significantly higher figure than that for the general population (1%).

INCORPORATION OF ISOLATED HUMAN METAPHASE CHROMOSOMES BY CULTURED HUMAN FIBROBLASTS. Mark W. Steele, Univ. of Pittsburgh Sch. of Med. and Children's Hosp., Dept. of Ped., Pittsburgh.

If normal human fibroblasts in culture would incorporate functionable metaphase chromosomes isolated from malignant human cells, a powerful technique could become available for studying the malignant process and for the chromosomal localization of human genes. Accordingly, tritium labeled metaphase chromosomes were isolated from a human malignant cell line (Det.98/AH_2) which was HGPRT$^-$,G6PD"A"; and then were added to monolayer cultures of a normal diploid human fibroblast strain (fetal lung)which was HGPRT$^+$,G6PD"B". After 8 hrs. intact metaphase chromosomes were observed in the cytoplasm of numerous fibroblasts; but disintegration of the chromosomes had already begun as evidenced by morphological changeswhich included loss of the radioactive label on autoradiography. After 24 hrs.diffuse radioactive label was observed in interphase and metaphase nuclei. After 48 hrs. disintegration of the cytoplasmic chromosomes was extensive. After 36 to 48 hrs. 3.4% of the fibroblast metaphase plates demonstrated extra intact and partially disintegrated unlabeled metaphase chromosomes; the same not being found for controls. After several weeks in culture there was no evidence of functioning malignant chromosomes within the monolayer culture; in that the latter retained its fibroblast morphology and G6PD"B" phenotype; and could not live in media containing purine analogues. Ways to prevent disintegration of isolated malignant metaphase chromosomes after fibroblast incorporation are now being explored.

NON-FLUORESCENT AND NON-HETEROCHROMATIC STAINING Y CHROMOSOME IN 45,X/46,XY MOSAICISM. L.Y.F.Hsu, H.J.Kim, L.Steinfeld, and K. Hirschhorn. Mt. Sinai Sch. of Med., Dept. of Ped., N.Y.C.

Caspersson demonstrated the bright fluorescence in the long arm of the Y chromosome stained with quinacrine (Q-banding). Arrighi and Hsu, using denaturation (C-banding), showed that the same region of the Y stained densely with Giemsa. Recently we have observed a Y chromosome with neither of these properties in a phenotypic female with 45,X/46,XY mosaicism and Turner's syndrome. In the cells with 46,XY, the Y chromosome was easily identified by its morphological characteristics.Q-banding showed only two faint bands in the long arm of the Y. The banding patterns of the other chromosomes were normal; one No. 22 had an enlarged satellite. C-banding of 46,XY cells also showed the absence of the dark staining area of the Y chromosome and the presence of two lightly staining bands in its long arm. Q-banding and C-banding of the father's chromosomes showed a normally fluorescent and heterochromatic Y and a No. 22 with an enlarged satellite as in the patient. Measurements of the patient's Y and the father's Y in comparison to their No.2 and four G chromosomes showed that the difference in length between the Y's is insignificant($0.3<p<0.5$). There was no evidence for chromosomal translocation or deletion in our case. In 10 previous cases of XO/XY mosaicism, 5 cases had non-fluorescent Y chromosomes. Four of their fathers had normal Y fluorescence. Although the mechanism of the loss of Y fluorescence is not yet known, it appears that such an altered Y may be predisposed to anaphase lag leading to mosaicism.

PARTIAL TRISOMY OF CHROMOSOME 1 IN A FAMILY WITH A t(1q-;4q+) TRANSLOCATION. Richard L. Neu and Lytt I. Gardner, State Univ. of New York, Upstate Med.Ctr.,Dept.of Ped.,Syracuse,NY

Few translocations have been reported involving translocations or partial trisomies for chromosome number 1. The case presented here is that of an infant trisomic for approximately one-third of the terminal portion of a number 1 chromosome. The extra 1q chromosomal material was attached to the ends of the long arms of a number 4 chromosome (46,XX,4q+). The parents were both 24 years of age. The proposita's mother, maternal grandfather and uncle were all balanced translocation carriers, 46,XX,t(1q-;4q+) or 46,XY,t(1q-;4q+). The uncle has a boy with typical Apert's syndrome who has an apparently balanced chromosome complement. The affected infant girl became jaundiced 3 days after birth, attributed to ABO incompatibility. The jaundice cleared, but a respiratory infection at age 6 weeks had a fatal outcome. Physical findings at that time included elfin facies, large head, low set large pinnae, harsh systolic-diastolic murmur, enlarged labia majora and rocker-bottom feet. Further clinical and necropsy investigation revealed pulmonary abcesses, hydrocephaly, marked subaortic stenosis with left ventricular hypertrophy, patent foramen ovale and small patent ductus arteriosus.

PARTIAL TRISOMY 7 AND PARTIAL MONOSOMY 7 IN CHILDREN OF A FATHER WITH A BALANCED CHROMOSOME TRANSLOCATION (46,XY,t(7q-;21q+))

Harold N. Bass, S. Michael Marcy, Barbara F. Crandall (Intr. by E. Richard Stiehm) Kaiser-Permanente,Panorama City, Ca91402

The importance of the new differential stains is emphasized by studies in one family. An 18 month old girl was referred because of failure to thrive, slow development and multiple physical anomalies including frontal bossing, low set ears, high arched palate, micrognathia, bilateral cataracts, scoliosis and pulmonic stenosis. Standard karyotyping done elsewhere was interpreted as normal. Trypsin-Giemsa banding studies revealed additional material on the long arms of one No.21 chromosome. The mother's karyotype was normal but the father had a balanced translocation involving the long arms of a No.7 and No.21 chromosome (46,XY,t(7q-;21q+)). The mother, who was seven weeks pregnant, aborted spontaneously at the time of these studies and chromosome analysis of the fetus, using the same stain, revealed deletion of material from the long arms of No.7 chromosome. Thus, a balanced translocation in one parent resulted in a child with partial trisomy for the long arms of the No.7 chromosome and a fetus with partial monosomy for the same chromosome. Amniocentesis for future pregnancies is clearly indicated and these studies emphasize the need for differential chromosome stains despite an apparently normal standard karyotype when faced with a child with similar findings.

CRANIOFACIAL DYOSTOSIS WITH FOOT ABNORMALITIES:A DISTINCTIVE AUTOSOMAL DOMINANT PHENOTYPE;Charles E. Jackson, Lester Weiss, James A.Peterson,William A.Reynolds,Tod F. Forman,Henry Ford Hospital,Detroit,Michigan and Elkhart Clinic,Elkhart,Indiana

A condition superficially resembling Pfeiffer's Syndrome(Acrocephalosyndactyly Type V)was encountered in an Amish population.Roentgen and clinical studies suggest that this condition represents a previously undescribed genetic disorder.In this kindred 62 affected individuals were observed and 43 reliably reported as being affected. All affected individuals had foot abnormalities consisting of a large medially deviated great toe and/or radiographic abnormalities of the tarsal bones.The craniofacial dyostosis varied from severe oxycephaly with proptosis to almost imperceptibal abnormalities.The distribution of cases within 6 generations is consistent with an autosomal dominant mode of inheritance apparently with complete penetrance.This disorder differs from previously described syndromes of craniostenosis(or facial dyostosis) and limb abnormalities by the particular roentgenographic findings of the feet.Tarsal abnormality was the consistent manifestation indicating the presence of the abnormal gene.At birth,the foot abnormalities are apparent on clinical examination.Intelligence testing suggests that even severely affected individuals do not have impaired intelligence.Therefore,surgery for the correction of craniostenosis in order to protect against impairment of intellectual function is rarely,if ever,indicated.

SHORT MUSCLE SYNDROME. C. Charlton Mabry and M. William Hutcheson. Dept. Pediatrics, Univ. Kentucky, Lexington.

In an Appalachia Head Start preschool examination, we found a boy with an inability to open his mouth and a flexion deformity of his fingers. Investigation of the kindred showed others affected, with variations of the anomaly.

Anomaly: Opening of the mouth is most limited in childhood (1/4 inch between incisors), with less difficulty in adults. Impairment is due to shortening of the muscles of mastication. When hands are dorsiflexed, there is ulnar deviation with the fingers tightly flexed; the fingers cannot be forced open. This is due to shortening of the flexor digitorum profundus and sublimis muscle-tendon units. Motion of the fingers is normal when the hand is flexed. Some affected individuals also have foot and toe deformities and shortening of gastrocnemiae.

Inheritance: The defect was verified in 24 family members spanning five generations. There were 15 males and 9 females affected, the living ranging in age from 4 to 85 years. Transmission of the defects was from affected parents to about half of their children without skipping obligatory carriers.

This new malformation syndrome is the same as that reported by Hecht, et al. and Wilson, et al. (Birth Defects:Original Article Series 5:96-102, 1969, preliminary notes). The greater number of affected individuals reported here permits a better appreciation of the phenotypic expression of the gene and the establishment of inheritance as autosomal dominant with complete penetrance.

EVALUATION OF A GENETIC COUNSELLING SERVICE.Elizabeth J.Ives, Pat M.Petersen and Sharon E. Cardwell.(Intr.by J.W.Gerrard) Univ. of Saskatchewan, Dept. Ped. Saskatoon, Sask.,Canada

A retrospective evaluation of a genetic service established in 1965 was initiated in 1969. Major objectives were 1.To determine reproductive behaviour subsequent to the counselling interview.2.To investigate the relative importance of factors influencing decisions about reproduction.3.To assess patient satisfaction.A questionnaire was designed and tested and criteria for inclusion in the study determined.To ensure a minimum lapse of one year since counselling family file 380 was taken as the cut off.164 files were eliminated and 30 families could not be located.The remaining 186 yielded information about 194 counselling enquiries.341 interviews were conducted: 224 to both of 112 couples,70 to one member of a couple and the rest to older siblings or offspring of propositi.Grouped as high risk (1:10<) and low risk (1:10>) there had been 12 further high risk pregnancies (2 affected) and 66 low risk pregnancies (2 affected).50% of these pregnancies were unplanned.Recall of information was most accurate when couples had been counselled together,the condition severe and the risks high.Overall socioeconomic factors in the study group did not differ significantly from the general population.Dissatisfaction was expressed at the lack of previous information the time interval between diagnosis and counselling and the lack of follow-up.These and other results have assisted in planning a more appropriate genetic service for the province.

GENETICS

Read by Title

AUTOMATED GENETIC COUNSELING IN SICKLE CELL ANEMIA, Ray M. Antley, Constance M.Burks and Lawrence C.Hartlage, Indiana Univ. Sch. of Med.,Dept. of Ped.,Indianapolis (Intro. By I.K. Brandt).

This project was aimed at developing and evaluating an approach to the delivery of large scale counseling in conjunction with sickle cell screening programs. There are insufficient numbers of trained geneticists to deliver content information about the inheritance of hemoglobin to the numbers of counselees who are identified by sickle cell screening programs. To study the effectiveness of an automated counseling procedure in providing content information for counselees, we produced a videotape for counseling individuals with HbAS. For formal evaluation of the program an extensive 50 item test was administered to 89 individuals before and after viewing the videotape. Based on pretest scores the overall improvement was 26.8% ($p<.001$). Counseled students gained in the knowledge of the genetics of hemoglobin 24.8% ($p.<.001$), of health of trait and affecteds 29.0% ($p<.001$) and of epidemiology of S hemoglobin 24.2% ($p<.001$). The six questions on genetic segregation all improved, four to a statistically significant level (all $p<.04$). These results demonstrate that videotape presentation can deliver carefully edited subject matter in an educationally effective manner. It is concluded that such an audiovisual counseling program can be of considerable value as an adjunct to genetic counseling in a sickle cell screening survey. (Supported by NIH Project No. 71-2)

SICKLE CELL TRAIT:AN INSTRUMENT FOR MEASUREMENT OF COUNSELEES UNDERSTANDING,Ray M.Antley and LarryC.Hartlage,Indiana Univ. Sch.of Med.,Dept.of Ped.,Indianapolis (Intr.by I.K.Brandt).

The purpose of this project was to help evaluate the effectiveness of genetic counseling in Sickle Cell screening programs,by developing an instrument to measure the content information learned by counselees from the counseling. Requirements for the instrument would involve its ability to measure relevant information,be applicable to a wide range of educational levels,and require a minimum of time to administer. To develop this instrument,a 50 item questionnaire was administered to inner city high school students. Their responses were used to construct a quartile for each question in each grade level.From the quartile ratings,20 questions covering the genetics of hemoglobin,health of trait and anemic individuals, and epidemiology of S hemoglobin were selected.The revised instrument was then used to measure the information gained by students who watched a film of an instructive genetic counseling session.The scores of 9th,10th,11th, and 12th graders all improved an average of more than 20% per student,in all informational content cagegories (all $p<.006$).Greatest improvement was found in knowledge of epidemiology (26%; $p<.001$).The results using the mini test indicate that it is sensitive for discriminating improvement in knowledge of S hemoglobin over a wide range of educational levels. This implies that it can be used for most genetic counselees without regard for their educational level and that is will provide criterion information for evaluating the effectiveness of genetic counseling.
Supported by NIH Project No. 72-7

THE EFFECTS OF GENETIC COUNSELING FOR DOWNS SYNDROME, Ray M. Antley and Lawrence C. Hartlage, Indiana Univ. Sch. of Med., Dept. of Ped., Indianapolis (Intro. by I.K. Brandt).

The purpose of this study was to evaluate the effectiveness of genetic counseling for Downs syndrome in imparting genetic information and understanding of probability. Thirteen families who had received genetic counseling at a university medical genetics department were compared to 9 families who had received no genetic counseling and 11 families who had been counseled by their family physician on such factors as knowledge regarding genetics of Downs, understanding of probability, and information about prenatal diagnosis. The three groups were compared on 15 items. The parents receiving genetic counseling scored higher on 13 of the items ($p<.004$). Analysis of individual items indicate counseled parents had a significantly better concept of what chromosomes are ($p<.02$); of risk for Downs in the general population, in their relatives, and in themselves (all $p<.05$); of the practicalities of prenatal diagnosis; and of probability ($p<.01$). In general families counseled by their private physician were better informed than the uncounseled group. This study indicates the greatest gain in knowledge for counselees is in regard to recurrence risk for themselves and their children. Counseled parents on the average had more children than the other two groups and indicated that this information was important to their decision to have future children. (Supported by NIH Project No. 72-7)

BIRTH WEIGHTS OF OFFSPRING OF MATERNAL AND PATERNAL IDENTICAL TWINS. Patricia I. Bader, Glenn J. Bingle, Pao-lo Yu, Walter E. Nance. Indiana University School of Medicine, Department of Medical Genetics, (Introduced by Ira K. Brandt), Indianapolis, Indiana.

Previous twin and family studies have indicated that genetic factors have a significant influence on birth weight. Furthermore the positive correlation between birth weights of maternal first cousins has been taken as evidence for a significant maternal effect. We have tested directly for this effect by an analysis of the birth weights of 84 offspring of 10 female, and 6 male monozygotic twins. The offspring of identical twins are related to each other in the same way as half-siblings and comparisons of full and half-sibling correlations in these families permit estimation of the additive and dominance components of the genetic variance. Maternal effects may be detected by comparing the variation between sibship means within male and female half-sibships. In the present study of birth weight, the variance of sibship means in male monozygotic half-sibships (derived from genetically unrelated mothers) was more than 4 times as great as the variance for female half sibships (derived from genetically identical mothers): The F value, (F=0.6671/0.1509=4.43) was significant at the 0.025 confidence level. The data provide evidence for the existence of genetically determined maternal effects on birth weight in man.

AMNIOCENTESIS FOR THE PRENATAL DIAGNOSIS OF GENETIC DISORDERS Barbara F. Crandall and Robert S. Sparkes (intr. by E. Richard Stiehm)UCLA Ctr.Health Sciences,Dept.Ped.L.A.,Ca90024

Amniocentesis is rapidly evolving into a very useful adjunct to genetic counseling and for the prevention of certain genetic disorders. Experience with this technique in most medical centers is limited, and for this reason we wish to report on the first 100 cases of amniocentesis by the Medical Genetics Unit at the UCLA Center for the Health Sciences. 90% of these studies were performed for possible chromosomal abnormalities, a previous child with trisomy 21 being the largest proportion. In approximately 25% of the patients the indication was maternal age greater than 35 years; a translocation in one parent or a previous child with another chromosome abnormality accounted for a further 20%. Sex linked diseases and metabolic disorders were minor categories. There were two "spontaneous" abortions following amniocentesis; one occurred four weeks later and was probably unrelated to the procedure. 82% of the first 50 and 98% of the second 50 studies were successful. There was one chromosomally unbalanced fetus, a D-G translocation Down's, therapeutically aborted at 20 weeks. Trypsin-Giemsa banding was necessary to identify a balanced translocation (46,XY,t(9p-;16q+)) in another fetus and this was confirmed on cord blood from the normal infant at birth. We believe that amniocentesis for prenatal diagnosis is a relatively safe procedure which will be more widely used especially for increased maternal age and specific metabolic errors.

BRAIN β-GALACTOSIDASE IN TYPES I AND II GENERALIZED GANGLIOSIDOSIS. Liang Chou*, Celia I Kaye* and Henry L Nadler, Northwestern University Medical School, Department of Pediatrics, Children's Memorial Hospital, Chicago.

GM_1 gangliosidosis, a lipid storage disease associated with a generalized deficiency of β-galactosidase (β-gal) activity, exists in two phenotypic forms. Type I (I) presents in early infancy with severe neurological, visceral and bony involvement and is rapidly fatal. Type II (II) is of later onset and primarily involves the central nervous system. Differences in the pH optima and thermostability of the residual β-gal in skin fibroblasts suggested I and II were distinct entities. Other investigators studying liver β-gal detected no differences.

In an attempt to resolve this question, β-gal from brains of normals (C) and patients with I and II were studied. The pH optimum of β-gal in C brain is 4.1 while the optimum in I and II is 3.1. Residual enzyme activity in I and II brain is approximately 10% (pH 4.1) and 30% (pH 3.1) of normal. The β-gal in both I and II is stable at 42° C whereas C is heat labile. Electrophoresis of β-gal from C and I brain on both cellulose acetate and starch gel demonstrated two distinct bands. In contrast, β-gal from II demonstrates only one band corresponding to one of the two bands in C and I brain. These data suggest that the basic defects in I and II are distinct. Some caution should be exercised in interpreting these data as artificial substrates were utilized. Studies using the natural substrate GM_1 ganglioside should provide the answer.

TRIGLYCERIDE AND ACID LIPASE DEFICIENCY IN TRIGLYCERIDE STORAGE DISEASE, A POSSIBLE VARIANT OF WOLMAN'S DISEASE. W.R. Den Tandt*, Michel Philippart, Seiji Nakatani* and Paolo Durand*. Neuropsychiatric Inst., Los Angeles and Inst.Gaslini, Genoa,It.

Fatty livers were obtained from a new-born with "fatty metamorphosis of the viscera" (J.Peremans), a 10 yr-old mentally retarded girl and a 32 yr-old who compained of chronic abdominal pain. These cases can be described as triglyceride storage disease (TGSD). Control livers included normal autopsy and biopsy material, and cases of Reye's syndrome (n=1), alcoholic cirrhosis (n=4), Wolman's disease (WD) (n=3) and cholesterol ester storage disease (CESD) (n=1). Triglyceride lipase activity, using 1-^{14}C-trioleate, was about 1 percent of normal in both TGSD and WD. All other controls, including fatty livers overloaded with triglyceride, had normal activity. Acid lipase activity, using o-nitrophenylpalmitate, was 10 percent of normal in TGSD, 4 percent of normal in WD and CESD and normal in all other controls. β-glucosidase, β-xylosidase and α-mannosidase were somewhat decreased in TGSD, WD and CESD, as well as in alcoholic cirrhosis. These findings suggest that TGSD, except for the lack of overt accumulation of cholesterol esters, is very similar to WD. Striking discrepancies between probands, but not between members of the same pedigree, emphasize genetic heterogeneity. A genetic factor, rather than a secondary metabolic inhibition of triglyceride lipase, is indicated by the normal activity of this enzyme in fatty livers from Reye's syndrome and alcoholics . No inhibitor was found in TGSD.

NEVOID BASAL CELL CARCINOMA SYNDROME: STUDY OF A FAMILY. Stephen D. Codish (Intr by I.A. Porter)
Department of Pediatrics, Albany Medical College, Albany, New York 12208

Evidence of the nevoid basal cell carcinoma syndrome (NBCCS) was seen in 9 members of a family in 4 generations. Five of the 6 children (3 female) in the fourth generation displayed osseous and ectodermal anomalies characteristic of the syndrome, the hallmark of which is multiple basal cell carcinomas. Roentgenograms depicted rib anomalies in 4 of these children, spina bifida in 2 and mandibular cysts in one. Characteristic facies (broad nasal root, frontal bossing, ocular hypertelorism) were uniformly present. Piezogenic papules appeared on the feet of 2 affected children, and intellectual development was slowed in all five. The family pedigree showed a vertical pattern of inheritance of basal cell carcinomas. Immunoglobulin D could not be detected in 6 of 7 family members; IgG, IgM and IgA levels were normal. This recently described syndrome appears to be inherited as an autosomal dominant condition with variable expression.
(Supported by the Kidney Disease Institute and Birth Defects Institute, N.Y.S. Department of Health)

THE INTRARENAL SOURCE OF URINARY MUCOPOLYSACCHARIDES AND LYSOSOMAL ENZYMES IN MUCOPOLYSACCHARIDOSIS. Robert P. Erickson William van B. Robertson, Robert Sandman, and Charles J. Epstein. Univ. of California, San Francisco Sch. of Med. and Stanford Univ. Sch. of Med., Depts. of Pediat., San Francisco and Palo Alto.

Although mucopolysaccharidurias has been the sine qua non of mucopolysaccharidosis (MPS), little attention has been paid to the origin of these glycosaminoglycans (GAG) or of the lysosomal enzymes which are frequently elevated in the urine of these patients. We have studied the clearance ratios (C_X/$C_{creatinine}$) of GAG, β-galactosidase (β-gal), β-glucuronidase (β-gluc) and N-acetylglucosaminidase (N-acet) in normal individuals and in patients with renal failure, a presumptive MPS without mucopolysaccharidurias, and MPS II (Hunter's). In the first three groups we find an inverse relationship between the clearance ratios and the serum levels of GAG or enzymes. This is what is expected if these substances are not cleared by filtration but are secreted at a constant rate by the kidney (presumably by epithelial desquamation). In cases of MPS II, urinary GAG, β-gluc, and N-acet are present in much larger amounts than is predicted by this relationship. It is suggested that GAG, β-gluc and N-acet are stored in abnormal amounts in renal cells (in MPS II skin, β-gal is decreased, β-gluc and N-acet are increased while serum β-gal is elevated) and are secreted in urine. Therefore, in cases of MPS which do not include abnormal storage in the kidney, we would not expect mucopolysaccharidurias.

CYSTATHIONINE SYNTHASE DEFICIENCY: ENZYMATIC AND ULTRASTRUCTURAL STUDIES OF LIVER FROM HETEROZYGOTES AND FROM HOMOZYGOTES TREATED WITH PYRIDOXINE: Gerald E. Gaull, Fenton Schaffner & John A. Sturman. Dept. Ped. Res., N.Y. State Inst. Basic Res. Ment. Retard., Staten Island, N.Y.; Depts. Ped. & Med., Div. Med. Genetics, Clin. Genetics Center & Clin. Res. Center, Mt. Sinai Sch. Med. of the City Univ. of N.Y.

Hepatocytes from B_6-unresponsive homozygotes for cystathionine synthase deficiency show striking abnormalities on electron microscopy: unusually-shaped mitochondria, increased smooth endoplasmic reticulum and increased numbers of lysosomes (Gaull & Schaffner, Ped. Res. 5: 23, 1971). We have now studied heterozygotes and additional homozygotes, both unresponsive and responsive. The same morphologic changes are present in four obligate heterozygotes. Treatment with massive doses of B_6 failed to change the morphologic findings in two unresponsive homozygous siblings and in two responsive but unrelated homozygotes.

Enzymatic evidence of genetic heterogeneity is presented: Two unresponsive patients had no detectable synthase activity before or after B_6. One responsive patient showed 9% normal synthase activity before B_6, but no significant increase after; another responsive patient showed 4% normal synthase activity before B_6, increasing to 16% after. A fifth patient, an atypical homozygote or a heterozygote, also showed a four-fold increase in synthase activity following B_6 treatment.

The genetic and metabolic significance of these findings will be discussed.

SEX RATIO IN THE PROGENY OF MOTHERS WITH TOXEMIA OF PREGNANCY. Richard C. Juberg, Louisiana State Univ. Sch. of Med. in Shreveport, Dept. of Ped., Shreveport.

Maternal-fetal incompatibility may be an essential component in the etiology of toxemia of pregnancy. An abnormally high sex (male:female) ratio suggests Y-dependent histoincompatibility; an exceptionally low sex ratio supports X-dependent histoincompatibility; prior studies have shown both.

Among 3,246 births in one hospital in 1969, 375 (11.5%) women manifested 1 or more of the following criteria of pre-eclampsia: (1) systolic blood pressure >140 mm. Hg, diastolic blood pressure >90, increase >30 systolic or increase >15 diastolic during pregnancy, (2) weight gain >5 pounds in any week after the 20th in gestation, and (3) proteinuria >30 mg./100 ml. (2+) one or more times.

The patients were grouped and sex ratio of the progeny determined for: (1) 104 (62 first pregnancy + 42 two or more pregnancies) women with any 2 of the criteria, the "pre-eclampsia" group, (2) 183 (90+93) with just elevated blood pressure during gestation, (3) 61 (30+31) with elevated blood pressure only on the day of delivery, (4) 23 (11+12) with just weight gain, (5) 4 (1+3) with only proteinuria, (6) all 375 with any manifestation, (7) 194 in their first pregnancy with any manifestation, and (8) 181 in their second or later pregnancy with any manifestation.

There were no significant differences for any group compared with the secondary sex ratio expected of the population (94.7% negro).

CHROMOSOME STUDIES ON 11,357 NEWBORN INFANTS - John L. Hamerton, M. Ray and Nolia Canning, The Children's Hospital of Winnipeg, Department of Genetics, Winnipeg, Canada.

Chromosome studies, using conventional staining and an initial two cell analysis, on 11,357 newborn infants born in one hospital between 1st Feb. 1970 and 31st Dec. 1972 have revealed 49 infants with major chromosome abnormalities (4.3/1000 births) and 192 infants with significant chromosome variants. Of the 49 major abnormalities, 14 involve sex chromosomes (3 - 47,XXX; 3 - 47,XYY; 6 - 47,XXY; 2 - mosaic); 14 are autosomal trisomics (12 - +21; 1 - +13; 1 - +18) and 21 are chromosome rearrangements (11 balanced and one unbalanced reciprocal translocation, 9 - t(DqDq) balanced Robertsonian translocations). This data will be compared with other neonatal surveys comprising 28,717 newborn infants. The results of characterization by means of Q- or G-banding of many of the major chromosome abnormalities will be presented. Preliminary results on G- and C-band variation in 2000 consecutive newborn infants will be presented.

NEPHROBLASTOMA (WILMS'S TUMOR) IN ONE OF MONOZYGOUS TWINS AND IN ANOTHER SIBLING. Richard C. Juberg, Eugene C. St. Martin, and J. Roderick Hundley, Louisiana State Univ. Sch. of Med. in Shreveport, Depts. of Ped. and Urology, Shreveport.

Genetic determination of nephroblastoma is suggested from its occasional occurrence in successive generations, in sibships, or in twins. Some evidence points to different etiologies of this childhood tumor--an "hereditary" form being autosomal dominant with high penetrance, early age of onset, and high incidence of multiple tumors, and a "nonhereditary" form occurring sporadically, with later age of onset, and single tumors. Two mutational events precede each; in the former one mutant is transmitted but in the latter both mutations arise in the same cell line.

A young nonconsanguineous couple has had 3 pregnancies: (1) spontaneous abortion, (2) twin males, (3) singleton male. At age 23 1/2 months one twin had a right nephroblastoma removed; 9 months later, at age 13 months the singleton male had a right nephroblastoma removed. By 4 1/2 years the unaffected twin showed no evidence of tumor, and both affected siblings had no further involvement.

Diagnosis of monozygosity is based on: (1) the twins' similarity in many physical features, (2) the probability of 0.99 from their similarity in 10 blood groups, and (3) bi-, homo-, and heterolateral comparisons of their dermatoglyphics.

So long as the twin remains unaffected and the two affected siblings show no evidence of multiple occurrence, the sibship seems contrary to hereditary determination. The nonhereditary form seems unlikely because of the occurrence in two siblings.

EVALUATION OF POTENTIAL SCREENING TESTS FOR HETEROZYGOUS β-THALASSEMIA (THAL). Glen H. Hoversten, and George R. Honig. Univ. of Illinois College of Medicine, Univ. of Ill. Hospital, Sickle Cell Center, Chicago, Illinois.

An extensive effort is being carried out to detect individuals genetically at risk of having offspring with symptomatic forms of sickle cell disease. Simple and inexpensive means are widely employed for detection of hemoglobins S, C, and D, but a readily applicable test has not been generally available to detect β-thal trait. Three determinations were evaluated for applicability as β-thal screening tests in black individuals: 1) erythrocyte osmotic fragility was measured using a Fragiligraph; 2) red cell indices were determined with a model S Coulter counter; 3) blood smears were examined for fetal hemoglobin-containing cells by a slide-elution procedure. The study included 32 black subjects of which 12 had heterozygous β-thal with elevated levels of hemoglobin A_2, and 20 had normal values for hemoglobins A_2 and F. In the osmotic fragility test all of the β-thal carriers showed times of at least 180" from the start of the study until the peak derivative value was reached, whereas 2 of the nonthal individuals exceeded this time. MCV values less than 75 μ^3 were obtained with all thal subjects and in 1 of the 20 controls. The MCH was less than 25 pg in all of the thal carriers and in 4 of the 20 control subjects. Fetal hemoglobin-containing cells were seen only in 3 β-thal patients. Determinations of the MCV or Fragiligraph osmotic fragility both appear to be suitable screening tests for β-thal trait in the black population.

LEIGH'S DISEASE: DETECTION OF THE HETEROZYGOUS CARRIER. Jerome V. Murphy (Intr. by Frederick J. Samaha) Univ. of Pittsburgh, School of Medicine, Children's Hospital of Pittsburgh, Dept. of Neurology, Pittsburgh, Pennsylvania.

When the test has been performed, all untreated patients with autopsy-proven Leigh's disease have had an inhibitor to the synthesis of thiamine triphosphate (TTP) in their urine, blood and CSF. In the past year urine and blood have been collected from the relatives of eight patients with Leigh's disease. In six patients the diagnosis was confirmed by autopsy and in two more patients the diagnosis has been made on clinical grounds. Utilizing an acetone extract of brain as the source of thiamine pyrophosphate phosphotransferase, control urine and blood inhibited the enzyme 0% to 10%; urine and blood from all 15 parents consistently inhibited more than 10%. When both members of a grandparent pair were available, only one person carried the inhibitor. The incidence of inhibitor in siblings, aunts and uncles is below that expected for a randomly distributed gene. Neither the amount of inhibition nor the chemical characteristics of the inhibitor could distinguish a carrier urine from the urine of a patient afflicted with Leigh's disease. It is suggested, therefore, that urine from all patients suspected of having Leigh's disease, as well as urine from both parents, be assayed for this inhibitor. Although the presence of inhibitor in all three urines will not prove the diagnosis in the patient, its absence in just one parent indicates that the patient does not have Leigh's disease.

IMPAIRED SULFATE TURNOVER IN CULTURED SKIN FIBROBLASTS AND AMNIOTIC CELLS FROM PATIENTS WITH MUCOPOLYSACCHARIDOSIS. Michel Philippart, Klaske Zeilstra*and Elsa Kamensky*. Mental Retardation Unit, Neuropsychiatric Institute, Los Angeles.

Using ^{35}S-sulfate we have developed a simple diagnostic procedure. Confluent cultures (T-30 flasks) are pulse-labeled for 2 days (20 μc). Cells are harvested at the end of the pulse and at 3, 7, 13, 28 and 42 day intervals. A first extract is obtained by freezing and thawing in water. Insoluble material is dissolved in 0.5 N NaOH. Proteins are precipitated with trichloroacetic acid (TCA 10)(10 percent w/v). Total counts were increased 3-fold at the end of the pulse and plateaued for a month in fibroblasts from patients with mucopolysaccharidosis type I and V and Leroy's I-Cell disease. Total counts had a half-life of 6 days in normal fibroblasts and 3 days in normal amniotic cells. In amniotic cells from Hurler's disease, incorporation was not increased but plateaued so that twice as many counts remained after one week and 3 times as many after 2 weeks. In amniotic cells and fibroblasts from patients with mucopolysaccharidosis type I,V and VI and I-Cell disease, 60 to 80 percent of the water-soluble counts were found in TCA 10, compared to 20 to 40 percent in control fibroblasts and 10 percent or less in control amniotic cells. Preliminary results with Sanfilippo disease type A indicated that this test is probably not applicable for the diagnosis of this condition. (Supported by the Dept. of Mental Hygiene, State of California and PHS grants NB 06938, HD 04612, MCH 927, HD 00345 and HD 05615).

IS MUCOLIPIDOSIS III A HURLER ALLELE? Meinhard Robinow and Shirley M. Soukup. Univ. Cincinnati Sch. of Med., Children's Med. Ctr. Dayton,O. and The Children's Hosp. Research Fndn. Cincinnati.

Observations by the authors suggest that mucolipidosis III (ML III) is a mucopolysaccharide (MPS) disorder and that the enzyme defect is closely related to that of Hurler's disease. Cultured fibroblasts from two brothers suffering from ML III accumulated MPS. The intensity of accumulation was within the range found in the mucopolysaccharidoses, as shown by staining with Toluidin blue, Alcian blue and aldehyde fuchsin and by the ^{35}S uptake.

MPS accumulation in subcultures could be prevented by mixing with fibroblasts of normal individuals and fibroblasts of patients with Sanfilippo's disease and two unclassified MPS disorders but not by fibroblasts of two Hurler patients. Mixing experiments with fibroblasts of other MPS disorders are in progress and will be reported. The findings suggest that patients with ML III and Hurler's disease lack a similar "corrective factor".

THE ORIGIN OF THE EXTRA CHROMOSOME IN TRISOMY 21. Hope H. Punnett and Mildred L. Kistenmacher, Temple Univ. Sch. of Med., St. Christopher's Hospital for Children, Dept. of Pediatrics, Phila., Pa.

The G group chromosomes of 10 children with Down's syndrome and of their parents have been analyzed in the initial phase of a study of factors influencing nondisjunction of chromosome 21.

Using satellite size, length of satellite stalk, and variations in G banding patterns as markers, the parental source of the extra chromosome 21 in Down's syndrome was identified in two informative families. We could also determine in which meiotic division nondisjunction of the marker region occurred.

In the first family, nondisjunction occurred in the father's second meiotic division, since two identical paternal 21 markers were seen in the male trisomy 21 child. This is the first reported case of paternal nondisjunction identified in a child with Down's syndrome. In the second family, second division nondisjunction occurred in the mother of a female trisomy 21 child as indicated by the presence of both maternal 21's. All 4 parents were under 30 years of age. Confirmatory studies with fluorescence and C banding are in progress.

A GENETIC SYNDROME OF ISOLATED "TOTAL" APLASIA OF THE ANTERIOR PITUITARY. RECOGNITION AND TREATMENT WITH SURVIVAL. A. Sadeghi-Nejad, A. Binkiewicz and B. Senior. Pediatric Endocrine-Metabolic Service, Tufts-New England Medical Center Hospitals, Boston

A male newborn infant of normal size developed hypoglycemia, collapsed and convulsed at 8 hours. The sole physical abnormality was a small penis, bifid scrotum and minute testes. He responded to glucose and glucocorticoids. Studies revealed

TRF Stimulation (100 μg)	0'	20'	40'	60'
TSH (μU/ml)	1.3	1.7	1.7	2.4
T4I (μg%)	.5	1.2	0	0
Prolactin (ng/ml)	<8	<8	<8	<8

Growth Hormone (ng/ml)	F	5'	15'	30'	45'	60'	90'
Arginine stimulation	.7	--	--	.8	.7	.8	.8
Glucose stimulation	.8	.7	.7	.7	--	.8	--

The electrolytes were normal. With replacement of glucocorticoids and thyroid hormone, he remained stable but growth approached normal only when growth hormone was added. A previous female sibling had died on the first day of life with similar symptoms. Isolated pituitary aplasia and atrophic adrenals were present.

Analysis of reports of this rare condition, the most severe form of anterior pituitary insufficiency, reveals a familial and recognizable entity transmitted as an autosomal recessive. Although all previous cases have died, the distinctive features permit diagnosis and, therefore, the possibility of effective treatment.

CLINICAL, RADIOGRAPHIC, HISTOLOGIC AND ULTRASTRUCTURAL DEFINITION OF THE KNIEST SYNDROME. D.L. Rimoin, D.W. Hollister, D. Siggers, R. Silberberg, R. Lachman, W. McAlister, R. Kaufman, V.A. McKusick and J. Dorst. UCLA-Harbor General Hospital, Torrance, Ca., Washington Univ., St. Louis, Mo. and The Johns Hopkins University, Baltimore, Maryland.

The Kniest syndrome is a newly defined chondrodystrophy consisting of disproportionate dwarfism with kyphoscoliosis, flat face with prominent eyes, myopia, cleft palate, hearing loss and limited joint motion. The radiographic features consist of marked platyspondyly with vertebral irregularity, shortening of tubular bones with metaphyseal flaring, and epiphyseal delay, irregularity and stippling. Clinical, radiographic, histologic and ultrastructural studies have been performed on 5 patients with this syndrome. Biopsies of costochondral junction and iliac crest reveal identical histological and ultrastructural abnormalities which differ from all other chondrodystrophies. The cartilage is friable and irregular in both cellular size and matrix staining. Throughout the cartilage are large hypertrophic cells whose surrounding matrix is loose and irregular in staining ability, with large holes resembling Swiss cheese. Electron microscopy reveals chondrocytes with markedly dilated cisternae of the endoplasmic reticulum containing finely granular electron opaque contents. Focal microscars containing large collagen bundles are seen. The Kniest syndrome represents a distinct chondrodystrophy associated with specific abnormalities in cartilage structure which suggest a defect in collagen metabolism.

AN ASSOCIATION BETWEEN DUFFY GENOTYPE AND GALACTOKINASE ACTIVITY: SUPPORT FOR A RACIAL POLYMORPHISM FOR THE Gk GENE. T.A. Tedesco, K. Miller, H. Wurzel, H. Boedecker, E. Rawnsley, and W.J. Mellman. Dept. of Human Genetics and Pathology, Univ. of Pa. Sch. of Med.

We recently reported quantitative evidence of a racial polymorphism for galactokinase (Gk) in man where significantly lower RBC Gk activity was found in blacks as compared to whites. Since the frequency of Duffy genotype Fy $a^+/Fy\ b^+$ approaches zero in African blacks, is nearly 50% in whites, and has been used to estimate genic admixture in U.S. blacks, we looked for an association between Gk activity and Duffy genotype. Blood samples from 370 blacks were typed for both Fy a and Fy b antigens. The same bloods were assayed for RBC Gk activity. The results in Table I suggest that the portion of this black population that is Fy $a^+/Fy\ b^+$ has significantly higher Gk activity than that found in the other three Duffy genotypes, approaching that found in whites. We consider this supporting evidence for the previously described racial polymorphism for galactokinase.

Table I

Group	Fy Genotype	n	$\bar{x}$	S.D.	S.E.
Blacks					
A	a^-/b^-	221	0.2397	0.0564	0.0037
B	a^-/b^+	86	0.2509	0.0567	0.0061
C	a^+/b^-	49	0.2474	0.0512	0.0073
D	a^+/b^+	14	0.2789	0.0660	0.0176
Whites		386	0.3160	0.0652	0.0033

CEREBRAL GIGANTISM (SOTOS' SYNDROME): EVIDENCE FOR RECESSIVE INHERITANCE. Philip L. Townes and Albert P. Scheiner. Dept. of Pediatrics, University of Rochester School of Medicine, Rochester, New York.

Cerebral gigantism is generally considered to be of sporadic etiology; neither parents nor sibs have been noted to be affected. Hook and Reynolds (J. Ped. 70:900, 1967) reported concordant twin boys but concluded that the twins "provided no definitive evidence concerning etiology since concordance could even occur if environmental factors were exclusively responsible".

Reported here are observations of a family which provides clear evidence of autosomal recessive inheritance of this syndrome. Affected are a boy and his monozygotic twin sisters. Their clinical and laboratory findings are characteristic of cerebral gigantism. Another sib is also mentally retarded. Parents are of below average height and family history is otherwise negative except that consanguinity is not excluded.

Except for this family and the one concordant twin pair cited above there has been no prior evidence of recessive inheritance in the small number of patients reported. This family provides clear evidence for recessive inheritance and thereby a reason for caution in counseling families regarding recurrence risk which has previously been considered to be negligible.

INFANTILE FAMILIAL AGRANULOCYTOSIS WITH A RING G CHROMOSOME. Robert Wilkinson, Darleen Powars, Stebbin Chandor, June Marshall, Charles Bacha (Intr. by Paul F. Wehrle); Univ. of So. Calif. Med. Ctr., Dept. of Ped., Los Angeles, Calif.

Morphologic abnormalities of G chromosome group have been repeatedly associated with a variety of myeloproliferative syndromes. A family has been observed during 10 years with six children, three of whom have died. The clinical complex consisted of acute enterocolitis, bacterial septicemia, massive intertriginous skin infections, severe oral moniliasis and a rapidly progressive downhill course. All patients had an absolute agranulocytosis. Variable hematologic abnormalities were thrombocytopenia, anemia and reticulocytopenia. Autopsies of two children showed severe membranous enterocolitis, stress thymic involution with cystic Hassels corpuscles, inadequate lymph node germinal follicle formation and essentially no myelocytic inflammatory response in all tissue including the bowel wall. Cytogenetic studies performed on ante mortum bone marrow of the last affected sibling revealed a consistent ring in the G chromosome group. There was abnormal myelopoiesis showing no normal maturation beyond the myelocytic stage. Immunoglobins, PHA lymphocytic transformation, white cell myeloperoxidase were normal in all family members studied, including the parents and non-affected siblings. Cytogenetic studies of the parents and non-affected siblings were normal. This family represents a previously unreported association of familial agranulocytosis and a G ring chromosome.

VARIATION IN THE FREQUENCY OF FLUORESCENT Y CHROMATIN DURING THE NEWBORN PERIOD. J.P. Welch, H. Wellwood, and C.L.Y. Lee. (Intr. by Richard B. Goldbloom) Dept. of Pediatrics, Dalhousie Univ., Halifax, Nova Scotia.

The frequency of X-chromatin in females is known to change during the first few days of life, but the frequency of Y chromatin over a similar period in males has not previously been investigated.

Buccal smears were obtained from 33 male infants at <24 h of age, then every 24 h for 4-7 days, also from 12 adult females and 10 adult males. Slides were stained with quinacrine HCl and examined by an observer who was unaware of the age and sex of the donor.

The frequency of Y chromatin was found to rise steadily over the first five days of life, attaining the mean adult level by day three. Analysis of variance over the first 5 days indicated a significant change from day to day ($F = 7.1$ for 4 d.f., $P<0.001$).

The changing frequency of Y chromatin during the neonatal period is thus similar to that of X-chromatin and may be influenced by similar factors. These findings also have important practical implications for the attempted ascertainment in neonates of mosaicism involving the Y chromosome.

SERIAL CYTOGENETIC STUDIES OF LEUKEMIC BONE MARROW - Wolf W. Zuelzer, Susumu Inoue, Ruby I. Thompson, Mark J. Ottenbreit, Child Res. Ctr., Childr. Hosp. of Mich. and Wayne State Univ., Dept. of Pediatrics.

Therapy of acute leukemia (AL) aims at total eradication of the malignant cell population, implying a finite autonomous stem line. Cytogenetic data are in general agreement with this concept. However, reported relapses involving transplanted donor cells are consistent with the alternative of persistent leukemogens. Serial analysis of AL bone marrow karyograms can shed light on this question. 56 cases of childhood AL were studied in consecutive relapses with intervening remissions ranging from 6 to 57 months (median 13, mean 18), total observations extending up to 80 months.

In 32 cases the original karyotype emerged unchanged after remission. In 13 others, new markers or numerical changes occurred, but the persistence of original markers allowed definite identification of the initial malignant line. In 10 of the remaining 11 cases numerical changes in mixoploid populations or new markers were seen, consistent with, though not provably due to, mitotic errors or clonal evolution. In only 1 case the karyotype findings changed drastically (from an initial 53/54 to a diploid line). The usual stability of the original stem line in these data fits the assumption that relapse, even after very long remission, represents persistence of AL cells, except in rare cases and under unusual circumstances when "new" leukemia may account for relapse and persistence of systemic leukemogens may be postulated.

FREQUENCY AND BIOLOGICAL SIGNIFICANCE OF CHROMOSOME ANOMALIES IN SELECTED SAMPLES OF THE GENERAL POPULATION. J.P. Welch, E.J. Winsor, S.K. Vethamany, C.L.Y. Lee, and J.A.R. Tibbles. (Intr. by Richard B. Goldbloom) Dept. of Pediatrics, Dalhousie Univ., Halifax, Nova Scotia.

Unusual stature is a common accompaniment of certain major chromosomal disorders (XXY, XYY, and XO) but the effect on growth and development of the possession of minor chromosomal variants is largely unknown.

Height was measured on 97.7% of a total urban school population of 11,165 children. Blood samples were obtained from 104/144 'tall' boys and 71/166 'tall' girls (each >2 SD above mean ht. for age) and from 83/109 'short' girls (>2 SD below mean ht. for age). Among the 'tall' boys, one was XXY, one XYY, and one was a balanced t(14q15q). There were no major anomalies among the 'short' girls and analysis of this group failed to disclose any evidence of XO mosaicism. Among the 'tall' girls one (sister of the corresponding male case) had a balanced t(14q15q). A total of 16 children with minor chromosome variants were found by routine staining; these were approx. equally distributed among the three groups. Subsequent banding techniques have shown that these include anomalies of chromosome 13, 14, 15, 21, and 22.

Evaluation of these children with a paired control group has not shown any indication of impaired neurologic or psychologic function, or significantly decreased birth weight in those children with 'minor' chromosomal variants.

HEMATOLOGY AND ONCOLOGY

First Session

B-CELL PROLIFERATION MANIFESTED AS ACUTE LYMPHOSARCOMA CELL LEUKEMIA AND LYMPHOMA IN ADOLESCENTS. Kazimiera J. Gajl-Peczalska, John H. Kersey, Mark E. Nesbit and Robert A. Good. Depts. of Pathology & Pediatrics, University of Minnesota, Minneapolis, Minnesota 55455. Two cases of lymphoma in adolescents which seem to represent monoclonal IgM-lambda B-cell proliferation were studied. The one had morphologic features of undifferentiated (Burkitt's type) lymphoma and presented as acute leukemia with 51.2% of membrane-bound IgM-lambda lymphocytes in peripheral blood. The other case was a lymphoma without peripheralization, of 4 years duration. The patient was successfully treated with x-rays and a lymph node removed 2 years after treatment did not show evidence of lymphoma on routine histological evaluation. However, the analysis of viable lymphocytes from the same node disclosed an abnormal increase in IgM-lambda bearing cells. The possible clinical implications of these findings will be discussed. (Aided by grants from The National Foundation-March of Dimes, American Cancer Society, U.S. Public Health Service, AI-00798, AI-08677, and a contract from NIH SVCP 71-2261).

PLATELET INFLUENCE ON NEUTROPHIL CHEMOTAXIS & PHAGOCYTOSIS. C.C. Clawson, (Intro by J.G. White), Dept. Ped., Univ. Minn., 55455. Human platelets have previously been shown to react in a physiologic manner to contact with a variety of common pathogenic bacteria. This interaction results in irreversible platelet aggregation in which bacteria are incorporated in large numbers in forming aggregates. A critical question raised by these observations is: What influence does platelet-bacterial interaction have on normal functions of human neutrophils in their role as an antibacterial defense agent? Neutrophil chemotaxis was studied under the influence of albumin-washed platelets alone and in various combinations with bacteria, pooled serum, and heat inactivated serum. Neutrophil phagocytic and bactericidal effects on Staph. aureus were quantitated in the presence and absence of platelets. These studies demonstrated that washed platelets or S. aureus alone were not a potent chemotactic stimulus, but in combination the platelet-bacterial aggregation reaction produced a strong chemotactic influence. The neutrophil response was quantitatively nearly as great in a serum-free system as when complement was present. In the phagocytic studies where presence or absence of platelets was the only variable there was a marked increase in bacterial killing over a 60 min period when platelets were present. The results of these studies provide further evidence that platelets may have a significant impact on the fate of intravascular bacteria. (Supported by Amer. Heart Assoc. and NIH, AI-10033 and CA-11996)

2,3-DIPHOSPHOGLYCERATE (DPG) AND THE CONTROL OF RED CELL GLYCOLYSIS. Frank A. Oski, Joan Urmson, Patricia O'Neal. S.U.N.Y., Upstate Medical Center, Syracuse, New York.

Red cell DPG, a potent modifier of hemoglobin-oxygen affinity, has been suggested to play a role in the control of red cell (RBC) glucose consumption. DPG was found to inhibit hexokinase, phosphofructokinase, phosphoglycerate kinase and pyruvate kinase activity of hemolysates prepared from both adult and newborn's RBC's. RBC's from newborns and adults were prepared at varying DPG levels, while ATP levels were kept constant, and measurements of RBC glucose consumption, pH, and glycolytic intermediates were performed. An inverse relationship between red cell DPG and glycolytic rate was observed. RBC glucose consumption, in the newborn, rose from 2.7 to 4.2 umoles/ml hour when DPG fell from 5-7 to 1 umoles/ml and glucose consumption fell to 1.05 when DPG rose to 10-12 umoles/ml. Adult cells showed similar qualitative responses. The apparent Ki for DPG inhibition of glycolysis in the intact cell was at 8.1 umoles. Analysis of intermediates indicated that DPG inhibition was at the hexokinase step and was not related to changes in intracellular pH. RBC glycolysis was similarly increased in a patient with pyruvate kinase deficiency as the high DPG level was reduced. These studies demonstrate that DPG plays a role in the control of red cell glycolysis and hemoglobin-oxygen affinity.

NEUTROPHIL (PMN) METABOLISM AND BACTERICIDAL FUNCTIONS IN CONGENITAL PHOSPHOGLYCERATE KINASE (PGK) DEFICIENCY. Ronald G. Strauss, Dennis J. McCarthy and Alvin M.Mauer. The Children's Hospital Research Fndn., Cincinnati, Ohio.

PGK is an enzyme in the Embden-Meyerhoff glycolytic pathway regulating ATP production. X-linked recessive deficiency of erythrocyte (RBC) PGK is associated with hemolysis and neurologic abnormalities. Although infections generally are not a problem, PMN dysfunction has been reported in this disease. ATP for normal bacterial engulfment was provided by increased Kreb's cycle activity, but a defect in postphagocytic oxidative metabolism (glucose-1-^{14}C oxidation and Staphylococcal iodination) was considered to cause inefficient killing. (Baehner, R. L., et. al: Blood, 38:833, 1971).

In two boys from a new kindred with nearly absent RBC and PMN PGK, however, PMN functions were normal. PMN $^{14}CO_2$ production from glucose-1-^{14}C (hexose monophosphate shunt) and formate-^{14}C (H_2O_2 production) increased more than fourfold following latex particle ingestion. PMN killing of Staphylococcus, Pseudomonas and Streptococcus, and monocyte Staphylococcus killing were normal at 120 min. In PGK deficient RBC the block in glycolysis is bypassed via the Rapoport-Leubering shunt. The increased Kreb's cycle activity and normal post-phagocytic oxidative events in PGK deficient PMN suggest the existance of a comparable pathway allowing normal bactericidal function. Thus, PMN dysfunction is not present in all patients with PGK deficiency.

DESSICYTOSIS ASSOCIATED WITH PRIMARY RBC POTASSIUM LOSS: A NEW CONGENITAL HEMOLYTIC SYNDROME. B. Glader, N. Fortier, M. Albala and D. Nathan. Harvard Medical School, Children's Hospital Medical Center, Boston, Mass. and Rhode Island Hosp., Providence, Rhode Island.

We have observed a 3-year-old boy and his 19-year-old mother who have a new and unusual hemolytic anemia with hepatosplenomegaly, hyperbilirubinemia, and reticulocytosis. Most of the RBC were large dehydrated appearing "dessicytes" in which hemoglobin was "puddled", occupying only part of the total cell volume. In addition there were many contracted dessicytes, and notably very few stomatocytes. The RBC dehydration was also manifested by an elevated MCHC (42 gm/100 ml) and by increased osmotic resistance. The hemoglobin was normal as were RBC glycolytic enzymes, and hexose monophosphate shunt activity. RBC cation concentrations were strikingly abnormal. RBC-Na^+ was only slightly increased to 18.6 mEq/L RBC (normal 5-12 mEq/L RBC) but RBC-K^+ was markedly decreased to 59.1 mEq/L RBC (normal 95-105 mEq/L RBC). RBC-K^+ flux, measured with ^{42}K, revealed that these cells are very leaky and exchange K^+ at 7.5 mEq/L/h (normal 1.7 mEq/L/h). Active (ouabain inhibitable) influx was 6.6 mEq/L/h (normal 1.1 mEq/L/h). The dessicytosis in these RBC is due to an unique membrane lesion characterized by K^+ loss in great excess of Na^+ gain. The K^+ pump operates at an accelerated rate, but cannot prevent the K^+ loss. The result is cation and hence water loss (or dessicytosis), increased viscosity and premature destruction.

PHOTOSENSITIZED SHIFT IN THE O_2 DISSOCIATION CURVE OF FETAL BLOOD by Enrique M. Ostrea, Jr.* and Gerard B. Odell, Johns Hopkins Univ., Dept. of Pediatrics, Baltimore.

In vitro exposure (15 min) of washed fetal erythrocytes to blue light (9 mW/cm^2/420-480 nm) in the presence of bilirubin was associated with a significant decrease in the affinity of the fetal cells for O_2 when compared to the paired control samples protected from light: ΔP_{50} at pH 7.4 = +3.17 $\pm$ 0.69 mmHg, (n=7 pairs, $p < .005$). This decrease in O_2 affinity was only observed with intact fetal red cells, and not with adult cells or hemolysates of fetal or adult cells. The photosensitized ΔP_{50} occurred in fetal red cells suspended in albumin-free phosphate buffer at concentrations of bilirubin as low as 2 mg%.

In vitro exposure of pre-exchange whole blood also exhibited a similar ΔP_{50} if the serum albumin was highly saturated with bilirubin (Salicylate Saturation Index $\geq$ 8).

The ΔP_{50} was associated with evidence of membrane damage characterized by a significant decrease in the Na^+K^+ATP'ase and leakage of K^+ and hemoglobin. The Mg^+ ATP'ase and red cell 2,3 DPG were unchanged, and no qualitative alterations were observed in the electrophoretic behavior of the fetal hemoglobin or its γ-chain. It is proposed that the shift in O_2 dissociation of fetal cells represents an additional manifestation of the photodynamic action of bilirubin on red cells and suggests that the membrane of the fetal erythrocyte has a significant influence on the greater affinity of fetal red cells for O_2.

FIELD SCREENING OF CHILDREN FOR LEAD POISONING WITH THE FEP TEST. Sergio Piomelli, Patricia Young and Giselle Gay. Department of Pediatrics, New York University School of Medicine, New York.

These studies were directed to evaluate the efficiency of the FEP test in the screening of children for Pb poisoning. The FEP test is a micromethod for free erythrocyte porphyrins (FEP) developed in this laboratory which shows an excellent correlation with blood Pb level.

A modification of this test (FEP spot test) was performed on samples collected on filter paper, of the type used for PKU. The hemoglobin concentration was simultaneously measured and the concentration of FEP expressed as a ratio FEP/Hgb. A value above 6.2 µg FEP/g Hgb was considered positive; a value over 10 µg FEP/g Hgb was considered strongly positive.

A total of 1226 children were screened with this technique; of these 60 were positive. All positive cases were studied with Serum Fe/TIBC, blood Pb, radiological examination of bones for Pb lines and abdomen for Pb flakes and, when indicated, EDTA provocative test.

These studies revealed that of the 60 FEP positive children, 17 had Pb poisoning and 32 had Fe deficiency (of the latter 13 were markedly anemic). There were 11 false positives (all in the 6.3-9 µg FEP/g Hgb range).

These investigations indicate that the FEP test is an excellent screening method for the detection of lead intoxication and of Fe deficiency.

HbS AND HbF-SPECIFIC RADIOIMMUNOASSAYS FOR QUANTITATION OF HbS AND HbF IN FETAL BLOOD. Richard A Doherty Peter T. Rowley, Elsa Cernichiari, Mary Keller, Cheryl Rosecrans (Intr. by Gilbert B. Forbes). Depts. Ped., Med., Rad. Biol./Biophysics, Div. Genetics, Univ. Rochester Sch. Med., Rochester, N.Y.

Radioimmunoassay provides the specificity and sensitivity required for quantitation of Hbs in fetal blood. Hb S,F and A were purified by column chromatography and injected with complete Freund's adjuvant into goats, rabbits and chickens. Antisera were tested for reactivity against Hb S,F and A by immunodiffusion, quantitative precipitation and/or specific radioimmunoassay. Cross-reactivity was removed by precipitation of antisera with Hbs other than the Hb used to stimulate antibody production. Antisera specific for HbS and HbF have been obtained. HbS,F and A labelled with ^{125}I (chloramine-T method) are stable for >1 month. The assays permit reliable, specific quantitation of as little as 1 ng. of HbS or HbF. Assuming 10% of Hb in fetal blood at 16 weeks gestation to be adult type, the assay will detect the amount of HbS in $<10^{-6}$ ml. of SS fetal blood. Prenatal detection of sickle cell anemia (SS) vs. trait (SA) also requires a method for demonstrating absence of HbA and a method for safely obtaining fetal blood.

RAPID DIAGNOSES OF SICKLE CELL DISEASE AT BIRTH BY COLUMN CHROMOTOGRAPHY. Darleen Powars, Walter Schroeder (Intr. by Paul F. Wehrle); Univ. of So. Calif. Med. Ctr., Dept. of Ped., Los Angeles, Calif.

Accurate specific diagnoses of sickle cell disease can now be performed at birth on routinely obtained cord blood samples using a newly perfected method of micro column chromatography. The method uses a small column of a cation ion exchange resin, CM - Sephadex and a single developer that rapidly washes the Hemoglobin F from the column and allows definitive rapid distinction of hemoglobin types AS, AC, SS, SC and CC within two hours. Fifty samples a day can be analyzed by one technician with minimal biochemical background. A pilot program screening all newborn infants born in a small municipal hospital is now in progress. In six months time, 749 consecutively born infants have been studied without regard to racial origin for hemoglobin type. Definitive diagnoses was made, using single anticoagulated cord blood samples of 37 AS, 8 AC, 1 SS and 703 AA.

Newborn diagnoses of hemoglobinopathies utilizing rapid column chromotography is now ready to be used by large perinatal programs and should materially decrease the early childhood mortality associated with undiagnosed sickle cell disease.

CONGENITAL HEINZ BODY ANEMIA DUE TO HEMOGLOBIN ABRAHAM LINCOLN, β32 LEU → PRO. George R. Honig, Mir Shamsuddin, Loyda N. Vida, R. George Mason, David J. Gnarra, and Helen S. Maurer, Univ. of Illinois College of Medicine, Univ. of Ill. Hospital, Department of Pediatrics, Chicago, Illinois.

An abnormal hemoglobin was identified in a black female with severe hemolytic anemia since infancy. The anemia was accompanied by red cell inclusion bodies and excretion of darkly pigmented urine. The hemoglobin concentration varied from 4.8 to 11.0 gm/100 ml with reticulocyte counts of 20-40%. A ^{51}Cr red cell survival study demonstrated a $t\frac{1}{2}$ of 2.4 days, indicating a greatly accelerated rate of red cell destruction. Methemoglobin formation occurred at an increased rate in red cells from the patient, and reduced glutathione was present in decreased amounts. From 16 to 20% of the hemoglobin precipitated when hemolysates were heated at 50°. Precipitation was prevented by addition of carbon monoxide, dithionite, or hemin, suggesting that heme-binding is defective in the unstable hemoglobin. The P50 O_2 and Bohr effect were normal. An abnormal β chain was isolated by precipitation with p-hydroxymercuribenzoate. Peptide mapping demonstrated an abnormal chromatographic position of βT4, and analysis of this peptide demonstrated a substitution of proline for leucine at β32. Globin synthesis studies demonstrated an excess of α chain synthesis as compared to β chain synthesis, beyond that attributable to early destruction of newly synthesized β chains. This imbalance may contribute to the severity of the hemolytic process.

HEMATOLOGY AND ONCOLOGY

Second Session

IDENTIFICATION OF THE CIRCULATING BLAST CELL OF "JUVENILE" CHRONIC GRANULOCYTIC LEUKEMIA (CGL) AS A MONOBLAST. Arnold J. Altman, Catherine G. Palmer, and Robert L. Baehner, Indiana University Medical Center, Indianapolis, Indiana

Peripheral blood (PB) and bone marrow (BM) from 3 children with "juvenile" (Ph^1 chromosome-negative) CGL were studied for in vitro leukocyte colony production. Two children had circulating blast cells; the 3rd had normal PB but 12% blasts in BM. Washed leukocytes from PB or BM were suspended (10^5 cells/ml) in McCoy's 2A medium containing 0.4% methyl cellulose and incubated in petri dishes at 37° C in 10% CO_2 in air. BM produced 50-100 colonies/10^5 cells while PB from patients with circulating blasts produced 40-50 colonies/10^5 cells and 10 colonies/10^5 cells developed from the patient with no apparent circulating blasts. Normal PB produced no colonies. As expected, BM colonies were of 3 types: 1) pure granulocytic, 2) pure monocytic, 3) mixed monocytic and granulocytic. In contrast, patients' PB colonies were always pure monocytic and never granulocytic; the cells were phagocytic, contained I_gG receptors, and lacked peroxidase. A marker chromosome demonstrable in spontaneously dividing PB and BM blasts of one patient was also identified in his PB monocyte colonies. Addition of normal PB Conditioned Medium (CM) did not affect quantity or cell type of colonies from patients, nor did addition of patient PB CM affect development of colonies from controls. Clinical differences between adult and "juvenile" forms of CGL may be explainable by the fact that the latter is actually a monocytic leukemia.

GRANULOPOIESIS IN CHILDHOOD LEUKEMIA. Abdel H. Ragab, Ellen Gilkerson, Martha Lindsay, Washington University, St. Louis Children's Hospital, Department of Pediatrics, St. Louis Missouri (Intr. by Teresa J. Vietti).

Cells from the bone marrows of children with acute leukemia were serially cultured in vitro by the agar technique. In this culture system granulopoietic stem cells will form colonies composed of granulocytes and macrophages. A total of 181 bone marrow cultures were performed on 62 children with acute leukemia. It was noted that at the time of diagnosis or during a relapse the number of colony forming units (CFU) in the bone marrow were decreased, when compared to either children who were in remission or to "normal" children. Fifteen children went from relapse to remission and in 14 cases there was an increase in bone marrow CFU despite chemotherapy. Fifteen children went from remission to relapse and in all cases there was a decrease in bone marrow CFU. A decrease in bone marrow CFU preceded the relapse in 5 instances. Three children with AML had an increase number of CFU initially compared to children with ALL. Peripheral blood of children with ALL or AML in relapse also contained colony forming cells, although these cells when used in underlayer were inadequate supporters of normal CFU. When bone marrow cells of children in relapse and remission were mixed in the same culture plates, there was no inhibition of CFU growth.

The study of in vitro colony forming units in childhood leukemia may be a useful parameter to follow in monitoring the response of patients to chemotherapy and their disease.

PROLONGED REMISSION WITH CHIMERISM AFTER BONE MARROW TRANSPLANTATION FOR ACUTE MYELOGENOUS LEUKEMIA. Martin R. Klemperer, Hahng Lee, George B. Segel, Kong-oo Goh, and Allyn May. Univ. Rochester Sch. Med., Depts. Ped., Med., and Surg., Rochester, NY.

A 4 y.o. girl underwent bone marrow transplantation one year ago and remains in hematological remission. The donor was an HL-A identical, MLC nonreactive brother. After preparation with cyclophosphamide (CYT) 50 mg/kg x 4 daily doses, 1.7 x 10^8 marrow cells/kg was administered intravenously. Modification of GVHR was attempted with CYT 7.5 mg/kg on days 1,3,5,7,9,11 and 18. Rash and icterus were noted on day 21. Biopsies of skin and liver were consistent with GVHR. Repeated analyses of bone marrow show 20-30% XY cells. Erythrocyte typing post transplant shows conversion from S(-) to S(+) which is of donor origin. Immunoglobulins have remained normal or elevated but tests of delayed hypersensitivity have been negative. Isohemaglutinin titers have been markedly diminished and CF and CMT titers against cytomegalovirus have decreased significantly. The patient has been suffering from progressive chronic obstructive lung disease and chronic HAA negative hepatitis. This case indicates that bone marrow transplantation may be effective in producing prolonged remission in leukemic individuals despite lack of 100% donor marrow take.

VALUE OF SERIAL DETERMINATIONS OF URINARY AMINOIMIDAZOLECARBOXAMIDE IN CHILDHOOD LEUKEMIA. Thomas E. Williams, University of Texas Medical School at San Antonio, Department of Pediatrics (intr. by M. J. Sweeney).

Urinary 5-amino-4-imidazolecarboxamide (AIC) correlates with the marrow blast cell content of children with acute leukemia (Lulenski, et al, Pediatrics 45:983, 1970). To assess the value of serial determinations for prediction of bone marrow and CNS relapse, AIC was measured weekly in 25 children with acute lymphoblastic leukemia and correlated with weekly hemograms, monthly marrow examinations and CSF evaluations when clinically indicated. The mean AIC for controls was 1.68 μg/mg creatinine/24 hrs. ± 0.86; leukemia patients in M_1 marrow remission (60 paired observations) 2.03 ± 1.01, M_2 marrow relapse (44) 7.79 ± 8.82, CNS relapse (8) 8.04 ± 6.40. At 2.80 μg/mg creatinine/24 hrs. 80% positive and negative correlation with marrow status was found. This value was observed one or more times during the month preceeding 19 of 22 marrow relapse episodes and preceeding CNS relapse in 5 of 6. Similarly one instance each of periosteal and ovarian involvement was preceeded by AIC elevation. AIC proved valuable in the differentiation of aseptic meningitis from CNS leukemia and pancytopenia of drug toxicity from leukemic replacement of the marrow. 44% of all AIC levels from patients receiving methotrexate were elevated. Prednisone may also cause elevation of AIC. AIC may be valuable in timing the periodic reinforcement of leukemic maintenance therapy and the prophylactic use of intrathecal chemotherapy.

RESULTS OF COMBINATION CHEMOTHERAPY INCLUDING "PROPHYLACTIC" INTRATHECAL METHOTREXATE IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA. Mahroo Haghbin, Charlotte Tan, and M. Lois Murphy. Memorial Sloan-Kettering Cancer Center, Department of Pediatrics, New York, N.Y.

A 10 drug treatment protocol "L2" was initiated in November 1969. There are 3 phases: I, Induction is prednisone, vincristine and daunomycin. II, Consolidation is arabinosyl cytosine and thioguanine, succeeded by L-asparaginase. III, The maintenance phase is a multidrug regimen administered as a 5 day cycle interrupted by a 1 to 2 week rest period. Intrathecal injections are given at the beginning of induction and consolidation, then every 2 months throughout the maintenance. Therapy is terminated after 3 years.

Sixty-five consecutive patients 6 months to 14 years of age are enrolled. All except one child with Down's syndrome achieved remission. Two died in remission with serum hepatitis and 7 have relapsed. In only one child relapse was confined to the central nervous system. The remaining 55 continue in complete remission up to 3 years.

This regimen is well tolerated. The main side effect which is hematopoietic depression is minimized by careful adjustment of the drug dosage to the individual tolerance. No adverse effect on the children's growth has been observed.

These encouraging results indicate that perhaps complete control of acute lymphoblastic leukemia can be achieved by chemotherapy alone.

Supported by Research Grants CA 08748 and ACS CI-67.

GRANULOCYTES (PMN) FOR TRANSFUSION: VIABILITY AND FUNCTION. Michael B. Harris, Isaac Djerassi, Elias Schwartz, and Richard K. Root, Depts. of Ped. and Med., Univ. of Penn., Children's Hosp. and Mercy Catholic Med. Ctr, Philadelphia,Pa.

PMN prepared for transfusion have been reported to exhibit decreased in vitro functional and metabolic activities. We have examined these properties of PMN prepared by an improved method of continuous flow filtration leukophoresis (CFFL). Each of 3 preparations studied were obtained from single donors during a 4 hour period with a yield of approximately $1x10^{11}$ PMN/donor. The PMN were compared to control PMN collected from the same donor prior to CFFL. Viability (trypan blue exclusion), morphology by phase microscopy, oxidation of ^{14}C-1-glucose during phagocytosis, and conversion of iodide to a protein-bound form were similar in both groups. Phagocytic rates for ^{14}C-Staph. aureus were comparable (3.4 ± 0.2% uptake/min. CFFL; 2.9 ± 0.15% uptake/min. controls, P>0.01). There was no difference in staphylocidal activity (502A S. aureus) expressed as % survival at the end of 60 minutes (3.2 ± 0.7% CFFL; 4.1 ± 1.1% controls, P>0.4). These are the first extensive studies on cells prepared by this improved method. The results indicate that these PMN have normal in vitro metabolic and bactericidal functions. These relatively easily prepared cells may be of benefit in the management of neutropenic patients with bacterial sepsis.

CHEMOTHERAPY, NOT EXENTERATION FOR PELVIC RHABDOMYOSARCOMA. Georges E. Rivard, Jorge A. Ortega, Ruprecht Nitschke, Myron Karon. Division of Hematology, Childrens Hospital of Los Angeles, University of Southern California School of Medicine, Los Angeles, California.

Although pelvic exenteration has been widely used for the treatment of pelvic rhabdomyosarcoma, the results have not only been physically and psychologically destructive, but therapeutically ineffectual, resulting in a 90% death rate. In an effort to improve these results, a pre-operative multidrug regimen followed by local radiation therapy and/or surgery was undertaken. Eight patients with pelvic rhabdomyosarcoma who had unresectable tumors without sacrificing other pelvic structures, were treated.

Chemotherapy consisted of repeated courses of Actinomycin-D 600 $\mu gm/M^2$ I.V. from day 1 through 4, Cytoxan 300 mg/M^2 I.V. on days 1,4, and 7, and Vincristine 1.5 mg/M^2 on day 1 and 7. Courses were repeated every 3-4 weeks.

Seven patients had measurable tumor and all showed a 75% or more decrease of the tumor volume after 2 courses of chemotherapy. Two patients had surgical removal of the remaining tumor without sacrificing the pelvic organs. Both are free of disease after 2 and 4 years respectively. One patient died of necrotizing hepatitis after 2 courses of treatment with no demonstrable tumor found at autopsy. The rest of the patients are alive at 5 to 18 months, only 1 has active disease. The estimated median survival of these 8 patients will be in excess of 2 years, while that of 17 patients treated prior to this study is 7 months. Intensive chemotherapy followed by radiation therapy and surgery is now the best approach for the treatment of rhabdomyosarcoma of the pelvis and can obviate the need for pelvic exenteration.

MAINTENANCE OF REMISSION IN CHILDREN WITH STEM CELL LEUKEMIA (ALL) BY INTRAVENOUS METHOTREXATE ADMINISTERED IN MAXIMALLY TOLERATED DOSAGE. Julius Rutzky, Charles A. Main, Hassan Yaish, Lalit J. Shah, Hadi Sawaf, and Inder Saini, (Intr. by Paul V. Woolley, Jr.) Wm. Beaumont Hosp., Royal Oak, Mich.

Since 1965, a total of 40 consecutive children with acute stem cell leukemia (ALL) were entered on a regimen based on induction of remission with corticosteroids (3 mg pred/K daily) and 6-MP (2.5 mg/K daily), maintenance of remission by progressively increasing pulsed doses of methotrexate intravenously (3 to 17 mg/K every 10 days to 3 wks) according to tolerance and annual reinduction with the same agents used initially. 37 patients (92%) achieved a complete remission in approximately one month. One patient was lost to follow-up. The first 10 of the 36 remaining were treated with the basic regimen without modification and 5 of these (50%) are still in their first complete (BM and CNS) remissions after 41 to 83 mos. The next 10 patients were given 4 doses of vincristine (.05-.075 mg/K) after remission in addition. Only 2 (20%) are still in their original complete remissions of 25 mos and 30 mos. The subsequent 16 patients received intrathecal methotrexate (5 doses) after remission (12 mg/M^2) in addition to the basic protocol. 15 are in complete remission 2 to 18 mos. All are in hematologic remission. In the entire group, the incidence of complete remission after 36 mos is level at 26%. The regimen involves an average of only 3.3 days of hospitalization/patient/year for either reasons of routine evaluation or for complications. One death (2%) was attributed to therapy.

PHOTODYNAMIC THERAPY OF MALIGNANT TUMORS. I.Diamond, S.F.Granelli, A.F.McDonagh, S.Nielsen, C.B.Wilson, and R.Jaenicke (Intr. by M.M.Grumbach), Dept.Pediatrics, Neurol.,Neurosurg., Medicine, Pathology, Univ. California, San Francisco.

Because many neoplasms are often treated by x-ray irradiation, there has been an extensive effort to find an effective radiosensitizing agent. Malignant tumors take up and retain hematoporphyrin to a greater extent than normal tissue. Porphyrins are powerful photodynamic agents which can sensitize biological preparations so that they are severely damaged when exposed to visible or near ultraviolet light. This study is a test of the idea that hematoporphyrin can serve as a selective photosensitizing agent to destroy malignant cells by exposure to light. Addition of hematoporphyrin ($10^{-5}M$) followed by light treatment with 8 20-watt fluorescent light bulbs proved lethal to glioma cells in culture after 15 min. Also, injection of 10 mg hematoporphyrin into rats bearing subcutaneous gliomas followed by light therapy directed at the tumor from a 150 watt light bulb produced massive destruction of the porphyrin-containing gliomas. In vitro or in vivo treatment with either light or hematoporphyrin alone was without effect. Photodynamic therapy offers a new approach to the treatment of brain tumors and other neoplasms resistant to existing forms of therapy.
(Preliminary studies were reported in Lancet,Dec.2,1972.)

HODGKIN'S DISEASE IN CHILDREN. Narendra K. Shah, Arnold I. Freeman, John F. Gaeta, Leon Stutzman, A. Rathnakar Rao and Moshe Friedman. Introduced by Lucius F. Sinks. Roswell Park Mem. Inst., Buffalo, New York.

Fifty-seven children were seen at RPMI with Hodgkin's disease from 1959 to 1971 and evaluated to detect any variation from Hodgkins in adults. On clinical staging there were 20 patients with Stage I, 21 with Stage II, 6 with Stage III and 10 with Stage IV. Nineteen of these patients presented with symptomatic (B) disease. Histology revealed an increased incidence of lymphocytic predominance type noted in 12 patients. Also there were 19 nodular sclerosis, 18 mixed cellular, 3 lymphocytic depletion and 5 not histologically classified. The initial treatment consisted of radiotherapy (RT) alone in 35 patients, chemotherapy (CT) alone in 10 patients and combined RT and CT in 10 patients. There was complete response in 37, partial response in 18 and no response in 2. Followup of 1 to 11 years show 24 alive without evidence of disease, 16 are alive with disease, and 16 are dead from disease. In the last 3 years laparotomies have been performed for staging. Four of 6 patients with clinical Stage I or II disease with laparotomy had Hodgkin's disease in their abdomen. Laparotomies performed during the later course of disease revealed abdominal disease in 7/10 patients. Because of clinically undetected high incidence of abdominal disease in children, exploratory laparotomy should be performed in Stage I and II disease in older children, unless routine abdominal RT and CT is given. The initial approach of combined RT and CT directed at all stages of disease in the hope of increasing the survival appears reasonable.

USE OF THE IN VITRO COLONY GROWTH ASSAY FOR THE DIFFERENTIAL DIAGNOSIS OF CHILDHOOD ACUTE MYELOMONOCYTIC LEUKEMIA. Yigal Barak and Nomie A. Shore. (Intro. by Myron Karon). Division of Hematology, Childrens Hosp. of Los Angeles and USC Sch. of Med., Los Angeles, California.

Controversy still exists as to the origin of the monocytic (Schilling) and the granulocytic (Naegeli) types of acute myelomonocytic leukemia (AMML). Morphologic, cytochemical and enzymatic methods often fail to differentiate between these two variants, which may differ in their clinical course, prognosis and response to therapy. The role of the in vitro bone marrow cultures in the diagnosis of AMML was studied in a 4 month old male with hepatosplenomegaly, whose peripheral blood and bone marrow aspirates contained 5-10% progranulocytes and 20-50% monocytes. The diagnosis of AMML was confirmed by a liver biopsy which showed infiltration with immature myelomonocytic cells. The patient expired two months after the commencement of combined chemotherapy. At autopsy, leukemic infiltration of the bone marrow, liver, spleen and lymph nodes was evident. In vitro cloning of the patients peripheral blood and bone marrow cells in soft agar cultures with or without leukocyte feeder layers, revealed excessive colony growth with the mean of 283 colonies per 2.10^5 cells seeded. Colonies were 200-1000 cells in size and were composed of large macrophages. In contrast, colony growth in cultures of marrow cells from 5 other patients with AMML or acute myelogenous leukemia ranged from 0-35 colonies, were smaller in size and were composed of granulocytes. These observations suggest that the monocytic (macrophage producing) and the granulocytic (granulocyte producing) forms of AMML arise from different progenitor cells, and that the in vitro bone marrow culture system can differentiate these two variants.

HEMATOLOGY AND ONCOLOGY

Read by Title

DISSEMINATED INTRAVASCULAR COAGULATION (DIC) AFTER A SINGLE DOSE OF ENDOTOXIN Drs. A. J. Aballi, G. Karayalcin, L. Silver, F. Costales and K. Y. Kim (Stony Brook School of Medicine, Department of Pediatrics, Nuclear Medicine and Pathology, and Queens Hospital Center Affiliation of The Long Island Jewish-Hillside Medical Center, Jamaica, New York 11432

A single dose of 0.1-0.3 mg. E. coli endotoxin per kg. given to rabbits regularly produced alterations of coagulation tests and increase in digestion products of fibrinogen (DPF). Of 30 animals, 24 died within 24 hours. Changes observed in prothrombin, partial thromboplastin and plasma recalcification times, as well as in factors II, V, (II-VII-X), fibrinogen and platelets showed alterations of 41 to 96% from initial values. Also there was a rise in titer in the Fi and staphylococcal clumping tests and higher O.D. in protamine titration. However, pathological studies in 6 rabbits failed to show fibrin thrombi in viscera (light microscopy). With epsilon aminocaproic acid given after endotoxin it was possible to demonstrate numerous fibrin thrombi in the lungs and liver. Administration of 25μCi of ^{125}I-fibrinogen and endotoxin in 6 rabbits was followed by a shortening of half life to 91 minutes as compared to 180 minutes for controls. Radioactivity of organs showed much higher counts of ^{125}I for animals receiving endotoxin than for controls. Figures observed were as follows: Liver 2502, spleen 3402, heart 733, lungs 1073 and kidneys 1585. In controls counts were liver 573, spleen 643, heart 517, lungs 825 and kidneys 486. These findings are indicative of DIC.

BONE MARROW LYMPHOCYTOSIS IN ACUTE LYMPHOCYTIC LEUKEMIA (ALL): ANOTHER LOOK: Hugh Bryan, Annick LePourhiet, Harvey Gralnick, and Brigid G. Leventhal, NCI, NIH, Bethesda, Md.

The significance of bone marrow lymphocytosis (BML) in ALL is uncertain. Its occurrence has been variously held to be a good or poor prognostic sign. BML has occurred in a group of children currently treated at the NIH and appears to be related to the therapy schedule employed. 22 children with ALL in remission received alternating phases of maintenance therapy. Phase I (PI) maintenance was 4 mos. of daily 6-mercaptopurine, weekly methotrexate (MTX) and pulses of vincristine and prednisone. They were randomized for 2 mos. of Phase II (PII) to either: 1) biweekly MTX; 2) MTX in 5 day courses with 10 day rest periods; or 3) immunotherapy with BCG and allogeneic leukemia cells. Pts. were considered to have BML in PII if the percentage of lymphocytes in the last marrow of that period was at least twice the highest percentage seen in PI and $>20\%$. By these criteria, 0/7 in group 1, 3/7 in 2, and 5/8 in 3 had BML; groups 1 and 3 differ significantly. Median BML was 10% in group 1; 24% in 2, and 25% in 3. Thus the no drug and intermittent drug therapy groups had lymphocytosis while the continous drug therapy group did not. An increase in BML has been reported by others in pts. <5 after cessation of long term chemotherapy; there was no correlation with age in our group. While status of BML as a prognostic sign remains uncertain, it is clear that in the month or two after completion of an intensive course of chemotherapy, bone marrow lymphocytosis is to be expected in the majority of patients.

HEMATOLOGIC STUDIES IN LEAD BURDENED CHILDREN IN A HIGH RISK COMMUNITY. Festus O. Adebonojo, Univ. of Pennsylvania Sch. of Med., Children's Hosp., Phila., Pa.

Severe anemia is the rule with lead poisoning. Little is known about the hematologic effects of lead burden at sub toxic levels. In a study of 575 high risk black ghetto children aged 1 - 19 years, two subgroups could be identified. One group of 136 children aged 1 - 3 years averaged blood lead value of 32 ug per 100 ml of blood; and had decreasing hemoglobin and hematocrit values with increasing blood lead values. With blood lead values under 10 ug per 100 ml of blood, mean hemoglobin was 12.9 gms% and mean hematocrit was 39%. With blood lead values between 50 - 60 ug per 100 ml of blood, the Hemoglobin was 11 gms% with a Hematocrit of 34%. Intermediate Hemoglobin and Hematocrit values were found for intermediate blood lead levels. For hemoglobin and blood lead values, correlation coefficient $r=0.35$, $n=136$, $p < 0.001$. MCV and MCH values were unaffected until blood lead values exceeded 40 ug per 100 ml of blood, when a significant drop occurred in both. WBC and RBC counts were unaffected by increasing blood lead values up to 60 ug per 100 ml of blood. Another group of 439 children aged 4 - 19 years showed a steady decline in mean blood lead values with increasing age. No deleterious effect on Hgb, Hct, RBC, MCV, MCH and WBC was found as the blood lead level rose. This study shows that deleterious hematologic effects can be demonstrated in the youngest population with the highest burden of lead.

MACROGLOBULINEMIA ASSOCIATED WITH ACUTE LEUKEMIA IN A CHILD Jan Cejka*, Robert O. Bollinger*, Wolf W. Zuelzer, Jeanne M. Lusher*. Child Research Ctr. and Children's Hosp. of Michigan, Dept.Ped., Wayne State Univ. Sch. of Med., Detroit

Monoclonal gammopathies are very rare in children. Three cases of IgG type paraproteinemia have been reported in childhood leukemia. We report a unique case of IgM (κ) macroglobulinemia in a previously healthy 12 year-old male with acute leukemia. The history and findings were typical of leukemia. No symptoms related to cryoglobulinemia were present. The leukemic cells in blood, marrow, and tissues were uniform and undifferentiated, though the abundant deep blue cytoplasm resembled that of plasma cells. The karyotype was 46XY. No remission was attained, and the patient succumbed only 10 weeks after diagnosis, following meningeal leukemia.

Serum IgM was high, IgG and IgA low. Agar gel electrophoresis of serum consistently showed a monoclonal band identified with specific antisera as IgM, type κ. The isolated IgM reacted only with anti-μ and anti-κ. Fluorescent labeled antisera showed the leukemic cells to react specifically with anti-μ and anti-κ. Electron microscopy showed many polyribosomes, but relatively scanty RER. The urine contained Bence-Jones protein, reacting with anti-free κ chain serum.

The case has marked similarities to Waldenström's macroglobulinemia and may be interpreted as an acute form of that condition and a link between it and leukemia-lymphoma.

THE EFFECT OF INHALED CLEANING AGENT "LPH" ON RED BLOOD CELL DESTRUCTION IN RABBITS. Juh-huey, Chen*, John M. Senior, and Carol L. Shaffer. (Intr. by M. Delivoria-Papadopoulos) Department of Pediatrics and Physiology, Sch. of Med., University of Pennsylvania, Philadelphia, Pennsylvania.

Previous studies in vitro have shown that "LPH", a commercial available cleaning agent, containing D-Bensyl-P-Chlorophenol 6.4% P-Tertiary-Amylphenol 3.0%, O-Phenylphenol 0.5% and Glycolic acid 12.6%, hemolized human adult blood. These studies are designed to investigate the "LPH" effect in vivo. Seven rabbits, 5-6 months old, weighing 2-3 Kg., were exposed for two hours in a 100 liters plastic box attached to a closed rebreathing system. The box was washed with "LPH" in the recommended dilution of 1:256. Blood samples were obtained before and immediately after exposure and at 24 and 48 hours for measurements of whole blood hemoglobin (Hb), hematocrit (Hct), and free plasma Hb level. There were no significant differences in total blood Hb and Hct between the control and post exposure to "LPH" blood samples, but free plasma Hb increased from a control value of 4.2 mg% to 19.6 mg% immediately after exposure, slightly decreased to 13.8 mg% at 24 and 7.1 mg% at 48 hours respectively. These data indicate that increased free plasma Hb levels may be the result of increased red blood cell destruction due to the phenolic substances inhaled by these animals. It is suggested that cleaning the nurseries, incubators and isolettes with this cleaning agent may be hazardous to newborn infants.

"PSEUDO" CENTRAL NERVOUS SYSTEM (CNS) LEUKEMIA. Ronald B. David, Harold M. Maurer, and Clare N. Shumway (Intr. by William E. Laupus). Dept. of Ped., Med. Col. of Virginia, Richmond, Virginia.

CNS leukemia is a frequent complication of acute leukemia in childhood. CNS manifestations are presumed to be related to leukemic infiltrates in the subarachnoid space, but similar signs and symptoms may occur with cerebral dural sinus occlusion. The purpose of this paper is to describe a patient with superior saggital sinus occlusion complicating leukemia. A 7 year old W/M with a 3-year history of lymphoblastic leukemia had done well despite documented CNS leukemia which was treated with intrathecal methotrexate. Prior to admission he developed severe headache and papilledema with no CSF abnormalities. Peripheral blood indicated relapse. Cerebral arteriography demonstrated partial occlusion of the superior saggital sinus. He was treated with dexamethasone and radiotherapy to the head, particularly to the superior saggital sinus; and he subsequently improved. Two months later, however, he died in marrow relapse. At the post-mortem the superior saggital sinus was partially occluded by leukemic infiltrates within the sinus wall, although the subarachnoid space was free of tumor. Other reports note hydrocephalus in the absence of leukemic infiltration in the subarachnoid space; however, cerebral venous occlusion has not been suggested as a possible cause. Our case suggests that cerebral dural venous sinus thrombosis may be an important factor in CNS leukemia independent of subarachnoid space disease.

LEVEL OF COAGULATION FACTOR XII IN SICK NEWBORNS. James J. Corrigan, Jr., Elsa J. Sell, and Cheryl Pagel. Dept. of Ped., Arizona Medical Center, Tucson, and Emory Univ., Atlanta.

Factor XII (Hageman factor) has been postulated to play a pivotal role in the initiation of many pathologic reactions ranging from inflammation to shock. When compared to older children normal full term newborns have lower levels of factor XII; the significance of which is unknown. Since reduced levels have been equated with evidence for factor XII activation by other investigators the purpose of this study was to determine if a relationship existed between low factor XII and neonatal disease. Factor XII levels were measured on citrated platelet poor plasma by a one stage technique using congenital factor XII deficient plasma as substrate. Mean factor XII levels were: 30% for 28 healthy prematures (36 wks G.A.); 47% in 25 healthy fullterms; 20% in 58 sick prematures and 41% in 26 sick fullterms. Significant reduction in factor XII was clearly most prominant in sick prematures particularly in those with severe RDS. Sequential data in 6 sick prematures suggested that the reduced levels were probably a result of a shortened half-life of factor XII. Low factor XII levels of the same magnitude were observed in infants with or without other coagulation evidence for diffuse intravascular coagulation. The data suggest that factor XII activation may play a role in the pathophysiology of certain neonatal disorders.

THE EFFECTS OF STEROID HORMONES ON ERYTHROCYTE 2,3 DIPHOSPHOGLYCERATE. J.N. Desai, L.M. Rao, S. Gunther, N.T. Shahidi. Department of Pediatrics, University of Wisconsin, Madison, Wisconsin.

We have previously shown that a significant increase in red cell 2,3-DPG occurs in patients with chronic renal failure treated with androgens. (NEJM, 287:381, 1972.) In this study we evaluated the effect of testosterone propionate (TP), 11-ketopregnanolone (11KP), and oestradiol benzoate (OB) in vivo on red cell 2,3-DPG in nine female rhesus monkeys (Macaca mulatta). The animals were divided in groups of three and after baseline studies they were given the above hormones at dosages of 2mg/kg IM three times a week. The mean 2,3-DPG level in TP treated group rose from 4211 ± 37 (S.E.M.) mμ mole/ml rbc to 7622 ± 500 (81% increase) within 4 weeks. In 11KP treated group the mean 2,3-DPG rose from 4459 ± 176 to 8323 ± 750 within the same period (85% increase). The administration of OB resulted in no change in the level of red cell 2,3-DPG. The increase in red cell 2,3-DPG was not accompanied by any change in hemoglobin concentration, red cell mass or plasma phosphorous. Since each 430 mμ mole of 2,3-DPG results in an increase in P50 of 1 mm Hg., the above findings indicate that both testosterone and certain 5 βH (non masculinizing) steroids are able to significantly increase the delivery of oxygen to the tissues.

COMPARATIVE PLATELET PHOSPHOLIPID(PL) ASSAYS OF ADULT AND NEWBORN(NWBN) RABBITS. M. Douglas Cunningham, Marie V. Kulovich, and M. Jean Westberg, Univ. of Cal., San Diego Sch. of Med., Dept. of Ped., Div. of Perinatal Med., La Jolla. (Intr. by Louis Gluck).

Human NWBN coagulation studies reveal transient functional platelet deficiencies. Of these, lowered platelet factor 3 activity implies decreased platelet PL availability. This investigation was carried out to compare NWBN and adult rabbit platelet PL fractions. Adult platelets were obtained from 23 males. NWBN platelets were obtained by pooling blood of mates from 12 litters. Platelets in suspension were disrupted by freeze-thaw and lyophilized. Lipids were extracted with chloroform-methanol 2:1, separated from protein, and re-extracted. Neutral fats and PL fractions were separated by DEAE column chromatography. Phosphatides were separated by thin layer chromatography on 6 percent $(NH_4)_2HPO_4$ silica gel plates. Percent ratios of phosphatides were determined by densitometry of charred plates and were as follows:

	Sph	Lec	PE	PS	PI
NWBN	8	58	34	1	99
ADULT	24	55	21	7	93

Total lipid extracts for NWBN and adult platelets were 1.6 μg and 4.7 μg; percent neutral fats and PL were similar. The NWBN data indicates less total available PL and different phosphatide proportions. These findings may imply an altered role of PL as a cofactor in NWBN coagulation.

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ABNORMAL GROWTH PATTERNS IN SICKLE CELL DISEASE(SCD) AND SICKLE CELL TRAIT(SCT). Lois Dicker, John McDaniel, Mae Caleb, Albert Humphrey, Eileen Buchert, Bertram Lubin, and Solomon Katz. Wm Krogman Ctr. for Research in Children and Dept. of Hematology, Children's Hosp. of Philadelphia, Philadelphia, Pennsylvania.

With the increasing availability of general health services for patients with SCD and SCT it is important for the clinician to have an adequate picture of the somatic growth and skeletal development of these children. Skeletal age SA, height-weight HW, and other somatic determinations were made in 77 children with SCD, 19 children with SCT, and 91 adolescents with SCT. Results were compared to 1000 normal Philadelphia black children and expressed as the percentage of patients 1 S.D. above↑ or below↓ the normal mean.

Type	Mean Age	SA	M Height	F	M Weight	F
SCD	8.5	60%↓	50% ↓	50%↓	50% ↓	50%↓
SCT	7.5	30%↓	Normal	67%↓	20% ↓	56%↓
SCT	15.0		22% ↑	22%↑	Normal	Normal
			5% ↓	5%↓		

The results demonstrate marked changes in growth patterns in SCD. In the trait, retardation in SA and HW determinations noted in the younger age group were reversed or corrected in the older children. This data is evidence that the child with SCT may have many differences from the child with normal hemoglobins which have been overlooked.

IN VIVO AGING OF TRANSFUSED ERYTHROCYTES AND 2,3-DIPHOSPHOGLYCERATE (2,3-DPG) LEVELS Joseph D. Dickerman, Enrique M. Ostrea, Jr., and William H. Zinkham, Johns Hopkins Univ. Sch. of Med., Dept. of Ped., Baltimore, Md.

Other investigators have shown that the activity of many red cell enzymes decreases during red cell aging; that old cells have a higher oxygen affinity than young cells; and that 2,3-DPG levels are elevated in young cells and reduced in old cells. One would predict, therefore, that in vivo aging of transfused red cells would impair the ability of these cells to oxygenate tissues. A patient with refractory, congenital hypoplastic anemia requiring transfusions every 6 to 8 weeks presented an opportunity to study the effects of in vivo cell aging on 2,3-DPG levels and oxygen transport. Reticulocyte counts varied between 0 and 0.1%. Ferrokinetic studies immediately after one of the transfusions revealed that the rate of erythropoesis was approximately 10% of normal. Values for 2,3-DPG and P_{50} obtained twice weekly during a 9 week intertransfusional period progressively increased as the hematocrit decreased. Immediately preceding transfusions on five separate occasions, values for 2,3-DPG were 25.7, 23.7, 26.9, 27.9 and 23.3μM/gm Hb (values for 52 normal adults - 15.2±5μM). P_{50} levels at these times were 37.8, 33.7, 35, 29.5 and 30 mm of mercury, and the hematocrits were 15.9, 14.9, 18.8, 16.7 and 15.2%. These findings indicate that in vivo aging of transfused erythrocytes does not prevent these cells from responding to an increasingly hypoxic environment by elevating 2,3-DPG levels and decreasing oxygen affinity.

SEPHADEX ADSORPTION OF CONJUGATED BILIRUBIN: A NEW OBSERVATION. Montgomery C. Hart and Wanda Woznicki, Perinatal Lab., St. Joseph's Hosp., Phoenix. (Intr. by W. J. R. Daily).

It is widely accepted that unbound unconjugated (free) Bilirubin (BR) is neurotoxic and that its presence on a Sephadex column is a criterion for exchange transfusion (ET). We have previously reported rapid ultramicro Sephadex column techniques for detection of free BR and for prediction of the critical total BR at which free BR will occur in newborn infant sera. Adsorption of conjugated BR by Sephadex has not previously been reported. Conjugated BR is not considered neurotoxic and its presence on a column should not indicate a need for ET. Sephadex adsorption of conjugated BR from infant serum in the absence of free BR could therefore result in unnecessary ET therapy. The presence of each of these BR fractions in column eluates is detectable by thin layer chromotography since they have different R_f values. BR was adsorbed from the sera of 22 hyperbilirubinemic infants in our nurseries. The column eluates of six of these exhibited conjugated BR alone or in addition to free BR. This occurred only when the conjugated BR fraction in serum exceeded 3 mg%. ET should be reserved for infants either with free BR or with a total BR near the critical level. Consequently, proper management of hyperbilirubinemic infants requires knowledge not only that Sephadex-adsorbed BR is present, but also whether it is conjugated or unconjugated.

RELATIVE DEFICIENCY OF Ca^{2+}-ATPASE IN HEREDITARY SPHEROCYTOSIS (HS). Stephen A. Feig, UCLA Sch. of Med., Dept. of Ped. and Harvard Univ., Dept. of Biochemistry and Biophysics, Los Angeles and Boston. (Intr. by Robert C. Neerhout).

HS is a congenital hemolytic anemia characterized by reduced red cell (RBC) membrane deformability. In view of several recent reports correlating membrane Ca^{2+}-ATPase activity, with contractile function in RBC ghosts, the activity of this enzyme was examined in HS. No significant difference was detected when 4 unsplenectomized (438±98 nmoles/mg ghost protein /hr.) and 11 splenectomized (497±71) HS patients were compared to 28 normal subjects (459±102) and 6 patients who had undergone splenectomy for non-hemolytic disease (505±146). HS and normal RBC's were separated into young and old cell populations by ultracentrifugation and Ca^{2+}-ATPase activity was shown to be age-labile. No difference was detected in either young or old cells when HS and normal ghosts were compared. Another age-labile marker, GOT, was always higher in HS cells. The expected effect of a young cell population was further demonstrated in 6 patients with elevated reticulocyte counts (5 to 50%) due to hemolytic diseases other than HS. These ghosts had elevated Ca^{2+}-ATPase activity (666±126) compared to both normal and HS ghosts ($p<0.01$). Since HS cells have a shortened RBC survival even after splenectomy, the activity of this enzyme would be expected to be elevated in HS ghosts. These data suggest that RBC membranes in HS are relatively deficient in Ca^{2+}-ATPase activity. This may be related to the pathogenesis of diminished membrane deformability in HS.

LONG-TERM SURVIVORS OF ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) OF CHILDHOOD. F. A. Hayes, K. Y. Wong, B. C. Lampkin, and A. M. Mauer, Children's Hosp. Research Fndn., Univ. of Cincinnati College of Medicine, Cincinnati, Ohio.

The following is a review of the results of sequential anti-leukemic therapy in 84 children diagnosed to have ALL from 1960 to 1967. All children with ALL were included in the study. The data were analyzed through December 31, 1972. Chemotherapy was the same for long-term survivors and non-survivors, and no central nervous system (CNS) prophylactic therapy was used. The results are as follows:

Years Followed	No. of Patients	No. of Survivors	No. of Survivors in at Least 1 Remission for 5 Years or More	No. in First Remission
5	84	18	11 (> 60 mos.)	4
7	61	13	7 (> 84 mos.)	2
10	33	7	5 (>120 mos.)	2

By statistical analysis there was no difference between survivors and non-survivors in age of onset, presenting signs and symptoms or initial blood counts. However, a longer median duration of first remission (40.5 mos. vs. 7 mos.) and a decreased incidence of CNS leukemia were found in the long-term survivors. From our results and results reported with the use of intensive combination chemotherapy and CNS prophylaxis with 1200 rads or less, approximately 20% of children with ALL survived 5 years or more. It would, therefore, appear that factors other than the method of administering chemotherapy determine long-term survival in ALL of childhood.

HODGKINS DISEASE AND FULMINATING HEMOLYTIC ANEMIA OCCURRING AFTER PROLONGED REMISSION IN ACQUIRED HEMOLYTIC ANEMIA. Gary R. Geller, Harry S. Jacob, William Krivit, Univ. of Minn. Hosp., Dept. of Ped. & Med., Minneapolis, Mn.

Idiopathic acquired (Coombs$^+$) hemolytic anemia (AIHA) has been successfully treated with steroids, immunosuppressive drugs, and splenectomy. The occurrence of malignancy and recurrence of hemolytic anemia, despite remission of the AIHA extending over a decade, prompts this report. One patient who had recurrent AIHA and thrombocytopenia from 3½ to 14 yrs. of age was extensively treated with steroids and a short course of 6-MP and had a splenectomy. He was asymptomatic until 22 yrs. of age when Hodgkins disease was diagnosed with histological features of immunoblastic lymphadenopathy (Luke's). Despite therapy he died in 6 mo. The second had AIHA for 9 yrs. and was similarly treated with steroids, splenectomy and a short course of HN_2. After 10 yrs. remission the AIHA became fulminating. He developed multiple thromboses, had a monoclonal gammopathy, had generalized lymphadenopathy and died in 5 wks.

Because of the analogy to the NZB mouse model (Coombs$^+$ AIHA with neoplasms of the lymphatic system) a note of caution is prescribed in the future care of patients with AIHA regarding prognosis. Reevaluation of the treatment as well as more intensive immunologic investigation of pathophysiology of AIHA becomes important. The review of our 20 yrs. experience of childhood AIHA indicates that it may not be a benign process.

LONG-TERM SURVIVAL IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) COMPLICATED BY RETICULUM CELL SARCOMA (RCS) AND CENTRAL NERVOUS SYSTEM DISEASE. F. A. Hayes, K. Y. Wong, B. C. Lampkin, and W. K. Schubert, Children's Hosp. Research Fndn., Univ. of Cincinnati College of Medicine, Cincinnati, Ohio.

The unusual course in an 11-year survivor of ALL is described. A now 19-year-old girl was diagnosed to have ALL in April, 1961. The first 2 remissions lasted 11 and 21 months respectively. A third remission was induced in April, 1965 after a relapse of 13 months. All therapy was discontinued in April, 1969 because of hemorrhagic cystitis due to cyclophosphamide. RCS of the mandible was diagnosed in January, 1970. RCS recurred in April and December, 1970 and in March, 1971. Complete remissions were obtained each time with irradiation or irradiation plus vincristine. In April and June, 1971 the patient presented with diplopia. The spinal fluid contained blast cells of undetermined type. It, therefore, was not possible to determine whether the CNS involvement was due to ALL or RCS. Cranial irradiation and intrathecal methotrexate were given on both occasions, resulting in clinical remission and clearance of the spinal fluid of cells. Bone marrow examinations have been normal since April, 1965. No therapy has been given since June, 1971 and no evidence of malignant disease has been present since that time. Long-term survival of ALL and clinical remission of RCS and CNS disease were obtained in our patient without intensive therapy. Thus, it would appear that host factors have played a prominent role in the favorable outcome of 2 primary malignancies in this patient.

ERYTHROCYTE COMPOSITION, IN SICKLE CELL ANEMIA. Stanley Hellerstein and Tasanaporn Bunthrarungroj. (Intr. by Herbert Wenner). Univ. of Mo. Sch. of Med. at K.C.; The Children's Mercy Hosp., Dept. of Ped., Kansas City, Mo.

Red cell solids, Na, K, Mg, and Cl have been examined on 62 occasions in 19 children with sickle cell anemia. Using the solids/kg RBC as the base of reference, the data divide the children with sickle cell anemia into three groups. Group 1 (5) Normal RBC solids (335.8 + 8.9 gm/kg RBC); Group 2 (7) Elevated RBC solids (>353.6 gm/kg RBC); and Group 3 (6) RBC solids normal on some occasions and elevated on others. RBC Na, K, Mg, and Cl are usually normal when RBC solids are in the normal range. Increased RBC solids are usually associated with increased RBC Na and decreased RBC K per kg RBC and per kg RBC H_2O. RBC Mg is usually normal while RBC Cl shows considerable variation. The observed abnormalities in erythrocyte composition are not those which would result from anomalous distribution of the ^{131}IHSA tracer for trapped intercellular plasma. Similarly, the observed alterations are distinct from those caused by in vitro sickling. Deoxygenation in vitro causes deformity of the cells, a decrease in RBC solids and RBC K, and an increase in RBC Na. There was no correlation between the alterations in red cell composition and the clinical state of the children, but there was positive correlation between the number of irreversibly deformed RBCs in the stained smears and red cell solids.

EFFECT OF CYANATE ON RED CELL SURVIVAL IN CONGENITAL HEINZ BODY ANEMIA. George R. Honig, R. George Mason, Loyda N. Vida, and Unsal Gunay. Univ. of Illinois College of Medicine, Univ. of Ill. Hosp., Dept. of Pediatrics, Chicago, Illinois.

The antisickling effect of cyanate is attributed in part to an increase in the O_2 affinity of the blood resulting from carbamylation of the hemoglobin. We have examined the effect of cyanate on red cell survival in a patient with an unstable hemoglobin. This variant (Hemoglobin Abraham Lincoln) undergoes intracellular precipitation by a series of events initiated by oxidation of the hemoglobin to the methemoglobin form. Because methemoglobin formation occurs most readily in partially deoxygenated hemoglobin, it seemed possible that an increase in oxygen affinity might reduce the rate of intracellular precipitation and thereby prolong red cell survival. Erythrocyte survival was studied as described by Alter et al. (Blood 40:733, 1972). Blood from the patient was incubated with L-leucine-^{3}H and subsequently with sodium cyanate, 50 mM. Approximately 50% of the hemoglobin was carbamylated. A second blood sample was incubated with L-leucine-^{14}C without cyanate. The cells were washed and infused into a splenectomized volunteer. Radioactivity of both ^{14}C and ^{3}H declined at a nearly linear rate. Red cell survival was 12.7 days with untreated cells and 14.3 days with cells incubated with cyanate. The presence of pre-existing precipitated hemoglobin in the red cells may have prevented a greater increase in survival following carbamylation. Treatment of early red cell precursors with cyanate may produce a greater effect.

DISEASE PATTERNS AND RESIDUAL ABNORMALITIES IN ACQUIRED APLASTIC ANEMIA OF CHILDHOOD. James D. Hilton, Melvin H. Freedman, E. Fred Saunders, Peter D. McClure, Div. of Hematology, The Hosp. for Sick Children, and Dept. of Pediatrics, Univ. of Toronto, Toronto, Canada (introduced by Henry Levison).

In the past 10 years, we have examined 23 children with acquired aplastic anemia. Two cases followed hepatitis and 3 followed chloramphenicol administration. In the others, there was no known precipitating factor. A review of the original marrow aspirates, hemoglobin values, leukocyte and platelet counts was not helpful in prognosis. The occurrence of sepsis was of significance as all 13 children with proven sepsis died. All patients received either prednisone alone or in combination with androgens. Ten children survived from 1 to 9 years and 8 of these were reassessed at this time. All have residual thrombocytopenia of 30,000 to 140,000/mm^3 (normal >150,000). One case has leukopenia and 2 have absolute neutropenia. None has anemia. Marrow fragments remain hypocellular in 3 children. The fetal hemoglobin has stayed elevated (>2%) in 3 children and appears to reflect the severity of residual disease in that the highest values (16.8% and 7.5%) are found in 2 patients with the most abnormal blood work. Serum iron values are normal in all. Unexpectedly, 4 cases have markedly elevated iron binding capacities. Surviving children with acquired aplastic anemia do not completely recover but may have persistent abnormalities of blood and bone marrow for an indefinite period of time.

UNBALANCED GLOBIN CHAIN SYNTHESIS IN CONGENITAL DYSERYTHROPOIETIC ANEMIA. Marilyn A. Hruby and George R. Honig. The Univ. of Illinois Col. of Med., Univ. of Illinois Hospital, Dept. of Pediatrics, Chicago, Illinois.

Hematologic evaluation of a five year old girl with life long transfusion-dependent anemia demonstrated the characteristic findings of congenital dyserythropoietic anemia (CDA) type II. Bone marrow abnormalities consisted of erythroid hyperplasia with karyorrhexis, multinuclearity and fragmentation of late stage normoblast nuclei. Ferrokinetic studies indicated a marked degree of ineffective erythropoiesis with rapid iron clearance ($t_{\frac{1}{2}}$ = 40 minutes), increased iron turnover (3.86 mg/day/100 ml), and decreased iron incorporation into hemoglobin (12.3% at 10 days). No red cell serologic abnormalities were detected. Globin chain synthesis studies performed by measuring the incorporation of L-leucine-^{14}C into globin by reticulocytes from the child demonstrated an abnormal balance between the synthesis of the α and non-α globin components of hemoglobin with α-chains being synthesized in excess. The β:α ratio was approximately 0.69. Heretofore findings of this kind have been found only in β-thalassemia and related disorders including hemoglobin Lepore disease, and in certain unstable β-chain-variant hemoglobinopathies. Our findings would suggest that this form of congenital dyserythropoietic anemia may represent a β-thalassemia disorder or may be an acquired β-thalassemia-like abnormality.

EFFECT OF FOLIC ACID ANTAGONISM ON SYNTHESIS OF β^A and β^S BY BONE MARROW CELLS. George R. Honig, R. George Mason, and Loyda N. Vida. Univ. of Illinois, Col. of Med., Univ. of Ill. Hosp., Dept. of Peds. and the Sickle Cell Center, Chicago, Ill.

The percentage of abnormal hemoglobin present in the blood of a heterozygous individual falls within a relatively narrow range for most variant hemoglobin forms, and appears to be primarily under genetic control. Nongenetic factors, including iron deficiency and folic acid deficiency have been reported to alter the percentage of the abnormal hemoglobin in the heterozygote, in some cases to a severe degree. In order to study this phenomenon in a controlled manner, synthesis of β chains of hemoglobins A and S was determined in bone marrow cells of a child with sickle cell trait before and after folic acid antagonism was effected by administration of methotrexate. Globin synthesis was studied by measurement of L-leucine-^{14}C incorporation into chromatographic globin fractions. Bone marrow samples were obtained prior to methotrexate treatment and again after the drug had been given for 6 weeks. At the time of the second study urinary excretion of formiminoglutamic acid was significantly elevated, providing biochemical evidence of folate antagonism. The percentage of hemoglobin S remained unchanged throughout the course of the study, and β^A and β^S synthesis was not affected by the methotrexate. Synthesis of these hemoglobin forms appears not to be influenced by folate antagonism due to methotrexate, but may be altered by deficiency of a folic acid cofactor whose formation is not affected by this drug.

CORRECTED CARBOXYHEMOGLOBIN (COHb) - A SENSITIVE INDEX OF HEMOLYSIS IN JAUNDICED NEWBORNS (NB). Stephen R. Kandall, Stephen A. Landaw and M. Michael Thaler. Univ. of California, San Francisco, Dept. of Pediatrics, and Univ. of California, Berkeley, Donner Laboratory.

COHb values reflect rates of heme degradation when corrected for contamination with ambient CO (aCO). Correction was possible in NB, who were found to equilibrate COHb and aCO extremely efficiently, compared with adults ($T_{\frac{1}{2}}$=10 to 15 min vs 4 to 5 hr). The relationship between COHb and aCO was highly significant ($p<0.01$). Corrected COHb in jaundiced NB was 0.71 ± 0.04% in fullterm (T), 1.79 ± 0.021% in premature (P) and 2.0 ± 0.15% in those with Rh disease (H). The difference between T and P or H was $p<0.001$; the difference between P and H was not significant. COHb declined during the 1st week in T and P, but remained higher in P. After 4 weeks, T and P had similar COHb (0.80 ± 0.08). The correlation between serum bilirubin (B) and COHb was not significant in T or P, but was significant in H. On this basis (COHb/B), T were clearly separable from H, whereas P frequently overlapped H. Phototherapy had no effect on COHb.

These results indicate that corrected COHb provides a sensitive index of hemolysis in NB. Excessive heme breakdown appears to be a major factor in jaundiced prematures. In contrast, jaundice in mature NB appears to reflect other mechanisms. Phototherapy does not contribute significantly to heme breakdown.

COAGULATION CHANGES AFTER ACUTE BLOOD LETTING

Drs. G. Karayalcin, K. Y. Kim and A. J. Aballi - Stony Brook School of Medicine, Pediatrics Department and Queens Hospital Center Affiliation of The Long Island Jewish-Hillside Medical Center, Jamaica, New York 11432

Coagulation studies were performed in 10 rabbits before and after removal of 15-25 ml of blood per kg. All animals in which 15-20 ml per kg. were removed survived. Hemoglobin and hematocrit values decreased by 30% of initial values in 30 minutes. Prothrombin, partial thromboplastin and plasma recalcification times and factors II, V and (II-VII-X) were essentially unchanged. Mean platelet counts decreased from 412,000 to 318,000 and fibrinogen level from 284 to 188 mg%. Withdrawal of 25 ml per kg. in 3 rabbits was fatal in 3 to 30 minutes. Hemoglobin and hematocrit values dropped only by 24% of original values. Coagulation studies before and after again were essentially unchanged except for fibrinogen and platelets. The former mean values dropped from 302 to 240 mg% and platelets from 482,000 to 333,000. No changes occurred in tests for split products of fibrinogen. Therefore rapid removal of blood even when fatal does not induce changes suggestive of DIC.

EFFECT OF CANCER CHEMOTHERAPEUTIC AGENTS ON FIBRINOGEN-FIBRIN CONVERSION AND STABILIZATION. Diane M. Komp, Robert L. Lyles*, Thomas H. Boyd*, Glenn E. Stoner* and Betty J. Cox*. Univ. of Va. Schools of Medicine and Engineering, Depts. of Pediatrics and Materials Science, Charlottesville, Va. 22901

In a variety of tumors, successful implantation of metastases is associated with fibrin formation. Anticoagulants have been reported to reduce the numbers of such metastases. Chemotherapeutic agents were studied to determine if any of these agents discouraged fibrin formation. Thrombin time, urea solubility and EM of fibrin formed were studied. Electrical resistance was measured during recalcification. Two patterns of abnormality were seen:1)prolongation of thrombin time, abnormal EM and urea solubility corrected by EACA;2)normal thrombin time, abnormal fibrin by EM and urea solubility not corrected by EACA. MTX, CTX and NH_2 produced abnormalities of the first type, interpreted as related to increased fibrinolysis. L-asparaginase, daunorubricin and adriamycin produced the second type, attributed to lack of fibrin stabilization. The defect produced by 5-FU and TIC mustard were profound and showed features of both types of abnormalities. It is concluded that high concentrations of some chemotherapeutic agents impair fibrin formation. In addition to anti-mitotic activity, prevention of metastases may reflect the inability to support a fibrin bed for the tumor.

SHOULD BLOOD LEAD LEVELS BE CORRECTED FOR ANEMIA? J. Kochen, Y. Greener, Albert Einstein Col. of Med., Montefiore Hosp. & Med. Ctr., Dept. Ped., Bronx, New York (Intr. by Laurence Finberg)

Blood lead (Pb) levels provide the best means for assessing Pb intoxication. It is generally accepted that the clinical evaluation of a given blood Pb level requires correction for anemia. This is based on the assumption that the capacity of the blood to carry Pb is limited by the volume of red cells. The following results question the validity of this assumption at hematocrit (hct) and Pb levels in the clinical range. (1) The uptake of Pb by red cells was determined in vitro at varying hct and Pb levels. Red cell volume was not a limiting factor in Pb uptake at hcts above 5% and Pb levels below 250 μg%. (2) Blood at varying hcts was dialysed against plasma containing Pb. At equilibrium no increased uptake of Pb was found at higher hcts. (3) Rats with varying hcts owing to bleeding or transfusion were injected I.P. with Pb. Subsequent uptake of Pb by blood did not correlate with hct. (4) Rats were exposed to 1% Pb in their drinking water for 4 months. No correlation between hct and Pb levels was found. (5) Pb was injected into yolk sacs of chick embryos. No correlation between hct and blood Pb was found after 14 days. (6) No significant difference could be found between maternal and newborn infant blood Pb levels despite the marked differences in hct between the two groups. It may be concluded that anemia is not a limiting factor in blood Pb uptake and that correction for low hct is unnecessary and may result in an incorrect estimation of soft tissue Pb burden. (Supported by United Cerebral Palsy Fndn.)

A COMPARATIVE STUDY OF RED CELL ADAPTATION TO HYPOXIA IN NEWBORN INFANTS AND OLDER CHILDREN. Michael S. Kramer* and Norman S. Talner, Dept. of Ped., Yale University School of Medicine, New Haven, Conn.

Red cell adaptation to hypoxia was assessed in newborn infants and children older than 6 months by measuring red cell 2,3-diphosphoglycerate (2,3-DPG), P_{50}, and Hb A and F ratios. In the older group (7 months-23 years), with over 95% Hb A, those children who were hypoxic due to congenital heart disease had red cell 2,3-DPG values of 6520±850 nmole/ml RBC (mean ± S.D.), and P_{50}'s of 29.5±1.1 mm Hg. Patients with acyanotic cardiac disease had values of 4530±610 nmole/ml RBC for 2,3-DPG and 26.0±0.6 mm Hg for P_{50}. In the newborn group, with predominantly fetal hemoglobin, the cyanotic infants had 2,3-DPG's of 7140±1140 nmole/ml RBC and P_{50}'s of 25.6±1.4 mm Hg as compared to control values of 4090±730 nmole/ml RBC and 21.0±1.1 mm Hg.

Thus, 2,3-DPG synthesis is indeed stimulated in hypoxic neonates to a degree at least as great as in older children. By taking into account the relatively weaker binding of 2,3-DPG to Hb F, i.e., by use of the concept of effective 2,3-DPG concentration (%HbA X DPG + 0.4 X %HbF X DPG), the relationship between P_{50} and 2,3-DPG can be shown to be identical in the 2 groups. These results cast serious doubt on the theory of end-product inhibition of 2,3-DPG synthesis.

ANIMAL MODEL SYSTEM FOR SICKLE CELL ANEMIA. J. Kochen, R. Kravath, S. Baez, Albert Einstein Col. of Med., Montefiore Hosp. & Med. Ctr., Dept. Pediatrics, Dept. Anesthesiology, The Bronx, New York (Intr. by Laurence Finberg)

An animal model system which permits direct microscopic observation of the effects of sickling on the microcirculation has been developed. Rats have been kept alive for up to one week following exchange transfusion with normal human blood and blood from patients with sickle cell anemia. An infusion pump connected to femoral artery and vein cannulas in the anesthetized animal was used to provide a continuous and balanced infusion and withdrawal of blood. The volumes exchanged were equivalent to the total rat blood volumes, resulting in a substantial replacement of rat red cells by human cells. The circulation of these cells through the microvasculature of the meso-appendix of the living animal was visualized by direct microscopy. Similarly, sickle blood was perfused through the isolated rat meso-appendix preparation following cannulation of the ileocolic artery and vein. The presence of sickled cells resulted in slowing of blood flow, red cell sludging and progressive plugging of the microvasculature by aggregates of sickled cells. Histologic examination of various tissues from rats transfused with oxygenated sickle blood and then subjected to hypoxia shows widespread intravascular sickling and plugging of microvessels with inspissated masses of sickled cells. These pathologic sections bear a strong resemblance to post-mortem findings in patients with sickle cell anemia. (Supported by U.S.P.H.S. grant HL-14808-01.)

URINARY HOMOVANILLIC ACID: A SENSITIVE INDICATOR OF NEUROBLASTOMA. Elwood H. LaBrosse, Priscilla A. Gilman and Audrey E. Evans, Univ. of Maryland Sch. of Med., Depts. of Surg. and Ped., Baltimore, and Children's Hosp. of Philadelphia, Dept. of Oncology, Philadelphia.

3-Methoxy-4-hydroxyphenylacetic acid (HVA) was first reported to be elevated in the urine of patients with neuroblastoma by von Studnitz (Klin. Wschr. 40:163, 1962); since that time there have been few additional reports on the urinary excretion of this metabolite by patients with neuroblastoma.

Our recent studies on urinary excretion of 8 catecholamine metabolites by these patients demonstrate that HVA is the most sensitive indicator for the tumor. HVA was significantly elevated in the urine of 11 of 15 patients with active disease. Vanilmandelic acid (VMA) was also elevated in 10 of these patients, but the levels of HVA were markedly elevated in 2 patients whose VMA levels were borderline. In 1 patient, HVA was excreted at over 8 times normal at diagnosis. His urinary HVA levels decreased to normal for 2 months following radiation and chemotherapy, then rose to 5 times normal 3 weeks prior to return of clinically apparent disease. During subsequent months, urinary levels of HVA closely paralleled exacerbation of disease and response to chemotherapeutic agents. These findings emphasize that urinary HVA can be a sensitive diagnostic and prognostic aid in patients with neuroblastoma, and they indicate that assay of urinary HVA should be routinely incorporated into the initial evaluation and follow-up of these patients. (Supported by NIH Grant CA-08726.)

JUVENILE CARCINOMA OF THE PANCREAS: EVIDENCE FOR IN VITRO AND IN VIVO DIFFERENTIATION. Vita J. Land and Milton N. Goldstein, Washington University School of Medicine, Departments of Pediatrics and Anatomy, St. Louis, Mo. (Intr. by Teresa J. Vietti).

A liver biopsy was obtained in 1968 from a 7 year old girl with an abdominal mass and hepatomegaly. A portion of the tissue from this specimen, which on histologic diagnosis was thought to be neuroblastoma, was explanted in vitro. Only sheets of epithelium grew out. Electron microscopic studies of these cells demonstrated that the outgrowth contained undifferentiated cells, some of which contained microvilli. Clinical response was obtained with radiotherapy and chemotherapy (vincristine, cyclophosphamide, and later adriamycin). A second liver biopsy was obtained at the time of laparotomy for bowel obstruction due to tumor in 1972, and again the outgrowth was composed of sheets of epithelium, some arranged in the forms of small ducts. E.M. studies of these cultures showed that the tumor cells were more differentiated, formed small lumen and that there were junctional complexes between the cells. The histology of the tumor was now compatible with a duct cell carcinoma of the pancreas. These observations were confirmed by histologic study of the tumor at other metastatic sites.

As tumor growth progresses, malignant cells usually become more anaplastic - that is, less differentiated. In this child, a reverse process occurred, and was confirmed by histologic study of the cultured cells.

ANTICIPATION AND AVOIDANCE OF ASPARAGINASE-INDUCED ANAPHYLAXIS Sue McIntosh, A.D. Schwartz, R.T. O'Brien, G.T. Aspnes and H.A. Pearson, Dept. of Pediatrics, Yale Univ. School of Med., New Haven. Remission induction in acute leukemia with l-asparaginase may be complicated by drug-induced anaphylaxis in 10 to 20% of patients. Circulating anti-asparaginase antibody can be detected by passive hemagglutination (Handschumacher, et al.), and reduction of sensitivity reactions with 6-mercaptopurine has been reported. Children with acute leukemia received 43 courses of E. coli l-asparaginase, either 500 units/Kg. 3 times weekly or 1,000 units/Kg. twice weekly. Two of 13 patients receiving l-asparaginase alone developed antibody prior to the sixth dose. Acute hypotension and dyspnea followed subsequent drug administration; both patients responded to intravenous epinephrine. A third child in whom antibody was detected reacted with acute fever, chills and vomiting. Thirty courses were given with oral 6-MP, 2.5 mg./Kg./day, concomittantly with l-asparaginase. All patients were tested for circulating antibody prior to each injection of l-asparaginase. No anaphylaxis occurred in this group. Two patients developed antibody and the drug was withheld. The concomittant use of 6-MP may reduce, but does not prevent, sensitization as indicated by production of hemagglutinating antibody. However, routine employment of the hemagglutination test for anti-l-asparaginase antibody has permitted anticipation of life-threatening drug reactions and appears reliable in avoiding clinical anaphylaxis.

PLASMA FIBRINOGEN GEL-FILTRATION CHROMATOGRAPHY IN DISSEMINATED INTRAVASCULAR THROMBOSIS (D.I.C.), Koon-Hung Luke, Jack Hirsh and Marilyn Johnston (Intr. by R.P.Bryce Larke), Depts. of Pediatrics and Pathology, McMaster University, Hamilton, Ontario, Canada.

Detection of left shift of plasma fibrinogen reactive material by Gel Filtration Chromatography has been advocated by Fletcher as a sensitive means of early detection of intravascular thrombosis. Using Sepharose 4B Chromatography and an immunodiffusion technique to detect fibrinogen reactive material we have characterized the fibrinogen chromatographic pattern of 25 normal plasma and 14 patients with suspected D.I.C. Void volume of the column with dextran blue was 10 ml. In 25 normal plasma studied, detection of fibrinogen reactive material was seen at elution volume of 1.70 ± 0.15 times the void volume. In 7 of the 14 patients D.I.C. was confirmed by thrombocytopenia high fibrin-split products in serum, positive paracoagulation tests and decreased fibrinogen. 5 of these 7 plasma samples showed left shift of detectable fibrinogen reactive material at an elution volume earlier than 1.35 times void volume. Protamin sulphate precipitation was positive in 3 of these 5 samples. Ethanol gelation test showed no correlation. In the other 6 patients, 2 showed a left shift of fibrinogen reactive material without other evidence of D.I.C. other than thrombocytopenia. Our data supports that plasma fibrinogen chromatography is a useful investigative test for the study of disseminated intravascular thrombosis.

INHIBITION OF ACTINOMYCIN D ACTIVITY BY RADIATION TREATED CELLS. J.Mendecki, C.Botstein, J.Kochen, Albert Einstein Col. of Med., Montefiore Hosp. & Med.Ctr., Dept. Radiotherapy, Dept. Pediatrics, Bronx, New York. (Intr. by Laurence Finberg) Actinomycin D (AMD) is used in combination with other chemotherapy agents and radiotherapy in a number of treatment regimens in childhood neoplasia. When combined with other forms of therapy, AMD may be used at a time when many tumor cells are no longer viable. We show that the activity of AMD may be markedly diminished in the presence of killed tumor cells. The inhibitory effect of AMD on the incorporation of tritiated-uridine into RNA of mouse sarcoma 180 cells was used as the index of AMD activity. Under standardized conditions, this inhibition was up to 98% effective. The presence of radiation or heat-killed tumor cells in numbers equal to those of viable cells resulted in a reduction of the AMD induced inhibition of RNA synthesis to 15%. The addition of native DNA, but not denatured DNA, resulted in a similar reduction in the AMD effect. The presence of DNAase reversed the dead cell and DNA-induced inhibition of AMD activity. Tritiated-AMD incorporation studies showed that the killed tumor cells had an affinity for AMD which was 3 times that of the viable tumor cells. These findings indicate that killed tumor cells and their products compete with living cells for AMD binding and thus decrease the capacity of AMD to inhibit RNA synthesis. Thus, tumors with relatively larger numbers of dead cells may be more resistant to the effects of AMD. The role of dead cells in protecting the living tumor cells is a factor to be considered when designing tumor treatment regimens. (Supported by the Lisa Casper Fund.)

THROMBOGENIC ACTIVITY OF ANTI-HUMAN THYMOCYTE GLOBULIN (ATG) H. M. Maurer, J. Thomas, F. Thomas, J. Caul and D. Hume Medical College of Virginia, Depts. of Ped. and Surg. Richmond, Virginia

ATG is widely used for immunosuppression in human organ transplantation. Intravascular thromboses have occurred in ATG recipients suggesting that it may be thrombogenic. 16 clinical preparations of horse ATG and 3 pools of rabbit ATG were studied for platelet aggregating activity (PAA) and coagulant activity. All ATG preparations had potent PAA which was not inactivated by heating to 56°C, adsorption with $BaSO_4$, dialysis against saline, and ultracentrifugation. PAA was not related to hemoagglutinin, microlymphocytotoxicity or microcomplement fixation titers of ATG. ATG also caused release of platelet ADP, activation of platelet factor 3, and shortening of the partial thromboplastin time of normal plasma without effect on the prothrombin or thrombin times. 100mM aspirin inhibited platelet aggregation and platelet factor 3 activation induced by ATG. Lower aspirin concentrations (1 and 10mM) were less inhibitory. We conclude that ATG preparations are thrombogenic and should be screened for PAA and coagulant activity prior to clinical use. I.V. administration of this material is not recommended until it is free of thrombogenic activity. The usefulness of aspirin clinically to prevent thrombotic complications warrants exploration.

DISSEMINATED INTRAVASCULAR COAGULATION ASSOCIATED WITH NEONATAL LISTERIOSIS. Ron Miller and C. Thomas Kisker, Children's Hosp., and Children's Hosp. Research Fndn., Cincinnati, O.

Petechiae may occur with neonatal listeriosis and are usually associated with a fatal outcome. A 2-day-old infant with listeriosis receiving penicillin and gentamicin developed petechiae and the diagnosis of disseminated intravascular coagulation (DIC) was established by absent platelets on blood smear, a decreased fibrinogen concentration, and a positive serial protamine sulfate dilution test (SPSD). There was also soluble circulating fibrin (SCF) in his plasma as measured by a technique based on the ability of fibrin stabilizing factor to incorporate ^{14}C glycine ethyl ester into fibrinogen after the alpha peptide of fibrinogen has been hydrolized by thrombin. The infant was treated with heparin and survived. During heparin treatment there was an initial increase of fibrinogen associated antigen in his serum. The disappearance of SCF from the plasma occurred within 12 hours. Within 24 hours, there was clinical improvement, a normal fibrinogen level, a normal SPSD test, and return of platelets on the blood smear. Heparin was then discontinued. Though no previous studies for DIC in neonatal listeriosis are known, the petechiae and purpura observed in this illness probably represent unrecognized episodes of DIC. The rapid clearance of SCF, the improvement of other coagulation tests, and the good clinical outcome suggest that a short course of heparin is beneficial when neonatal listeriosis is accompanied by DIC.

IN VITRO INTERFERON PRODUCTION BY LEUKOCYTES OF PATIENTS WITH SICKLE CELL DISEASE. Anthony B. Minnefor and Thomas R. Walters, Dept. of Ped., New Jersey Med. Sch., Newark, N. J.
(Intr. by Franklin C. Behrle)

The propensity to develop frequent, often life threatening, bacterial infections is a well documented complication of many patients with sickle cell disease. Various studies have reported humoral, cellular and mechanical defects which, together, probably account for most of these episodes. In contrast, sparse information is available concerning the effect of viral infections on this disease in general, or the response of individual patients to viral agents. As a preliminary attempt to pursue the latter, an in vitro technique was used to measure production of interferon, a protein with broad antiviral activity.

Leukocytes were isolated from 16 patients with SCD and incubated at 37°C. for 18 hours with high titered New Castle Disease virus. Aliquots of the supernatant from each reaction were inhibitory to the replication of Sindbis virus in human embryonic fibroblast culture. Reciprocals of the supernatant dilution which inhibited growth of the Sindbis virus ranged from 16 to 1024. This inhibitor had the following properties of interferon; it was species specific, non-dialyzable, sensitive to trypsin, stable at acid pH and did not directly inactivate the test virus. While this suggests an appropriate in vivo response, this and other aspects of viral infections in sickle cell disease should be pursued.

HEMOGLOBIN LEVELS IN PRESCHOOL CHILDREN BY AGE, RACE AND TRANSFERRIN SATURATION by George M. Owen, A. Harold Lubin and Philip J. Garry. Ohio State Univ., Col. of Med., Children's Hosp., Dept. of Ped., Columbus, Ohio.

In a national probability sample for 205 black children and 275 white children between 24 and 71 months of age and of comparable socioeconomic status, mean hemoglobin concentrations were 12.1 and 12.5 gm/dl, respectively ($p < .001$). Hemoglobin electrophoresis was performed on all samples and abnormal hemoglobin types were excluded. We assumed that differences in serum iron would account for these differences in hemoglobin levels. However, holding transferrin saturation and age constant, we found black children had lower hemoglobin concentrations than did white children.

Age & Race	TRANSFERRIN SATURATION (Fe/TIBC x 100) 10% N	Hgb(SD)	10-14% N	Hgb(SD)	15-19% N	Hgb(SD)	20% N	Hgb(SD)
24-47								
Black	11	11.3(1.1)	22	12.2(0.9)	18	11.8(0.8)	47	12.6(1.0)
White	17	11.6(0.9)	19	12.4(1.1)	29	12.6(0.8)	93	12.7(1.0)
48-71								
Black	13	12.1(1.0)	18	11.7(1.0)	19	11.8(1.0)	57	12.4(1.0)
White	11	12.4(0.9)	22	12.6(0.9)	22	12.8(0.8)	68	13.0(1.0)

These data indicate that even with non-limiting amounts of iron available in serum (transferrin saturation >15%), hemoglobin levels in black children were significantly ($p < .01$) lower than in white children.

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DIAGNOSIS OF HEMOGLOBINOPATHIES BY AGAR GEL ELECTROPHORESIS OF CORD BLOOD. Richard T. O'Brien, Sue McIntosh, Gregg T. Aspnes and Howard A. Pearson, Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut.

Although abnormal hemoglobin states occur with quite high frequency, detection at birth is difficult because of the presence of large amounts of Hgb F. The use of agar gel electrophoresis at pH 6.2 obviates technical problems. A program of routine screening of cord blood specimens from black and Puerto Rican newborns has been conducted for the past seven months. The following diagnoses have been established:

	Number	%
Hgb AA (normal)	420	86.5
Hgb AS (sickle cell trait)	40	8.3
Hgb AC (HgbC trait)	12	3.0
Hgb SS (sickle cell anemia)	5	1.0
Hgb SC (Hgb S-C disease)	1	.2
Hgb A-Barts (α thal. trait)	4	.8
Hgb A+fast Hgb	5	1.0

Identification of infants with major hemoglobinopathies at birth permits intensive follow up in an attempt to anticipate and hopefully prevent serious morbidity and mortality from overwhelming pneumococcal sepsis and profound anemia. Further, only by identification and careful follow up of a group of new born infants with sickle cell anemia, can a true assessment of prognosis and survival of sickle cell anemia in the United States be determined - information which is not now available.

MYCOPLASMA-LIKE ORGANISM IN HODGKIN'S DISEASE. Richard T. Parmley, Samuel S. Spicer, Samuel K. Morgan, and H. Biemann Othersen. (Intr. by Milton Westphal). Depts. of Pediatrics, Pathology, and Surgery. Medical University of South Carolina, Charleston, South Carolina.

In four consecutive children with untreated Hodgkin's disease ultrastructural examination of lymph nodes revealed unique spherical structures which resembled mycoplasma. The patients, whose ages ranged from 4 to 10 years, were staged as $CSIAPSI_{S-H-N-M-}$, $CSIIA_3PSIII_{S-N+H-M-}$, $CSIIIBPSIII_{S+N+H-M-}$, $CSIVBPSIV_{L+H-M-}$. All showed some degree of mixed cellularity. There was no clinical evidence of mycoplasma infection in any of the patients. Ultrastructurally, discreet spheroids were identified singularly and in groups attached extracellularly to the plasmalemma and within intracellular vacuoles of enormous tumor cells, reticular cells, and macrophages. The spheroids were approximately 200 to 600 millimicrons in diameter, were limited by a unit membrane, and contained from 20 to 50 closely packed ribosome-like particles evenly distributed throughout the cytoplasm. The pathologic nature of the spheroid was indicated by the apparent damage to infected cells in which the microorganism appeared to multiply and undergo developmental changes. The results of this study do not determine if a mycoplasma-like organism is primarily or secondarily involved in Hodgkin's disease. Such organisms have not been previously observed in human lymph node tissue or in Hodgkin's disease.

BONE PAIN: A SIDE EFFECT OF METHOTREXATE THERAPY IN CHILDHOOD LEUKEMIA. Sean O'Regan, David K. Melhorn, Arthur J. Newman, introduced by Samuel Gross. Case Western Reserve University Sch. of Med., University Hospitals, Dept. Ped.,Cleveland,Ohio.

Bone pain has long been a recognized symptom of leukemia in childhood. This report confirms the observation that bone pain and associated xray changes can also be produced by long term methotrexate (MTX) therapy during complete hematologic remission. Five patients (age range at onset of disease 3-7 years) maintained on MTX for periods of 7 to 14 months developed incapacitating leg and ankle pain, entirely refractory to a variety of treatments which included bed rest, radiation, bracing and splinting. Xray studies revealed severe osteoporosis of the lower extremities, as well as distal fractures and microfractures. Cessation of MTX resulted in prompt disappearance of symptoms, while hematologic remission was maintained on alternate drugs. The radiologic abnormalities persisted for much longer periods. Although xray abnormalities in several patients were described as "scorbutic changes," no alterations in calcium, phosphorus, or ascorbic acid metabolism were identified. MTX toxicity should be considered when leukemia patients on this drug develop bone pain in the lower extremities.

LYMPHOEPITHELIOMA IN CHILDREN. T.E. Pick, H.M. Maurer, and N.B. McWilliams,(Intr. by W.E. Laupus) Medical College of Va. Dept. of Pediatrics, Richmond, Virginia

Clinical features and therapy of lymphoepithelioma, a rare tumor in childhood,are incompletely reported.Between 1962-1972 9 children,4 boys and 5 girls,all black,ages 28 mos.-17 yrs.,were diagnosed as having lymphoepithelioma of the nasopharynx.Symptoms included painful unilateral cervical mass (9/9),weight loss (7/9),trismus (5/9),epistaxis (4/9),torticollis (2/9),and voice change (2/9).Abnormal physical findings were tender unilateral cervical adenopathy (9/9),nasopharyngeal mass (7/9) with soft palate depression (4/9),and 6th nerve palsy (1/9).Radiographs revealed mastoiditis (2/9), basilar skull erosion (2/9) and nasopharyngeal mass(3/9).None of the tumors was resectable. 7 were treated initially with radiotherapy (R.T.) alone (4000-6000 r),and 2 with R.T. (4550, 6000) plus daily oral cytoxan. 8 children showed complete tumor regression for 2 mos. to 6+ yrs. 3 of the 8 responders had local recurrences or distant metastases (lung,bone,nodes) at 2-6 mos. following R.T. alone.These 3 were treated with additional R.T. and oral cytoxan was added.There was partial response in the 2 with metastases and complete tumor regression for 3+ yrs. in the child with local recurrence.Other antitumor agents were of no benefit in those with metastases. 6 children remain in remission for 2 mos. to 6+ yrs. 3 of these are maintained on oral cytoxan. The 3 deaths occurred after metastases developed.

INTERACTIONS OF G6PD DEFICIENCY AND SICKLE CELL ANEMIA. Sergio Piomelli, Laurence Corash and Mohamed Arzanian. Department of Pediatrics, New York University School of Medicine.

The interactions between the African type of G6PD deficiency (GdA^-) and sickle cell anemia (SCA) were studied.

In SCA patients the G6PD genotype cannot be assessed solely by the biochemical assay, which might appear normal in GdA^- individuals as a result of the young mean red cell age. In 56 unrelated male SCA patients the correct G6PD genotype was established by a combination of assay, electrophoresis and cytochemical staining of individual red cells.

Of 56 SCA males 15 (27%) were GdA^- and 6 (11%) GdA^+, of 207 Black males 22 (11%) were GdA^- and 22 (11%) GdA^+. The frequency of GdA^- among SCA patients was significantly increased: $\chi^2 = 8.12$; $p < .01$. There was a tendency to increasing frequency with age.

Age Group	1-10 Years	11-20 Years	Over 20 Years
GdA^-/total	6/28 (21%)	4/16 (25%)	5/12 (41%)

The genes for GdA^- and Hgb S are on two different chromosomes. At conception, the frequency of GdA^- in SCA offspring must be identical to that in the general Afro-American population. The increased frequency of GdA^- among SCA patients indicates that concomitant inheritance of GdA^- enhances the probability of survival of the SCA patient. The increasing frequency with age suggests a protective effect of GdA^- in SCA throughout the patient's life.

A RAPID, SIMPLE TEST FOR DETECTION OF SICKLE HEMOGLOBIN (Hb S). Elias Schwartz, Toshio Asakura and Shlomo Friedman, Univ. of Pennsylvania, Children's Hosp., Depts. of Ped. and Biophysics, Philadelphia, Pa.

The most commonly used tests for Hb S rely on abnormal properties of deoxy-Hb S or on electrophoretic mobility of the hemoglobin. We have found that oxy-Hb S is abnormal in its ease of denaturation by mixing. This property is useful in detecting Hb S in hemolysates. The mechanical instability of oxy-Hb S is easily demonstrated by the turbidity which develops on shaking one drop of sickle cell disease (Hb SS) blood in 2 ml of water for a few minutes. An extension of this test distinguishes between sickle cell trait (Hb AS) and Hb SS. The hemolysate is shaken for 15 minutes. A Hb SS solution loses most of its color during this time due to precipitation of Hb S. A Hb AS solution will also become turbid, but it only loses one-half of its color. Further studies have shown that denaturation is maximal at pH 8.0, and is increased by raising ionic strength. A quantitative test may be performed by diluting hemolysates in 0.1 M Tris HCl buffer at pH 8.0 and shaking on a Vortex mixer. After 10 minutes, 10% of Hb AA, 44% of Hb AS and 90% of Hb SS are precipitated. Hb CC acts similarly to Hb AA, and Hb SC to Hb AS. Hb F is similar to Hb A and does not protect Hb S from denaturation. This abnormal property of oxy-Hb S may serve to confirm the presence of Hb S, or as a rapid bedside screening test.

SCREENING TEST FOR ALPHA-THALASSEMIA IN NEWBORNS. Alvin H. Schmaier, Harold M. Maurer, Charles L. Johnston and Robert B. Scott. Medical College of Virginia, Depts. of Ped., Path., and Med., Richmond, Virginia

Although α-thalassemia trait may be diagnosed in the newborn by the presence of Hgb Barts, no simple screening test for this disorder is available. Our aim was to determine whether the mean corpuscular volume (MCV) and mean corpuscular Hgb (MCH) could serve this purpose. Capillary blood samples from 200 full-term black infants, 1-3 days of age, were drawn for determination of RBC indices (Coulter S) and Hgb electrophoresis (cellulose-acetate).

Of the 200 infants, 9 had MCV's of $\leq 94u^3$ and MCH's of $\leq$ 29.5uug and of these, 6 (67%) had Hgb Barts ranging from 3 to 6.6% of total Hgb. No infant with Hgb Barts had indices in the normal range (normal values expressed as mean minus tolerance limit: MCV 106.4 - 9.4; MCH 34.5 - 3.5).

We conclude that α-thalassemia may be easily detected through MCV and MCH screening of newborn populations at risk. An MCV $\leq 94u^3$ and MCH $\leq$ 29.5uug in the newborn is abnormal and should be followed by a Hgb electrophoresis. Our 3% incidence of α-thalassemia is consistent with its reported incidence in blacks.

LONG-TERM MANAGEMENT OF FAMILIAL ERYTHROPHAGOCYTIC LYMPHOHISTIOCYTOSIS. Narayan Shah and James A. Wolff. Coll. of Physicians & Surgeons, Columbia Univ. and the Babies Hosp. Dept. of Ped. New York City.

Familial erythrophagocytic lymphohistiocytosis is a disease involving the reticuloendothelial system, manifested clinically by hepatosplenomegaly, fever and pancytopenia. This entity has been described under various names. No case reported to date has survived more than six months after diagnosis.

We describe two cases in two different families, in which the first sibling died of the disorder within six months of diagnosis, whereas the second affected sibling in each family has survived thus far. In the first family, the patient is in good health after four years and in the other family the disease in the second affected sibling has been under control for 7 months. Both living subjects have been treated with oral Methotrexate and Prednisone daily. When the Prednisone dosage has been greatly reduced or discontinued, relapse of the disease has occurred rapidly.

The etiology of the disorder is not known. Our cases have shown immunoglobulin deficiency. During remission splenic function, as tested by Tc-99 scan, has been normal in one patient. Immune deficiency may play a role. No support for an infectious or malignant origin has been advanced.

ERYTHROBLASTOSIS FETALIS SECONDARY TO MATERNAL AUTOIMMUNE HEMOLYTIC ANEMIA. (AIHA) Julian B. Schorr, Richard Fass, Phyllis Schorr. Intr. by Murray Davidson. Albert Einstein College of Medicine-Bronx Municipal Hospital Center, The Bronx-Lebanon Hospital Center, Bronx-New York.

AIHA rarely causes erythroblastosis. The infant of a 25 year old primigravida became anemic, hematocrit falling to 19% at 12 days with normoblastosis and positive direct antiglobulin test.

The mother had thrombocytopenia and AIHA due to anti-e, at age 13. Her illness, initially responsive, became stormy and unresponsive both to steroids and splenectomy, then remitted with 6 MP. She remained well for 8 years, receiving no therapy for the last 6 years.

Late in this pregnancy, a slight fall in hemoglobin was noted. At term a circulating panagglutinin was found. Following delivery the mother developed progressive pallor and weakness and on the 12th post-partum day was rehospitalized with hematocrit 11%, reticulocytes 0.0% and antiglobulin test strongly positive. Following transfusion and 4 months of steroid and 6 MP therapy, the process remitted; she has been well for 8 months, off all therapy.

Reappearance of AIHA during pregnancy in a woman who has been well for 8 years is unusual as is the presence of hemolytic disease in her offspring. Review of the literature suggests that pregnancy may cause exacerbation of AIHA, and that women with this disorder should be so informed.

PRESENCE OF ANTI-NUCLEAR ANTIBODY (ANA) ON THE RED CELL SURFACE IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE). Ashok Shende, Dario Perez & Arthur Sawitsky. Long Island Jewish-Hillside Medical Center, New Hyde Park, N.Y. (Intr. by Philip Lanzkowsky)

The presence of ANA on the red cell surface in SLE has not been reported. Nine patients with SLE were studied: 7 had positive red cell eluates for ANA, 6 of these had positive serum ANA & 5 had positive direct anti-globulin test (Coombs). A positive ANA test was found in the red cell eluate of one patient whose serum ANA tests were negative although serum ANA tests in the patient were positive 3 months previously. In the remaining 6 patients both serum and red cell eluates were positive. In vitro absorption elution experiments were performed using blood group compatible patient and control serum and red cells. When normal serum was incubated with the patient's red cells, ANA could be detected in the normal serum. When the patient's serum and the patient's eluted red cells were incubated recoating of the patient's red cells with ANA took place. When control red cells were incubated with patient's serum, ANA could be eluted from saline washed post-incubation control cells. It is concluded that ANA is absorbed on the red cell surface in patients with SLE whose serum ANA may be positive or negative at the time of testing. In vitro studies demonstrate the passive absorption of ANA to the red cell surface in patients with SLE. Tests for ANA in red cell eluates of patients with negative serum ANA may be valuable in the diagnosis of subtle cases of SLE.

PROGNOSIS OF ACQUIRED APLASTIC ANEMIA IN CHILDHOOD: Stuart E. Siegel, Yigal Barak, Jorge A. Ortega, Carol B. Hyman, Kenneth O. Williams, Gussie R. Higgins, Nomie A. Shore, and Myron Karon. Children's Hosp. of Los Angeles, USC Sch. of Med, Los Angeles, California.

While combined androgen and corticosteroid therapy in acquired childhood aplastic anemia is widely used, the efficacy of such therapy is presently the subject of controversy. To clarify the role of these agents in altering the natural history of this disease, data were analyzed from 58 patients seen and followed at this institution in the past 23 years. Thirteen (22.4%) of the patients survived. The remaining 45 patients succumbed to infectious and/or hemorrhagic complications; 75% of these patients died within 6 months of diagnosis. Three of 17 patients receiving no specific therapy, 8 of 26 receiving corticosteroid alone, and 2 of 15 patients receiving androgen and corticosteroid survived. Factors correlated with unfavorable prognosis included age at diagnosis > 5 years; initial bone marrow demonstrating marked hypocellularity with < 5% erythroid precursors, < 10% myeloid precursors, >60% lymphoid precursors, and/or increased numbers of tissue mast cells, plasma cells, and macrophages. Presenting signs and symptoms, initial hemogram and reticulocyte count, and increased bone marrow fat were of no prognostic value. These data support the conclusion that the prognosis in acquired childhood aplastic anemia is not improved by the addition of androgen therapy. Further improvement in outcome awaits the use of newer methods of preventing infectious and hemorrhagic complications, and the development of more effective therapeutic agents.

IDIOPATHIC AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA) IN CHILDHOOD: DIAGNOSIS, TREATMENT AND OUTCOME IN 13 PATIENTS. M. Stuart, T. Kinney, S. Murphy and F. Oski. The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, and the S.U.N.Y., Upstate Medical Center, Syracuse, New York.

Between 1966-1972, 13 patients, ages 4 months to 7 years, were seen with AIHA. Onset was acute in 9 and insidious in 4. The hemoglobin averaged 4.7 gm% (range 2.0 - 8.4 gm%) with a mean reticulocyte count of 16.4% (range 0.8 - 97%). Four children demonstrated relative or absolute red cell aplasia at the time of diagnosis. In all cases the direct Coombs' test was positive although in one patient it could be demonstrated only with the use of anti-IgA antisera. Antibodies were frequently multiple, warm type panagglutinins: 5 examples of anti-e and 2 of anti-C were encountered. Neither a viral etiology or an immune defect could be demonstrated. All patients were treated with prednisone, 2 - 10 mg/Kg/day. Patients refractory to a dose of 2 mg/Kg responded when higher doses were employed. Treatment was necessary for an average of 8 months (range, one month to two years). One child required splenectomy. Transfusions were employed sparingly. One child died within 48 hours of diagnosis. Three of the four patients with a chronic course of more than one year's duration had associated thrombocytopenia. This survey illustrates the nature of AIHA in children and contrasts its course with those of adults.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY (G-6-PD) IN HEMOPHILIA B. Lance Sieger, Jack Lazerson, Gussie Higgins. (Intr. by Myron Karon). Division of Hematology, Childrens Hosp. of Los Angeles and Department of Ped., USC School of Medicine, Los Angeles, California.

Both the genes associated with G-6-PD and Factor IX are located on the X-chromosome. To date, no evidence for close association between these two genes has been described. We have studied a family in which red cell G-6-PD deficiency and Hemophilia B appear to be inherited together. The family of the propositus consists of 4 brothers and 1 sister. Three of the boys manifest Hemophilia B clinically and have markedly decreased Factor IX activity levels by assay. The mother of this family has an intermediate level of G-6-PD activity consistent with a heterozygous state and has normal Factor IX. Her father was a "bleeder". All 3 brothers with Hemophilia B have very low levels of G-6-PD in their red cells. The sister has a decreased level of erythrocyte G-6-PD activity, intermediate between the levels of her mother and of her hemophiliac brothers, and normal Factor IX activity. The normal brother has normal red cell G-6-PD and Factor IX activity.

It appears likely that both the abnormal genes for Factor IX and G-6-PD are located on the same X-chromosome in the mother and, furthermore, that these are linked in view of the absence of evidence of crossing over in any of the 5 children in this family. These data would imply that the sister is an obligate carrier of Factor IX deficiency.

IMPAIRED PLATELET AGGREGATION IN SICKLE CELL ANEMIA. M.J. Stuart, J.A. Stockman and F.A. Oski. Dept. of Pediatrics, SUNY, Upstate Medical Center, Syracuse.

Platelet aggregation was studied in 20 normal controls and 14 patients with SS Disease (6 when asymptomatic, and 8 in "vaso-occlusive" or "painful" crises). Platelet aggregations were performed using 0.8 micromolar adenosine diphosphate (ADP), and the slope of the curve determined, in both the patients and the controls. When compared to 20 normal controls a significant reduction in the rate of ADP induced platelet aggregation (p value $<.0005$) was present in 7/8 patients with SS Disease during their vaso-occlusive crises. These changes were, however, temporary, reverting to normal within a 2 to 4 day period, and coincided with relief of their pain. Platelet aggregation was normal in all 6 sicklers studied when asymptomatic. The impairment of ADP induced aggregation was independent of the analgesic used, and the presence of either Fibrin Degradation Products, or serum bilirubin concentrations.

This demonstrated temporary impairment of ADP induced platelet aggregation, or "refractory" state in SS Disease with vaso-occlusive crises, is analagous to that seen in the post-operative patient, and may be due to ADP released from red cells or damaged tissues. The possible role of the platelet in the production or maintenance of the vaso-occlusive crisis requires clarification - ADP induced platelet aggregation may prove a useful tool in the initial differentiation of the infected patient from one in vaso-occlusive crisis.

NUCLEOTIDE METABOLISM IN PLATELET FUNCTIONAL DISORDERS
Clive C. Solomons, William E. Hathaway, and Edward A. Millar, Univ. of Colo. Med. Ctr., Dept. Ped., Denver and Shriners Hospital, Chicago, Ill.

This investigation correlates the nucleotide biochemistry of platelets with their physiologic function in several disease states. The incorporation of U-C-14 adenine into ATP, ADP, AMP, IMP, cyclic-AMP, and hypoxanthine was determined in platelet-rich plasma (PRP) of 82 subjects. Platelet aggregation to ADP, l-epinephrine and collagen was done concurrently. The % of the nucleotide pool present as cyclic-AMP+hypoxanthine for normal children was 4.6 ± 1.7, n=14 and 3.8 ± 1.0 n=11 for adults. The C-AMP+hypoxanthine proportions in subjects who showed abnormally-low platelet aggregation were: normal newborns 26.3 ± 17.4 n=10 p 0.005; osteogenesis imperfecta 9.7 ± 5.4 n=37 p 0.005; congenital failure to release ADP 22.3 ± 13.3 n=4 p 0.005; neurofibromatosis 12, 18; chronic ITP 16; post-ASA 8.6, 3.0; von Willebrand 17, (normal aggregation but decreased adhesion). Other nucleotides were also affected, with prominent decreases in ATP and increases in ADP, AMP and IMP. In general, changes in the proportion of C-AMP+hypoxanthine and other nucleotides correlated best with decreased platelet aggregation to l-epinephrine in subjects with abnormalities of platelet function. The relatively simple determination of C-14 adenine incorporation appears to be a useful tool for studying a variety of platelet functional defects.

THE CHANGING MANAGEMENT OF CHILDHOOD HODGKIN'S DISEASE. Charlotte Tan, Philip Exelby, Giulio D'Angio, Jagdish Sidhu, and M. Lois Murphy. Mem. Sloan-Kettering Cancer Ctr., Dept. of Ped., N. Y.

Between 1928-1972, 140 children with Hodgkin's disease were treated. Before 1959, lymphangiography (LAG) was not used for staging, radiation therapy (RT) was limited to clinically involved areas and chemotherapy consisted of alkylating agents and adrenal steroids. The median survival of 54 patients treated was 3 years from diagnosis. Use of LAG, extended field RT and of vinblastine and procarbazine increased the median survival of 62 children, treated during 1960-1969, to 4+ years. From 1970-1972, 24 children had splenectomy and biopsy of the liver and abdominal lymph nodes for more accurate staging. This led to more precise definition of involved field RT. Since 1970, 16 previously treated children with stage IV disease, diagnosed between 1967-1972, were put on a multiple drug regime, consisting of adriamycin to induce remission, followed by combined prednisone, procarbazine, and vincristine, then by cyclophosphamide. Of these, 15 achieved remission. After initial remissions of 2 to 18 months, 4 died and 3 are on other therapy. Eight are continuing free of disease: 4 for 1-8 months, 2 > 12 months, and 2 > 24 months. The median duration of remission was 12+ months, as compared with 2 months in patients treated with a single drug. As in acute leukemia, this newer multiple drug regimen has given longer remissions; and RT directed precisely to involved fields, facilitated through improved diagnostic techniques, should increase survival.

HEMOGLOBIN CONCENTRATION (Hgb) OVERESTIMATES OXYGEN CARRYING CAPACITY ($C_{MAX}O_2$) DURING FAVIC CRISES. Paul M. Taylor, Leonidas G. Morphis and K. Mandalenaki. Univ. of Pittsburgh, Sch. of Med., Magee-Womens Hosp., Dept. of Ped. and Athens Univ. Med. Sch., St. Sophia Hosp., Dept. of Ped.

The acute hemolytic anemias triggered by ingestion of fava beans in certain individuals with G-6-PD deficient red blood cells and by phenylhydrazine in dogs are similar as to time-course, severity and the presence of Heinz bodies in peripheral red blood cells. We tested the possibility that $C_{MAX}O_2$ (ml O_2/100 ml) might also fall relatively more than Hgb (cyanmethemoglobin method) in children during favic crises as it does in dogs following injection of phenylhydrazine (P.M.Taylor, et al.,Proc.Soc.Exper.Biol.Med., in press). In each of 11 G-6-PD deficient Greek infants and young children with favism (Hgb 3.4-9.1 g/100 ml) $C_{MAX}O_2$ of whole blood ranged from 61-92% (mean, 76%) of that expected from Hgb and the O_2 binding capacity (ml O_2/g Hgb) of non-anemic G-6-PD deficient children. $C_{MAX}O_2$ of dialyzed, buffered, aqueous solutions of the Hgb of these patients ranged from 75-92% (mean, 80%) of that expected from Hgb. After removal of Heinz bodies by ultracentrifugation, $C_{MAX}O_2$ of the supernatant increased (in all cases) to a mean of 96% of that expected from Hgb. Heinz bodies thus seem to account for most of the non-O_2 carrying apparent Hgb during favic crises.

NITROBLUE TETRAZOLIUM TESTING IN SICKLE CELL ANEMIA. Thomas R. Walters and B. Narasimha Reddy, Dept. of Ped., New Jersey Med. Sch., Newark, N. J.
(Intr. by Franklin C. Behrle)

Polymorphonuclear leukocyte defects may play a role in the increased susceptibility of the patient with sickle cell anemia to bacterial infections. Inconsistent results have been obtained when NBT tests have been performed in this disease. This study reports the results of NBT tests in our patients with sickle cell anemia.

A histochemical technique was used. The results were recorded as percent and absolute number NBT positive cells. The results were correlated with the clinical, radiologic and bacteriologic findings. Forty-three patients have been evaluated. A positive test (25 - 67 %) was found in seven patients; one each with pneumococcal septicemia, H. influenza septicemia, meningitis with possible brain abscess, pneumonia with lung abscess and three children with pneumonia. Two children, who in painful crisis had negative tests, became NBT positive when they developed pneumonia. The test was negative (i.e. $<$10 %) in thirty-four patients who were either in a painful crisis with no evidence of bacterial infection or ambulatory.

These findings indicate that the NBT test has been useful as an additional parameter in evaluating the possibility of bacterial infection in patients with sickle cell anemia. Granulocytic function, as evaluated by the NBT test, was appropriate for patients with bacterial infections.

KINETICS OF THE SICKLING PROCESS. Harold S. Zarkowsky, Robert M. Hochmuth and Steven Springrose (Intr.by Alan Robson), Washington University, Department of Pediatrics and Chemical Engineering, St. Louis Children's Hospital, St. Louis Mo.

The kinetics of sickling were studied in a parallel-plate flow-channel in which rapid equilibration of erythrocytes with ambient pO_2 is achieved. Sickle cells were introduced into the flow-channel and allowed to settle onto the glass surface. Deoxygenated buffer was passed through the channel and morphologic changes were recorded at 30 second intervals by phase photomicrographs of a constant field of 200 cells. The change in pO_2 within the flow-channel during passage of deoxygenated buffer was measured with microelectrodes positioned in the channel. The rates of sickling were determined for cells exposed to the passage of buffers with pO_2's between 5 and 40 mmHg. Deoxygenation of the channel began within 7 seconds after initiation of flow and by 40 seconds the pO_2 was within 2% of buffer pO_2. However, the full extent of sickling at a given pO_2 was not reached before 120 seconds, thus indicating a lag in the sickling process. When sickled cells were exposed to oxygenated buffer, unsickling occurred simultaneously with the pO_2 increase. These studies indicate that the hemoglobin aggregation and cell deformation of sickling are not instantaneous processes.

IMMUNOLOGY

First Session

COMBINED IMMUNODEFICIENCY DISEASE AND ADENOSINE DEAMINASE DEFICIENCY, AN INBORN ERROR OF METABOLISM. B. Pollara, R.J. Pickering and H.J. Meuwissen (Intr. by M.L. Cowger). Dept. of Ped., Albany Med. Col., Albany, N.Y. and Depts. of Ped. and Microbiol., Dalhousie Univ., Halifax, Nova Scotia.

We previously reported an association (Lancet, Vol. II, #7786, p. 1067, 1972) of absent adenosine deaminase activity (ADA) in erythrocytes of two females with combined immunodeficiency (CID). We have now studied more than 18 patients with a variety of immunodeficiency diseases and discovered two additional patients (one male, one female) with ADA deficiency and CID. One male and two females lacking ADA activity had severely impaired cellular and humoral immunity. The history of the affected male suggested autosomal inheritance of his disease. The group of patients with CID and normal levels of ADA included two males with definite evidence of X linked disease. ADA deficiency has not been found thus far in patients with other forms of immunological deficiency disease or in large numbers of normal subjects. The autosomal recessive transmission of ADA deficiency is shown by studies of ADA levels in the blood of carriers: all parents of affected patients investigated so far have decreased levels of ADA activity when compared to a normal population control group. The biologic role of purine salvage in immunocompetence has not yet been defined but our findings suggest that some immunodeficiency states may be reflections of inborn errors of metabolism. (Supported by N.Y.S. Kidney Disease and Birth Defects Institutes and Canada Council.)

MATERNAL MARROW TRANSPLANT IN A PATIENT WITH COMBINED IMMUNE DEFICIENCY DISEASE (CID) AND ADENOSINE DEAMINASE (ADA) DEFICIENCY. H.J. Meuwissen, E. Moore, B. Pollara (Intr. by M.L. Cowger). N.Y.S. Birth Defects and Kidney Disease Insts. and Dept. of Ped., Albany Med. Col., Albany, N.Y.

We have described (Lancet 2:1067, 1972) a patient with CID and a genetically determined deficiency of ADA in her red cells. This girl had all clinical hallmarks of CID. She was lymphopenic and her lymphocytes responded poorly to PHA. IgM and IgG were very low but IgA was normal in serum, and absent in saliva; antibody to some antigens was produced. In addition, she had intestinal lymphonodular hyperplasia and mild neutropenia. ADA activity was absent not only in RBC but also in her lymphocytes; her parents were heterozygous for both red cell and lymphocyte defects. The patient was HLA non-identical with her mother, but MLC identical. She was transplanted three times with increasing doses of maternal bone marrow cells. Five weeks following the last transplantation (19 x 10^6 bone marrow cells per KG) fever, hepatomegaly, and diarrhea began. Atypical lymphocytes appeared in the blood in great numbers and CMV infection was documented in blood and urine. Now, three months after the last transplant, immunoglobulins have become normal, and the number of lymphocytes have increased, but cell mediated immunity has not been normalized. The maternal marrow cells may have repopulated the patient's tissues, or alternatively, may have provided sufficient ADA for expansion of this child's own lymphoid cell population. (Supported by N.Y.S. Dept. of Health.)

ONTOGENY IN THE HUMAN FETUS OF CERTAIN IN VITRO CORRELATES OF CELL MEDIATED IMMUNITY. Martin C. Carr, Daniel P. Stites, H. H. Fudenberg, Univ. of Calif. Sch. of Med., Depts. Ob.-Gyn. and Med., San Francisco, Calif. (Introduced by M. Thaler).

The purpose of this study was to determine the precise order of acquisition by various human fetal lymphoid tissues of in vitro reactivity to phytohemagglutinin (PHA) and to mitomycin treated adult allogeneic cells (MLR) both of which are considered to be attributes of lymphoid cells possessing cellular immunity. Reactivity of these tissues to PHA or in a MLR was measured by ^{3}H-thymidine incorporation. PHA reactivity was seen in the thymus after 11 1/2 wks fetal age, with 16 of 16 reactive; next in the blood by 14 wks, 8 of 9 reactive; followed by the spleen at 15 wks, 8 of 9 reactive. Activity was not evident in 14 of 18 hepatic or in 8 of 8 marrow preparations. The reactive livers were minimally positive. Reactivity in the MLR was seen in the liver by 5 wks fetal age, 18 of 21 reactive; in the thymus at 12 1/2 wks fetal age, 12 of 12 preparations reactive; followed at 13 1/2 wks by both blood, 4 of 4, and spleen, 6 of 7. Marrow cells (4 of 4) did not react. These results show that in the human fetus MLR reactivity precedes PHA reactivity; however, in the thymus PHA reactivity appears before the MLR. They are consistent with the current belief that PHA reactivity is thymus derived, but introduce the possibility that MLR reactivity arises elsewhere.

MACROPHAGES AND THE DEVELOPMENT OF IMMUNOCOMPETENCE, R. Michael Blaese, NIH, Bethesda (Intr. by John L. Sever).

Neonatal animals of almost all species are relatively immunodeficient despite wide differences in physiologic maturity and thymic development at birth. Neonatal Lewis rats are unresponsive to most antigens and are particularly susceptible to fatal infections with Listeria Monocytogenes. Newborn rats given adult splenic lymphocytes or thymocytes remain immunodeficient, while neonates given adult peritoneal exudate macrophages (MØ) become resistant to Listeria infection and produce antibody to several antigens. Neonates irradiated before MØ transfer fail to produce antibody indicating that the macrophage inoculum is actively inducing the neonate's lymphoid cells to produce the antibody found. The neonate's own MØ can be activated by a variety of materials so that adult MØ need not be given to accelerate the development of immunocompetence. Brucella vaccine, pertussis vaccine, endotoxin, BCG, alum and poly A·U are all capable of promoting antibody synthesis in the neonate through MØ activation, and these same agents also confer resistance to Listeria infection via this mechanism. The relative ease with which neonatal animals develop immunological tolerance is also a function of MØ immaturity, and tolerance can be prevented by providing the neonates with adult MØ or MØ activating substances. Thus, many aspects of the immunodeficiency of the neonate including failure of antibody production, susceptability to infection with facultative intracellular microorganisms, and ease of immunological tolerance induction, can be attributed to immaturity of the macrophage-afferent limb of immunity.

DEFICIENCY OF LYMPHOCYTES FORMING ROSETTES WITH SHEEP ERYTHROCYTES IN PARTIAL DIGEORGE SYNDROME. Harold W. Lischner, Dale S. Huff, Suhung Hann and Angelo N. DiGeorge, Temple Univ. Sch. of Med. and St. Christopher's Hosp. for Children, Philadelphia.

Lymphocytes which form rosettes with sheep RBC are thought to be thymic dependent. Enumeration of these was evaluated as a means of establishing minimal thymic deficiency in partial DiGeorge syndrome. The assays were performed by the method of Jondal, Holm and Wigzell (J. Exptl. Med. 136:207, 1972) on mononuclear cells obtained on a ficoll-hypaque gradient. A 3 year old girl with a complex of anomalies known to be associated with DiGeorge syndrome, absence of thymus shadow on chest X-ray at age 3 days, mild lymphopenia and depletion of deep cortex in a lymph node biopsy was the first subject. Although her immunologic function was essentially normal by most criteria including skin allograft rejection, 3 to 16% of her peripheral mononuclear cells formed rosettes with sheep RBC as compared with 40 to 58% in simultaneous controls. In addition, a 5 day old girl with anomalies of the great vessels and only 0.8 gm of thymus, all located high in the neck, was studied postmortem. Although 60% of the mononuclear cells suspended from her thymus formed rosettes, only 3% of those from lymph node and 0.5% of those from spleen formed rosettes. There was a compensatory increase in proportion of cells giving membrane immunofluorescence with antiimmunoglobulin sera. This rosette assay is the most promising of several parameters evaluated by us for identifying thymic hypoplasia during life. (Supported by grants # AI-00863, RR-75 and RR-5624 from the USPHS.)

PANHYPO-γ-GLOBULINEMIA AND DYS-γ-GLOBULINEMIA IN FAMILIES WITH X-LINKED B-CELL DEFICIENCIES. Randall M. Goldblum, Ralph A. Lord, Max Cooper and Armond S. Goldman, Dept. of Pediatrics, Univ. of Texas Medical Branch and Shriners Burns Inst., Galveston, Texas and Univ. of Alabama in Birmingham, The Medical Center, Birmingham, Alabama.

Two unrelated families, each with two male siblings with an X-linked antibody deficiency, were investigated. The clinically more severely affected sibs were panhypo-γ-globulinemic, while those with a more benign course demonstrated an unusual dys-γ-globulinemia characterized by decreased serum IgG (275 and 380 mg/100 ml), IgM (22 and 0 mg/100 ml) but normal IgA (98 and 110 mg/100 ml). Cell-mediated immunity and pokeweed mitogen (PWM) lymphocyte stimulation were normal. However, all four boys exhibited marked deficiencies of surface immunoglobulins by immunofluorescent staining (< 3.2% of blood lymphocytes).

The studies indicate that serum immunoglobulin levels are variable manifestations of the same X-linked genetic defect, whereas, the consistent expression of the genetic abnormality is the deficiency of surface immunoglobulins on blood lymphocytes. This suggests that the development of immunoglobulin-bearing cells is more closely related to the action of this gene than are levels of serum immunoglobulins. Studies of PWM stimulation and surface immunoglobulins of lymphocytes suggest that female carriers in these two families may manifest a partial B-cell deficiency. The inheritance pattern in both families indicates that the B-cell deficiency locus is widely separated from that of deutans color-blindness.

ANTIBODY TO THYMUS CELLS IN LUPUS ERYTHEMATOSUS. Robert L. Levy, Sheldon Horowitz, Doris Lattos and Richard Hong, Dept. of Pediatrics and Laboratory Medicine, Univ. of Wisconsin Health Sciences Center Madison, WI.

Antibody of the IgM and IgG classes directed against human thymic cells was found by immunofluorescence in 9 of 14 patients with systemic lupus erythematosus. IgA antibodies were not detected. The antibody was optimally cytotoxic at 4°C in the presence of complement but was not correlated with the lymphotoxin recently described in viral infections. The antibody was not found in other collagen diseases surveyed, nor in normal controls.

Thymus deficiency is intimately related to the pathogenesis of SLE. Thymectomy is known to hasten the progress of SLE in NZB/NZW mice and thymus transplantation delays the onset. Thymus weights at autopsy are markedly decreased in patients dying of SLE. Recently a naturally occurring cytotoxic thymus antibody was described in NZB/NZW mice. Thus, antibody mediated thymic destruction may play a significant role in the pathogenesis of SLE.

Supported by AM 15086.

EFFECTOR PHASE ABNORMALITIES OF CELL MEDIATED IMMUNITY IN FAMILY MEMBERS OF A CHILD WITH HEREDITARY T-CELL IMMUNODEFICIENCY. Flossie Cohen and James J. Lightbody. Wayne State Univ. Sch. of Med., and Children's Hosp. of Michigan - Detroit.

It has been shown that peripheral blood lymphocytes sensitized by allogeneic cells in the one way mixed leucocyte culture (MLC) are rendered specificially cytotoxic to lymphocytes autologous to the sensitizing cells, or to lymphocytes posessing the same or cross-reacting histocompatibility antigens as the sensitizing cells. The degree of cytotoxicity is measured by the chromium release from the target cells and is thought to be a measure of the effector limb of cell mediated immunity. We have used this system in conjunction with MLC to study the family members of a patient with hereditary T-cell immunodeficiency. In the absence of consanguinity, and apparently independent of the HLA typing, it was found that the lymphocytes of the parents failed to demonstrate cytotoxicity against one another, despite their ability to stimulate one another in MLC. In addition, their lymphocytes were only minimally cytotoxic to non-members of the family despite a strong stimulation in MLC. These findings suggest a possible defect in the effector phase of cellular immunity in the parent's lymphocytes with relative integrity of the affector phase. However, their lymphocytes were capable of both sensitizing and inducing a strong cytotoxic response in the lymphocytes of non-members of the family. The data suggests the possible application of the cytotoxicity assay for the detection of heterozygotes in immunodeficiency syndromes. (Supported by Am. Cancer Soc. Grant IC 82).

ABNORMALITIES IN DISTRIBUTION OF SERUM IMMUNOGLOBULIN CONCENTRATIONS IN JUVENILE RHEUMATOID ARTHRITIS (JRA). James T. Cassidy, Ross E. Petty & Donita B. Sullivan (Intr. by William J. Oliver). University of Michigan Medical School, Ann Arbor.

In addition to the well documented arthritis found in children with hypogammaglobulinemia, an association between JRA and selective IgA deficiency has been reported by this laboratory. In the present study, we explored the validity of this relationship by examining discrete immunoglobulin responses in 200 children with JRA. Probit distribution of the serum concentrations of IgA showed marked deviation from normal, not all of which was accounted for by the 4% of patients with undetectable serum and salivary IgA. In contrast, relative frequency distributions of serum IgG and IgM approached log-normal curves. In order to permit further intragroup comparisons, serum immunoglobulin concentrations on these patients were standardized by comparison to 520 sera from children stratified for age and sex, so that individual values expressed the extent of deviation from normal. There was concordance of IgG with IgA in JRA and IgG with IgM ($p<0.001$). The degree of hypergammaglobulinemia in these children was not related to sex. Females had significantly lower levels of IgA ($p<0.003$) and IgM ($p<0.01$) than males. Serum IgG levels were comparable in both sexes. The high prevalence of selective IgA deficiency and the relative inability of girls (in whom JRA is twice as common as in boys) to develop high levels of serum IgA, may reflect an underlying immunodeficiency predisposing to development of inflammatory joint disease.

QUALITATIVE DIFFERENCES IN ANTIBODY TO GROUP A STREPTOCOCCAL CARBOHYDRATE AMONG RHEUMATIC AND NON-RHEUMATIC INDIVIDUALS. Stanford T. Shulman and Elia M. Ayoub, Dept. of Ped., Univ. of Florida, Gainesville, Fla. 32601

Previous studies of antibody response to group A streptococcal antigens have focused on quantitative aspects of this response. The present study was undertaken to investigate the presence of qualitative differences in antibody to group A streptococcal carbohydrate (A-antibody) produced by rheumatic and non-rheumatic individuals. Using a H^3-labelled hapten derived from group A carbohydrate, binding affinity of A-antibody was studied as one qualitative parameter. Affinity studies were performed on serial sera from 27 patients with acute rheumatic fever (ARF), including 16 with carditis (RHD), as well as from 8 patients with post-streptococcal nephritis (AGN) and 16 with uncomplicated streptococcal infection (SI). Significantly lower affinity values were obtained for ARF sera compared to sera of patients with SI ($p < .01$). In contrast, no significant difference in affinity was found between AGN and SI. No increase in affinity of A-antibody was observed in serial specimens obtained from RHD patients up to 30 months after the acute attack, despite the presence of quantitatively elevated levels of A-antibody. This finding differs from animal studies which show increasing antibody affinity with time after immunization. These data demonstrate that the A-antibody produced by rheumatic individuals is qualitatively different from that produced by non-rheumatic patients and that, in RHD, this antibody does not show an increase in affinity with time.

USE OF TRANSFER FACTOR IN THYMIC DYSPLASIA WITH IMMUNOGLOBULIN SYNTHESIS. L.M. Pachman, Northwestern Univ., Children's Mem. Hosp. (Chicago), R. M. Rothberg, Univ. of Chicago, Wyler Children's Hosp. (Chicago), D. B. Kaufman, Mich. State Univ., Dept. Human Dev. (East Lansing, Mich.) and C. H. Kirkpatrick, N.A.I., N.I.H. (Bethesda).

Three males and one female, each having normal parathyroid function, and deficient thymic-dependent cell function are described. All patients had normal immunoglobulin levels and serological or histological responses to antigenic stimulation. Only the currently living child was Schick non-reactive following immunization. All patients were delayed skin test nonresponsive and had deficient in vitro lymphocyte responsiveness to PHA, C. albicans, streptococcal M protein and blastogenic factor produced by normal cells. Lymph node biopsies showed depletion of thymic-dependent areas. Three had persistent C. albicans infection, 2 had severe atypical mycobacteria infection and 1 had P. carinii. Administration of active transfer factor to three patients did not alter their clinical course, skin tests or in vitro lymphocyte responses. It is speculated that isolated thymic deficiency may occur in variable degrees and that transfer factor was ineffective in these patients because of the relative absence of thymic-influenced lymphocytes.

IMMUNOLOGY

Second Session

CHRONIC ACTIVE HEPATITIS (CAH) IN CHILDHOOD: FAVORABLE RESPONSE TO INTENSIVE IMMUNOSUPPRESSIVE THERAPY. A.R. Lawton, V.W. Persky, G.F. Scofield and M.D. Cooper, Dept. of Pediatrics and Pathology, Univ. of Ala. Med. Ctr., Birmingham, Ala. (Introduced by J. W. Benton)

Despite reduced morbidity and early mortality, immunosuppressive therapy has not been considered to improve long-term prognosis of CAH because of persistent histologic activity and progression to cirrhosis in many treated patients. We have treated 15 patients (11 girls, 4 boys) during a 5 yr period. Diagnosis was established by biopsy in 14/15 patients jaundiced more than 2 mos. All had increased SGOT (620->5000) and hypergammaglobulinemia; 3/12 had anti-nuclear antibody. Ascites occurred in 4 and encephalopathy in 3. Prednisone (40-100 mg daily) induced improvement by 2 wks and reduction of SGOT to < 100 by 8 wks in 14/15. One patient presented with advanced cirrhosis, having responded twice to short courses of steroids, and died shortly after admission. Therapy (alternate day steroid, 6-mercaptopurine, or both) has been continued 1-5 yrs in 14 patients. All are clinically well, growing normally, and have normal hepatic function; 10/13 had no histologic abnormality on last biopsy, and 1 had borderline fibrosis without CAH. Treatment was stopped in 4 patients; 2 had definite relapses, 1 had a slight increase in bilirubin, and 1 has remained well for 6 mos. Our results suggest that (1) intensive immunosuppressive therapy can abolish inflammation of CAH and prevent cirrhosis and (2) steroids on alternate days are effective and safe for long term suppression of CAH in children.

ENHANCED BACTERICIDAL ACTIVITY OF LEUKOCYTES FROM CHILDREN WITH CHRONIC GRANULOMATOUS DISEASE (CGD) IN THE PRESENCE OF SULFISOXAZOLE (SSX). R.B. Johnston, Jr., C.M. Wilfert, L.S. Webb, R. Buckley, L.R. DeChatelet, and C.E. McCall. Dept. of Ped., Univ. of Ala. Med. Ctr., Birmingham, and Duke Univ. Med. Ctr., Durham, and Dept. of Med., Bowman Gray Sch. of Med., Winston-Salem.

We have observed apparent suppression of the frequency and severity of bacterial infections in 4 of 5 children with CGD on long-term sulfonamide therapy. The response has been out of proportion to demonstrable direct antibacterial effects of the drug. In an attempt to determine the mechanism for this apparent protective effect, ingestion and killing of E. coli and S. aureus by leukocytes from 4 patients with CGD was studied in the presence or absence of 5-65 µg/ml SSX added to pooled normal serum or in serum from a patient taking SSX. Growth of the bacteria used was not inhibited by SSX or serum. With leukocytes from 3 of 4 patients there was modest but reproducible enhancement of bactericidal activity in the presence of SSX (40-250% more bacteria killed after 120 min.). In the fourth patient, whose infections have not been clearly suppressed by SSX, there was only slight enhancement. Study of O_2 uptake, iodination, hexose monophosphate shunt activity, and NBT reduction by one patient's cells did not reveal a metabolic basis for improved killing. These results suggest that SSX enhances the ability of CGD phagocytes to kill bacteria and that further clinical and laboratory investigations of the effects of sulfonamides in CGD are indicated.

TRANSFER OF CELL-MEDIATED IMMUNITY IN SEVERE COMBINED IMMUNODEFICIENCY. Ralph A. Lord, Randall M. Goldblum, Elton Dupree and Armond S. Goldman, Dept. of Pediatrics, The Univ. of Texas Medical Branch and Shriners Burns Institute, Galveston, Texas.

Severe combined immunodeficiency has been treated successfully only when histocompatible bone marrow could be grafted. A five-month-old male was found to have an X-linked form of this disorder, characterized by deficiencies of all immunoglobulins, absent delayed hypersensitivity (DHS) and lack of lymphocyte transformation to mitogens and antigens. However, blood lymphocyte counts and surface immunoglobulin staining were normal.

Because no histocompatible donor was available, treatment with transfer factor (TF) was undertaken. Afterwards, lymphocytes transformed in response to phytohemagglutinin and Candida albicans in culture, but DHS skin tests remained negative. With the administration of more TF and cell-free plasma, DHS tests became positive and clinical improvement occurred. After one month, cell-mediated immunity decreased and despite further TF and plasma, the patient quickly succumbed to Pneumocystis carinii and cytomegalovirus infections.

Autopsy revealed no Hassall's corpuscles, lymph nodes, plasma cells or germinal centers. However, the thymic-dependent regions of the spleen appeared normal. A defect in T- and B-cells, other than a stem cell deficiency, is suggested. Because of the period of improvement in this case, TF should be considered when histocompatible bone marrow is unavailable for patients with severe combined immunodeficiency.

CELLULAR IMMUNODEFICIENCY IN HUMAN PROTEIN-CALORIE MALNUTRITION. Glenn J. Lawlor, Jr., Charlotte G. Neumann, Marion Swenseid, Carter Newton, Jennifer Herbert, Arthur J. Ammann, and E. Richard Stiehm (Intr. by Arthur J. Moss). UCLA Sch. Med., Depts. Ped. and Nutrition, Los Angeles, Calif.

The antibody and cellular immune function was assayed on 71 malnourished Ghanaian children ages 7 months to 6 years and 47 age-matched controls. Subjects with protein-calorie malnutrition (PCM) were divided into severely malnourished (26) and moderately malnourished (45) patients based on anthropometry, overt signs of kwashiorkor or marasmus and serum proteins. Serum immunoglobulins (IgG, M, A, D and E), antibody response to keyhole limpet hemocyanin and polyvalent pneumococcal polysaccharide and cutaneous delayed hypersensitivity response to monilia, streptokinase-streptodornase and phytohemagglutinin (PHA) were determined. Also, in vitro lymphocyte response to several dilutions of PHA was determined in 24 severely malnourished patients, 14 moderately malnourished and 12 controls.

Increased immunoglobulin levels were found in all 3 groups; levels were somewhat higher in the malnourished groups. Antibody responses were equal in all 3 groups. Cutaneous delayed hypersensitivity was decreased in both malnourished groups as compared to controls. The lymphocytes of 5 of 24 severely malnourished children gave an abnormally reduced response to PHA in vitro. All controls and moderately malnourished children responded in a normal fashion; however, the maximal level of response was higher in the controls.

This study indicates that cell-mediated immunity is depressed in relation to the degree of PCM and may be a contributing factor in the severity of infection seen in these patients.

MEASUREMENT OF SPECIFIC IgE ANTIBODY TO ^{14}C RAGWEED ANTIGEN IN RAGWEED SENSITIVE CHILDREN. John Santilli, William C. Rees, Robert T. Scanlon and Joseph A. Bellanti. Dept. of Ped., Georgetown Univ. Sch. Of Med., Washington, D. C.

Recently a simplified method for the labeling of whole ragweed pollen with ^{14}C was developed in our laboratory. A pollen extract of this preparation contained 40 mg/ml of protein (21 μg of AgE/ml) and a specific radioactivity of 2600 cpm/μg of AgE. The extract was shown to be biologically active by its histamine releasing capacity from leukocytes of ragweed sensitive subjects and is stable upon prolonged storage at -70°C.

Employing this ^{14}C labeled ragweed antigen (RA) a radioimmunoassay was developed to demonstrate specific IgE antibody. Sera were obtained from 5 ragweed sensitive and 5 normal children whose ages ranged from 5-8 years. A 0.1 ml dilution of serum was added to 0.2 mg of the radioactive RA for 24 hr at 4°C. Subsequently, goat antihuman IgE was added and the reaction mixture reincubated for 24 hr at 4°C. At 72 hours, immune precipitation was facilitated by the addition of rabbit antigoat antibody and the total amount of radioactivity was measured in the precipitate. All allergic children, thusfar tested, have shown maximum radiobinding at a serum dilution of 1:100; no radiobinding is detected in control sera. This assay combines simplicity, ease of performance and stability of reagents and may find clinical application in the diagnosis and evaluation of ragweed sensitivity in children.

ASSOCIATION OF Gm AND Au ANTIBODIES IN ADOLESCENT DRUG ADDICTS. G.Nathenson, M.Cohen, I.Millman and B.Blumberg, Albert Einstein Col. of Med.,Montefiore Hosp. & Med. Ctr.,Dept. Ped. The Bronx, New York and Cancer Research Inst., Phila., Pa.

Gm antibodies often result from sensitization by incompatible Gm antigens of heavy chains of IgG. They may also be found in rheumatoid disease. In a group of 298 adolescent heroin addicts 7.7% possessed Gm antibodies of either the Gm (1), Gm (4), or Gm (5) types, compared to 0.9% in 103 nonaddicted controls. Forty-six percent of the anti-Gm (+) addicts sera were associated with positive latex fixation reactions. To explore the possibility that Gm antibodies are induced by the injection of incompatible blood contained in needles used in common,rbc antibodies were sought in the sera of addicts. No increase in incidence of these antibodies were found in addicts when compared to normals. Furthermore, isoimmunization fails to explain those latex (+) sera of some addicts with Gm antibodies of the same Gm phenotype as the host.

That Gm antibody production may be the result of repeated innoculation of infectious material,particularly the hepatitis virus is suggested by a significant association of Anti-Au and Anti-Gm in the sera of addicts. Eight of 23 addicts sera containing anti-Gm also possessed anti-Au, while only 10 of 80 addicts sera lacking anti-Gm contained anti-Au [p=.02]. Though the mechanism of development of Gm antibodies as a response to hepatitis infection is not clear, the situation may be analogous to the induction of anti-A and anti-B blood group antibodies by microbial agents.

LEUCOCYTE MIGRATION INHIBITION BY ANTI-IMMUNOGLOBULINS
Walter L. Henley and Sirje Okas (SPON: H. L. Hodes).
Department of Pediatrics, Mount Sinai School of Medicine, C.U.N.Y., New York, New York 10029

Human leucocyte migration inhibition (LMI) occurs when the donor's leucocytes are exposed to an antigen to which he is sensitive. LMI was obtained with goat antisera directed against human γ, α, μ, ε, κ and λ chains and fragments. There was no correlation between the serum level of the immunoglobulin class and the extent of LMI produced by antisera in normal individuals and patients with disorders of autoimmunity. The LMI pattern may indicate which light chain of the individual is the predominant one. Antisera to Fab and Fc of human IgG caused significant LMI usually greater with the former. It was occasionally significant with normal goat serum and goat antisera to human albumin and transferrin. Cells from a long-term lymphoid cell line (PGLC33H) showed LMI with the antisera against the various heavy and light chains and IgG fragments. In these experiments the cause of LMI may be the formation of antigen-antibody complexes. The interaction would take place at the cell membrane where the immunoglobulin is the antigen. Another possible mechanism is pinocytosis of the complex by B as well as T cells with release of mediators of cellular immunity including migration inhibitory factor(s).

Supported in part by USPHS Grant EYA100145.

DEFICIENT RANDOM MOBILITY,NORMAL CHEMOTAXIS AND IMPAIRED PHAGOCYTOSIS.A NEW ABNORMALITY OF NEUTROPHIL FUNCTION.Michael E. Miller and Richard Dooley,Charles R. Drew Postgraduate Medical School, Los Angeles, California 90059.

Previously described defects of polymorphonuclear leukocyte(PMN) movement have involved either chemotaxis(C)and random mobility(RM),or C alone.We now describe a new abnormality of PMN movement,deficient RM with normal C in 2 male sibs with pyoderma gangrenosum.In vitro measurements of PMNS from each patient demonstrated normal C by Boyden chamber migration,but completely absent RM as measured by capillary tube migration.In addition to the unique abnormality of PMN movement,PMNS from the patients were also deficient in phagocytosis of baker's yeast particles,a finding which has not been observed previously despite many years of experience with the yeast assay in this laboratory.We conclude the following: 1)These findings lend clinical support to previous in vitro data indicating that C and RM are separable PMN activities; 2)Yeast phagocytosis and RM do not appear to be interdependent processes.This is supported by:a)The demonstration of different metabolic requirements in vitro for the two processes;b)Phagocytic activity of PMNS is decreased upon incubation with glycolytic or chemotactically active inhibitors, while RM is unaffected by these agents;c)PMNS which are passaged through cellulose acetate hollow fiber dialysis membranes retain normal phagocytic activity while being rendered totally inactive in RM;3)The PMN defect in these patients is highly specific and not secondary to other factors.

MITOGENIC RESPONSE DIFFERENCES BETWEEN ADULT AND NEWBORN LYMPHOID CELLS. John R. Montgomery, Edward O. Mason and Mary A. South. Baylor College of Medicine, Dept. of Pediatrics, Houston, Texas.

During immunologic evaluation of patients with congenital cytomegalovirus, we had the opportunity to study the response of normal newborn cord blood lymphoid cells to phytohemagglutinin (PHA) and pokeweed mitogen (PWM). Lymphocytes of the newborn differ from those of adults in their response to in vitro stimulation. One hundred and eight heparinized cord blood specimens were tested. The 19 adult controls consisted of heparinized blood from 10 normal individuals. All cultures were performed by micro-technique in RPMI 1640 medium without serum supplement and labeled with C^{14} thymidine after 4 days. A stimulation index was obtained by comparison of the uptake of radioactive label by stimulated and unstimulated cells.

As previously reported, background counts of unstimulated cells were high in cord blood samples. While adult cells responded to both mitogens to a greater degree than did cord cells, the response toPHA was always higher than the response to PWM. In contrast, cord blood lymphocytes responded to PWM to a greater degree than PHA. If PHA:PWM stimulation indices of the two groups were compared, adults average 0.6 while newborns average 3.2. This difference was found to be significant ($p < .005$). Further studies using both mother's and baby's serum failed to implicate the presence of a suppressive factor. These findings suggest that there are age dependent differences in lymphoid cell populations (T & B cells).

IMMUNOLOGY

Read by Title

MIF OR PNIF PRODUCTION INDICATES PASSAGE OF CELLULAR IMMUNITY FROM MOTHER TO FETUS. Stephen H. Astor, M.D. and Oscar Frick,MD Univ. California, San Francisco, California.

Cellular immunity to 3 antigens (PPD, Candida,Streptokinase-Streptodornase (SK/SD) were compared in 6 maternal-infant pairs. A direct MIF assay measuring migration of human leukocytes from mother's blood and infant's cord blood was compared with maternal skin tests. The assay compares ratios of areas of circular migration of cells from wells cut into agarose in a petri dish with and without antigen. An antigen inhibition ratio of 0.8 or more is considered positive.

There was complete correspondence in the 6 pairs' cellular immune status for PPD; 5 were tuberculin positive and one negative, For Candida and SK/SD, 2/6 infants had positive tests, and 4 were negative, despite the mothers' positive tests which indicates that passage of cellular immunity is incomplete. How such transfer occurs is unknown, but could be due to placental crossing of maternal lymphocytes, or transfer of antigen, transported IgG or transfer factor.

The migrating cells are polymorphonuclear leukocytes and some eosinophils but not monocytes. For this reason, we call this polynuclear inhibition factor or PNIF.

TRANSFER FACTOR AND 5-FLUROCYTOSINE IN THE TREATMENT OF CHRONIC MUCOCUTANEOUS CANDIDIASIS. Richard J. Bonforte, Photini Papageorgiou, Linda Cahill, Susan Tannenbaum, and Philip R. Glade. Mount Sinai Sch. of Med., Dept. of Ped., New York, N.Y.

Chronic mucocutaneous candidiasis (CMC), a persistent fungal infection of skin and mucous membranes, is not erradicated by antifungal agents alone. Recently this disease has been associated with a variable impairment of cell-mediated immunity. Immunologic reconstitution with multiple doses of transfer factor was attempted in 3 children with CMC associated with altered in vivo and in vitro responses to Candida extract. Dialyzable transfer factor was prepared by the method of Lawrence and was obtained from donors with marked skin reactivity to Candida extract. Each child received the equivalent of at least 1×10^9 lymphocytes. Transfer of skin reactivity to Candida extract was effected in only 1 child, without any demonstrable change in his clinical course. In an attempt to lessen the antigenic load and thereby increase the effectiveness of transfer factor, 5-flurocytosine (5-FC) was administered to 2 of the children. 50-150 mg/Kg/day of 5-FC was given for 2 weeks. Transfer factor was then added to the regimen at biweekly intervals. Skin reactivity to intradermal Candida extract was effected in both children, more intensely in the child who had previously responded to transfer factor alone. No lasting changes in their clinical courses have been observed. Specific therapy of CMC awaits a clearer understanding of this disease entity.

A DEFECT IN HUMORAL IMMUNITY IN CHRONIC MUCOCUTANEOUS CANDIDIASIS. Linda T. Cahill, Eugene Ainbender, Philip R. Glade. (Intr. by A.J. Steigman). Mount Sinai Sch. of Med., Dept. of Ped., New York, N.Y.

Chronic mucocutaneous candidiasis is associated with an immunodeficiency involving T lymphocytes with failure of cell mediated immunity. Routine immunologic screening of two such patients revealed that despite recent immunization, both had strongly positive Schick tests, suggesting deficiency of humoral immunity as well. These children possessed normal salivary IgA and serum immunoglobulins by radial immunodiffusion and both had circulating titers of isohemagglutinins. Although Schick positive, they both had antibody to diphtheria antigen as measured by hemagglutination inhibition. Their plasma neutralized the Schick response in rabbits, confirming the presence of antitoxin activity. It is likely that neutralizing activity demonstrable by the Schick test is a function of IgG antibody since little IgM normally leaves the intravascular space. Plasma was separated by DEAE column chromatography. The IgG fractions failed to neutralize Schick antigen in rabbits. Fractions containing IgM showed neutralizing activity. Recent studies suggest that T lymphocytes are necessary for the conversion of IgM to IgG synthesis by B lymphocytes. It is likely that the deficiency in T lymphocytes in our patients extends beyond their capacity to mediate cellular immune function.

T AND B LYMPHOCYTES IN BLOOD AND BONE MARROW AFTER CESSATION OF ANTILEUKEMIC THERAPY. Luis Borella and Luisa Sen. St. Jude Children's Research Hosp., Memphis, Tenn. 38101.

When immunosuppressive therapy is stopped in children with acute lymphocytic leukemia (ALL) *in complete remission* (CR) there is an increase of lymphocytes first in the bone marrow (BM) and secondly in the peripheral blood (PB) ((Borella *et al.*, Blood *40*: 42, 1972). To establish whether this cell rebound was selective for a particular class of lymphocytes, we investigated the kinetics of recovery of T (thymus dependent) and B (thymus independent) lymphocytes in PB and BM. Forty-five children with ALL who were in CR for 3-7 years were studied before and after cessation of 3 years of chemotherapy, consisting of mercaptopurine, cyclophosphamide and methotrexate given in combination. Immunofluorescence of immunoglobulins (Igs) on viable lymphocytes and assay of rosette-forming lymphocytes (RFL) were used to identify B and T cell surface markers, respectively. The results indicate: 1) Combination chemotherapy depressed B cells more than T cells. 2) Within 3 months after stopping therapy there was a rebound of B cells; the intensity of Ig fluorescence and the proportion of Igs bearing lymphocytes rose above normal levels. Conversely, the proportion of T cells (RFL) was normal during therapy and decreased 3 months after cessation of therapy. Thus, lymphocytes bearing T and B markers had different kinetics of recovery. 3) Simultaneous assays of BM and PB in patients who had not received therapy for 1-6 months demonstrated that most BM lymphocytes did not possess T or B cell markers. Median value of Igs bearing lymphocytes was 13.3% in PB and 2.8% in BM. The proportion of RFL was three-fold higher in PB than in BM. Thus, the early lymphocyte rebound in BM is mostly of undifferentiated lymphocytes or stem cells.

EXPERIMENTAL CRYOGLOBULINEMIA IN THE RABBIT. William J. Chernack, Michael N. Koss, William R. Griswold and Rawle M. McIntosh. Columbia Univ. N.Y.

Cryoglobulins have been described in several diseases frequently associated with infectious processes or immune complex states. It has been suggested that proteins with unusual physico-chemical characteristics may be less soluble at reduced temperatures. We have postulated that neuraminidase, a microbial enzyme, by altering the chemical composition of immunoglobulins may lead to the formation of complexes of immunoglobulins or altered immunoglobulins which cryoprecipitate. In this study neuraminidase was administered to rabbits intravenously and the detection and characterization of serum cryoproteins were performed as previously described. Cryoproteins were detected from 4 hours to 31 days after administration of the enzyme. All contained slow migrating IgG, IgM and B1C. IgA and fibrinogen were present less frequently. Total cryoprecipitable protein ranged from 5-35mg/100cc serum. Proteinuria and hematuria were found in most animals. Pulmonary and to a lesser extent hepatic and renal morphologic alterations were present. In vitro studies suggest that cryoprecipitation associated with neuraminidase is a function of alteration of immunoglobulins and the milieu of the blood.

DIFFERENCES IN C^{14}-ACETATE INCORPORATION IN ANTIGEN AND PHA STIMULATED HUMAN LYMPHOCYTES. E.J. Brandt and Margaret H. MacGillivray. Children's Hosp., SUNY at Buffalo, N.Y.

Human lymphocytes stimulated with phytohemagglutinin (PHA) in vitro manifest increased C^{14}-acetate ($C^{14}A$) incorporation prior to RNA, protein and DNA synthesis. On the basis of this observation, acetylation of nuclear histones has been proposed as a possible regulatory mechanism for gene activation.

In this study, a comparison was made of the in vitro responses of human lymphocytes to the non-specific mitogen, PHA, and to the specific antigens, tuberculin (PPD) and Candida albicans (Monilia). Incorporation of $C^{14}A$ and H^3-thymidine (H^3T) was quantitated utilizing liquid scintillation counting, and the results were expressed as the ratio of cpm in mitogen stimulated cultures to cpm in unstimulated control cultures.

Following stimulation by PHA, PPD and Monilia, the lymphocytes exhibited increased DNA synthesis. Peak H^3T uptake occurred after 3 days of incubation with PHA (ratio=248) but not until after 5-6 days of exposure to PPD (ratio=25.3) and Monilia (ratio=50.8). $C^{14}A$ incorporation was significantly increased only in the PHA treated lymphocytes (ratio=4.2) but remained at or below baseline throughout the entire incubation period for the lymphocytes exposed to PPD or Monilia. The finding of DNA synthesis in PPD and Monilia treated lymphocytes which is not preceded by a detectable increase in C^{14}-acetate incorporation suggests that acetylation of nuclear histones may not necessarily be a primary regulator of DNA template activation in mitogen stimulated cells.

PHAGOCYTOSIS AND cAMP: A. Constantopoulos and V. A. Najjar. Division of Protein Chemistry and Department of Pediatrics, Tufts University School of Medicine, Boston, Massachusetts.

The occurence of adenyl cyclase in polymorphonuclear (PMN) cells and the rise of cyclic adenylic acid (cAMP) during phagocytosis have been the subject of recent controversy. Our interest in the phagocytosis stimulating tetrapeptide, tuftsin (BBRC 47, 172, 1972), prompted us to investigate the problem and study the effect of tuftsin on cAMP levels. Our results confirm the presence of membrane adenyl cyclase in rabbit peritoneal PMN. No rise of cAMP during phagocytosis of latex particles occurs nor in response to mM concentrations of tuftsin 0.01-10, epinephrine 0.1-1 and glucagon 0.1-0.2. Fluoride 10 mM stimulates the enzyme in either intact PMN or purified membranes. Prolonged homogenization or sonication of cells destroys the enzyme. (PHS AI-09116 and NSF GB-31535 X.)

Time course (minutes) of cAMP accumulation (*p* moles)

PMN	0'	5'	10'	15'	20'	30'
cells alone	9.0	24.0	26.0	25.0	19.0	15.0
" + latex	12.0	25.0	26.0	24.0	--	--
" + tuftsin	10.0	27.0	26.0	23.0	20.0	14.0
" + NaF	10.0	27.0	63.0	74.0	61.0	56.0
" + epiniphrine	12.0	24.0	26.0	26.0	25.0	14.0
" + glucagon	10.0	27.0	28.0	26.0	20.0	15.0
membrane alone	3.0	3.5	3.1	2.2	1.4	1.0
" + NaF	3.1	4.3	22.0	28.0	31.0	15.0

GRAFT VERSUS HOST FOLLOWED BY HOST VERSUS GRAFT: EVIDENCE FOR ACCELERATED INDUCTION OF T-CELL COMPETENCE. Don W. Fones, Alan S. Levin, Stanley N. Mogerman, Lynn E. Spitler, Daniel P. Stites, Henry R. Shinefield, & H. Hugh Fudenberg. Kaiser Foundation Hospital/U.C. Medical Center, San Francisco, California.

A newborn female was discovered to have the DiGeorge Syndrome after over 200 ml of whole blood was transfused during cardiac surgery. 48 hours later, a GVH reaction developed. The child was treated symptomatically over the next 4 months and recovered with an immunologic status which appears to be normal. In vitro response to the mitogens phytohemagglutinin, concanavalin A and poke weed mitogen were performed. Response to allogenic cells was assessed in one way mixed leukocyte reactions. Immunoglobulin levels, skin test reactivity, granulocyte function, rosettes, and parathyroid hormone levels were also followed. Skin tests and in vitro studies reveal progressive acquisition of very strong cellular immune reactivity against several antigens to which normal children of this age rarely react. These specificities conform to those of the transfusion donor and not to those of the child's parents. HLA and red cell typing indicate no evidence of chimerism. Chromosome karyotype indicate normal female. On the basis of clinical and laboratory findings, we conclude that this child, who previously lacked T-cell competence, now has reasonably normal T-cell function, and that her present state of competence is a result of the patient's own T-cells. We feel that the immunologic specificities expressed by this child were endowed upon her T-cells by sub-cellular substances secreted by graft cells during the Graft Versus Host/Host Versus Graft reaction.

A PLASMA INHIBITOR OF 'MOTILITY' AND ADHERENCE OF HUMAN LYMPHOCYTES. Armond S. Goldman, Beth Rudloff and Randall M. Goldblum, Dept. of Pediatrics, The University of Texas Medical Branch and Shriners Burns Inst., Galveston, Texas.

The in vitro effect of human plasma upon the development of the motile configuration and glass-adherence of human blood lymphocytes was investigated. The motile configuration of blood lymphocytes was examined by Nomarski interference contrast microscopy of living cells. Glass-adherence was determined by the use of glass bead columns.

Motile forms were seven times more frequent when the cells were incubated without plasma ($p < 0.001$). Plasma also markedly inhibited the adherence of lymphocytes to glass beads ($p < 0.001$). The inhibition was readily reversed by washing the cells in tissue culture media which lacked plasma. The factor was not consumed during coagulation since serum was as active as plasma. The inhibition was not due to protein per se since neither albumin nor Cohn's Fraction II were inhibitory. The inhibitor appears to be a heat stable protein with a molecular weight which exceeds 160,000 daltons.

CORRELATION OF IMMUNOLOGIC DEFICIENCIES WITH FAMILIAL MANIFESTATIONS OF CANCER, ALLERGY AND INFECTION. Robert L. Ganaway, William T. Kniker, John S. Harvey, Jr. Univ. Texas Med. Sch., Dept. Ped., San Antonio, Texas 78248.

An evaluation of immunologic function in an 8 year old female with osteogenic sarcoma, her identical twin and their parents was performed prior to utilizing the healthy twin's lymphocytes therapeutically. In vitro lymphocyte responses to mitogens were found to be poor. A detailed family history revealed a high incidence of cancer, recurrent infection and allergy. Studies on the original family (parents and 6 children) are almost complete. Serum immunoglobulin and hemolytic complement levels were normal in all. Delayed hypersensitivity skin tests were positive to phytohemagglutinin (PHA), SK-SD and mumps in all but were negative to pokeweed mitogen (PWM) and monilia. On 24 hour Rebuck skin windows both mitogens induced fair to good mononuclear cell responses although the polymorph response was poor in 5. In 7 individuals mitogen induced lymphocyte transformation, markedly reduced in the prescence of autologous serum, was significantly increased using heterologous serum. A discordance in in vitro and in vivo responses to mitogens and antigens is apparent, and a serum inhibitor to PHA induced transformation of lymphocytes has been demonstrated. As part of a comprehensive analysis of immunologic competence in selected family members over 3 generations, we hope to correlate specific immunological deficiencies with susceptibilities to oncogenic, infectious and atopic agents.

IN VITRO DIAGNOSIS OF IMMEDIATE HYPERSENSITIVITY IN ATOPIC CHILDREN BY RADIOIMMUNOASSAY. Zack H. Haddad* and Donald R. Hoffman,* Intr. by Paul F. Wehrle, Los Angeles County-Univ. of So. Calif. Med. Ctr., Dept. of Ped., Los Angeles

The diagnosis of immediate hypersensitivity reactions to suspected allergens has thus far been made on subjective clinical history correlated with skin tests. 100 children (1-16 yrs.) with diagnoses of hay fever, asthma, urticaria, angioedema and eczema had a full clinical evaluation including skin testing, and a serum sample was obtained prior to any therapy. Serum IgE level was determined by competitive radioimmunoassay, and a sandwich radioimmunoassay using crude allergen extracts (RAST) was used to determine specific IgE antibodies. All of the patients had significantly positive skin tests and their serum IgE levels ranged from 10 to 10000 I. U. per ml. The correlation between skin tests and RAST with grass pollen extract was about 98%. The correlation for other pollens was statistically significant but not as good as for grass. The correlation for the mold, alternaria, was poor. Peanut, fish, egg, walnut, wheat and corn gave excellent correlations between RAST and skin test. Milk and chocolate did not yield as good a correlation between RAST and skin test. Serum IgE levels are of little practical use in identifying atopic children, however RAST with crude extracts of some pollens and foods could substitute for skin tests for routine diagnostic purposes. Other allergen extracts need further fractionation and purification before they are of practical diagnostic use.

POLYCLONAL GAMMOPATHY FOLLOWING TRANSFER FACTOR (TF) IN SEVERE COMBINED IMMUNODEFICIENCY DISEASE (SCID). E.W. Gelfand, R. Baumal, J. Huber, M.C. Crookston, K.H. Shumak. Depts. of Immunology & Pathology, Hospital For Sick Children & Dept. of Hematology, Toronto General Hospital, Toronto, Canada. (Intr. by J.R. Hamilton).

A 6-month old girl was diagnosed as SCID on the basis of lymphopenia, hypogammaglobulinema (IgG <200 mg%, IgA 0, IgM 0) and absent delayed skin test reactivity and lymphocyte transformation to specific and non-specific mitogens and allogeneic cells. No immunoglobulin (Ig) was detected, by surface immunofluorescence, on peripheral blood and bone marrow cells; B and T cell rosettes were absent. Because there was no HL-A compatible donor, she received one dose of TF (from 10^9 leukocytes). On Day 14 the lymphocyte count, skin tests, Ig and lymphocyte studies were unchanged. On Day 21, the leukocyte count began to rise, reaching 115,000 by Day 26. These cells were predominantly blast-like, but mature plasma cells were also seen. Ig levels were: IgM 2,000 mg%, IgA 0, IgG 160 mg%. The IgM contained κ and λ chains. Further evidence that the IgM was polyclonal was provided by the finding of three specific antibodies: anti-A, anti-B and potent anti-i. The patient died on Day 28; autopsy findings were consistent with the diagnosis of SCID. Although the relationship of TF to the polyclonal gammopathy may be coincidental, the possibility is raised that, in SCID, TF may induce uncontrolled B cell proliferation.

(Supported by the Medical Research Council of Canada)

SALIVARY IgA DEFICIENCY WITH NORMAL SERUM IgA IN COMBINED IMMUNODEFICIENCY DISEASE (CID). R.J. Laffin, B. Pollara, E. Moore, H.J. Meuwissen (Intr. by E. Hook). N.Y.S. Kidney Disease and Birth Defects Insts. and Depts. of Microbiol. and Ped., Albany Med. Col., Albany, N.Y.

We report a patient with CID and hypogammaglobulinemia G and M, who since early life had normal or elevated levels of IgA. Over a 2-year period, and in multiple specimens, this girl showed only occasional traces of IgA in highly concentrated saliva specimens. This IgA appeared not to be bound to transport piece. Immunofluorescence data indicated absence of IgA containing cells in the rectal submucosa. In addition, the patient had nodular lymphoid hyperplasia of the small and large intestines. The nodules had the classical appearance of germinal centers, but again were devoid of immunoglobulin-containing cells (including cells containing IgE) with the exception of very few cells staining for IgM. Transplantation of maternal bone marrow normalized all immunoglobulins with a maximum serum IgA of 150 mg%, but despite this, no IgA appeared in saliva.

Because this patient also has a deficiency of the enzyme adenosine deaminase, we postulate that these abnormalities of the IgA system may be related to the genetically determined enzyme defect. (Supported by N.Y.S. Dept. of Health.)

IMMUNOLOGIC PARAMETERS IN HISTIOCYTOSIS X

Sanford L. Leikin, Gregorio Purugganan, Anne S. Frankel, Ruth Steerman, and Roma D. Chandra, Children's Hospital of D.C., Department of Child Health & Development, George Washington University School of Medicine, Washington, D.C.

Histiocytosis X is a disease of unknown etiology occurring in infancy and childhood. A relationship between susceptibility to infection and this disease has been observed and it has been suggested that some of the patients with histiocytosis may have immune defects. Further, malignant reticuloendotheliosis has arisen in patients with various immunodeficiency diseases. We therefore evaluated the immunocompetence of six infants with the clinical form of Letterer-Siwe Disease and seven older children in various stages of histiocytosis. In most of the patients delayed hypersensitivity, lymphocyte blastogenesis to mitogens and allogeneic cells, bactericidal killing and leukocyte nitroblue tetrazolium dye reduction were found to be within normal limits. One patient with the infantile form of histiocytosis and one older child could not be sensitized to DNFB. The lymphocytes from 2 infants were hyporeactive to mitogen induced stimulation. These reverted to normal after chemotherapy was instituted. Decreased immunoglobulin levels were found in two infants and several patients exhibited elevated immunoglobulin levels. In general, the immunoglobulin abnormalities improved following chemotherapy. No evidence of a combined immunodeficiency disorder was found in either group of patients.

The results of these studies and autopsy examinations indicated that the immunologic abnormalities which were found were either secondary to malignant cell infiltration or immunosuppressive chemotherapy rather than the immunologic defect having a causal relationship to the histiocytosis X.

SUPPRESSION OF MATERNOFETAL TRANSFER OF HUMAN IgG IN THE RHESUS MONKEY BY HIGH MATERNAL SERUM CONCENTRATIONS OF IgG.

D. Lee Miller, Donald L. Hutchinson and David Gitlin. Univ. of Pittsburgh Sch. of Med., Depts. of Ped. and of Obs. and Gynec.

This laboratory previously demonstrated that the transfer of human IgG in the mouse was mediated by two different mechanisms: by diffusion and by active transport. The amount of IgG actively transported across the maternofetal barrier was dependent upon the maternal serum concentration of IgG and was markedly inhibited by high maternal IgG concentrations. Maternofetal transfer of IgG in the pregnant woman also occurs by active transport. To determine if active IgG transport in the pregnant woman might be dependent upon the maternal serum concentration of IgG, pregnant rhesus monkeys near term were given ^{131}I-labeled IgG intravenously 2 to 5 days prior to Caesarean section. The ratio of the fetal to maternal serum concentrations of ^{131}I-labeled IgG was then determined. Some of the monkeys were given 1 g/kg of human IgG subcutaneously at least 24 hours prior to the intravenous injection of the radioiodinated IgG. It was found that maternofetal transfer of human IgG was depressed by the administration of exogenous IgG. The data indicate that a carrier system for the maternofetal transport of IgG sensitive to and inhibitable by the maternal serum concentration of that protein is present in primates.

VARIABLE IMMUNODEFICIENCY WITH MICROCEPHALY AND DEFECTIVE GROWTH.

Harold W. Lischner, Suhung Hann, Mildred L. Kistenmacher and Hope H. Punnett, Temple Univ. Sch. of Med. and St. Christopher's Hosp. for Children, Philadelphia, Pa.

Immunodeficiency syndromes in which there are associated congenital anomalies offer a unique opportunity for study of the development of lymphoid tissues relative to that of other organs. Two unrelated girls, SB and DM, of East-European descent have congenital microcephaly, mental retardation, short stature and variable deficiency of humoral and cell-mediated immunity. At 7 and 8 years, respectively, IgG levels were 1.4 and 1.2 mg/ml, IgM 6 and 0.2 mg/ml and IgA undetectable. The 15 year old brother of SB has microcephaly without increased susceptibility to infection or hypogammaglobulinemia. His IgA level was 8 mg/ml. DM has an abnormal C group chromosome (46, XX,Cq+). Her parents have normal karyotpes but the mother has isolated IgA deficiency. There was no evidence that intrauterine infection had occurred in either child, but this could not be ruled out. Immunologic studies on DM revealed depletion of lymphocytes from far and deep cortex of lymph node. In peripheral blood there was virtual abscence of lymphocytes with immunoglobulin markers on their surface, absolute deficiency of lymphocytes forming rosettes with sheep erythrocytes (presumably T cells) and of cells responsive to mitogens, and adequate numbers of macrophage precursors. Congenital microcephaly associated with a defect in regulation of immunologic development may represent a syndrome which is identifiable by performing immunoglobulin assays on children with microcephaly. (Supported by grants #RR-75, AI-09863, and RR-5624.)

DEVELOPMENT OF SERUM IgE DURING THE FIRST YEAR OF LIFE.

J.R. Miller*, M.A. Lenoir*, T. Groshong*, W.Wallace*, M. Bazaral*, H.A. Orgel*, and R. N. Hamburger. University of California, San Diego, Calif. 92037 and U.S. Naval Hospital, San Diego, Calif. 92134.

Serum IgE levels of 43 infants have been measured at frequent intervals from birth to one year of age. These infants have been followed for general development, infections, and manifestations of atopy.

The data show that serum IgE levels start to increase from the usual very low level at birth (median 2.9, range 0-11 units/ml) at very different ages and at different rates in individual babies. At nine months of age the median IgE level is 10.0 units/ml (range 0-525 units/ml) and shows the wide range and multimodal distribution characteristic of adult values.

Those babies who showed a rapid, early increase in serum IgE level maintain this rate of increase up to one year of age. Of these children, 83% had a history of allergic disease in the immediate family, and 25% of these children had already developed atopic disease by 9 months of age.

Measurement of total serum IgE in infancy may be a useful diagnostic test to identify babies with a high risk of developing allergy. It will be necessary to follow these infants for a longer period to determine the true prognostic value of this test.

CHRONIC MUCOCUTANEOUS CANDIDIASIS (CMC) - PROLONGED REMISSION AND ENHANCED DELAYED HYPERSENSITIVITY INDUCED BY AMPHOTERICIN B (AmB) THERAPY.

Harold W. Lischner, Manjit K. Sharma and Angelo M. DiGeorge, Temple Univ. Sch. of Med. and St. Christopher's Hosp. for Children, Philadelphia.

The pharmacology of AmB suggests that prolonged use in low doses might be effective and safe. A 12 year old boy with autoimmune Addison's disease had CMC involving all of his nails and over 70% of his body surface. He received a total of 18 mg/kg of AmB intravenously over a 12 week period. The dose was only 0.2 mg/kg every other day for the last 7 weeks because of uncontrollable renal tubular loss of potassium at higher doses. Clearing of cutaneous lesions was seen 4 days after therapy began, and that of the nails was evident 4 to 7 weeks later. Remission was complete and renal function normal, but onychitis of one nail and thrush recurred. The skin and remainder of the nails are still clear after 22 months. Prior to therapy 1 mg of Candida albicans antigen was needed to induce a perceptible delayed skin test reaction; after therapy 3 cm of induration was induced by 0.1 mg of the same antigen. In CMC: 1) the effectiveness of AmB therapy may be more related to the duration of therapy than to the dose administered; 2) it is not necessary to surgically remove infected nails in order to obtain satisfactory therapeutic results with AmB; 3) delayed skin test responses are depressed by cutaneous disease or by excess total body antigen load; 4) the results of transfer factor therapy must be interpreted with caution if candidacidal drugs are incorporated into a therapeutic program. (Supported by grant #RR-75 from the USPHS.)

THE REBUCK SKIN WINDOW RESPONSE RE-VISITED.A DICHOTOMY OF IN VITRO AND IN VIVO RESPONSES.

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The Rebuck skin window technique has been a long recognized aid in the study of the inflammatory response.In the evaluation of patients with defects of leukocyte movement involving chemotaxis(C) and/or random mobility (RM),the Rebuck window has been regarded as a test of in vivo chemotaxis,and it has been assumed that deficiencies of in vitro leukocyte movement should be reflected by abnormal Rebuck windows.This data now indicates that the correlation between in vivo Rebuck windows and in vitro activities of C and/or RM is both inconsistent and unpredictable.Studies of patients have revealed normal skin windows with 1)primary in vitro defects of C,as in juvenile diabetes and two families with a familial defect of C; 2)primary defects of RM,as in two sibs with pyoderma gangrenosum,and 3)primary defects involving both,as in the normal human neonate. Further, neutropenia is not a consistent finding among these patients. It is concluded that:1)The Rebuck window need not reflect in vitro abnormalities of cell movement;2)The Rebuck window may not be a diagnostic essential in clinical abnormalities related to defects of cell motility.3)The relationship between Rebuck windows,peripheral neutrophil counts and existing in vitro assays of leukocyte movement is highly complex and incompletely understood.The criteria upon which one may reasonably establish cause and effect relationships between in vitro and in vivo abnormalities involving leukocyte movement must,therefore, be re-evaluated.

LONG TERM RESULTS OF MARROW TRANSPLANTATION IN WISKOTT-ALDRICH SYNDROME. Ellen C. Moore, Dong S. Kim, Fritz H. Bach, Hilaire J. Meuwissen (Intr. by Ian H. Porter). N.Y.S. Birth Defects Inst. and Dept. of Ped., Albany Med. Col., Albany, N.Y. and Dept. of Med. Genetics, U. Wisconsin, Madison, Wisconsin.

Little is known of the long term results of marrow transplantation. This patient was transplanted with MLC compatible sibling marrow in 1968 and is one of the two longest surviving marrow transplant recipients. He has remained in a chimeric state. Reconstitution was incomplete and involved primarily lymphocytes, with variable proportions of donor and recipient lymphocytes in both marrow and peripheral blood.

He has had no clinical evidence of chronic graft-versus-host disease. Despite low platelet counts, bleeding since transplantation has been confined almost entirely to epixtaxis. Eczema has not been a problem since the transplant. He has had numerous minor respiratory and middle ear infections, but no major bacterial, viral or fungal infections. Immunoglobulin-M and antibody responses to diptheria and polio immunizations have remained low. He has shown strong delayed hypersensitivity to SKSD, candida, mumps, trichophyton and PHA, but does not respond to challenge with DNCB, despite repeated sensitizations. His response to PHA and PWM in vitro has been persistently low, but the response to antigens and allogeneic lymphocytes is normal.

Transplantation in this patient has, therefore, caused marked clinical amelioration, but has not completely corrected the basic defects. (Supported by N.Y.S. Dept. of Health.)

CELL-MEDIATED IMMUNE DEFECTS IN CHRONIC MUCOCUTANEOUS CANDIDIASIS. Photini S. Papageorgiou, Richard Bonforte, Michael Lilis, Philip R. Glade. Mount Sinai Sch. of Med., Dept. of Ped., New York, N.Y.

Chronic mucocutaneous candidiasis (CMC) is associated with deficiency of cell-mediated immunity. We have investigated the cell-mediated immune responses of three patients with CMC by (1) in vivo skin reactivity to candida extract (CE), streptokinase-streptodornase (SK-SD), trichophyton, and dinitrochlorobenzene (DNCB); (2) in vitro lymphocyte responses to phytohemagglutinin (PHA) and CE; and (3) release of migration inhibitory factor (MIF) by lymphocytes in response to PHA and CE. No patients had cutaneous reactivity to CE. One patient responded to SK-SD and DNCB. All patients responded normally to PHA with the release of MIF. The patient with some cutaneous reactions had minimal response to CE in vitro with normal release of MIF. Administration of dialyzable transfer factor (extracts of at least 1 x 10^9 lymphocytes) resulted in transfer of skin reactivity to CE only in the minimally responsive patient without any effect on his in vitro reactions. These studies suggest that chronic mucocutaneous candidiasis is not a single entity, but rather a syndrome associated with a variety of defects in cell-mediated immunity.

CELL-MEDIATED IMMUNITY IN CAT-SCRATCH DISEASE, Martin L. Schulkind and Elia M. Ayoub, Dept. of Ped., Col. of Med., Gainesville.

The in-vitro cell-mediated immune responses of 6 patients with cat-scratch disease (CSD) and 6 age-matched control individuals were studied by measuring ^{3}H-thymidine (^{3}H-T) incorporation by peripheral lymphocytes cultured in the presence of cat scratch antigen (CS), phytohemagglutinin (PHA), and C. albicans. All 6 patients had positive cutaneous reactions to CS while none of the controls responded to this antigen. The CSD lymphocytes were collected from patients during their acute illness and after a 4 to 6 week interval, and assayed concomitantly with control lymphocytes.

The results indicate that despite the presence of cutaneous reactivity to CS, CSD lymphocytes failed to incorporate increased ^{3}H-T in the presence of CS. In addition, the magnitude of the response of CSD lymphocytes collected during the acute illness to PHA and C. albicans was significantly less than the response of control lymphocytes. However, when assays were performed following recovery from the illness CSD lymphocytes responded as well as control lymphocytes to PHA and C. albicans.

These findings indicate that despite cutaneous reactivity to CS, CSD lymphocytes fail to respond in-vitro to CS by incorporating ^{3}H-T. In addition, they show that during the acute phase of the disease, CSD patients may have a temporarily depressed state of in-vitro responsiveness to PHA and C. albicans akin to the immunosuppression described in certain viral illness.

THYMUS TRANSPLANTATION AND TRANSFER FACTOR: REPEATED TEMPORARY RECONSTITUTION IN A PATIENT WITH CELLULAR IMMUNE DEFICIENCY AND HYPERIMMUNOGLOBULINEMIA. Diane Wara*, Arthur J. Ammann, Sydney Salmon*, Glenn Lawlor, Jr.* and E. Richard Stiehm. University of California, San Francisco, Dept. of Pediatrics and University of California, Los Angeles, Dept. of Pediatrics.

Reconstitution of cellular immunity was achieved repeatedly in a patient with thymic hypoplasia following thymus transplant and transfer factor. Although thymus transplant and transfer factor resulted independently in temporary normalization of in vitro lymphocyte stimulation with PHA, only thymus transplantation was associated with clinical improvement. The patient's ability to respond to both thymic dependent and thymic independent antigens was partially restored.

Reconstitution by thymus transplant occurred within 72 hrs, lasted approximately 3 months, was not associated with lymphocyte HL-A chimerism and was probably due to the effect of a thymic humoral factor on an already existent cell population. The temporary nature of reconstitution is best explained on the basis of discrepant HL-A antigens between donor and recipient and of the long term requirement for circulating thymic "hormone". The similarity between responses following thymic transplant in this child and in the nude mouse suggests that the nude mouse model more closely resembles this patient than the Di George syndrome where transplant of a non HL-A identical fetal thymus results in permanent reconstitution.

THYMUS TRANSPLANTATION: PERMANENT RECONSTITUTION OF CELLULAR IMMUNITY IN A PATIENT WITH SEX-LINKED COMBINED IMMUNODEFICIENCY. Diane Wara*, Arthur J. Ammann, Sydney Salmon* and Herbert Perkins*. University of California, San Francisco, Dept. of Pediatrics and Cancer Research Institute and Irwin Memorial Blood Bank, San Francisco, California.

Successful reconstitution of cell mediated immunity was achieved in a 4 week old infant with sex-linked combined immunodeficiency. A 14 week gestational age fetal thymus was given intraperitoneally. The first sign of reconstitution was complete resolution of tinea 1 week post-transplant. Simultaneously, a mild graft versus host reaction was observed. Ten days post-transplant, a new HL-A antigen was found in the patient that was present in the phenotype of the mother of the thymus donor. Two months post-transplant, the patient's total lymphocyte count was normal; 9 months post-transplant, in vitro lymphocyte response to PHA and PWM were normal and the only HL-A antigens found were 2 of those represented in the phenotype of the mother of the thymus donor. The patient lost the 4 initial HL-A antigens accounted for by inheritance. Ten months post-transplant, lymphocytes reacted normally in mixed leukocyte culture but not to KLH following sensitization.

In contrast to patients with Di George syndrome and with thymic hypoplasia, reconstitution was achieved in our patient by lymphocyte repopulation as evidenced by length of time required for normal cellular responses to develop and by the development of lymphocyte HL-A chimerism. There was no reconstitution of antibody mediated immunity.

INFECTIOUS DISEASE

First Session

SYSTEMIC CAPSULAR ANTIGEN, CLINICAL COURSE AND ANTIBODY RESPONSE IN HEMOPHILUS INFLUENZAE b MENINGITIS. David L. Ingram, Richard J. O'Reilly, Georges Peter, Porter Anderson and David H. Smith (Intr. by Charles A. Janeway) Children's Hospital Medical Center, Boston, Mass.

Capsular antigen (PRP) is released from H. influenzae b in systemic infections. PRP levels were studied in relation to hospital course and antibody response in 70 patients aged 1-136 mo. Serial serum and CSF samples were obtained and titered for PRP by latex agglutination and counterimmunoelectrophoresis. Anti-PRP antibody was measured by radioimmunoassay. Patients with prolonged antigenemia (>6ng/cc,>5 days) tended to have more severe symptoms ($p<0.09$), prolonged fever (>5 days, $p<0.001$), and lower antibody responses (<50ng/cc, $p<0.025$). In contrast, high levels of PRP (>67ng/cc) were not significantly associated with any of these parameters. Although severity and poor immune response were more frequent in younger patients, both were better predicted by duration of antigenemia than by age (>12 mo.).

On admission, antibody levels were generally unmeasurable, and were lower than those in age-matched control children. 40 of 46 patients tested had antibody rises within 7 weeks. Rises were detected even in children under 13 mo. (17/20) and under 7 mo. (5/5), but these responses were usually small and transient. The 6 children with no acute antibody response were aged 8-19 mo.; 3 have since developed antibody. Of 13 convalescent patients later vaccinated with purified PRP, all had antibody responses similar to those of non-diseased children.

HETEROIMMUNIZATION OF PRIMATES TO H. INFLUENZAE TYPE B CAPSULAR POLYSACCHARIDE (HITB) BY GASTROINTESTINAL COLONIZATION WITH A NON-ENTEROPATHOGENIC E. COLI (075:K147:H5 STRAINS EASTER AND "89") POSSESSING A CROSS REACTING CAPSULAR ANTIGEN (CRA) TO HITB

Z.T. Handzel, R. Schneerson, J.C. Parke, Jr., M. Argaman, and J.B. Robbins, NICHD, NIH, Bethesda, Md.

20 adult Rhesus were fed 10^9 and 5 were fed 10^{11} live strain Easter without untoward reactions. Colonization was assayed by stool cultures on antiserum agar. 4 of 20 fed 10^9 had a single positive culture compared to 4 of 5 fed 10^{11} with positive cultures for 1 to 2½ months. Other bacteria with CRA (strep. staph. proteus) were isolated from stools of 5 controls and 7 fed animals. Serum HITB antibodies (radioimmunoassay) are summarized:

# Rhesus	Dose E. coli	Pre	Peak	2½-3 mos. (µg Ab.ml)
20	10^9	0.18	0.41	0.25
5	10^{11}	0.13	0.80	0.61
5	None	0.09	0.43	0.33

3 newborn Rhesus were fed 10^9 strain Easter and 3 fed 10^9 strain "89". Easter-fed newborns were colonized 1-7 weeks and "89" for 8-10 weeks. Growth of the fed and 5 newborn controls was similiar. Their serum HITB antibodies are under study and will be reported.

11 of 996 human newborns had E. coli with CRA in their stools. The avg. serum HITB antibodies of these babies at 6 mos. was 0.31 µg/ml compared to 0.20 of controls. All 11 newborns had detectable serum HITB in contrast to 51% with undetectable levels in the controls. Heteroimmunization may be a safe and effective method for inducing HITB antibodies in the neonate.

IMMUNOGENICITY OF THE GROUP A AND GROUP C MENINGOCOCCAL POLYSACCHARIDES IN INFANTS

Martha L. Lepow*, Irving Goldschneider, Ronald Gold, Emil C. Gotschlich; Univ. of Conn. Health Cntr., Sch. of Med., Depts. of Ped., Path., Farmington; Rockefeller Univ., NY.

250 infants 10 weeks to 21 months of age were injected subcutaneously on 1 to 3 occasions with 12.5 µg to 100 µg of meningococcal group A or group C polysaccharide. No local or systemic side effects were observed. 3 and 7 month old infants produced geometric mean antibody concentrations of 0.50 to 0.72 µg/ml serum within 4 weeks of primary immunization with the group A or group C polysaccharide. There was no clear dose-response relationship over the range of antigen concentrations that were administered. 18 month old infants produced geomtric mean antibody concentrations of 8 µg/ml and 3 µg/ml within 4 weeks of primary immunization with the group A and group C antigens respectively. Antibody responses to secondary and tertiary injections with the meningococcal polysaccharides at 7 or 18 months were equivalent to those produced by primary immunization at these ages, indicating that neither immunological tolerance nor immunological memory had been induced by prior immunization at 3 months of age. Rather, the immunological responses of infants to the meningococcal group A and group C polysaccharide vaccines appeard to be age dependent.

HEPATITIS ASSOCIATED WITH INFLUENZA A_2 (HONG KONG) INFECTION.

Ilya Spigland, Charles Grose and Albert Aharon. Albert Einstein Col. of Med., Montefiore Hosp. & Med. Ctr., Dept. of Ped., Bronx, New York (Intr. by Laurence Finberg)

Although the pathogenesis and clinical features of Influenza have been studied extensively, there is little mention of the association of this infection with hepatitis. In the winter of 1972, 23 hospitalized patients presented viral or serologic evidence of A_2 (HK) infection. 8 of these patients had various hepatic manifestations. 3 patients,(4 mos.,18 mos., and 12 years old) developed jaundice,hepatomegaly,elevated bilirubin and abnormal liver function tests following an acute attack of Influenza. They were thought initially to have primary liver disease.5 adult patients had acute respiratory involvement in addition to hepatitis,with 2 of these also presenting neurological manifestations of encephalitis.In only 3 cases was the respiratory illness the primary reason for the hospitalization.All our patients recovered with complete regression of clinical and biochemical signs.Creatinine phosphokinase assays on 4 of the 8 patients revealed marked elevations suggestive of myositis and may serve as a useful marker in the diagnosis of Influenza.These studies suggest that A_2 (HK) virus infection may be related to clinical manifestations of neurological,muscular and hepatic involvement. Although the precise relationship of A_2 (HK) to the syndrome is not clear, it is probable that it occurs more frequently than initially appreciated since most attention has been focused on the respiratory complication.

EXPOSURE TO HEPATITIS VIRUS, TYPE B AND DEVELOPMENT OF THE CHRONIC CARRIER STATE IN CHILDREN.

Robert J. Gerety, Richard D. Krugman, Lewellys F. Barker, Paul D. Parkman and Harry M. Meyer, Jr. Food and Drug Admin., Bureau of Biologics, Rockville, MD. Five to 15% of U.S. adults have antibodies to hepatitis B antigen (anti-HBAg) as a result of infection with hepatitis B virus (HBV). Approximately 0.5% are chronic carriers of hepatitis B antigen (HBAg). In contrast, anti-HBAg occurs more frequently and adult HBAg carriers exceed 20% in certain overseas countries. Several reports suggest that HBV infection in early childhood more frequently produces HBAg carriers. To test this hypothesis, serum samples from healthy children 7 months to 6 years of age from the U.S., West Africa, and two Pacific islands were assayed for anti-HBAg by passive hemagglutination and for HBAg by radioimmunoassay. As expected, 100 sera from U.S. children were devoid of both antibodies and antigen. Tests on 937 comparable sera from the overseas groups showed antibodies in 3 to 29% and antigen in 3 to 10.4%, with the highest prevalences being found in a country with a high HBAg carrier rate in the adult population. HBAg subtype determinations revealed 53% AYw, 15% ADw and 32% ADr. In conclusion 1) Infection with HBV in childhood is common in these overseas populations; 2) HBV infections in childhood predispose to the chronic carrier state, irrespective of virus subtype; 3) High rates of HBV infections in childhood may account for the high prevalence of HBAg carriers in some adult populations.

PROTEIN CALORIE MALNUTRITION: A HOST DETERMINANT FOR *PNEUMOCYSTIS CARINII* PNEUMONITIS.

Walter T. Hughes, Robert A. Price, Frank Sisco, W. Samuel Havron, and Anthony G. Kafatos, St. Jude Children's Res. Hosp., Memphis, Tenn.; and Mary Schonland and P.M. Smythe, Univ. of Natal, Durban, South Africa. (Intr. by Donald Pinkel.)

Pneumocystis carinii pneumonitis (PCP) occurs almost exclusively in patients with certain serious underlying diseases. Outbreaks have appeared in debilitated infants in European nurseries. The infection also occurs spontaneously in the cortisone-treated rat. Since emaciation is a common feature of susceptible hosts, we explored the role of protein calorie malnutrition in PCP in three separate studies.

Of 44 children with cancer and PCP, 62% had serum albumin values of less than 3.0 gm%, compared to 11% in 44 patients with malignancies of the same type, duration and therapy but who did not have PCP ($p < 0.001$). Body weight loss was also greater in the PCP group ($p < 0.05$).

Lung sections from 60 South African children were examined for *P. carinii*. Of the 39 who died with severe Kwashiorkor, 3 (7.7%) were found to be infected with the organism. In the lungs of 21 well-nourished and geographically matched children, no *P. carinii* organisms were found.

In comparative experiments 135 Sprague-Dawley rats divided into groups of 15 were fed one of three basic diets with and without vitamin supplements. With a 23% protein diet for normal growth, 0/15 acquired PCP; whereas, 13/15 fed a protein-free diet died with PCP. With 8% protein diet rats did not have PCP but 28% harbored the organism. Cortisone-treated animals on 0%, 8% or 23% protein diets died with PCP. After half of one group of animals fed a protein-free diet had died with PCP, the emaciated survivors were treated with amino acids and protein rescue diets and recovered.

We conclude that protein calorie malnutrition is an important factor in predisposing both rats and children to PCP.

E. coli TYPING BY HeLa CELL INVASION

Eugene Ainbender, Helen D. Zepp, Magda Hevizy, Horace L. Hodes Mt. Sinai Schl. of Med., Dept. of Ped. NYC

In 1964, Labrec et al reported that certain strains of Shigella were capable of invading HeLa cells and that these invasive strains were associated with clinical disease. E. coli also have the ability to invade HeLa cell monolayers. This property of the E. coli is stable and is not altered by various treatments of the organism or the cell monolayer.

The epidemiology of these organisms is at present unknown. A survey was made to determine the distribution of these organisms in a normal population. There was a striking absence of invasive E. coli in the stools of babies under 3 months of age. Only 1 infant among the 37 well babies studied carried an invasive strain. In contrast, of 27 well children between the ages of 3 months and 6 years, 15 carried invasive strains. Above the age of 6 years, the number of invasive strains showed a marked decrease. Only 2 were recovered from 21 children. These results suggest that the colonization with these strains takes place after the 2nd month of life; and that after 6 years of age the carrier rate is low.

Enteropathogenic E. coli, certain Shigella and various viruses cause infantile diarrhea. We have attempted to determine whether the invasive strains of E. coli may be another etiological agent. In a small series of infantile diarrhea, there was a significant increase of invasive strains as compared with well infants.

SEPTRIN-INDUCED STIMULATION OF GRANULOCYTE METABOLISM IN CHRONIC GRANULOMATOUS DISEASE (CGD). Allan F. Pyesmany and Dorothy L. Cameron, Dalhousie Univ., Dept. of Ped., Halifax, N.S. (Intr. by Richard B. Goldbloom)

In-vitro studies to assess specific effects of Septrin (trimethoprim and sulphamethoxazole) on the metabolism of normal and CGD granulocytes were done after a 2 1/2 year-old boy with CGD had 2 episodes of multiple hepatic abscesses due to Serratia marcescens which cleared dramatically when treated with Septrin. He has remained completely free of infection for periods of 8 and 10 months during which he has received Septrin prophylactically. The bactericidal capacity (J. Lab. Clin. Med. 72:136, 1968) of granulocytes from the boy and his carrier sister and mother for S. marcescens and Staph. aureus were improved with Septrin when compared to other antibiotics. Significant increases ($p<0.01$) in measurable H_2O_2 production and O_2 uptake were obtained during phagocytosis of heat-killed Staph. aureus by granulocytes of normal controls, carriers, and the patient when Septrin was added; in contrast to penicillin-streptomycin and other antibiotics. Measurements of CO_2 production from glucose-1-C^{14} during phagocytosis were inconclusive. These studies suggest that Septrin has a stimulating effect on granulocyte metabolism which may be related to its unique ability to permeate phagocytes. Septrin may be a useful antibacterial agent in the treatment of bacterial infections in CGD and in other conditions attended by defective bactericidal capacity of granulocytes.

THE NITROBLUE TETRAZOLIUM (NBT) SLIDE TEST IN GRANULOCYTE DYSFUNCTION. Hans D. Ochs, Robert P. Igo and Seymour J. Klebanoff, Dept. Ped., Univ. of Washington, Seattle, WA.

A modification of the histochemical NBT-slide test of Gifford and Malawista in which coverslips were coated with endotoxin to stimulate NBT reduction was used to screen individuals with unusual susceptibility to infection. The results are summarized as follows:

Patients [number in ( )]	NBT-positive cells
Normal controls (100)	>90%
Chronic granulomatous disease (CGD) (50,♀)	0
CGD variant (2)	10-30%
X-linked CGD carriers (7)	43-73%
Hereditary myeloperoxidase deficiency (1)	>90%

Granulocyte dysfunction was confirmed in the 9 patients with CGD by bactericidal assay, glucose C-1 oxidation, formate oxidation and iodination. Granulocytes from 2 girls with Job's syndrome and 7 boys with X-linked agammaglobulinemia were normal in NBT reduction and other leukocyte function tests. In 1 patient with myeloperoxidase deficiency, normal numbers of NBT-positive cells were found despite the presence of a granulocyte microbicidal defect. 2 boys aged 4 and 8 (CGD variant) with repeated staphylococcal skin infections but no apparent systemic manifestations had intermediate values in the NBT reduction test and in other granulocyte function tests. The simple NBT-slide test is useful for the detection of CGD; it clearly delineates the carrier state and has made possible the detection of a variant form of CGD.

HYPERACTIVITY OF NEUTROPHIL LEUKOTACTIC AND METABOLIC RESPONSES DURING BACTERIAL INFECTION. Harry R. Hill and Paul G. Quie. Univ. of Minnesota Medical School, Dept. of Pediatrics, Minneapolis, MN.

The neutrophil granulocytes of 16 patients with acute bacterial infections and 16 age matched controls were compared for leukotactic activity, random mobility and nitroblue tetrazolium reduction. The mean leukotactic index observed in patients with bacterial infection (177 ± 68) was markedly higher than that of controls (72 ± 9). In most patients increased leukotactic activity was associated with a high percentage of nitroblue tetrazolium positive neutrophils. After 7 to 10 days of appropriate therapy with clinical and bacteriological response, leukotactic activity returned to normal values. A hyperactive leukotactic response continued, however, in patients with persisting bacterial infection. Random mobility of leukocytes from patients with bacterial infection was not different from that of controls and could not be correlated with nitroblue tetrazolium reduction. The neutrophil leukocytes of patients with bacterial infection are hyperactive in unidirectional movement toward a chemotactic stimulus as measured in the leukotactic assay, and have elevated metabolic responses as demonstrated by increased nitroblue tetrazolium reduction. The hyperactive leukotactic response appears to be an early event in the inflammatory cycle stimulated by bacterial infection and may contribute to rapid localization of bacterial invasion.

INFECTIOUS DISEASE

Second Session

HYPOREACTIVITY OF INFECTION: OCCURRENCE IN EXPERIMENTAL VIRUS INFECTIONS AND TRANSFER BY A SERUM FACTOR. Lowell A. Glasgow and Dale A. Stringfellow. Dept. of Pediatrics and Microbiology, Univ. of Utah Col. Med., Salt Lake City.

One approach to therapy of viral infections has been the development of interferon (IF) inducers. In most circumstances, however, efficacy has been limited to the prophylactic use of these agents. To investigate the mechanism for this failure, the effect of experimental virus infections on the capacity of the host to produce IF was examined. Mice infected with a picorna-(EMC), arbo-(SFV), or herpes-(CMV) virus progressively developed a state of hyporeactivity to IF induction (90% decrease in serum IF) in response to 4 different inducers (Tilorone, Poly I:C, Newcastle Disease [NDV], and Chikungunya virus). Macrophages and lymphocytes from animals infected with EMC manifested a similar degree of hyporeactivity in vitro in response to 2 viral inducers (1:20→85% decrease; 1:400→50% decrease). More significantly, hyporeactivity could be transferred by serial dilutions (IF was reduced from 3900 to 150 u/ml with NDV and 1000 to <100 u/ml with Chikungunya virus) of serum (containing no IF or virus) from hyporeactive animals to cultures of mouse embryo fibroblast or peritoneal exudate cells challenged with NDV.

These results suggest: (1) an explanation for failure of IF inducers in therapy, (2) hyporeactivity is mediated by a factor present in the serum during certain viral infections, and (3) macrophages and lymphocytes from hyporeactive animals manifest a similar degree of hyporeactivity in vitro.

PHAGOCYTOSIS IN DOWN'S SYNDROME. R.R. Kretschmer, M. Lopez-Osuna, S. Armendares, and L. De la Rosa (Intr. by F.S. Rosen). Secciones de Inmunologia y Genetica, Departamento de Investigacion Cientifica, Instituto Mexicano del Seguro Social, Mexico, D.F.

Patients with Down's syndrome have an increased infection rate. In the present study, the circulating phagocytes of 25 patients with Down's syndrome (Trisomy 21, HAA negative) were evaluated by means of the quantitative nitroblue tetrazolium (NBT) reduction test; and their bactericidal capacity against Streptococcus pyogens and Staphylococcus aureus (Coagulase +), measured by the method of Quie. A significant decrease in NBT-reducing capacity was found in phagocytes of patients with Down's syndrome when stimulated with latex particles, as compared to normal, age-matched controls. This finding was not related to age, sex, or immunoglobulin levels in the patients. Reduced bactericidal capacity against staphylococci was found in these patients, whereas killing of streptococci remained normal.

The degree of phagocyte dysfunction is similar to that in heterozygous carriers of chronic granulomatous disease (CGD). The distinct partial leukocyte dysfunction against staphylococci (Catalase +) in Down's syndrome contrasts with the normal bactericidal capacity against streptococci (Catalase -) in the same patients, a discrepancy that is also observed in CGD.

DEFICIENT KILLER FUNCTION IN NEWBORN AND YOUNG INFANT LYMPHOCYTES. Gary S. Rachelefsky, Peter R. McConnachie, Paul I. Terasaki, and E. Richard Stiehm. UCLA Sch. Med., Depts. Ped. and Surgery, Los Angeles, Calif.

Because the newborn has decreased ability to respond to delayed hypersensitivity skin test antigens and has increased susceptibility to infection, a defect in cellular immunity has been suspected. Prior in vitro studies of lymphocytes indicate near normal response to allogenic cells, PHA and other antigens, indicating proliferative ability. We evaluated another indicator of cell-mediated immunity using direct cell to cell interaction measuring lymphocyte killer function.

We studied 53 medically normal patients, including 10 term newborns, 8 infants aged 2-6 months, 4 infants aged 6-12 months and 8 children 1-5 years old and 23 adult controls. Isolated blood lymphocytes were incubated with normal adult Cr^{51}-labelled allogenic lymphocytes coated with specific HL-A antibodies (target system) for 5 hours. The ratio of killer to target cells was 24 to 1. Killing was expressed as a percent of Cr^{51} released after subtracting the spontaneous target cell release; maximal killing occurred by 5 hours.

The mean value for the 10 newborns, 2.33% ± 1.25 (S.D.) was significantly different ($p < .05$) from the 23 adult values of 19.5% ± 5.46; low intermediate values were noted through 6 months (5.16% ± 6.34) with adult levels being achieved by 12 months indicating maturation of killer function.

The defective lymphocyte killer function in the young infant suggests a possible mechanism for increased susceptibility to certain infections in young infants.

SOME UNEXPECTED FINDINGS IN A COMPREHENSIVE 2-YEAR STUDY OF STREPTOCOCCAL INFECTIONS IN AN ISOLATED COMMUNITY. Bascom F. Anthony, Edward L. Kaplan, S. Stephen Chapman, and Lewis W. Wannamaker, Univ. of Minnesota Medical School, Minneapolis.

During a prospective year-round investigation of group A streptococcal infections over 7200 patient visits were made among 200 children (1-10 yrs) in an isolated community. Weekly cultures were obtained from nose, throat and skin lesions (when present). During the 2 yrs >95% had strep recovered from upper respiratory tract and >85% from skin lesions.

Streptococcal pyoderma (while reaching a peak incidence in late summer and fall) occurred during all seasons, affecting 10% even during mid-winter. No influence of age or sex on development or recurrence of pyoderma was evident.

This study afforded a unique opportunity to compare pattern of spread of classical "respiratory" and "skin" serotypes in the same population at the same time. Certain serotypes (M-4, 6,12,13) were almost exclusively recovered from the upper respiratory tract (URT) but seldom from skin lesions. So-called "pyoderma strains" (M-31,41,49,52,54,T-5/27/44), however, were isolated with equal frequency from skin and URT.

Type 49, a nephritogenic strain, was frequently isolated in this population but spread slowly and produced relatively little pyoderma in young children. The low incidence of acute nephritis (AGN) in this population, in contrast to a nearby population, who also experienced spread of type 49, suggests that factors other than the presence of a nephritogenic strain played a role in the epidemiology of AGN.

AN INTERFAMILIAL OUTBREAK OF YERSINIA ENTEROCOLITICA (Y. e.) ENTERITIS. L. T. Gutman, E. Ottesen, T. Quan, W. Bradford (Intr. by S. L. Katz). Depts. of Ped. and Path., Duke Univ. Med. Sch., Durham, N. C. and C.D.C., E.I.P., Ft. Collins, Colorado.

In 4 families of 21 persons, 16 experienced infection with Y. e.. Two deaths occurred and 2 patients underwent appendectomy. Sequential onset of disease suggested person-to person transmission following initial infection of a bitch and her sick puppies. The index case expired after presenting with fever, diarrhea, exudative pharyngitis, and leukocytosis. Autopsy findings included sero-sanguinous peritonitis, and ulcerations with extensive adenopathy throughout the gastrointestinal tract. Y. e. was isolated from the spleen.

The child was a member of a rural family which, with a neighboring family and 2 related urban families, were experiencing gastrointestinal disease. The 16 symptomatic persons had fever (87%), diarrhea (69%), abdominal pain (62%), vomiting (56%), pharyngitis (31%), or headache (18%). The 4 hospitalized children had hypoalbuminemia which resolved spontaneously within 6 weeks. Clinical response to antimicrobial therapy was slow; fever and diarrhea continued more than 5 days in all patients.

All 18 sera demonstrated a positive response in the passive hemagglutination test to Y. e. antigen. Two pairs of sera showed 4 $\log_2$drops; one remained constant. Eight patients had significant titers ($\geq$ 1:512) and all were clinically ill.

This is the first reported epidemic of Y. e. enteritis, a disease widely recognized in Europe to mimic other acute abdominal disorders.

PROTECTION AGAINST GROUP A STREPTOCOCCUS INFECTION BY AN M PROTEIN VACCINE. Eugene N. Fox, Robert H. Waldman, Masako K. Wittner, and Albert Dorfman, La Rabida-Univ. of Chicago Institute, Dept. of Pediatrics, Univ. of Chicago and Univ. of Florida, Dept. of Medicine, Gainesville.

Adult male volunteers were immunized subcutaneously by three monthly doses of aluminum hydroxide precipitated purified M protein from group A streptococci, type 1. Control subjects received a placebo of the aluminum hydroxide adjuvant. (All procedures were conducted on a double blind basis.) Thirty to 50 days after the last injection the vaccinees and control subjects were infected by applying a virulent strain of type 1 streptococci to the pharynx. Throat cultures, white blood cell counts, temperatures, and signs and symptoms were monitored daily. Benzathine penicillin (1.2 million units) was administered no later than 6 days after infection to all subjects. Illness was judged by the appearance of exudative pharyngitis and cervical adenopathy accompanied by a positive throat culture. By these criteria, 9 of the 19 controls and one of the 19 vaccinees were ill. No residual illness or clinical complications were observed following treatment. It is concluded that the alum-precipitated M protein vaccine afforded protection against an upper respiratory type 1 streptococcal infection. Supported by PHS Contract PH 43 NIAID-68-83.

CELL-MEDIATED IMMUNITY TO RUBELLA VIRUS FOLLOWING IMMUNIZATION. Russell W. Steele, Sally A. Hensen, Monroe M. Vincent, David A. Fuccillo, and Joseph A. Bellanti. Georgetown Univ. Sch. of Med., Dept. of Ped., Washington, D. C.; Microbiological Associates and NIH, NINDS, Infectious Diseases Branch, Bethesda, Md.

A microassay procedure for the measurement of cell-mediated immunity (CMI) to rubella virus was recently developed in our laboratory. The technique employs cell lines chronically infected with rubella virus and utilizes the release of ^{51}Cr to detect lymphocyte-mediated cytotoxicity. The present studies were performed to measure the development of CMI and rubella serum hemagglutination inhibition (HAI) antibody responses in children immunized with rubella vaccine. Following immunization of 12 children with the HPV 77 strain of rubella virus, CMI was demonstrated as early as 3 days after immunization; serum HAI responses did not appear until after 2 weeks post-immunization. The development of CMI appeared to correlate with gestational age; children born full term reached maximal levels at 7 days whereas children born prematurely attained maximal levels at 14 days; after 28 days the levels of CMI were comparable. The delayed development of CMI seen in premature infants, even at 2 years of age, may be responsible in part for their increased susceptibility to viral infections. This in-vitro microassay provides a simplified procedure for the measurement of CMI following administration of viral vaccines or natural infection.

MODIFICATION OF ENDOTOXIN SHOCK IN MICE WITH ANTIHISTAMINES. Donald M. MacQueen, Teresa Rittmanic, and Heinz J. Wittig. Univ. Fla. Col. Med., Dept. Ped., Gainesville, Fla. (Intr. by Gerold L. Schiebler).

The prevention of fatal gram-negative endotoxin shock was studied in mice by pre-administration of two antihistamines at varying dosage levels.

The LD_{80} of Westphal (Difco) gram-negative endotoxin was first determined by injection into the tail-vein as being 36 mg/kg body weight of mouse. Subsequently, groups of 10 mice each were pretreated with varying doses of diphenhydramine and hydroxyzine intravenously one hour prior to the administration of the LD_{80} of endotoxin.

With diphenhydramine, a dose of 5 mg/kg mouse gave 60% protection, while with hydroxyzine, doses of 5 mg and 2.5 mg/kg mouse protected 100% of the animals from fatal shock. In subsequent studies, simultaneous administration of hydroxyzine and of endotoxin still protected 100% of animals. Treatment at 6 hours before and 3 hours after endotoxin gave only 50-60% protection. Higher doses of diphenhydramine and hydroxyzine (greater than 30 mg per kg mouse) proved fatal in more than 80% of the animals, death occurring with convulsions.

The protective properties of hydroxyzine in gram-negative bacteremia appear to deserve further investigation in other species. Mechanisms and rationale of the protection against endotoxin shock by antihistamines are as yet poorly understood.

SUBCLINICAL CONGENITAL CYTOMEGALOVIRUS (C-CMV) INFECTION: A MICROBIOLOGIC AND CLINICAL LONG TERM STUDY. David W. Reynolds Sergio Stagno, Kathern G. Stubbs, and Charles A. Alford, Univ. of Ala. in Birmingham, Dept. of Ped., Birmingham, Alabama.

Subclinical C-CMV infection is exceedingly common, about 1% of live births, yet the natural history of this condition has not been adequately determined. Eighteen such cases have been longitudinally studied for an average of 38 months. No significant abnormalities of intrauterine growth or neonatal clinical status were observed; however, 4 had minor aberrations in CSF cell count, protein level, or both. The duration of virus excretion ranged from 3 to 50 months with 11 excreting beyond 24 months of age. Immunoglobulin G production began earlier and has been maintained at a significantly higher level than expected; further, CMV-CF antibody has persisted in all the patients. At birth and during the first few months of life, IgM levels were also increased. Despite the evidence for prolonged active infection and the resultant antigenic stimulation, physical growth, general health status, and more specifically the occurrence of liver and pulmonary disease have been within normal limits as were intelligence and social quotients when compared to matched pair controls. Two infected patients, however, have moderate to severe mental incapacitation. Importantly, some degree of sensori-neural hearing loss occurred in 50% and 2 with concomitant brain damage require hearing aids. In order to assess for a continued low grade pathologic process, these children will be tested serially for CNS development and immune complex disease.

EVALUATION OF PASSIVE IMMUNIZATION AGAINST VARICELLA USING A NEW SEROLOGIC TECHNIQUE. Anne Gershon and Philip Brunell. NYU Sch. of Med., Dept. of Ped., NY

Zoster Immune Globulin (ZIG) has been used to passively immunize children with no history of previous varicella. The management of these high risk patients would be facilitated by the availability of a serologic method which could be used to evaluate their immune status. When ZIG recipients were tested for antibody against membrane antigens (AMA) of V-Z infected cells it was found that 6 patients with antibody at the time of household exposure failed to develop clinical varicella or rises in AMA. Eight of 12 children without detectable AMA developed vesicular lesions and titers of AMA of 1:8 or greater during convalescence. Four seronegative ZIG recipients did not develop clinical evidence of infection but 3 of these 4 subsequently developed barely detectable levels of antibody. ZIG used in these studies was found to have 20 times the concentration of V-Z AMA as regular immune serum globulin. AMA determinations may be useful in measuring potency of ZIG preparations and identifying those high risk children who are truly susceptible to varicella and require ZIG.

INFECTIOUS DISEASE

Read by Title

DIAGNOSIS OF PERTUSSIS IN EPIDEMICS, INCLUDING CASES NEGATIVE BY CULTURE. Rouben V. Aftandelians and James D. Connor. Univ. Cal.,San Diego Sch. of Med., Dept. of Peds.

A majority (86%) of children with B. pertussis infection develop gel precipitating antibody (PGPA). Antibody prevalence can be used to identify outbreaks and occurrence of infection in culture-negative cases. The test is markedly superior to the standard agglutination test. Of 112 clinical cases studied, 91 (81%) had PGPA, including 31 of 36 culture-positive cases (86%) and 20 of 29 culture-negatives (68.9%). Of 76 cases from several outbreaks occurring in different locations, including 20 culture negatives from San Diego, 60 (78.9%) were positive. In comparison, 57 of 200 adults (28.5%) and 134 of 367 DPT immunized children (36.5%) had PGPA. The higher prevalence of PGPA in clinical pertussis is significant ($p=<.01$) when compared to either immunees or adults and may be used to identify the occurrence of an outbreak. The character of the gel precipitin response is also diagnostic of an outbreak. Of 91 PGPA-positive cases, 21 of 31 (35.4%) culture-positives and 21 of 60 (35%) culture-negative or culture-unidentified cases had 3 or more PGPA bands in one or more sera, in comparison to only 3 of 57 PGPA positive adults (5.2%) and 16 of 134 (11.9%) immunees had 3 or more PGPA bands ($p=<0.01$).

AN ANALYSIS OF ELEVEN CONSECUTIVE RESPIRATORY SYNCYTIAL VIRUS (RSV) OUTBREAKS IN CHILDREN. Carl D. Brandt, Hyun Wha Kim, Julita O. Arrobio, Robert M. Chanock and Robert H. Parrott. Research Fndn. Children's Hosp. of D.C. and George Washington University Medical School.

More than 15,000 infants and young children were studied for virus infection during 11 consecutive RSV outbreaks. Data obtained at monthly intervals during the outbreaks were combined to plot a composite epidemic curve, which showed a "normal" distribution. Of more than 1,000 respiratory disease patients who yielded an RSV isolate, 39.6% shed virus during the peak epidemic month and 81.4% shed virus in the 3 mid-epidemic months. During the peak month of the composite epidemic, RSV was recovered from 46.2% of all inpatients with bronchiolitis, from 33.6% of all inpatients with respiratory disease, and from 32.1% of all respiratory disease outpatients. Control subjects who were free of respiratory disease rarely yielded RSV. By virus recovery and/or complement-fixation serologic response, 70.3% of bronchiolitis patients and 56.4% of all respiratory disease inpatients showed evidence of RSV infection during the peak epidemic month. A similar epidemic wave was seen in males as compared to females, and in black as compared to non-black children. More than half of all hospitalized patients who yielded an RSV isolate were less than 6 months of age. Among bronchiolitis patients, about half of those with RSV isolates were 3 months of age or younger. The age distribution analysis negates the hypothesis that RSV bronchiolitis in infancy represents an "allergic" reaction resulting from the later of at least two infections.

BACTERIOPHAGE TYPES AND ANTIBIOTIC SUSCEPTIBILITIES OF S. AUREUS FROM PEDIATRIC PATIENTS. Nancy Byrd, Fred F. Barrett, Dorothy J. Clark, and Peter B. Smith (Intr. by Martha D. Yow). Baylor College of Medicine, Dept. of Pediatrics, Houston.

Since the early 1960's Staphylococcus aureus has been a less prominent cause of nosocomial infections. However, it remains an important pathogen, especially among pediatric outpatients. This study compares 459 strains of coagulase positive S.aureus isolated from two different socioeconomic groups of pediatric patients. Isolates from various sources were collected over a 6 month period from patients at a private (222 strains) and a county (237 strains) hospital. Susceptibilities to 9 antibiotics were determined by the innocula-replicating method. Bacteriophage typing was performed by the CDC. Among outpatient strains, phage groups 2 and 3 were most common. Phage group 3 was also prevalent among inpatient strains from both hospitals, while phage group 1 occurred more frequently among isolates from private inpatients. Antibiotic therapy was employed prior to culture in 51% of private and 17% of county patients. However, susceptibility patterns were very similar for isolates from both patient groups. Penicillin G resistance occurred in 75% of all outpatient strains. No methicillin resistant strains were isolated. No significant emergence of resistance to the other antibiotics tested was detected. Thus, penicillin G is not efficacious against the majority of outpatient staphylococcal isolates. Pediatric nosocomial infections with S.aureus were infrequent among the study patients. However, the significance of the increase in phage group 1 isolates among inpatients at the private hospital remains to be elucidated.

THE VARIETY OF SERRATIA FOUND IN A PEDIATRIC INTENSIVE CARE UNIT DURING A SINGLE MONTH. Stephen F. Cardos, Alfred L. Florman, Michael S. Simberkoff and Lillian Larrier. Depts. of Ped., Med. & Path. New York Univ. Sch. of Med. NYC.

Non pigmented Serratia marcescens are among the bacteria which are now being associated more frequently with hospital acquired infection. During July 1972 this organism was recovered from 7 of 20 patients at risk in an ICU. Four of the 7 children died. Serratia was isolated from the blood of 3 and the CSF of one of the fatal cases. Epidemiological investigations, which included efforts to characterize the isolates more precisely, failed to reveal a common source, but did show that there were at least 5 different strains in the unit during the month. Each of the first 4 isolates was different. Each had unique antibiotic sensitivities. They were serotypes 014 H12, 014 H11, 0 undetermined H12 and 014 H4. The last 3 isolates were all recovered during a single week and appeared to be the same. (014 H12 but with an antibiotogram like that of the 014 H4 strain). Only one of the strains was sensitive to the bactericidal activity of normal serum. It was recovered repeatedly from the endotrachial secretions of a child who remained in the unit during the entire month and never from any of the other patients. This is consistent with the observation that serum sensitive strains are likely to be non virulent. Each of the other 4 strains was associated with a fatal illness.

MODIFICATION OF EXPERIMENTAL HERPESVIRUS HOMINIS ENCEPHALITIS BY HUMORAL ANTIBODIES. C.T. Cho, K.K. Feng, N. Brahmacupta and C. Liu, Dept. of Ped. & Med., U. of Kansas Med. Center, Kansas City, Kansas.

Three-week-old Swiss Webster strain mice were infected with a strain of HVH (type 1). The mortality rate of intracerebrally (I.C.) infected mice was 60% and 95% for an inoculum of $10^{0.5}$ and $10^{2.5}TCID_{50}$ respectively. Mice recovered from HVH encephalitis exhibit increased resistance to reinfection. Antisera to HVH prepared in rabbits (SN titers 1:128) administered subcutaneously (S.C.) before (24 h.) or shortly after (24 h.) infection gave a mortality rate of 20-23%, in contrast to 57% for control mice receiving normal rabbit sera. Low or no protective effects were seen when antisera were given after 48 h. of infection.

Human immune globulin (HIG; SN titers 1:512) also showed protective effects on HVH infected mice. Mice inoculated with $10^{2.5}TCID_{50}$ had a mortality rate of 95% in controls vs. 45-50% in mice given HIG before (24 h.) or shortly after (24 h.) infection. HIG were ineffective when given 48 h. after infection. Mice inoculated with $10^{0.5}TCID_{50}$ had a mortality rate of 60% in controls and 5% in mice treated within 24 h. after infection. Preliminary studies suggest a slight prolongation of average survival time in HVH infected marmosets treated with HIG. Our findings suggest the protective effects of humoral antibodies on HVH encephalitis in mice and, perhaps, in marmosets. Such protective mechanism warrants further study.

LACK OF CORRELATION BETWEEN IN VIVO TOXICITY AND IN VITRO GELATION TEST FOR ENDOTOXIN. James J. Corrigan,Jr. and James F. Kiernat.Dept. of Ped., Univ. of Arizona Medical Center, Tucson, Arizona.

Endotoxin toxicity induced in animals is manifested by fever, leukopenia, thrombocytopenia, intravascular coagulation, shock, the Shwartzman reactions, tumor necrosis and/or death. Recently, the development of the horseshoe crab lysate gelation test has provided a sensitive in vitro test for endotoxin and when positive has been equated with endotoxicity. In the present study 10 white rabbits of either sex were given 0.1 mg/kg E. coli 0127:B8 endotoxin intravenously. These animals developed leukopenia, thrombocytopenia, intravascular coagulation, and positive lysate tests on their plasmas. Polymyxin B sulfate was mixed with endotoxin, ratio 50:1, incubated for 15 minutes at 37°C and injected into 10 other rabbits. Although no animal developed leukopenia, thrombocytopenia or intravascular coagulation, the polymyxin-endotoxin mixture was consistantly positive in the lysate test. Polymyxin B is known to neutralize endotoxin toxicity probably by a detergent effect. These data suggest that sub-units of the endotoxin molecule may be capable of reacting with the lysate but are incapable of causing toxicity. The results further suggest that the technique may not be useful in testing materials for possible endotoxin toxicity.

QUANTITATIVE MEASUREMENT OF ENDOTOXIN ACTIVITY. James J. Corrigan,Jr. and James F. Kiernat. Dept. of Ped., Univ. of Arizona Medical Center, Tucson, Arizona.

Amebocytes in the hemolymph of the horseshoe crab contain a material which gels when reacted with endotoxin. In this reaction the endotoxin-lysate mixture becomes cloudy and a change in percent light transmission or optical density can be measured. In this study the change in light transmission produced by various concentrations of endotoxin was determined by using a photoelectric nephelometer (Chrono-Log platelet aggregometer) and recorder (Heath). Hemolymph was collected into sterile siliconized chilled glass tubes and the cells harvested by centrifugation in the cold. Lysate was prepared by adding sterile distilled water to the cells and sonicated. The average protein content of the lysate was 25 mg/ml. The reaction mixture contained 0.2 ml lysate and 0.2 ml test material, incubated at 37°C without stirring and a recorder speed of one inch per 10 min. The results show that a straight line relationship occurs with concentrations of endotoxin (S. typhosa) ranging from 1.0 to 0.001 microgram/ml, 3 min to 27 min respectively. Reproducible, sensitive results were similarly obtained with human, rabbit and dog plasmas and human CSF. This technique allows the use of small volumes (0.2 ml) of test substance, accurate recording of reaction time and minimal technician time.

ISOLATION OF CYTOMEGALOVIRUS FROM FECES AND TEARS. Frederick E. Cox and Walter T. Hughes (Intr. by Donald Pinkel). St. Jude Children's Research Hosp., Memphis, TN.

Acquired cytomegalovirus (CMV) infection in children may present with symptoms of pneumonitis, hepatitis or gastrointestinal (GI) disease. Focal or diffuse ulcerations may occur at various sites in the GI tract, from esophagus to rectum, but there have been no attempts at antemortem virus isolation from these sites. Thirty-six children with complement-fixing antibody to cytomegalovirus had human embryonic lung fibroblast cultures of urine and rectal swabs performed at monthly intervals for 2 months. This included 31 children with acute leukemia, two with solid neoplasms, one with sickle cell anemia, one with systemic lupus erythematosus and one congenitally-infected infant. Twenty-eight patients were found to be excreting virus in their urine. Virus was also isolated from the feces in two of the 28. One was a patient with acute lymphocytic leukemia in remission and disseminated cytomegalovirus infection with viremia, chorioretinitis and isolation of virus from tears. The other was a congenitally-infected infant with hepatosplenomegaly and thrombocytopenia as the only manifestations of disease. Both patients had simultaneous fecal, saliva and urine cultures positive for CMV. No signs or symptoms of GI disease were present in either patient. The data demonstrates that asymptomatic fecal excretion of CMV occurs and that a possible method of spread of infection via the fecal-oral route should be investigated further. This is the first known cultural isolation of CMV from tears and feces.

PSEUDOMONAS AERUGINOSA: SENSITIVE TO ANTIBIOTICS IN VITRO AND RESISTANT TO THERAPY IN VIVO. Starkey D. Davis and Antoinette Iannetta, Univ. of Wash. Sch. of Med., Dept. of Ped., Seattle.

Physiologic concentrations of calcium antagonize the effects of colistin, polymyxin, gentamicin, and tobramycin on Pseudomonas aeruginosa (Ps) in vitro (Antimicrob. Agents Chemother. 1:466, 1972). The degree of antagonism by calcium varies with the strain of Ps, the concentration of calcium, and the antibiotic. The order of antagonism by calcium is polymyxin and colistin > gentamicin > tobramycin. For in vivo studies, 10 strains of Ps were selected that had known patterns of antagonism of antibiotics by calcium. All strains were sensitive to antibiotics in vitro and all were virulent for mice. Mice were inoculated intraperitoneally with 10^9 bacteria in mucin, and antibiotic was given subcutaneously as a single dose 1 hour later. Survivors were counted after 48 hours. Results for 3 antibiotics were (the ED_{50} is the effective dose that would save 50% of the animals from death):

ED_{50} mg/kg	Tobramycin	Gentamicin	Colistin
>120	2	3	4
15-120	3	4	5
<15	5	3	1

There was a good correlation between the degree of antagonism by calcium in vitro and the results of therapy in vivo. This study directly demonstrates a dissociation between in vitro antibiotic susceptibility tests and efficacy of antibiotics in vivo.

EVALUATION OF ANTIGENICITY AND A 2 YEAR PROSPECTIVE FOLLOWUP OF JOINT, MUSCULAR, AND NEURITIC COMPLAINTS IN CHILDREN FOLLOWING IMMUNIZATION WITH AN INVESTIGATIONAL HPV77DK12 RUBELLA VACCINE. Jerry J. Eller, Eleanor A. Eller, Kenneth J. Moran, Univ. of Texas Med. Sch. at San Antonio, San Antonio, Texas. (intr. by W.T. Kniker).

This prospective study included a random sample of the population given a placebo. The findings in our study are in marked contrast to recent reports of uncontrolled field trials with the licensed HPV77DK12 rubella vaccine showing high reaction rates and recurrent arthritis. Our close followup group consisted of 496 patients aged 1-15 from 3 private pediatricians and a residential school. Vaccine was given to 399 and 97 received a placebo. Ninety-nine % of seronegative vaccinees seroconverted with a GMT of 80. Followup was accomplished by questionnaire, telephone and doctor visits at 3 and 10 weeks. Eleven (2.8%) vaccinees and 3 (3.1%) controls had joint, muscular and neuritic complaints. Two (.5%) of the vaccinees had neuritis, but none had arthritis. The gross safety group consisted of 1497 children from local preschools followed by questionnaires and telephone. There was 99.5% followup. Twenty (1.4%) children reported reactions. Two (.1%) had arthritis. In the 2 year period after immunization, 29 of 31 vaccine reactors were followed. There were no instances of recurrent arthritis or neuritis. Canine kidney cell culture should not be discarded as a system for rubella viral vaccine production. This HPV77DK12 vaccine was immunogenic, yet acceptably attenuated for clinical use.

ATTACK RATES FOR HOSPITALIZED ACUTE BACTERIAL MENINGITIS DUE TO HEMOPHILUS INFLUENZA, MENINGOCOCCUS, AND PNEUMOCOCCUS IN CHILDREN IN A MILITARY POPULATION. IMPORTANCE OF THE FIRST 4 MONTHS OF LIFE. Jerry J. Eller, and Daniel C. Plunket. Univ. of Tex. Med. Sch., San Antonio, Tex., and Fitzsimons General Hospital, Denver, Colo. (intr. by W. T. Kniker).

The number of infants and children hospitalized at Fitzsimons General Hospital with acute bacterial meningitis from January 1960 to January 1970 were determined by record review. H. flu type b caused the most (27) cases, with 2 deaths. Patients were hospitalized during each of the 10 years. Meningococcus (M.C.) caused 4 cases during 4 separate years with 2 deaths. Two of the cases were type c. Six pneumococcal (P.C.) cases were spread over 6 years with 2 deaths. There were 8 cases with etiology undetermined. Although the majority of cases occurred in the age group from 6 mos to 3 yrs, the highest attack rates occurred in the 0-3 mos age groups for all major pathogens. Age specific attack rates per 10,000 children at risk are given for H. Flu and P.C. based on the 10 year surveillance, and for M.C. based on the "epidemic" 4 years. (H.F.) 0-3 mos: 4.7, 4-5 mos: 1.4, 6 mos-3 yrs: 1.1, 4-12 yrs: 0.2. (P.C.) 0-3 mos: 1.2, 4-5 mos: 0.7, 6 mos-3 yrs: 0.1, 4-12 yrs: 0.1. (M.C.) 0-3 mos: 3.0, 4-5 mos 1.7, 6 mos-3 yrs: 0.2, 4-12 yrs: 0.2. These data suggest that recommendations should be made for immunization of neonates or very young infants with purified capsular antigens of pathogens causing acute bacterial meningitis, especially H. Flu type b.

ANTIBODY LEVELS TO POLIOVIRUS IN CHILDREN AGED 1-4 YEARS RELATED TO THE HISTORY OF IMMUNIZATION. Eli Gold and Alfred Fevrier, Case Western Reserve Univ. Sch. of Med. at Cleveland Metropolitan General Hospital, Dept. of Pediatrics, Cleveland, Ohio and the Nat. Communicable Dis. Ctr., Atlanta, Georgia.

A survey of immune status among 1-4 year old children residing in Cuyahoga (Cleveland) County revealed that over 50% had antibody levels less than 10 to one or more serotypes of poliovirus. Vials of poliovaccine in use in offices where many of these children had received their immunization were tested; there was no evidence of loss of potency. Among a group of children with a documented history of 3 or more OPV and no antibody at a dilution of 10 to one or more serotypes of polio, retesting a serum showed that 16 of 17 had antibody titers of 2 or greater! Serum antibody levels following OPV may fall to levels of < 10 in a large proportion of immunized children. Such children probably can respond to wild poliovirus reinfection with an anamnestic immune response not accompanied by clinical disease or significant virus excretion. However, the result of reinfection probably depends on the level of immunity which in turn may be related to the interval between immunization and challenge. The high proportion of young children living in the inner city who have not been immunized against poliomyelitis as determined by the United States Immunization Survey plus the evidence of the short duration of antibody persistence in those who have been immunized makes it imperative to urge more effective use of polio vaccine.

BACTERICIDAL CAPACITY OF HUMAN BLOOD PHAGOCYTES. Martha Greenwood, Joe Jawad, Erwin Jones, Charlotte Lubawy, and Phillip Holland, Univ. of Ky. Med. Sch., Lexington, Kentucky.

The bacterial kill capacity of neutrophils (PMN) and monocytes (MN) against S. aureus and E. coli was determined using purified populations (Ficoll-Hypaque gradient centrifugation) of each cell from normal subjects and compared with the MN functional capacity of a 15 month old male with virtual absence of blood and bone marrow PMN yet minimal infection since birth. Results are summarized as follows:

CELL(C)	BACTERIA(B)	RATIO (B:C)	%SURVIVAL 60"	%SURVIVAL 120"	RATIO (B:C)	%SURVIVAL 60"	%SURVIVAL 120"
Control MN	E. coli	1:1	3	1	3:1	30	14
Control PMN	E. coli	1:1	1	1	3:1	6	3
Control MN	S. aureus	1:1	12	6	3:1	64	50
Control PMN	S. aureus	1:1	8	4	3:1	30	16
Patient MN	E. coli	1:1	6	2	3:1	38	17
Patient MN	S. aureus	1:1	29	10	3:1	60	48

These findings indicate that the bacterial kill capacity of blood phagocytes is most clearly defined using pure populations of MN and PMN and varied bacteria:cell ratios. Studies of MN function in this child with no PMN are as follows: normal MN kill of S. aureus and E. coli, normal in-vitro MN to macrophage transformation with intact macrophage "Fc" receptor sites for IgG. These results indicate that the MN serves as a compensatory mechanism in host defense and that the clinical course and survival of these children may depend on the functional capacity of their MN and macrophage system.

QUANTITATIVE NITROBLUE TETRAZOLIUM(NBT) REDUCTION IN HYPERTHYROIDISM. **Elena R.Grimes,Elizabeth M.Smithwick & Qutub H.Qazi, S.U.N.Y.,Downstate Medical Center, Dept. of Ped.,Brooklyn.**

Quantitative NBT reduction correlates with leukocyte oxidative metabolism. The present study sought to determine whether the increased metabolism of hyperthyroids would be reflected in their neutrophils as increased resting NBT reduction. NBT reduction was measured by the method of Baehner & Nathan,incubating for 30 min. at 37C. The results are summarized below, expressed as mean optical density (OD) at 515mμ for $2.5x10^6$ phagocytes, resting and latex-stimulated.

	Controls(13)	Hyperthyroid(13)	p
Resting	0.133±0.045	0.227±0.079	<0.0025
Phagocytosing	0.284±0.058	0.379±0.103	<0.005
Δ OD	0.151±0.061	0.144±0.076	N.S.

The data reveal that both resting and phagocytosing NBT reduction are significantly increased in hyperthyroids. The increment of phagocytosing over resting value(ΔOD) is unchanged. The histochemical NBT technique of Park et al. also showed a higher number of positive cells in hyperthyroids(7.9%) than in controls(4.6%). Three hyperthyroid patients,restudied when euthyroid,had normal mean resting(0.158)and phagocytosing(0.314) levels. Two hypothyroid subjects had depressed resting(0.092 & 0.101) and phagocytosing(0.196 & 0.262)values. Further studies are planned to determine if the alterations in NBT reduction correlate with bactericidal capacity. There is recent evidence that iodinated thyroid hormones may be utilized in the microbicidal system consisting of myeloperoxidase,H_2O_2 and halogen.

DISSEMINATED ECHOVIRUS TYPE 3 INFECTION IN COMBINED IMMUNODEFICIENCY. Suhung Hann, Dale S. Huff, Adamadia Deforest, Manjit K. Sharma and Harold W. Fischner (Intr. by Victor H. Auerbach), Temple Univ. Sch. of Med. and St. Christopher's Hosp. for Children, Philadelphia, Pa.

Patients with combined immunodeficiency (CID) exhibit undue susceptibility to viral infections, but Echovirus-3 infection has not been reported in these patients. A 4 month old Caucasian girl with history of recurrent upper repiratory infections, intractable diarrhea and persistent moniliasis presented with ascites, electrolyte imbalance, severe lymphopenia, generalized hypogammaglobulinemia and absence of isohemagglutinins. Delayed hypersensitivity skin responses and lymphocyte proliferative response to phytohemagglutinin could not be elicited. Postmortem examination confirmed the diagnosis of CID. The ascites was chylous but contained almost no lymphocytes. There were multifocal necrosis with round cell infiltration in the liver and diffuse intra-alveolar hemorrhage in the lungs. Echovirus-3 was recovered from a rectal swab taken 11 days premortem and from the liver and lung tissues obtained at autopsy (in January). Pneumocystis carinii was found in the lungs. Hepatic lesions of this kind have not been described in Echovirus-3 infection although fatty metamorphosis has been reported. The lesions seen in this patient are reminiscent of those of neonatal Herpes simplex infection and of adenovirus infection in one case of CID. It is assumed that defective cell-mediated immunity is primarily responsible for viral dissemination in this patient. (Supported by Grants #1F03-CA-5433, AI-09863 and RR-75 from the USPHS.)

FAMILIAL CARRIAGE OF ASCARIS. J.O. Hendley*, D. Williams*, G. Burke*. Dept. of Ped. Univ. of Va. Sch. of Med., Charlottesville, Va. (intro. by W. G. Thurman)

Although parasites are reported to be present in more than 10-20% of Southern children, the number usually detected with stool exams in a hospital laboratory is much lower. The incidence of helminth carriage in children up to 12 yrs residing in both Charlottesville and the surrounding county was determined. Lower income families in a C&Y project were tested at the yearly checkup. Fresh and concentrated samples of stool specimens from all children in the family were examined with iodine stain.

Ascaris lumbricoides was detected in 10% and Trichuris trichuria in 8% of 146 children. Pinworms were present in 20%, Entamoeba coli cysts in 18%, and Giardia lamblia cysts in 6% of 78 children examined.

Since the eggs of ascaris and trichuris are infective only after incubation in the soil for 2 weeks, it is accepted that these parasites are not family associated. Unexpectedly, familial aggregation of ascaris and trichuris infestation was very prominent in this population. Nine (82%) of 11 siblings of children with ascaris also had this parasite. Seven (64%) of 11 siblings of children positive for trichuris had trichuris. The 8 families with helminth infestations resembled the 64 negative families in urban vs rural residence and indoor vs outdoor plumbing. Recommendations for therapy of ascaris infection should be altered to include treatment of the siblings of a child with this parasite.

SUPPRESSION OF HOST RESPONSE TO STREPTOLYSIN O BY SKIN LIPIDS AND ITS CLINICAL IMPLICATIONS. Edward L. Kaplan and Lewis W. Wannamaker, University of Minnesota Medical School, Department of Pediatrics, Minneapolis.

Lipids, such as cholesterol, which are abundant in skin, are known inhibitors of two biologic properties of streptolysin O (SO), the hemolytic and cardiotoxic effects. This study was designed to investigate the possibility that skin lipids may also modify the antigenicity of SO and may thereby be responsible for the feeble ASO response following streptococcal pyoderma.

2 groups of rabbits were injected intravenously twice weekly with SO (33 units), DNase B (700-1000 units) and streptococcal NADase (1700 units). Group I (15) received the antigens in physiologic saline. Group II (13) received the same doses of antigen, but mixed with lipid extracts of rabbit dermis and epidermis (chloroform-methanol extract suspended in saline). Sera were obtained during the 3rd, 5th, 7th and 9th weeks. Mean ASO titers from Group I were significantly higher ($p<.01$) for every bleeding than were the corresponding mean ASO titers for Group II (e.g. 1.6 and 0.2 log respectively at 3 weeks). In contrast, anti-DNase B and anti-NADase titers for the 2 groups were almost identical for every bleeding.

These observations are of special interest since--by suppressing the antibody response or by inhibiting the toxicity of SO--skin lipids may alter the host response in streptococcal pyoderma, a finding which may explain the failure of rheumatic fever to develop after infection at this site.

A CONTROLLED EVALUATION OF TRIMETHOPRIM-SULFAMETHOXAZOLE, AMPICILLIN AND NO THERAPY IN SALMONELLA GASTROENTERITIS IN CHILDREN. M. Kazemi, M.I. Marks (Intr. by M.E.Avery). McGill Univ.-Montreal Children's Hosp. Research Inst., Montreal, P.Q.

A prospective, random controlled study was designed to evaluate the role of antibiotics in the therapy of salmonella gastroenteritis in children. Children (age 10 mos. to 16 yrs.) were randomly assigned to three groups: I. trimethoprim-sulfamethoxazole (TMP-SMZ) 20 mg/kg/day and 100 mg/kg/day respectively, II. ampicillin 100 mg/kg/day, III. no therapy. Antibiotics were given orally for 7 days. In vitro minimum inhibitory concentrations were performed on all isolates and pharmacokinetic studies of TMP-SMZ were also done. There were no significant differences in the clinical features, duration of illness (2.6-4 days), bacteriological cure rates (63-72% at 1 week) or carrier states (0 at 8 Wks.) among the three groups followed for 6 months. All isolates were sensitive in vitro to the antibiotics used and no resistance developed. The combination drug TMP-SMZ was well absorbed and serum and urine concentrations were adequate. Although highly active (and synergistic) in vitro the new combination drug TMP-SMZ is no more effective than ampicillin in vivo; neither antibiotic treatment offered any advantage over no antibiotics in the therapy of salmonella gastroenteritis in children.

AMPICILLIN VS. CHLORAMPHENICOL IN HEMOPHILUS INFLUENZA TYPE B (H. FLU B.) MENINGITIS. A REAPPRAISAL OF PAST EXPERIENCE WITH 74 PATIENTS. Najwa Khuri-Bulos (Intr. by C. Henry Kempe). Yale New Haven Med. Ctr., Dept. of Ped., New Haven, Conn.

A retrospective study of 74 patients with H. flu b meningitis admitted to Yale N.H. hosp., between 1964-70 was done. 32 patients received chloramphenicol, 32 received ampicillin and 10 received a combination of both. Average duration of hospitalization, fever and complication rates were similar in the ampicillin and chloramphenicol groups but increased in the combination group. 29/74 patients had 50 complications. 21 patients had seizures, 8 developed subdural effusions. 5 inappropriate ADH, 3 G.I. bleeding, 2 neuronal deafness and 8 had other neurologic sequelae. 2 patients who received ampicillin and 1 patient on combination therapy died. Patients with CSF glucose <15 mg% did not have a significant increase in morbidity or mortality. Of 7 patients who were afebrile or hypothermic on admission 3 died and 3 others developed major complications. Total duration of therapy was 10 days for both groups. The average duration of I.V. therapy was 7.9 days for the ampicillin group and 4.3 days for the chloramphenicol group. These data suggest that in spite of more intensive ampicillin therapy, the eventual outcome remains the same. For this reason a trial of prolonged I.V. therapy with chloramphenicol may be indicated.

NITROBLUE TETRAZOLIUM TEST IN CHILDREN WITH MALIGNANT DISEASE, Diane M. Komp, Renee Charette* and Joseph J. Braintwain* Univ. of Va. School of Medicine, Dept. of Pediatrics, Charlottesville Virginia 22901

The nitroblue tetrazolium (NBT) test is useful in otherwise normal children in distinguishing between bacterial infection and fever of other etiology. Defects of white cell function that would interfere with the interpretation of the test have been reported in children with leukemia undergoing craniospinal irradiation and patients receiving hydrocortisone.

Unstimulated and andotoxin-stimulated NBT tests were performed in 74 instances on children with metastatic solid tumors or acute lymphoblastic leukemia. No child had received radiotherapy for at least one month prior to study.

Children with tumors receiving no chemotherapy had normal studies. Those receiving vincristine, cyclophosphamide, TIC mustard or CCNU had very low unstimulated values and failed to respond to endotoxin stimulation. Untreated leukemics in relapse had abnormally low endotoxin-stimulated NBT's. Patients in relapse or remission had low values while receiving vincristine, prednisone, daunorubricin or 6-MP. Particularly low values were seen in patients with septicemia.

These data suggest: 1)The NBT test is not useful in supporting or refuting the diagnosis of bacterial infection in a child with malignancy receiving certain chemotherapeutic agents; and 2)Chemotherapeutic agents may induce a qualitative granulocytic defect that may enhance the propensity towards infection.

INAPPARENT CONGENITAL CYTOMEGALOVIRUS INFECTION: A FOLLOW-UP STUDY. Mary L. Kumar, George A. Nankervis, Eli Gold. Case Western Reserve University School of Medicine, Cleveland Metropolitan General Hospital, Department of Pediatrics, Cleveland, Ohio.

A follow-up study on a group of children with clinically inapparent congenital CMV, identified at birth as part of a prospective study, was undertaken in order to assess their physical and mental development in their first four years of life. Of a group of 25 children described by Starr in 1968, 14 were available for study, as well as 8 children from a control group originally selected on the basis of sex, race, and birth weight. Assessment of the children included a complete physical examination, psychological testing, and virologic studies. None of the children exhibited any remarkable physical abnormalities. The mean IQ of the congenitally infected children was 84.1 while the mean IQ of the control children was 86.5. Urine viral cultures were positive in eleven children. All 7 males tested were positive, as compared to 4 out of 7 females ($P<0.05$). CMV CF titers on the virus negative children did not differ significantly from the titers on the virus positive children. In summary, children with congenital CMV infections who appeared normal at birth had no evidence of physical abnormalities at age 4 and their performance on the Stanford-Binet was comparable to a control group. Viruria was present in 78% of the children, most of whom had circulating antibody.

ORAL ATTENUATED STREPTOMYCIN-DEPENDENT (SmD) SHIGELLA VACCINES: IN VIVO STABILITY AND TRANSMISSABILITY. Myron M. Levine and Eugene J. Gangarosa. (Intr. by Marvin Cornblath) Ctr. for Disease Control, Atlanta and Univ. of Maryland Sch. of Med. Division of Infectious Diseases, Baltimore.

Oral shigella vaccines are being investigated as a means of controlling shigellosis in custodial institutions. Previous studies demonstrated their safety and stability; person-to-person transfer of vaccine strains was not recorded. In Feb. and again in Aug. 1972, 4 doses of SmD S. sonnei and S. flexneri 2a vaccine were given to high-risk institutionalized children. In Feb. 106 children received SmD S. sonnei vaccine and 101, SmD S. flexneri 2a. In Aug., 109 received sonnei and 99, flexneri vaccine. SmD S. flexneri was recovered by rectal swab culture from 91%of the flexneri vaccinees in Feb. and 82% in Aug.; SmD S. sonnei was recovered from 87% of sonnei vaccinees both times. From 18 sonnei vaccinees (18%) given the Feb. lot of sonnei vaccine, streptomycin-independent S. sonnei revertants were isolated; the revertants remained attenuated (non-invasive) and the excretors were not ill. Children given a later lot of sonnei vaccine excreted no revertants. Person-to-person transfer of vaccine strains was observed: 13 of 207 vaccinees in Feb. and 40 of 208 in Aug. excreted the other vaccine strain, and vaccine strains were recovered from 18 of 68 non-vaccinated controls. Despite the safety of SmD shigella vaccines, the documentation of their transmissability and reversion has significance regarding the design of future field trials.

POLIOVIRUS IN THE CARIBBEAN. Myron M. Levine, Michael Hattwick, Joao Risi, Milford Hatch. (Intr. by Marvin Cornblath). Ctr. for Disease Control, Atlanta.

In the past 2 years epidemics of type 1 poliomyelitis have occurred in 3 Caribbean countries. The first outbreak was in the Dominican Republic (eastern Hispaniola), and affected children less than 5 years old. An enteroviral culture survey and a serosurvey were performed in contiguous Haiti (western Hispaniola) at the time of the Dominican epidemic to determine immune status and prevalence of poliovirus in Haitian children. Samples were taken in a mountain village and the clinic of Schweitzer Hospital from 147 children of the following ages: infants-16, 1 yr-29, 2 yrs-34, 3 yrs-27, 4 yrs-21, and 5 yrs or >-20. Only 27% of children had neutralizing antibody to poliovirus type 1 (pv1); 8% to pv2; and 3% to pv3. Ten of 35 cultures were positive: one infant had pv1; 8 children <3 yrs had Echo 4, 7, 19 or 33, and an older child had Echo 33. Results in the two populations of children were similar. These data are in contrast to other surveys of children of poorly-sanitated developing areas in which early acquisition of antibody to all 3 pv types was universal. The relative isolation of the surveyed population or change in ecology of poliovirus in the Caribbean can explain these data.

THE COMPUTER AS AN AID IN MONITORING IN VITRO ANTIBIOTIC SENSITIVITY TESTING. Gilbert W. Mellin and Marion E. Hosmer. Columbia University College of Physicians and Surgeons, Columbia-Presbyterian Medical Center, Department of Pediatrics, New York, N. Y.

Standard methods and interpretation of antibiotic sensitivity testing enables more meaningful reports. Monitoring the laboratory reports allows one to compare laboratories and technicians as well as alerting one to significant change. Beginning in 1966 IBM cards were keypunched for input into a magnetic tape data bank for all reports issued by the pediatric bacteriology laboratory. In 1969 the Kirby-Bauer* method of single high concentration antibiotic discs on Mueller-Hinton agar and the measurement of the zone of growth inhibition became standard procedure for antibiotic sensitivity testing in the same laboratory. For the years 1969 through 1972, single disc sensitivity tests were done on more than 8000 clinical isolate strains of bacteria. Analysis of these bacterial strains and the antibiotics tested confirmed the Kirby-Bauer standards for zone of inhibition. Fluctuation in sensitivity patterns monitored against previous experience has revealed no abrupt change.

* Bauer, A.W., Kirby, W.M.M., Sherris, J.C., and Turck, M: Am. J. Clin. Path. 45:496 (April) 1966

RACIAL AND ENVIRONMENTAL FACTORS IN HEMOPHILUS MENINGITIS -- A TALE FROM THREE CITIES. R.H. Michaels, W.F. Schultz, and F.E. Stonebraker (Intr. by T.K. Oliver, Jr.). The Children's Hospitals of Pittsburgh, Birmingham and Washington, D.C.

A medical record study of over 600 recent cases of hemophilus meningitis and a like number of hospitalized controls (matched for age, date and place) showed that a significantly larger number of meningitis cases from each of three cities had siblings, as compared to controls. An excess of blacks was noted among meningitis cases from Birmingham and Washington, but not from Pittsburgh; the disproportion was eliminated in Washington by excluding out-of-District patients.

The first 78 parental interviews from a case-control study in Pittsburgh show that hemophilus meningitis cases under 15 months often come from crowded homes (>1 person/room) and also nearly always have siblings -- differing significantly from controls, and from other infants with meningococcal meningitis. Upper respiratory infection in the two weeks prior to admission was common among infants with hemophilus meningitis (found in 2/3 cases) but equally common among controls.

Bacteriologic study of 9 Pittsburgh families with a child with hemophilus meningitis show one or more of the siblings to be intensely colonized with type b, H. influenzae (>1,000 organisms/throat swab). These and the above epidemiologic data are consistent with the thesis that the development of hemophilus meningitis is related to dose as well as to age, but not to race per se -- nor to prior viral respiratory infection, contrary to what has often been suggested.

STUDIES ON THE PATHOGENESIS OF H. INFLUENZAE b MENINGITIS. Edward R. Moxon, Damon R. Averill, Arnold L. Smith, David H. Smith (Intr. by William B. Berenberg) Children's Hospital Medical Center, Boston, Mass.

The challenge of reducing the morbidity of bacterial meningitis would be aided by an animal model facilitating the study of the pathophysiology. Intranasal inoculation of 5 day old rats with 10^7 H. influenzae b produces histologically documented meningitis. Bacteremia was detectable 3 hours after inoculation and always preceeded the appearance of meningitis. Two days after inoculation meningitis was present in 70% of animals with bacteremia. Animals without bacteremia did not have meningitis. There were no significant lesions in any other organ system. Animals inoculated with an untypable strain of H. influenzae had neither detectable bacteremia nor meningitis. There was a marked age dependent susceptibility to H. influenzae b infection which was independent of serum bactericidal antibody titers. Decreasing the size of the inoculum to 5 day old animals resulted in a lower incidence of bacteremia, meningitis and death. Inoculation of 10^7 H. influenzae b into older infant rats resulted in a progressive decrease in the incidence of bacteremia and meningitis. Studies with flourescent-labelled type b antiserum indicated that the organisms entered the blood stream through the nasal mucosa. The first observed intracranial localization of bacteria was in the dorsal longitudinal and lateral sinuses, the area with the most intense acute inflammatory exudate. Some rats developed otitis media without evidence that organisms entered from that site.

IMMUNOLOGIC RESPONSE TO H. INFLUENZAE EPIGLOTTITIS AND MENINITIS. Carl W. Norden, Richard H. Michaels. University of Pittsburgh School of Medicine, Departments of Medicine and Pediatrics, Pittsburgh, Pennsylvania.

This study reports the antibody responses in children with epiglottitis due to H. influenzae, type b, and compares them with children with meningitis due to the same organism. Thirteen children with epiglottitis (ranging in age from fifteen months to 49 years-median 31 months) were studied. Of ten patients over two years of age all had increases in antibody activity as measured by hemagglutination (HA) or bactericidal (BA) assay. Of three patients under two years of age, two had significant increases in antibodies by HA or BA, while a third child age seventeen months showed no rise in antibody titer. A significant correlation (r=.64) was seen comparing age with the increase in antibody response.

Thirteen of 63 patients with meningitis had increases in either HA or BA titers. However, only three of 49 children under two years of age with meningitis had increases in antibody titers as contrasted with ten of fourteen patients with meningitis over two years of age. Examining only the children over two years of age, one finds that children with meningitis had greater increases in antibody titers than children with epiglottitis. In both diseases there was correlation between increasing age and the magnitude of the antibody response.

REDUCTION OF MORTALITY IN ACUTE BACTERIAL MENINGITIS. John C. O'Bell, Samuel J. Horwitz, and Bernard Boxerbaum. (Intr. by Leroy W. Matthews). CWRU Sch. of Med., Univ. Hosp. of Cleveland, Department of Pediatrics, Cleveland, Ohio.

Mortality of acute bacterial meningitis, excluding neonates, is generally 5-10% with current antibiotic therapy. Cerebral edema with herniation is a major factor in the mortality associated with this disease. During the 5-year period (1967-71), 140 patients with H. influenzae, pneumococcal, or meningococcal meningitis were treated. Those demonstrating signs of impending herniation were acutely treated with intravenous mannitol followed by intravenous steroids. Two deaths were directly attributable to meningitis (1.4%). Another died of neuroblastoma complicated terminally by pneumococcal meningitis, and one died of meningococcemia with minimal meningeal infection. Of 11 patients treated with mannitol for impending herniation, 10 showed improvement of abnormal neurologic signs and recovered without major sequellae. Six improved within minutes and 4 more slowly. Both of the meningitis deaths demonstrated clinical signs of herniation with fixed pupils, coma, and respiratory failure. One received mannitol after herniation was diagnosed without benefit. The other received steroids alone. The low mortality of 1.4% cannot be attributed solely to efficacious antimicrobial therapy. Rather it is concluded that early recognition and vigorous therapy of cerebral edema and impending herniation utilizing mannitol substantially reduces mortality in this disease.

VACCINE INDUCED RUBELLA IMMUNITY: RESPONSE TO RE-INFECTION AND DEVELOPMENT OF NASOPHARYNGEAL ANTIBODY. P.L. Ogra*, R.B. Wallace*, D. Kerr-Grant*, and G. Umana*. Sch. of Med., State Univ. of N.Y. at Buffalo (Intr. by D.T. Karzon).

The effects of re-infection with live attenuated rubella vaccines was studied in groups of children 3-6 months after natural infection or primary immunization with HPV-77, Cendehill or RA27/3 rubella vaccines. The antibody response was determined by hemagglutination-inhibition and radioimmunodiffusion. Primary infection or immunization resulted in a significant serum antibody response in all subjects. However, nasopharyngeal antibody was regularly detected only after natural infection or intranasal (I/N) immunization with RA27/3, and frequently after subcutaneous (S/C) immunization with RA-27/3 vaccine. Nasopharyngeal re-infection with RA27/3 resulted in booster serum antibody response, and transient nasopharyngeal virus excretion in 85% of HPV-77, 70% of Cendehill, and 40% of RA27/3 vaccinees immunized S/C, 15% of RA27/3 vaccinees immunized I/N, and 5% of naturally infected subjects. Significantly, nasopharyngeal re-infection with RA27/3 resulted in the appearance of rubella antibody in the nasopharynx of most subjects who had failed to manifest such a response after initial S/C immunization with HPV-77, Cendehill or RA27/3. No response was observed after S/C re-infection with RA27/3 and S/C or I/N re-infection with HPV-77 and Cendehill vaccines. These data suggest that immunization with RA27/3 vaccine may provide more effective immunity against rubella, and its role should be considered in current immunization programs.

POLIO ANTIBODIES DURING PROLONGED LACTATION IN AN UNACCULTURATED SOCIETY. William J. Oliver, Joseph V. Baublis, and James V. Neel, University of Michigan, Departments of Pediatrics and Human Genetics, Ann Arbor.

Breast milk and serum samples were obtained from women of the Yanamama Indians, an unacculturated tribe of N. Brazil and S. Venezuela. Based on estimated ages of the nursing children, duration of lactation extended from 1 month to 2½ years. We sought to determine whether the prevalence of polio neutralization by breast milk related to sero-immunity in this population sample. Our findings document persistent secretion of immunoglobulins during prolonged lactation. Concentrations by radial immuno-diffusion were: IgG 4.8 mgm% S.D. 2.9; IgA 11.7 mgm% S.D. 5.9; IgM 2.2 mgm% S.D. 3.2. Breast milk samples neutralized 100 tcd 50 of polio virus at dilutions of 1/8 or higher in 5/30 for Type I, 7/30 for Type II and 4/30 for Type III. Only two neutralized all three types of virus, but 5/30 neutralized Types I and II. Although polio neutralization by the breast milk samples was roughly proportionate to the level of maternal sero-immunity, it was demonstrable in only 4/25 mothers immune to Type I, 7/22 immune to Type II, and 3/15 to Type III. Thus, neutralizing activity to polio of post-colostral milk from these primitive women was not universal. The findings emphasize the need for devising specific approaches for protection of infants of primitive people in transition to civilized patterns of life. These observations are also relevant to parents of the new generation, aspiring to a healthy "natural" life.

MORTALITY FROM MENINGITIS, U.S.A. 1950-1967 J.M. Ryan, Indiana University Hospitals, Indianapolis, Dept. Peds. (Introduced by M. Green)

This study is a preliminary analysis of mortality from tuberculous meningits(tbc), meningococcal infections(mn), and non-meningococcal non-tuberulous meningitis(nmnntb) as reported on death certificates by the U.S. Center for Vital Statistics, 1950-1967. Death rates were calculated using estimates of population for each year of age for the first year of life for mn and nmnntb, but the highest death rate from tbc occurs during the second year of life. Males have a higher death rate than females andnon-whites greater than whites for all types of meningitis and at all ages. The secular trends for deaths from tbc has been one of steady decline for all males, females, white and non-white, but most notably in children less than 5 years. The secular trend for deaths from mn has been a slight increase followed by a decrease in all categories. In contrast, the secular trend for deaths from nmnntb had a tendency to increase in males, females, whites, non-whites less than one year of age and to a lesser extent in those 1-4 years of age. There was a decline in death rates for nmnntb in all persons 5 years of age and older.

Death rates for tbc and mn have shown a definite decrease from 1950-1967, while deaths from nmnntb have shown an increase especially in children less than one year of age.

DEVELOPMENT OF EXPERIMENTAL MODELS FOR EVALUATION OF ANTIVIRAL CHEMOTHERAPEUTIC AGENTS, J.C. OVERALL, Jr., D.A. Stringfellow, E. Kern, L.A. Glasgow, Depts. of Ped. & Micro., Univ. of Utah Col. Med., Salt Lake City.

A series of animal models have been developed to (1) simulate potentially treatable virus infections of humans, (2) analyze the efficacy of therapy in terms of the sensitivity of the virus, the pathogenesis of the infection, and the distribution of the drug. A systemic picornavirus (EMC) and a disseminated herpesvirus (genital HVH) infection have been studied in mice treated with one or more agents (Poly I:C, IUDR, Ara A, Ara C). The sensitivity of the virus to the drug was defined and, where appropriate, compared with clinical isolates. In EMC virus infections, induction of interferon following early therapy with Poly I:C was partially effective. Successful therapy was characterized by suppression of viremia and lack of seeding of target organs. Failure to maintain antiviral activity resulted in development of a viremia, seeding of target organs and death. In contrast, in generalized HVH infection in suckling mice exposed by the intranasal route IUDR was not effective. Lack of efficacy was characterized by (1) sensitivity of virus to the drug, (2) suppression of HVH in lung, liver, spleen, and prevention of viremia, (3) failure to inhibit nerve transmission or virus replication in the CNS, (4) correlation of the presence of antiviral activity with suppression of virus multiplication. These results illustrate the importance of defining the effect of therapy on pathogenesis in the evaluation of new antiviral agents.

CANDIDA ENDOCARDITIS AND ACUTE CANDIDIASIS IN GASTROINTESTINAL DISEASE. Mildred S. Seelig, Philip J. Kozinn, Robert S. Holzman. (Intr. by Howard A. Joos) Downstate Med. Ctr., Maimonides Med. Ctr., Brooklyn.

Analysis of 13 cases of candida endocarditis, following gastrointestinal surgery suggest clues to earlier diagnosis. Such patients, who commonly are treated with multiple antibiotics and indwelling intravenous catheters, often with hyperalimentation, are at a particular risk of developing acute or subacute forms of systemic candida infection. Overwhelming invasion can produce acute disease that mimicks gram-negative sepsis. This is frequently associated with disseminated microabscesses. The disease may progress insidiously, even when iatrogenic factors are removed and antifungal therapy is instituted. Our findings suggest that serodiagnostic tests for candida antibodies are a more frequent and reliable index of infection than positive blood cultures for candida. These serodiagnostic tests in conjunction with characteristic physical findings enable the clinician to evaluate the presence of infection.

DO VIRUSES CAUSE JUVENILE RHEUMATOID ARTHRITIS (JRA)? Paul E. Phillips, Wan N. Lim, Paul D. Parkman and Yashar Hirshaut. Cornell Univ. Med. Col., Depts. Med. and Ped., New York and Food & Drug Admin., Bur. Biol., Rockville, Md.

Increased virus antibody in JRA might causally implicate a specific virus. In 50 JRA compared to age, sex and race-matched controls with other diseases, rubella and measles antibodies were not significantly higher, but parainfluenza type 1 (Para-1) and Epstein-Barr virus (EB) antibodies were ($\log_2$ mean titers 6.2 vs 5.7, 7.0 vs 5.3 respectively), as was IgG (15.9 mg/ml vs 10.4). Significance was not affected by excluding virus antibody negatives, but the EB elevation was partly due to fewer such JRA (12 vs 23). Prior rubella history did not affect rubella antibody. Nor did sex or race, type, activity or duration of disease, ESR, ANA or rheumatoid factor significantly influence virus antibody or IgG levels, except for higher IgG in more active JRA ($p<.05$). Significant direct correlations ($p<.05$ or less) were found between age and rubella antibody, measles antibody and IgG levels, and also between IgG and rubella, measles and EB antibodies. These 3 virus antibodies also intercorrelated significantly. Correlations were stronger for JRA than controls, and those with IgG were improved by excluding antibody negatives. The unexplained increase of Para-1 antibody seemed too small to implicate this virus etiologically. As in adults, moderately increased virus antibodies may be secondary to increased IgG in diseases like JRA. Although a virus was not implicated in JRA, such data do not exclude the hypothesis.

IMMUNIZATION OF CHILDREN WITH PRP. David H. Smith, Georges Peter, David L. Ingram and Porter Anderson. Children's Hospital Medical Center, Boston, Mass.

141 children of 5-59 months of age have been immunized with a single intramuscular dose of 0.67, 3.3, 17 or 67 µg polyribophosphate (PRP), the capsular antigen of Hemophilus influenzae b. The immunizations were well tolerated. Antibody activity was measured by radioactive antigen binding, using ^{3}H-labelled PRP. Doses of 3.3 and 17 µg produced significant antibody rises in nearly 90% of recipients; 0.67 and 67 µg in approximately half. The geometric mean titers (GMT) were similar at 3 and 6 weeks after immunization and were greater with the middle doses. Preimmunization GMT ranged from 14 (1st yr) to 400 (5th yr) nanograms of antibody/ml. Net antibody increase in responders did not correlate with these titers but was strongly age dependent: 23 (1st yr); 280 (2nd); 1100 (3rd); 2400 (4th); 4900 (5th). To put these data into perspective, anti-PRP antibody concentrations were measured in individuals considered "immune" to H. influenzae b. Anti-PRP concentrations in 111 mothers was 218 ng/ml; that of their newborn infants 63 ng/ml; agammaglobulinemic boys protected by gamma globulin had a mean of 290 ng/ml. Furthermore, 40 ng of human anti-PRP protected infant rats from experimental bacteremia and meningitis. These observations indicate the need for continued evaluation of PRP as a vaccine, of the age-dependence of antibody response to polysaccharides, and of the quantity of anti-PRP antibody required for host resistance.

CELLULAR DETERMINANTS OF ORGAN SUSCEPTIBILITY TO MUMPS VIRUS. Joseph W.St.Geme, Jr.,Hawley L.Martin,Catherine W.C.Davis, Robert C.Neerhout,James B.Peter, and James F.Mead. UCLA Sch. Med.,Harbor Gen.Hosp.,Ctr.Health Sciences,Depts.Ped.,Med.,and Biochem. Torrance and Los Angeles.

The chick embryo provides a unique system for the study of critical factors which determine the relative susceptibility of cells and tissues to a paramyxovirus. Heart cells infected in vitro are susceptible(S)and produce the greatest amount of mumps virus(MV). Liver cells are the least susceptible(LS) and produce 10-fold less MV. Following early embryonic infection in ovo, maximum titers of MV are found in the heart, with 100-fold less MV in the liver. Neither rates of MV attachment and penetration nor interferon production explain the discrepant cellular susceptibility. Non-interferon proteins may attenuate late MV replication in LS cells. The ratio of virogenic to hemadsorbing S cells is 1.0, while the ratio with LS cells is <1.0 suggesting inefficient MV release.

Purified plasma membranes(PM)of S and LS cells were analyzed to determine the role of lipids in the release of MV.Klenk and Choppin(Virol.40:939,'70)demonstrated that the S cell(monkey, bovine)for parainfluenza SV5 virus possesses a PM lipid pattern characterized by a higher molar ratio of cholesterol to phospholipid, and higher phosphatidylethanolamine and lower phosphatidylcholine content than the LS cell(hamster). Identical differences in PM lipids were observed for the S and LS cell of MV. Our data support the notion that PM lipid composition is the crucial determinant of paramyxovirus yield from host cells.

Prognostic Indicators for Severe Cases of Pneumococcal Meningitis. Edward Tabor* and Katherine Sprunt, Dept. of Pediatrics, Columbia College of Physicians and Surgeons, NYC.

The mortality rate in pneumococcal meningitis still exceeds that of any other common form of meningitis. The records of 56 children during 20 years were examined for evidence present on admission which would indicate poor prognosis and need for special therapy. These factors (and their mortality rates) were: < 9 mos. of age (43%); many pneumococci and <3 wbc/OIF on direct smear of CSF or <1000 wbc/mm^3 (89% mortality or neurologic sequelae rate), CSF sugar <10 (58%), peripheral absolute pmn <5000 (64% mortality vs. 14% among those with >9000), patient not seen by M.D. prior to admission (67% vs. 9% of those seen for early symptoms) even if those receiving antibiotics are eliminated. Five of 29 patients measured were microcephalic on admission; four died.

Factors not statistically related to mortality were race, height and weight, existence of siblings, parents' ages, social class, month of onset, presenting symptoms, hemoglobin, blood lymphocyte or monocyte counts, ESR, positive blood cultures, type of pneumococcus, CSF protein, CSF cell count (above 1000) or differential, or urine output within twenty-four hours of admission.

All patients 3 years old or older had a basic problem (sickle cell disease, immunologic defect, etc.) or contiguous site of infection (cellulitis, mastoiditis). In this age group, one of the thirteen patients died.

CONGENITAL CYTOMEGALOVIRUS INFECTION (C-CMV): CONSECUTIVE OCCURRENCE WITH SIMILAR ANTIGENIC VIRUSES. Sergio Stagno, David W. Reynolds, Alfred W. Lakeman, Leigh J. Charamella, Charles A. Alford, Univ. of Alabama in Birmingham, Dept. of Ped., Birmingham, Alabama.

C-CMV with marked clinical, serologic, and immunologic differences was detected in 2 siblings born consecutively 3 years apart. The first child was born with severe disease resulting in devastating brain damage. His Ig (M,A,G) development and specific (C-F) antibody response were strikingly increased from birth onward. In contrast, all these parameters, clinical included, were normal in the 2nd infected sibling indicating a much reduced antigenic load. To determine if antigenic heterogenicity between viral strains caused the differences in virulence and load, serial sera from mother and babies were simultaneously examined for C-F and neutralizing antibodies using antigens prepared from the viruses obtained from both babies as well as 2 prototypes. The results were identical suggesting viral homology. Though previously negative, the mother became viruric at 33 weeks in the second pregnancy in spite of circulating antibody which persisted throughout gestation. Chronic infection of the fetus was established even with normal levels of maternal antibody in cord serum. Clearly, reinfection with homologous virus or more likely reactivation of CMV with transmission to the fetus can occur in immune pregnant women and result in repeat bouts of C-CMV. Therefore, successive pregnancies in mothers known to have had previous C-CMV babies should be considered at risk.

IMPAIRED IN-VITRO CELL-MEDIATED IMMUNITY TO RUBELLA VIRUS DURING PREGNANCY. Y.H. Thong, Russell W. Steele, Monroe M. Vincent and Joseph A. Bellanti. Georgetown Univ. Sch. of Med., Dept. of Ped., Washington, D.C.; Microbiological Associates, Bethesda, Md.

Cell-mediated immunity (CMI) represents an important mechanism of defense against viral infections. Several reports have noted a transient depression of CMI during pregnancy as demonstrated by decreased phytohemagglutinin (PHA) and mixed-lymphocyte culture (MLC) responsiveness. The present studies were performed to measure both specific CMI to rubella virus using a ^{51}Cr release microassay, recently developed in our laboratory, and to compare these with PHA and MLC responses. Thirteen pregnant rubella seropositive women in various stages of gestation showed significant reduction of ^{51}Cr release as compared to controls ($P < 0.001$). Lymphocytes from 11 of these subjects studied for PHA and MLC responsiveness also showed a significant reduction in reactivity ($P < 0.05$). The ^{51}Cr release values were found to return to normal as early as 3 days postpartum although no exposure to rubella was noted and no significant changes in HAI titers were observed. The findings of a depressed viral CMI may be an important biologic marker of susceptibility to viruses which are known to be particularly severe in pregnancy. It is ironic that such a biological adaptation of survival advantage to the fetus may carry with it a deleterious outcome in terms of susceptibility to certain viral infections.

CONCURRENT EPIDEMICS OF COXSACKIE B5 ASEPTIC MENINGITIS ON AN INTENSIVE CARE NURSERY AND THE COMMUNITY AT LARGE. Phillip T. Swender, Roger J. Shott and Margaret L. Williams (introduced by Frank A. Oski). State University of New York, Upstate Medical Center, Syracuse, New York.

Coincident with a community outbreak of aseptic meningitis, a similar epidemic was observed in an intensive care nursery that provided an opportunity to compare and control the manifestations of Coxsackie B5 disease in these two population groups. The infants ranged in age from 2-8 days at the time of initial symptomatology (weights 1075 to 2640 grams). Initial symptoms included irritability, lethargy, apnea and jaundice. In 12 older infants and children, ages 3 weeks to 12 years, initial symptoms included fever, headache and vomiting. Spinal fluid findings showed a higher mean concentration of both protein and cells in the newborns (244 mg% vs. 67 mg% and 1070 cells/mm^3's 779 cells/mm^3). In both groups initial cell counts revealed a high percentage of polymorphonuclear leukocytes averaging 67% in the neonates and 41% in the older group. One of the seven infants died from associated severe respiratory distress. In the remaining infants and children, no recognizable neurologic sequelae have been observed. No illness was observed in the mothers of the infants. Routine isolating procedures were unsatisfactory in stopping nursery spread of the virus epidemic. These observations underscore the need for total community epidemiologic surveillance in dealing with diseases manifesting themselves in the newborn period.

DIAGNOSTIC VALUE OF THE WHITE BLOOD CELL AND DIFFERENTIAL COUNTS IN CHILDHOOD INFECTIOUS DISEASE. James K. Todd (Intr. by C. Henry Kempe). Univ. of Colo. Sch. of Med., Dept. of Ped., and Denver Children's Hospital, Denver, Colo.

The white blood cell count and differential are laboratory tests which are performed frequently when infectious illness is suspected. Although normal values are established, there are no accepted criteria for the appropriate interpretation of abnormal values in children. The white blood cell parameters of 244 children with documented acute bacterial infections (8 types) were compared with those of 397 children with documented nonbacterial infections (7 types). Statistical analysis demonstrated the absolute number of PMNs and the absolute number of nonsegmented PMNs to be significantly ($p < 0.01$) better at predicting the presence of bacterial infections than the more conventional absolute number of WBCs, % PMNs, or % nonsegmented PMNs. Patients whose white blood cell parameters exceeded 10,000 PMNs/mm^3 or 500 nonsegmented PMNs/mm^3 had an 80% chance of having a severe bacterial illness. Exceptions to this occurred in patients with superficial bacterial infections, osteomyelitis, or diseases caused by coagulase positive Staphylococcus or beta hemolytic Streptococcus. No significant age variation was noted. These results were subsequently confirmed in a study of 298 consecutive admissions to a pediatric infectious disease ward and intensive care unit.

HEPATITIS B ANTIGEN IN BLOOD AND SALIVA. R. Ward, P. Borchert, A. Wright and E. Kline. Childrens Hospital, L.A., U.S.C. School of Medicine and Pacific State Hospital, Pomona, Calif.

Serum samples from 2011 patients at an institution for mentally handicapped persons were tested for Hepatitis B antigen (HB Ag) and antibody (HB Ab); HB Ag was measured by complement fixation (CF) and hemagglutination inhibition (HAI). HB Ab was measured by passive hemagglutination (HA). The incidence of HB Ag and HB Ab in the patient population was 12.5% and 27.8%, respectively; the sum of the 2 figures indicates that 40% of the patients had experience with HB Ag. The incidence of HB Ag and HB Ab seemed to be higher in patients with Down's syndrome than in the others.

Parenteral modes of spread of HB Ag include injection of blood and blood products, unsterilized needles, syringes, etc. Although evidence of spread by nonparenteral routes is increasing, the precise routes are ill-defined. The copious drooling of patients led to testing saliva and mouth washings (SMW) for HB Ag; SMW of 22 patients (51%) yielded positive tests for HB Ag. Titers of HB Ag in SMW ranging from 1:32 to 1:256 and persistence of HB Ag for 5 to 6 months obtained in half the positive patients. The presence of HB Ag in saliva suggests a possible mode of spread of Hepatitis B.

IN VITRO VIRAL STUDIES OF CELLS GROWN FROM HUMAN AMNIOTIC FLUIDS. Catherine M. Wilfert, Duke Univ. Med. Ctr., Ped. Dept., Durham, N. C. (introduced by Samuel L. Katz).

Eight amniotic fluids were obtained for study in the 2 year period, 1970-72. Six patients underwent therapeutic abortion for reasons other than infection. One pregnancy was interrupted because of maternal rubella infection. One pregnancy in a woman with ECHO 18 viral meningitis was terminated by C-section at 40 weeks gestation.

Amniotic fluid cells were grown and multiple passages of cells from 3 of these patients were accomplished. Confluent monolayers of cells were inoculated in duplicate with each of 15 viruses. One cell line from the mother with rubella infection was compared to 2 cell lines from therapeutic abortions. The cells were observed for cytopathic effects, hemadsorption, and susceptibility to challenge with Coxsackie A21. Amniotic fluid cells supported the replication of HSV, Coxsackie A21, ECHO 4, adenovirus 7, measles, CMV, vaccinia, VSV, and RSV, whereas no replication of rubella, influenza, parainfluenza II, or mumps viruses could be detected.

Immunodiffusion assays of the immunoglobulin content (IgG, IgM, IgA) of the amniotic fluids were performed. Specific antiviral activity against 22 viral antigens was measured by CF, HI, and/or neutralization. There were no correlations of the levels of immunoglobulins or specific anti-viral activity of the amniotic fluids with the ability of the amniotic fluid cells to support viral replication.

NEONATAL OSTEOMYELITIS: RECENT EXPERIENCE. Eleanor Weissberg, Arnold L. Smith and David H. Smith (Intr. by William B. Berenberg) Children's Hospital Medical Center, Boston, Mass.

Twenty infants with osteomyelitis in the first months of life seen between 1965 to 1972 were reviewed. In contrast to the epidemiological pattern seen with neonatal sepsis, low birth weight, shortened gestational age and distant infectious foci were not prominent. However 75% (15 of 20) of the mothers had complications of pregnancy and/or delivery. The majority of the infants were females and 85% (17 of 20) were subjected to some potentially infective procedure in the immediate neonatal period. Seventy percent (14 of 20) had exposure to infectious illness in family members or other close contacts. Twelve of 14 infants had a "benign" presentation with only local manifestations of disease. Radiographic evidence of osteomyelitis was present on admission in 19 of 20 infants with deep soft tissue swelling being the most frequent early finding. Half of the patients had multiple bones involved, with the femura and humerus occurring most frequently. Staphylococcus aureus was the most common isolate (7 pts) with Treponema pallidum the next most common organism identified (3 pts). Enteric gram negative bacilli were identified in 4 patients. A poor eventual outcome, deformity or loss of function, corelated with joint (and epiphyseal) involvement rather than etiologic agent.

AN EPIDEMIC OF VIRAL MENINGITIS ASSOCIATED WITH ECHOVIRUS 18. C. M. Wilfert, M. Cohen, M.L. Costenbader, G. Meyers, and B. Lauer. Duke Univ. Med. Ctr., Ped. Dept.,Durham, N. C. and Ctr. for Disease Control, Public Health Service, Atlanta, Ga. (introduced by S. L. Katz).

An outbreak of viral meningitis associated with the isolation of ECHO 18 was studied epidemiologically, clinically, and by viral isolation in cell culture. The outbreak began in June, 1972, peaked in July and August and ended by October, 1972. It was primarily limited to Durham County, N. C. . The recognized affected population was predominantly black with an equal distribution of male and female patients. The patients' ages ranged from 6 weeks to 63 years with the largest number between the ages of 10 and 30 years. The high incidence of disease in young adults suggests that this agent is new to the community.

Symptoms included headache, nausea, and/or vomiting, nuchal ridigity and fever. There were no recognized deaths and a minority of patients required hospitalization. Cell counts of the CSF's ranged from 0-1400 cells/mm3 with the majority having less than 500 cells/mm3.

Viral isolation was attempted on 132 CSF's, 29 throat and 16 rectal swabs from 150 patients. Cytopathic effects consistent with an enterovirus were produced by 66 CSF's, 8 throat and 9 rectal swabs when inoculated into cell culture. To date, 25 of the 66 CSF isolates have been identified as ECHOVIRUS 18 by tissue culture neutralization studies employing standard bovine antiserum prepared against the reference Metcalf strain.

This is the first reported outbreak in the U. S. of viral meningitis associated with ECHO 18.

NEONATAL CYTOMEGALOVIRUS HEPATITIS AND TYROSINEMIA. M. Weitzman and A. J. Schneider. Department of Pediatrics, State University of New York, Upstate Medical Center, Syracuse, New York. (Introduced by F. Oski.)

An infant, born after 38 weeks gestation, to parents of French-Canadian ancestry developed hyperbilirubinemia and thrombocytopenia during the first week of life. Urine cultures grew cytomegalovirus (CMV). The patient failed to thrive and hepatic failure ensued. At age 6 weeks blood phenylalanine, tyrosine, methionine, and alanine levels were found to be elevated while other amino acids were within normal limits. At this time it was learned that blood phenylalanine and tyrosine levels had been elevated during the first week of life. Institution of a low tyrosine, low phenylalanine diet produced a dramatic decrease in blood levels of these two amino acids but the course of the hepatitis was unremitting and the child died of a massive gastrointestinal hemorrhage. Pathologic specimens grew CMV from all tissues. Islet cell hyperplasia, previously observed in patients with tyrosinemia, was present. This case is of special interest because of the unique association of CMV hepatitis with congenital tyrosinemia. It serves to illustrate that the finding of progressive liver disease with CMV should alert the physician to the presence of other possible disorders and also underscores that the presence of an elevated phenylalanine level during the first week of life may signify the presence of diseases other than PKU.

IMPAIRED ANTIBACTERIAL ACTIVITY OF LEUKOCYTES OF SICK NEWBORNS William C. Wright, Jr., Bonnie Ank, Jennifer Herbert, and E. Richard Stiehm. UCLA Sch.Med., Dept.Ped., Los Angeles, Calif.

Impairment of polymorphonuclear (PMN) cell function under stress may contribute to the high incidence and mortality of neonatal sepsis. The phagocytic and bactericidal capacity of PMN's from 32 newborns with a variety of illnesses were compared with that of 9 normal newborns and 15 adults, using pooled adult serum as an opsonin source, an organism/cell ratio of 0.6/1.0, and S. Aureus 502A and E. Coli as test organisms.

PMN's from 16 of 32 sick newborns killed a significantly reduced percent of organisms (> 2 S.D. below the mean of the well newborn or adult groups). 13 showed reduced killing of S. Aureus, 8 showed reduced killing of E. Coli, and 5 showed reduced killing of both organisms. The sick newborn group killed 83.6% ± 1.96 (S.E.M.), significantly less than the well newborns (94.6% ± 1.57) or adult controls (93.1% ± 1.69). Defective function included both phagocytic and bactericidal activity. Repeat studies in 2 sick newborns remained abnormal and 2 became normal. Although there was a suggestive relationship of defective PMN function to severity of illness, there was no definite relationship to diagnosis, type of therapy, birth weight, gestational age, age of testing, or sex.

Impairment of antibacterial function of PMN's from infants stressed by illness, particularly when accentuated by the defective opsonic function of prematurity, may provide a mechanism which subjects these infants to further bacterial invasion.

ISOLATION OF BACTEROIDACEAE FROM NEONATAL BLOOD CULTURES. Terry Yamauchi, Anthony W. Chow, Rosemary D. Leake, Bascom F. Anthony, and Robert M. Rosenblatt, UCLA Sch. of Med., Harbor Gen. Hosp., Depts. of Ped. and Med., Torrance, Calif.

Over a 39 month period 8.5% of all positive blood cultures at Harbor General Hospital were due to Bacteroidaceae. The patients from whom these cultures were obtained included 15 newborns, an approximate incidence of 1.1 per thousand live births. Ten of the 15 mothers of these infants had rupture of membranes greater than 24 hours prior to delivery, with a mean of 73 hours. Eight infants were premature. All infants were cultured because of maternal amnionitis and/or suspected sepsis. Only one died and he had multiple congenital anomalies (trisomy 13). Eleven neonates were treated with antibiotics for 5-10 days, the 4 others were untreated. Of the 4 isolates speciated, 3 were B. fragilis and subspecies and one was B. capillosus.

In several cases Bacteroidaceae was recovered from other culture sites such as the ear or gastric aspirate as well as the blood. In 3 newborns anaerobic streptococcus and Bacteroidaceae were recovered simultaneously from the blood. Matched blood specimens under aerobic conditions failed to reveal any organisms.

Although the significance of these positive cultures is difficult to determine, it is conceivable that these organisms may have played a role in neonatal infection. Anaerobic organisms should be sought in suspected cases of sepsis when the routine aerobic blood cultures are negative.

TRANSMISSION OF CYTOMEGALOVIRUS INFECTION TO NEWBORNS BY MEANS OF BLOOD TRANSFUSION. Anne S. Yeager (Intr. by Reuben S. Dubois). Univ. of Colo. Sch. of Med., Dept. of Ped., Denver, Colo.

Urines were obtained from all infants admitted to the Sick Newborn-Premature Nursery for isolation of cytomegalovirus (CMV). At 6-7 months of age, the infants were evaluated for serological evidence of infection with CMV. All infants with positive complement fixation titers to CMV were also documented to be excreting virus. Of 38 infants who had not received a transfusion, 4 showed evidence of having had a CMV infection. Of these infants, 2 were small for gestational age, 1 had neonatal thrombocytopenia and 1 had pneumonia in the perinatal period. Of 33 infants who had received one or more blood transfusions, 9 showed evidence of having had a CMV infection. Of these 9 cases, 2 appeared to be congenital and 2 acquired. An additional infant began excreting virus at 37 days of age and showed a four-fold rise in titer to CMV at three months of age. The mother showed a four-fold rise in titer six months after the infant's discharge. This infant probably acquired a CMV infection in spite of a passively transferred antibody titer of 1:32 to CMV as measured by the complement fixation test. In the remaining 4 cases, the time of origin of infection could not be determined with accuracy; however, these 4 infants as a group had a total of 7 urine cultures submitted during the newborn period all of which were negative for CMV. It is likely that some of these cases were also acquired.

METABOLISM

First Session

LETHAL NEONATAL HYPERAMMONEMIA SECONDARY TO CARBAMYL PHOSPHATE SYNTHETASE DEFICIENCY. Thomas D. Gelehrter, and Philip J. SNODGRASS, Yale Univ. Sch. of Med., Depts. of Human Genetics, Med. and Ped., New Haven,Conn., and Harvard Med.Sch.,Dept. of Med., Boston,Mass. (Intr. by Leon E. Rosenberg).

Selective hereditary deficiency of three of the five urea cycle enzymes (ornithine transcarbamylase, argininosuccinate synthetase, and argininosuccinate lyase) has been described as the cause of fatal hyperammonemia in infancy. We report here the first case of lethal neonatal hyperammonemia secondary to virtually complete deficiency of the first enzyme in the urea cycle, carbamyl phosphate synthetase I (CPS I). The baby appeared normal at birth; but after beginning breast feedings he developed increasing irritability and rigidity, and expired 75 hours after birth. A male sibling had also died with an identical clinical picture. The parents are unrelated, and gave no history of protein intolerance.

Laboratory studies of the proband revealed a blood ammonia of 1480μg/100ml (normal <150μg/100ml) and BUN of 4mg/100ml. Serum electrolytes, pH, and sugar were normal. Assay of the urea cycle enzymes in liver obtained at autopsy revealed selective deficiency of CPS (<10% of the lowest newborn autopsy control); activity of the other four urea cycle enzymes was normal. The residual CPS activity was not dependent on N-acetylglutamate suggesting it represented cytosol CPS II and that mitochondrial CPS I activity was completely absent. Thus deficiency of CPS I must be added to the causes of lethal hyperammonemia in the newborn.

ROLE OF CARNITINE IN SUBCUTANEOUS (WHITE) ADIPOSE TISSUE FROM HUMAN NEWBORN INFANTS. Milan Novak, Peter Hahn, Duna Penn, Ellen F. Monkus and Josef P. Skala, Dept. of Ped., Univ. of Miami, Sch. of Med., Miami, Fl., and Dept. of Ped. & Obst. and Gynec., Univ. of British Columbia, Sch. of Med., Vancouver,Can.

The transfer and oxidation of free fatty acids in the mitochondria are necessary for heat production in brown adipose tissue. Data in newborn rats show these processes are dependent on an adequate supply of carnitine and the presence of carnitine transferases. Carnitine is believed to aid in the transfer of acylCoA across the mitochondrial membrane. The reaction is catalysed by Carnitine acyl(palmitoyl)transferase (CPT). Carnitine acetyltransferase (CAT) is involved in ketone oxidation and transport.

Carnitine content of newborn adipose tissue was 26 nmoles/gm, considerably less than in rat brown adipose tissue but sufficient to enhance oxidation of fatty acids. CPT activity was higher in mitochondria from newborn adipose tissue($p<0.01$)than in the adult. Mean mitochondrial CAT did not change with age. In adipocytes isolated from newborn adipose tissue, norepinephrine stimulated respiration was increased by l-carnitine and blocked by deoxycarnitine, an inhibitor of the carnitine transferases. These effects were not seen in the adult.

These findings suggest increased capability for fatty acid metabolism in newborn adipose tissue. This might mean that subcutaneous (white) adipose tissue of the human newborn is capable of heat production and might play a role in non-shivering thermogenesis.

AUTONOMIC AND ENZYMATIC CONTROL OF GLUCOSE PRODUCTION IN THE ISOLATED PERFUSED CANINE FETAL AND NEONATAL LIVER. Rowan Chlebowski,Satish Kalhan,Michael Lowry,and Peter Adam.Case Western Reserve Univ.,Dept.of Ped.,Cleve. Metro. General Hospital.

In order to determine the effects of birth and norepinephrine(NE)on net hepatic glucose production(HGP)and gluconeogenesis,29 isolated livers of canine fetal, premature and normal neonatal dogs were perfused in pairs with a recirculating medium containing 6mM glucose(G)plus 10mM lactate-3-C^{14}($L3C^{14}$). The changes of HGP and $L3C^{14}$ incorporation into G were correlated with changes of mitochondrial CO_2 fixation(CO_2 fix)and with the activities of pyruvate carboxylase(PC)and phosphorylase,active and total.HGP by the fetuses and puppies delivered prematurely by cesarean section(P3hr)was low,but rose rapidly to a plateau during the first day, as tabulated (mean ± SD):

	Glucose Production (μmoles/min. g liver)			
Group(n)	Fetus	P3hr	1 day	5 days
Control	0.4(2)	0.4(3)	1.2 ± 0.4(5)	1.3 ± 0.2(5)
NE(10^{-6}M)	0.4(2)	1.5(3)	2.1 ± 0.6(4)	2.0 ± 0.2(5)

Although NE did not raise HGP by the fetal liver,NE effect was evident 3 hours after premature delivery. The developmental changes of both HGP and $L3C^{14}$ incorporation correlated only with CO_2 fix and PC activity.NE effects were not explained by enzymatic activities,but occurred only after induction of mitochondrial PC and CO_2 fix.Apparently,initiation of gluconeogenesis immediately after birth is required for the maintenance of a physiological hepatic glucose production rate and for its autonomic regulation by norepinephrine.

DEFECTIVE GLUCONEOGENESIS (GNG) IN SMALL FOR GESTATIONAL AGE INFANTS (SGAI). MOREY HAYMOND, IRENE KARL, ANTHONY PAGLIARA, Washington U. School of Med., St. Louis Children's Hosp. Dept. Ped., St. Louis (Intr. Ralph Feigin).

Hypoglycemia occurs frequently in SGAI. Five normal infants (AGAI) and 7 SGAI were studied during the first 24 hours of life. Bloods were obtained for lactate (L), ketone bodies (KB), glucose (G), cortisol (C), growth hormone (GH), insulin (I), and amino acids (a.a.). Three SGAI had G of 21.4±9 mg% (mean± SEM) as opposed to 71.3±16.5 mg% for the remaining SGAI($p<0.01$) and 51.5±3.5 mg% for the AGAI ($p < 0.01$) at 2 hours of age. With exception of G, no other parameters separated the hypoglycemic and non-hypoglycemic SGAI; thus, all subsequent SGAI data were compared to AGAI. Cord, 2, and 24 hour L(7.8±0.9, 6.9±1.1, and 3.1±0.4 mM) were significantly elevated in the SGAI when compared to AGAI(2.2±0.6, 2.2±0.3 and 2.2±0.2 mM respectively). At the same times, alanine was significantly elevated 614±56, 568±64, and 433±58 uM in the SGAI and 307±26, 254±22, and 235±43 uM in the AGAI. All potential GNG a.a. entering at the level of pyruvate were significantly elevated in the cord and 2 hour plasmas of SGAI. SGAI plasma C and GH were elevated ($p<0.01$) in the cord and 2 hour samples. No differences in KB were observed. Elevated concentrations of L, alanine and other a.a. entering at pyruvate suggest a functional delayed maturation in a rate limiting hepatic GNG enzyme(s). Hypoglycemia in 3/7 of SGAI may reflect in part defective GNG observed in all SGAI superimposed upon other undefined factors which may deplete limited hepatic glycogen stores.

METABOLIC RESPONSE TO I.V. GLUCAGON IN SMALL FOR GESTATIONAL AGE INFANTS (SGA).
B. Schiff, E. Colle, J. Aranda, A. Pappageorgiou, S. Reisner, C. Scriver and L. Stern. Dept. of Paediatrics, University of Alberta, Edmonton. McGill University-Montreal Children's Hospital Research Institute.

Glucose, Insulin, Growth Hormone (HGH) and Amino Acids (AA) responses to I.V. Glucagon (300 µg/Kg) on the 1st day of life, were measured in 7 SGA infants and 10 Normal Controls of comparable gestational age. The fasting glucose was significantly lower (24.6 ± 5.0 mg%, vs. 48.6 ± 4.9 mg%; P<0.005); although the response pattern was similar, the levels achieved were different (P<0.01). Fasting insulin was similar (9.6 ± 3.1 µu/ml, vs. 1.9 ± 0.5 µu/ml; P>0.05); the response pattern was significantly different (P<0.01), with the SGA infant reaching a maximum at 1 minute of 190.0 ± 60.9 µu/ml, vs. 44.1 ± 10.3 µu/ml; P<0.05. Fasting HGH was significantly higher (46.4 ± 12.3 ng/ml, vs. 19.8 ± 4.8 ng/ml; P<0.05); although the two groups responded similarly, there was a difference in the levels achieved (P<0.01). The AA levels were significantly lower in the SGA group (1,867 ± 210 µmoles/L, vs. 2999 ± 147 µmoles/L, P<0.005), and where the normal infant did not demonstrate any response to glucagon, the SGA infant did (P<0.05). This data suggests that the glucose instability seen in SGA infants may be related in part to insulin secretion, and that AA gluconeogenic pathways are turned on at an earlier stage than in the normal.

ENZYME AND COENZYME FUNCTION IN MAPLE SYRUP URINE DISEASE.
L.J. Elsas, F.B. Wheeler, B.A. Pask, J.H. Priest, Emory Univ. Sch. of Med., Dept. of Ped., Atlanta (Intr. by R.W. Blumberg).

Maple Syrup Urine Disease (MSUD) results from genetically determined defects in branched chain α-ketoacid decarboxylase. Little is known regarding the genetic control of at least 3 enzymes and several cofactors implicated in this catalytic reaction. Broken cell suspensions of cultured skin fibroblasts were derived from 6 normal, 2 parents and a homozygous affected patient with MSUD. This system decarboxylated αketoisocaproic-1-^{14}C (KIC); αketoisovaleric-1-^{14}C (KIV) and αketo-β-methylvaleric-1-^{14}C (KMV) and was stimulated by the addition of cofactors. TPP, Mg^{++}, CoASH and NAD^+ increased CO_2 formation from KIC, KIV and KMV 10-20 fold in normal, 5 fold in heterozygous and 3 fold in homozygous affected cells. Qualitative differences in cofactor requirements were found for each of the 3 ketoacids. TPP and Mg^{++} alone enhanced KMV decarboxylation by all three genotypes to identical levels, but failed to stimulate KIC or KIV. By contrast CoASH and NAD^+ alone stimulated normal cells to 91% of maximum from KIC, 60% from KIV, but only 17% from KMV. The 3 genotypes were distinguishable using KIC or KIV but not KMV as substrate when CoASH and NAD^+ were added alone. We conclude that KIC, KIV and KMV have different cofactor requirements for decarboxylation, that the MSUD mutation studied here does not affect a TPP responsive KMV decarboxylase but does impair a CoASH-NAD^+ responsive decarboxylase common to all three substrates.

STUDIES OF SERUM IMMUNOREACTIVE PARATHYROID HORMONE (IPTH) IN NORMAL AND HYPOCALCEMIC NEWBORN INFANTS. Louis David and Constantine Anast, Univ. of Missouri, Columbia, Mo.

Serum IPTH, total calcium (Ca), ionized calcium (Ca^{++}), Mg and P were determined in 84 normal newborns from birth to 7 days of age and in 36 hypocalcemic neonates 5 hrs. to 7 days of age. Cord blood levels of IPTH were usually non-detectable (ND) or low. In normal newborns during the first 3 days there was a progressive decrease in Ca and Ca^{++}, while most serum IPTH levels remained ND (63%) or low; after 3 days there were parallel increases in serum Ca, Ca^{++} and IPTH (only 12% ND). In the hypocalcemic infants the serum Ca and Ca^{++} were less than 7.5 and 4 mg/100 ml respectively; 94% of these infants, including those older than 3 days, had ND or inappropriately low serum IPTH.

Serum IPTH was determined in 18 newborns in which a fall in serum Ca^{++} was induced by exchange transfusion with citrated blood. In 8 infants younger than 24 hours serum IPTH did not increase in response to low Ca^{++}; by contrast, in 8 of 10 infants older than 56 hours there was a sharp and usually sustained increase in IPTH.

Direct evidence obtained from these studies indicates that (1) parathyroid reserve is low in the early newborn period and (2) impaired parathyroid function, characterized by ND or low serum IPTH levels, is present in most infants with neonatal hypocalcemia. The fact that low blood calcium levels do not necessarily accompany depressed newborn parathyroid function suggests that additional unknown factors contribute to the development of neonatal hypocalcemia.

ABNORMAL LEUCINE/ISOLEUCINE RATIO AND THE ETIOLOGY OF ACRODERMATITIS ENTEROPATHICA-LIKE RASH IN MAPLE SYRUP URINE DISEASE (MSUD). John H. DiLiberti, Angelo M. DiGeorge, and Victor H. Auerbach, Temple Univ. School of Medicine, St. Christopher's Hosp. for Child., Dept. of Ped.,Phila., Pa.

An erythrodermic rash has been reported in several infants with MSUD while on dietary treatment. This complication has been attributed to a possible vitamin deficiency. We have studied an infant with MSUD who developed on two occasions scaling red areas over the extensor surfaces of the extremities, the cheeks, and the diaper area. Angular stomatitis glossitis and failure to thrive at this time were pronounced. The appearance of the rash was strikingly similar to that seen in acrodermatitis enteropathica (AE).

A pellagra-like syndrome with angular stomatitis, glossitis, etc. has been reported in humans on diets with a high leucine/isoleucine (L/I) ratio. High L/I dietary ratios causes growth retardation in rats which is reversible by isoleucine supplementation. With this in mind we re-examined the L/I ratios of our patient and found that the rash was most intense when this ratio was 10-20/1 and disappeared when the ratio was normal (1-2/1). The major change was in the isoleucine. Healing was complete with isoleucine supplementation (100 mg/Kg).

We suggest the etiology of the rash in MSUD is a high L/I ratio. These observations may have more far reaching significance. For example, some of the rashs observed in parenterally hyperalimented patients and the rash of AE may be related to aberrations of L/I ratios.

ISOVALERIC ACIDEMIA: A DEFECT OF LEUCINE METABOLISM IN CULTURED FIBROBLASTS. Kay Tanaka, Roseann Mandell and Vivian E. Shih. Depts. of Med. and Neurol., Harvard Med. Sch. and Mass. Gen. Hosp.,Boston.

The enzyme defect in isovaleric acidemia (IVA) has been assumed to be at the level of isovaleryl CoA dehydrogenase from its metabolite pattern and from the depressed ability of patients' white cells to oxidize [1-^{14}C]isovaleric acid in vitro. We attempted to further elucidate the derangement of leucine metabolism in IVA using cultured fibroblasts: harvested fibroblasts ($2x10^7$ cells) were incubated in K-R-bicarbonate buffer containing glucose and [1-^{14}C]-or [2-^{14}C]leucine (0.3 µc/1.5µmoles). IVA cells are distinguished from normal and MSUD cells by $^{14}CO_2$ production as follows:

Substrates	1-^{14}C-leucine (CPM)	2-^{14}C-leucine
Control (skin fibroblasts)	46600±10800(5)	9280±2550(5)
Control (amn. fl. cells)	22500± 3000(5)	5000±1000(3)
Control MCPA	9400 (2)	350± 80(4)
Isovaleric acidemia	9500± 1000(3)	100± 40(3)
Maple syrup urine disease	340 (2)	300 (2)

A high radioactive isovaleric acid peak was demonstrated in the incubation media from [2-^{14}C]leucine by radio GLC with IVA cells, confirming that the block in IVA is located at the level of isovaleryl CoA dehydrogenase. These results were identical to those observed with normal cells incubated with methylenecyclopropylacetic acid (MCPA), a specific inhibitor of isovaleryl CoA dehydrogenase.

EFFECT OF COPPER AND CERULOPLASMIN IN KINKY HAIR SYNDROME. William E. Bucknall, Neil A. Holtzman. The Johns Hopkins Univ. Sch. of Med., Dept. of Ped., Baltimore. A 3 month old male with clinical findings of Kinky Hair Syndrome, plasma copper (Cu) of 10 µg%, and undetectable ceruloplasmin, was given copper salts and ceruloplasmin and the chemical and clinical response measured. A seven day oral course of cupric sulfate, 300 µg Cu/kg/day, did not change plasma copper or ceruloplasmin concentration. Intravenous cupric acetate, 90 µgm Cu/kg administered over 2 hours, was followed by detectable ceruloplasmin (oxidase) activity at 22 hours, and peak activity at 48 hours corresponding to 4.5 mg% ceruloplasmin. A second dose of 180 µg Cu/kg resulted in a peak of 7.1 mg%. This dose was calculated to raise plasma copper acutely to normal and was considered the highest permissible dose, since hypercupremia can cause intravascular hemolysis. There was no hemolysis with the doses given. Purified, pyrogen-free human ceruloplasmin, 17 mg/kg administered intravenously, raised plasma ceruloplasmin to normal. The in vivo half life of this ceruloplasmin was within the range observed in nutritional copper deficiency. Thus the defect in this disorder does not appear to involve the synthesis or decay of ceruloplasmin. A defect in intestinal absorption of copper (Danks, D.M. et al, Lancet I:1100, 1972) is supported by these observations. The patient's clinical condition did not change significantly after Cu or ceruloplasmin and his E.E.G. pattern worsened. The possibility remains that earlier administration of copper may be effective.

METABOLISM

Second Session

TERMINATION OF PKU DIETARY THERAPY IN 62 PATIENTS
William B. Hanley & Lydia Linsao (Intr. by Donald Fraser)Dept. of Paed.,Univ.of Toronto, Research Institute, Hosp. for Sick Children, Toronto, Canada.

We have discontinued low phenylalanine dietary therapy at about age 5 yrs.(range 3½-8½ yrs.) in 62 cases of classical phenylketonuria (PKU). Mean time off diet has been 4.45 yrs. (range 6 mos.-9½ yrs.) 4 have been off diet for less than one year & 23 for over 5 years.

I.Q.	@ TERMINATION	NOW
over 90	13	13
80 - 90	13	12
70 - 79	7	8
below 70	29	28

- 49 children have had no change in I.Q. since diet termination. 5 have had an I.Q. drop (over 10 points) & 7 have had an I.Q. rise (over 10 pts.) There has been no follow up in one patient. 3 of the 13 with normal I.Q. are failing regular school.

- 52 had no behavioural changes, 4 deteriorated & 5 had improved behaviour.

- None of the patients have had seizures. 49 had follow up E.E.G.'s - 43 showed no change, 4 had slight change & 2 developed definite abnormal patterns.

- 9 developed lighter hair colour, none developed skin rashes.

This data suggests that the majority of PKU patients can be safely taken off diet at age 5 years.

EVIDENCE FOR EPINEPHRINE DEFICIENCY AND ALTERED ALANINE AVAILABILITY IN "KETOTIC HYPOGLYCEMIA".
Pierre C. Sizonenko, Luc Paunier, Michel B. Vallotton, Guy S. Cuendet and Errol B. Marliss (Intr. by Melvin M. Grumbach and Pierre E. Ferrier). Univ. of Geneva Med. Sch., Hôpital Cantonal, Dept. of Ped., Geneva. Switzerland.

Five children with ketotic hypoglycemia aged 2½ to 9½ years and six control children were given : 1. an infusion of 2-deoxy-D-glucose (2DG, 50 mg/kg during 30 min), to test the catecholamine response, 2. intravenous glucagon prior to and following a 28-hour-fast. In controls, the infusion of 2DG increased plasma glucose from 78 ± 5 to 156 ± 13.5 mg/100 ml, and plasma renin activity (PRA) from 2.0 ± 0.5 to 5.8 ± 1.2 ng/ml/h. In hypoglycemics, plasma glucose and PRA remained identical (77 ± 6 ng/100 ml and 1.3± 0.3 ng/ml/h) suggesting no epinephrine response. Plasma glucagon and cortisol levels were similar in both groups after 2DG. After the 28-hour-fast, plasma glucose and insulin decreased, β-hydroxybutyrate and glucagon increased similarly in both groups. Cortisol levels were higher in hypoglycemics. Fall in plasma alanine was significantly greater during fasting in hypoglycemics (151 ± 13 μM) than in controls (200 ± 13 μM, $p < 0.05$) and after the second glucagon administration ($p < 0.01$).

Absence of response to 2DG in the hypoglycemics suggests impaired catecholamine response. Since epinephrine has been shown to raise plasma alanine in man, a connection may exist between defective catechol secretion and alanine mobilization in "ketotic hypoglycemia".

PANCREATIC RESPONSE TO ACUTE AND CHRONIC GLUCOSE INFUSIONS IN OBESE CHILDREN. Robert H. Fiser, George A. Bray, Delbert A. Fisher, Depts. of Ped & Med, Harbor Gen. Hospital, UCLA School of Medicine, Torrance, California.

Carbohydrate intolerance and hyperinsulinism have been reported in obese children as in the obese adult; and peripheral insulin resistance has been documented in the obese adult. It is not known whether these abnormalities are present early in childhood obesity. Moreover, the mechanism(s) responsible for these phenomena are not clear. To further clarify this problem, we have investigated acute and chronic release of immunoreactive insulin (IRI) in response to short term and prolonged infusions of glucose (G) in obese children aged 3 to 13 years. Fasting plasma G and IRI levels were increased and G/IRI ratios decreased in obese children when compared with lean controls. In addition, relative hyperglycemia and hyperinsulinemia were observed in response to both stimuli in obese children; when plotted as a change from control values, the responses were similar to those in lean children during chronic infusions. G/IRI ratios remained similar to fasting ratios at all times in both lean and obese children. After acute infusions the ratios changed similarly in both groups. G/IRI ratios were lower in the older as compared with the younger obese children.

These data substantiate 1) glucose intolerance 2) peripheral resistance to glucose disposal and 3) hyperinsulinism in the obese child. The latter involves both acute and chronic insulin pools in a quantitatively similar manner.

TREATMENT OF PEDIATRIC FAMILIAL TYPES II AND IV HYPERLIPOPROTEINEMIA. C.J. Glueck, R. Fallat, R. Tsang (Intr. by J. Sutherland). Univ. of Cincinnati Coll. Medicine, Cincinnati General Hospital, GCRC and Dept. Pediatrics, Cincinnati, Ohio.

Diagnosis of pediatric familial types II and IV offers opportunities for therapy and primary atherosclerosis prevention. Low dietary cholesterol (LDC) (<300 mg/day, P/S 2:1) was given to 39 children with familial type II (ages 5-20). In 11 of the 39, after 6 months on LDC, mean index cholesterol (C) (249±14) and beta lipoprotein cholesterol (BLP) (188±20mg%) normalized; C(210±17), BLP(138±20). In 28 of the 39, after 6 months on LDC, index C (332±53) and BLP (257±42mg%) fell slightly; C(306±39), BLP (234±41mg%). With C and BLP high after 6 months LDC, cholestyramine (CST) (12 gm/day) was given to 14 type II children. On CST for 6 months, C (onLDC 311±34), and BLP (231±33mg%) fell in 9 of 14 to 262±36 and 182±32mg%. On CST for 12 months, C was 270±38, BLP was 196±40mg%. In 5 of 14, C (on LDC 306±41) and BLP (230±33mg%) did not fall after 6 months on CST, C(310±31), BLP (238±33mg%). On CST for 12 months, C in these 5 was 300±22, BLP 220±8mg%. Weight reduction and N.I.H. type IV diet were used in 23 children with familial type IV, ages 3-20. Mean triglyceride (TG) before diet was 289, range 158-1320, and fell to 128, range 72-262mg% after 6 months. TG fell below 140mg% in 91%, 21 of 23 children with type IV. C and BLP fell to normal in 28%, 11 of 39 children with type II on LDC alone. C and BLP fell notably in 64%, 9 of 14 children with type II on LDC and CST. Treatment of pediatric types II and IV often normalized C, BLP and TG.

ANOREXIA NERVOSA: CLINICAL AND METABOLIC OBSERVATIONS. JOSEPH A. SILVERMAN Columbia Univ. Col. of Physicians and Surgeons, Babies Hosp., Dept. of Ped., New York City. (Intr. by Robert H. Winters.)

This poorly understood disease has been extensively studied in 27 girls and 8 boys, ages 10 to 17. Impressive metabolic aberrations were detected in significant numbers: EKG abnormalities in 80%, hypovitaminosis A in 67%, bizarre oral glucose tolerance curves in 62%, decreased levels of luteinizing hormone in 58%, hypercarotenemia in 57%, absence of diurnal variation of plasma glucocorticoids in 55%, decreased follicle stimulating hormone levels in 54%, elevation of blood urea nitrogen in 48%, bone marrow hypoplasia in 41%, decreased xylose excretion in 33%, decreased serum thyroxine iodine levels in 27%, leucopenia in 24%, and decreased serum iron levels in 23%. Levels of plasma electrolytes, blood acid-base status, plasma proteins, plasma immunoglobulins, vitamin B12, serum folate, plasma total calcium and inorganic phosphorus, xray studies of the GI tract, and EEG, were nearly always normal. With restoration of proper nutrition, almost all abnormalities, (except FSH and LH) were corrected in about one month. FSH and LH levels became normal 3 to 6 months after metabolic recovery, with resumption of menses occurring 6 to 12 months after hormonal levels became normal. An effective method for inpatient medical treatment with zero mortality has been developed, (as an adjunct to psychotherapy), and stresses correction of malnutrition as well as a unified medical-nursing regimen to expedite the patient's recovery.

INDUCTION OF GAUCHER'S "LIKE" DISEASE IN NORMAL CULTURED FIBROBLASTS
Irwin A. Schafer, Norman S. Radin, Julia S. Sullivan, Mary Petrelli and Kenneth Rabenowitz, Department of Pediatrics and Pathology, Cleveland Metropolitan General Hospital, Case Western Reserve University and the Mental Health Research Institute, University of Michigan.

Fibroblasts cultured from Gaucher patients are deficient in B-glucosidase activity (5-10% normal) and accumulate glucosyl ceramide (2-3X normal). The chemical and morphologic effects of N-hexyl-O-glucosyl sphingosine, an inhibitor of rat brain glucosyl ceramide B-glucosidase were studied in normal cultured fibroblasts. This compound competitively inhibits the activity of B-glucosidase in normal fibroblasts to levels of less than 1% of the activity found in untreated controls. The activity of other lysosomal glycosidases is not affected. Inhibition of B-glucosidase activity is associated with the formation of lysosomes filled with myelin figures. Lipid analyses which are in progress indicate that the treated cells accumulate glucosyl ceramide. The chemical lesions produced by the inhibitor resemble those found in the mutant Gaucher fibroblast but are more severe. If the specificity of the inhibitor is confirmed, it would provide a powerful tool to study the chemical evolution of lysosomal storage in Gaucher's disease.

THE SANFILIPPO A SYNDROME: A SULFAMIDASE DEFICIENCY. Reuben Matalon and Albert Dorfman, University of Chicago, Department of Pediatrics, Chicago, Illinois 60637

The Sanfilippo syndromes, types A and B, (Kresse et al, Biochem. Biophys. Res. Commun. 42, 892, 1971) are autosomal recessive mucopolysaccharidoses characterized by severe mental retardation and excretion in urine and deposition in tissues of heparan sulfate. O'Brien (Proc. Nat. Acad. Sci. 69, 170, 1972) demonstrated an α-N-acetylglucosaminidase deficiency in cells and tissues of Sanfilippo B patients. In order to elucidate the enzymic defect in the Sanfilippo A syndrome, cultured skin fibroblasts were utilized. Cells were suspended in acetate:NaCl buffer, pH 5.0, containing Triton X-100, sonicated and centrifuged at 10,000 xg. The supernatant fluid was assayed for sulfamidase activity using heparin labeled with ^{35}S *in the N-sulfate group (Amersham/* Searle) as substrate. Following 18 hr incubation, the release of inorganic sulfate was determined by chromatography on Sephadex G-25. One to three per cent of the radioactivity was released as inorganic sulfate by extracts of normal, Hurler, Hunter and Sanfilippo B fibroblasts while extracts of Sanfilippo A fibroblasts released less than 0.1% of the radioactive sulfate. These results indicate that Sanfilippo A disease results from a sulfamidase deficiency. Supported by USPHS Grant Nos. AM-05996, HD-04583, and RR-305.

ENZYME THERAPY IN β-GLUCURONIDASE DEFICIENCY MUCOPOLYSACCHARIDOSIS. William S. Sly, Frederick E. Brot, Pra-on Chavalitdhamrong, and Philip D. Stahl, Washington Univ. Sch. Med., St. Louis Children's Hosp., Dept. Ped., Med., and Physiol. St. Louis, Mo.

We recently reported the clinical, radiological, and biochemical features of a mucopolysaccharide storage disease associated with deficiency of the lysosomal hydrolase β-glucuronidase. (Sly, et al. J. Pediat. Feb. 1973) The affected patient shows no β-glucuronidase activity in leukocytes and cultured fibroblasts.

Neufeld's group demonstrated abnormal accumulation of ^{35}S-mucopolysaccharide by cultured skin fibroblasts from this patient, and that this defect was corrected by addition of partially purified bovine β-glucuronidase to the patients fibroblasts.

Enzyme substitution therapy on two occasions with fresh frozen plasma selected for high levels of β-glucuronidase produced an apparent clinical response with x-ray documented reduction in hepatosplenomegaly. We have since purified human β-glucuronidase from urine, plasma, and platelets with an antibody-sepharose column containing goat antibody to purified rat preputial gland β-glucuronidase. Fibroblasts in culture show selective uptake and prolonged retention of the purified human enzyme which corrects the biochemical defect in ^{35}S-MPS accumulation. These results and progress of clinical trials with purified enzyme will be presented.

EFFECT OF SERUM PROTEINS IN MPS II. N. DiFerrante, B.L. Nichols, G. Coppa and J. Singh. Baylor Col. of Med., Houston, Texas. A 50% ammonium sulfate saturation precipitate of fresh human serum, (dialyzed against saline, ultrafiltered, pyrogen and viruses-free) was injected I.V. (2.1 g in 160 ml volume) into a 2½ year old boy with mild Hunter disease. Large mol. wt. urinary glycosaminoglycans, degradation fragments and their ratio were measured. The ratio decreased from 5.0 to 1.9 after the infusion. Pre- and postinfusion degradation fragments, absorbed on Dowex 1x2 column were eluted with NaCl. Most of the material was eluted with 0.4 and 0.8 M NaCl and represented disaccharides and tetrasaccharides, containing iduronate, with ratios to reducing groups of 1:1 and 2:1 respectively. The Dowex eluates were fractionated on Biogel P-2 (96x2 cm). The percentage of iduronate-containing material excluded and retarded by the gel respectively decreased and increased after the infusion. In particular, two iduronate-containing peaks, eluted 25 and 50 ml after the void volumn, had a ratio hexuronate/reducing group/S of 1:1:2. After the infusion they disappeared while the percentage of iduronate-containing material completely retained increased correspondingly. Thus this Hunter patient excretes abnormal disulfated disaccharides which become desulfated after serum protein infusion. These disaccharides, not found in normal human urine, may represent the material being accumulated because of deficiency of a specific sulfatase. (Supported by USPH grants AM-10811, HL-05435, RR-00188, and the National and the Welch Foundations.)

FAILURE OF PLASMA INFUSION THERAPY IN MUCOPOLYSACCHARIDOSES. Maurice J. Mahoney and Y. Edward Hsia. Yale Univ., Depts. of Human Genetics and Pediatrics, New Haven. (Intr. by Charles D. Cook).

Two pairs of siblings were chosen for plasma infusion therapy: brothers with Hunter syndrome and sisters with Sanfilippo B syndrome. A therapeutic trial was designed to compare, in each child, the effects of infusing plasma containing corrective factor with plasma devoid of that factor, and to compare within each sibship, the response in one child who received corrective factor with the response in his untreated sibling. Plasma infusions, averaging 20 ml/kg, were given at 5-7 week intervals for 6 months. Each child first received plasma from his own sibling by exchange plasmapheresis. Later plasmaphereses used plasma from an unrelated donor presumed to have the missing corrective factor.

Neither clinical nor biochemical assessment documented significant changes correlated with the giving of plasma. No change was found in liver and spleen size, measured by isotope scan; or in behavior, in skin thickness, skin histology, or bone marrow morphology. One of the boys with Hunter syndrome had equivocal improvement in range of motion of small hand joints; his brother showed no change. Total urinary glycosaminoglycan (GAG) excretion and small molecular weight GAG excretion varied considerably and could not be correlated with receiving corrective factor.

We have been unable to find evidence of significant improvement in these children under the conditions of this trial.

METABOLISM

Read by Title

ADIPIC ACID EXCRETION IN KETOTIC HYPOGLYCEMIA-A RAPID SAFE SCREENING TEST. Kyrieckos Aleck, Morey Haymond, and Richard E. Hillman (Intr. by P. R. Dodge) Washington Univ. Sch. Med., St. Louis Children's Hosp., St. Louis, Missouri.

At present diagnosis of ketotic hypoglycemia (KHG) requires a prolonged fast. GLC of urinary organic acids from these patients during fasting revealed a marked increase in dicarboxylic acid excretion with time. Although this finding is not specific and has been reported with glycogen storage disease and fatal lactic acidosis, the time course of excretion allows use of dicarboxylic acid measurements (particularly adipic acid) as a simple screening test for KHG. Urine from 7 patients with KHG was studied during fasting and compared with 10 age matched controls. Adipic acid excretion with normal feeding did not differ. However, after an overnight fast (12 hrs) patients excreted 10 times as much adipate as did normals. In the 2nd voided urine after the fast the excretion was over 20 times as great. These data form the basis of a simple screening test for this disorder and provide further evidence for the presence of methyl terminal (omega) oxidation of fatty acids in man.

Adipic acid excretion μg/mg creatinine

	Fed	12 hour fast 1st void	2nd void
Normal Adults	trace→1 (6)	1.60±.47(6)	2.40 (1)
Normal Children	1.61±.47(10)	2.32±.45(10)	2.35±.95(4)
Ketotic hypoglycemia	2.10±.52(7)	21.63±3.8(7)	78, 54 (2)

GROWTH HORMONE (GH) SECRETION IN JUVENILE DIABETES MELLITUS FOLLOWING GLUCAGON (GL) AND GLUCAGON-PROPRANOLOL (GP) ADMINISTRATION. T.W. AvRuskin, S. Tang, and C. Juan. (Intr. by S. Castells). The Brookdale Hosp. Med. Ctr., Dept. of Ped., New York.

IM or IV GL provokes GH release in normal subjects, less in hypothyroidism, and none in hypopituitarism. Juvenile diabetes provides an *in vivo* hyperglycemic model for evaluation of GL action. Eleven non-obese juvenile diabetics had 30 min. IV single and repeated pulse GL infusions(0.03 mg/kg, max.1mg) and 7 patients had GL infusions alone and with prior propranolol priming (40 mg).

Fasting and sustained hyperglycemia were noted in all infusions. Blood sugar(BS)increments in single, double dose, and repeated GL pulse studies were 35±8.6, 95±16.4, 82±18.2 mg/100 ml, and BS decrements were 77±15.9, 51±14.9, 54±9.2. Fasting and mean max. IRGH were 1.5±0.7, 17±6.2; 3.2±1.8, 22.7±7.0; 4.0±1.9, 18.3±6.5 ng/ml, respectively. Max. IRGH responses were similar in patients who had GL(11.9±1.5) and GP tests(17.8±5.0). Total and increment areas under IRGH curves, and ratios of increment IRGH area to BS decrement were similar in all infusions. GL provokes GH release in the diabetic state, propranolol does not augment GL-induced GH release in diabetes and hypoglycemia is not essential for GL-mediated GH release.

HYPOKETONEMIC HYPOGLYCEMIA: A NEW SYNDROME. Lester Baker, Rex S. Clements, Jr. and Albert I. Winegrad. Child. Hosp. of Phila. and Hosp. of the Univ. of Penna.

A 5½ yr old girl has had repeated admissions since age 16 mo for vomiting, lethargy and coma, usually associated with hypoglycemia. Growth and development and physical examination are normal. Muscle symptoms are absent. On a ketogenic provocative diet blood glucose (BG) fell to 32 mg%, FFA rose to > 3.5 mM; however, total ketones were only 0.18 mM. Plasma IRI and HGH responses were appropriate. BG did not respond to glucagon. Prolonged fasts (26 and 32 hr) produced hypoglycemia and the clinical syndrome; plasma FFA rose to 4.08 mM but total ketones never exceeded 0.45 mM. Plasma triglycerides rose from 84 to 185 mg%. Plasma alanine was .132 mM and glucose rose in response to alanine administration. Following the administration of medium chain triglycerides (MCT) the rise in plasma ketones was markedly impaired.

These data clearly distinguish this syndrome from ketotic hypoglycemia, and suggest a defect in hepatic fatty acid (FA) metabolism. The rise in plasma triglycerides during fasting is abnormal, and suggest a diversion of FA to triglyceride synthesis as a consequence of impaired mitochondrial FA oxidation and/or ketogenesis. The poor ketogenic response to MCT suggests that the defect is not restricted to long chain FA metabolism, and does not involve carnitine-acyl-CoA transferase. This syndrome illustrates the importance of ketogenesis in the adaptation to fasting.

HYPOINSULINISM IN CHILDREN WITH KETOTIC HYPOGLYCEMIA DURING FAST. J.L.Chaussain, M.Georges, M.F.Diebler and J.C.Job. (Intr. by M.M.Grumbach), Hosp. St-Vincent-de-Paul, Paris, France.

Metabolic and hormonal changes during a 24-hour fast were studied in 6 children with a recent history of ketotic hypoglycemia (KH) and in 6 controls of comparable ages. Mean blood glucose level dropped more in KH children (78 to 28 mg%) than in controls (81 to 54 mg%). The variations of serum growth hormone, lactate, pyruvate and FFA during fast were similar in the two groups, while only KH children exhibited a significant rise of plasma cortisol (11.5 to 29 ug%). Serum alanine dropped similarly in controls (37 to 17 uM%) and in KH children (28 to 15 uM%). All KH children exhibited at the end of the test a significant rise of branched amino acids (+35% for valine, +70% for leucine, +80% for isoleucine) not observed in controls. Responses to intramuscular glucagon (0.03 mg/kg) were studied before and after fasting. At time 0, both groups had a similar rise of blood glucose and insulin. At time 24 hr, these responses were suppressed in KH children, still present in controls. High levels of branched amino acids and lack of blood insulin and insulin reserve indicate that hypoinsulinism, as well as hypoalaninemia, is probably one of the major biological features of KH children during fast.

SULFONYLUREAS INHIBIT PROSTAGLANDIN E_1. Hans H.Bode, Patricia A. Meara, Helen S.Jones, John D.Crawford, Harvard Med.Sch., Massachusetts Gen. Hosp., Shriners Burns Inst., Boston.

Sulfonylureas alter cAMP mediated responses in many tissues but their exact mode of action is still unknown. Inhibition of phosphodiesterase is an unlikely explanation because: a) in equimolar concentrations chlorpropamide (CPM) and tolbutamide (TOL) were far less effective than theophylline (THEO); b)TOL effect was not reduced by its competitive antagonist diazoxide nor did TOL augment the influence of THEO at submaximal doses; and c)TOL in concentrations sufficient to show phosphodiesterase inhibition (10^{-2}M) consistently inhibited hormone stimulated adenylcyclase (HSA)activity in rat renal cortex and medulla ($p<.005$). In sulfonylurea pretreated rats potentiation of HSA was observed in renal medulla and cortex after vasopressin (VP) or parathyroid hormone stimulation, $p<.005$ and $p<.025$. Because all observed sulfonylurea actions could be explained by PGE_1 inhibition, slices of renal inner and outer medulla of CPM pretreated rats were exposed for 15 minutes to VP 10^{-3}U/ml, PGE_1 10^{-8}M, or both, in the presence of THEO 10^{-2}M; and cAMP accumulation compared with controls. PGE_1 had no effect and VP caused a >3-fold rise in cAMP in both groups. Addition of VP and PGE_1 together caused decline in cAMP in the controls while in the CPM pretreated animals the nucleotide content of both medullary tissues was increased ($p<.05$ and $p<.01$). Similarly, PGE_1 did not reduce high HSA in renal medulla of CPM pretreated rats, but suppressed HSA of controls. These data suggest that sulfonylurea effects depend on PGE_1 inhibition.

DYSMETABOLISM AS A POSSIBLE CAUSE OF GROWTH FAILURE IN CHILDREN WITH FAMILIAL DYSAUTONOMIA. Harold S. Cole, Dept. of Pediatrics, New York Medical College, New York, N.Y. (Intr. by Miriam Lending)

Children with familial dysautonomia (Riley-Day Syndrome) have marked retardation in height and weight. Almost all dysautonomics are below the 10th percentile for both parameters, the precise causes of which are unknown. Nine children with dysautonomia and seven control children were studied by means of intravenous glucose tolerance tests. The glucose disappearance rates (K_G) and changes in growth hormone and insulin were determined in order to detect abnormalities which might be responsible for growth failure. There were no significant differences in the K_G or growth hormone between the groups. The insulin values of the dysautonomics were lower than those of the control children at every one of the 8 sampling periods during the 1 hour test. The insulin areas above the fasting values were also determined. The mean $\pm$ SEM for the controls was 27.64 ± 8.88 compared with 9.88 ± 1.43 uU per ml per hour for the dysautonomics. This difference was significant ($p < 0.025$). The lower levels of serum insulin might reduce its effectiveness as an anabolic agent and result in growth impairment. The hypoinsulinemia might be caused by the poor nutrition of the dysautonomics or an insufficiency of acetylcholine. Under physiologic conditions the alpha adrenergic action of the catecholamines on the pancreatic beta cell predominates and inhibits insulin secretion. Acetylcholine is a known antagonist to catecholamines and its insufficiency might significantly diminish insulin output.

EFFECTS OF SYNTHETIC SALMON CALCITONIN IN OSTEOGENESIS IMPERFECTA (O.I.). Salvador Castells, Chun Lu, Richard K. Baker and Stanley Wallach State University of New York Downstate Medical Center, Departments of Pediatrics and Medicine, Brooklyn, New York

We have shown that porcine calcitonin caused retention of Ca and P and decreased hydroxyproline excretion in O.I. The present study was done to ascertain whether synthetic salmon calcitonin, more potent than porcine, given I.M. three times a week reduces the increased bone resorption of O.I. In five patients with O.I., after 1 week equilibration, 2 or 3 weeks of control metabolic Ca and P balance were followed by 2 to 5 weeks of salmon calcitonin. In two patients calcitonin caused an increase in positive Ca balance of greater than 10%. In three net gastrointestinal absorption of calcium increased by 317-37 mg/day. ^{47}Ca Kinetic studies before calcitonin showed a 25 hr. exchangeable calcium pool in O.I. 2 to 3 times the mean value of normals. Administration of calcitonin reduced the exchangeable calcium pool size. Quantitation by a digital computer program based on a four-compartment open model of Ca exchange, further confirm the ability of calcitonin to inhibit skeletal resorption. No toxic effects were found. The data indicates that calcitonin reduced skeletal turnover and thereby increased Ca accumulation by the skeleton. These observations suggest a role for salmon calcitonin in the management of O.I. (Supported by NIH Grant RR-318).

TRYPTOPHANE LOADING TESTS IN OBESE AND NEW DIABETIC CHILDREN Platon J. Collipp, Shang Y. Chen, Joseph Thomas, Viswanathan Balachandar and Vaddanahally T. Maddaiah. Nassau County Med. Ctr., Dept. of Ped., East Meadow, N.Y.

A microassay method for urinary xanthurenic acid (XA) and kynurenic acid (KA) has been developed as a modification of the method of Satoh and Price. 0.2 to 0.5 ml of urine mixed with 3 ml 0.2 NHCl are applied to Dowex 50 W columns (1 cm in height). Columns are washed with 5 ml 0.2 NHCl, 5 ml 0.5 NHCl and 1.5 ml 2.0 NHCl, and then XA and KA are eluted with 40 ml water. Recoveries of XA and KA by this method are 90-105%, and both internal and external standards are run daily. XA and KA are then determined fluorometrically in saturated NaOH or concentrated H_2SO_4.

Obese, new (1-3 weeks of diagnosis) diabetic and normal (healthy non-obese) children were given 2 gm oral l-tryptophane, and 24 hour urine collections were obtained on the day before and same day as the dose. Results (mean ± SD) expressed as mg/gm creatinine were:

	Before Tryptophane		After Tryptophane	
	XA	KA	XA	KA
normal (13)	8.8± 3.9*	16.0±10.1	15.5± 7.2*	30.2± 17.9*
obese (9)	22.3±31.9	16.3±19.8	54.0±42.6	58.3± 34.9
diabetes (5)	24.0±10.5	72.4±27.8*	39.0±28.7	70.0± 42.7

*$p<0.05$ from other two values

Xanthurenic acid has been reported to bind strongly to insulin; to reduce the hypoglycemic effect of insulin; and to be diabetogenic.

KINSHIP WITH ELEVATED LACTATE AND PYRUVATE. Angel R. Colón, Douglas H. Sandberg, Paul M. Tocci, William W. Cleveland. Univ. of Miami, Sch. of Med., Dept. of Ped., Miami, Florida.

A family with 11 children, 7 of whom have had grossly elevated blood lactate (L) and pyruvate (P) concentrations, has been examined. The proband is a 9 y/o BM with early growth failure (GF) who has been studied extensively without demonstration of a specific enzymatic defect. The siblings have signs and symptoms which overlap into both syndromes of congenital lactic acidosis (CLA) and hyperpyruvicemia with hyperalaninemia (PA).

	Age	Cardio-myopathy	Skeletal myopathy	Neuro-pathy	Mental Retard	Seizure	GF	L ↑	P ↑	A ↑
Proband	9	++	+++	++	+++	+++	+	+	+	+
Sister		+++	died at 16: severe fatty liver				+	+	+	
Brother	17	+++					+	+	+	
Brother	15		+	++		+++	+	+	+	
Sister	7						+	+	+	
Sister	2							+	+	
Brother	5m							+	+	

Only the proband with skeletal myopathy demonstrates hyperalaninemia, supporting the contention of Marliss et al (1972), that elevated alanine with lactic acidosis is consistent with increased peripheral release (muscle) and impaired hepatic disposal. The 5 m/o sibling has elevated plasma phenylalanine and tyrosine without alaninemia. Although considered to be separate syndromes, CLA and PA may represent one disease with varying expression. This kinship, the largest known with CLA and/or PA, tends to support this concept.

CYSTINE INTAKE AND THE PLASMA AMINOGRAM. L.J. Filer, Jr., Lewis D. Steginк, B. Chandramouli, Department of Pediatrics, University of Iowa College of Medicine, Iowa City, Iowa 52242.

Inability on the part of low birth weight (LBW) infants to convert methionine to cystine may make the latter an essential amino acid. Both Snyderman and Gaull consider cystine essential for LBW infants. Formulas that provide increased amounts of cystine have been advocated for feeding LBW infants. To test this concept 8 healthy growing infants, 11 to 35 days of age, weighing 1.3 to 1.8 kg at birth, were fed in a Latin Square Design four formulas providing 67 Kcal, 1.5 gm protein and 28 (I), 13 (II), 6 (III) and 4 (IV) mg cystine per 100 ml, respectively. Each infant was fed each formula for 72 hours before a 2-hour postprandial plasma sample was obtained at 8 a.m. Plasma concentrations of cystine were statistically significantly higher on Formula I versus III and IV but not I versus II. Plasma concentrations of taurine, a metabolite of cystine, were statistically higher on Formula II than IV. No differences were found in plasma concentrations of methionine. These observations are consistent with estimates of cystine requirement in early infancy.

SERUM IMMUNOREACTIVE CALCITONIN (ICT) IN NEWBORNS, Louis David and Constantine Anast, Univ. of Missouri, Columbia, Mo.

The physiologic significance of calcitonin in man is uncertain. Studies in experimental animals suggest that the hormone may play a relatively more important role during early life. Further, the bioassay studies of Miller and Copp demonstrated high "calcitonin activity" in human cord blood (Pro. Can. Ped. Soc., p 15, 1968) and it has been speculated that hypercalcitoninemia may play an etiologic role in neonatal hypocalcemia. In an effort to gain more direct knowledge of this hormone in early life, serum ICT levels were determined by radioimmunoassay in cord blood, normal and hypocalcemic newborns, mothers at parturition and normal adults. In studies conducted to date in 24 normal adults and 6 parturient mothers the ICT levels have been uniformly non-detectable (ND<50 pg/ml). In 6 cord bloods and in 18 normal newborns during the first week of life, approximately 50% of serum ICT levels were ND, while the remaining 50% were in the detectable low range (60-180 pg/ml). In studies of 3 hypocalcemic neonates the serum ICT levels were either ND or in the low detectable range (60-70 pg/ml).

The ND and low levels of ICT found in cord blood suggests that the hypocalcemic activity detected by bioassay is not calcitonin. The low levels of ICT found in the infants with hypocalcemia indicates that hypercalcitonin secretion was not an etiologic factor. The higher incidence of detectable ICT levels in newborns as compared to adults is of interest, but the significance of this observation is presently unknown.

HOMOCYSTINURIA DUE TO CYSTATHIONINE SYNTHASE DEFICIENCY: *DETECTION OF HETEROZYGOTES AND HOMOZYGOTES USING CULTURED SKIN FIBROBLASTS.* L.D. Fleisher, N.G. Beratis, H.H. Tallan, K. Hirschhorn, and G.E. Gaull. Dept. Ped., Mt. Sinai Sch. Med.; Dept. Ped. Res., N.Y. State Inst. Basic Res. Ment. Retard., Staten Island, N.Y.

Homocystinuria due to cystathionine synthase (CS) deficiency is an inborn error of metabolism transmitted in an autosomal recessive manner. Although deficiency of CS has been demonstrated in cultured skin fibroblasts, detection of heterozygotes (heteroz.) has not been reported. We have increased the sensitivity of the CS assay and have compared the kinetics of CS in cultured skin and fetal fibroblasts and amniotic fluid cells. Enzyme activities have been determined in fibroblasts derived from 8 controls; 3 homozygotes; 4 obligate and 2 potential heteroz.; and 5 fetuses. Activities (nmoles cystathionine/mg prot./hr) in the fibroblasts were as follows: Controls, mean 19.13, range 12.04 to 31.11; Patients, 0.74, 0.83, 2.29; Obligate heteroz. mean 5.03, range 2.84 to 8.19; Potential heteroz., 6.76, 21.60; Fetal, mean 44.99, range 19.62 to 61.90. The relatively high CS activity found in one of the patients, clinically atypical, was also seen in extracts from his liver. The finding that heteroz. demonstrate intermediate CS activity in cultured skin fibroblasts provides an easy method for the detection of the heterozygous state, essential for genetic counseling, and strongly suggests that prenatal detection of normals, patients, and heterozygotes for CS deficiency can be accomplished.

GLUCOSE INTOLERANCE IN INFANTS LESS THAN 1000 GRAMS: THE EFFECTS OF A DOUBLE DOSE OF GLUCOSE. H. Dweck, Y. Brans, R. Milstead, G. Cassady. Univ. Ala. Sch. Med., B'ham, Ala.

Two immediately successive intravenous glucose tolerance tests (GTT; 0.5 gm/kg) were performed within 8½ hours of birth with sampling at 3-120 minutes after injection. The 5 babies had received no prior oral or parenteral glucose, weighed ≤ 1000 g (640-1000) and were ≤27 weeks gestation (23-27). The rate of disappearance (K_t) in %/min was calculated from the slope of the line best fitting the log serum glucoses at 10-60 minutes. Mean glucoses at each sampling time were also compared.

Contrary to all previous reported studies in adults, infants and mature newborns, these babies failed to demonstrate an increased rate of glucose disappearance in the second test. Mean K_t of the initial GTT was 1.14 ± 0.41 (range 0.42-1.39) whereas mean K_t of the second GTT was 0.68 ± 0.32 (range 0.37-1.17; $0.1 > p > 0.05$). Mean serum glucoses (mg%) and in fact, tended to be higher in later samples (mean ± S.D. shown): ($*p < 0.025$)

MIN	*0	3	5	10	20	30	60	120
GTT#1	49±15	280±73	213±34	167±22	140±26	122±11	96±9	80±19
GTT#2	80±19	277±36	211±17	168±26	155±36	144±32	125±29	95±44

These data, complemented by previous observations from this laboratory of hyperglycemia following parenteral glucose and diminished insulin response to a glucose load, confirm the fragile nature of carbohydrate metabolism in these tiny babies.

TRICHOPOLIODYSTROPHY (MENKES' KINKY HAIR SYNDROME): A COPPER DEPENDENT DEFICIENCY OF MITOCHONDRIAL ENERGETICS. J.H. French, C. L. Moore, N. R. Ghatak, I. Sternlieb, S. Goldfischer, and A. Hirano, Albert Einstein Col. of Med., Montefiore Hosp.& Med. Ctr., Dept. Neurology, Dept. Pediatrics, Dept. Pathology, Dept. Medicine, Dept. Biochemistry, The Bronx, New York.

Quantitative determination of mitochondrial cytochrome ata_3 content reveals a five-fold reduction in 4 patients with this sex-linked recessive neurodegenerative disorder. Oxygen consumption rates of isolated mitochondria in 3 patients with trichopoliodystrophy (TPD) exhibit a gross failure of respiratory control. This lack of a state 4-3 transition is least in the youngest case. The serum and caeruloplasmin incorporation of oral ^{64} copper is deficient in 3 patients with TPD when compared to a neonatal control. Ultrastructural abnormalities of liver mitochondria were no longer visible in one TPD patient following parenteral treatment with copper calcium EDTA. Study of brain vasculature and the neuropathologic findings in 3 cases of TPD fail to substantiate a vascular etiology for the nervous system abnormalities. Therefore, our findings suggest the feasibility of newborn diagnosis of TPD and support a failure of tissue energetics secondary to diminished body copper content as the probable basis of the hypoplastic neuropathology of this disorder.

STUDIES OF CERULOPLASMIN IN MENKES' KINKY HAIR DISEASE.
Adolfo Garnica, Jaime Frias and Owen Rennert. Univ. Fla. Col. Med., Dept. Ped., Gainesville, Florida.

Defective copper absorption has been implicated as the cause of Menkes' Kinky Hair Disease (MKHD). Low serum copper levels are not corrected by oral copper sulfate but have been reported to be raised following intravenous cupric chloride administration. Following an oral dose of labelled copper, accumulation of copper was found in jejunal mucosal cells.

Depressed serum ceruloplasmin levels have also been reported in MKHD. This glycoprotein has been shown to participate in copper transport, specifically as a special carrier for the transport of copper from the liver to the sites of cytochrome oxidase synthesis. Mitochondrial disorganization and decreased cytochrome a + a_3 have been demonstrated in liver, brain and muscle in MKHD.

A three-month-old male with MKHD was treated with intravenous cupric acetate in doses of 22 µg/kg per day. Response to therapy was monitored with serial serum copper and ceruloplasmin determinations. Serum copper was assayed by atomic absorption while cerulosplasmin by acrylamide gel electrophoresis and by measurement of oxidase activity. After ten days of intravenous therapy no increase in ceruloplasmin or copper was demonstrated.

Our findings suggest a possible defect in either synthesis or structure of ceruloplasmin.

NORMAL VITAMIN A AND E LEVELS IN CHILDREN WITH TYPE II HYPERLIPOPROTEINEMIA ON DIET AND CHOLESTYRAMINE. C.J. Glueck, S. Buxton, D. Boggs, R. Fallat, R. Tsang. (Intr. by J. Sutherland) Univ. Cincinnati Col. Med., Cin. Gen. Hosp., GCRC and Dept. Ped.

To determine whether cholestyramine (CST) and/or diets low in cholesterol (LDC, <300mg/day) and rich in polyunsaturates (P:S of 2:1) result in decreases in fat soluble vitamins A and E (A,E), we measured plasma A (fluorometrically) and E (colorimetrically, corrected for caroteinoids) in children ages 6-18 with type II. A and E were measured on an ad lib diet and serially during 12 months of LDC which produced a 14% fall in plasma cholesterol(C); and again during 12 months of both LDC and CST(12g/day) which led to a further 18% fall in C. In 20 children on an ad lib diet A(mean±S.D.) was 3.1±.1 µM/liter and E was .9±.4mg%. In 12 children after 3 months on LDC, A was 3.8±1.6 and E had increased to 1.4±.6($p<.005$). After 6 months on LDC, A in 12 children was 3.4±1.9 and E in 20 was 1.1±.4. After 12 months on LDC, A in 10 children was 3.3±.5 and E in 7 was 1.0±.4. After 3 months on LDC and CST, in 9 children A and E were 3.7±1.1 and 1.3±.4. On LDC and CST for 6 months, 5 children maintained A(3.7±.9) and E(1.5±.5) at levels ≥ A and E on ad lib diet. On LDC and CST for 12 months, 10 children maintained A(4.0±.8) and E(1.6±.6) both > A and E on ad lib diet($p<.05$). Neither LDC alone nor LDC and CST for periods of up to 12 months led to reduced levels of A or E in these children who did not take vitamin supplements. Normal plasma A and E levels were maintained in the face of an overall 32% fall in C on LDC and CST.

PREMISES FOR DEVELOPMENT OF INTRAVENOUS AMINO ACID SOLUTIONS
Ghadimi, H.
Department of Pediatrics, Methodist Hospital, Brooklyn, N.Y.

Tolerance response to constant-rate injection of amino acids (a.a.'s) was studied by administering a.a. mixtures in the central vein and analyzing femoral venous blood for a.a.'s. The response to different rates of administration (mg of a.a./kg body weight/24 hrs) was determined. Under such conditions, with hepatic catabolism substantially by-passed, observed changes in a.a. levels following infusion mainly reflect the overall anabolic requirements of tissues. Upon analyzing over 120 samples, dose limits consonant with normal plasma range were determined and an infusate consistent with tissue needs was formulated. Other criteria in determining optimal a.a. proportions included: enzymic immaturities of the newborn, sparing action of some a.a.'s, metabolic pathways of each a.a. with emphasis on the economy of the substance, the role of different tissues in a.a. metabolism, the side effects of administration of some a.a.'s, and total nitrogen requirements. "Minimal" and "safe" requirement of essential a.a.'s under conditions of oral administration was also taken into consideration. A range was formulated and working examples selected. With oversimplification for the sake of a terse presentation, the a.a.'s fall into 3 categories: Group I: branched-chain a.a.'s and arginine, making up over 50% of the formula. Group II: none or small amount, e.g., ornithine, glutamic and aspartic acids. Group III: 12 a.a.'s with major metabolic pathways in the liver.

CORD BLOOD LOW DENSITY LIPOPROTEIN CHOLESTEROL: ESTIMATION VERSUS MEASUREMENT WITH THE PREPARATIVE ULTRACENTRIFUGE. C.J. Glueck, V. Leuba, P. Steiner, R. Tsang (Intr. by J. Sutherland) Univ. Cincinnati, Coll. Medicine, Cincinnati General Hospital, GCRC and Dept. of Pediatrics.

Determination of low density lipoprotein cholesterol (LDL) in neonates and infants should provide the most accurate approach to diagnosis of pediatric familial type II hyperlipoproteinemia. In 39 unselected neonates (mean cord plasma cholesterol 71mg%), LDL was quantitated by ultracentrifuge (LDL-u), and estimated (LDL-e), by the formula of Friedewald et al (Clin. Chem. 18:499, 1972) as follows: LDL=total cholesterol (C)-(alpha lipoprotein cholesterol (HDL)-triglyceride (TG)/5). LDL-e requires measurement of C, HDL (by heparin-manganese precipitation), and TG, while LDL-u requires all the above and ultracentrifugation and measurement of C in the density fraction D>1.006. Mean ±SD LDL-u and LDL-e were similar (26±13mg% and 28±13mg%) and were highly correlated (R=.934, P<.001). The mean difference between LDL-u and LDL-e was 1.8mg%, and 95% confidence intervals for differences were 0.26-3.3mg%. Restrictions on the use of LDL-e (chylomicrons, type III hyperlipoproteinemia, TG>400mg%) did not apply in 500 consecutive cord blood samples. In cord blood samples (which have relatively lower C and LDL as compared with adults), the close agreement of LDL-u and LDL-e should confirm the usefulness of LDL-e, obviate need for ultracentrifugation, and reduce the requirement for large blood sample collection at birth or in early infancy.

THE RESPONSE TO 1,25-DIHYDROXYCHOLECALCIFEROL (1,25-DHCC) IN X-LINKED HYPOPHOSPHATEMIA.
F. Glorieux, C. Scriver, M. Holick & H. DeLuca. Dept. of Pediatrics, McGill Univ., Montreal & Dept. of Biochemistry, Univ. of Wisconsin, Madison.

1,25-DHCC was chemically synthesised (Tetrahedron Letters 40, 4147, 1972), dissolved in propylene glycol and administered (1µg/dose) to an untreated 6-yr male with X-linked hypophosphatemic rickets, and 3 control subjects (2 normal male adults and one 13-yr male with non-familial hypophosphatemic rickets of renal origin). Intravenous 1,25-DHCC given at 10AM in the fasted state did not change serum inorganic phosphorus (P) or calcium, or raise the tubular reabsorption of phosphate (TRP) in all 3 control subjects. I.V. 1,25-DHCC raised endogenous TRP by 13% within 30 min. of infusion in X-linked hypophosphatemia (pre infusion TRP: 79,65,75%; post infusion TRP: 87,82,88,86%, $p<0.02$); and restored responsiveness to intravenous bovine PTH (200 units), allowing immediate depression of TRP to endogenous phenotypic levels (post PTH, TRP: 72,74,72,76%, $p<0.001$). Serum P and calcium, GFR and calcium excretion did not change consistently. By contrast daily 1,25-DHCC (1µg/day I.V. at 10AM x 5 d, or by mouth x 5 d) did not produce long term elevation of TRP, or raise endogenous serum P. The acute TRP response was not apparent after oral 1,25-DHCC.

TYPE V HYPERLIPOPROTEINEMIA IN AN INFANT. Carole-Ann Hamly* and Avedis K. Khachadurian* (Intr. by H. L. Nadler) Clinical Research Center, Children's Memorial Hospital, Northwestern University Medical School, Chicago, Illinois.

A five-month-old caucasian male presented with fever, eruptive xanthomatosis, hepatosplenomegaly and severe hyperchylomicronemia. Plasma total cholesterol (TC) was 670, triglycerides (TG) 8175 and phospholipid phosphorus 28.5 mg/100 ml. Lipoprotein electrophoresis (LE) was type V with marked trailing. Studies failed to reveal known causes of secondary hyperlipidemias. Carbohydrate tolerance and serum immunoglobulins were normal. After 6 wks on a diet containing 4 gm of fat per day TC was 165, TG 1194, LE type V. On a 2 gm/day fat diet TC was 116, TG 477, LE type IV. During a five-month follow-up on the low fat diet the infant developed normally and was symptom-free. Parents were not related. Father's TC was 227, TG 289, LE type IV. Mother's TC was 188, TG 434 and LE type IV. TG after a high carbohydrate diet 174 and 151 respectively. 12 hrs after fat loading, father's TG 741, mother's TG 108.

Plasma post-heparin lipolytic activity (PHLA) done by Dr. R. Krauss at NIH gave following results in mM free fatty acid/ml/hr. Propositus 2.0; father 3.1; mother 2.0; control males 4.2 ± 1.7, females 3.5 ± 1.3. Findings in this patient illustrate that massive chylomicronemia can coexist with only moderate reduction of PHLA as measured presently and that a type IV LE pattern may be associated with fat induced hyperlipemia.

HORMONAL AND SUBSTRATE RESPONSES TO FASTING IN NORMAL AND KETOTIC HYPOGLYCEMIC (KH) CHILDREN. MOREY HAYMOND, IRENE KARL, and ANTHONY PAGLIARA. Washington U. School of Med., St. Louis Children's Hosp. Dept. Ped., St. Louis. (Intr. Ralph Feigin.)

Glucose (Glu), lactate (L), pyruvate (P), ketone bodies(KB), glycerol (Gly), plasma amino acids (a.a.), cortisol (C), growth hormone (GH), insulin (I), and glucagon (Glc) were determined at frequent intervals throughout a fast in 10 KH and 6 age matched control children. In KH children, G was 27.4± 2.6 mg% (mean± SEM), KB was 5950±855uM, and Gly was 232±34uM as compared to 75.7±2.9 mg%, 1116±188uM, and 121±13uM respectively at 20 hours fasting ($p < 0.01$ for all values). After 6 hours fasting, little or no I was detectable in either group. Total gluconeogenic a.a. were significantly lower ($p < 0.01$) in the KH children when compared to normals at all points examined (3024±118 vs 2354±121uM at 0 time and 1743±112 vs 1161±69uM at 20 hours fasting). Branched chain a.a. were significantly higher in the KH children ($p < 0.05$) at 20 hours fasting (384±35 vs 567±41uM). No differences in L or P were found. At the time the KH children were hypoglycemic, C was 27±3 vs 8.8±2 µg% for controls ($p < 0.01$), GH was 11±4 vs 2±.2 ng/ml ($p > 0.05$), and Glc was 340±74 pg/ml vs 92±22 pg/ml ($p < 0.05$). With hypoglycemia, low I and elevated KB and Gly are appropriate responses. Elevated C, GH, and Glc reflect normal responses to the hypoglycemic stress, further evidence against a hormonal etiology for this disorder. Low gluconeogenic a.a. would appear to be the rate limiting event due either to decreased a.a. mobilization or increased Glu utilization.

HYPERAMINOACIDEMIA AND REYES SYNDROME. Milo D. Hilty, Carolyn A. Romshe, Paul V. Delamater. Med. Col. of Ga., Dept. of Ped., Augusta, Ga., and Ohio State Univ., Col. of Med., Childrens' Hosp., Dept. of Ped., Columbus, Ohio. (Intr. by Juan F. Sotos)

Sera from 13 patients with the clinical, liver biopsy or autopsy findings of Reyes Syndrome were analyzed for amino acids by column chromatography. Specimens were obtained from all patients prior to treatment and from 5 patients during the course of treatment. Treatment consisted of exchange transfusion, insulin and glucose or supportive only.

In 12/13 patients there was a marked hyperaminoacidemia. Those amino acids with the greatest and most consistent elevations included alanine (> 5 fold), αamino butyrate, lysine, glutamine and glutamic acid. In two patients the amino acids returned to near normal by the 6th and 7th hospital day. One survived and one expired; therefore, the improvement in amino acid levels appears to have no significance in predicting the outcome. The degree of hyperalaninemia on day 1 or 2 may have some relationship to outcome. In 1/5 survivors and in 6/8 patients that expired, serum alanine was increased 5-8 fold.

Quantitation of amino acids was also obtained in sera from patients with hepatitis, diabetic ketoacidosis, salicylism and encephalitis. The pattern of hyperaminoacidemia observed in Reyes Syndrome appears to be unique and may reflect the action of either an endogenous or exogenous toxin on amino acid metabolism.

CYSTATHIONINE EXCESS IN CHILDHOOD TUMORS. Lawrence Helson, June L. Biedler, and M. Lois Murphy, Memorial Sloan-Kettering Cancer Center, Department of Pediatrics, New York.

Increased excretion of cystathionine in urine (12 - 20 x normal) was detected in children with disseminated neuroblastoma (16/19) and with hepatoma (6/6). Assay was by automated column chromatography. Tumor tissue from patients with neuroblastoma and their cell cultured lines (2) by thin layer chromatographic analysis had high concentrations of cystathionine. This increased intracellular content is attributed to decreased breakdown (cystathionase defect) and may reflect a fetal biochemical character in malignant cells. After 0.04 mCi ^{75}Se-selenomethionine (SEM) was administered intravenously, total body scan showed nuclide uptake in areas of tumor. Radioautography of neuroblastoma cells aspirated from bone marrow did not demonstrate uptake of isotope. Radiochromatography of urine did not show metabolism of SEM to radiolabeled cystathionine. Increased cystathioninuria has also been detected in children with Wilms' tumor (1/1), teratocarcinoma (1/1) and intrahepatic biliary cirrhosis (1/1). Cystathioninuria appears to be an established metabolic abnormality associated with several cancers.

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INCREASED PHOSPHORYLASE ACTIVITY IN LIVER HOMOGENATE WITH AMP: POSSIBLE PITFALL IN INTERPRETING LIVER BIOPSIES. Jean Holowach-Thurston, Elizabeth M. Jones, Hulda J. Wohltmann, and Richard E. Hauhart, Wash. Univ. Sch. of Med. St. Louis, Mo.

Measuring enzyme activity in the direction of glycogen breakdown we have found that phosphorylase in liver homogenate can be significantly stimulated by AMP. Five litters of normal 17-23 day-old mice were studied, total 39 animals. AMP-stimulation varied inversely with the level of active enzyme. At high levels, 7.16 ± 0.13 µmoles $g^{-1}min^{-1}$ AMP increased activity only 10%, p=0.016. At low levels, 2.43 ± 0.31 µmoles $g^{-1}min^{-1}$ (after phenobarbital) AMP almost doubled the velocity 4.40 ± 0.31 p=0.003 - a value equal to that found in littermates injected with epinephrine, (4.20 ± 0.28 and 3.77 ± 0.39 µmoles $g^{-1}min^{-1}$ with and without AMP, respectively). This finding appears to be important in the evaluation of levels of phosphorylase and phosphorylase kinase activity. There are published reports of increased phosphorylase activity with AMP in the leukocytes of patients with low kinase activity. Although the observed AMP effect reported here may indeed be due to activation or stabilization of the kinase, in interpreting the results of liver biopsies in patients with suspect glycogenosis, it must be realized that AMP stimulation of low phosphorylase activity in liver homogenate can be a normal finding.

OMEGA OXIDATION OF MEDIUM CHAIN FATTY ACIDS. Richard E. Hillman, Pra-on Chavalitdhamrong, and James P. Keating (Intr. by W. S. Sly) Washington Univ. Sch. Med., St. Louis Children's Hospital, Dept. Ped., St. Louis, Missouri.

Indirect evidence for the presence of omega (methyl terminal) oxidation of medium chain (C6-C12) fatty acids has come from studies of patients with various causes of ketosis. To study this process more directly, urinary organic acids from children fed diets high in medium chain triglycerides (MCT) were analyzed using gas-liquid chromatography and mass spectrometry. 1-2% of the daily intake of MCT was excreted in the urine as dicarboxylic acids and hydroxy acids (C6-C10). The total excretion exceeded 4 grams per day in older children. The presence of large amounts of C7 and C9 acids indicates that chain length can be shortened from the C8 and C10 acids fed to the patients. In addition, the presence of several previously undescribed branched-chain fatty acids raises the possibility that these products may alter normal fatty acid synthetic pathways. These data indicate that omega oxidation is an important pathway in man. Because the toxicity of many of the products of omega oxidation is not known, MCT's should be used with caution in children.

PROPIONICACIDEMIA:BIOCHEMICAL CHARACTERIZATION OF MUTANT PROPIONYL-CoA CARBOXYLASE. Y. Edward Hsia,Katherine J.Scully and Leon E. Rosenberg,Depts. of Human Genetics and Ped.,Yale Univ. Sch. of Med., New Haven, Conn.

Propionyl-CoA carboxylase (PCC) activity in crude lysates of cultured skin fibroblasts from 5 patients with propionicacidemia was 4-10% of that in control lines. PCC from control lysates was purified 300-fold by the sequential application of detergent treatment, differential centrifugation, ammonium sulfate fractionation and glycerol density gradient centrifugation: specific activity (SA)-0.5 nmoles/min/mg protein (u) in the crude lysate; 148 u in the final preparation. The partially purified PCC had: a pH optimum of 8.3-8.6; a K_m for propionyl-CoA of 1.4 mM; and a K_m for bicarbonate of 0.6 mM. With this schema, mutant PCC was purified more than 50-fold, although with less efficient yield (SA of crude lysate-.03 u; SA of final preparation-1.7u). This activity was not due to cross-specificity with acetyl-CoA carboxylase (ACC).ACC activity disappeared during purification of PCC,and anti-ACC antibody abolished ACC activity without altering PCC activity. Mutant PCC had a normal pH optimum and affinity for propionyl-CoA and bicarbonate, but differed in two ways:detergent treatment, which doubled normal PCC activity, failed to enhance mutant PCC activity; and it was more thermolabile: heating at 54° for 10 minutes, which reduced normal PCC activity by 20%, reduced mutant PCC activity by 60%. We conclude that residual PCC activity in mutant cell lines represents structurally altered PCC.

PRENATAL DIAGNOSIS AND FETAL PATHOLOGY OF INFANTILE GLYCOGENOSIS TYPE II(GSD IIa). George Hug, Shirley Soukup, William K. Schubert and Clarence R. McLain. The Children's Hospital Research Foundation, Cincinnati, Ohio.

Prenatal diagnosis of GSD IIa on the basis of acid α-glucosidase deficiency(AGD) in cultured amniotic fluid cells has been reported previously as has our observation that such diagnosis can be made by the electronmicroscopic demonstration of "abnormal lysosomes" (i.e. membrane surrounded accumulations of glycogen) in uncultured and cultured amniotic fluid cells. This morphologic technique led to the termination of 2 pregnancies by saline infusion and hysterotomy, respectively. Extensive biochemical and electronmicroscopic tissue analyses of both fetuses confirmed the prenatal diagnosis of GSD IIa by demonstrating (a) AGD in the presence of normal (fetal) activity of 10 other lysosomal enzymes and (b) "abnormal lysosomes". Increased glycogen concentration and electronmicroscopic appearance of fetal cardiac and skeletal muscle in GSD IIa were not distinguishable from those of heart and muscle of infants with GSD IIa or those of heart and muscle of age matched fetuses without GSD IIa. The condition of infantile cardiac and skeletal muscle in GSD IIa could thus be interpreted as the postnatal persistence of the normal fetal state; this hypothesis suggests that the study of normal fetal heart and muscle and of their glycogen metabolism may clarify the pathophysiology of GSD II.

ASPARTYLGLUCOSAMINURIA, BIOCHEMICAL AND ULTRASTRUCTURAL STUDIES OF A VISCERAL STORAGE DISEASE MASQUERADING AS A MUCOPOLYSACCHARIDOSIS.

J. Nevin Isenberg and Harvey L. Sharp, Univ. of Minn. Hosp., Dept. of Ped., Minneapolis, Minn.

The observation of vacuolated lymphocytes in a coarsely featured 2 yr. old female with hepatosplenomegaly, mitral insufficiency and mild psychomotor retardation ultimately resulted in the first diagnosed case of aspartylglucosaminuria in the U.S. Her bone roentgenograms were consistent with a mucopolysaccharide disorder but repeated urine analysis showed no MPS elevation. Enzyme deficiencies for the lysomal accumulation of ganglioside or mannoside were not found. Peripheral blood and bone marrow cells, as well as liver, lymph node, and skin biopsy specimens demonstrated large vacuoles not readily stainable by histochemical techniques. Hepatic ultrastructure revealed these vacuoles to be large single membrane lined organelles containing an amorphous granular material resembling the contents of lysosomes in mucopolysaccharidosis, but different because of additional small neutral lipid droplets. Thin layer chromatography of the patients urine showed an abnormal ninhydrin positive spot not present in control urines. In several solvent systems, this spot had staining and migratory characteristics similar to 2-acetomido -1-(L-s-aspartamide)-1,2-dideoxyglucopyranoside, a bimolecular compound which accumulates in aspartylglucosaminuria. Further definition by tissue and bile extraction with results of the previously reported enzyme deficiency B-aspartylglucosylamine amido hydrolase (Lancet 2:253, 1968) will be presented.

ADULT VERSUS INFANTILE GLYCOGENOSIS TYPE II(GSD II): DIFFERENCES UNEXPLAINED BY ACID α-GLUCOSIDASE DEFICIENCY(AGD). George Hug, Margery R. Sper and William K. Schubert. The Children's Hospital Research Foundation, Cincinnati, Ohio.

Of 22 patients(P) in 13 families(F) with GSD II we found that 20 P in 12 F had the classical form of the disease with death in early childhood (GSD IIa) and 2 brothers (9 and 23 y) in 1 F had GSD IIb. On PE the 9 y boy with GSD IIb is healthy; his brother had progressive hypotonia in his teens and died at 23 y. Necropsy allowed biochemical, light and electronmicroscopic comparison of 14 tissues with those of GSD IIa and with biopsy specimens of the 9 y boy. Results are:

	GSD IIa	GSD IIb
AGD	marked	marked
Glycogen	marked increase	normal-slight increase
Abnormal lysosomes	many and large	few and small
Prognosis	early death	slow progression

AGD was as severe in GSD IIb, as in GSD IIa. The findings are consistent with the hypothesis that GSD II has abnormal synthesis of glycogen in addition to AGD. In GSD IIb, abnormal synthesis is negligible and deficiency of the lysosomal pathway can be tolerated. In GSD IIa, abnormal synthesis is fully expressed. Deficient lysosomal glycogen degradation is manifest with excessive glycogen, abnormal lysosomes and dismal prognosis. This hypothesis may be supported by our observation that the 9 y boy has AGD as severe as in GSD IIa; and shows morphologic muscle changes viz: altered Z-bands and myofilaments, and excessive glycogen outside abnormal lysosomes.

ELECTRON MICROSCOPY AND CHEMICAL STUDY OF CULTURED SKIN FIBROBLASTS IN STORAGE DISORDERS. Elsa Kamensky*, Michel Philippart, Pasquale A. Cancilla* and Stephen Frommes*. Univ. California, Dept. of Ped., Neur., Psych. and Path. Los Angeles

A number of storage disorders resulting from specific deficiencies of lysosomal hydrolases have been recognized. As a rule such deficiencies can be demonstrated in most tissues, including skin. To a large extent the storage process depends on the ability of a given tissue to synthesize an undegradable substance. Qualitative and quantitative differences in synthetic enzymes reflect tissue differentiation. Electron microscopy and incorporation of labeled chemical precursors represent the most sensitive techniques to detect a process of storage. In a first group of diseases (Fabry's, Niemann-Pick's A and C, Hurler's, Sanfilippo B, Sandhoff's and Leroy's Inclusion-Cell) one can demonstrate an absolute increase in specific substances which upon microscopy appear as more or less characteristic lysosomal inclusions. On the basis of these features neuraminic acid storage disease, first reported as "sialuria," may represent another example of a lysosomal disorder. In a second group of diseases (sulfatidosis, Krabbe's, Gaucher's and Sanfilippo A) an enzyme deficiency is found in fibroblasts, but no ultrastructural abnormalities and little or no chemical storage is demonstrable. In a third group of diseases without a known enzyme deficiency, fibroblasts appear normal in all respects (Batten's disease with curvilinear bodies in the brain) or contain a seemingly nonspecific increase in triglycerides (lipofuscinosis).

GLYCOGENOSIS TYPE IX(GSD IX): DEFICIENT LIVER PHOSPHORYLASE KINASE(LPK) WITH NORMAL k_m (GSD IXa) AND WITH INCREASED k_m (GSD IXb). George Hug, Linda Walling, Gail Chuck and William K. Schubert. The Children's Hospital Research Foundation, Cincinnati, Ohio.

Following our description of deficient LPK in Science 153: 1534;1966, others used WBC to diagnose the condition. We find that normal WBC phosphorylase activity (in μmP/mmN/min) is 1.1 ± 0.9(S.D.); thus WBC studies could not distinguish between normals and/or phosphorylase system defects as may (or may not) exist in WBC of GSD VI, VIII, IX, X (for details: "The Liver", Int.Acad.Path. Monograph No. 13: The Williams & Wilkins, Co. 1972).

We find that LPK of normal human liver (nm rabbit muscle phosphorylase a produced/g/min) is 27.0 ± 6.8 whereas that of liver of GSD IX is <4. Phosphorylase kinase of normal human muscle is 4.8 ± 2.4 and is indistinguishable from muscle of GSD IX. Thus, GSD IX affects liver but not muscle, and is not a generalized disorder.

In our 9 patients with GSD IX inheritance is autosomal recessive in 4 (GSD IXa), and sex-linked recessive in 2 (GSD IXb). We find that apparent k_m of LPK (substrate: rabbit muscle phosphorylase b) is $3.9\pm1.8\times10^{-6}$ in normal controls and indistinguishable ($p>0.1$) from $3.3\pm0.9\times10^{-6}$ of GSD IXa, but is distinguishable ($p<0.01$) from $8.2\pm1.8\times10^{-6}$ in liver of GSD IXb. This observation is consistent with the hypothesis that GSD IXa results from the deficiency of a regulatory mechanism; and GSD IXb from that of a structural gene.

EFFECT OF INTRAVENOUS FAT-FREE ALIMENTATION (IVFFA) ON PLASMA LIPOPROTEINS AND ADIPOSE TISSUE COMPOSITION Avedis K. Khachadurian* Joseph O. Sherman* and Frank S. Kawahara* (Intr. by H. L. Nadler) Clinical Research Center, Children's Memorial Hospital, Northwestern University Medical School, Chicago.

Infants and children unable to maintain adequate oral nutrition were maintained on a fat-free intravenous solution containing 3.3% amino acid mixture, 20% glucose and providing 120 cal/Kg/d. In addition they received fresh plasma, 22 ml/Kg/week.

In 7 newborn infants there were no changes in the plasma lipoprotein electrophoresis (LE) pattern during the IVFFA (mean duration 20 days). The plasma cholesterol (C) was 124 before and 128 mg/100 ml after therapy. The corresponding values for triglycerides (TG) were 40 and 31 and for phospholipid phosphorus (PLP) 8.2 and 6.5. Four infants receiving a combination of intravenous and oral fat-free solutions for a mean duration of 18 days did not show changes in their plasma LE pattern.

In 4 children aged 10-15 yrs. treated for a mean of 11 d. C was 131 before and 110 after, TG were 129 and 135 and PLP 7.8 and 7.0. However 2 of the patients showed a transient rise in TG to 282 and 252 and a prebeta band on LE.

The fatty acid composition of subcutaneous adipose tissue was studied by gas liquid chromatography in 4 infants 20-34 days after initiation of therapy. In two infants the fatty acid pattern was normal but in the other two there was a marked increase in stearate.

ABNORMAL BRANCHED CHAIN AMINO ACID METABOLISM IN VALINE SENSITIVE NON-KETOTIC HYPERGLYCINEMIA (NKH). Ingeborg Krieger, Zwi H. Hart, Jesse F. Goodwin. Children's Hosp. of Mich., Detroit.

Trials with different Tx regimen, begun in a newborn with NKH, showed that glycine control with Na-benzoate and C_1-donors prevented life threatening neurological symptoms, which occurred on 3 occasions as pl. glycine rose gradually over 3 to 6 days while off Tx. These glycine levels were low (4.3 to 5.5 mg%) compared with the maximum after a large oral glycine load (26.3 mg%). Neurological deterioration was more severe after the gradual rise than after the load. The most dramatic clinical response followed a valine load of 150 mg/kg although pl. glycine remained low (valine rose to 7.7 mg%). Toxicity of a smaller load, 50 mg/kg, was enhanced by glycine, since somnolence and a valine rise to 7.3 mg% were only seen when baseline glycine was high. Pl. valine, leucine, and isoleucine were significantly lower than in normal controls; all other AA were normal. Other evidence of an abnormality affecting branched chain AA was the finding of allo-isoleucine in urine. Pl. allo-isoleucine rose after a leucine-isoleucine load. Pyridoxine, which facilitates conversion of isoleucine to allo-isoleucine in vitro, caused a rise in urine excretion from 4.3 to 69 mg/day. High urine values and an allo-isoleucine load were not associated with clinical change. A low valine diet and good glycine control did prevent recurrence of acute neurologic symptoms but not severe mental deficiency. The data indicate that an abnormal branched chain AA metabolism exists in NKH. Hyperglycinemia seems to enhance valine toxicity.

HIGH DOSAGE INTRAVENOUS CALCIUM THERAPY FOR OSTEOPOROSIS AND OSTEOMALACIA IN ANTICONVULSANT TREATMENT WITH HYPOMOBILIZATION. Humbert Latorre and Frederic M. Kenny, Univ. of Pgh. Sch. of Med., Dept. of Ped., Pittsburgh, Pa.

Twenty-five-30% of those receiving the anticonvulsants diphenylhydantoin (DP) and phenobarbital (PB) have low serum Ca and elevated alk. phos. secondary to hepatic hydroxylase induction and inactivation of principal vitamin D metabolites. We found hypocalcemia, hypophosphatemia, and X-ray evidence of rickets in 4 children. Healing occurred in 3 continuing on anticonvulsants during 2-4 mos. of vitamin D 1,000 IU/day and Ca and P supplement. Another patient had seizures treated with DP and PB, and spastic quadriplegia with hypomobilization from infancy. At 9 years she had osteoporosis, osteomalacia, and spontaneous fracture. Rickets and secondary hyperparathyroidism were characterized by Ca 6-8 mg%, P 1.2 - 2.7 mg%, elevated alk. phos., elevated phosphate clearance and low % TRP. Intravenous Ca gluconate (2 g elemental Ca/24 hrs--total 120 g) over several 2 - 3 week periods resulted in healing of rickets and osteoporosis and normal chemistries without deleterious side effects. Intravenous Ca therapy suppresses endogenous parathormone and stimulates thyrocalcitonin. It deserves trial use in juvenile osteoporosis when rapid correction is necessary.

CONVERSION OF MONOSODIUM GLUTAMATE (MSG) TO ACETYLCHOLINE (ACh) IN RAT BRAIN

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Because of striking clinical similarity, and on the basis of biochemical data, we earlier identified Chinese Restaurant Syndrome as transient acetylcholinosis. A peripheral effect of MSG injection in humans has been established (Schaumberg, H.H., et al. Science 163:826, 1969). The present animal study shows that under conditions similar to loading tests in humans, a central conversion of MSG to ACh occurs.

Adult rats were given by stomach tube a solution containing 15 mg of MSG/100 gm body weight, together with 25 μc of L-C^{14} glutamic acid, and sacrificed at 0, 1, 2, 5, 10, 20 and 30 minutes. ACh was isolated from whole brain and biological activity as well as specific radioactivity was measured. At 20 minutes after ingestion, a rise in the biological activity of almost 45% above baseline occurred. More significantly, there was a steady rise in specific radioactivity amounting to a sevenfold increase at 30 minutes. This increase in specific activity occurred in the face of expanded pool size and resultant dilution in the administered labeled substance. The study therefore demonstrates that ingestion of MSG results in increased synthesis of ACh in the rat brain. Whether the conversion of MSG to ACh follows the same sequence of reactions in vivo as in vitro - glutamate to α-oxoglutarate, citrate, acetyl CoA and finally ACh - or some other pathway, can only be surmised at the present.

HOMOCYSTINURIA DUE TO BACTERIAL CONTAMINATION IN PYRIDOXINE-UNRESPONSIVE CYSTATHIONINEMIA

Harvey L. Levy and S. Harvey Mudd. Dept. of Neuro., Harvard Med. Sch., and the Neuro. Ser., Mass. Gen. Hosp.; State Lab. Inst., Mass. Dept. of Pub. Hlth., Boston, Ma.; and the Nat. Inst. of Ment. Hlth., Bethesda, Md.

In an infant with pyridoxine-unresponsive cystathioninemia some urines were strongly nitroprusside positive and contained homocystine whereas other urines contained cystathionine and gave negative or weakly reacting nitroprusside tests. Initially this infant was believed to have a unique metabolic disorder in which homocystine and cystathionine alternately accumulated. However, of these two sulfur amino acids only cystathionine was present in blood. Cultured skin fibroblast studies indicated that methionine remethylation was normal as was cystathionine synthase activity. It was then discovered that those urines not containing a preservative and that were contaminated with bacteria contained homocystine while the urines with preservative contained cystathionine. Subsequent studies proved that bacterial contamination was the cause of the homocystinuria. This is understandable since bacterial cleavage of cystathionine yields homocysteine whereas mammalian cleavage of cystathionine yields cysteine. Thus this artefact could be the cause of the positive nitroprusside tests and the homocystinuria reported in several other patients with cystathioninemia.

OXIMATION OF KETO ACIDS; RELEVANCE TO MAPLE SYRUP URINE DISEASE (MSUD) PHENOTYPE. G. Lancaster, O.Mamer, P.Lamm & C.Scriver. MRC Genetics Group, McGill University, Montreal, Quebec.

The plasma conc. of branched-chain keto acids in MSUD has been rarely studied. Extraction of keto acids as hydrazones is adequate but tedious. Ether extraction of free keto acids for silylation and gas chromatography (GC) coupled to mass spectrometry (MS) yields erratic and low recoveries (<15%). We use hydroxylamine to form oximes before silylation, and to improve recovery and identification. The respective recoveries of α-ketoisovaleric, α-keto-β-methylvaleric and α-ketoisocaproic acids are 66, 94 and 98% from aqueous solutions; and GC/MS identification under various conditions is excellent. Their respective concs. in plasma, in 2 untreated infants with MSUD were up to 0.5, 0.75 and 3mM respectively (50-100 x normal) which was 2/3 that of the corresponding amino acids, valine, isoleucine and leucine. MSUD keto acid conc. is sufficient to inhibit pyruvate dehydrogenase (McArthur & Bowden, Int. J. Biochem. 3, 193, 1972) and during the acute uncontrolled phase of MSUD it may be essential to supply lipid precursors of acetyl CoA to bypass acquired and inherited blocks in the synthesis of this critical metabolite.

PYRIDOXINE-UNRESPONSIVE CYSTATHIONINEMIA

Harvey L. Levy, S. Harvey Mudd, and Phyllis M. Madigan; Harvard Med. Sch., Dept. of Neuro., Mass. Gen. Hosp., State Lab. Inst., Mass. Dept. Pub. Hlth., Boston, Ma.; Nat. Inst. Ment. Health, Bethesda, Md.

An infant girl was discovered to have a cystathioninuria at 3 weeks of age on the basis of a routine urine screening test. She has had constant cystathioninemia and cystathioninuria and in addition excretes an unidentified sulfur-containing ninhydrin-positive compound that contains cystathionine.

In contrast to most individuals with persistent cystathioninemia she shows no biochemical response to pyridoxine (B_6) even when given as much as 500 mg/day (Table). Thus she represents only the second reported case of pyridoxine-unresponsive cystathioninemia (uria). In contrast to this first case our patient is clinically well at 8 months of age. In our experience the magnitude of cystathionine accumulation before pyridoxine supplementation seems to be greater in the infant with pyridoxine-unresponsiveness than in infants with pyridoxine-responsiveness (Table).

	PT		B_6 responsive		Normal
	Before B_6	After B_6	Before B_6	After B_6	
Plasma Cysta. (mM)	0.08	0.09	0.04	0	0
Urine Cysta. (mmoles/gm creat)	18.0	21.8	7.6	0.3	0

ALTERATIONS OF Ca METABOLISM AND RICKETS ASSOCIATED WITH ANTICONVULSANT THERAPY (AT). Fima Lifshitz, Noel Maclaren*, Cornell Univ. Med.Col., North Shore Hosp., Manhasset, N.Y., Univ.of Md.Sch.of Med. &Rosewood State Hosp., Baltimore, Md.

In 288 children institutionalized for mental retardation a fasting serum Ca, P, alkaline phosphatase (alk phos), Na, K, Mg and osmolality and a random urine, Ca, P, Na, K, Mg, creatinine, and aminoacid nitrogen were measured. In 75 pts roentgenograms of long bones were also obtained. Of the 288 pts, 134 received AT of various types and dosages. Pts receiving AT had lower serum Ca and P, lower urinary excretion of Ca and P, higher serum alk phos, and urinary aminoacids than those pts receiving no AT. These changes were correlated with the duration of AT for >1 year but not with dosage employed. The 68 pts receiving combinations of AT had marked reductions in Ca and P and increases of alk phos. Eight of these 68 pts had definitive biochemical and roentgenological rickets and 20 had severe osteoporosis (? osteomalacia). Five of the 8 pts rapidly responded to treatment with 50u of daily oral 25-hydroxycholecalciferol (increased serum P by one week and roentgenologic healing by 1 month). The other 3 pts persisted with rickets when given less than 6000 u of oral vitamin D_2 daily but healed with higher dosages. None of the 66 pts receiving phenobarbital or dilantin alone had rickets but some had significant Ca or alk phos changes. The rapid response to 25-hydroxycholecalciferol suggest that the mechanism for rickets induction in pts on long-term AT is one of interference of vitamin D metabolism at the stage of hepatic conversion from cholecalciferol to its 25 hydroxy metabolite. Lack of sunlight, relative inactivity and chronic recurrent infections may also be factors.

IMPORTANCE OF CONTROLLING FASTING PERIOD AS PART OF THE STANDARDIZATION OF THE ORAL GLUCOSE TOLERANCE TEST. Murthy, D.Y.N., Carl, R. and Jackson, R.L. Department of Pediatrics, University of Missouri Medical Center, Columbia, Missouri.

The fasting level of blood sugar and serum insulin have been found to influence considerably the levels reached at subsequent time intervals during a standard oral glucose tolerance test (OGTT). In 224 non-obese children (4 to 17 years) without a known family history of diabetes in preceding 2 generations (normal children) who were fasted for varying periods of time (10 to 16 hours) the fasting blood sugar levels were 81±24mg/100ml (mean ± 2S.D.) and in 77 of these normal children the fasting levels of serum insulin (median, 97th and 3rd percentiles) were respectively 15μU/ml, 36μU/ml and 1μU/ml. In another group of 47 normal non-obese children of comparable age, a small standard meal depending upon the age of the child was given at 10 p.m. before the commencement of the 10 hour fast. In these 47 children the fasting blood sugar levels were 79±11mg/ml (mean ± 2S.D.) and fasting serum insulin levels (median, 97th and 3rd percentiles) were respectively 23μU/ml, 39μU/ml and 12μU/ml. Standardization of the OGTT should include a controlled intake of food preceding the fast and a fasting period of 10 hours.

INDEPENDENT MODIFICATION OF EFFECTS OF PROSTAGLANDIN E_1 (PGE_1) AND ISOPROTERENOL (IPT) ON CYCLIC AMP LEVELS IN HUMAN FIBROBLASTS. V. Manganiello, J. Breslow, M. Vaughan, Bethesda, Md.

In confluent subcultures of human fibroblasts 2 μM IPT and 5.6 μM PGE_1 increased intracellular cAMP about 10-20 and several hundred fold, respectively, in 15 min (JCI 51: 60a, 1972). In contrast, during the first 2-4 days after initiating cultures with $16\text{-}22\times10^4$ cells, the effect of PGE_1 on cAMP content was only twice that of IPT. During the next 7-10 days the magnitude of the effect of PGE_1 increased and that of IPT decreased such that by 2 weeks the responses to the two agents were similar to those observed in confluent cultures. In cells studied 3 days after subculture, the relative effects of PGE_1 and IPT on cAMP content of cultures initiated at high density ($2\text{-}4\times10^6$ cells) were as observed in confluent or 2 week old cultures. In cultures initiated at lower densities ($1\text{-}3\times10^5$ cells), the effect of IPT on cAMP was greater and that of PGE_1 smaller. These studies were performed on several different uncloned lines which had grown <30 generations. Both PGE_1 and IPT markedly increased the cAMP content of a single line of aging cells (>60 generations), and during the period of senescence the relative effects of PGE_1 and IPT were like those observed in the younger cells. Thus cultured fibroblasts, presumably in response to cell density or another variable correlated with it can within days alter their capacity to accumulate cAMP in the presence of PGE_1 or IPT. Such a system may prove useful in investigations of mechanisms for development and modulation of adenylate cyclase-receptor interactions.

ARSENIC POISONING SIMULATING HEMOLYTIC UREMIC SYNDROME. James E.Musgrave, Yeshawant B.Palwalkar, Robert A.Campbell, and David Linder (Intr.by Richard W.Olmsted) Dept.Peds., Univ.of Oregon Medical School, Portland.

A 10 month old male infant presented with vomiting, bloody diarrhea, oliguria and seizures. Urinalysis showed 3+ albumin, 20-30 RBC, 5-6 WBC and granular casts. BUN rose from 65 to 100 mg%, creatinine 3.5 to 4.1 mg%. Hematocrit fell from 38 to 25.5%, prothrombin and proconvertin was 45% and bilirubin 9 mg%. Peripheral smear showed tear drop, helmet and disintegrated RBCs. Platelets were 180,000 and protamine test was negative. CSF was normal. Despite supportive care, blood transfusion and peritoneal dialysis, his neurological status progressively deteriorated, the EEG becoming isoelectric 48 hours before death. At autopsy the kidneys showed acute tubular necrosis, interstitial edema and mild round cell infiltration. There was fatty metamorphosis with focal necrosis of the liver, gastric ulceration and cerebral edema. Postmortem tissue arsenic levels from the kidney were 2100 mcg/kg and liver 500 mcg/kg which approximate levels found in previously described cases of acute arsenic intoxication. Urine and hair samples from family members did not show significant increase in arsenic levels. It would appear that acute arsenic poisoning may simulate the hemolytic uremic syndrome symptomatically, biochemically and hematologically during infancy.

ETIOLOGIC MECHANISMS IN HYPOCALCEMIC TETANY. James F. Marks, Charles E. Mize, and Angela Fairney. Dept. of Ped., Univ. of Texas Southwestern Med. Sch., Dallas and Westminster Med. Sch. London.

Urinary cyclic-AMP excretion and serum parathormone levels were studied in healthy and hypocalcemic low-birth-weight infants. Healthy prematures of less than 48 hours of age showed a diminished urinary excretion of cyclic-AMP both in comparison to children over 120 hours of age and in comparison to children age 6-14 years. The increase was significant at $P < 0.01$. Specimens obtained on 13 well, low-birth-weight infants under 2 days of age and again at 5-7 days further confirmed the increase with age. There was no difference between well, low-birth-weight infants and full-term infants under 48 hours of age. Infants with symptomatic hypocalcemic tetany under 48 hours of age excreted 0.67 ± 0.47 μmoles/24 hrs, significantly increased over healthy, low-birth-weight infants of the same age, 0.30 ± 0.43 μmoles/24 hrs ($P < 0.05$). There was no statistically significant increase in the hypocalcemic infants after treatment. Preliminary parathormone assay data, by a radioimmunochemical method suited for use in infants, does not show a rise in concentration with age in paired specimens taken at 0-3 days of age and again at 5-7 days of age in healthy, eucalcemic infants. The two sets of data provide a basis for understanding the relationship between parathormone levels and renal tubular responsiveness to parathormone in the development of neonatal tetany.

SERUM ALPHA-L-FUCOSIDASE ACTIVITY IN THE DIAGNOSIS OF FUCOSIDOSIS. Won G. Ng, George N. Donnell and Richard Koch. Divisions of Biochemistry and Medical Genetics, Childrens Hosp. of Los Angeles and the Depts. of Biochemistry and Pediatrics, Sch. of Med., University of So. California.

Fucosidosis is an inborn error of glycolipid metabolism. The clinical manifestations include progressive cerebral degeneration, spasticity, cardiomegaly and bony deformities. Deficiency in alpha-L-fucosidase has been demonstrated in liver, serum, leucocytes and cultured skin fibroblasts. In the present study, the validity of the serum enzyme assay as a diagnostic procedure has been examined. The serum enzyme activity of more than 350 individuals was measured. Included were normal newborns, children and adults and hospitalized patients. For comparison, two affected siblings and their parents were studied. A number of corresponding leucocyte preparations were assayed. There was absence of activity in both plasma and leucocytes for the two affected children. Both of the parents were found to have normal serum activity and half-normal activity in leucocytes. Several normal individuals were found to have extremely low serum alpha-L-fucosidase activity, but normal activity of this enzyme in their leucocytes. The low serum activity appeared to be an inherited characteristic. It is concluded that the serum assay alone of alpha-L-fucoside, cannot be employed for diagnosis. Confirmation must be based upon the leucocyte assay for this enzyme.

BLACK-WHITE DIFFERENCE IN PLASMA TRIGLYCERIDES by George M. Owen, A. Harold Lubin and Philip J. Garry. Ohio State Univ., Col. of Med., Children's Hosp. Dept. of Ped., Columbus, Ohio.

Determinations of total cholesterol and triglycerides (TGC) were made on 1944 plasma samples obtained from 1-6 year old, non-fasting children in 36 states. There were no significant differences between black and white children with respect to cholesterol concentrations. TGC (mg/dl) data are summarized:

Age	Black			White			(p)
(mos)	N	Mean	(SD)	N	Mean	(SD)	
12-23	52	94	(46)	211	118	(72)	< .05
24-35	50	75	(37)	317	112	(61)	< .001
36-47	62	68	(29)	334	103	(63)	< .001
48-59	65	76	(44)	337	104	(69)	< .005
60-71	65	70	(39)	334	97	(54)	< .001

If, from these groups (above), we, 1) eliminated children with TGC levels >125 mg/dl, and, 2) matched groups socio-economically, mean plasma TGC was 76 (SD 24) for 589 white children and 67 (SD 25) for 247 black children (p <.001). White children had 14 percent higher energy intakes than did black children and carbohydrate accounted for 63% of the difference in energy intake. By measurements of height, weight and skinfold thickness, white children were fatter than black children of corresponding ages. Subsequent determinations of plasma TGC from 16 black and 117 white children (fasting) gave mean values of 59 and 69 mg/dl, respectively. Supported by Grant MC-R-390050-06-0 from MCHS, DHEW.

METABOLISM OF TRITIATED 25-HYDROXYCHOLECALCIFEROL (^{3}H-25HCC) IN UREMIC CHILDREN BEFORE AND AFTER SUCCESSFUL RENAL HOMOTRANSPLANTATION. C.F.Piel, B.S.Roof and L.V.Avioli. Dept.Ped. Univ.California,San Francisco, and Washington Univ.,St.Louis.

The metabolism of ^{3}H-25HCC was evaluated before and after successful renal transplantation in two 10-yr old males with progressive uremia and elevated plasma parathyroid hormone (PTH) levels (130 and 250 µl eq/ml--normal 25 ± 3.25). In both children pretransplant derangement in ^{3}H-25HCC metabolism was characterized 12 and 24 hrs following its intravenous injection by: 1) an increase in circulating total radioactivity as well as in ^{3}H-25HCC water-soluble metabolites; 2) higher circulating levels of unaltered ^{3}H-25HCC with no lipid-soluble metabolites identified by Sephadex chromatography; 3) an increase in urinary lipid-soluble radioactivity, identified chromatographically as unaltered ^{3}H-25HCC. Repeat ^{3}H-25HCC studies either 8 or 24 days following renal transplantation, when circulating PTH levels were normal, demonstrated a normal plasma disappearance of ^{3}H-25HCC and the appearance of 3 lipid soluble plasma ^{3}H-25HCC metabolites, one of which was identified as ^{3}H-1,25 dihydroxycholecalciferol. There was also a 60-fold decrease in excreted lipid-soluble radioactivity and no detectable urinary ^{3}H-25HCC.

These observations provide clinical evidence that 25-HCC metabolism and excretion are abnormal in end-stage renal disease and demonstrate the rapidity with which the defect can be reversed following successful renal transplantation.

PROFILES OF SERUM CHOLESTEROL IN (I) NORMAL INFANTS AND CHILDREN (II) CHILDREN WITH CYSTIC FIBROSIS (III) CHILDREN WITH CONGENITAL HEART DISEASE.
Richard G. Pearse,Vera Rose,Michael J. Godman, John D. Keith. Univ. of Toronto,Dept.of Paed.,The Hospital for Sick Children, Res.Inst.and Dept.Cardiology, Toronto, Canada.

Serum Cholesterol levels were estimated in 1923 patients attending the Hospital for Sick Children,Toronto. Children with Renal,Liver,Endocrine or Malignant disease were excluded, leaving 1351 in the study. Normal values were determined for the following age groups:0-3 months(116mg%S.D.29.3);4-11months (129mg%S.D.33.3);1-4 years(150mg%S.D.31.9);5-9 years(160 mg% S.D.31.1);10-14 years(161mg%S.D.33.1);15-20 years(162mg%S.D. 36.0). There were 5 cases of Fredrickson Type II hyperlipoproteinemia and 3 cases of Type IV identified in this study.

Determination of the normal variation of cholesterol levels with age is basic to the identification of the child at risk of developing premature atherosclerotic disease.

Serum cholesterol levels were also determined in 121 cases of Cystic Fibrosis. These showed a statistically significant decrease in cholesterol levels when compared with normals of the same age, although no such decrease was found in the triglyceride levels. Children with congenital heart disease were also studied. Those who were cyanosed(102)were compared with an acyanotic group(282)matched for age. Although there was a marked disparity between the growth percentiles of children with congenital heart disease and normals, no significant difference was detected in serum cholesterol.

SERUM LIPID PATTERN IN REYE'S SYNDROME: ELEVATED TOTAL FREE FATTY ACIDS. J.D.Pollack, M.D.Hilty, R.E.Haynes, N.M.Flynn, and H.G.Cramblett. Depts. of Med. Microbiol. and Ped., Col. Med., Ohio State Univ., Columbus, and Med. Col. Ga., Augusta.

Serum lipids were determined in 12 children with Reye's Syndrome (RS) and 54 children fasting overnite and prior to minor surgery. Assays included: total lipid (TL), triglyceride, total cholesterol (TC), lipid phosphorus (LP), total free fatty acid (TFFA), and a new lipid screening thin-layer chromatographic technique. Specimens from children 3-7 and 8-19 years were examined separately. Levels of triglyceride and LP in children with RS were not different from controls. The 7 children with RS in the 3-7 yr. group had a marked depression of TL and TC compared to 31 controls. TFFA levels in children with RS in both groups were elevated 3 fold compared to controls. The depressed sensorium and elevated serum TFFA levels in our patients are consistent with the view of several workers that FFA, presumably short chained, may induce coma in animals. Our work corroborates the findings of Bourgeois et al, who found that Thai children with RS had elevated serum FFA and apparently hypocholesterolemia. They did not examine sera for TL or LP and concluded that the disease was associated with a mycotoxin. Our data with that of others suggest that in RS there is an impaired release of hepatic lipid and a concomitant mobilization of fatty acids presumably from hepatic or extra hepatic depots. These alterations in lipid metabolism may be relatable to the fatty viscera, vomiting and the altered sensorium characteristic of the disease.

IMPAIRED TURNOVER OF LIPID, PROTEIN AND MUCOPOLYSACCHARIDE IN LEROY'S INCLUSION-CELL DISEASE. Michel Philippart,Seiji Nakatani* Elsa Kamensky* and Klaske Zeilstra* Mental Retardation Unit, The Neuropsychiatric Institute, Los Angeles.

Incorporation and turnover of 1-^{14}C-acetate, 1-^{14}C-galactose and ^{35}S-sulfate were studied in cultured skin fibroblasts from 5 unrelated patients with I-Cell disease and 3 obligatory heterozygotes. Confluent cultures (T-30 flasks) were pulse-labeled for 2 days without serum. Cells were analyzed at regular intervals for 6 weeks. In agreement with the deficiency of a variety of lysosomal hydrolases, the turnover of the different precursors was markedly decreased in cultures from the 3 patients who clinically were the more severely affected. Mucopolysaccharide, neutral lipid, phospholipid, glycolipid and protein kept most of the label incorporated during the pulse throughout the 6-week experiment. The impairment of glycolipid turnover was especially noticeable. Hematoside and globoside incorporated about as much galactose as trihexosyl ceramide, which is the major glycolipid in normal confluent fibroblasts. Heterozygote cells had a normal turnover. Label incorporation into ethanolamine phospholipids was consistently decreased in both homozygote and heterozygote cells. Total phospholipids were increased severalfold, but ethanolamine phospholipids were only 80 percent of normal. Phospholipid concentration and distribution was normal in brain and liver obtained at the autopsy of another patient. In agreement with the pathological findings, the disease appears to be essentially limited to the fibroblasts.

ENZYMATIC ALTERATIONS IN REYE'S SYNDROME: PROGNOSTIC AND EPIDEMIOLOGIC IMPLICATIONS. Charles R. Roe, Lawrence Schonberger, S. H. Gehlbach, and James B. Sidbury, Jr. Duke University Medical Center, Department of Pediatrics, Durham, North Carolina.

A recent epidemic of seven cases of Reye's syndrome were studied at Duke Medical Center. Analyses of serum LDH and CPK isoenzymes and total activities of LDH, CPK, SGOT and SGPT were performed on serial samples from these patients. Although CPK has been normal in Reye's cases only one of the seven presented with a normal level. During the course of the illness all cases had elevations up to 30 times normal. The SGOT/SGPT ratios and tissue specific isoenzymes of LDH and CPK verified the extensive skeletal muscle involvement. These analyses further revealed three types of admission enzymes profiles. Type A: Extensive muscle and liver involvement; Type B: Muscle injury without hepatic indexes; and Type C: Severe hepatic involvement without muscle contributions. Type A was uniformly fatal. There were no fatalities in Types B and C. Similar analyses of siblings and playmates demonstrated qualitatively similar serum enzymatic abnormalities in the Reye's contacts which were not characteristic of control cases. These evaluations may have useful epidemiologic and prognostic significance for further characterization of Reye's syndrome.

LEAD INTOXICATION:DISPLACEMENT OF LEAD FROM RAT ERYTHROCYTES BY IONIZED CALCIUM IN VITRO. John F. Rosen and Asher Haymovits. Albert Einstein Col.of Med.,Montefiore Hosp. & Med.Ctr.,Dept. Ped.,and The Rockefeller Univ.,New York (Intr. by L. Finberg)

Previous studies indicated that ionized calcium (Ca^{++}) and, to a lesser extent, calcitonin, have a potent effect in lowering erythrocyte (RBC) lead content. To define this phenomenon, the effect of Ca^{++} on fresh RBC's from Pb-intoxicated rats was studied in vitro. RBC's were collected, washed, and suspended in a Ca^{++}-free, balanced salt solution at pH 7.40. Varying concentrations of Ca^{++},as $CaCl_2$, were added to the cell suspensions, which were then incubated at 37° C for 15-30 minutes. Ca^{++} concentration was measured by an ion-specific electrode, and Pb was determined by nonflame atomic absorption. After incubation with Ca^{++} in final concentrations of 0,2,4,6, and 8 mg%, RBC-Pb decreased by 0,0,12,24, and 40%,respectively. The highly significant decreases in RBC-Pb occurred within 15 minutes, only above a threshold Ca^{++} concentration of 4 mg%, and followed a linear dose-response curve. Experiments with radioactive Pb^{210} yielded similar results.

These results suggest that Ca^{++} at physiological levels competes with Pb for RBC receptors; and thus, Ca^{++} may control, partially at least, Pb transport from RBC to plasma.Furthermore, fluctuations in Ca^{++} concentration may thereby influence both the dynamics of Pb movement and the toxic activities of Pb. (Supported in part by The John A. Hartford Foundation, Inc.)

LACTATE KINETICS IN PATIENTS WITH TYPE I GLYCOGENOSIS (VON GIERKE'S DISEASE). A. Sadeghi-Nejad, H. Hochman, A. Binkiewicz, E. Presente and B. Senior. Pediatric Endocrine-Metabolic Service, Tufts-New England Medical Center Hospitals, Boston.

Elevated levels of lactate, characteristically present in patients with Type I glycogenosis, could reflect increased formation, decreased utilization or both. To determine the mechanism, a constant infusion of a tracer dose of ^{14}C lactate (0.18 μCi/kg/hr) was administered to four patients with this disorder and to normal volunteers. The specific activities of lactate and of glucose and the percentage of glucose derived from lactate were determined. The rates of inflow and outflow of lactate were calculated by application of Steele's equation.

	Patients (Mean ± SEM)	Normals	p
Glucose concentration (mg%)	49 ± 3	79 ± 2	<.001
Lactate concentrations (mg%)	43.6 ± 3.6	5 ± 0.2	<.001
Glucose from lactate (%)	24 ± 2.5	13 ± 1.3	<.001
Lactate inflow (mg/kg/hr)	242 ± 41	108 ± 9	<.005
Lactate outflow (mg/kg/hr)	276 ± 45	110 ± 11	<.005

The results indicate that the elevated lactate represents a dynamic pool with increased rates of inflow and outflow. Any error introduced by recycling of the labeled lactate, if present, would lower the calculated flow rates; therefore, the true rates could well be even higher than were observed. Furthermore, proportionately more glucose is derived from the lactate. The findings are compatible with a rapid cycling between glycogen and lactate, presumably generating branch points for release as glucose.

LEAD INTOXICATED CHILDREN: PLASMA LEVELS OF 25-HYDROXYCHOLECALCIFEROL (25-HCC). John F. Rosen and Martin Roginsky,Albert Einstein Col. of Med.,Montefiore Hosp. & Med.Ctr.,Dept. Ped., and Nassau County Med. Ctr.,New York. (Intr. by L. Finberg)

Speculations concerning the high incidence of lead (Pb) toxicity during the summer have focused upon enhanced intestinal absorption of Pb, resulting from a relative excess of sunlight-induced formation of vitamin D. Plasma levels of 25-HCC were measured by competitive radioassay in 3 groups of children: I - Blood Pb<40 μg%, II - 40-60 μg%, and III - 60-126 μg%. The levels of 25-HCC, in ng/ml ± S.D., were: I - 27 ± 5, II - 25 ± 5, III - 26 ± 5. The mean concentration of 25-HCC was similar within groups and between groups,regardless of the time of year. Curiously, in vitamin D-deficient, Pb intoxicated rats, strikingly high blood and plasma Pb levels were observed; and, in such animals, a more gradual, less dramatic decrease in erythrocyte Pb content was noted after calcium challenge, compared to similarly intoxicated-challenged rats without vitamin D deficiency.

These results suggest that a theorized excess of vitamin D measured as 25-HCC,cannot account for the seasonal variation in Pb toxicity; and,an alternative explanation is not readily apparent. The experimental data suggest that, in the intestine, Pb-Ca^{++} competition for specific binding proteins (receptors) may prove to be the cardinal factor in Pb absorption. (Supported by The John A. Hartford Foundation, Inc.)

METABOLIC RESPONSE TO I.V. GLUCAGON IN INFANTS OF DIABETIC MOTHERS (IDM).
David Schiff, Jacob V. Aranda, Eleanor Colle, Apostolus Pappageorgiou, Solomon H. Reisner and Leo Stern. Dept. of Paediatrics, University of Alberta, Edmonton. McGill University-Montreal Children's Hospital Research Institute.

Glucose, Insulin, Growth Hormone (HGH) and Amino Acids (AA) responses to I.V. glucagon (300 μg/Kg) on the 1st day of life was measured in 10 IDM's and 10 Normal infants of comparable gestational age. Fasting glucose was lower in the IDM group (28.7 ± 5.4 mg%, vs. 48.6 ± 4.9 mg%; P<0.02). The response pattern to glucagon was similar, but the levels achieved were significantly different (P<0.01). The fasting insulin levels were greater (65.1 ± 22.4 μu/ml, vs. 1.9 ± 0.5 μu/ml; P<0.02), and the response pattern was similar; although HGH fasting levels were similar (21.7 ± 6.3 ng/ml, vs. 19.8 ± 4.8 ng/ml; P>0.1), and the levels achieved were similar, the pattern of response was different (P<0.01). The AA levels were similar, as were the responses to glucagon. 5 IDM were hypoglycaemic, and these had significantly attenuated GH responses when compared to the 5 normoglycaemic IDM's and normal control infants (P<0.01). In addition to hyperinsulinism as a basis for hypoglycaemia, this data suggests that HGH may play an important role in the maintenance of normal glucose hemeostasis in IDM's.

PHENFORMIN IN CHEMICAL DIABETES IN CHILDHOOD. Arlan L. Rosenbloom, Chandra M. Tiwary, Raymond F. Bianchi, and Frank T. Chin. Univ. Fla. Col. Med., Dept. Ped., Gainesville, Florida.

The recognition of stable chemical diabetes in children and young adults raises questions of prophylaxis to prevent or delay progression to overt disease and/or the development of vasculopathy. Ten 5- to 20-year-old patients who had persistently abnormal glucose tolerance were given drug or placebo in a double-blind study. The total dose of 2-3 mg per kg per day was divided into 3 doses with meals. Treatment period was 3 months. Those on placebo were given drug for a second 3 months in single-blind design. Glucose and insulin levels during oral glucose tolerance testing on 2 occasions before treatment and after the 3-month courses were compared. There were 7 drug treatment and 6 placebo periods. No differences existed between mean glucose or insulin responses during tolerance testing of any of the groups (placebo and drug baseline, post-placebo, post-phenformin). Four courses of placebo were associated with improvement of glucose tolerance, none with worsening. Three courses of phenformin were associated with improvement, slight in 2 who subsequently developed insulin dependence and substantial in 1; 2 patients had worsened tolerance on drug. Several patients had improved tolerance 3 months after the study on no treatment. Gastrointestinal side effects in 2 patients were corrected by dose reduction. Conclusion: phenformin has no apparent beneficial effect on the natural history of chemical diabetes in children and young adults.

THE CELLULAR CHARACTERIZATION OF I-CELL DISEASE AND THE IDENTIFICATION OF THE STORAGE SUBSTANCE BY SULFATE LABELING AND ENZYMATIC HYDROLYSIS. Roy D. Schmickel, Jack J. Distler, and George W. Jourdian (Intr. by William J. Oliver). Univ. of Michigan Medical Center, Univ. Hosp., Dept. of Ped., Ann Arbor, Mich.

I-Cell disease has stimulated intense investigative interest because of its pivotal position in the manifestation of storage diseases. The physical and radiological characteristics of this disease are typical of the mucopolysaccharidoses, yet the pathognomonic urinary findings are absent. Fibroblasts from I-cell disease were incubated in the presence of $^{35}SO_4^{--}$. The cells accumulated high levels of sulfate in comparison to normal cells while both types excreted similar levels of mucopolysaccharides into the media. With the removal of $^{35}SO_4^{--}$ from the media, intracellular sulfate was retained within the I-cells at levels much higher than normal. A portion of the labeled substance in the cells had a characteristic size not seen in other mucopolysaccharide cells. The retained,sulfated intracellular substances were characterized as mucopolysaccharides by electrophoresis and chondroitinase hydrolysis. Additionally, the retention of sulfated substances was not "corrected" by co-culture with normal cells. I-cells contained no βgalactosidase activity and were not corrected by the addition of purified galactosidase.

THE KINETICS OF PHENYLALANINE DISPOSAL IN PKU AND PKU-VARIANTS
A.J. Schneider, Upstate Medical Center, Syracuse, N.Y.
(Introduced by Frank Oski)

Data from 21 cases of phenylketonuria (PKU) and 10 PKU-variants have been analysed mathematically. The time course of fall in blood phenylalanine (Phe) concentration was fitted by least-squares to the exponential equation:

$$Phe = L + Ae^{Bt}$$

B is the 1st order rate constant, L is the asymptote at infinite time, and A is the zero time elevation above L. The fraction, $1-e^{B}$, gives the daily fractional decline in blood Phe when it is measured in days. The values of $1-e^{B}$ ranged from .05 to .43 in PKU, with a mean of 0.24. Corresponding values in PKU-variants were .47 to .96 with a mean of 0.76. The standard deviation for a single value was 0.13. The difference in means is highly significant statistically and provides a practical guide to early diagnosis. Thus if a newly discovered infant is placed initially on a diet containing 200 mg. Phe, 4 gm "protein"/kg, and 120 calories/kg/day, the child with PKU and the PKU-variant will generally differ in response as shown in the table if both start with blood levels of 35mg/dl. and eventually end with levels of 5 mg/dl.

Time (days)	0	1	2	3	5	Infinite
PKU	35	27.9	22.5	18.4	12.8	5.0
PKU-Variant	35	13.3	7.3	5.6	5.04	5.0

It is noteworthy that a child who had been treated for 7 years as a case of PKU showed a fractional daily decline of 0.7 when data from the first year of life were examined in retrospect. Without diet restriction his levels now run 13-14 mg/dl.

A VARIANT OF HUMAN GALACTOKINASE WITH ELEVATED ACTIVITY.
T.A. Tedesco, K. Miller, P. Rabin, R. Diamond, E. Rawnsley, and W.J. Mellman. Dept. of Human Genetics, Univ. of Pa. Sch. of Med., Philadelphia.

A 29 year old black female has been found with RBC galactokinase (Gk) activity that is three times the mean activity of the adult black population. Since umbilical cord RBC's have 3 to 4 times the Gk activity found in adults, we are investigating the possibility of a persistent fetal galactokinase in this patient. Preliminary data suggests that this patient's RBC galactokinase has an elevated Km for galactose and a faster moving electrophoretic component than the enzyme in control adult RBC's and consistent with that found in fetal RBC samples. The mean galactokinase Km for 10 adult RBC samples is 0.159 (S.D. 0.0289, S.E. 0.0087), and that for 12 cord RBC samples is 0.315 (S.D. 0.1442, S.E. 0.0398). The patient's Km for galactose is 0.255.

SUCCINYL-CoA:3 KETOACID CoA-TRANSFERASE DEFICIENCY: A 'NEW' PHENOTYPE?
Matthew W. Spence, Mary G. Murphy, Harold W. Cook, Beverly A. Ripley, and Juan A. Embil. (Intr. by Richard B. Goldbloom) Dept. of Ped. & Bioch., Dalhousie Univ., Halifax, CANADA.

A previously healthy boy was first hospitalized at 7 mo. and again at 15 mo. with acute onset of severe metabolic acidosis and ketonuria. Two sibs (1m.,1f.) had died aged 6 mo. in acute severe metabolic acidosis; the parents share common ancestors through all 4 grandparents. Between acidotic episodes the child remained at home symptom-free on a high-carbohydrate, low-fat diet. A third acute episode, at 21 mo., was corrected biochemically, but the child died. Outstanding findings were: serum β-hydroxybutyrate (β-HyB) and acetoacetate (AcAc), 29 and 1.3 μmoles/ml respectively during acidosis and <0.2 μmoles/ml during symptom-free periods; only these acids were significantly elevated in the urine. $^{14}CO_2$ production by WBC and cultured fibroblasts from labeled leucine, propionic acid, glucose and succinate was normal; fibroblasts metabolized β-HyB only slightly. Liver had normal β-HyB dehydrogenase activity. Kidney, brain and fibroblasts had *normal* AcAc thiolase activity but *absent* (<5% of *normal*) succinyl-CoA:3 ketoacid CoA-transferase activity. This case differs from the only other reported (J.Clin.Invest. 51, 493, 1972), in older age of clinical onset, different clinical course, and different glucose metabolism by cultured fibroblasts. These differences and continuing family studies suggest a 'new' variant with a more optimistic prognosis.

25-HYDROXYCHOLECALCIFEROL (25OHCC) LEVELS IN PRECOCIOUS PUBERTY PATIENTS RECEIVING MEDROXY-PROGESTERONE ACETATE (MPA)
Joseph Thomas, Viswanathan Balachandar, Platon J. Collipp, Martin Roginsky, Vaddanahally T. Maddaiah, and Shang Y. Chen. Nassau County Med. Ctr., Dept. of Ped., East Meadow, N. Y.

It is now well-established that Vitamin D must be hydroxylated in the 25 position by the liver initially before it can function. The possible influence of sex hormones in this pathway has been implicated in the pathogenesis of osteomalacia of pregnancy. We studied the blood levels of 25 OHCC by competitive radioassay in nine normal children, seven patients (6 girls, 1 boy) with precocious puberty receiving MPA. The mean value of 25 OHCC in serum of normal children (2-17 years) was 32.1 ± 8.9 ng/cc, in serum of patients receiving MPA 50.2 ± 12.2. The difference was highly significant $p < .005$. No significant difference in the serum calcium, phosphorous, or alkaline phosphatase values was observed. The serum level in 1 male patient receiving MPA was 66 ng/cc. In another study 60% of pregnant patients had significantly low levels of 25 OHCC in the blood. The low levels in pregnancy together with the high levels in one male patient receiving MPA suggest that the high levels observed with MPA treatment might be related to the MPA itself rather than estrogen. The possible mechanisms are:

(1) **increased absorption of Vitamin D**
(2) **increased production of 25 OHCC by induction of the hepatic hydroxylating enzyme**
(3) **increased half life of 25 OHCC.**

DIMINISHED RESPONSE TO PHYTOHEMAGGLUTININ BY PERIPHERAL LYMPHOCYTES CULTURED FROM PATIENTS WITH CYSTIC FIBROSIS.
Mark W. Steele, Joan B. Rodnan, Ona L. Wood, Univ. of Pittsburgh Sch. of Med. and Children's Hosp., Dept. of Ped. Pittsburgh.

It has been suggested that Cystic Fibrosis (C.F.) may reflect a lysosomal disorder whereby there is an inability to metabolize excessive amounts of cellular substrate. Bartman et al (J.Ped.76:430,1970)found increased lysosomal size in fibroblasts cultured from C.F. patients. Antonowicz et al (Ped.Res.6:803,1972) found elevated levels of lysosomal α-glucosidase in lymphocytes cultured from C.F. homozygotes. We have previously reported increased accumulation of exogenous heparin in peripheral lymphocytes cultured from C.F. patients compared either to their parents or normal controls. We now report that after peripheral lymphocytes are cultured for 72 hrs. in media containing phytohemagglutinin(PHA)and heparin, there is a 3 fold lesser response($p<0.01$)to PHA(measured by incorporation of tritiated thymidine into DNA) with cells cultured from C.F. homozygotes compared to cells cultured from either normal controls or C.F. heterozygotes. When heparin is absent from the media, this difference lessens somewhat. Hirschhorn et al(Sci.147:55,1965)have suggested that PHA stimulates lymphocyte mitosis by inducing lysosome formation - the latter being an essential preliminary to cell division. Our data suggest that in lymphocytes cultured from C.F. patients, the lysosome function necessary for mitosis is being interfered with; at least in part by excessive accumulation of exogenous heparin.

EFFECT OF A DIET CONTAINING SMCS IN TYPE II HYPERLIPOPROTEINEMIA AND THE POTENTIAL DIAGNOSTIC VALUE OF SERUM SURFACE TENSION DETERMINATIONS.
Chandra M. Tiwary and Owen M. Rennert, Univ. Fla. Col. Med., Dept. Ped., Gainesville, Florida.

Hypercholesterolemia affects 1/200 newborns. Current modes of treatment to prevent development of atherosclerosis and coronary artery disease are unsatisfactory. We describe the apparent beneficial effect of S-methyl-L-cysteine sulfoxide (SMCS) dietary therapy in an 18-month-old white female with type II hyperlipoproteinemia. The patient was first noted to have xanthomata at age 11 months. These increased in size and number during the next 5 months. Her height was 79 cm (<10%), weight 9.6 kg (<3%). Xanthomata were present on her palms, soles, elbows and abdomen. Cardiac exam was normal and she had no hepatosplenomegaly. Serum cholesterol level was 1292 mg%, serum triglycerides 191 mg% and serum electrophoresis revealed increase in β-lipoproteins. Serum T_4 and BUN were normal. A diet low in cholesterol and high in polyunsaturated fatty acid was instituted. Her cholesterol fell to 800 mg% in 3 months; no subsequent further fall was observed. A 6-month trial of clofibrate was not effective. A diet of cabbage, cauliflower and raddish containing about 350 mg of SMCS was instituted; within 5 weeks the serum cholesterol fell to 300 mg% and subsequently the xanthomata regressed. We are investigating the use of surface tension methods to follow cholesterol levels, which are rapid and require only 0.1 ml of serum.

NORMAL GLUCONEOGENIC SUBSTRATE AND HORMONAL RESPONSE AND EFFECTS OF EPHEDRINE (EPH) IN IDIOPATHIC GLUCAGON (GLG) UNRESPONSIVE (KETOTIC) HYPOGLYCEMIA (IGUH). Chandra M. Tiwary, Arlan L. Rosenbloom and Owen M. Rennert. Univ. Fla. Col. Med., Dept. Ped., Gainesville, Fla. (Intr. by Gerold L. Schiebler).

Defective gluconeogenesis or deficiency of the substrate alanine (Ala) has been implicated in IGUH. Four controls and 9 IGUH had measurements of blood glucose (G), insulin (I), free fatty acids (FFA), amino acids, lactic acid (LA), cortisol (C), renin activity (RA) and urinary catecholamine (UC) at various times during oral G tolerance test, 24-hour fast and fed and fasting GLG stimulation. In IGUH studies were repeated after oral Eph treatment for 2 days. Glycemic response to GLG was normal at 24 hrs. of fast in controls and absent in IGUH. Changes in FFA, I, LA and RA levels and UC were not significantly different between the 2 groups. Mean C level was 21 µg % ± 2 (SEM) at the beginning and 44 ± 11 at the end of 24 hrs. of fast and 60' after GLG rose to 72 ± 14. Ala level at the beginning of fast was 3.27 mg% in controls and 1.86 in IGUH; at 24 hrs. of fast Ala fell to .68 mg% in controls and to .54 mg% in IGUH. After GLG there was a variable rise in Ala in controls and in IGUH unrelated to glycemic response in controls and its absence in IGUH. On therapy 4 of 7 IGUH showed normalization of fasting G and GLG response peak Ala level after GLG was similar in a responsive (2.02 mg%) and in an unresponsive (2.30 mg%) IGUH. Maturational delay of enzymes involved in glycogen synthesis or degradation is postulated as the pathogenesis. Eph probably acts through enzyme induction.

DISTURBED PROTEIN METABOLISM IN CEREBRAL GIGANTISM. Chandra M. Tiwary, Arlan L. Rosenbloom, and Jaime L. Frias. Univ. Fla. Col. Med., Dept. Ped., Gainesville, Fla. (Intr. by G. Schiebler)

We have studied a tall 15-year-old boy with mental retardation, coarse facial features, large head, hypertelorism, prognathism, large hands and feet, advanced bone age, abnormal EEG and clumsiness. Several members of the paternal family had a similar phenotype also associated with low intelligence. GH response was normal following oral glucose, IV arginine, insulin, vasopression and sleep, but there was no response to propranolol-primed glucagon stimulation. GH response to all stimuli except sleep was suppressed by chlorpromazine (CPZ). Other hypothalamopituitary responses (ACTH, TSH, FSH-LH) were normal. ADH-stimulated cortisol rise was abolished on CPZ. Arginine-induced insulinemia was augmented by CPZ with no difference in glucose levels, a paradoxical response in view of depressed GH levels. Nonessential to essential plasma amino acid ratios were significantly low; glycine/valine .14 (nl .83), glycine/isoleucine .62 (nl 2.28), glycine/leucine .36 (nl 1.23), alanine/valine .4 (nl 1.35), alanine/isoleucine 1.8 (nl 3.71), alanine/leucine 1.03 (nl 2.04). Total protein was at the upper limit of normal (8.5 g%), IgM was elevated (315 mg%, nl 30-190) and α_1-antitrypsin was low (150 mg%, nl 212± 32). Low levels of α_1-antitrypsin without hepatic or pulmonary dysfunction, high IgM without evidence of infection and high total proteins and essential amino acids imply a general abnormality in protein metabolism. Low nonessential to essential amino acid ratio is probably related to accelerated growth.

THE EFFECT OF DITHIOTREITOL ON CYSTINOTIC AND CONTROL FIBROBLASTS IN CULTURE. Francoise M. Verroust and Jerry A. Schneider, Univ. of California, San Diego, Sch. of Med., Dept. of Ped., La Jolla.

Dithiotreitol (DTT, Cleland's Reagent) causes the loss of free-cystine from cystinotic fibroblasts (Cys.F.) and has been suggested as a treatment for this disease (Lancet 1:811, 1970). In 1.0mM DTT (added every 8 hours), the free-cystine content of Cys.F.'s decreased from 6.7 to 1.3nmole ½cystine/mg protein in 2 days, but growth ceased and the cloning efficiency was 0. Lower concentrations of DTT caused less loss of free-cystine, but led to a surprising enhancement of growth rate (about 20%) and cloning efficiency (see table). These effects have not been reported previously and indicate that sulfhydryl compounds may play an important role in the regulation of cell growth.

DTT	Cystine in Cys.F. (nmole ½cystine/	Cloning Efficiency control F.	cys.F.
(mM)	mg protein)	(%, mean±S.D.)	
---	6.7	33±12	25± 8
0.2	5.3	91± 9	86±14
0.5	3.4	35± 9	49±12
1.0	1.3	0	0

NEONATOLOGY

First Session

RESPIRATORY DISTRESS SYNDROME WITH AMNIOTIC FLUID LECITHIN/SPHINGOMYELIN RATIOS GREATER THAN TWO. Richard D. Zachman; Earl B. Olson, Jr.; Thomas A. Frantz; Margaret E. Bergseth; and Stanley N. Graven, Univ. of Wis., Dept. of Peds., St. Marys Hosp. Med. Ctr., Madison, Wis.

Numerous articles have stressed the validity of the amniotic fluid Lecithin/Sphingomyelin (L/S) ratio in prediction of fetal lung maturity and respiratory distress syndrome (RDS) in the newborn. Until now, there have been no apparent cases of RDS occurring with a L/S of >2.0. We are reporting in detail the characteristics of 8 patients with L/S >2.0 that developed RDS. Four patients had severe RDS, requiring continuous positive airway pressure (CPAP), four had moderate RDS.

The total lecithin ranged from 1.5-3.9 µMoles/100 ml and sphingomyelin from 0.5-1.5 µMoles/100 ml. The L/S ratio ranged from 2.3-3.3. A control fluid is run in parallel with each new sample. The range of values of this control is within ±10% of the mean. In the 4 patients with severe RDS, the L/S of the cold acetone precipitable phospholipids was 2.1-3.5 in 3 cases, 1.8 in the other. The foam stability was positive in 3, intermediate in the fourth. The RDS index (Hobel, et al: J. Peds. 81:1178, 1972) was >4 at 4-7 hours of age and the severity score was 4-7. The gestational ages were 33-37 wks. Low APGAR scores, hypoglycemia, hypotension, or hypothermia were not present. Two were born by cesarean section. The RDS severity score was still 5-7 at 48 hours of life, and 3 of 4 were on CPAP at that time (indication, p_aO_2 <50 mmHg in F_iO_2 90%). The 4th needed CPAP at 60 hours of age.

THYROID FUNCTION IN NEWBORN RESPIRATORY DISTRESS SYNDROME (RDS) Ralph A. Redding, Celina Pereira, and John T. Barrett (Intr. by L. Stern) Lying-In Hospital, Memorial Hospital, and Brown Univ., Providence, R.I.

Animal studies have shown that thyroid hormones stimulate lung surfactant production, increase lamellar inclusions within type II pneumonocytes, and accelerate lung maturation in utero. Because lung surfactant deficiency is believed to play a primary role in human RDS, thyroid function was assessed in 79 premature newborns with or without RDS, in 22 normal full term babies, and in their respective mothers. At birth, cord blood total serum thyroxine (T_4) was significantly lower in 40 babies with RDS when compared with 39 other prematures (No RDS), both as a group (7.2 vs 8.9 µg%) and when compared by gestational age. Normal term babies displayed a higher mean value than either group (9.4 µg%). Differences in thyroid-binding proteins did not account for this difference since "Free Thyroxine Index" ($T_4 \times T_3$ uptake/100) was also significantly lower in the RDS compared with No RDS prematures (2.10 vs 2.53). Two days post-delivery the surviving RDS babies mean T_4 rose less than No RDS prematures (8.2 vs 10.3µg%). No significant difference in intrapartum venous T_4 was found among the mothers of premature and full term babies. We conclude that the lower T_4 levels in the RDS prematures appear to be independent of maternal thyroid status, reflects either a decreased secretion or increased utilization of T_4, and may be related to inadequate lung surfactant production. (Supported in part by a Hines Estate Grant).

PROLONGED RUPTURE OF MEMBRANES ASSOCIATED WITH A DECREASED INCIDENCE OF RESPIRATORY DISTRESS SYNDROME (RDS). Charles R. Bauer, Leo Stern and Eleanor Colle. McGill Univ.-Montreal Children's Hosp. Research Inst., Montreal, Quebec.

Total blood corticoid (TBC) levels were measured using a protein binding method in 14 premature infants without RDS. They were divided into two groups by duration of rupture of membranes (ROM) > or < 16 hours. Results were as follows:

		TBC µg% ± SEM				
No.	Dur.ROM	4 hrs	12 hrs	24 hrs	48 hrs	72 hrs
8	>16 hrs.	36.4±13.5	46.2±10.6	19.0±1.6	12.6±0.9	11.5±1.8
6	<16 hrs.	18.1± 2.8	14.3± 1.6	12.5±1.7	15.7±2.0	12.9±2.2

Subsequently, TBC and cortisol (F) levels were measured in both mother and infant in 11 additional premature deliveries with results shown below:

		TBC µg% ± SEM	
No.	Dur.ROM	Maternal	Infant(1 hr. of age)
5	>16 hrs.	48.6 ± 10.3	26.8 ± 5.5
6	<16 hrs.	29.5 ± 1.7	15.2 ± 2.4

TBC were higher in maternal and infant plasmas when ROM occurred > 16 hours before delivery. Cortisol paralleled TBC, with highest values after longest ROM. Clinically no RDS occurred in the 5 infants born > 16 hours after ROM. In contrast, 3 of the 6 infants born < 16 hours after ROM had RDS. In addition, of 44 consecutive infants with RDS, none were born > 16 hours after ROM. This suggests a relationship between prolonged ROM, elevated corticoid levels and a protective mechanism against the development of RDS.

SIMPLE DEVICE FOR PRODUCING CONTINUOUS NEGATIVE PRESSURE IN INFANTS WITH IRDS. Eduardo H. Bancalari, Tilo O. Gerhardt, and Ellen F. Monkus (Intr. by William W. Cleveland), Dept. of Pediatrics, Univ. of Miami, Sch. of Med., Miami, Florida.

The beneficial effects of continuous transpulmonary positive pressure in idiopathic respiratory distress syndrome (IRDS) are well established. Continuous positive and continuous negative pressure appear to be equally effective in raising PaO_2. The major advantage of negative pressure (CNP) is that it does not require an endotracheal tube or a tight face mask.

A simple inexpensive unit for applying CNP has been developed using a plastic box, 22 x 22 x 8.5 cm, provided with two plastic iris diaphragms (Air-Shields) in the opposing larger faces. Distance between the diaphragms is 15 cm. One is placed on the neck of the infant and the other seals the box over the pelvic area. CNP is developed by connecting the box to a simple vacuum pump, or even to the wall suction. The box is equipped with an aneroid manometer and a safety valve. It is placed in a regular Isolette or warmer unit. Nursing care can be provided without difficulty and without interrupting the negative pressure.

Eighteen patients with IRDS have been treated with these new units. Results were comparable to those found in forty patients previously treated with CNP using a modified Air-Shields Incubator Respirator. The safety and simplicity of this new device has changed our criteria for starting CNP and presently we use CNP in any patient requiring more than 70% oxygen to maintain an arterial PO_2 over 60 mm Hg.

CONTROLLED TRIAL OF CONTINUOUS POSITIVE AIRWAY PRESSURE (CPAP) IN RDS AND A SIMPLIFIED APPLICATION BY THE NASAL ROUTE. John Kattwinkel, Avroy Fanaroff, Chul Cha, David Fleming, Roberto Sosa, Robert Crumrine, and Marshall Klaus. CWRU Sch. of Med., Depts. of Ped. and Biomed. Eng., Cleveland.

Previous reports of CPAP in the treatment of the infant respiratory distress syndrome (RDS) have shown improved oxygenation and suggested increased survival; there are no controlled studies. We have performed a sequential controlled trial of CPAP vs. O_2 alone in severe RDS (PaO_2 <60 mm. Hg in $FiO_2 \geq 70\%$). 29 infants paired for birthweight and age at admission to study had been randomly assigned to either CPAP or O_2 control when trial halted. Failure was defined as PaO_2 <50 mm. Hg in $FiO_2 = 100\%$. Study success: CPAP, 10/15 (67%); O_2 control, 2/14 (14%); $p < .05$. CPAP improves oxygenation and significantly reduces duration of exposure to high FiO_2.

To simplify CPAP we developed a Silastic® device which cannulates the nares for 1 cm. and provides CPAP while eliminating previously described complications. Pressures of 10-12 cm. H_2O are easily obtained before significant "blow-off" through the mouth becomes evident. Nasal CPAP was instituted in another 22 consecutive RDS infants fulfilling above criteria. 18 (82%) were treatment successes and survived. There were no pneumothoraces, perhaps due to mouth blow-off. In summary, a controlled trial has shown CPAP to be an effective treatment for RDS; application by the nasal route is simple, safe, and available to any intensive care nursery.

ABNORMAL PARTIAL THROMBOPLASTIN TIME AND SURVIVAL IN RESPIRATORY DISTRESS SYNDROME: EFFECT OF EXCHANGE TRANSFUSION. R.A. deLemos, G.W. McLaughlin, H.F. Koch, and H.W. Diserens. Dept. of Pediatrics, Wilford Hall USAF Medical Center and University of Texas Medical School, San Antonio, Texas. Intr. by M.J. Sweeney.

Recent advances in the therapy of infantile respiratory distress syndrome have markedly reduced mortality (Ped Res:6,146, 1972), but deaths from intracranial and/or intrapulmonary hemorrhage remain a major problem. Coagulation screens consisting of prothrombin time, partial thromboplastin time and platelet count were performed every 12 hours in 50 consecutive infants with respiratory distress syndrome. 16 of 20 with PTT > 90 seconds died; all of these had recognizable antemortem hemorrhage. 15 of 17 infants with PTT < 60 seconds survived; both of the fatalities had intracranial hemorrhages.

Attempted connection with platelets (count < 30,000), fresh frozen plasma and Vitamin K failed to result in any significant decrease in mortality from hemorrhage.

Therefore, 40 newborns with PTT > 90 seconds were divided into two treatment groups. One had attempted connection of PTT and low platelet counts by platelet infusion and transfusions of fresh frozen plasma. The second group underwent 2 volume exchange transfusion with fresh heparized blood every 12 hours if the PTT was > 90 seconds. 15 of the 20 infants in the exchange transfusion group survived as compared to 4 of 20 controls.

It is not cleat which of the possible factors in fresh heparinized blood was responsible for the marked decrease in fetal hemorrhage.

CORD BLOOD COAGULATION STUDIES - ASSOCIATION WITH MORBIDITY IN HIGH-RISK INFANTS. Wm. E. Hathaway, Chularatana Mahasandana, Edgar L. Makowski, and Frederick C. Battaglia. University of Colorado Medical Center, Denver; University of Southern California-Los Angeles County Medical Center, Los Angeles.

A prospective study of cord blood for coagulability, circulating anticoagulants, viscosity, and evidence for disseminated intravascular coagulation was done in a group of high-risk infants in order to determine the relationship of hematologic changes to neonatal diseases associated with thrombotic and hemorrhagic complications. Thrombelastograms, antithrombin III (AT-III) levels, blood hematocrit and viscosity, platelet counts, and assays for factors I, V, VIII, and fibrin split products were done in 75 normal control infants and 110 infants delivered from high-risk pregnancies. Results: (1) Preterm infants are hypercoagulable and AT-III deficient. (2) The most marked hypercoagulability, AT-III deficiency, and highest incidence of IRDS were seen in infants delivered from mothers with premature labor or third-trimester bleeding (47%). (3) In contrast, infants undergoing intrauterine distress (acute fetal distress or pre-eclampsia) often showed high AT-III, V, and VIII levels. (4) Offspring of third-trimester bleeders were anemic. In conclusion, hypercoagulability and AT-III deficiency in the cord blood of certain high-risk infants is associated with neonatal complications such as IRDS, intracranial hemorrhage, necrotizing enterocolitis, and localized large vessel thrombosis.

CARBON MONOXIDE DIFFUSING CAPACITY IN HEALTHY AND DISTRESSED NEWBORNS. D.B. Klain, A.N. Krauss, P.A.M. Auld., Dept. of Ped., Cornell Univ. Med. Coll., New York City.

Hypoxemia may be due to shunting, maldistribution of ventilation/perfusion ratios, or alveolar-capillary diffusion block. The role of diffusing capacity in the hypoxemia of the neonate was examined with serial studies of 21 healthy newborns, birth weight 765 to 4720 grams, and 8 infants with hyaline membrane disease. Arterial Po_2, Pco_2 and pH were measured in room air or 100% O_2. End-tidal CO_2 was measured and alveolar-arterial (A-a) gradients calculated. Functional residual capacity was measured by helium dilution. CO diffusing capacity (D_{Lco}) was measured by the steady-state method of Bates. Dead space/tidal volume ratios were determined from collected gas. D_{Lco} was significantly related to weight (r=0.6), body area (r=0.6), and lung volume (r=0.8), and poorly related to Pao_2 (r=-0.4) and A-a oxygen gradient (r=0.4). Significant reductions in D_{Lco} were seen only in the two most severly ill infants. This data suggests that low D_L is not a major cause of hypoxia in newborns, and that loss of lung volume (atelectasis) is the major cause of lowered Pao_2 in neonates. The reduced D_{Lco} found in HMD thus appears to be due to reduced pulmonary area available for diffusion due to collapse and not to a specific diffusion block.

PLACENTA TO FETUS TRANSFUSION IN UTERO DURING ACUTE HYPOXEMIA IN FETAL LAMB. K. Omori, D.L. Phelps, G.C. Emmanouilides, & W. Oh, UCLA Sch. of Med., Harbor Gen. Hosp., Torrance, Ca.

Ten studies on 6 fetal lambs (120-140 days gestation) were performed to evaluate the effect of acute hypoxemia (H) on the placental blood flow (PBF), distribution of blood volume in the placenta (PBV) and the fetus (FBV) in utero. Hysterotomy was performed for femoral artery (FA) and umbilical vein (UV) catheter placement. PBF and PBV were determined by dye dilution method with injection of indocyanine green into the FA catheter (below renal arteries) and continuous sampling via the UV. Total placental fetal blood volume (TPFBV) was measured by I^{131} RISA. FBV was the difference between TPFBV and PBV. Following a control period, the maternal inspired O_2 conc. was reduced to 10%. Maternal and fetal PaO_2 dropped from 82.0± 6.0 (M± SEM), to 47.0± 4.3 and from 19.2± 0.6 to 11.7± 0.9mmHg respectively. Blood pH and $PaCO_2$ were unchanged in both mother and fetus. During H the PBF remained unchanged. The PBV was reduced (86.8± 3.9 to 66.8± 2.0 ml/kg, $p < .05$ at baseline and 15-25 min. post H period respectively) and remained low (71.0± 3.0 ml/kg) at the end of the 30 min. recovery (R) period. TPFBV remained unchanged during H and R periods. Thus, FBV was elevated ($p < 0.05$) from baseline of 74.6± 8.0 to 85.7± 10.0 ml/kg at 15-25 min. post H period. These observations suggest that intrauterine transfusion from placenta to fetus may occur during fetal H and the fetus may retain the transfused blood up to half an hour after the relief of the hypoxemic insult.

INCREASED UTERINE ACTIVITY & FETAL DETERIORATION DURING EXPERIMENTAL HYPERTHERMIA. H.O. Morishima, B. Glaser, W.H. Niemann, S.S. Daniel & L.S. James, Division of Perinatology, College of Physicians & Surgeons, Columbia University, N.Y., N.Y.

Maternal urinary tract infection has been related to premature labor & birth asphyxia. Experiments have been performed in 22 pregnant baboons to determine whether maternal hyperpyrexia in the absence of infection might be related to increased uterine activity and fetal deterioration. After placing catheters into maternal & fetal arteries, & thermocouples in maternal colon & fetal esophagus, maternal temperature was raised gradually to 42° C in 11 animals by applying external heat. Hyperthermia was maintained for one hour, then slowly decreased. 11 animals served as normothermic controls. Temperature gradient between fetus & mother increased significantly from control value of 0.47° C to 0.77° C after the mother had stabilized at 42° C. Hyperpyrexia was accompanied by hypotension & tachycardia in both mother & fetus. A moderate maternal metabolic acidosis developed & all fetuses became significantly acidotic & hypoxic, pH falling from 7.34 (control) to 7.16. Uterine activity increased significantly during hyperthermia, accompanied by late deceleration of the fetal heart rate which occurred at a higher fetal pH and PO_2 than is seen under normothermic conditions. When normothermia was restored, uterine activity & fetal tachycardia decreased, but fetal hypotension & acidosis persisted. These experiments demonstrate that maternal hyperthermia alone causes an increase in uterine activity & fetal deterioration.

Supported by NIH grant GM09069

A QUANTITATIVE METHOD FOR DETERMINATION OF NON-ALBUMIN-BOUND BILIRUBIN IN NEWBORN SERUM. Jorgen B. Jacobsen and Richard P. Wennberg, (intro. by W. Alan Hodson) Dept. Ped., Univ. of Wash., Seattle, WA.

A new binding test is developed which is sensitive to nanomolar concentrations of unbound bilirubin (B_u), requires only 25 µl serum, and is easy to perform in about five minutes. The principle for the method is as follows: B_u is oxidized by ethyl hydroperoxide (EtOOH) and peroxidase, whereas albumin-bound bilirubin (AB) is not oxidized. Thus, the velocity of oxidation is proportional to the concentration of B_u. Since the establishment of the equilibrium $AB \rightleftarrows A + B_u$ is very fast compared to the velocity of the oxidation B_u + EtOOH → X + EtOH, the velocity is measured as the initial decrease in total bilirubin concentration.

We have examined sera with bilirubin concentrations from 5 to 30 mg/100 ml. The concentrations of B_u were very low, ranging from .001 to .003 mg/100 ml.

The albumin binding capacity was determined by adding bilirubin to the serum in vitro and measuring B_u. B_u rises slowly until the primary site becomes saturated, and then increases more rapidly with further increments in total bilirubin. Binding capacities of the primary binding site in both cord and post-natal sera ranged from 0 to 30% less than would be expected on the basis of albumin concentration, suggesting that a variable fraction of the newborn albumin is incapable of binding bilirubin at the primary site.

NEONATOLOGY

Second Session

A FLUX DAY--OBSERVATIONS ON FACTORS INFLUENCING THE LIGHT ENVIRONMENT OF INFANTS. Jerold Lucey, Thomas Wolk, James Bottoggi and Richard Klein. Depts. of Ped. and Botany, Univ. of Vt., Med. Ctr. Hosp. of Vt., Burlington.

Little is known about the normal light environment of the newborn infant. Nothing is known about the optimal light environment for an infant. Concern has been expressed that phototherapy might expose infants to risks from excessive radiant energy exposure. Observations in our nursery using a spectroradiometer indicate that a normal infant in an incubator near a window exposed to 6 hours of summer sunlight receives approximately 4700 microwatts/cm^2 at 450 mu in 24 hours (a flux day). An infant receiving 24 hours of conventional phototherapy receives 3800 microwatts/cm^2. A number of factors (skin surface area, thickness, and fetal age) profoundly affect the actual light dosage an infant receives. Transmission of light through the skull of 2 premature infants was studied at postmortem and found to be about 10% at 450 mu. These and other important environmental factors affecting phototherapy will be discussed. The hypothesis that a component of physiologic jaundice is due to "light deprivation" will be presented.

Study supported by NIH Grant PHS R01 05561, United Cerebral Palsy Grant and Easter Seal Foundation Grant.

SUBSEQUENT GROWTH OF LOW BIRTH WEIGHT INFANTS FOLLOWING PHOTOTHERAPY. Joan E. Hodgman, Annabel J. Teberg, Paul Y.K. Wu. (Intr. by Paul F. Wehrle) Los Angeles County-USC Medical Center.

A one-year follow-up study of the effect of phototherapy in our nursery on subsequent growth of low birth weight infants revealed that treated infants were significantly smaller than controls. These infants (Group-I: 29 infants) were followed for an additional year, and a second group of infants (Group-II: 57 infants) treated with continuous and intermittent phototherapy in the nursery with concurrent controls have also been followed for two years. Weight, length, and head circumference (H.C.) were recorded as percentile of expected growth for chronologic age on standard curves. At two years, a significantly greater number of treated infants in Group-I were still below the 10th percentile in all measurements; however, the number with H.C. below 2 S.D. was no longer different from controls. (3/14 or 21% vs. 2/15 or 13%). There were no significant differences in measurements at two years between the continuously or intermittently treated and control infants in Group-II (H.C. below 2 S.D.: 2/19 or 10% vs. 0/18 vs. 2/20 or 10%). In addition, the two control groups, although not concurrent in time, were not different. Although the differences in growth between treated and control infants in Group-I remain unexplained, this study clearly demonstrates that phototherapy in the nursery has no deleterious effect on later growth.

BIOLOGIC RHYTHM IN LOW BIRTH WEIGHT NEWBORNS: PLASMA HUMAN GROWTH HORMONE. Thomas R.C. Sisson, and Allen W. Root. Temple University School of Medicine, Philadelphia. (intr. by Angelo Di George).

Plasma HGH was measured in 37 newborn infants 1500 to 2500 Gm. birth weight and <38 weeks' gestation, at 8 hour intervals from 48 to 96 hours of age. Infants were placed in 4 groups, depending on time of birth, so that sampling included each 2 hour interval around the clock: Group A 0800, 1600, 2400 hrs.; Group B 1000, 1800, 0200 hrs.; Group C 1200, 2000, 0400 hrs.; and Group D 1400, 2200, 0600 hrs. Subjects were in ordinary nursery lighting from 0800 to 2200 hrs., and in the dark from 2200 to 0800 hrs.

There was no significant difference in HGH between infants 1500-2000 Gm. and those 2000-2500 Gm., nor in mean HGH related to hours after birth. However, differences were found in relation to time of day - peak levels were observed at 0000-0200, 1000, and 1600 hrs., minima at 0600, 1200, and 2000 hrs.

Rhythmic alteration of plasma HGH occurred under conditions of cycled lighting by at least 48 hours of age. The rhythm was ultradian in character, but appropriate analysis indicates an underlying circadian periodicity similar to adults. Since onset of sampling occurred in both light and dark cycles it would appear the rhythm is endogenously not exogenously entrained. As HGH does not cross the placenta, it is unlikely that maternal HGH periodicity itself set the neonatal rhythm, although they are nearly identical in time.

INSENSIBLE WATER LOSS IN PRE-TERM INFANTS EXPOSED TO NONIONIZING RADIANT ENERGY. Paul Y.K. Wu, Joan E. Hodgman. (Intr. by John A James.) Dept. of Pediatrics, LAC-USC Medical Center, Los Angeles.

Current use of nonionizing radiant energy for thermoregulation in neonates produces changes in insensible water loss (IWL). Using a Potter's Electronic Scale with sensitivity of 250-500 mg, IWL (expressed as ml/Kg/hr) was measured in 112 well pre-term infants. The IWL study consists of 4 periods of 30 min. each. Skin temperature was maintained at 36.5°C with servo-control, ambient relative humidity ranged from 35-55%. Heart rate, respiration rate, ambient and rectal temperature was recorded. IWL was found to decrease progressively from mean=2.5 in infants with B.W. 800 gm. to mean=0.6 in infants with B.W. ≥2000 gm. Mean IWL values on exposure to 3 different radiant heat sources were:

Birth Wt. (Gm)	Incubator	Heat Shield	Nichrome Wire	Infra Red Lamps
1001-1500	1.6	2.3	2.5	3.1
1501-2000	0.7	1.4	1.5	2.2

In 10 infants IWL was found to increase from a mean of 0.7 before phototherapy to 2.14 during phototherapy. In 3 infants with RDS, IWL was found to average 3.5 ml/Kg/hr. The observed differences in IWL points to the need for IWL studies in calculating fluid requirements and balances in neonates under varying states of health and modes of management.

Supported in part by a Grant from Mead-Johnson Company

INTRALIPID: USE IN THE NUTRITIONALLY COMPROMISED PREMATURE INFANT. Betty Bernard and Salvador J. Garcia. (Intr. by Paul F. Wehrle). USC School of Medicine, L.A. Calif.

Four premature infants (780, 940, 1200, 1320 gm.) with hyaline membrane disease on assisted ventilation & gastrointestinal dysfunction or surgery required parenteral nutrition (PN) for 5 to 24 days at the 7th, 8th, 12th and 20th day of life. This protocol specifically tailored each component of the PN fluid to the patient on a daily basis. PN components in gm/Kg/d were: Dextrose 10-15, Amino Acids 1-3, Fat 1-3, plus Na, K, Ca, Mg, Phos, Vitamins and trace elements (Cu, Fe, Zn, I, Mg and Co). This regimen required continuous surveillance of balances (Na, K, Ca, Phos, Nitrogen & fluids). Positive nitrogen balance could initially be attained on 1 gm. of protein and fat and 10-12 gms of dextrose per Kg/d. Weight gain averaged 17.8, 11.7, 42 and 17 gm/d for the 4 infants. No adverse clinical changes were noted during continuous 24 hr. Intralipid administration in temperature, respiratory rate, pulse rate, ambient O_2 requirements, blood pH, pO_2, or pCO_2. Hyperlipemia was not noted in any case. Whether this is due to 24 hr. fat administration or adequate trace element (Ca & Zn) supplementation is not known. The 3 smallest infants succumbed to infection. Autopsies included special fat staining of lungs, liver, spleen and kidneys. No consistent pattern of pathology could be associated with use of intravenous fat.

EFFECTS OF CORRECTION OF LATE METABOLIC ACIDOSIS ON GROWTH AND MINERALS IN VERY LOW BIRTHWEIGHT (<1.3 kg) INFANTS (VLBW). Ingeborg C. Radde, Graham W. Chance, Kathryn Bailey, John O'Brien, Gillian M. Day, Josepha Sheepers (Intr. by Paul R. Swyer). Depts. Neonatology & Endocrinology, Research Inst., Hosp. for Sick Children, Toronto, Canada.

Because insufficient correction of late metabolic acidosis may lead to arrested weight gain we compared two regimens of $NaHCO_3$ treatment in VLBW infants between days 14 and 60: Group "A"-sporadic correction of base deficit (BD) to <-8meq/l and Group "B"-continued maintenance of BD within 1 SD of normal (-3.2±1.7 meq/l). The infants were matched for birthweight and gestational age. Infants were weighed daily and from day 14 received "20 cal" Enfalac (200 ml/kg/day) with added vitamin D (400 IU/day)."A" babies had lower blood pH than "B" babies and higher plasma ionic Ca and total Mg between days 20 and 39 ($p<0.05$). Body weight increments did not differ but daily length gains were higher in "B" than in "A" babies ($p<0.05$), both in babies appropriate for gestational age (AGA) and small for gestational age (SGA). 7/12 AGA babies showed radiological evidence of mild osteoporosis. In 9/21 instances "A" babies showed higher net excretion of Ca than intake. Hypercalciuria did not occur in spite of high Ca^{++}/total Ca ratio in plasma resulting from hypoproteinemia. Thus (1) careful longterm control of late metabolic acidosis in VLBW infants increases their growth in length; (2) AGA babies fed "20 cal" Enfalac retain inadequate Ca to allow for their more rapid bone growth despite high doses of vitamin D; (3) with this formula malabsorption of Ca, Mg and fat is common in VLBW infants.

TRACHEAL SUCTION IN MECONIUM ASPIRATION
Pauline Ting and June P. Brady, Children's Hosp., Dept. of Pediatrics, San Francisco, (Intro. by S. Giammona)

One hundred and eleven meconium-stained infants were admitted to the Intensive Care Nursery from 1970-1972. 109 were term and 103 weighed over 2500 gm. 5% of infants with meconium staining weighing over 2500 gm died of meconium aspiration syndrome. This was 20 times the mortality of infants over 2500 gm (N=5515) delivered during this period.

Comparison was made of gestational age, maternal age, type of anesthesia and analgesia, duration of meconium staining, type of delivery, cord complications, abnormalities of fetal heart rate, Apgar Score, method of resuscitation, and x-ray findings with signs of respiratory distress and mortality. Thirty-eight infants (34.2%) developed respiratory distress and 8 died. Infants who died (mean survival time 72 hours) received multiple therapies including mechanical ventilation. At autopsy typical meconium aspiration was found without evidence of sepsis. There were no significant differences in any parameter evaluated between affected and non-affected infants, except for the use of tracheal suction. 86 infants had adequate tracheal suction immediately after delivery 27% had respiratory distress and only one died, 25 infants did not receive immediate tracheal suction 60% had respiratory distress and 7 died ($P< 0.001$).

Tracheal suction immediately after delivery in this study reduced the incidence of respiratory distress and neonatal mortality.

IMPAIRED ARTERIAL OXYGENATION AFTER FEEDING IN NEWBORN INFANTS. Max Klein*, V.C. Harrison* and H. deV. Heese* (intr. by W.H. Tooley). Dept. of Pediatrics, University of Cape Town, S. Africa.

We studied the effects of gavage feedings on arterial blood pH and oxygen and carbon dioxide tensions (Pao_2 and $Paco_2$) in 15 babies (mean age 119 hrs., mean weight 2401 gm). Most babies were recovering from respiratory distress and all were receiving supplemental inspired oxygen. A technique was used which enabled feedings to be given and arterial blood to be sampled without disturbing the baby or his environment. Arterial blood was drawn immediately before, and again 10 minutes after feeding with 8 ml/kg body weight of 5% dextrose in water.

Mean Pao_2 fell 29.4 mm Hg ($p < 0.002$ by paired t test) and significant hypoxemia ($Pao_2 < 60$ mm Hg) occurred in 3 babies. $Paco_2$, pH and bicarbonate were not affected, indicating that hypoventilation did not cause the drop in oxygen tension. Pulse and respiratory rates were also unchanged.

The results suggest that oxygenation of newborn babies may be impaired by relatively minor degrees of abdominal distension. This finding may have particular importance for babies with borderline oxygenation and could explain the occurrence of some apneic spells after intermittent feeds in such babies.

A WALKING BLOOD DONOR PROGRAM FOR REGIONAL NEONATAL INTENSIVE CARE NURSERIES (ICN's). Braden E. Griffin, Mohamad R. Sedaghatian, Belton P. Meyer, Montgomery C. Hart, William J. Daily. St. Joseph's and Good Samaritan Hosps., Phoenix.

Because present techniques for banking and distribution of blood products fail to meet urgent and frequent small volume requirements of critically ill neonates, a walking donor program for the rapid collection and transfusion of fresh heparinized whole blood was established. Donors meeting AABB standards are screened from hospital personnel and pertinent data is registered in the blood bank and nursery. Hepatitis-associated antigen screening is done monthly. Heparinized blood is collected and administered through an in-line filter. During a six-month interval 82 of 550 infants admitted to two regional ICN's received 294 transfusions. Sixty infants received 79 transfusions for shock verified by central arterial and venous pressure and hematocrit.

Type of Shock	# Infants	# Transfusions	# Survivors
Hypovolemic	43	59	36
Hemorrhagic	10	13	2
Septic	7	7	2

Three infants required 8 transfusions for accidental acute blood loss. There were 10 transfusions for concurrent losses. One hundred ninety-seven transfusions were given for symptomatic, chronic anemia. No transfusion complications have been noted. An effective program has been developed for the immediate transfusion of small volumes of fresh heparinized blood to neonates.

NEONATOLOGY

Read by Title

THE VALUE OF TOTAL PARENTERAL ALIMENTATION (TPA) IN NEWBORNS WITH SURGICAL PROBLEMS
Abaci, F.U., Arulanantham, K., Klotz, D., and Ghadimi, H. Department of Pediatrics, Downstate Medical Center, State University of New York and Methodist Hospital, Brooklyn, New York

Ten neonates with signs of intestinal obstruction received TPA for periods varying from 1½ to 18 days. All but one underwent major abdominal surgery and two underwent 2 operations. Six were term and 4 had gestational ages of 30, 32, 35 and 36 wks. The birthweight ranged from 1410 to 4470 gm. Four died and 6 survived. Because of the small number of the patients and the heterogeneity of the clinical material, statistical evaluation of morbidity and mortality is of limited significance. However, certain trends are obvious. Five patients received commercially available amino acid solution and developed clinical complications concomitant with abnormal biochemical parameters. Three with birthweight of 4470, 2440 and 3400 gm died at 3, 4 and 5 wks of life respectively. Five counterparts were treated with a newly developed amino acid formula (Ghadimi Formula, G.F.). Only 1 patient in this group died: this infant received TPA for only 34 hrs postop while showing signs of a second perforation (increased subdiaphragmatic gas shadow). The GF group also included the smallest baby (1140 gm). This patient was subjected to 2 laparotomies (ileal perforation at 21 days, adhesions at 58 days), followed by TPA therapy of 8 and 14 days duration. In general, the final outcome had a better correlation with nutritional and biochemical management than with the magnitude of surgical trauma.

UMBILICAL ARTERY, UMBILICAL VEIN, AND MATERNAL BICARBONATE CONCENTRATION AT BIRTH. B. D. Ackerman and P. J. Chou, Univ. of Calif., Irvine College of Med.,(Intr. by Philip Lanzkowsky)

Umbilical artery pH and PCO_2 were determined by the Astrup technique for 41 newborn infants. Plasma HCO_3^- was calculated from the pH and PCO_2, and from these data a neonatal pH-PCO_2-HCO_3^- buffer line was constructed. This buffer line intercepts HCO_3^- values of 18.6 and 22.4 mEq/L at PCO_2 values of 40 and 60 mm Hg. For 40 infants simultaneous samples were obtained of umbilical artery (UA) and umbilical vein (UV) blood, and for 7 of these infants a simultaneous sample of maternal venous blood was obtained. Oxygen saturation (SO_2) was measured for each sample. For each infant, the points representing the pH-PCO_2-HCO_3^- coordinates for UA and UV were connected by a line on a buffer diagram. These lines had a slope different from the buffer line described above, such that the UV HCO_3^- and pH were higher than would be predicted solely from the drop in PCO_2 from UA to UV. The "pathway" from the UA to the UV values was separated into 3 components: the measured decline in PCO_2 with the "expected" HCO_3^- and pH changes predicted from the buffer line; a decline in HCO_3^- and pH due to increased SO_2; an "unexpected" increase in HCO_3^- and pH bringing the blood to the measured UV values. The mean "unexpected" increase in UV HCO_3^- was 1.7 mEq/L. The magnitude of this "unexpected" increase in UV HCO_3^- was significantly related to the maternal HCO_3^- concentration. These data indicate that, between birth and cord clamping, partial correction of the infant's metabolic acidosis may occur as a result of HCO_3^- transfer from maternal to fetal circulation.

AN ELECTROPHORETIC METHOD FOR DETECTION OF FREE BILIRUBIN BINDING CAPACITY IN SERUM OF NEWBORNS. S. Athanassiadis, D.R. Chopra, M. Fisher, J. McKenna. Dept. of Pathology and Dept. of Pediatrics, Valley Medical Center, Fresno, Calif.

A new method has been developed by which we can detect the "free" bilirubin in serum, as well as predict the reserve bilirubin capacity, and the available binding sites of serum under study. In this method, the patient's serum is electrophoretically separated on a cellulose acetate membrane, along with a series of increasing concentrations of bilirubin added to the patient's serum. The addition of bilirubin ranges from 1 mg.%-16 mg.%. On the same membrane an application of crystalline bilirubin solution has been used as a positive control of free bilirubin. Following that, the membrane is stained with ICTOTEST Reagent, Ames Labs, giving a positive reaction at the points where bilirubin is present.

At normal levels of total bilirubin the only protein fraction to give a positive bilirubin reaction on the plain serum specimen, is albumin. As the concentration of bilirubin increases, more binding sites of other protein fractions appear to be saturated with bilirubin (a_2-B_1-globulins), and only after that point does free bilirubin appear on the membrane, at the next highest concentration.

DATA ANALYSIS ON INFANTS BORN AT RISK. Billy F. Andrews (Intr. by William Thurman) Univ. of Louisville Sch. of Med., Dept. of Ped., Louisville, Kentucky.

One of the major areas of interests for physicians and lay people is the area of development of health care research. Before adequate research into care can be performed, problems must be defined and data collection systems devised. Over the past few years our center for high risk infants and children has been involved in developing such forms. A data collection system for maternal, labor and fetal conditions as well as further conditions after birth will be shown. Forms for yearly evaluation of health status by medical and paramedical disciplines will be discussed. A form for parental evaluation of health and development of their infants will be presented. All forms have been adopted to computer analysis and can be used or altered to suit the needs or aims of other institutions.

FACTORS IN THE REGIONALIZATION OF INTENSIVE CARE FOR NEWBORN INFANTS, A.G.M. Campbell (intr. by Charles D. Cook), Yale Univ. Sch. of Med., Dept. of Pediatrics, New Haven.

Through 1970-72, 702 infants were referred to a central intensive care nursery from community hospitals. Overall utilization of the center increased by 8% in spite of a 20% fall in live births. The pattern of referral indicated an increase of 29% for premature infants; 16% for infants with severe respiratory difficulty; and 18% for infants with severe congenital abnormality excluding congenital heart disease. There was a reduction in admissions for congenital heart disease which paralleled the fall in birth rate. Factors in regionalization include: educational programs in the medical center and community hospitals, personal contact between center staff and community, physician experience and facilities within each community hospital, and distance from the center. Most important was the initiation of an infant transportation service staffed by physicians and nurses from the central nursery. The current referral "rate" for the region is 9.2 per 1000 live births which represents less than 1/3 of the infants who could benefit from transfer (calculated from our data to be 30/1000 LB). Two major priorities require special emphasis to improve access to adequate care and facilities for all infants. There must be consolidation of obstetric services to eliminate (as geographic locations permit) those with fewer than 1000-1500 annual births. Referral to the regional center for certain categories of high risk pregnancy and neonate should be considered mandatory to satisfy minimal acceptable standards of care.

HEMODYNAMIC ALTERATIONS DURING SLOW AND FAST EXCHANGE TRANSFUSION. Jacob V. Aranda* and Avron Y. Sweet. Case Western Reserve Univ. at Cleveland Metropolitan Gen. Hosp., Dept. of Pediatrics, Cleveland, Ohio.

Blood withdrawal and infusion during exchange transfusion (ET) result in fluctuation of venous return to the heart, blood volume and blood pressure (BP). The latter might be minimized or prevented by adjusting the rate of ET. To test this possibility, aortic pressure (P_{Ao}) of 2 pre-term infants was recorded continuously with a strain gauge transducer attached to an umbilical artery catheter during ET done via an umbilical vein catheter. A 2 volume ET was divided into 5 phases: 3 Slow (10cc in and out every 3 min) alternating with 2 Fast (10cc in and out every 45-60 sec). Baseline P_{Ao} was recorded between phases and changes in P_{Ao} were calculated. The changes from baseline during withdrawal follow:

	Changes in Pressure (Mean±SE mmHg)			
	mean aortic	systolic	diastolic	pulse
Slow	- 4.9±0.6	-11.0±1.0	- 3.3±0.7	- 5.0±1.0
Fast	-14.5±1.6	-22.5±2.3	-10.4±1.4	-12.2±1.7

Pressures return to or near baseline during infusion of the Slow phase but do not during the Fast infusion. Recovery to baseline occurs after 1½-2 min., but since Fast phase withdrawal is done before recovery, BP drops further. The data show some rate dependent BP alterations occurring during ET and that Slow ET causes negligible changes making it preferable to Fast ET. The marked fall in BP during Fast ET may be a determinant in the morbidity/mortality associated with ET.

HYPOVOLEMIA RESULTING FROM A TIGHT NUCHAL CORD AT BIRTH William J. Cashore and Robert H. Usher, Neonatal Unit, Royal Victoria Hospital, and Dept. of Pediatrics, McGill University, Montreal.

Infants with tight umbilical cord loops around the neck may shift blood into the placenta as pressure on the cord obstructs the umbilical vein earlier than the umbilical artery. If these infants require cord ligation and transection to deliver the body, significant blood loss may result.

Blood volume determinations (I^{125} Albumin) have been performed after birth in 11 such infants, with the following results: Blood Volume 75.3 ± 6.4 ml/kg, Red Cell Volume 25.8 ± 4.1 ml/kg, Plasma Volume 49.5 ± 5.1 ml/kg, and Venous Hematocrit 39.0 ± 4.6%. The value for Red Cell Volume is significantly lower ($p<0.005$) than that found previously in this Unit by Saigal for infants with immediate cord clamping (32.4 ± 2.6 ml/kg). This difference represents a blood loss at birth of 20% (or about 50 ml in a full-term infant), with a resulting decrease in body iron content.

Tight nuchal cord accounts for a large proportion of otherwise unexplained cases of neonatal anemia occurring in this hospital. Affected infants are pale and somewhat hypotensive after birth, sometimes with a systolic heart murmur, but otherwise appear to be unaffected by the blood loss.

POSTNATAL GROWTH OF LOW BIRTHWEIGHT INFANTS GIVEN EARLY INTRAVENOUS NUTRITION, William J. Cashore, Reza Sedachatian and Robert H. Usher, Neonatal Unit, Royal Victoria Hospital, and Dept. of Pediatrics, McGill University, Montreal.

A 2-year experience with glucose, Amigen, and Intralipid infusions in prematures under 1500gm and of normal weight for gestation now includes 25 patients. Scalp vein infusions of up to 100 cal/kg/day have been used to supplement milk feeds, with Amigen and Intralipid started after 24 hours of age. Seven infants weighing 700-1000gms survived 1 week, and 4 remain alive. One of 18 weighing 1000-1500gms has died.

Of 18 new patients studied since our abstract submitted last year, the following observations were made in 15 who survived after 5 to 30 (median 13) days of parenteral nutrition: mean birthweight was 1170gms and gestation 28-5/7 weeks. Birthweight was regained in 8 days; average gain over birthweight was 295gm at 21 days and 555gm at 30 days of age. At 36 weeks gestation, average weight was 2425gm, length 45.4 cm, and head circumference 33.3 cm, all approximating values for normal intrauterine growth.

Transient hyperglycemia, electrolyte imbalance, and hyperlipemia occasionally developed, especially in babies of 1000gm or less. No infections resulted from intravenous nutrition in these infants, all of whom had their scalp vein needles changed every other day. With careful monitoring, it is possible to supplement inadequate milk intake in small premature infants, using a balanced parenteral diet without need for indwelling central catheter.

COLONIZATION OF STAPHYLOCOCCUS AUREUS AND HEXACHLOROPHENE BATHING OF NEWBORN INFANTS. C.T. Cho, H.C. Miller, D.C. Jenkins and P. Leung. Dept. of Ped. & Path., U. of Kansas Medical Center, Kansas City, Kansas.

A diluted hexachlorophene (8 ml. of 3% solution diluted in about 120 ml. of warm water with a final concentration of approximately 0.2%) has been used for bathing newborn infants in our newborn nursery. During the hospital stay the normal full term infants were washed once soon after arriving in the nursery, generally within the first 4 hours after birth; infants requiring extended care were washed 3 times per week.

On Dec. 17, 1971, "Ivory soap" substituted hexachlorophene for bathing of babies, but hexachlorophene was retained for personnel hand washing. The average rate of staphylococcus aureus colonization was 3.4 (0.8-9.7) % for each quarter of 1968-1971. An abrupt increase in colonization had occurred two weeks after the discontinuation of hexachlorophene. In 1972, the rate was 15.3% for its first quarter and a case of severe staphylococcal abscess was observed in a premature infant. Hexachlorophene was reinstituted and "Ivory soap" was discontinued on April 1, 1972. Followup studies showed a high, but gradual reduction of colonization rate.

Our findings suggest that 1) it seems possible to maintain low rate of staphylococcal colonization with a relatively low concentration (0.2%) of hexachlorophene for bathing of newborn infants, 2) when using such low concentrations of hexachlorophene an extended period is required in order to reduce an established staphylococcal colonization.

TOLERANCE OF THE NEWBORN INFANT FOR INTRAVENOUS LIPIDS William J. Cashore and Robert H. Usher, Neonatal Unit, Royal Victoria Hospital, and Dept. of Pediatrics, McGill University, Montreal.

Tolerance for 10% Intralipid suspension was studied in 13 newborns receiving peripheral intravenous nutrition. When 1.5 - 2.0gm/kg of fat were given in 2 hours, mean value for serum triglycerides rose from 66mg% to 1030mg%, then fell to 238mg% within 6 hours after the infusion was stopped. In the same time period, free fatty acid levels rose from a mean of 0.6 meq/L to 2.3 meq/L (range 1.1 - 5.2), then fell again to 0.6 meq/L within 6 hours. Cholesterol levels did not change significantly. Clinically significant alterations in clotting factors and in blood O_2 or CO_2 tensions have not been found in these infants. Borderline hypoglycemia (31mg%) was seen in 1 infant and a mild compensated metabolic acidosis in 4; the highest triglyceride levels reached were in 2 infants small for gestational age. These data are consistent with those of Gustafson et al (Acta Paediat. Scand. 61: 149, 1972).

Preliminary results suggest that maintaining a constant lipid infusion over the whole day, rather than giving the daily dose over a short period, usually prevents marked elevations of serum triglycerides and fatty acids. Constant infusions of 3-4gm/kg/day of Intralipid have been given for 5-30 days in premature infants of 1000-1500gms without lipemia. Infants under 1000gm often show reduced tolerance, with lipemia developing at 2-3gm/kg/day of constant infusion.

DETECTION OF FREE BILIRUBIN IN NEWBORN HYPERBILIRUBINEMIA - INFLUENCE OF DIRECT BILIRUBIN AND INFUSED ALBUMIN IN VIVO. D.R. Chopra, S. Athanassiadis, J. McKenna, M. Fisher, Dept. of Pediatrics and Dept. of Pathology, Valley Medical Center, Fresno, Calif.

One hundred samples of serum were tested for the presence of free bilirubin by electrophoresis as described by Athanassiadis and Chopra et al. Free bilirubin was detected in 32 of the specimens. In 20 of the specimens with free bilirubin (total bilirubin more than 15 mg.%) the ratio of total bilirubin/total protein was 3.8 or greater, which fits with the suggestion of Odell based on the stoichiometric method developed by him. (This ratio suggests saturation of available binding sites in the albumin.) In the remaining 12 samples (total bilirubin less than 15 mg.%), however, free bilirubin was seen where the total bilirubin/total protein ratio was less than 3.8. This was also true when there was a high direct bilirubin present in the serum, i.e. free bilirubin was present at lower levels of total bilirubin with high direct bilirubin levels.

In the presence of increased levels of direct bilirubin there was no apparent binding of bilirubin to the globulin fractions, as was seen with indirect hyperbilirubinemia. Free bilirubin was seen at 9, 10 and 12 mg. % in babies weighing less than 1000 grams. I.V. administration of salt free albumin increased the total serum bilirubin by 1.2 mg.% and serum albumin by an average of 0.7 grams %. The effect of phototherapy is being investigated.

BLOOD VOLUME STUDIES IN COOMBS' POSITIVE INFANTS. G. Cassady, R. Milstead, H. Dweck and Y. Brans. Univ. Ala. Sch. Med., Birmingham.

Blood volume was estimated prior to exchange transfusion in 32 infants with non-hydropic Coombs'-positive disease. Rh (22) or ABO (10) disease was commonly accompanied by asphyxia (Apgar < 7; 10 patients), anemia (hct. < 35%; 11), elective prematurity (< 37 weeks; 11), and operative delivery (13). Studies were performed within 4h. of birth in 13 babies, within 12h. in 21, and ranged from 24-120h. in the remaining 11. Blood volume was estimated from the 10-min. T-1824 albumin space and venous hematocrit.

The mean blood volume of 86.7ml/kg (± sd 13.4) in the prematures studied was nearly identical to the mean of 87.9ml/kg ± 13.6, concurrently obtained in 69 prematures without hemolytic disease. Mean blood volume in the 17 mature subjects (86.9ml/kg ± 9.86) was significantly less than the mean value in 16 matures without disease (98.3 ± 7.7; $p < 0.001$).

The direct relation of blood volume to hematocrit was significant ($r = + 0.69$, $p = < 0.001$). Blood volumes of < 80 ml/kg were present in 5 of 11 subjects with hct. < 35% but in only 2 of 21 with hct. > 35% ($p < 0.02$).

NATURALLY OCCURRING PNEUMOTHORAX IN HYALINE MEMBRANE DISEASE. Daniel Cohen,* William Cochran,* Thorne Griscom* and G.B. Clifton Harris* Dept. of Pediatric, Harvard Med. Sch. and Boston Hosp. for Women, (intr. by - Charles A. Janeway).

A retrospective 5 year study of all chest radiograph reports of infants born at the Boston Hosp. for Women turned up 234 cases of hyaline membrane disease (HMD) in which the 1) clinical course, 2) blood gases and 3) chest radiograph were consistent with that diagnosis. Of those 234 cases there were 24 with associated pneumothorax, 5 of these occurring after IPPB had been utilized. the rest (19) occurring "naturally" in the course of the disease.

When the 19 were studied as to hour of onset, birth weight and gestational age of the infant interesting relationships were noted. No pneumothorax associated with HMD occurred under 12 hrs. of age and only 1 after 72 hrs. of age. The peak number, 9 cases, occurred between 25-48 hrs. of age, 6 occurred between 48-72 hrs. of age and 3 between 12-24 hrs. of age. Although HMD was recognized in infants born as early as 23 wks. no cases of associated naturally occurring pneumothorax showed up until the 33 wk. of gestation. There were 5 cases of HMD with pneumothorax at 39-40 wks. of gestation yet there were only 19 cases of HMD during that 2 wk. gestation period, an incidence of 26%.

GLUCAGON STIMULATES HEPATIC BILIRUBIN PRODUCTION IN NEWBORNS. Nancy H. Dawber and M. Michael Thaler. Univ. of California, San Francisco, Dept. of Pediatrics, San Francisco.

Hypoglycemic infants frequently develop neonatal hyperbilirubinemia. Hepatic heme oxygenase(HO), the enzyme which converts heme to bilirubin, is stimulated by hormones released in response to hypoglycemia. To determine whether this process may result in increased bilirubin formation in vivo, we studied the effects of glucagon on conversion of heme to bilirubin in normal and congenitally jaundiced rats (littermates). Glucagon was administered intraperitoneally(IP); controls received saline IP. Hepatic hemes were labeled with the specific precursor, δ-ALA (^{3}H or ^{14}C) given IP with glucagon or saline. At sacrifice, 2½ hr after injection, HO activity was measured, and hepatic bilirubin content was assayed after chloroform extraction, chromatography and spectrophotometry. Specific activities of labeled bilirubin were then determined in liver and serum.

HO activity (mμM bilirubin/mg protein/10 min) increased significantly ($p<0.005$) in glucagon-treated rats (0.244) compared with controls (0.155). Bilirubin specific activity in liver and serum of glucagon-treated rats were doubled during the 2½ hr post-injection period. The bilirubin content of the liver was 5.1 μg/g in controls and 8.4 μg/g in treated rats.

These results indicate that glucagon rapidly stimulates bilirubin production in newborn liver, and suggest a possible mechanism for hyperbilirubinemia associated with hypoglycemia.

TRIIODOTHYRONINE (T3) IN THE HUMAN FETUS AND NEWBORN. Allen Erenberg, Dale Phelps, Calvin J. Hobel and Delbert A. Fisher, Depts. of Pediatrics and Obstetrics & Gynecology, Harbor General Hospital, Torrance, California.

We have reported a high rate of thyroxine (T4) secretion in the fetus and a TSH surge in the newborn which stimulates T4 secretion and produces a state of neonatal thyroidal hyperactivity. The present study characterizes T3 levels in the human fetus and newborn. T4, T3, free T4 (FT4) and FT3 were measured in cord blood of fetuses 13 to 40 wks. gestation and in newborn infants between birth and 120 hrs. of age. T3 was not detected in fetal blood before 24 wks. and the mean level between 25 and 34 wks. (34 + 5.9 ng%) was less than that at 35-40 wks. (77 + 9.7 ng%). All values were below the mean maternal conc. (193 + 7.7 ng%) between 13 and 40 wks. FT3 levels also were lower in fetal than in maternal sera; the mean fetal FT3 at term was 234 pg%, whereas the maternal value was 447 pg%. T4/T3 and FT4/FT3 ratios were higher in fetal than maternal sera throughout gestation. Serum T3 and FT3 concentrations increase 6 fold during the first hr. of extrauterine life and peak at mean levels of 420 ng% and 1260 pg% by 24 hrs. after which they gradually fall to mean values of 228 ng% and 620 pg% at 120 hrs. These data indicate that the fetus is T3 deficient and that the newborn becomes chemically hyperthyroid during the first 3 days, due predominantly to T3 hypersecretion. Thus, a state of fetal T3 deficiency is abruptly transformed to a state of T3 toxicosis by the events of parturition.

EFFECT OF EXCHANGE TRANSFUSION WITH BLOOD OF "HIGH" OR "LOW" HEMATOCRIT ON TISSUE OXYGENATION OF INFANTS WEIGHING LESS THAN 1250g AT BIRTH. M. Delivoria-Papadopoulos, L. D. Miller, P. A. Branca, R. E. Forster and F. A. Oski. Univ. of Pennsylvania, School of Medicine, Philadelphia, and the Upstate Medical Center, Syracuse, New York.

During the course of evaluating the therapeutic benefits of early exchange transfusion in 21 infants weighing less than 1250g at birth an opportunity was provided to study the role of the oxygen carrying capacity of the donor blood. Measurement of pH, P_{O_2}, P_{50} and O_2 saturation were made before and at 3 and 24 hours post-exchange. Arterial-venous oxygen content (AVD) difference was calculated. In 14 infants (Group 1) the mean hemoglobin fell from 16.5 to 12.5 gm% following exchange while in 7 infants (Group 2) the hemoglobin value was unchanged. Prior to exchange the mean AVD for the entire group was 3.2 ml. The AVD fell to 1.8 ml in Group 1 infants and decreased to only 2.6 ml in infants in Group 2. For both groups, the mean P_vO_2 rose from 42 mm Hg pre-exchange to 48 mm at 3 hours and to 52.0 mm at 24 hours, reflecting the decrease in hemoglobin-oxygen affinity produced by the exchange. Two infants died during the first post-exchange day and 3 had serious complications; all were in Group 1. These data indicate the importance of maintaining the O_2 capacity of the blood for exchange transfusion. This will result in no change in cardiac output for a constant oxygen consumption.

PHOTOTHERAPY FOR NEONATAL HYPERBILIRUBINEMIA - A DOSE:RESPONSE RELATIONSHIP. Marcello Estrada, LeRoy C. Mims, David S. Gooden and Robert V. Kotas, (Intr. by George Cassady). William K. Warren Med. Research Ctr., St. Francis Hosp., Tulsa, Ok.

Phototherapy for neonatal hyperbilirubinemia has received widespread acceptance and application without standardization or individualization. Evidence has established that bilirubin undergoes photodecomposition by absorption of energy emitted from the blue region of the visible spectrum (420-490 nm). Since many physical and biological phenomena follow exponential decay, it was hypothesized that the decrease in bilirubin levels could be approximated by this function. Forty-four neonates with serum bilirubin levels > 9.0 mgm% were grouped (I-IV) according to energy received (3.5-17 $\mu W/cm^2$). Radiation was measured with an I.L. 155 Color Radiometer. No significant difference occurred between the groups as to weight, age, sex or level of bilirubin at onset of therapy. The only significant difference between the groups was the energy received and the 24-hour bilirubin response. The 24-hour bilirubin decrease showed a + correlation ($r = 0.723$) when compared to energy received. A nearly linear response is demonstrated between the 24-hour bilirubin decrease and energy received.

Data is presented that offers standardization and rationale in the use of phototherapy for neonatal hyperbilirubinemia.

FETAL UMBILICAL BLOOD FLOW MEASUREMENTS: COMPARISON BETWEEN ANTIPYRINE METHOD AND ELECTROMAGNETIC FLOWMETER TECHNIQUE. G. C. Emmanouilides, K. Omori, W. Oh, T. Oikawa, and R. Rosengart. Dept. Peds.,UCLA Sch. Med.,Harbor Gen. Hosp.,Torrance, Calif.

To validate the commonly employed antipyrine(AP) method for measuring fetal umbilical blood flow(FUBF), experiments were performed in 9 fetal sheep preparations with simultaneous flow measurement by the electromagnetic flowmeter(EMFM) technique. After spinal anesthesia and pentobarbital sedation, the fetus was delivered by hysterotomy maintaining intact placental circulation. Polyvinyl catheters were inserted in a fetal femoral vein(FV) and artery and in a peripheral branch of the umbilical vein(UV). The common umbilical vein was isolated for placement of the electromagnetic flowprobe. Antipyrine solution was infused at a constant rate into the FV. After 60 min. equilibration period, FUSF was calculated by the Fick's principle using the antipyrine gradient across the aorta and UV. Simultaneous measurements of FUBF were obtained by EMFM at various intervals. Forty-five paired measurements ranging from 200 to 800 ml/min. were obtained.

The results indicate that at low FUBF rates(200-400 ml/min) good correlation exists between the two methods. However with higher FUBF the AP method overestimates significantly the actual flow as measured by the EMFM. Since diffusion of AP across the placenta is a time dependent process, the reason for the discrepancy may be a result of a relative decrease in AP diffusion rate at high umbilical flow rates.

N.Y.CITY INFANT TRANSPORT SYSTEM (ITS): A PROGRAM OF REGIONALIZED NEWBORN CARE IN AN URBAN SETTING. Angelo Ferrara, and Sanford N. Cohen, N.Y.U. Sch. of Med. and Bellevue Hosp., Dept. of Ped., New York, New York

The ITS transported 1712 infants between 4/71 and 12/72. These high-risk infants originated at 60 different hosps. and were received by 16 neonatal centers. 80% were accompanied in transit by nursing personnel and the remainder by physicians. Most infants were less than 38 weeks gestation (82%). Mean birth weight was 1742 ± 532(1 SD). Two comparable 9 month periods were analyzed (4/71-12/71 and 4/72-12/72). Significantly more infants (especially >2500 g) were moved during 1972 and there was a decrease in transit time ($P<0.05$). Overall mortality was unchanged (16.5%) but there was a significant decrease in mortality among the larger infants in 1972 ($P<0.05$). This difference was related to the type of hosp. of origin, i.e., there was a significant decrease for infants born at voluntary hosps., but no decrease for infants born at municipals.

Two major changes in ITS occurred between 12/71 and 4/72: special vehicles replaced older, less adequate ones; and intensive area-wide educational programs were conducted. These preliminary data indicate that a well organized regional ITS can have an impact upon the survival of high-risk newborn infants in an urban setting.

FATTY ACID COMPOSITION OF LECITHINS FROM AMNIOTIC FLUID RELATED TO GESTATION AND DEVELOPMENT OF RDS. Thomas A. Frantz, Trygve Lindbach and Stanley N. Graven, Rikshospital, Oslo, Norway and Univ. of Wis., Dept. of Ped., Madison, Wis.

The lecithin fatty acid (FA) composition of 61 amniotic fluid samples was determined. These included 17 from diabetic mothers, 36 from Rh- mothers and 8 from normal or pre-eclamptic mothers at gestational ages from 26-42 weeks.

The % of saturated FA increased from 58-70% at gestational ages of 26-31 weeks to 75-90% after 37 weeks. Palmitic acid comprised 24-51%, myristic acid 0-4%, stearic 18-44% and oleic 16-32% of the lecithin FA between 26-31 weeks. With increasing gestational age, the % of palmitic and myristic acids increased, while the % of stearic and oleic decreased.

Five of 27 infants delivered within 7 days of obtaining the samples and 34-39 weeks gestation developed RDS. The lecithins from the 5 patients with RDS had no myristic acid and decreased palmitic acid (4 of 5 <60%) compared to healthy infants of similar gestational age. Amniotic fluid lecithin from the 5 infants with RDS had 16-35% stearic acid while only 2 of 22 healthy infants had >9% stearic acid (11 & 17%). The ratio of stearic acid to oleic acid was >1.3 in all 5 infants with RDS and <1.3 in all healthy infants.

Amniotic fluid from infants born between 34-38 weeks gestation who develop RDS has lecithins with characteristic FA patterns. The % of stearic acid more closely parallels pulmonary maturity than the concentration of lecithin or the L/S ratio.

GROWTH & DEVELOPMENT OF LOW BIRTH WEIGHT INFANTS (1 Kg OR LESS); PRELIMINARY OBSERVATIONS. Richard G. Grassy, Rudolph A. Barta, Richard D. Zachman, and Stanley N. Graven, Univ. of Wis., Dept. of Peds., Madison, Wis.

From August 1968 through December 1971 87 infants weighing 680 grams to 1000 grams were admitted to the Regional Neonatal Intensive Care Unit of St. Marys Hospital, Madison, Wisconsin. Twenty one of 27 survivors have been followed for periods up to 4 years. Of the 6 lost to follow-up one died of Sudden Unexpected Death Syndrome at age 4 months.

Neurological development was normal and no handicaps were noted in 15/21 infants. Major neurological handicaps were observed in 2/21 (hemiparesis; spastic CP). Minimal delay in language development alone was noted in 2/21. Minimal delay in gross motor development alone was seen in 2/21. Ophthamological abnormalities were observed in 3/21 (RLF 2; squint 1).

The majority of children reached or exceeded the 3% tile for height and weight by 12 months of age. All infants attained head circumferences above the 3% tile by 12-18 months of age.

USE OF CONTINUOUS POSITIVE AIRWAY PRESSURE IN BILATERAL DIAPHRAGMATIC PARALYSIS. Tilo O. Gerhardt, Eduardo H. Bancalari, and Lee D. Mockrin (Intr. by William W. Cleveland), Dept. of Pediatrics, Univ. of Miami, Sch. of Med., Miami, Florida.

The effects of continuous positive pressure breathing (CPPB) were studied in a newborn infant with bilateral diaphragmatic paralysis who required four months of mechanical ventilation through a tracheostomy tube for respiratory insuffiency. Without mechanical ventilation or CPPB for one hour, there was clinical deterioration with a $PaCO_2$ greater than 100 mmHg, and a PaO_2 of 38 mmHg on 70% O_2. The respiratory rate (RR) was 30 and the tidal volume (V_T) was 19 ml. Expiratory time was prolonged, and after 50% of the V_T was expired the flow rate decreased suddenly and the esophageal pressure became positive. These findings suggest lower airway obstruction. After one hour on 6 cm H_2O CPPB, there was improvement in the clinical condition and the $PaCO_2$ was 73 mmHg and PO_2 75 mmHg on 70% O_2. The RR increased to 70 and the V_T to 22 ml. The expiratory time and the expiratory flow rate became normal and there was no positive esophageal pressure. The negative esophageal pressure required to produce the same V_T was less than that needed when there was no CPPB. The increase in minute ventilation observed during CPPB in this patient is the opposite of what has been described in normal individuals. This, we believe, is due to facilitation of inspiration which is impaired in diaphragmatic paralysis and to increase in lung volume toward normal with resultant decrease in airway resistance and improvement in lung compliance.

INGUINAL HERNIA: A SURGICAL COMPLICATION OF BELOW 1000 GRAMS PREMATURE INFANTS. Rita G. Harper, Alfredo Garcia and Concepcion Sia. Department of Pediatrics, Downstate Medical Center, Brooklyn, N.Y. (Introduction by Dr. M. Robinson)

Multiple medical problems have been reported in under 1000 grams infants. Surgical problems however have not been commonly noted. We were surprised therefore to find that 11 of 38 consecutive surviving below 1000 grams infants (30%) developed bilateral or unilateral inguinal hernia, most between 10-16 weeks of age. Those infants with both respiratory and gastrointestinal disturbances appeared especially prone; 2/3 of this group developing inguinal hernia. Females were affected disproportionately, 7 out of 21 females (33%) being affected while 4 out of 16 males (25%) were affected. Transillumination was negative in all. The hernial sac contained ovaries in 28% (2/7) of the affected females. Incarceration occurred in 18% of the cases. Mortality was 9% of those affected. Eight of the 11 cases were confirmed to be bilateral at surgery.

Our study indicates that the incidence of inguinal hernia is high in below 1000 grams infants. Contrary to expectation females are particularly susceptible as are those with both respiratory and gastrointestinal disturbances in this weight group.

RISK OF NEONATAL SEPSIS FOLLOWING RUPTURE OF MEMBRANES (ROM). Barry Goldberg, Norman J. Siegel and A.G.M. Campbell (Intr. by C. D. Cook) Yale Univ. Sch. of Med., Dept. of Ped., New Haven.

235 infants with ROM>12 hours were studied at birth to identify those at greatest risk from sepsis (during first 48 hrs.) A simple evaluation score for some major risk factors was combined with examination of the gastric aspirate. Each infant scored 1 each for prematurity (<37 weeks) and maternal pyrexia>100.5°F; and 2 each for membrane rupture >24 hrs. and Apgar score<7 at 1 min. The aspirate was considered positive with >5 polymorphs per H.P.F. Sepsis (clinical signs + pos. blood culture) did not occur in any infant with a neg. aspirate (0/132). Of 103 pos. infants 12 (11.7%) developed sepsis. The relationship to the sepsis score is shown:

Group	Score	Infants	Pos. asp.	Sepsis	Risk (%)
1	0-2	130	44	0	0.0
2	3-4	76	38	4	5.3
3	5-6	29	21	8	27.6

If the sepsis score was combined with a pos. aspirate, the risk of sepsis in groups 2 and 3 increased to 10.5% and 38.1%.

A systematic approach to identification of risk and use of antibiotics can be outlined. Infants who score <2 require no action. Examination of aspirate is indicated for all other infants. If Pos., close observation is maintained and if the sepsis score is >2, a blood culture and chest x-ray are advisable. The infant's clinical condition should determine the use of antibiotics but "prophylaxis" for group 3 may be justified if facilities for close observation do not exist.

AMNIOTIC FLUID PHOSPHOLIPID CONCENTRATIONS AND PULMONARY RETRACTIVE PROPERTIES OF THE NEWBORN. John W.C. Johnson, Henry H. Lim, H. Lorrin Lau, (Intr. by Gerard B. Odell), Johns Hopkins Univ. Sch. of Med., Dept. Ob-Gyn, Balto., Md.

There is considerable controversy regarding the predictive significance of amniotic fluid phospholipid concentrations to respiratory function in the newborn. However, little attention has been given to the relationships between these amniotic fluid components and the mechanical recoil properties of the newborn lung. The purpose of this newborn lamb study was to determine the relationships between amniotic fluid lecithin (AFL) and sphingomyelin (AFS) concentrations to 1) gestational age, and 2) specific pulmonary retractive properties at delivery (lung volumes and extract surface tensions).

The correlation between AFL/AFS and gestational age was not statistically significant. However, the AFL/AFS was always less than 0.8 in pregnancies below 136 days duration. AFL/AFS values of 0.8 or greater were observed in 1/2 the pregnancies over 136 days duration. Significant correlations were observed between pulmonary distensibility and deflation properties and lung extract surface tensions values. However none of these parameters of pulmonary elasticity were found to correlate with AFL, or AFL/AFS when gestational age was considered in analyses of partial correlation coefficients. The results indicate that lamb AFL/AFS values as determined by our methodology are helpful as indices of pulmonary maturity. However, these values are not indicative of specific alveolar surface tension properties.

VITAMIN E & RETROLENTAL FIBROPLASIA(RLF) L.Johnson,D.Schaffer, T.R.Boggs,UofPa Med Sch,Penn & Childrens Hosp,Phila.Ped. Dept.

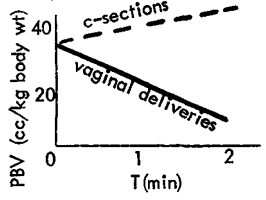
Immature human infants develop RLF in the absence of oxygen (O_2) abuse, though fortunately less frequently and to a less severe degree than in its presence. The vasculature of the immature retina is peculiarly sensitive to changes in O_2 tension. Human retinal vessels normally develop entirely in the intrauterine O_2 environment. Therefore, in the prematurely born, these vessels are exposed to abnormally high O_2 tensions even in the absence of O_2 therapy. Premature infants are, to a greater or lesser degree, deficient in Vit E, the antioxidant of biologic membranes. The possibility that Vit E deficiency predisposes to the development of RLF is being assessed by a clinical trial in which alternate infants are started on Rx with E or a placebo (Hoffman LaRoche parenteral preparations) within 4 to 24 hours of birth. Retinae are examined weekly during the period of vessel immaturity and/or proliferative RLF and bi-weekly or monthly during stabilization and regression. The incidence of cicatricial RLF and sequelae will be assessed by examination under anesthesia at age 1 year. The results of the first 8 months experience is reported below:

Surviving infants < 2500gm	Total #	Vitamin E	Placebo
Incidence of active RLF	11/45 (24%)	3/22 (14%)	8/23 (35%)
Duration of active RLF			
less than 7 days		2	0
less than 14 days		1	3
more than 14 days		0	5
Max severity active RLF		Grade 1	Grade 2

LACK OF PLACENTA TO INFANT TRANSFUSION WITH DELAYED CORD CLAMPING AFTER CESAREAN SECTION DELIVERY. F. Kleinberg*, R.Phibbs and L. Dong*. Dept. of Ped. and Cardiov.Res. Inst,NHLI Sp. Ctr.of Res.-Pulm, Univ of California, San Francisco,Ca.

To estimate the infant blood retained in the placenta after cord clamping, we measured total and fetal hemoglobin in the whole homogenized placenta and in cord blood. We calibrated the method by adding known amounts of fetal and adult hemoglobin to the homogenate. Observed and predicted values correlated well (r=.991,S.D. = .6cc). Placental blood volume (PBV) is expressed as cc/kg infant body weight and plotted against time (T) between delivery and cord clamping which varied from 5 to 115 sec.

The figure shows the regression lines for changes in PBV with T in 30 normal vaginal and 18 normal cesarean section deliveries. The 2 regressions are significantly different ($P<0.05$) by analysis of co-variance. When T<30 sec, the mean PBV of the 2 groups were the same. When $T \geq 30$ sec, the mean PBV of the vaginal group was half that of the c-sections ($P<.001$). PBV for infants of diabetic mothers born by c-section are the same as the normal c-section group. PBV for preterm infants and asphyxiated infants are the same as the normal vaginal deliveries when plotted against T. These results imply that infants born by c-section under the usual circumstances do not receive the placenta transfusion that occurs in normal vaginal deliveries.



THE GASLESS ABDOMEN IN THE INTUBATED NEONATE. E. George Kassner, Erlinda L. Koo and Rita G. Harper. Dept. of Ped. and Radiology. Downstate Medical Center, Brooklyn, N.Y. (Introduction by Dr. M. Robinson)

Complete absence or marked decrease of intestinal gas was observed by x-ray in 64% of 40 consecutive neonates who underwent orotracheal intubation and were maintained on the Bird respirator. This phenomenon was not encountered in other non-intubated sick newborn controls.

Bowel gas disappeared in as little as 6 hours after intubation. Many infants continued to have bowel movements while the abdomen remained gasless. Studies with radiopaque media and instilled air showed that propulsive bowel activity was present. Bagging through the endotracheal tube generally did not reintroduce significant amounts of gas into the bowel. A normal bowel gas pattern returned in the small number of patients who were successfully extubated.

Mortality was not significantly different in those who did or did not develop a gasless abdomen. Below 1000 gms. infants tended to develop a gasless abdomen more often than larger infants. No constant relationship between intestinal gas disappearance and blood pH, pCO_2 or pO_2, or the level of spontaneous somatic or respiratory activity was noted.

Our data indicates that the most likely mechanisms for the gasless abdomen is obstruction of the infant's oropharynx by the orotracheal tube. Gas already present in the gut is expelled as flatus and to a lesser extent absorbed across the bowel wall.

CARDIAC PERFORATION BY UMBILICAL OXYGEN CATHETER IN NEWBORN PUPPY. Kenneth R. Kollmeyer, Reginald C. Tsang, Leonard I. Kleinman, and Irwin J. Light,Univ. of Cinti Col. of Med., Dept. of Ped., Cincinnati.

Intravascular catheters are being utilized for continuous measurement of oxygen tension in neonates. One of these catheters uses a metal oxygen sensing electrode which extends 1 cm beyond the catheter tip into the bloodstream. As part of a study of respiratory chemoreceptors, such catheters were positioned in the right ventricle of three neonatal puppies via an external jugular vein. A 1.1 kg, 27-day-old puppy had a cardiac arrest 20 minutes after insertion of the catheter. At autopsy, cardiac tamponade was found to be the cause of death. The Hydron^R coated metal oxygen sensor tip was found protruding from a perforation in the anterior right ventricular myocardium. In 30 other puppies when a similar catheter without the oxygen electrode was used there was no evidence of myocardial damage. The anterior wall of the right ventricle in the paraseptal region was found to be exceptionally thin (<1 mm) in both canine and human neonates. Accordingly, although complications have not yet been reported in human infants, extreme caution should be employed in positioning continuous recording intravascular metal oxygen electrodes.

RESPIRATORY SENSITIVITY TO CO_2 IN NEONATES. D.B. Klain, A.N. Krauss, P.A.M. Auld, Dept. of Ped., Cornell Univ. Med. Col, New York City.

Respiratory sensitivity to carbon dioxide in adults with chronic lung disease has been shown to be correlated with timed vital capacity, indicating that the apparently reduced sensitivity in these patients is due to mechanical limitations of pulmonary function. Eleven AGA premature infants of birth weight between 964-1920 gm. and 5 healthy full term infants underwent studies of respiratory sensitivity to CO_2. Five per cent CO_2 was rebreathed in 40% oxygen and the slope of the line minute volume vs. CO_2 determined (method of Read). All infants underwent determinations of compliance, total pulmonary resistance, and functional residual capacity. Term infants had a mean response of 361 cc/min/mm. Hg Pco_2 during their first week of life. This was significantly greater than infants born at 28-32 weeks gestation or 33-36 weeks gestation when studied at birth. Low birth weight infants increased their CO_2 sensitivity with increasing post-natal age, reaching that of term infants by 40 weeks post-conception. No correlation (r=0.2) was found between mechanical factors and CO_2 sensitivity, indicating that neurological maturation is a major factor in determining the CO_2 sensitivity of low birth weight infants.

SIGNIFICANCE OF C-REACTIVE PROTEIN (CRP) IN CORD BLOOD. Irving Kushner, Avron Y. Sweet, Belinda Yen-Watson, William N. Ribich, and Judith Merk, Case Western Reserve Univ. Sch. of Med. at Cleveland Metropolitan General Hosp., Depts. of Med. and Ped., Cleveland.

Inflammation and tissue necrosis result in CRP formation, and this protein does not cross the placenta. Accordingly, cord sera were analyzed for CRP content to determine its relationship to abnormal clinical states in the newborn. A radial diffusion technique sensitive to 1.5 µg/ml was used.

At this county hospital 1420 samples were studied (97.6% of births during a 5 1/2 month period). CRP was detected in 68 (4.8%). CRP was more frequently found ($p < .005$) in infants with the following diagnoses: birth weight less than 2500 grams (25/173, 14%); gestation less than 36 weeks (19/81, 23%); amnionitis (12/26, 46%); maternal fever during labor or delivery (10/22, 45%); and prolonged rupture of membranes (17/68, 25%); as well as hyaline membrane disease, neonatal asphyxia and neonatal death. Cord blood was positive in 8 of 19 infants who died in the neonatal period. CRP-positive cord bloods were found 2 1/4 times more frequently at this hospital than in 1772 samples obtained during the same period from a voluntary hospital with a middle class population.

The findings suggest that intrauterine fetal inflammation and tissue necrosis are related to some underlying causes of premature onset of labor as well as to low socio-economic status.

CHANGING PATTERNS OF STAPHYLOCOCCAL COLONIZATION IN NEWBORNS: Irwin J. Light, Harry D. Atherton, James M. Sutherland, Univ. of Cinti. Col. of Med., Dept. of Ped., Cincinnati.

Epidemics of staphylococcus aureus 80/81 which plagued newborn nurseries a decade ago are rare in present day nurseries. In contrast group II staphylococci are frequently encountered. To examine changing patterns of staphylococci 25,662 surveillance cultures obtained from 9216 newborn infants between 1960-72 have been reviewed. Hexachlorophene handwashing has been employed throughout the study period; daily hexachlorophene bathing was used in the full-term nursery from 1965-67. In the full-term nursery S. aureus decreased from 17.8% to 6.5% ($p<.001$). This decrease occurred prior to the introduction of hexachlorophene bathing, did not change during the hexachlorophene bathing period and remained low during the four years following cessation of this procedure. A similar decrease in S. aureus 80/81 from 10.7% to 6.4% ($p<.02$) occurred in the premature nursery although daily hexachlorophene bathing was never used in this nursery. In the newborn nursery a similar decrease in group II staphylococci began prior to and persisted during the period of hexachlorophene bathing but rebounded to pre-hexachlorophene levels following cessation of this procedure. The data suggest that a spontaneous decrease of 80/81 staphylococci has occurred independent of hexachlorophene bathing. In contrast an increase in group II staphylococci occurred following cessation of hexachlorophene bathing.

ORAL THERAPY OF VITAMIN E DEFICIENCY IN PREMATURE INFANTS. David K. Melhorn and Samuel Gross, Dept. Ped., CWRU Sch. of Med. Cleveland.

Vitamin E deficiency with anemia in the small premature infant is often neither prevented nor corrected by oral administration of commonly-used fat soluble forms of E. This study compares the responses to fat-soluble α-tocopherol acetate (FSE) and water-soluble (WSE) α-tocopherol polyethylene glycol 1000 succinate in 159 infants of gestational age from 28 to 36 wks. Data obtained at 6 weeks of age, the most pronounced period of E deficiency include:

Groups	E Level	RBC H_2O_2 Fragility%	MDA (nm)	HCT (%)	Retic (%)
1. No supplement	0.32	76	345	25.8	9.6
2. FSE oral suppl.	0.58*	44*	290	28.3*	6.0
3. WSE oral suppl.	1.28* **	15* **	188* **	32.4*	3.2* **
4. Formula with FSE	0.42	55*	256*	27.0	5.6*
5. Formula with WSE	0.82* **	23	210* **	31.0	3.2* **

*P <0.01 (vs no suppl.) **P <0.05 (vs FSE oral supplement)

Satisfactory gut absorption of FSE is not attained until gestational maturity is reached, and elevated H_2O_2 fragility and MDA indicate increased RBC lipid peroxidation during E deficiency. In contrast, WSE is efficiently absorbed, as shown by E tolerance index (ETI=8 hr. tocopherol level—Baseline level; 25I.U E/Kilo given): FSE=3.0, WSE=7.3; $P<0.01$. Thus, WSE as α-tocopherol polyethylene glycol 1000 succinate is a preferable form of oral E supplementation.

THE EARLY NEONATAL PERIOD OF 100 LIVE-BORNS OF MOTHERS ON METHADONE. Philip J. Lipsitz and Saul Blatman, Mt. Sinai Sch. of Med., Beth Israel Med. Ctr., Dept. of Ped., N.Y.C.

From Jan. 1967 to Jan. 1973, we have taken care of 100 newborns delivered to mothers taking methadone. The dose of methadone taken was from 40 to 120 mg. daily. Only 5 pregnant patients were on doses of 50 mg. or less. All the newborns at delivery had Apgar scores greater than 6 and the majority scored more than 7 at one minute. The mean birthweight was 2786 grams (range 1176 to 4338 grams). Gestational age was 30 to 40 weeks. Twenty-six weighed 2500 grams or less and of these 54% were A.G.A. and 46% S.G.A. The sex ratio was male: female 1.2:1. A major congenital anomaly was noted in 1 newborn and 2 had supernumerary digits. There were 2 neonatal deaths in this group. Symptomatology of narcotic withdrawal was graded according to the system of Kahn, et. al., (J. Ped. 75:495, 1969). Grade II symptoms occurred in 53% and grade III in 5%. Three newborns had seizure-like activity. We propose a numerical scoring value of withdrawal symptoms so that everyone dealing with these newborns uses a standard grading system. The onset of symptoms occurred in the first 4 days of life in 78% of the symptomatic newborns. Bilirubin levels $\geq$ 13 mg% occurred in 14%. The newborns were observed in the hospital for a mean of 16 days range (7-78). All but 8 were discharged in care of the mother.

STATEWIDE REDUCTION OF NEONATAL MORTALITY THROUGH EFFECTIVE REGIONALIZATION OF NEWBORN INTENSIVE CARE. Belton P. Meyer, Thomas C. Harris, William J. R. Daily, and Frederick K. Baum. Good Samaritan, St. Joseph's Hosps. and Arizona State Health Dept., Phoenix; and Tucson Med. Ctr. and the Univ. of Arizona Col. of Med., Tucson.

We have previously reported (SPR, 1971) a reduction in neonatal mortality among infants born in, or transported from rural hospitals to, centers. In 1970, state subsidization of costs of transport to and care in 4 designated newborn centers in 2 cities permitted uniform statewide distribution of care. Five years experience with systematic regional newborn care in Arizona can now be reviewed. Eleven percent of newborn infants in Arizona are compromised annually by prematurity or significant illness. Following establishment of a regional intensive care and transport system in 1967, the percentage of these infants cared for in centers increased from 0 to 50.7%. Concomitantly, state neonatal mortality rate has declined from 17.3 to 11.5, while the U.S. neonatal mortality rate declined from 17.2 to 13.8 deaths/1000 live births. This decline in neonatal mortality has been sufficient to improve the state rank of the Arizona infant mortality rate from 43rd of 50 states (1966) to 11th of 50 states (1972) despite a failure of the state rank for post-neonatal infant mortality rate to improve during the same interval. Uniform regional distribution of effective newborn care will result in significant reduction of neonatal mortality.

FINGERNAIL NITROGEN ACCRETION IN THE FOETUS AND NEWBORN. W. Lockard, Y. Brans, J. Sumners*, H. Dweck, and G. Cassady. Univ. Ala. Sch. Med., Birmingham.

Fingernail samples were collected within 69 days of birth from 26 infants who were normally-grown prematures (NG-P), 30 intrauterine growth retarded infants (IGR), 11 infants of diabetic mothers (IDM), and 89 normally grown mature infants (NG-M). Fingernail nitrogen contents (FNC) were determined on a total of 163 samples by a micro-Kjeldahl technique.

Mean FNC for each group showed no increase during the first 21 days of life. During that period, there was no difference between NG-P's and NG-M's but IGR's and IDM's had lower values than their NG peers ($p< 0.005$ and $p< 0.001$, respectively). After 21 days of age the mean FNC of IGR's increased significantly ($p< 0.01$) and became similar to that of NG.

These data confirm previous observations from this laboratory that the mean FNC in certain compromised newborns is lower than in their normal peers. They point to intrauterine growth impairment as a factor affecting the pattern of foetal nail nitrogen accretion and suggest that FNC at birth may be a reflection of the quality of foetal cell development. They further suggest that recovery occurs within two months of birth.

Mean FNC (g N/100g sample):

Age	$\leq$ 21 days	21 - 69 days
NG	13.3 ± 1.34	14.0 ± 1.72
NG-M	13.4 ± 1.29	13.5 ± 1.45
NG-P	13.0 ± 1.46	14.6 ± 1.97
IGR	12.3 ± 1.46	13.7 ± 0.99
IDM	11.6 ± 1.31	

(*Dr.Sumners:Naval Reg.Ctr.,Jacksonville,Florida)

DIRECT MONITORING OF ARTERIAL PRESSURE IN DEPRESSED AND NORMAL TERM NEWBORNS DURING THE FIRST HOUR OF LIFE. H. Modanlou, B. Siassi and E.H. Hon. (Intr. by P.F. Wehrle). Dept. of Ob. and Peds., L.A. County-USC Medical Center.

Direct monitoring of arterial pressure through an indwelling aortic catheter is part of the treatment in acutely ill newborn infants. A prospective project was designed to study high-risk and normal pregnancies during labor and the immediate post-natal life. In 150 term neonates systolic, diastolic and mean aortic pressures were recorded continuously and the data were analyzed at 4, 8, 16, 32 and 64 minutes of age.

Patients were divided into 4 groups according to their Apgar scores at 1 and 5 minutes as follows:

Group I (34 pts.): Apgar score (1 min.) $\leq$6
Group II (116 pts.): Apgar score (1 min.) $\geq$7
Group III (13 pts.): Apgar score (5 min.) $\leq$6
Group IV (137 pts.): Apgar score (5 min.) $\geq$7

The umbilical cord was clamped prior to the first breath in all groups. The low Apgar score groups (Groups I and III) had generally higher systolic, diastolic and mean arterial pressure during the entire period of observation compared to high Apgar score groups (Group II and IV).

Comparison of Group I and II showed statistically significant differences ($p<0.05$) at 4, 8 and 16 minutes for systolic; and 4 minutes for diastolic pressures. Such comparison between Group III and IV showed statistically significant differences ($p<0.001$) at 4 minutes for diastolic blood pressure. No statistically significant differences of mean arterial pressure were found between Group I and II or between Group III and IV during the entire period of observation.

This study shows that depressed newborns at birth have higher systolic, diastolic and mean arterial blood pressure during the first hour of life and these differences are more evident during the first 16 minutes.

FETAL AND NEONATAL BIOCHEMISTRY AND UMBILICAL ARTERIAL pH: H. Modanlou, S-Y Yeh and E.H. Hon. (Intr. by P.F. Wehrle). Dept. of Ob. and Peds., L.A. County-USC Medical Center.

A prospective project was designed to study high-risk and normal patients during labor and the neonate immediately after birth. In 143 term pregnancies scalp pH measurements were done in early labor, at 5 cm and full cervical dilatation and immediately before delivery. pH measurements were also done on umbilical artery and venous blood and on umbilical arterial blood at 4, 8, 16, 32 and 64 minutes of age.

Patients were divided into 3 groups based on their umbilical arterial pH as follows: Group I (11pts.) pH≤7.12, Group II (26pts.) pH: 7.13 to 7.18, Group III (106pts.) pH≥7.19.

Group I had a generally lower pH during the fetal and neonatal period than Group II. Group II in turn had lower pH than Group III. Comparison of Groups I and II revealed statistically significant differences at full cervical dilatation (p=0.04), umbilical artery (p=0.001), umbilical vein (p=0.002). Comparison of Group I and III showed statistically significant differences at full cervical dilatation (p=0.001), before delivery (p=0.04), umbilical artery (p=0.001) and vein (p=0.001), at 4, 8, 16 and 32 minutes of age with respective P values of 0.02, 0.002, 0.003 and 0.01. Comparison of Group II and III showed statistically significant differences at full cervical dilatation (p=0.006), before delivery (p=0.002), umbilical artery (p=0.001) and vein (p=0.001), at 4, 8 and 16 minutes of age with respective P values of 0.001, 0.04 and 0.003.

Since umbilical arterial blood pH reflects the biochemical (acid-base) status at birth, this study supports the idea that labor and delivery are asphyxiating processes and that depressed newborns recover from acidosis at a much slower rate.

TOLERANCE AND METABOLISM OF INTRAVENOUS INFUSION OF A TRIGLYCERIDE EMULSION IN NEWBORN LAMBS. A. Otten, E. Dolanski, L. Victorin, H.C. Meng, M. Stahlman. Depts. Physiology & Pediatrics, Vanderbilt Univ. Sch. Med., Nashville.

High caloric source, essential fatty acid content and isotonic nature of a triglyceride emulsion make it an ideal intravenous nutrient for newborns, if the triglyceride provided is metabolized without adverse effects. Soybean oil (10%) and egg-phosphatides (1.2%) was administered intravenously as Intralipid in 8 newborn lambs on the 1st and 7th day of life. Two dosages 3 hrs apart of fat 0.5 g/kg were given in 5 min. Infused triglycerides and phospholipids were eliminated from the circulation at the end of the observation period; on the 1st day of life after 120 min; on the 7th day after 60 min. Free fatty acid levels increased from 0.9 μmol to 2.0 μmol in 10 to 30 min. On the 7th day of life this increase was faster than on the 1st day. Ketones rose from 0.1-0.2 μmol/ml to 0.5 μmol/ml in 30 to 120 min. There was no significant change in blood glucose, pyruvate or lactate. There was a tendency for decreasing PO_2 in the first 30 min after infusion, but no significant change in pH or PCO_2. Temperature was stable. Platelets decreased between 30-50% but returned to normal after 2-3 hrs. Transaminases and bilirubin remained normal. The infused triglycerides were tolerated at the first day of life, but the elimination of the administered fat from the circulation was faster on the 7th day. No toxic side effects could be observed. These results suggest that Intralipid may be well handled by the normal newborn, and could be a useful and safe source of calories when parenteral alimentation is the route of choice.

PERSISTENT ELEVATED GLYCOGEN CONTENT IN SUBCUTANEOUS ADIPOSE TISSUE FROM OFFSPRING OF DIABETIC MOTHERS AND SMALL-FOR-GESTATIONAL AGE NEWBORN INFANTS. Ellen F. Monkus, Duna Penn, and Milan Novak, Dept. of Ped., Univ. of Miami, Sch. of Med., Miami, Florida.

Glycogen content of adipose tissue of normal fullterm human infants, like the blood glucose, falls rapidly in the first hours of extrauterine life. Lipolysis, as evidenced by in vitro glycerol release, increases to a maximum at a few hours of age, remains elevated but gradually decreases in the first days of life. Glycogen content (mg/gm wet weight) of adipose tissue from offspring of diabetic mothers (IDM) have previously been shown to be increased in the first hours of life and also to disappear much more slowly than in the normal infant. This finding was not due to intravenous glucose therapy. Present investigations show adipose tissue from small-for-gestational age infants (SGA) following the same pattern. The highest glycogen levels were found in two SGA. Certain IDM and SGA also had increased in vitro glycerol release from intact fragments of adipose tissue.

Both IDM and SGA tend to suffer hypoglycemia in the first hours and days of life, IDM because of hyperinsulinism and SGA presumably because of inadequate liver glycogen secondary to intrauterine malnutrition. They may also be hyperinsulinemic (LeDune). These studies show that at least the adipose cells of IDM and SGA are not suffering a lack of carbohydrate for energy requiring processes, in spite of hypoglycemia. They imply that the utilization of carbohydrate stores in these two groups of abnormal infants may be faulty.

FETAL AND MATERNAL PLACENTAL BLOOD FLOW IN THE BABOON. John B. Paton, David E. Fisher and Boyd E. Metzger. (Intr. by Samuel P. Gotoff.) Univ. of Ill. Coll. of Med., Dept. Ped., and Northwestern Univ. Med. Sch. Dept. Med., Chicago.

Fetal and maternal placental blood flows were estimated in 6 pregnant baboons between 160-170 days gestation (term 184 days); both fetal and maternal intraplacental distribution were measured simultaneously for the first time to evaluate the efficiency of placental exchange. Fetal placental blood flow was measured by the antipyrine equilibration technique and its intraplacental distribution estimated using radioactive microspheres. Maternal placental blood flow was measured following a left ventricular injection of differently labelled radioactive microspheres and the intraplacental distribution estimated by the same method. The mean maternal placental blood flow was 46.2 ± 10.2 ml/min and the mean fetal placental blood flow was 76.8 ± 16.6 ml/min. When blood flow to placental lobes (cotyledons) was examined the ratio of maternal to fetal flow was 0.66 ± .40 (range 2.19 - 0.09). From these data the effective placental blood flow was calculated as 45.9 ± 6.6 ml/min (range 35.2 - 53.3) on the basis that the optimal functional placental perfusion ratio between maternal and fetal circulation is one to one. When compared with a functional measurement of effective placental blood flow (antipyrine clearance - mean 34.8 ± 2.9 ml/min) the lobar maldistribution of maternal and fetal placental blood flow accounted for 71% (range 35 - 90%) of the functional inefficiency of fetal placental blood flow.

REPEAT PERINATAL DEATHS. Richard L. Naeye, William A. Blanc, Depts. of Pathology, Pennsylvania State University College of Medicine, Hershey, Pa. and Columbia University College of Physicians & Surgeons, New York City.

The current study jointly analyzed maternal and fetal factors in repeat unsuccessful pregnancies in a series of 783 consecutive fetal and neonatal deaths. Multiple maternal factors were found to interact in their effects on fetal and neonatal disorders so multivariant analysis was used. The following factors were included in the analysis: number of unsuccessful pregnancies, gestational age, smoking habits, number prenatal clinic visits, late gestational vaginal bleeding, cord prolapse, interval since last pregnancy, whether pregnancy was wanted, sex of newborn, family economic status, race, work during pregnancy, marital status, mother's height and age. The amniotic fluid infection syndrome was identified in 23% of cases with no previous unsuccessful pregnancies, 31% of first pregnancies, 55% of pregnancies with two or more previous losses, and 47% of cases with previous premature infants who survived. The incidence of cytomegalovirus lesions increased from 1% in the first to 7% in the third group. As expected, Rh erythroblastosis fetalis increased in incidence with unsuccessful pregnancies and previous premature deliveries. Of ten other relatively common fetal and neonatal disorders, none had a significant association with previous premature deliveries and perinatal losses.

INCUBATOR CONTROL AND PREMATURE SURVIVAL. Paul H. Perlstein, Neil K. Edwards, Harry Atherton, and James M. Sutherland, Univ. of Cinti. Col. of Med., Dept. of Ped., Cincinnati.

Using a computer to establish a stable neutral thermal incubator environment resulted in a significant improvement in survival of 1001-2001 gm prematures. 44 infants are included in this study. Their selection was based on 1) a birth weight of less than 2001 gms, 2) arrival in the special care nursery when a study incubator was available, and 3) random assignment to a computer controlled environmental study group by sealed envelope selection. Sex, race, and birth weight were used to match each study infant with a routinely cared for control infant. Of the 44 study infants 12 died (27%). 20 (45%) of the 44 control babies died. The difference between these groups is not significant (X^2=3.143, p=0.25). 33 of the study infants weighed between 1001 and 2001 gms. 4 of these infants died (12%) whereas 11 (33%) of the 33 matched control babies died. The difference between these two groups is significant (X^2=4.23, p less than .05). The improved survival for infants in the computer controlled incubator may be related to a lower number of apneic spells and greater cardiac rate stability.

HOMEOTHERMIC ADAPTATION IN THE NEWBORN. Paul H. Perlstein, Carol B. Hersh, Charles J. Glueck, and James M. Sutherland, Univ. of Cinti. Col. of Med., Dept. of Ped., Cincinnati

Three-day-old cold stressed infants have an enhanced ability to maintain their rectal temperatures if they have previously experienced cold challenges. Nine full-term infants were exposed naked in a 74-75° F room for 20-minute periods during the first 3 days of life (the adapted group). Thirteen other infants (the unadapted group) were similarly cold stressed, but only on the third day of life. Plasma glycerols and rectal temperatures were measured before and after cooling in both groups of babies. The rectal temperature after exposure was significantly higher in the adapted (98.2 ± SE .2° F) than in the unadapted infants (97.4 ± SE .7° F) ($p<.05$). Moreover, the mean fall in rectal temperature of 0.27 ± SE .09° F in the adapted infants was significantly less than the 0.64 ± SE 0.1° F fall in the unadapted babies ($p<.05$). Changes in plasma glycerol correlated significantly with changes in rectal temperature ($r=.6280$, $p<.05$). Whereas homeothermic adaptation has been reported in older babies, this study reveals that adaptation to mild environmental stress occurs even in the first 3 days of life.

HYALINE AND RUPTURED--THE MEMBRANE DILEMMA. C. Joan Richardson, Jeffrey J. Pomerance, M. Douglas Cunningham and Louis Gluck, Univ. of Cal., San Diego Sch. of Med., Dept. Ped., Div. of Perinatal Med., La Jolla.

Traditionally pregnancies with premature rupture of membranes (PROM) are terminated by 24 hours of rupture regardless of fetal maturity. This study evaluated the effect of PROM on the subsequent development of respiratory distress syndrome (RDS). Criteria for admission to the study included singleton birth, less than 36 weeks gestation, weight under 2268 grams (5 lb.) and no maternal complications other than PROM. Of 206 prematures born at Univ. Hosp., San Diego, from Jan. 1, 1970, to Dec. 31, 1972, 64 qualified for the study. Results are tabulated as follows:

Duration of Rupture	≤ 24 hours	> 24 hours
Number	42	22
Mean birth weight	1666 grams	1440 grams
Mean gestation	32.4 weeks	30.4 weeks
Mean duration of rupture	3.9 hours (range 0 to 24)	93.5 hours (range 29 to 524)
Number with RDS	27	7
Incidence of RDS	64%	31%
RDS deaths	13	5
Total deaths	14	8
Survival rate	67%	64%

There was no difference in maternal morbidity or mortality in the two groups. This data suggests that PROM > 24 hours accelerates lung maturation by factors as yet undefined.

PAH CLEARANCES AND SODIUM BALANCE IN ACIDOTIC NEWBORN LAMBS TREATED WITH SODIUM BICARBONATE. D. L. Phelps, K. Omori, and W. Oh, UCLA Sch. of Med., Harbor Gen. Hosp., Dept. of Ped., Torrance, Ca.

Six near term lambs were artificially ventilated during the first 5 hours of life. Arterial blood gases, blood pressure, PAH clearances (C_{PAH}), serum sodium (Na), and osmolarity (Osm) were monitored. Metabolic acidosis was induced (pH 7.42 ± 0.01 to 7.19 ± 0.02, M ± SEM) with 0.3 M HCl infusion in 4 animals, and corrected with 4.8 to 6.6 mEq/kg of 0.9M $NaHCO_3$ at 2 ml/min (pH corrected to 7.41 ± 0.02). In 2 untreated control lambs, no significant changes in the values measured were observed. Serum Osm and Na were unchanged during acidosis and post $NaHCO_3$ infusion, but were elevated at the 2 hr. follow-up period ($p < .05$). C_{PAH} was unchanged during acidosis, rose significantly following $NaHCO_3$ (0.9 ± 0.1 to 1.6 ± 0.2 ml/min/kg, $p < .025$), and returned to baseline during follow-up. Na excretion (μEq/min) rose from a pre-infusion level of 22.7 ± 4.3 to 59.2 ± 13.8 ($p < .025$) for 40 min. following $NaHCO_3$ and returned to baseline in 2 hrs. These lambs excreted approximately 25% of the infused Na in the following 2 hrs. Autopsy showed no evidence of cerebral hemorrhage.

The renal extraction ratios of PAH were determined in 5 of the animals and showed a result of 0.29 ± .013, $n = 27$.

These data indicate that in the near term lamb, effective treatment of a metabolic acidosis without hypoxia by $NaHCO_3$ was accompanied by a transient rise in C_{PAH} and urinary Na excretion without evidence of cerebral hemorrhage.

SYSTEMS MODEL FOR ASSISTED VENTILATION (AV). Rosan, Robert, C., Champagne, Gloria, J., Nixon, Joseph, M. McEwan, Sherri, N., White, Wilma, L. Cardinal Glennon Mem. Hosp. for Childr., St. Louis, Mo.63104.

AV is often modified for neonatal intensive care (NIC) without chronic animal experiments, prospective controls, or systems approaches. Our data imply that O_2 is one likely cause of AV sequelea; thus, we chose it as the dependent variable in a systems approach comprising: newborn guinea pigs; growth data; technology for ventilation/perfusion study; servo-regulated ventilator; radiographic followup; cell/organ function tests; statistical histologic study. Novel aspects are: fast continuous non-invasive O_2 analysis suitable for servo input; automated 2-2ave-length blood lactate micro-analyses; emphasis on bronchiolar control aspects of pulmonary lobular function. We conclude that AV technology can be modeled in simulated NIC; small rodents yield significant cardiorespiratory data if analyzed by clinical laboratory methods; postnatal growth affects rodent molecular and functional lung development. Nothwithstanding differences in comparative gross and cellular anatomy, the guinea pig AV system is a useful infant model.

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THE EFFECT OF ABORTION "ON DEMAND" UPON PERINATAL STATISTICS. Alistair G. S. Philip (Intr. by J. F. Lucey), University of Hawaii, Kapiolani Hospital, Department of Pediatrics, Honolulu.

In March 1970 a liberalized abortion law was passed in the State of Hawaii. A reduction in the number of "sick" infants which occurred 9 months later, resulted in the closure of a new intensive care nursery (located at a distance from the main nursery), and reorganization within the main nursery. As a result, the perinatal statistics in the 2 years preceding the effect of liberalized abortion (1969-70) were compared with the 2 succeeding years (1971-72).

	L.Births	Low B.Wt.	Stillborns	N.N.D.	Perinatal
1969-70	10,105	889 (8.8%)	86 (0.84%)	125 (1.24%)	207 (2.03%)
1971-72	9,714	799 (8.2%)	95 (0.97%)	98 (1.01%)	190 (1.94%)

rates in parentheses

Despite a maternal population which is very representative of the State, and a significant reduction in the neonatal mortality rate, there was only a slight reduction in the perinatal mortality rate. However, there were other factors operating to reduce neonatal mortality (use of fetal monitoring; neonatologist; and new pediatric pulmonary center).

On the other hand, the total number of "sick" neonates seemed to be noticeably reduced, as evidenced by a reduction in the number of infants a) with respiratory distress syndrome, b) in the I.C.N., c) undergoing umbilical artery catheterization, and d) requiring roentgenographic examinations. It is concluded that abortion "on demand" may have reduced perinatal morbidity but not perinatal mortality.

ABNORMAL BLEEDING DURING THE FIRST WEEK OF LIFE. S. Sarraf, H.N. Maurer, D. Draper, and N. McWilliams. Medical College of Virginia, Department of Pediatrics, Richmond, Virginia

Although the entity of Dissem. Intrav. Coag. (DIC) has been recognized clinically for several years, its relative incidence among causes of bleeding in the first week of life has not been reported. Records of 26 babies with significant external bleeding in the first 6 days of life, seen between 1969-1972, were reviewed. Of these, 14 were male, 16 black, and 25 weighed more than 2500g at birth. Causes of bleeding were the following:

Diagnosis	No.	%	No. died
Vit.K deficiency	6	23.0	2
DIC	4	15.3	1
Cow's milk colitis	3	11.6	0
Trauma	3	11.6	0
Infectious and nonspecific colitis	2	7.7	0
Thrombocytopenia	2	7.7	0
Hemophilia	1	3.8	0
Unclassified	2	7.7	0
Incompletely studied	3	11.6	2

We conclude that neonatal hemorrhage may result from a variety of pathologic processes, more frequently acquired than inherited. Although DIC is an important cause of severe neonatal bleeding, bleeding due to Vit.K deficiency remains a significant problem despite the recommendation that Vit.K be given prophylactically at birth. Milk colitis is a definite cause of localized GI bleeding that has not been recognized in studies already reported.

BIRTHWEIGHT/GESTATIONAL AGE RELATIONSHIPS IN A POPULATION ENROLLED IN THE COLLABORATIVE PERINATAL STUDY. Thomas F. McNair Scott, Olin B. Van dyck. The Children's Hospital of Philadelphia, Department of Pediatrics, University of Pennsylvania.

The availability of data collected prospectively using standardized procedures on 44,744 single born infants, about equally divided between Black & White has allowed reexamination of relation of birthweight to completed gestational week. Using histograms of weight distribution in 100 gram increments by gestational week and other statistical procedures it has been possible to prepare a "normal" intrauterine growth curve from ~~the 21st~~ to 50th week by race and sex. Associated with this curve of "normal" population, 2 other populations were observed, (1) too heavy before the 34th week (2) small for dates, defined as more than 2 standard deviations below the normal mean. Racial differences in mortality rates appeared; that for Whites was less than for Blacks for term infants, while that for Blacks was less than for Whites for too heavy early and premature (normal weight before 37th week) infants. Correlations were made relating these infant populations to maternal characteristics which included physical measurements, pregnancy health, previous pregnancy experience and socio economic environment. The records on the surviving children will be available through their 8th year and will provide the opportunity for relating outcome to the intrauterine characteristics of these same children.

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PREVENTION OF HYALINE MEMBRANE DISEASE WITH ACTH INFUSION IN FETAL LAMBS. H. Sundell, A. Tsiantos, J.P. Relier, E. Dolanski, L. Victorin, A. Otten, C. Strott, D. Orth, F. Chytil, M. Stahlman. Newborn Lung Center, Vanderbilt Univ. Sch. Med., Nashville.

The ability of ACTH infusion to protect premature lambs from HMD was studied in 8 sets of twins delivered by C-section at 114-135 d gestation. Catheters were inserted into CA and JV (0.12 mg/24 hr) for 5 days. Blood gases and pressures, ACTH, cortisol, estradiol, progesterone and testosterone were monitored from lambs and ewes. 4 sets of lambs were killed at section and 4 sets resuscitated. In 3 of the latter, ewes were previously made hypotensive. 2 treated lambs were well and 2 had moderate respiratory distress but not HMD. 3/4 control lambs had severe HMD. Treated lambs' lungs appeared more mature on light microscopy in 6/8, had more lipid inclusions on EM in 4/7; normal or lower minimum surface tension in 5/7; higher conversion into total lipids and CO_2 and a higher percentage of converted phosphatidyl choline of total and phospholipids when incubated with ^{14}C-1-acetate and ^{14}C-1-palmitate in 4/7. There was no difference in choline kinase activity. Treated lambs had increased cortisol blood levels, smaller thymuses and larger adrenals. ACTH infusion to the lambs had no effect on the ewes' ACTH or cortisol levels. High ACTH blood levels were needed to elevate cortisol values in lambs. Although ACTH seemed to offer some protection from typical HMD the effect was not dramatic nor consistent either in the clinical outcome or as judged by the biochemical data, which were not able to explain its mode of action.

ISLET CELL FUNCTION IN THE HUMAN NEWBORN. Mark A. Sperling, Dale Phelps, Paul V. DeLamater, Robert H. Fiser and Delbert A. Fisher. Dept. of Peds., Harbor Gen. Hosp., Torrance, Calif.

We have developed sensitive radioimmunoassay systems for total "glucagon like immunoreactivity" (GLI) and for pancreatic glucagon, and have used these to study glucose-glucagon-insulin interrelationships during the first days of life in normal full term infants. Thirty minutes after birth plasma glucagon (GLU) averaged 224 ± 27 (SEM) pg/ml and no significant changes in concentration occurred over the ensuing 12 hours although plasma glucose fell from 77 ± 6 to 57 ± 5 mg% during the first 4 hours. Plasma immunoreactive insulin (IRI) did not change significantly. With the introduction of oral feeding at 12 hours GLI rose to a mean of 2000 pg/ml and was maintained at this level for the first 96 hours. However, 10 samples which averaged 2982 ± 449 pg/ml GLI averaged 370 ± 57 pg/ml of pancreatic GLU. Thus the major proportion of circulating GLI in the newborn is of gut origin. In 5 newborns, infusion of arginine (0.5 Gm/Kg over 30') into the umbilical vein resulted in a prompt increment in plasma GLU (averaging 280% of fasting values) and IRI (193% of fasting values). The results indicate 1) the pancreatic α cell like the β cell in the newborn is sluggish in its response to changes in blood glucose, 2) the α cell, like the β cell responds to changes in amino acid concentration, 3) the gut in the newborn period is capable of secreting large amounts of GLI and 4) lack of pancreatic GLU secretion may be a contributing factor to the hypoglycemia of the newborn.

THE RISKS OF HEXACHLOROPHENE BATHING. O. Ward Swarner, Jr., Harry Powell, Peter Lampert, Louis Gluck, Univ. of Cal., San Diego Sch. of Med., Dept. Ped. and Pathology, La Jolla.

CNS lesions have been reported in infants bathed with hexachlorophene (HCP). The brains of 69 infants autopsied during 1969-72 were examined for these lesions and clinical correlations drawn to identify risk factors in HCP toxicity. Light and electron microscopy studies revealed changes in the midbrain myelinated tracts of 7 infants. This characteristic status spongiosa consisted of wide separation of the myelin lamellae. These alterations are identical to the findings in experimental animals exposed to HCP.

In affected infants: (1) 6 of the 7 had 8 or more exposures to HCP; one only 4 HCP baths. (2) All 7 had severe acidosis. (3) All were less than 34 wks gestation. (4) The birth weights of the 7 ranged from 964 to 1332 grams.

Birth Wt. (gm)	250-500	501-750	751-1000	1001-1250	1251-1500	1501-1750	1751-2000	2001-2500	2500+
Lesions			1	5	1				
No Lesions	2	4	4	8	2	4	3	12	23

22 unaffected infants had > 4 HCP exposures and 10 had > 8. The life spans of the babies were comparable. No spongioform myelinopathy was found in infants > 1400 gm, > 34 wks gestation, or who had < 4 baths with 3% HCP.

No. of HCP Washes	0-3	4-6	7-8	9-10	11-15	16+
Lesions		1		2	3	1
No Lesions	42	9	3	5	2	3

A NON-INVASIVE APPROACH TO BODY COMPOSITION IN THE NEWBORN: DYNAMIC SKINFOLD MEASUREMENTS. J. Sumners*, Y. Brans, H. Dweck, W. Lockard and G. Cassady. Univ. Ala. Sch. Med., B'ham.

Midtriceps (MT) and subscapular (SS) skinfolds were measured within 24h. of birth on 12 growth retarded (IGR) and 16 premature (P) infants. Gestations were < 30 wks. in 2 IGR and 14 P infants. Measurements were recorded 15 and 60 seconds following application of a Harpenden caliper. A rapid initial decrease occurred in measured skinfold thickness (SFT) but readings stabilized by 60 secs. Tissue compressibility (estimated by the difference between 15 and 60 sec. readings)was expressed as percent (% Δ) of the 15 sec. reading.

Tissue compressibility was directly related to gestational immaturity. For SS measurements, 5 of 14 infants less than 30 wks. gestation had % Δ > 18 while all 10 infants 30 or more wks. gestation had % Δ < 18 ($p < 0.025$). For MT measurements, 9 of 16 infants less than 30 wks. gestation had % Δ > 18 while 10 of 11 infants 30 or more wks. gestation had % Δ < 18 ($p < 0.02$). These trends were the same for both P and IGR infants.

Tissue compressibility also correlated directly with maximum postnatal wt. loss (expressed as % birth wt.) in IGR infants. Three of 4 IGR's with > 10% Δ MT lost > 7.5% birth wt. while all 5 infants < 10% Δ MT lost < 7.5% birth wt. ($p < 0.02$).Both gestational immaturity and postnatal wt. loss have previously been related to extracellular water volume. The relation of tissue compressibility with both these parameters suggests that % Δ SFT may provide a useful, non-invasive clinical estimate of subcutaneous interstitial water.

*Dr. Sumners is currently on active duty attached to the Naval Regional Medical Center, Jacksonville, Florida.

RELATIONSHIP OF AMNIOTIC FLUID SURFACE ACTIVE MATERIAL AND GESTATIONAL AGE TO RESPIRATORY DISTRESS SYNDROME. D.W. Thibeault, & C.J. Hobel (Intro. by W. Oh), UCLA Sch. of Med., Harbor Gen. Hosp., Depts. of Peds. and Obstetrics, Torrance, Ca.

Amniotic fluid surface active material (AFSM) as assessed by the Foam Stability Test (FST, Clements et al, N.E.J.M., 286: 1077, 1972) was measured in 97 high risk pregnancies (gestation 26-37 wks.) and the infants followed for the subsequent development of the respiratory distress syndrome (RDS). The FST relates the presence of stable bubbles to AFSM and reads as negative (absence), intermediate (borderline) and positive (presence of AFSM). All amniotic fluid was collected within 24 hrs. of delivery. No significant correlation was observed between gestational age and the AFSM. RDS was significantly related to AFSM. Twenty-one/22 infants with negative, 12/23 with intermediate, and 17/52 with positive FST developed RDS. Therefore, RDS may occur with positive FST, and infants < 34 wks. gestation with pos. FST had significantly more RDS than infants > 34 wks. with pos. FST ($P < .01$) indicating that gestational age is also an important factor in RDS. Mortality of infants with RDS was related to a decreased AFSM ($P < .01$), and to low 5 min. apgar score (<5) ($P < .001$). The latter is significantly related to the intrapartum complications: third trimester bleeding, breech presentation, fetal bradycardia, twins, and maternal hypotension. This study suggests that surfactant is not the only determinant of RDS and that gestational age and intrapartum events are of major importance in determining the outcome of RDS.

NEONATAL HYPOCALCEMIA (NHC), HYPOMAGNESEMIA AND HYPERPHOSPHATEMIA IN BIRTH ASPHYXIA: Reginald C. Tsang, William Hayes, William Atkinson, Harry D. Atherton, Ivy Chen, Neil K. Edwards (Intro. by Irwin J. Light), Univ. of Cinti. Col. of Med., Dept. of Ped., Cincinnati.

Infants with birth asphyxia (BA, 1-min Apgar ≤ 6) have been reported to have an increased risk for NHC. Prematures also have an increased risk for NHC. Since BA infants are often premature, it has been unclear whether NHC in BA is related to prematurity or BA. Forty-two infants with BA, gestation 29-43 wks, were matched prospectively (sex and gestation) with 42 control infants with Apgar ≥ 7. Infants were studied from birth to 72 hrs. Serum Ca (12, 24 hrs) in BA infants was lower than serum Ca in controls (paired t, $p<0.025$). Serum P at 48 hrs was higher in infants with BA (paired t, $p<0.01$); serum Mg was lower from 12 to 48 hrs ($p<0.005$). Urinary Ca, Mg and P loss was not different from controls. Parathyroid extract 5 units/kg was given to 17 additional BA infants at 24 and 48 hrs of age, compared with 17 untreated matched infants with BA: in treated infants a calcemic response occurred (paired t, $p<0.01$). In conclusion, NHC occurs in infants with birth asphyxia: factors such as urinary Ca and Mg loss and end organ unresponsiveness to parathormone are unlikely causes of NHC; the triad of hypocalcemia, hypomagnesemia and hyperphosphatemia could be due to functional hypoparathyroidism.

RISK OF PERINATAL DEATH WITH INTRAUTERINE GROWTH RETARDATION (IUGR), Robert H. Usher and Frances H. McLean. Royal Victoria Hospital and McGill University, Montreal.

Among 44,259 consecutive deliveries, perinatal mortality rose in a geometric fashion from 13 to 687 per 1000 with increasing degrees of IUGR. Deaths of infants more than 2 S.D. underweight for date were due to anomalies in 24%, to other specific causes (primarily abruptio and infection) in 20%, and to obscure cause (mainly unexplained intrauterine or perinatal asphyxia) in 56%.

Deaths from obscure cause of IUGR fetuses after 33 weeks gestation are always potentially preventable. The frequency of such deaths increases progressively from 2 to 400 per 1000 with increasing degrees of IUGR. In all, 62 out of 1038 fetuses who were 2 S.D. or more underweight died of obscure cause in the last 6 weeks of pregnancy, accounting for 8% of the total over-1000g perinatal deaths. Such deaths are preventable by intrauterine diagnosis of IUGR and delivery as early as is indicated by the severity of the fetal deprivation process.

	PERINATAL MORTALITY PER 1000	
	ALL CAUSES ALL GESTATIONS	OBSCURE CAUSE AFTER 33 WEEKS
+1 to -1 S.D.	13	2
-1 to -2 S.D.	24	5
-2 to -3 S.D.	124	29
-3 to -4 S.D.	385	150
> -4 S.D.	687	400

RELATIONSHIP BETWEEN DURATION OF RUPTURE OF THE MEMBRANES AND THE DEVELOPMENT OF RESPIRATORY DISTRESS SYNDROME. Jing J. Yoon and Rita G. Harper Dept. of Pediatrics, Downstate Medical Center, Brooklyn, N.Y. (Introduction by Dr. M. Robinson)

The effect of rupture of the membranes on the development of respiratory distress syndrome was studied. Two hundred eleven consecutive infants (between 1001 and 2165 grams at birth and less than 38 weeks gestation) were investigated. The incidence of respiratory distress syndrome in infants with rupture of the membranes more than 24 hours prior to delivery was lower (12.5%:6/48) than in infants with rupture of the membranes less than 24 hours prior to delivery (23.3%:38/163).

Infants with factors which are known to predispose to respiratory distress syndrome (maternal hemorrhage, maternal diabetes, fetal asphyxia, twin B, history of a sibling with respiratory distress syndrome and cesarean section) were then eliminated. One hundred twenty infants remained. The incidence of respiratory distress syndrome in infants with rupture of the membranes more than 24 hours prior to delivery remained lower (3.2%:1/31) than in infants with rupture of the membranes less than 24 hours prior to delivery (19.1%:17/89).

From this study it appears that rupture of the membranes for more than 24 hours prior to delivery protects against the development of respiratory distress syndrome.

NEPHROLOGY

First Session

THE HETEROGENEOUS PATHOLOGY OF THE HEMOLYTIC UREMIC SYNDROME. Lorenzo C. Aschinberg, Boyce Bennett, Chester M. Edelmann, Jr., Adrian Spitzer, and Ira Greifer. Department of Pediatrics and Pathology, Albert Einstein College of Medicine, Bronx, New York.

It is generally accepted that the pathology underlying the hemolytic uremic syndrome (HUS) is renal thrombotic microangiopathy (RTM). The present report is based on 8 children varying in age between 8 months and 13 years fulfilling stringent criteria for the HUS: all presented with acute renal failure, hemolytic anemia, and thrombocytopenia. Renal biopsy performed between 10 and 42 days after clinical onset revealed RTM (3 patients), cortical necrosis (1 patient), tubular necrosis (2 patients) and proliferative nephritis (1 patient). The remaining patient, who was the only one that died, was found at autopsy to have thrombotic thrombocytopenic purpura. The clinical course in the patients with RTM, cortical necrosis and proliferative glomerulonephritis correlated well with the severity of the renal involvement. The two patients with tubular necrosis recovered promptly and completely. It appears, therefore, that HUS cannot be considered a histological entity. The heterogeneity of the renal lesions suggests variability in pathogenesis with important implications in regard to treatment and course of the disease. Reports on prognosis and results of therapeutic trials in this condition are meaningful only in the context of the underlying renal histology.

RENAL LESION IN THE HEMOLYTIC UREMIC SYNDROME(HUS): A CLINICAL ENTITY? Morrison Hurley, Pierre Dery, M. Bernadette Hogarty & Keith E. Drummond. Depts. of Nephrology & Radiology, McGill Univ. Montreal Children's Hosp. Research Inst., Montreal.

In only 17 cases of HUS reported from 1936-70 have renal abnormalities been mentioned. Renal pathologic findings in these patients were heterogenous and often an incidental finding at post mortem.

We have examined 7 families in which 9 children had HUS. All 9 patients had abnormal urographic findings. The salient features were a prolonged nephrogram, decreased concentration of contrast medium, parenchymal scarring and calyceal distortion. Of these patients 3 have died in renal failure, 3 have uremia and hypertension, 2 are well and 2 are not available for examination at present. Renal tissue was obtained by biopsy in 4 patients and at autopsy in 5 patients. In the 2 with abnormal radiologic findings only, with or without mild azotemia, mesangial proliferation and sclerosis without interstitial involvement was seen. This suggests that the glomerulus is the primary site of injury. The lesion in those with more advanced renal insufficiency consisted of interstitial scarring with pericelomerular fibrosis, glomerular sclerosis, chronic inflammatory cell infiltration and the presence of cortical and medullary cysts. These latter changes resemble those seen in medullary cystic disease.

Renal disease is a major feature of HUS and in our experience is a key determinant of mortality and morbidity.

PROPRANOLOL, A NEW DIAGNOSTIC AND THERAPEUTIC TOOL IN CHILDHOOD HYPERTENSION. A.M. Yuceoglu, Sarla Inamdar, Edward Wasserman. New York Medical College, Renal Service and Laboratories, Depts. of Medicine & Pediatrics, New York, N.Y.

Propranolol, a beta-adrenergic blocking agent was found to lower the blood pressure in adults with renovascular and essential hypertension. This action is closely linked to its suppressive effect on renin and aldosterone secretion. Two children, eleven weeks and 26 months, with hypertension of undetermined etiology and a 16-year old girl with chronic renal disease and hypertension were treated with Propranolol. Two to 8 mg/kg/day were administered for 2 months to 1.5 years. The two infants had markedly elevated serum renin values (7.2 and 20 nanogram/ml/hour) prior to therapy. No renin determination was made on the adolescent. The blood pressure returned to normal in one infant (from 146/90 to 90/60) and in the adolescent (from 180/120 to 110/70). A fall of 40mm Hg in systolic and 30mm Hg in diastolic was observed in the youngest infant, yet blood pressure remained at a hypertensive level for the age. Propranolol was tolerated well with no side effects by all patients. The serum renin level was repeated in the older infant 3 weeks after Propranolol therapy and was normal (1.1 nanogram/ml/hour). We thus believe that Propranolol is an effective antihypertensive drug for long-term therapy in renin-dependent hypertension. In addition, specificity of action of Propranolol may permit its use as a diagnostic aide in the identification of renin-dependent hypertension.

JUVENILE HYPERTENSION CAUSED BY OVERPRODUCTION OF RENIN BY A RENAL SEGMENT. S.Bennett,L.Levine,J.Lewy,M.Susin,R.Peterson,M.New.Cornell Univ.Med.Col.,Depts Ped.,Med.,& Pathology,New York

A 7 y.o. boy with severe hypertension (160/130) was found to have elevated peripheral plasma renins and elevated 24 hr urinary aldosterone(aldo) excretion.

Regimen	Peripheral renin	(normal)	24 hr urinary aldo	(normal)
Regular salt	17 ng/ml/hr	(0.5-5.0)	37.0 µg/24hr	(2.7-8.2)
Low salt	19 "		108.1 "	
High salt	8.5 "		29.1 "	
Propranolol	1.7		16.9 "	
Post-op	5.5		2.7 "	

The plasma renin and urinary aldo levels increased with low Na+ diet and decreased with high Na+ diet. Only propranolol decreased the renin to normal. IVP,BUN,electrolytes and creatinine clearance were normal. Renal arteriogram showed a very small aneurysmal dilatation of intrarenal right lower pole renal artery,but renal vein renins were equal bilaterally.Renin levels at the time of right lower pole nephrectomy were measured as 19.6 ng/ml/hr from the right upper pole renal vein and 56 ng/ml/hr from the lower pole renal vein. Biopsy of the upper pole of right kidney and left kidney were normal. Pathology of the removed right lower pole demonstrated marked juxtaglomerular hyperplasia and increased granularity. The patient became normotensive post-operatively without treatment and renin levels normalized. This is the first example of segmental renal renin overproduction as a cause of childhood hypertension which responded to removal of the source of renin overproduction.

EFFICACY OF FUROSEMIDE IN TREATMENT OF EDEMA IN CHILDREN WITH NEPHROTIC SYNDROME. Winston C. Wong and John E. Lewy. Cornell Univ. Med. Col.-New York Hosp., Dept. of Ped., New York.

Furosemide was administered orally to 12 children with the nephrotic syndrome accompanied by marked generalized edema. Serum protein averaged 3.5 gm% and albumin 0.9 gm%. Furosemide (2 mg/kg) was given as a single oral dose at the start of the periods. Urine volume increased from 0.24 ± 0.04 (mean ± 1 S.E.) in the 12 hrs. before, to 0.86 ± 0.12 ml/min/M^2 in the 12 hrs. after furosemide ingestion. Urinary sodium increased from 6.3 ± 2.1 to 63.7 ± 14.4 µEq/min/M^2. Urinary potassium increased from 19.0 ± 3.8 to 37.1 ± 2.6 µEq/min/M^2 and urinary Na/K ratio increased from 0.33 ± 0.18 in the control period to 1.72 ± 0.37 after furosemide. With subsequent doses, volume increased from a pre-furosemide value of 0.36 ± 0.08 to 1.10 ± 0.25 ml/min/M^2, urinary sodium increased from 12.7 ± 6.5 to 94.6 ± 26.6 µEq/min/M^2, and urinary potassium from 20.7 ± 4.7 to 43.9 ± 9.8. GFR was not significantly altered in any period after furosemide. Four children with an initial GFR of 50.0 ± 8.1 ml/min/1.73M^2 had as effective a natriuresis as 8 others with a GFR of 105.7 ± 11.8 ml/min/1.73M^2. The average weight loss after 3 days therapy was 38% of edema as judged by subsequent dry weight.

Furosemide was thus shown to be an effective diuretic and natriuretic agent in the nephrotic syndrome in children despite severe hypoproteinemia and decreased GFR. Undesirable side effects were not evident in patients in this short term trial.

RENAL TUBULAR RESPONSE IN VIVO TO PTH AND DIBUTYRYL-cAMP (db-cAMP).R.McInnes & C.Scriver.McGill Univ.-Montreal Children's Hosp.Res.Inst.,Montreal,Quebec.

PTH modulates the inhibition of tubular reabsorption in certain conditions (Morris et al, PNAS 68, 132, 1971) and our previous data show that tubular responses differ after acute and chronic exposure to PTH. Tubular reabsorption of Pi (TRP) and of non-metabolizable ^{14}C-α-aminoisobutyrate (TRAA) was measured in anesthetized volume-expanded rats perfused with ^{3}H-inulin over 6-8 hr. Plasma and renal tissue amino acids were measured chromatographically. In the intact rat, rapid bovine PTH infusion (12-50µ/kg/hr) immediately decreases TRP but inconsistently inhibits TRAA. In thyroparathyroidectomized, normo-calcemic rats, PTH inhibits only TRP, whereas db-cAMP immediately inhibits TRP and stimulates TRAA. While TRAA stimulation by db-cAMP is immediate, plasma AA remains unchanged for 2 hr, then falls 30%. This contrasts with in vitro studies (Weiss et al, JBC 247, 760, 1972) where db-cAMP increased renal cortex AA's only after 2 hr incubation. It is evident that Pi and AA transports are independent in energy coupling as well as binding sites, and that db-cAMP may act differently on the luminal and capillary surfaces of the tubular cell.

THE RELATIONSHIP OF BODY AND KIDNEY SIZE TO RENAL FUNCTION IN SUCKLING RATS. 1. RESPONSE TO NH_4Cl LOADING. E.S. Moore, M.S. Ocampo and E.C. Lyons. (Intr. by G. Honig.) Univ. of Ill. Coll. of Med., Dept. of Ped., Chicago.

Excretory needs, dietary intake and kidney size are thought to influence the rate of maturation of renal function during the first year of life. This hypothesis was studied by measuring the renal response to NH_4Cl loads in normal and malnourished suckling rats. Newborn infant rats were separated into unequal liter sizes within the first 24 hours of life. Larger liters consisted of 17-19 babies to one mother while small liters were 4-6 babies. At the end of 21-28 days, the babies from the small liters averaged 60.7 gms while those from the large liters averaged 38.7 gms ($p<.001$). NH_4Cl loads were given intrapertoneally to the infants with the following results:

INFANTS	URINE pH	TA uEq / min	NH_4^+	C_{cr} uml/min	DRY KIDNEY WGT.
Small	5.62	0.02	0.23	70.47	0.099
Large	5.51	0.02	0.29	164.58	0.160
p	—	—	—	<.05	<.001

Although the infants were the same age, the larger infants had statistically higher C_{cr} and kidney weights. However, there was no significant difference between UpH and excretion of TA and NH_4^+. These studies suggest that maturation of specific tubular functions is related to age and may proceed independent of excretory needs, dietary intake and kidney size.

EFFECT OF CYCLIC AMP ON RENAL TUBULAR PERMEABILITY. William B. Lorentz, Jr. (Intr. by Luther B. Travis), Dept. of Ped., Univ. of Texas Med. Br., Galveston, Texas.

The effect of cyclic AMP on renal tubular permeability has been studied utilizing micropuncture techniques in the rat kidney. Nanoliter quantities of a solution of ^{3}H inulin and ^{14}C mannitol in normal saline were microinjected into surface nephrons, and urine from both kidneys analyzed for radioactivity. During control periods inulin (99.4 ± 2.7%) and mannitol (97.9 ± 2.8%) recovery from the experimental kidney was essentially complete. Following an infusion of cyclic AMP or dibutryl cyclic AMP into the aorta, reinjections were performed at the same puncture site. Inulin (99.1 ± 1.7%, $p>0.9$) recovery was not different from control. Mannitol recovery was significantly decreased following both early proximal (79.0 ± 6.9%, $p<0.001$) and late proximal (89.7 ± 2.6%, $p<0.001$) injections. There was also a highly significant difference ($p<0.001$) in mannitol recovery between early and late proximal injection sites. However, there was no significant loss of either mannitol or inulin following distal tubular injections. These results indicate that cyclic AMP induces a change in proximal tubular permeability to a usually impermeable non-electrolyte, mannitol. These studies suggest that cyclic AMP may inhibit reabsorption of sodium and phosphate by altering the permeability characteristic of the proximal tubular epithelium. This alteration would allow for increased back-flux of sodium and phosphate into the tubular lumen and decrease net reabsorption.

EFFECTS OF ACETATE ON THE DEVELOPING KIDNEY TUBULE. Barbara R. Cole, Kathy G. Bartlett and Alan M. Robson. Wash.Univ.Sch.of Med., St.Louis Children's Hosp., Dept. of Ped., St. Louis.

The patterns of development of the proximal tubular mechanisms for PAH uptake and maintenance of intracellular/extracellular fluid spaces, Na, and K gradients have been studied in the developing rabbit using the slice technique of Cross and Taggart. PAH uptake was low in fetal slices, increased post-partum to above adult levels by 4 weeks of age and declined to adult values by 10 weeks. Fetal slices had increased $(Na)_I$ but as early as 1 week of age $(Na)_I$ was maintained at adult levels. ECF and ICF spaces progressively decreased to adult values by 4 and 8 weeks respectively.

Eliminating acetate from the medium had little effect on PAH uptake in the fetus, whereas uptake was reduced 50% or more in animals 4 weeks or older. Cell sizes and ICF/ECF cation gradients were preserved in the absence of exogenous substrate. Increasing the acetate concentration in the medium from 10 to 30 mEq/L did not further stimulate PAH uptake in the immature kidneys nor were cell size and cation gradients changed.

The data illustrate that individual transport mechanisms in the proximal tubule mature at different rates. In addition the acetate studies suggest the existence of two mechanisms of PAH uptake; one unaffected by acetate is active during gestation; the other, acetate-dependent, matures after birth. The immature kidney's ability to maintain cell integrity in the absence of exogenous substrate indicates the presence of adequate endogenous energy sources.

CHLOROTHIAZIDE AND LOW SOLUTE DIET: ITS ROLE IN TREATMENT OF NEPHROGENIC DIABETES INSIPIDUS. Sandra L.H. Davenport, Moira Feeney, David B. Shurtleff, C. Ronald Scott. Dept. of Ped., Univ. of Washington, Seattle, Washington.

Accepted medical therapy for Nephrogenic Diabetes Insipidus has not usually resulted in normal growth and development. We have followed 10 boys for 7 mo. to 12 yr. using a modification of standard regimens, which emphacizes intermittent chlorothiazide, low solute diet, and ad libitum water intake. This therapy has reduced urinary volume by 32-60% [$\bar{x}$ = 47%, P = <.001]. Long-term results can be divided into 3 groups: I. Combined therapy with ad lib. water begun before 6 mo. of age, II. combined therapy with water started after 6 mo., and III. ad lib. water begun prior to 6 mo. but combined therapy started after 6 mo. Analysis of the groups indicates that, in patients started on treatment after 6 mo., intellectual impairment was uniformly present. Institution of ad lib. water in 7 of 10 boys at less than 6 mo. of age (Groups I. & II.) preserved normal intelligence [P < .01]. When low solute diet and intermittent chlorothiazide was also begun less than 6 mo, normal growth was achieved.

The effect of early treatment of males with N.D.I. is striking. Of patients started on ad lib. water, low solute diet, and intermittent chlorothiazide prior to 6 mo. of age, all have achieved normal growth and development.

NEPHROLOGY

Second Session

EFFECTS OF GANGLIONIC BLOCKADE UPON KIDNEY AND CARDIOVASCULAR FUNCTION IN BURNED DOGS. Hugo F. Carvajal, Daniel L. Traber and John A. Reinhart (Intr. by Luther B. Travis), Univ. of Texas Med. Br. and Shriners Burns Inst., Depts. of Ped. and Physiol., Galveston, Texas.

In order to determine the role played by an overactive sympathetic system in the genesis of postburn oliguria, kidney and cardiovascular function studies were performed in conditioned mongrel dogs with and without ganglionic blockade (chlorisondamine). Glomerular filtration rate (GFR) and renal plasma flow (RPF) were measured utilizing sodium iothalamate I^{-125} and sodium para-aminohippurate clearance techniques. Tests of cardiovascular function included cardiac output (CO), total peripheral resistance (TPR), left ventricular peak dp/dt/p and end diastolic pressure (LVEDP). All studies were performed simultaneously at 20 minute intervals for 1 hour prior to the burn and 4 hours after a 20% standard full thickness flame burn was induced. Maintenance and replacement fluids were given during the whole procedure. In the untreated animals after the burn, there was a fall in urine output, GFR, RPF, CO and LVEDP; TPR and peak dp/dt/p increased. In the ganglionic blocked animal, the burn induced no changes in urine volume but only a slight and often transient drop in GFR, RPF and cardiovascular function. These studies support the contention that a hyperactive sympathetic system is partly responsible for the state of renal hypoperfusion and antidiuresis that characterizes the immediate postburn period and opens several therapeutic avenues that need to be further explored.

KININS IN THE PATHOGENESIS OF RENAL DISEASE. Donald B. Kaufman & Joan Mattson (Intr. by William B. Weil), Mich. State Univ., Col. of Human Medicine, Dept. of Human Development & Path., East Lansing.

The Kallikrein-Kinin system has been implicated in the pathogenesis of inflammation. Hori et.al. demonstrated proteinuria in rabbits after injection of a crude kallikrein extract. We have recently evaluated the effects of kinin activation on the male rabbit kidney. 2Kg. male albino rabbits were anesthetized with sodium pentobarbitol. Control collections of blood and urine were made at timed intervals for 1/2 hr. Six units of purified plasma kallikrein were given IV. urine and blood were collected at 30 sec, 1 min, 2 min, 5 min, 10 min, 15 min and then every 15 min for 2 hrs. The rabbits were sacrificed and the kidney examined by E.M., fluorescent and light microscopy. Plasma kininogen and kinin levels were measured as were urinary kinin levels. Urine protein was measured quantitatively. Within 1 min. after injection proteinuria was noted followed by hematuria and oliguria. Maximal hematuria and proteinuria was noted between 1/2 and 1 hr, oliguria persisted up to 1 1/2 hrs. These findings were associated with an elevation of 8 fold in serum and 10 fold in urinary kinin levels and decreased kininogen levels. Histologic sections of the kidneys revealed increased numbers of eosinophilic polymorphonuclear leucocytes.

These findings suggest that activation of the kinin system may play a role in the pathogenesis of glomerulonephritis.

(Supp. GRS and So. Cal. Kidney Fdn.)

BERGER'S DISEASE. Bernard Gauthier, Eli Friedman, Edmund Whang In-Tai Cheong, Anthony Nicastri and Melinda McVicar. Dept. of Ped., Med. and Path., Downstate Medical Center, Brooklyn, N.Y. (Introduction by Salvador Castells)

In 1969 Berger reported finding IgA in the glomeruli of 55 patients who had persistent microscopic hematuria and bouts of gross hematuria. Histological findings were variable. 52 had normal renal function and 1 progressed to renal failure. The table summarizes our experience with 12 patients with mesangial deposits of IgA. None had SLE or Henoch's purpura. 8 had minimal to moderate mesangial proliferation. The findings in the others were variable. 11 had recurring bouts of gross hematuria and 8 had persisting microscopic hematuria.

Pat.	Sex	Age	GFR	Proteinuria	Prodrome	Duration (years)
1.	F	50	↓	0.9G/24Hr	0	6
2.	F	9	↓	2+	Pharyngitis	0.6
3.	M	16	↓	3.3G/24Hr	URI	8
4.	M	6	N	4+	URI	1.6
5.	M	11	N	2+	Fever	0.3
6.	M	23	N	0.6G/24Hr	Pharyngitis	5
7.	F	8	N	0.2G/24Hr	Fever	1.7
8.	M	10	N	0.4G/24Hr	0	3
9.	M	16	↓	3+	Pharyngitis	2.3
10.	M	12	↓	0.3G/24Hr	Flank Pain	4
11.	M	7	↓	0.2G/24Hr	Pharyngitis	3.8
12.	M	8	↓	0.05G/24Hr	Pharyngitis	2.3

Patients 3 and 9 developed end stage kidney disease. Our experience suggests that Berger's disease has a male preponderance and a guarded prognosis.

GLOMERULAR FUNCTION AND MORPHOLOGY IN CHILDREN WITH DIABETES MELLITUS(DM).R.Morrison Hurley,Mimi M.Belmonte,Sharon Johnson & Keith N.Drummond.McGill Univ.-Montreal Children's Hosp. Research Inst.,Depts.of Nephrology & Metabolism,Montreal.

Glomerular functional,structural and immunopathological changes were studied in 19 DM patients with no evidence of renal disease.Duration of DM was 13-133 months(median 62).The glomerular filtration rate(GFR)by the cumulative integral method,micro-proteinuria in a 12 hr resting specimen and renal biopsy were done.Results were compared with duration of DM,degree of control,age,puberty staging,insulin dose and body size.All but 2 patients had an increased GFR compared to normals(95&71 ml/min/m^2 respectively,p < .001).Increased GFR does not correlate with duration of DM,degree of control,insulin dosage or severity of changes on renal biopsy.All biopsies showed mild to moderate increase in mesangial matrix,6 showed thickened peripheral glomerular basement membrane(GBM).None of the latter were well controlled.Morphologic changes were not related to age,duration of DM,or insulin dose.In none was GBM deposition of IgG,B_{1C} or fibrin present,a finding at variance with those of other authors;however in some there were focal vascular deposits of B_{1C} and fibrin.Increased protein excretion was seen in 1 patient.

This study shows early renal functional changes in DM - increased GFR;this and the morphological changes are not correlated with duration of DM suggesting that some aspects of the renal manifestations may be independent of known or controllable features of this disease.

THE SIGNIFICANCE OF CRYOPROTEINS IN RENAL DISEASE
William R. Griswold, William J. Chernack, and Rawle M. McIntosh
Columbia University, New York

We have previously reported the presence of serum cryoproteins in patients with renal disease and suggested that they are of clinical and immunopathological significance. The present study reports our five year experience on the nature, incidence, prognostic and diagnostic significance of serum cryoproteins in 213 patients with renal disease as well as 30 controls. Pretransplant, posttransplant and hemodialysis patients were studied. All forms of renal disease except SLE were included. Cryoproteins were isolated and characterized as described previously; their presence was correlated with morphologic, functional and immunologic data. Excluding hemodialysis patients, cryoproteins were detected only in patients with immune deposit nephritis. They were associated with immune deposit nephritis even in patients with apparent benign hematuria and proteinuria. All cryoproteins contained IgG: IgM, IgA, B1C and fibrinogen were variably present. The persistence of cryoproteinemia and the presence of fibrinogen were poor prognostic signs. In hemodialysis patients Australian antigen and specific antibody were demonstrated in cryoprecipitates. Mixed cryoglobulins with rheumatoid factor activity were also detected. Cryoprotein detection is a useful tool in diagnosis, prognosis and management of renal disease.

ROLE OF SOMATOMEDIN AND RENAL FUNCTION IN GROWTH AFTER RENAL TRANSPLANTATION. Paul Saenger, Eckehart Wiedemann, Sigrun Korth-Schutz, John E.Lewy, Robert R.Riggio, Albert L.Rubin, Kurt H.Stenzel, Ernest Schwartz and Maria I. New. Depts.of Ped.,Surg.and Biochem.,Cornell Univ.Med.Col.,New York and Dept.of Med., V.A. Hospital, Bronx, N.Y.

Hormonal and metabolic factors influencing growth were studied in 9 uremic children who received renal homografts. While virtual growth arrest was present prior to transplantation in all, except one, post-transplant growth velocity based on bone age (GVBA) became normal in 4 (88-103%) accelerated in 2 (127-139%), and remained subnormal in 3 (18-50%). Serum somatomedin (SM), now recognized as an important factor in linear growth, was very low in all children before transplant (0.39 $\pm$ 0.12 U/ml) with levels in the hypopituitary range, but rose in each child after transplantation (0.83 $\pm$ 0.16 U/ml), reaching the normal range in all except 2. Post-transplant GVBA was not related to glucose tolerance, growth hormone response to arginine-insulin stimulation, thyroid function and plasma FSH and LH levels, but showed significant positive correlations ($p < 0.05$) with both serum SM activity and creatinine clearance. No significant correlation existed between post-transplant creatinine clearance and SM activity. The data suggest that growth failure in severe chronic uremia is at least in part due to lack of serum SM. In addition, even moderate azotemia may be associated with a state of resistance to somatomedin action.

GROWTH FAILURE IN UREMIC RATS: THE ROLE OF CALORIE DEFICIENCY. Robert C. MacDonell, Jr., Macario M. Buzon, and Malcolm A. Holliday. Dept. of Ped., Univ. of California, San Francisco.

Food intake and growth were studied from 25-60 days of age in 21 female rats subjected to 7/8 nephrectomy at 30 days (U) and 9 sham controls (C). Both ate ad libitum a diet providing over 150% of recommended essential nutrients, including protein (34% of calories). Significant differences in food intake appeared by 35 days, body weight (BW) by 40, and tail length by 45. Mean values (+SEM) for BUN, average food intake (Kcal/d), and average weight gain (Gm/d) are given (* = $p < .01$):

	BUN	Kcal/d	Gm/d
C	24(0.7)	55(0.9)	4.7(0.16)
U	72(1.7) *	46(0.9) *	3.8(0.14) *

Significant correlation existed between food intake and weight gain ($r = .89$); no correlation was found between BUN and either food intake or weight gain.

To test the hypothesis that calorie deficiency in uremia limits growth, 8 additional controls (PF) were pair-fed with 8 uremics (U) from 25-50 days. Growth of PF and U was the same and was significantly less than in 9 controls allowed free access to food (C). Six uremic rats (LP) fed an isocaloric low protein (13% of calories) diet showed the same calorie intake and growth as U and PF, despite 60% less protein intake. Initial BW=58 Gm; BW at 50 days: C=181, U=148, PF=146, LP=146.

The results demonstrate the importance of reduced food intake in the growth retardation of these uremic rats. Total calorie intake, rather than protein intake, appears to be the more critical nutrient.

THE RENAL RESPONSE OF THE NEWBORN TO HYPOXIA
Marc I. Rowe and Jose Strauss Div. Ped. Surg. and Ped. Nephrology, Univ. of Miami Sch. of Med.

To determine the renal response to hypoxia 30 newborn piglets were studied under Ketamine anesthesia. The following variables were measured every 30 min: arterial and venous blood pressure, pulse rate, cardiac index, body temperature, arterial blood pH, pCO_2 and pO_2, serum and urine electrolytes, urea nitrogen and total osmolality. The animals were separated into two groups balanced for age, weight and litter. The control group spontaneously breathed room air for 4 hrs, the experimental group 10% oxygen. There were no changes in cardiac index, pulse rate, blood pressure or U/P_{osm} ratio in either group. Body temperature, $paCO_2$ and paO_2 fell in the hypoxic animals. The total urinary output during the 4 hr period was 25.5 ml in the hypoxic piglets and 14.1 ml in the controls. The control animals' urinary flow rate and osmolar clearance remained stable (average urinary flow rate = 0.0587 ml/min, average C_{osm} = 0.06 ml/min). In the hypoxic animals urine flow rate and C_{osm} was higher reaching a peak during the first 60 min - urine flow rate = 0.151 ml/min; C_{osm} = 0.22 ml/min. The T^C_{H2O} of the hypoxic piglets was seven times greater than the controls - 0.01 ml/min versus 0.07 ml/min. It is concluded that urine flow rate, C_{osm} and T^C_{H2O} increase with moderate hypoxia and these changes occur although the U/P_{osm} ratio and cardiovascular function remain stable.

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NEONATAL CHANGES IN RENAL BLOOD FLOW DISTRIBUTION IN PUPPIES. Lorenzo C. Aschinberg, David I. Goldsmith, Herman Olbing, Mark Hardy, Adrian Spitzer, Chester M. Edelmann, Jr., M. Donald Blaufox. Dept. of Ped. and Med., A. Einstein Col. of Med., Bronx N.Y.

Morphologic evidence and data from this laboratory suggest that redistribution of renal blood flow is an important component of maturation during the first 6 weeks of life. In this study intrarenal blood flow distribution was determined in 30 puppies (age 1-69 days). ^{133}Xe washout was monitored for 45 min. after a left renal artery injection (0.3 ml, 250μc). Curves were analyzed as multiexponentials; anatomical correspondence was established by autoradiography. The functional integrity of the left kidney was validated by injecting ^{203}Hg systemically and comparing the uptake of both kidneys. Mean blood flow increased linearly from 0.6 (day 1) to 2.2 ml/g/min (day 69). Inner cortical flow was 0.7 ml/g/min at birth and remained unchanged throughout the study. Outer cortical blood flow increased abruptly from 0.12 (day 1) to 3.7 ml/g/min at day 10 and then remained unchanged. The fraction of total renal blood flow expressed as the ratio of outer cortex/inner cortex was 1.1 at birth, 0.27 at 3 weeks, and 4.3 at 10 weeks. These findings suggest that the deeper nephrons are functionally dominant during the first 3 weeks of life. The superficial nephrons appear to play little functional role during the first two weeks, and thereafter assume a progressively increasing role, mature functional balance being attained at 8 to 10 weeks.

EFFECT OF OSMOTIC DIURESIS ON RENAL SOLUTE GRADIENT IN THE FETAL LAMB. E.S. Moore, M.B. Galvez, J.B. Paton and C.W. deLannoy. (Intr. by S.P. Gotoff.) Univ. of Ill. Coll. of Med., Dept. of Ped., Chicago.

The effect of osmotic diuresis on renal solute gradients was studied in fetal lambs. Slices of cortex, medulla and renal papilla were analyzed for urea, Na^+, K^+, and osmolar concentrations. In 16 control studies, the [Na] in the fetal renal cortex, medulla and papilla were 96.5, 134.2, and 168.4 mEq/l of tissue water; the calculated osmolarities were 327.9, 399.3, and 473.1 mOsm/l of tissue water. Simultaneous fetal arterial and urine osmolarities were 310.0 and 152.8 mOsm/kg. Thus in the control state, although the urine was hypotonic to plasma there was a sodium and osmotic gradient that increased from cortex to papilla. After control collections for UV, $U_{Na}V$, C_{H_2O} and GFR, 10% mannitol and 10% urea were infused rapidly into 2 additional groups of fetuses. After mannitol and urea, UV, $U_{Na}V$ and C_{H_2O} increased significantly. At the height of increased UV during infusion of mannitol and urea, the kidneys were immediately removed. After 10% mannitol and urea, [Na] in the cortex, medulla and papilla fell but not significantly. Calculated osmolarities also fell but not significantly and the medulla and papilla remained hypertonic to plasma. These studies suggest that although tubular fluid [Na] is lowered during osmotic diuresis, active Na^+ reabsorption continues and renal solute gradients are preserved in the fetal kidney.

NEPHROLOGY

Read by Title

IMPROVED GROWTH IN CORN OIL SUPPLEMENTED UREMIC RATS. Raymond D. Adelman and Malcolm A. Holliday. Dept. of Ped., Univ. of California, San Francisco.

Male Sprague Dawley rats on a diet of 43.5% protein, 15% fat, 38% dextrose, and 3.5% Jones Foster salts with vitamins, underwent 7/8 nephrectomy at 35 days of age. At 52 days of age the nephrectomized rats differed significantly from sham operated controls in weight (152 gm vs. 200 gm, $p < .01$), calories ingested/100 gm body weight/da (100 gm BW/D), (41.5 kcal vs. 45.5 kcal, $p < .05$), and BUN (84 mg% vs. 23 mg%, $p < .05$). The nephrectomized uremic rats, paired according to weight, tail length, age, and BUN, were continued on stock diet and gavaged daily with 2.2 cc/100 gm BW/D of either H_2O (control unsupplemented) or corn oil (supplemented). After 18 days of gavage, the supplemented group differed significantly from the control group in the following:

	SUPPL	CONTROL	p
Gm of weight gain/100 gm BW/D	2.28	1.52	<.05
Calories ingested/100 gm BW/D	33.0	30.8	<.01
Gm of protein ingested/100 gm BW/D	1.78	2.68	<.01
Gm weight gain/100 kcal	6.9	5.0	<.01
Tail length gain--mm/100 mm tail/D	1.02	0.77	<.05

Corn oil supplementation increased caloric intake, decreased protein intake and significantly improved growth of young uremic rats as measured by weight and tail length. The specific contribution to growth of each variable--calorie intake, fat intake, protein intake, and modified feeding pattern--has not yet been determined.

RENAL GLUCOSE AND SODIUM EXCRETION IN THE NEWBORN DOG. Jeffrey T. Baker and Leonard I. Kleinman (Intro. by Irwin J. Light), Univ. of Cinti. Coll. of Med., Dept. of Ped., Cincinnati.

Factors affecting renal glucose and Na excretion were studied in 22 puppies, 1-14 days of age and in 7 adult controls. In 9 puppies and 2 adults in which glucose titration curves were obtained, greater glucose splay and lower plasma threshold were observed in the puppy. During glucose loading in 13 puppies and 5 adults, total Na excretion (ueq./min/g.kid) and fractional Na excretion (FE_{Na}) were higher in the puppy (10.6 and .16 respectively) than in the adult (3.4 and .03) $p<.01$. At any level of glucose excretion, Na excretion was higher in the puppy. Those puppies with higher maximum glucose reabsorption/ml filtrate $(T_G/GFR)_M$ had lower values of FE_{Na}, suggesting a relationship between Na and glucose reabsorption. Distal blockade of Na reabsorption (DB) was accomplished with ethacrynic acid and chlorothiazide. In the puppy, when DB followed glucose loading, FE_{Na} increased from .158 to .598, and when glucose loading followed DB, FE_{Na} increased from.409 to .561. During antidiuresis, FE_{Na} in puppies was .005. Since the increment of FE_{Na} due to glucose loading before DB (.158-.005) was the same as after DB (.561-.409), Na loss during glucose loading probably came from the proximal tubule. *These results support the following conclusions:* compared to the adult, a) the puppy spills glucose at lower plasma levels, b) the puppy loses more Na for any glucose loss, c) the puppy has a greater depression of proximal tubular Na reabsorption in response to a glucose load.

NEPHROSONOGRAPHY IN THE EVALUATION OF RENAL FAILURE IN INFANTS. Frank G. Boineau, Jeffrey P. Rothman, John E. Lewy. Cornell Univ. Med. Col.-New York Hosp., Dept. of Ped. and Radiology, New York.

Nephrosonography has been shown to be a useful non-invasive adjunct in the investigation of renal tract pathology in children and adults. The availability of a probe with a 0.5 cm diameter allowed this technique to be applied to five infants with non-visualizing kidneys on intravenous pyelography. These were all carried out and interpreted without knowledge of the underlying pathology. In one infant with renal failure, a non-visualizing IVP and normal cystogram, the nephrosonogram demonstrated renal hypoplasia. This was later confirmed by retrograde pyelography. In a second infant, hydronephrosis was suggested prior to and confirmed at exploration. IVP and cystogram were unrevealing. A third infant had a large flank mass. The nephrosonogram suggested an upper pole cystic mass. At operation a double collecting system with hydronephrosis of the upper system was demonstrated. In two infants, the ultrasound study revealed the absence of one kidney. This was confirmed at operation in the one who was explored.

This technique appears to be a useful adjunct to conventional radiography in the differential diagnosis of the infant with urinary tract anomalies. It is of especial value when a kidney does not visualize by IVP and may appropriately guide therapeutic decisions without the need to employ invasive techniques with their attendant increased morbidity.

OSTEOPETROSIS (O) AND RENAL TUBULAR ACIDOSIS (RTA). J. Baluarte, L. Hiner, A. Root and A. Gruskin. Dept. Ped. Temple Univ. Sch. Med. and St. Christopher's Hosp. Child. Phila., Pa.

A male infant with normal acid phosphatase was found to have O and RTA at 9 mos. of age. The HCO_3^- threshold was low (14.7-15.3 mM/l) and more than 19% of the filtered HCO_3^- load was excreted at a plasma HCO_3^- 19.7 mM/l. These data and the dose of alkali (>10 mEq/kg/day) needed to correct the acidosis document a rate-defect in HCO_3^- reabsorptive capacity (Type II RTA). NH_4Cl loading failed to establish a steep urine/plasma pH gradient (urine pH >7) even though serum HCO_3^- fell 3 mM below the threshold. This is indicative of a gradient-defect (Type I RTA). Determination of Cl^-space did not show volume expansion, although $PO_4^=$ and lactate clearances increased, and uric acid clearance fell after the HCO_3^- threshold was reached. Increased uric acid, $PO_4^=$ and lactate clearances occurred in other HCO_3^- titrations when threshold was associated with ECF expansion. Inactivation and/or resistance to parathyroid hormone (PTH) have been postulated as etiologic factors in O.Circulating high levels of PTH could cause RTA since PTH increases urinary excretion of HCO_3^- and decreases the NH_4 excretion.During Ca^{++} infusion serum Ca^{++} rose,but the PTH levels were undetectable; $PO_4^=$ clearance was normal. Conclusions: 1)No relationship between RTA, PTH levels and O was found. 2)The relationship between O and RTA may constitute a genetically determined syndrome. 3)The attainment of the HCO_3^- threshold is associated with changes in clearances of $PO_4^=$ and lactate but not of uric acid.

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TREATMENT OF ALPORT'S SYNDROME WITH CYCLOPHOSPHAMIDE (CPA) AND PREDNISONE (P): A FIVE YEAR STUDY. Robert A. Campbell, James E. Musgrave, James C.M. Chan, Elsa Y. Jacinto, and Yeshawant B. Talwalkar. (Intr. by Richard V. Olmsted) Univ.of Oregon Med. School, Portland

A 15 year old Mexican-American boy was found at age 9 years to have severe high-tone neural deafness, hematuria, proteinuria (Pr), subclinical nephrotic syndrome, hyperchloremic acidosis and large kidneys on I.V.P. Two close relatives were noted to have hematuria. Open renal biopsy revealed widespread interstitial nests and, in some cases, cords of foam cells. Bowman's capsules showed periglomerular fibrohyaline thickening and fibrosis. Over a 5 year period the patient was hospitalized six times for study. Following the first course of combined therapy (incremental CPA from 2 mg/kg p.o./day and P2 mg/kg p.o., q.O.D.) and the induction of leukopenia, Pr fell from 11 and 24 gm/24 hrs. to 0.5 gm. Creatinine clearances (Ccr) increased from 54 and 66 mm/min/1.7 M^2 to 80 and 127. Lost to follow-up for 2 years, the patient then, despite progressive low-grade uremia, responded to treatment during three additional hospitalizations.

Rx courses		1	2	3	4
↓ Pr	gm	73	80	55	35
↑ Ccr	%	31	9	19	32

A sixth admission, during which no CPA was administered, compared with previous courses of therapy suggested no improvement, there being no change in serum creatinine or BUN.

CELLULAR RESPONSE TO STREPTOCOCCAL ANTIGENS IN PATIENTS WITH ANAPHYLACTOID PURPURA. Rajesh M. Bhatnagar, Aaron R. Rausen, Stanley E. Read, and John B. Zabriskie. The Rockefeller University, New York and Mt. Sinai Medical School, New York.

In view of the hypothesis that previous exposure to streptococcal antigens may play a role in the development of the clinical and laboratory symptoms of patients with anaphylactoid purpura, the cellular response to Group A streptococcal cellular antigens and human renal antigens was studied in 14 patients with this disease. Using the in vitro technique of capillary migration inhibition of leucocytes as our index of cellular reactivity to these antigens, the cellular response to streptococcal and renal antigens was carried out in all patients.

The results demonstrate that patients with evidence of documented renal disease had a heightened response only to streptococcal membrane antigens (30.4%) as compared to results obtained in children with uncomplicated streptococcal infections (11.6%). The response to streptococcal cell walls was the same for both groups (23% and 20% respectively). Further analysis of these patients indicated that this heightened cellular response to streptococcal antigens was not related to the level of the ASO titer. Patients with ASO titers of <100 had 22% inhibition. Patients with ASO >500 had 29% inhibition. Migration inhibition values for renal antigens were the same for both groups (11% and 8% respectively). These studies suggest that prior streptococcal sensitization may play a role in the development of renal disease in these patients.

LONG-TERM FOLLOW-UP OF CYCLOPHOSPHAMIDE(C)THERAPY IN FREQUENT RELAPSING MINIMAL LESION NEPHROTIC SYNDROME(MLNS).Jane M.Chiu & Keith N.Drummond.McGill Univ.-Montreal Children's Hosp. Research Inst.,Dept. of Nephrology,Montreal.

Prospective controlled studies have demonstrated the value of C in reducing relapse rate and prolonging length of remission.There are no reports on the long-term follow-up of such pts.We have followed 36 such pts.At initiation of C the mean period from onset of MLNS was 5 yr with a mean of 7.3 episodes of NS/pt.Mean follow-up is 3.3 yr.At end of follow-up, relapse rate is 27% and minimal interval of relapse(MIR)2.2 yr.Both values are significantly different($p < 0.001$)from those of pts treated with prednisone alone(11 pts:follow-up 2.3 yr;relapse rate 91%;MIR 0.9 yr).No serious complications of C therapy were observed.Of the 5 girls who reached the age of puberty,each has normal menses and sexual development.In the 10 pts who have relapsed following C we found:1)when pt was treated with steroid alone again(9 pts),frequency of relapse was the same as before C.Thus there was no subclinical persistent effect of C enabling sustained remission during subsequent relapses.2)6 pts received 2 courses of C.The 2nd remission was as long as or longer than the 1st in 3 pts;3 other pts have just completed a 2nd course of C.It thus appears that refractoriness to C does not develop.3)3 pts have demonstrated both responsiveness and resistance to C therapy in different episodes of MLNS suggesting that these terms are relative and depend on the state of the host at time of relapse.

SECRETION OF RENIN BY RAT KIDNEY SLICES. William A. Corsini and Michael D. Bailie (Intr. by William B. Weil). Michigan State Univ., Dept. Human Develop. and Physiol., E. Lansing.

Rat kidney cortical slices have been used by several investigators to study control of renin secretion. The data suggest it is difficult to stimulate renin secretion in this preparation. We have studied the effect of furosemide (F), which is known to stimulate renin secretion in vivo, on the rate of secretion by kidney slices. Slices were incubated in Robinsons medium with 100% O_2, and the rate of renin secretion estimated from the amount of angiotensin I formed after incubation of the slice medium with excess renin substrate. Control slices were compared to slices from animals pretreated with F or to slices from untreated animals with F added to the medium. Effects of NaCl were studied in a similar manner.

Treat. Group	N_2 Atmos.	NaCl in Medium*	High Na Diet	F in Medium**	F I.P.***
Contr.	2.0±.2(5)	2.8±.6(10)	1.2±.3(10)	1.6±.1(5)	1.8±.3(10)
Expt.	0.3±.1(5)	2.8±.4(10)	0.3±.1(8)	2.0±.5(4)	1.4±.2(10)

*200mM;**0.0mg/ml;***4mg/kg. Secretion in ng/min/100mg wet wt.

Slices from untreated rats incubated in N_2 and slices from rats on high Na diet had decreased secretory rates. Addition of F or excess NaCl to the medium did not alter secretion. Pretreatment of animals with F had no effect on the rate of renin secretion. Addition of aldosterone, epinephrine or PGE1 to the medium also had no effect. The data suggest renin secretion by kidney slices approaches a maximal rate for a given set of incubation conditions.

DETECTION OF URINARY PATHOGENS BY DIP-SLIDE TECHNIQUE. Mohsen Gharib, Pierce Gardner, David H. Smith, Children's Hospital Medical Center, Boston, Mass.

A dip-slide technique and standard quantitative cultures were compared experimentally and clinically for detection of a variety of common urinary tract pathogens. Growth of enterococcus, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella aerogenes, Escherichia coli, Proteus mirabilis, and Enterobacter diluted in urine at concentrations of 10^4 and 10^6 was the same in both systems when incubated at 27°C or 37°C and read at 18, 24 or 48 hrs. The dip-slide consistently underestimated growth of low concentrations (10^3) of enterococcus, S. aureus and P. aeruginosa incubated at room temperature but results for all organisms were comparable when cultures were incubated at 37°C.

4036 consecutive clean-voided urine specimens simultaneously evaluated by the two techniques showed that by the dip-slide method, growth of urinary pathogens was overestimated in 0.07% and underestimated in 0.09% of specimens. Discrepancies were more common (3.6%) for organisms considered non-pathogens (diptheroids, lactobacilli, S. epidermidis, alpha-streptococci other than enterococcus). The Dip-slide technique yields bacteriologic data comparable to standard quantitative cultures and has the advantage of being easily read by lay and paramedical personnel.

CHRONIC DIALYSIS IN CHILDREN - EIGHT YEARS' EXPERIENCE. S.Counts, R.Hickman, A.Garbaccio, H.Tenckhoff (intr. by D. Shurtleff). Depts. of Pediatrics & Medicine, University of Washington, Seattle, Wa.

Seventeen patients under age 16 at onset of dialysis have been on chronic hemodialysis (HD) (5 pts.) or peritoneal dialysis (PD) (9 pts.) or both (3 pts.) for periods of 3 mos. to 6 yrs. All but one dialyzed at home thrice weekly or on alternate days. Nine prepubertal children, 4 to 14 yrs. at onset and dialyzed 9 mos. to 6 yrs. have grown from 2 to 6 cm/yr. (2 to 22 cm/pt.). No growth occurred in 5 females 14-10/12 to 15-9/12 at onset: 2 were postpubertal, 1 has Turner's, and 2 had arrested puberty. Of the latter, 1 had menarche after 16 mos. of PD and 1 shows advancing puberty after 4 mos. PD. One male 14-1/2 has grown 6 cm in 12 mos. of HD and shows advancing puberty. A prepubertal male 11-10/12 has completed puberty and grown 22 cm. in 6 yrs. HD and PD. Seven patients had nephrectomies, 4 for hypertension. Two had parathyroidectomies and 3 others have evidence of severe secondary hyperparathyroidism. Fourteen of 17 patients are alive, and 5 of 17 received a transplant. Nine remain on PD and 1 on HD. One died posttransplant, 1 death was a complication of HD, and 1 died unrelated to PD. Growth and development of children have occurred on chronic dialysis. It offers a reasonable alternative to transplantation in children with congenital urologic problems or when growth suppressive steroid doses are anticipated.

GLOMERULAR PERMEABILITY IN EXPERIMENTAL GLOMERULONEPHRITIS. Joseph Giangiacomo, Frederick G. Germuth, Saiyid T. Naqvi, Randy A. Kienstra and Alan M. Robson. Wash. Univ. Sch. of Med., St.Louis Children's Hosp., Dept. of Ped., St. Louis, Mo.

Experimental glomerulonephritis was induced in 30 rabbits using goat anti-GBM antibody. One week later glomerular permeability was measured using polydisperse polyvinyl pyrollidone I^{131} (PVP); C_{in} and C_{PAH} were measured and renal biopsy was performed, the tissue being studied by light, immunofluorescent and electron microscopy. 12 normal rabbits were similarly studied. Mild glomerulonephritis (assessed by clearance and biopsy data) showed virtually normal permeability. With more severe lesions permeability to PVP molecules of radius $\geq$ 30 Å was increased. With the most severe lesions there was increased permeability to large PVP molecules but decreased permeability to molecules with radius < 30 Å. Untreated, the glomerulonephritis resulted in progressive deterioration of renal function, death occurring within 2 weeks of induction in 33% of animals. Administration of high dose steroids during the second week of disease modified both the clinical course and PVP permeability, the latter returning to normal in 3 of the 5 animals studied. Although the explanation for these findings is not yet determined, the data indicate that it is the severity rather than the nature of the lesion that modifies permeability and that permeability can be restored to normal by therapy. This method may provide a means to quantitate and assess response to therapy in the course of human glomerulonephritis.

A SIMPLIFIED METHOD FOR ESTIMATING GFR FROM THE BLOOD DISAPPEARANCE OF INULIN. Jehoshua Earon, M.Donald Blaufox, Chester M.Edelmann,Jr., and Adrian Spitzer. Departments of Pediatrics and Medicine, Albert Einstein College of Medicine, Bronx,N.Y.

Compartmental analysis of the plasma disappearance of an appropriate radionuclide provides an accurate estimate of GFR. The major disadvantages of this method are irradiation of the patient and need for a large number of blood samples. In the present study GFR was estimated from plasma disappearance of "cold" inulin, given i.v. as a single dose of 100 mg/kg B.W. Blood samples were obtained during the subsequent 120 minutes. Correlation between values obtained by this method and by standard endogenous creatinine clearance was excellent ($r=+0.93$, $n=24$). In an effort to simplify the method, comparison was made between conventional two-compartmental analysis using 8 blood samples (C_8) and one in which the late slow component was determined from the terminal three points of the curve, and the early fast component was derived empirically (C_3). A time zero intercept of the rapid component calculated on the basis of a volume of distribution of 20% B.W. and crossing the axis at 40 min. provided the best fit of the data ($C_3=0.96\ C_8 + 1.8$). This approach avoids exposure to radioactivity and allows estimation of GFR from a disappearance curve obtained from only three samples of blood.

CHLORAMBUCIL IN A CONTROLLED STUDY OF STEROID DEPENDENT NEPHROTIC SYNDROME. Warren E. Grupe, Dept. of Ped., CWRU School of Medicine, Cleveland, Ohio.

Chlorambucil in combination with cortiosteroids has been used in the treatment of steroid-responsive nephrotic syndrome to assess its effect on the duration of remission and the rate of relapse. Eighteen children, in whom steroids were required for 6 months or longer to maintain a remission or when relapses occurred three times per year or more, were assigned to two groups. Group I continued to receive 1.5-1.8 mg/kg prednisone daily until urine was free of protein for two weeks, then an intermittent regimen of twice the daily dose given as a single morning dose every other day for an additional 2 months. Group II received the same prednisone therapy along with 0.3-0.4 mg/kg/day chlorambucil for 7-12 weeks. Results are summarized below:

	Group I	Group II
number of patients	10	8
aver. months between relapses	1.3	20.6
aver. number of relapses/pt.	4.3	0
relapse rate/pt. month	0.25	0
aver. months followed	19.8	20.6

No significant complications were seen in any child in this study, although the leukopenia intentionally induced by chlorambucil took > 3 weeks to return to normal in 3/8. It would appear that chlorambucil is of significant value in reducing the rate of relapse in the steroid-responsive, frequently relapsing patient.

INTRARENAL BLOOD FLOW IN CHILDREN: CHANGES DUE TO ANGIOGRAPHY AND CONGENITAL HEART DISEASE. Alan B. Gruskin, Victor H. Auerbach and Iain F.S. Black. Dept. Ped. Temple Univ. Sch. Med and St. Christopher's Hosp. Child. Phila., Pa.

Xenon^{133} determinations of fractional flow (FF) and blood flow to the outer cortex (BF/OC) and inner cortex (BF/IC) were performed in 22 children ages 1½-9½. In 4 normal kidneys BF/OC averaged 376±34 (S.E.M.) ml/min/100gm (FF/OC 84±5%); BF/IC was 54±19 ml/min/100gm (FF/IC 12.4±4%).

In 11 patients before angio BF/OC was 259±23 ml/min/100gm and fell post angio in 10/11 to 184±26 ml/min/100gm. Pre angio BF/IC was 27±4 ml/min/100gm. Post angio, excluding 3 cases in which components I and II fused, BF/IC was 21±3 ml/min/100gm. FF/OC of 76±4% and FF/IC of 24±4% was not changed by angiography.

Nine children with congenital heart disease had a pre angio BF/OC of 238±23 ml/min/100gm (FF/OC 70±6%) and BF/IC of 29±5 ml/min/100gm (FF/IC 31±6%). Eighteen cardiacs had a post angio BF/OC of 176±21 ml/min/100gm (FF/OC 67±5%) and BF/IC of 29±3 ml/min/100gm (25±4%).

Conclusions: 1) Children with congenital heart disease have diminished renal blood flow to the outer cortex. 2) The decreases which may occur in renal blood flow post angio, if severe, may result in the development of renal insufficiency. 3) The degree of change in blood flow post angio is not related to the dose of contrast media.

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EVIDENCE FOR GONADAL TOXICITY OF CYCLOPHOSPHAMIDE IN PRE- AND POSTPUBERTAL CHILDREN WITH RENAL DISEASE. Ronald J. Kallen, Robert L. Rosenfield, and Victor S. Fang. Univ. of Chicago-Pritzker Sch. of Med., Depts. of Ped. and Med., La Rabida Children's Hospital and Research Ctr., Chicago.

Serum FSH and LH estimation by radioimmunoassay was done in 15 children and adolescents treated with cyclophosphamide (C) for lipoid nephrosis (LN), membranous nephropathy (MN), and systemic lupus erythematosus (SLE). Normal pubertal values for serum LH are: mean, 40 ng/ml (range, 10-70 ng/ml); for serum FSH: mean, 200 ng/ml (range, 70-480 ng/ml). 9 with LN received C between ages 4 and 9 years and 3 have elevated LH (100, 102, and 116 ng/ml), although FSH is normal. 3 males with LN were given C at age 12-14 years, and have borderline or elevated FSH (470, 590, and 850 ng/ml) and high LH (128, 204, and 224 ng/ml) when tested at age 15-17 years. All 3 are pubertal but the state of spermatogenesis is not known. A prepubertal 17 year-old female with Sjøgren's syndrome and MN has an inappropriately high serum FSH, 226 ng/ml. A 17 year-old male with MN has a borderline FSH (440 ng/ml). A 13 year-old pubertal female with a recent course of C for severe SLE nephropathy has normal FSH and LH. Conclusion: Of 6 patients treated with C after 12 years of age, 3 have high LH and borderline or elevated FSH suggesting primary gonadal failure; elevated LH in 1/3 of young patients suggests that the prepubertal gonad may be damaged by C.

EVIDENCE FOR AN IMPAIRED SODIUM REABSORPTION BY THE PROXIMAL TUBULE IN RENAL ALLOGRAFTS: Cidio Jaimovich, Mohammad H. Malekzadeh, Richard N. Fine and Carl M. Grushkin. U.S.C. Sch. of Med., Dept. Pediat. and Childrens Hosp. of Los Angeles, Ca.

The effect of 0.45 normal saline diuresis on renal sodium (Na) and water (H_2O) excretion was examined by clearance technique in 3 groups of subjects: 5 children 14-50 months post kidney transplantation (PT) with serum creatinine < 1.2 mg%, normal urinary concentration and no evidence of rejection; 3 healthy kidney donors (D) 12-30 months after nephrectomy and 5 normal children (C). The free water clearance (C_{H_2O}) plus sodium clearance ($C_{H_2O} + C_{Na}$) were used as an approximate index of Na delivery to the distal nephron and the $C_{H_2O}/(C_{H_2O} + C_{Na}) \times 100$ as an estimate of Na reabsorption at the diluting segment. The GFR was corrected to 1.73 M^2 and the clearance values to 100 ml of GFR.

	PT	D	C	P VALUES
GFR (ml/min/1.73 M^2)	83.0 ± 11	123 ± 9	124.0 ± 26	-0.01
Cosm (ml/min)	4.5 ± 0.5	4 ± 1	4.9 ± 1	NS
$C_{H_2O} + C_{Na}$ (ml/min)	19.4 ± 4.7	10 ± 1	12.7 ± 1.6	< 0.05
$C_{H_2O}/(C_{H_2O} + C_{Na}) \times 100$	82.0 ± 4.6	75 ± 5	82.0 ± 7.9	NS

These findings indicate that after extracellular volume expansion, fractional reabsorption of Na at the proximal tubule is significantly lower in PT patients than in control groups; and that osmotic diuresis cannot account for this abnormality. Our results suggest that there may be impaired proximal tubular reabsorption of Na in recipients of kidney allografts.

METABOLIC STUDIES ON ISOLATED RAT GLOMERULI IN AMINONUCLEOSIDE(AN)NEPHROSIS. Bernard S.Kaplan, Louise Renaud & Keith N. Drummond. McGill Univ.-Montreal Children's Hosp. Research Inst., Dept. of Nephrology, Montreal.

Proteinuria in AN nephrosis may be due to direct damage to the glomerular basement membrane(GBM)or result from damage to the GBM-producing cells. We have studied the metabolism of isolated rat glomeruli, thus including GBM-producing cells, in the AN model. Substrates used were G-U-C^{14}(G-U), G-6-C^{14}(G-6), G-1-C^{14}(G-1)and alanine-U-C^{14}(A)in an in vitro system. Glomeruli from experimental rats given 15 mg AN/100 gm i.p. once and controls given water were incubated. By day 7 experimental rats had the nephrotic syndrome (mean protein excretion 208 mg /24 hrs). Results were expressed as nM substrate-C oxidized/mg DNA/3 hrs:

	Controls	Day 7	Day 14
nM G-U	789(n=14)	447(n= 8, p < .02)	728(n= 8, p > .5)
nM G-6	320(n=10)	288(n=10, p > .4)	-
nM G-1	280(n=10)	213(n=10, p < .05)	234(n= 6, p > .1)
nM A	315(n=18)	231(n=17, p < .02)	111(n=10, p < .001)

A reduction in G-U and G-1 oxidation is seen by day 7 with a return to control values by day 14. Decreased oxidation of G-1 suggests a defect in direct oxidative pathway metabolism. No change is seen with G-6. A progressive decrease in A oxidation is found. The significance of these findings is the demonstration in AN nephrosis of a reduction in glomerular oxidative metabolism, and by inference in the cells which produce or maintain the GBM.

EFFECTS OF ANGIOTENSIN II INHIBITOR (p-113) ON NEONATAL RENAL FUNCTION. Pedro A. Jose, Gilbert M. Eisner, Julio V. Medina and Philip L. Calcagno, Depts. of Ped. and Med., Georgetown Univ. Sch. of Med., Wash., D. C. 20007

Young puppies have low renal blood flow, low glomerular filtration rate (GFR) and high peripheral renin activity (PRA). To examine the relationship between the renin angiotensin system (RAS) and renal hemodynamics, p-113 was administered to 6 anesthetized puppies 20-30 days old. P-113 dose was calculated to inhibit the hypertensive effects up to 5 ug of exogenous angiotensin II (AII). GFR, clearance of p-aminohippurate (C_{PAH}), PRA and mean arterial pressure (MAP) were measured before, during and after intravenous p-113. The results are shown in the following table:

	MAP mmHg	GFR ml/min	C_{PAH} ml/min	PRA ng A_1/ml/hr	
control	65.83	2.87	7.37	11	
p-113	64.17	1.47*	3.95*	21	
recovery	68.33	1.38*	3.27*	21	*p<.02

Others have shown that inner cortical flow is preserved and size of juxtamedullary glomeruli(JMG)increased by AII. This study suggests a role of AII in maintaining perfusion of the more developed JMG since GFR and C_{PAH} declined after inhibition of AII. The RAS is mature in puppies and is involved in intrarenal hemodynamics but not in the regulation of MAP.

PROTEIN TURNOVER AND PLASMA UPTAKE RATES IN THE UREMIC RAT. J. Keitges, C. Chantler, R.C. MacDonell, Jr., and M.A. Holliday. Dept. of Ped., Univ. of California, San Francisco.

The following studies were done to estimate tissue protein turnover rates and plasma protein uptake rates (PPuP), dpm bound/gm plasma/da, of control and uremic rats in the post-absorptive state. Female rats were subjected to 7/8 nephrectomy (U) or sham-operation (C) at 30 days of age and fed a 38% protein diet to 50 days, when they were studied using the constant infusion method of Waterlow (Clin. Sci. 35:287, 1968) in which ^{14}C-Leucine (331 μCi/μmol) replaced Lysine. Specific activities of free and protein-bound Leucine of plasma samples and of liver and muscle homogenates after a 5-6 hr infusion were measured using high-voltage electrophoresis and Lowry's determination of protein. Total body protein turnover (TPT), gm/100 gm rat/da, and fractional turnover rates for liver (k2L), muscle (k2M), and plasma proteins (k2PP) were estimated.

	TPT	k2L	k2M	k2PP	PPuP x 10^3
C (5)	1.82	.780	.106	1.42	808
U (4)	1.61	.743	.109	2.11	1185
p value	NS	NS	NS	<.05	<.05

The results of the controls agreed with those of Waterlow. Uremia had no discernible effect on liver or muscle but did increase plasma protein turnover. A new basis for assessing protein metabolism in uremia can be developed from these results, particularly with respect to the effect of dietary alterations upon protein turnover.

PROXIMAL TUBULAR FLUID TRANSFER IN AMINONUCLEOSIDE NEPHROSIS. John E. Lewy. Cornell Univ. Med. Col.-New York Hosp., Dept. of Ped., New York.

A micropuncture study of the relationship between glomerular filtration and proximal tubular reabsorption of fluid was carried out in 6 rats 142-144 hr. after intravenous injection of an aminonucleoside of puromycin in order to clarify the role of these factors on fluid accumulation. By 6 days ascites was evident. Urinary protein excretion averaged 46.0 ± 2.4 µg/min compared to 8.9 ± 2.0 µg/min in 5 control rats. Serum protein was 5.3 ± 0.1 gm% in aminonucleoside rats (AMN) and 6.5 ± 0.1 gm% in controls. Single nephron glomerular filtration rate was diminished to 22.5 ± 2.0 mµl/min (Control: 32.6 ± 2.8 mµl/min) while reabsorption to the end of the proximal convolution of the same nephrons was 14.6 ± 1.5 mµl/min (Control: 14.3 ± 1.9 mµl/min). TF/P inulin ratios were thus increased to 2.9 ± 0.1 (Control: 1.8 ± 0.1). Whole kidney GFR was also diminished in AMN rats while renal blood flow was preserved and filtration fraction was therefore diminished. Absolute proximal tubular reabsorption of fluid was preserved in surface nephrons despite diminution in serum protein and total kidney filtration fraction implying a change in intrarenal blood flow distribution or tubular hydraulic conductivity. The observed decrease in glomerular filtration rate was thus the principal factor leading to decreased delivery of fluid to more distal parts of the nephron. This partial imbalance between a change in glomerular load and tubular reabsorption may contribute to the retention of fluid and solute which characterizes this disease.

SEQUENCE OF ULTRASTRUCTURAL AND BIOCHEMICAL CHANGES IN AMINO NUCLEOSIDE (A.N.) NEPHROTIC SYNDROME (N.S.) Melinda McVicar, Chun-Kuo Chen, Ahmad Dakroury, Bernard Gauthier and Anthony Nicastri. Depts. of Ped. and Path. Downstate Med. Ctr. B'klyn, N.Y. (Introduction by Salvador Castells, M.D.)

Rats weighing 200 gms were given daily subcutaneous injections of 1.5mg per 100gm body weight of a 0.5% pruine A.N. There were 6 experimental and 4 control animals in each group. Animals were pair-fed and one group sacrificed each day from day 2-12. Increase in proteinuria (P values between .10 and .05) was first noted on days 5, 6, 7. On days 8, 9 and 10 P values for proteinuria became significant. ($P<.05$, $<.02$ and $<.01$ respectively).

There were no changes in serum albumin or cholesterol through day 7. A.N. rats had marked increase in cholesterol on day 8 ($P<.01$) and serum albumin was significantly decreased ($P<.05$). From day 9 all differences were highly significant ($P<.01$)

By slow induction of N.S. we have demonstrated the coincidence of decrease in serum albumin and increased cholesterol, both preceded by albuminuria. Electron microscopic examination of glomeruli showed onset of changes parallel to onset of proteinuria beginning of day 5. There were minimal foot process fusion, protein globules and intracytoplasmic vacuoles noted in the podocytes. With progression of N.S. these vacuoles enlarge. Their intracytoplasmic origin was demonstrated by their failure to stain with colloidal iron distinguishing them from invaginations of the podocytic cell membranes.

EFFECT OF INTENSIVE NUTRITIONAL REHABILITATION AND RETRAINING ON CALORIE INTAKE AND GROWTH OF UREMIC CHILDREN ON HEMODIALYSIS. Howard E. Maltz, Darla Erhard, Donald E. Potter, and Malcolm A. Holliday. Dept. of Ped., Univ. of California, San Francisco, and San Francisco General Hospital.

Growth failure in the uremic child is due in part to calorie deficiency secondary to appetite failure. Delivery of adequate calories to induce growth usually is difficult. This study tested the influence of a program of Intensive Nutritional Rehabilitation and Retraining (INRR) upon calorie intake and growth. Two anuric, cachectic patients undergoing hemodialysis were admitted to a metabolic study ward under the supervision of a nutritionist, a physician, and nurses. One was a 16 year old girl with systemic lupus erythematosus; the other was a 3 year old with the congenital nephrotic syndrome. A wide variety of appropriate foods were prepared with much attention to individual taste preferences. The staff conveyed a positive attitude regarding the patients' recovery potential. Good nutritional input was rewarded. The 16 year old's daily intake rose from 400 to 2000 kcal; her affect improved. Marked hypertension necessitating nephrectomy interrupted the study. The 3 year old thrived. Cell mass, which had decreased 970 gm over the 3 mos prior to the study, increased 264 gm during the 4 weeks of the study. Calorie intake improved by 50%; linear growth rate increased from .17 to .63 cm/wk. The patient's level of activity and social interaction improved. The increased food intake persisted at home. INRR appears to be a workable mode for increasing calorie intake and inducing growth in the uremic, cachectic child on hemodialysis.

EVIDENCE FOR A VASOPRESSOR SUBSTANCE (RENIN) IN HUMAN FETAL KIDNEYS. A. Molteni, W. J. Rahill and J. Koo. Dept. of Pathology, Univ. of Kansas Medical Center, Kansas City, Kansas and Dept. of Ped., State Univ. of New York at Buffalo, New York.

Granulated cells, stained as those of the juxtaglomerular apparatus have been seen in the cortical blastema of human fetal kidneys (Lyungquist, Acta Path. Microbiol. Scand. 67:257,1966). A vasopressor substance was evident in fetal rat kidneys three days before renin granules were visible (Tsuda, Lab. Invest. 25:23,1971). In our laboratory, renin activity was measured in the kidney, heart, liver, spleen, pancreas and amniotic fluid of 14 human fetuses from 4.6 to 14.8 cm. in length (crown-rump) (gestational age 58-108 days) by an angiotensin 2 generating method (Lever, Biochem. J. 91:346,1964). Renal cortical renin was 89 (±18 sem) ng/4 mg tissue. Renin levels in other tissues were $<$ 1 ng/4 mg tissue or /ml amniotic fluid. Linear regression analysis of renal renin on fetal length showed a significantly ($p<.05$, t test) positive slope. A few juxta-glomerular granules (Bowie stain) were seen in the fetal glomeruli but not in other organs. The increase in renal vasopressor activity during this period of fetal growth correlates with a fall in renal cation concentration during this period. (Supported by Grants HE-06975 and AM-05626, NIH)

RELAPSE RATE OF IDIOPATHIC NEPHROTIC SYNDROME (INS) AFTER CYCLOPHOSPHAMIDE. T. Marr, P. R. Lewy, D. Hall, Y. Ahmadian, and G. Z. Given (Intr. by Henry L. Nadler). Northwestern Univ. Med. School, The Children's Memorial Hospital, Dep't. of Pediatrics, Chicago

The relapse rate of INS following treatment with Cyclophosphamide (C) has not been reported. Seventy-one children with INS have been followed for 1 to 13 years (mean 4 years). All had serum creatinine between 0.4-1.3 mg% (mean 0.8 mg%). None had hematuria or hypertension. Forty-one (27 males, 14 females) have experienced an average of 1 relapse/year and responded regularly to Prednisone (P) 2 mg/kg/day. Thirty patients (17 males, 13 females) experienced frequent relapses of nephrotic syndrome at an average rate of 3.9 relapses/year. These patients received 34 courses of oral therapy with C, 2.5-3 mg/kg/day, and were treated for a mean of 4 months (range 1-15 months), after renal biopsy was performed. All 30 renal biopsies were normal by light microscopy. During C therapy patients also received P, 2 mg/kg q.o.d. Eighteen courses of C therapy ended 4-44 months (mean 18.7 months) ago without subsequent relapse. However, 16 courses of C, ending 1-45 months (mean 18.2 months) ago, have been followed by a relapse at 1-22 months (mean 8.3 months). In nine instances >1 relapse occurred. The relapse rate after these 16 courses of C is 1.8/year. Side effects of C included leukopenia-10, alopecia-2, cystitis-2, infections-3. Male fertility has not been assessed. One girl delivered normal infant 2 years after C.

SHORT, FREQUENT HEMODIALYSIS IN CHILDREN. Melinda McVicar, Bernard Gauthier, In-Tai Cheong, Winifred Critchlow and Salvadore Castells. Dept. of Ped. Downstate Med. Ctr. B'klyn, N.Y.

Shorter more frequent hemodialysis may promote better growth in children with renal failure. We tested the feasibility of 3 hrs hemodialysis 5 days per week over a period of two weeks in 4 children ages 12 to 16 yrs. Protein intake was constant for each patient during the 6 hr and 3 hr regimens. Mean pre and post dialysis values for serum urea nitrogen were compared on the traditional 6 hrs/day, alternate day, 3 days/wk vs. 3 hrs 5 consecutive days/wk.

Pat.	6 Hrs. (18 hrs/wk.)		3 Hrs. (15 hrs/wk.)	
	Pre	Post	Pre	Post
S.S.	80 mg%	22 mg%	58 mg%	32 mg%
P.P.	60	21	32	16
M.E.	78	36	64	31
P.V.	-	-	62	26

After 3 hrs hemodialysis plasma amino acids were reduced 54% of 6 hr values. Shorter more frequent dialysis was associated with good electrolyte balance and ultrafiltration. Advantages are: Greater flexibility of dialysis schedule and better biochemical homeostasis with potential for improved growth and sexual maturation. Disadvantages are: More "set-up" time, possibly shortened fistula survival, probable need for home dialysis. The latter two disadvantages can be controlled. The regimen is effective and feasible. It remains to be seen if this regimen can improve growth and sexual maturation of children on chronic hemodialysis.

SPONTANEOUS CHEMOTAXIS (CT) IN ACUTE GLOMERULONEPHRITIS (AGN): DEMONSTRATION OF A POSITIVE CORRELATION WITH DISEASE ACTIVITY. M. Norman & M. Miller, U. of Pa., Sch. of Med., Dept. Ped., Phila., & Charles R. Drew Postgrad. Med. School, Los Angeles

We have previously reported an abnormality of CT in several types of glomerulonephritis and the nephrotic syndrome (Ped. Res.,6:155, 1972). This was characterized by: 1) Increased "spontaneously" generated chemotactic activity (SCT) in sera of patients (PS); 2) Inability to generate additional chemotactic activity (ACT) upon incubation of PS with immune complexes; 3) Demonstration of deficient CT by neutrophils (PMNs) from patients with increased SCT. No correlation of CT abnormalities was found with C3 and CH50 complement levels in PS. In the studies now reported, a positive correlation has been found between clinical status of 17 children with AGN, and the degree of CT abnormality noted above. When measured in PS of 6/17 pts at 6 months and 13/17 at 12 months, SCT and ACT equalled that found in control sera (CS). Pt PMNs showed slightly less CT towards CS than did control PMNs. Serum C3 returned to normal in 13/15 and CH50 in 12/12 pts. All pts. were well at 12 months. These data indicate: 1) Humoral and cellular CT abnormalities in AGN improve with clinical recovery; 2) Serum complement levels parallel CT improvement in most but not all AGN patients.

CARBONIC ANHYDRASE FUNCTION IN THE FETAL KIDNEY. Jean E. Robillard, Fred G. Smith, Jr., Claudius Kulvinskas, and Esther Braden. UCLA Sch. Med., Dept. of Ped., Los Angeles, Calif.

It has been well documented that the physiologic acidosis in the infant and newborn puppy is due to a low renal plasma bicarbonate threshold. This low bicarbonate threshold could be markedly increased, in newborn puppies, by modifying the stimulus for reabsorption of sodium.

In order to investigate the development of mechanisms regulating the bicarbonate reabsorption in the fetal lamb kidney, the carbonic anhydrase enzyme was assayed by an improved histochemical method (Hanson, 1967) in 6 fetal sheep (125-135 days gestation). Each kidney specimen was taken under local anesthesia and immediately frozen at -198°C without fixation.

In all tissue samples, after 10 minutes floating in a freshly mixed incubation medium, carbonic anhydrase was shown by a specific deposition of cobalt near the histologic locus of the enzyme. This reaction could be stopped by the addition of acetazolamide 10^{-6}M or by exclusion of bicarbonate in the medium.

It thus appears that the complete absence of carbonic anhydrase alone cannot explain the limited capacity of fetal proximal tubule to reabsorb bicarbonate.

PROTECTION FROM BILIRUBIN NEPHROPATHY IN GUNN RATS BY AGAR by Gerard B. Odell, Julio O. Cukier,* and Shatchai Seungdamrong,* Johns Hopkins Univ., Dept. of Pediatrics, Baltimore.

Homozygous Gunn rats show deposition of bilirubin in the renal papillae and deficiency in $T^{c}_{H_2O}$ and C_{H_2O}. Agar feeding can reduce serum bilirubins in these rats by increasing its fecal excretion. Homozygous (jj) and heterozygous (Jj) animals from 6 litters were separated at weaning age. jj rats (17) were fed a diet containing 2% Amend agar, and the remaining jj and Jj littermates were fed the same diet without agar. After 4 months, measurements of C_{H_2O} were made by gavage of 5% of body weight as distilled H_2O. Urine was collected under oil in calibrated tubes for 60 mins, and tail vein blood was subsequently collected. Creatinine and osmolality were determined in the serum samples and C_{H_2O} calculated.

	Agar fed	Control fed	
	jj (I)	jj (II)	Jj (III)
n	17	13	8
C_{H_2O}	+0.50 ± 0.49	+0.19 ± 0.48	+0.72 ± 0.59
t-test	I vs II $p < .05$,	II vs III $p < .01$,	I vs III $p > .1$

The serum and adipose bilirubins were both significantly lower in the agar fed animals: 8.1 vs 10.2 mg/dL and 0.8 vs 1.0 mg/gm fat ($p < .01$), when the animals were sacrificed one week after the clearance studies.

Thus, agar feeding was associated with lower carcass bilirubins and essentially normal papillary function.

REVERSIBILITY OF GLOMERULAR LESIONS FOLLOWING CONGENITAL SYPHILIS. Inge Sagel, Nirmal Khurana, Ayse M. Yuceoglu & Edward Wasserman. New York Medical College, Renal Service & Laboratories, Depts. of Medicine & Pediatrics, New York, N.Y.

Three infants, aged 2-3 months with signs and symptoms of congenital lues were found to have edema, elevated blood pressure, proteinuria, hematuria and lowered serum complement activity. Percutaneous renal biopsy showed granular deposition of gamma globulin and complement on the patients' glomeruli by immunohistology, subepithelial electron-dense deposits on electronmicroscopy and hypercellularity on light microscopy. All patients were treated with penicillin which resulted in prompt improvement of the syphilitic and urinary abnormalities. Two of the patients had a repeat renal biopsy 6 months after onset of overt syphilitic symptoms. Physical and laboratory examinations at this time were normal with the exception of persisting mild hypertension in both. Renal biopsies were now normal by all parameters. It is suggested that the finding of nephropathy in infants with congenital lues is not uncommon. Immune complex deposition seems to be responsible for the glomerular lesions as demonstrated by immunofluorescence studies. After therapy the lesions were reversed in two infants studied 6 months later. Mild hypertension, however, persisted in both.

ALTERNATE DAY PREDNISONE ADMINISTRATION IN CHILDREN AND ADOLESCENTS POST-RENAL TRANSPLANTATION. E.W. Reimold. University of Texas Southwestern Medical School, Dallas.

Retardation of growth and maturation is a frequently observed complication post-renal-transplantation in children. It is attributed predominantly to the presently used immunosuppressive therapy.

In four girls who were followed up to 28 months after transplantation the dose of prednisone was reduced to 1 mg/kg/day within 6 weeks. Between 3-1/2 and 5 months it was further changed to an alternate day treatment schedule provided no signs of rejection were found and a stable renal function was present.

With this treatment the incidence of rejection was not increased, all steroid side-effects gradually subsided and the renal function remained normal. All four patients were growth retarded before operation. They continued or started to grow post-transplantation; two girls reached the 50th height percentile within 12 months; the other two have shown considerable growth. Alternate day prednisone therapy is an important modification of the presently used immunosuppressive treatment facilitating medical and social rehabilitation of children and adolescents with a decreased rate of complications.

EXAGGERATED NATRIURESIS IN THE COURSE OF POSTSTREPTOCOCCAL GLOMERULONEPHRITIS. Robert G. Schacht, John M. Steele, Jr., David S. Baldwin. (Intr. by Alfred L. Florman). Depts. of Ped. and Med., New York Univ. Sch. of Med. New York.

We have found evidence of continuing disease as manifested by various combinations of hypertension, minimal proteinuria, sclerosis of glomeruli and reduction in filtration rate years after acute poststreptococcal glomerulonephritis (PSGN). In the present study the mechanism for sodium homeostasis was explored in 21 patients 1-15 years after the onset of PSGN by examining the response to an acute sodium load. Exaggerated natriuresis (EN) which began during the first 15 minutes of 2 1/2% saline infusion was induced in 19 patients whose filtration rates ranged from 156 to 26 ml/min. Sodium excretion rates after administration of 1 liter of infusion averaged 1846 μeq/min compared to 428 μeq/min in a group of normal volunteers. Volume expansion with dextrose or albumin infusions did not induce EN. The occurrence or magnitude of natriuresis was not dependent on the absolute level of filtration rate. The renal mechanism for EN could not be attributed to increase in filtration rate during saline infusion, decrease in peritubular oncotic pressure, or increase in intrarenal pressure. Although unexplained, this exaggerated natriuresis in PSGN may be related to the mechanism by which day to day sodium balance is maintained in the course of glomerulonephritis.

THE EFFECT OF SODIUM DIET ON INTRARENAL HEMODYNAMICS AND ACUTE RENAL FAILURE. Norman J. Siegel, Robert A. Feldman, John P. Hayslett and Michael Kashgarian (Intr. by Howard A. Pearson) Yale Univ. Sch. of Med., Depts. of Ped., Urol., Med. and Path., New Haven.

Variations in sodium intake have been shown to modulate the functional alterations in acute renal failure. The mechanism of this effect on intrarenal hemodynamics was studied in normal rats and after dichromate induced renal injury. The distribution of outer to total cortical blood flow (OC/TC), was estimated with radioactive microspheres and total renal blood flow (TRBF) from the clearance and extraction of inulin. On a normal sodium intake, the TRBF was 5.15 ± 0.14 ml/min/100g and OC/TC was 1.67 ± 0.03. These parameters were unchanged by either a high sodium or low sodium diet for 21 days.

After administration of dichromate, the OC/TC was reduced to a similar value, 1.30 ± 0.04 ($p < 0.001$) irrespective of sodium intake. TRBF, however, was maintained at 5.27 ± 0.75 ml/min/100g in the high-sodium group but reduced to 1.48 ± 0.32 ml/min/100g in sodium deficient animals.

These studies show (1) that sodium balance plays an important role in determining changes in renal vascular resistance during acute injury and (2) that functional changes are more dependent upon overall vascular resistance than cortical flow distribution.

THE ONTOGENESIS OF THE RENIN-ANGIOTENSIN SYSTEM IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE (SHR) WISTAR RATS. Alan R. Sinaiko and Bernard L. Mirkin, University of Minn., Dept. of Ped. and Pharm., Div. of Clin. Pharm., Mpls., Minn.

Essential hypertension appears to be a genetically determined disease which may be identified early in development. The renin activity of renal tissue from fetal rats (18 and 21 days gestation), neonates (1,7,14,21 days) and adult rats of both a genetically developed hypertensive strain of Wistar rat and its normotensive counterpart was compared using a modification of the radioimmunoassay for Angiotensin I developed by Haber, et al (1969). The renal tissue was homogenized in distilled water (4°C) and the supernatant used as the renin source. Plasma from sheep nephrectomized 72 hrs prior to bleeding was used as the substrate source for this assay.

These data demonstrate the presence of increasing amounts of renin activity (ng Angio I/cc/hr/µg tissue) with age. An apparent exception was noted in the one day neonates in which the renin activity was greater than in the 7 day neonates. In addition, greater amounts of renin activity per µg tissue were found in the hypertensive than in the normotensive neonates (1,7,14,21 days).

This evidence would suggest that essential hypertension is genetically influenced and that additional studies in the developing animal are warranted in an attempt to elucidate the etiologic mechanisms of this condition. (Supported by USPHS grants GM 01998 and GM 15477).

RENAL GLOMERULAR LESIONS IN PATIENTS WITH SICKLE CELL DISEASE Jose Strauss, Kjell Koch, Victoriano Pardo, Helmut Kramer and Donald F. Levi Depts. of Ped. and Path., Univ. of Miami Sch. of Med., Miami, Florida.

It is generally assumed that patients with sickle cell disease may develop hypothermia, unilateral hematuria and hemosiderosis but not any other functional or histological renal impairment. A retrospective study was undertaken to evaluate our experience in the last two years with patients under 30 years of age. Biopsy and autopsy material was examined by light and electronmicroscopy; biopsy material was also examined by immunofluorescense microscopy. Seven cases (10 - 27 years of age) were found with sickle cell disease and various degrees of renal disease. Three had biopsy, two had autopsy and the remaining two had biopsy initially and then autopsy. All deaths were in end-stage renal disease. All seven cases presented changes of membrano-proliferative glomerulonephritis - biopsy material exhibited deposits of IgG and C'3. Diagnosis of sickle cell disease was made between 6 months and 17 years of age. Serum albumin was 0.49-3.6 g%, cholesterol 115-425 mg/100 ml, BUN 5-180 mg/100 ml and creatinine 0.3-13 mg/100 ml. Creatinine clearance was 2-91 ml/min/1.73 m^2. Other evidence indicates that patients with sickle cell disease may have an altered immunological status and therefore might form soluble immune-complexes which induce glomerulonephritis. A careful prospective evaluation of renal functions and histology in sickle cell disease patients seems indicated.

CYCLOPHOSPHAMIDE IN CHRONIC RENAL DISEASE IN CHILDREN Mitsuki Suzuki and Francis X. Fellers, Dept. of Ped., Harvard Med. Sch. and Children's Hosp. Med. Ctr., Boston.

41 patients with chronic renal diseases have received high-dosage cyclophosphamide (C), 3 mg/Kg for 3 mos, followed by 5 mg/Kg for 3 mos. Toxicity frequently limited this dosage. 32 were minimal-change childhood nephrosis (CN), 5 were membrano-proliferative nephritis (MPN), and 4 were other non-lupus nephritis (NLN). Of 24 CN patients, all frequent relapsers, 14 also received steroids (S) concomitantly. S were tapered within the first 2 mos of C therapy in all. Efficacy of C was evaluated in the frequent relapser CN by comparing the length of S-free periods before and after C period: each patient served as his own control. C was effective in 17 of 24 CN, duration of remission pre-C was 5 mos, duration of remission post-C was 23 mos, minimum 17 mos. Total C dosage was 470 mg/Kg. C was ineffective in 2 due to short courses, and in 1 who relapsed in 5 mos post-C. In 4 remaining CN, evaluation was not possible due to long remissions prior to C, 32 mos duration, compared to post-C of 20 mos. In 8 S-resistant CN, 5 MPN, and 4 NLN, no change in proteinuria or course of disease was observed. The total dosage of C, 285 mg/Kg, resulted from increased toxicity in these patients.

ACUTE POSTSTREPTOCOCCAL GLOMERULONEPHRITIS IN CHILDREN WITH RHEUMATIC FEVER. Antonia Ty, Ayse M. Yuceoglu Rajanee Sensirivatana, and Kurt Lange, Depts. of Ped., New Jersey Med. Sch., Newark, N.J. and New York Med. Col., New York, N.Y.
(Intr. by Franklin C. Behrle)

A form of "glomerulitis" has been described in kidney biopsies performed in patients with acute rheumatic fever (ARF). The incidence of the simultaneous occurrence of ARF with the classical type of acute glomerulonephritis(AGN)has not yet been clearly documented. Two children who were admitted with ARF with carditis developed gross hematuria and markedly depressed serum complement activity. Kidney biopsy performed on each patient studied by light microscopy electron microscopy and immunohistology revealed the typical pathologic features of AGN. The streptococcal plasma membrane has been implicated as being the caustive agent in both diseases. The fact, however, that patients may have repeated episodes of ARF, but not of AGN, and the rare incidence of these two diseases together have led investigators to postulate different pathogenic mechanisms for these diseases. Acrylamide gel electrophoresis of solubilized streptococcal plasma membrane revealed the presence of more than 10 protein components. Our report clearly shows the simultaneous occurrence of ARF and AGN and suggests the possibility that the multi-component streptococcal plasma membrane may contain different antigens for the two diseases.

NEUROLOGY

FATTY ACID SYNTHETASE OF DEVELOPING MAMMALIAN BRAIN AND LIVER. Joseph J. Volpe, Thomas O. Lyles, Daniel A. Roncari, P. Roy Vagelos (Intr. by Philip R. Dodge). Depts. of Ped., Neurol., & Biol. Chem., Washington Univ. Sch. of Med., St. Louis, Mo.

The regulation of the fatty acid synthetase (FAS) of rat brain has been compared to that of rat liver synthetase during development and in various nutritional states. Immunochemical techniques have been utilized to study the content, synthesis and degradation of this multienzyme complex as well as the turnover of the prosthetic group of the acyl carrier protein, a central component of the FAS. All of the changes of FAS activity during development and in various nutritional states were shown to be related to changes in content of enzyme. During postnatal development of brain the rate of FAS synthesis decreases, while in developing liver at the time of weaning there is a dramatic acceleration of synthesis of enzyme. The rate of FAS degradation in brain of adult animals differs considerably from that in liver; the half lives are 6.4 and 2.8 days, respectively. In young suckling animals the rate of FAS degradation in brain is markedly faster ($t_{1/2}$ 1.9 days), while in liver there is little difference. Thus, in developing brain the changes in enzyme content are accompanied by an alteration of both enzyme synthesis and degradation. In both tissues prosthetic group turnover is much more rapid than turnover of the whole complex and may be important in short-term regulation. These observations represent the first direct measurements of rates of synthesis and degradation of an enzyme in mammalian brain.

CEREBRAL UTILIZATION OF ALTERNATIVE SUBSTRATES TO GLUCOSE: AN EXPLANATION FOR ASYMPTOMATIC NEONATAL HYPOGLYCEMIA. Lynne L. Levitsky, John B. Paton, and David E. Fisher, (Intr. by Samuel P. Gotoff). Abraham Lincoln Sch. Univ. of Illinois College of Medicine, Dept. of Pediatrics, Chicago, Illinois.

In 7 baboon infants delivered at term by C/section, and studied on the first two days of life, cerebral uptake of endogenous glucose, β-Hydroxybutyrate, acetoacetate, lactate, and glycerol was derived from A-V differences obtained from simultaneous catheter samplings of left ventricular outflow and sagittal sinus. If the observed A-V differences are converted to A-V O_2 equivalents, the observed cerebral oxygen uptake (6.15 ± 0.46 mM/L) agrees well with the calculated requirement for oxidation of these substrates (5.96 mM/L).

Substrate	Mean A-V Difference (mM/L)	Conversion Factor	Calculated O_2 Equivalent (mM/L)
Glucose	.594	6	3.56
Acetoacetate	.070	4	.28
β-Hydroxybutyrate	.258	4.5	1.16
Lactate	.288	3	.86
Glycerol	.028	3.5	.10

Thus 40% of cerebral O_2 consumption is utilized for metabolism of substrates other than glucose. Additional evidence of non-glucose substrate utilization was obtained by the measurement of significant cerebral production of $^{14}CO_2$ during steady state infusion of 3-^{14}C-DL-β-Hydroxybutyrate. Apparent neonatal tolerance of low blood glucose levels may be explained on the basis of non-glucose substrate utilization.

CEREBRAL CARBOHYDRATE METABOLISM IN HEMOPHILUS MENINGITIS. Edward R. Moxon, Arnold L. Smith, David H. Smith, Children's Hospital Medical Center, Boston, Massachusetts

Hypoglycorrhachia occurs commonly in bacterial meningitis and presumably reflects a disturbance in cerebral carbohydrate metabolism. The basis of this disturbance has been studied in 5 day old rats with H. influenzae b meningitis produced by intranasal inoculation. Control animals consisted of littermates inoculated with untypable H. influenzae. Glycogen and glucose content of cerebral cortex was increased significantly in meningitic animals at 2 and 3 days, but not 5 days, after infection when compared to controls. At 2 days the mean brain glycogen values were 4.6 μmoles/g in meningitic and 3.1 μmoles/g brain in control animals. Brain: blood glucose ratio 2 days after infection in meningitic rats was 0.37 compared to 0.21 in controls. Brain lactate concentration was also increased in meningitic animals, but there was no significant difference in brain pyruvate concentration. Total brain ATP content was decreased in the early phases of the meningitic process. These changes could not be accounted for by changes in brain water content, sequestration of blood, or inflammatory cell infiltrate. These findings are consistent with the hypothesis that cerebral carbohydrate metabolism is altered in the early phase of bacterial meningitis.

BETA HYDROXYBUTYRATE UTILIZATION OF THE INTACT INFANT RAT BRAIN. Thomas J. Moore, Armand P. Lione, and David M. Regen. Columbia Univ. Col. Phys. & Surg., St. Luke's Hosp. Ctr., Ped. Serv., New York; and Vanderbilt Univ. Sch. of Med., Dept. of Physiology, Nashville, Tenn.

Since nonglucose substrate is held to be the prime energy source for cerebral metabolism in newborn rats (Moore, T.J., et al, Am. J. Physiol. 221:1746, 1971) cerebral utilization of beta hydroxybutyrate (BOHB) throughout infancy in the fed intact rat was investigated. Ketonemia with plasma values ranging from 1.3 to 0.5 mM for BOHB exists in the first three postnatal weeks. Transport of BOHB across the blood brain barrier occurs very slowly during the first day after birth. The influx coefficient is 0.014 ml/min · g. A steady increase in transport activity follows so that at day nine the influx coefficient has increased tenfold to 0.14 ml/min · g. Cerebral utilization of BOHB is 0.016 μmole/min · g in one day old rats. In nine day old animals it is 0.12 μM/min · g compared to 0.17μM/min · g for glucose. At 23 days BOHB falls to 0.003 μM/min · g. These results indicate 1) blood brain barrier transport is rate limiting to BOHB utilization in the newborn, 2) BOHB transport maturation is independent of cerebral circulation or maturation of glucose transport, and 3) predict that a substrate other than glucose or BOHB is the prime source of energy for the rat's cerebral metabolism in early infancy.

LATE EFFECTS OF EARLY DIETARY PROTEIN INTAKE IN LOW BIRTH WEIGHT INFANTS. Herbert I. Goldman, Joan S. Goldman, Ira Kaufman, and O. Bernard Liebman. (Intr. by Philip Lanzkowsky). Long Island Jewish-Hillside Medical Center, Department of Pediatrics, New Hyde Park, New York.

Three hundred four infants of birth weight below 2000 gm were randomly assigned to either a 2% (Diet A) or 4% (Diet B) protein diet. As previously reported, infants fed Diet A had less fever and lethargy but more edema and hypoproteinemia than infants fed Diet B. Eighty percent of the infants were recalled at 3-3.5 and 5-7 years of age. An excess of visual and neurologic sequelae was found in the infants who were fed the high protein diet: Strabismus (18 B infants, 6 A infants, $P < .05$), Spastic Diplegia (3 B, 3 A), Hydrocephalus (3 B, 0 A), I.Q.< 70 (11 B, 7 A), Infants with Any of the Above (29 B, 14 A, $P < .05$). In infants with birth weight below 1300 gm, 17 of 23 B infants had I.Q. scores below 90, as compared to 6 of 26 A ($P < .01$). These data help explain the very low incidence of sequelae in surviving very low birth weight infants reported by Dr. Hess in the 1930's and '40's (human milk), the high sequelae rates reported in the '50's and early '60's (largely high protein diets), and several recent reports of improved results (largely low protein diets).

DIFFERENTIAL INCORPORATION OF ^{14}C DERIVED FROM GLUCOSE AND ACETATE IN DEVELOPING BRAIN: THE POSSIBLE "METABOLIC COMPARTMENTATION" OF BRAIN PROTEIN SYNTHESIS. Arthur L. Prensky and Harish C. Agrawal, Washington Univ. Sch. of Med. and St. Louis Children's Hospital, Dept. of Ped., St. Louis, Missouri.

The concept of two glutamate pools or metabolic compartmentation of amino acids in the CNS was originally proposed by Waelsch et al, to explain the anomalous labeling of glutamate-glutamine when 1-^{14}C-acetate was used as a precursor as compared to U-^{14}C-glucose. We have studied the rate of incorporation into developing rat brain of ^{14}C derived from these precursors into myelin and microsomal proteins and lipids. Rats were sacrificed 24-hrs. after injection of isotope at 14, 18, 21 and 40 days of age and myelin or microsomes isolated. Maximal incorporation occurred between 14 and 18 days of age. The decrease thereafter was more pronounced in myelin proteins and lipids labeled with 1-^{14}C-acetate. The rate of incorporation into myelin protein of ^{14}C-amino acids derived from U-^{14}C-glucose was higher at all ages when compared to 1-^{14}C-acetate. The glucose/acetate ratio was 3.1 at 14 days of age and increased to 5.9 at 40 days of age. The ration of incorporation into myelin cholesterol on the other hand was 0.61 and rose to 2.3 at 40 days of age. Preferential use of ^{14}C from glucose for protein synthesis was even greater in microsomes. The glucose/acetate ratio was 7.4 at 18 days of age and 16.3 at 40 days. The incorporation of ^{14}C into microsomal proteins of 18 day old rat brains was examined at intervals of time from 30 mins. to 24-hrs. after injection with regard to the possible "metabolic compartmentation" of brain protein synthesis.

EXPERIMENTAL LEAD ENCEPHALOPATHY: MODEL OF FOCAL BRAIN EDEMA. Gary Goldstein and Ivan Diamond (Introd. by M.M.Grumbach), Dept. Neurol. and Pediatrics, Univ.California, San Francisco.

Lead encephalopathy with acute cerebellar edema was produced in 4-wk old suckling rats by feeding the mother lead carbonate after the litter was 2 wks old. During the evolution of this encephalopathy, brain regions were sequentially analyzed for lead, water and electrolyte content and 3 distinct stages were identified. The first stage, day 14-24, is characterized by growth retardation without neurological symptoms. The rate of brain lead accumulation is the same in babies and mothers and brain water and electrolyte content are normal. During the second stage, day 24-26, brain lead deposition reaches a plateau in the mothers (3.1 μg/g) but continues to increase in the babies. Focal edema, indicated by elevated dry weight sodium (358 mEq/kg ± 38 S.D.), appears in the young cerebellum. In the third stage, day 26-30, the young rats develop ataxia and obtundation. Although cerebellar hemorrhages are found at death, the lead concentration in the cerebellum is the same as that in the unaffected cerebrum (6.2 μg/g). Blood lead levels during this third stage are 5.4 μg/cc in the babies and 3.4 μg/cc in the mothers.

These results suggest that lead in the brain is in equilibrium with lead concentration in the blood. However, despite an even distribution, only the cerebellum exhibits a pathological reaction. This selective effect should make it possible to identify specific metabolic abnormalities underlying lead encephalopathy.

CARDIAC ORIENTING OF 6-WEEK-OLD-INFANTS TO SPEECH AND NON-SPEECH STIMULI. L.A. Leavitt, P.A. Morse, J.W. Brown, and F. K. Graham. (Intr. by J. Opitz), Depts. of Ped. and Psychol., Univ. of Wisconsin, Madison, Wisconsin.

Recent studies suggest that speech is a species-specific trait of humans and that adult humans perceive acoustic differences in speech in a fundamentally different manner than other auditory stimuli. Four experiments with 6-week-old-infants studied their ability to attend to and discriminate between two speech and two non-speech sounds. The procedure used repeated presentations of one sound followed by presentation of a second sound. If an infant attends to a stimulus, its initial presentation should elicit an orienting response (OR) of cardiac deceleration. Discrimination would be shown if the OR habituated (decreased) with repetition of the same sound but returned with presentation of a changed sound. Experiments 1 and 2 used tones modulated in frequency between 150-250 or 1500-2500 Hz. Experiments 3 and 4 used computer-synthesized speech stimuli /BA/ and /GA/. All stimuli were presented at an intensity of 75 dB. The infants' orienting responses did not indicate discrimination between the contrasted speech or non-speech stimuli. However, on the initial presentations, speech stimuli elicited large, statistically significant heart rate deceleration whereas the non-speech stimuli did not. These data indicate that different acoustic stimuli may vary widely in their ability to evoke the cardiac OR. They suggest that speech stimuli may be especially potent elicitors of attention in very young infants.

EPISODIC BLINDNESS IN CHILDHOOD. Peggy A. Hanson and Mark P. Dentinger. (Intr. by Dr. Ian Porter), Albany Medical College, Dept. of Peds. and Neur., Albany, New York

We wish to call attention to episodes of transient blindness accompanied by headache in children. Within 1 year we have seen 3, possibly 4 such cases. All demonstrated a posterior temporal spike and slow wave focus. In 2 cases this was unilateral, in 2 the discharges were bilateral but with unilateral preponderance.

The first child was 10 years old and presented with episodes of headache and visual loss of 20-100 minutes duration. She subsequently developed generalized seizures. The second was 4 years old and had blindness lasting almost 4 hours following a frief "febrile" seizure. The third was 3 years old and complained of episodes of headache and brief loss of vision. All have been controlled with anti-convulsant medication. Only one patient had a family history of migraine.

The children were well and neurologically intact. The seizures when present were brief so that an anoxic orgin is unlikely. The discharge itself is thought to account for the loss of vision. Review of the literature on childhood migraine shows sharp wave foci to be rare in the inter-ictal electroencephalogram, and blindness is not described.

In light of these cases we believe that transient visual loss accompanied by sharp wave discharges on the electroencephalogram is a manifestation of epilepsy and that it is not uncommon in childhood. The relationship to migraine is unsettled.

QUANTIFICATION OF TOUCH-PRESSURE SENSITIVITY IN THE NEONATE Bradley T. Thach & James M. Weiffenbach, (Intr. by James F. Bosma) NIH, NIDR, Oral Development Section, Bethesda.

Morphologic studies indicate that neural receptors in skin and mucosa may not be fully mature in the human and other primate neonates. Quantitative studies of touch-pressure sensitivity in infants are lacking. A newly described reflex characterized by a discrete, ipsilateral movement of the tongue in response to a non-nociceptive local stimulus (light stroke, touch or 5µl fluid drop) provides an opportunity for quantification. 32 medically uncomplicated infants (32 to 40 wks. est. gest. age; mean = 36.4 wks.) were studied within 4 days of birth and every 2 weeks thereafter. Each of 8 calibrated nylon filaments was applied twice to each side of the tongue in a random order at 30 sec. intervals. The strongest stimulus (3.632gm.) elicited 95% responses and the weakest (.0677gm.) 15%. For averaged scores of all infants, response frequency was an S-shaped function of stimulus intensity. In both term and premature infants the same stimulus intensity (.4082gm.) gave responses close to 50% of the time. In spite of individual differences in test scores (total number of responses) there was a significant correlation of the neonatal score with the score at 2 weeks of age ($p.<01$).

In comparison, adult subjects (12) had 100% recognition at side of tongue for the weakest intensity stimulus tested in infants. Recognition of a .0230gm. stimulus occurred in 50% of trials for each of 5 adults tested. The stimulus intensity that gives 50% reflex elicitation in infants is 17 times greater than that giving 50% recognition in adults.

NEUROLOGY

Read by Title

ACUTE MYOCLONUS AND ATAXIA--AN EARLY WARNING SIGN OF NEUROBLASTOMA. Patrick F.Bray, M.Eugene Lahey, Dale G.Johnson. Univ. of Utah Col. of Med., Dept. of Ped., Salt Lake City.

Several years ago the authors and several other workers reported anywhere from one to three patients who developed a violent and abrupt movement disorder in association with a neuroblastoma. The designation of the movement disorder appears in the literature under a variety of titles including acute cerebellar ataxia, opsoclonus, polymyoclonus, "dancing eyes and dancing feet" and myoclonic encephalopathy (a term that we prefer). The significance of this nonmetastatic cancerous cause of brain damage impressed us at the time, but some skepticism has surrounded the observation.

During the past six months we have seen two more cases, and a recent survey of the literature discloses a total of 23 similar documentations as well as at least 10 additional unreported cases, by personal communication.

The purpose of this report is to emphasize this early warning sign of neuroblastoma to members of this Society because of the tumor's potential curability and because we believe the relation between the disorder of movement and a hidden tumor is still not widely enough appreciated. A short 16 mm film strip depicting the striking clinical picture and its improvement with time and treatment will be shown. We shall also discuss the current diagnostic workup and speculate briefly about the pathogenesis of the remote damaging effect of neuroblastoma upon the brain.

CEREBRAL METABOLISM DURING CARDIAC SURGERY WITH DEEP HYPOTHERMIA AND CIRCULATORY ARREST James A. Brunberg, Edward L.Reilly, and Donald B. Doty (Intr. by Ronald M. Lauer) Dept of Peds, Neuro, Psych and Surg. Univ of Iowa Hosp. Iowa City.

Total circulatory arrest and deep hypothermia are currently utilized in the surgical repair of congenital cardiac defects in infancy. Aspects of cerebral metabolism were studied in six infants undergoing such surgery with blood sampling from brachial artery and jugular bulb and continuous electroencephalographic (EEG) recording. During cooling with cardiac bypass, mean jugular pO_2 (mmHg) progressively increased (27.1 @ 24-30°C, 84.7 @ 18-23°C) while the arterio-venous difference (AVD) of pO_2 fell(+216 @ 24-30°C, +126 @ 18-23°C) suggesting diminished cerebral oxygen utilization. AVD pCO_2 indicated cerebral CO_2 retention (+15 @ 24-30°C, +4.9 @ 18-23°C) compatable with increased arterial bypass pCO_2 added to promote cerebral vasodilation, and with decreased cerebral CO_2production. With cooling to 18-23°C the AVD of acetoacetic acid (AAA) (+.237mg%) suggested cerebral AAA uptake while the AVD of lactate (-2.40mg%) and pyruvate (-.025mg%) indicated cerebral production. Efflux of free fatty acids (FFA) persisted throughout cooling (AVD=-328ueq/L @ 24-30°C, -81ueq/L @ 18-23°C).

EEG activity fell rapidly to 2-10uv late in cooling or early in circulatory arrest; this persisted throughout arrest in two patients (41 & 25 min.) while four patients developed cerebral electrical silence after 2-28 minutes of circulatory arrest.

During rewarming jugular pO_2 remained constant (25 @ 20°C, 30.8 @ 30°C) despite continued high arterial pO_2 values suggesting cerebral oxygen depletion or diminished cerebral blood flow. Mean jugular pCO_2 was 72 post arrest and fell to 29 at 35°C. AVD lactate post arrest (-5.3mg%) and at completion of warming (-3.0mg%) suggested continued anaerobic metabolism. AVD of AAA post arrest (+.154mg%) and after warming (+.505mg%) suggested continued cerebral AAA utilization. The AVD of FFA with warming (33ueq/L) demonstrated accumulation of FFA. Continued cerebral metabolism during hypothermic arrest was suggested by persisting EEG activity; and after circulatory arrest by increased jugular pCO_2 and lactate, and apparent cerebral oxygen depletion.

X-LINKED SCHILDER'S DISEASE: A GENERALIZED DISORDER OF CHOLESTEROL METABOLISM? Barbara K. Burton* and Henry L. Nadler, Northwestern University Medical School, Department of Pediatrics, Children's Memorial Hospital, Chicago.

Schilder's disease (SD) with adrenal insufficiency (AI) is a fatal degenerative disorder inherited as an X-linked recessive trait. On the basis of (1) birefringent crystals in ballooned cells in adrenal cortex of SD males with or without AI and (2) abnormal cholesterol (chol) esterification in the brains of patients with SD, we may propose that SD represents a generalized defect of chol metabolism. To test this hypothesis, fibroblasts were cultivated from a patient with SD and AI and from controls (C). Chol metabolism was investigated by studying 2-hour accumulation and 7-day accumulation and degradation of cholesterol-4-C^{14} (chol-C^{14}) and cholesteryl palmitate-1-C^{14} (CE-C^{14}). The uptake of both chol-C^{14} and CE-C^{14} over 2 hours was similar in C and SD. C and SD took up chol-C^{14} and CE-C^{14} equally for up to 24 hours; however, SD accumulated greater amounts upon continuous exposure to chol-C^{14} for 7 days. In addition, SD retained significantly greater amounts of label when grown in cold medium after a 24-hour exposure to chol-C^{14}. CE-C^{14} was retained equally in C and SD. These data are consistent with SD being a generalized defect in cholesterol metabolism.

SEVERE ENCEPHALITIS DUE TO HERPESVIRUSES IN CHILDREN. Lawrence T. Ch'ien, John W. Benton, James H. Halsey, Jr., and Charles A. Alford, Jr. Univ. of Ala. in Birmingham, Sch. of Med., Depts. of Ped. & Med., Birmingham, Ala.

To better understand the role of herpesviruses as a cause for severe encephalitis in children, 22 patients were examined virologically and serologically. Brain biopsies were performed in 9 children whose CNS disease was rapidly progressive with increasing intracranial pressure requiring surgical decompresion. Type 1 herpesvirus hominis (HVH) was isolated within 48 hours from brain biopsy obtained from 2 of 9 and was excreted simultaneously in urine and from the throat of 2 others not biopsied. Thus, incidence of proven HVH involvement was 9% and possibly as high as 18%. Reactivation or reinfection as opposed to primary infection was suggested by high levels of neutralizing and C-F antibody found in acute serum of HVH cases. Two of the biopsy-proven cases had more fulminant disease course and died acutely in spite of ara-C therapy while 2 not treated nor biopsied recovered slowly with only minimal brain damage.

Cytomegalovirus (CMV) was also recovered from the brain of 1 of the biopsied patients, yielding a 33% incidence of herpetic infection in this subgroup. The peculiar nature of this encephalitis and the unique biologic characteristics of the CMV isolated suggest a new viral encephalitic syndrome.

Data indicate that herpetic infection may be just as important as a cause for severe encephalitis in children as it is in adults, emphasizing the need for new diagnostic and therapeutic approaches.

A THREE YEAR STUDY OF 5-HYDROXYTRYPTOPHAN ADMINISTRATION IN DOWN'S SYNDROME; RESULTS OF A DOUBLE BLIND STUDY. Mary Coleman, Ann Lodge, Ann Barnet, Leon Cytryn, (introduced by Phillip L. Calcagno.) Georgetown University School of Medicine

In 1967, increased muscle tone in newborns with trisomy 21 following administration of 5-hydroxytryptophan (5HTP) was reported to this Society. No prognostic inferences regarding intelligence were made.

A subsequent three year double blind study of the effect of 5HTP on 19 patients with trisomy 21 comprise the results of this report. Neurological, psychological, psychiatric and biochemical evaluations show the following: the initial increased muscle tone noted with 5HTP was recorded for 3 months with a negative effect thereafter; utilizing the Bayley Mental Development Quotients, 5HTP patients showed no difference from placebo patients; psychiatric evaluation did not differentiate between the two groups; however, of the catecholamines and their metabolites (epinephrine, norepinephrine, HVA, VMA, and MHPG) 24 hour urine determinations of epinephrhe showed a statistically significant elevation (5HTP patients: 4.08ng/M^2; placebo patients: 16.61 ng/M^2. p=.003). Acute and chronic side effects of diarrhea, hyperactivity, infantile spasm syndrome, and EEG changes were observed.

This result suggests a previously undescribed direct effect of 5HTP on the induction of increased epinephrine excretion. The relationship of the pathways and binding sites will be presented.

RELATIONSHIP OF ACETYLCHOLINE SYNTHESIS AT THE NEUROMUSCULAR JUNCTION TO CHANGES IN MUSCLE MASS AND FUNCTION. Gary Franklin and Ivan Diamond, (Intr. by M.M.Grumbach), Dept.Neurology and Pediatrics, University of California, San Francisco.

Choline acetyltransferase (ChAc) catalyzes acetylcholine synthesis in presynaptic endings from motor neurons and is a convenient enzymatic marker of neural innervation in muscle. This is a biochemical study in rats to determine whether ChAc activity at the neuromuscular junction participates in the physiologic response of muscle to development, work and stress.

Each muscle fiber has one endplate. During development, ChAc activity per muscle increased directly with individual muscle fiber growth, suggesting that neural innervation of muscle continued to develop as muscle mass increased. However, a different form of growth, work hypertrophy, did not cause a concomitant increase in ChAc activity per muscle. Also, another acquired change in muscle mass, steroid atrophy, did not alter ChAc activity per muscle. These results are in contrast to the striking increase in ChAc activity with development, and suggest that ChAc maturation and regulation may be independent of muscle fiber size. This was confirmed in hypophysectomized rats which had a growth arrest of muscle. Here ChAc activity per muscle increased normally with age although there was little change in muscle mass.

These observations suggest that ChAc activity at the neuromuscular junction is independent of growth hormone, muscle mass and function, and is probably regulated by central factors.

THE PROGNOSTIC SIGNIFICANCE OF NEONATAL EEG TRACINGS AND AUDITORY EVOKED RESPONSES RECORDED FROM PREMATURE INFANTS. Leonard J. Graziani, Leonard Katz, Roger Q. Cracco, Elliott D. Weitzman

The prognostic significance of EEG tracings and auditory evoked responses (AERs) recorded in the neonatal period was determined in a prospective study of low birth weight infants. Neonatal EEGs and summed AERs were recorded biweekly from 24 premature infants between 28 and 41 weeks post-conception; all had a normal neurologic and psychological examination at 5 to 7 years of age. Each of 78 tracings was visually analyzed for relative amounts of 6 EEG patterns using objective criteria. Neonatal recordings from 14 infants with neurologic sequelae or IQ below 70 at 2 to 8 years of age were compared to those recorded in the normal group. Similar relationships between AER characteristics (latency and amplitude of N_1 and P_2) and 6 EEG patterns were found in both the normal and the abnormal groups of infants. Only 1 of the 6 EEG patterns in each of 3 neonatal tracings from 2 abnormal children were abnormal when compared to the 95% confidence limits of the normal neonatal regression curves. However, the EEG abnormalities were probably due to neonatal complications rather than to irreversible brain injury. These findings suggest that EEG patterns and their relationship to AER characteristics are influenced by age post-conception and by neonatal complications, but the neonatal recordings are of no prognostic significance in low birth weight infants.

STUDIES ON THE PATHOGENESIS OF ENCEPHALOMYELITIS: A ROLE FOR AN OBLIGATORY MICRO NUTRIENT? E.R.Hughes, M.L.McNatt*, B.S. Wingfield*, and M.J. Elders. Dept. of Ped. UofA Med. Ctr. Little Rock, Ark. and U of W.Va. Sch. of Med. Morgantown, W.Va.

Manganese is an essential micro nutrient. We have shown that Mn deficiency protects against avian encephalomyelitis in chicks, and that embryonic chick brain incorporates ^{54}Mn into a slow-turnover pool if the isotope is given early in development. These findings suggest an essential structural or metabolic role for Mn in brain development and function.

The encephalitogenic protein described by Eylar et al (Arch. Biochem. Biophys. 132:34, 1969) was isolated from embryonic chick brain labeled in embryo with ^{54}Mn. Throughout purification, including extensive dialysis, 26% of incorporated ^{54}Mn remained associated with protein. The basic protein recovered from DEAE-cellulose with water elution contained all of the ^{54}Mn and was shown to induce symptoms of experimental allergic encephalomyelitis in rats. All of the ^{54}Mn eluted from Sephadex-G25 with a single distinct protein fraction representing 10% of the total basic protein and shown by acrylamide gel electrophoresis to be a homogeneous Mn-containing protein. The identity of this protein in relation to known encephalitogens has not been determined. However, the requirement for manganese for development of encephalomyelitis in chicks suggests that the metal may be an integral part of or be essential for synthesis of an encephalitogenic protein, or it may be necessary for initiation of antigen-antibody reactions.

THE PREGNANT EPILEPTIC AND HER OFFSPRING, S. Iosub, N. Bingol, E. Wasserman, Ped.N.Y.M.College

Twenty six pregnant epileptic patients and 50 pregnant non epileptic controls and their offspring have been followed up to 2 years in a prospective study. Of these, 19 epileptics were receiving various anticonvulsants (phenlyhydantion, phenobarbital, chlordiazepoxide, and primidone) singly or in combination, while 7 received no medication during their gestational period. The study group had a significantly higher number of small for date infants compared to the control group (19% and 7% respectively). While all infants born to epileptics were full term 11% of babies born to controls were premature. Most of the small for date infants (3 out of 5) were born to mothers who were not receiving any treatment during pregnancy because of infrequent convulsive episodes. The mean head circumference was consistently smaller in the offspring of epileptics who were taking both phenylhydantion and phenobarbital than in any other group. There was no significant difference in other parameters of growth and development between the study group and the controls. Minor congenital malformations consisting mostly of deformities of the lower extremities, were more frequent in the study group and again highest in the offspring of mothers receiving combined phenylhydantion and phenobarbital.

PATTERN VISION IN NEWBORNS: VISUAL FOLLOWING OF FACE-LIKE STIMULI. Carolyn G. Jirari, Merrill Sarty, and Paul Y.K. Wu. (Intr. by Paul F. Wehrle). Dept. of Pediatrics, USC School of Medicine, Los Angeles.

Controversy exists as to whether human neonates are capable of visual discriminations and pattern perception with minimal prior visual experience. In order to test this, schematic faces consisting of Face (F), Mod. Scrambled Face (M), Scrambled Face (S), and Blank (B), were shown to 36 newborns ($\bar{X}$ age 12.6 hrs.) Responses were measured as degrees of head-turning as each stimulus was moved before the infant's face. Order of following (head-turning) was F > M > S > B, with significant differences between all pairs ($p<.025$). Similar stimulus preferences were obtained from 40 younger infants ($\bar{X}$ age 10.75 min.) for both head-turning and eye-turning. Differences between F and M and between S and B were significant at $p<.0005$. In a third experiment, a different series of 5 stimuli (Face, 6 Eyes, 2 Large Eyes, 2 Normal Eyes and Mouth) was presented to 25 neonates ($\bar{X}$ age 13.6 hrs.) Again, Face was followed more than any of the other stimuli ($p<.01$).

The first 2 experiments demonstrate that human neonates can discriminate a series of visual stimuli on the basis of pattern (faceness) when complexity of stimuli is equal. The third experiment suggests that the "gestalt" of the entire face is more effective in eliciting attention than parts of the face, even when these parts are exaggerated by number or size and are of similar complexity (e.g. Face vs. 6 Eyes).

PRIMARY HYPOVENTILATION SYNDROME (ONDINE'S CURSE): A METABOLIC DEFECT AFFECTING AUTOMATIC RESPIRATION, Derrick Lonsdale, and Robert D. Mercer, Intr. by Robert Schwartz, Cleveland Clinic Foundation, Dept. of Peds., Cleveland.

Primary hypoventilation syndrome has been known to be associated with diseases which interfere with automatic respiration. It has been recognized also as occurring spontaneously in infancy (Mellins, et al. Medicine 49:487, 1970) and is a common occurrence in Leigh's encephalopathy (Montpetit, et al. Brain 94:1, 1971). We have had experience with 11 unrelated patients in whom life threatening apnea or prolonged cessation of automatic respiration was the presenting clinical phenomenon. Of the 11 patients 6 were infants, 1 was age 2, 2 were adolescents and 2 were adults. Clinical features included short stature(3), scoliosis(2), abdominal distension (4), choking episodes(6), and severe neurological signs(6). Two of the infants fit the acceptable clinical pattern of Leigh's encephalopathy. Clinical features of another infant were characteristic of those in the H-type T-E fistula but surgical exploration was negative. The discovery of TPP inhibitor substance in the urine led to a clinical trial with thiamine with complete abolition of bulbar symptoms. Biochemical findings included variable amino aciduria(10), TPP inhibitor in urine(4), hyperuricacidemia/uria(5), hypertriglyceridemia(4), and increased output of low SG urine(7). The findings, though heterogeneous and possibly dependent upon biological adaptation suggest a defect in intermediary carbohydrate metabolism interfering with brain stem function.

STUDIES OF MYONEURAL JUNCTION IN FULLTERM, PREMATURES & HYPERMAGNESEMIC NEWBORN INFANTS. M.R. Koenigsberger,* B.M. Patten, & R.E. Lovelace, Division of Perinatology & Dept. of Neurology, Col. of Physicians & Surgeons, Columbia Univ., N.Y. Introduced by *L. S. James.

The newborn's immature neuromuscular junction (N.M.) may be an important contributing factor in his peculiar susceptibility to bulbar and respiratory failure. This immaturity may be additive to well known causes of N.M. failure such as neonatal myasthenia gravis, hypermagnesemia and antibiotic administration. (Kanamycin). This report contrasts N.M. transmission in immature and term infants and in turn compares their myoneural function to hypermagnesemic infants.

The methodology is rapid and safe. A square wave supramaximal stimulus at rates of 1,2,5,10,20, & 50 impulses per second (ips) was applied by surface electrodes to the median nerve at the wrist for 15 seconds. The evoked muscle action potential (MAP) was recorded by surface electrodes taped to the thenar muscles of the hand. In 11 fullterm and 6 immature infants no change in MAP was observed at 1,2, & 5 ips;at 10 ips, 50% showed potentiation of MAP; while at 20 ips,70% showed a decrease. Strikingly, only the immature infants showed post tetanic exhaustion. Unlike most adults, at 50 ips all newborns showed a decrease in the MAP of at least 10% during stimulation. By contrast, 3 of 6 hypermagnesemic infants showed a decrease in MAP at 2,5, & 10 ips.Decreased myoneural reserve was noted when serum Mg. was higher than 4.5 meq/l or at lower levels with concurrent hypocalcemia.

ADVERSE SEQUELAE IN TRANSIENT TYROSINEMIA OF THE TERM NEONATE. Peter Mamunes, Paul E. Prince, Patricia A. Hunt, Elizabeth S. Hitchcock and David E. Hoffman, Dept. of Ped., Med. Col. of Va. and Bureau of Child Health of the Va. State Health Dept., Richmond, Virginia (Intr. by Harold Maurer)

Thirty six term infants with severe tyrosinemia (mean 33.5 ± 15 mg%) were diagnosed by Guthrie testing for phenylketonuria in Va. from 1967-1971. All were on evaporated milk at the time of their initial test (mean age 14±6 days). Their tyrosinemia (TNT) resolved within a few days after lowering the protein intake and/or Vit. C supplementation. Fourteen of these 36 infants are now 3-5 years of age and were available for careful developmental testing by a single examiner. Their performance was compared to a group of 14 matched controls who were fed a low protein formula during early infancy. Whereas there was no significant difference in mean IQ's by the Sloosson Intelligence Test, TNT children had significantly lower scores ($p < 0.05$) in the Peabody Picture Vocabulary Test (76.1±6.5 for TNT vs. 94±3.4 for controls) and in the Valett Developmental Survey (210±64 for TNT vs. 282±91 for controls). Valett subtests with significantly lower scores for the TNT children evaluated visual discrimination, verbal fluency, conceptualization and auditory discrimination.

These results indicate that severe tyrosinemia in the term neonate may be injurious to the developing central nervous system. If further studies confirm this finding, public health measures to reduce the occurrence of this disorder are urgently needed.

CENTRAL NERVOUS SYSTEM DISEASE ASSOCIATED WITH MYCOPLASMA PNEUMONIAE INFECTION. Robert J. Lerer, Steven M. Kalavsky, and Bennett A. Shaywitz (Intr. by C.D. Cook). Yale Sch. of Med., Yale-New Haven Hosp., Dept. of Ped., New Haven.

Four children with encephalitis and two with polyradiculitis, seen during a three year period, have shown fourfold or greater changes in cold agglutinin titer (four patients) or complement fixation titer to Mycoplasma pneumoniae (five patients). Prior to the onset of neurologic symptoms, three children had pneumonia, one had rhinitis, and two had no respiratory illnesses. Two patients with encephalitis were deeply comatose to the point of apnea requiring ventilatory assistance. Both children with polyradiculitis had prolonged respiratory paralysis and needed artificial ventilation for over six weeks. Focal neurologic signs were present in three patients. Four children had increased intracranial pressure. All children survived, but four were left with significant neurologic deficit three months to two years after discharge. Treatment with tetracycline or lincomycin did not seem to alter the course of the neurologic disease. Central nervous system disease associated with Mycoplasma pneumoniae may be more common than generally recognized in children. The pathogenesis of this problem is unknown, but may represent a neurotoxic or immunogenic process.

A POSSIBLE DE LANGE SYNDROME VARIANT? Helouise C. Mapa and Qutub H. Qazi, Dept. of Ped., Downstate Medical Center, Brooklyn, New York

We have observed two unrelated 15-month old female infants, who presented with extreme failure to thrive, and, in addition, had identical physical abnormalities. Both infants were small for gestation (3 lbs. at 8 months), and weighed only 8 and 9 pounds at the time of examination. The linear growth was less affected than the weight. The infants shared the following physical abnormalities - microcephaly, hirsutism localized to head and forehead, prominent hyperteloric eyes, large low-set ears, fish-like mouth, prominent nipples, scanty subcutaneous tissue, congenital heart disease and developmental retardation. The laboratory studies revealed normal skeletal maturation, absence of aminoaciduria, normal thyroid and adrenal function, normal growth hormone response to arginine and normal karyotypes. One infant had marked dermal hypoplasia and the other had an increased atd angle and a radial loop on the left thumb. There was no consanguinity.

Although the patients do have features in common with De Lange syndrome, they differ in not having the characteristic facies, skeletal abnormalities, delayed bone age, and generalized hirsutism.

THE EFFECT OF THYROID HORMONE ON CEREBRAL GLUCOSE METABOLISM IN THE INTACT INFANT RAT. Thomas J. Moore, Armand P. Lione, and David M. Regen. Columbia Univ. Col. Phys. & Surg., St. Luke's Hosp. Ctr., Ped. Serv., New York; and Vanderbilt Univ. Sch. of Med., Dept. of Physiology, Nashville, Tenn.

Sugar Transport activity at the blood brain barrier of the rat was estimated from measurements of ^{14}C-3-O-methylglucose in serum and brain at various times following intraperitoneal injection. In the newborn, the transport rate was about 1/4 that of the adult and remained low for 12 days at which time it increased steeply for 8 days nearly to the adult value. Daily injections of thyroxine caused the transport rate to rise earlier and eventually to attain higher values between 20 and 30 days. At 15 days, the thyroxine treated animals showed sugar transport rates about 40% greater than controls and cerebral glucose utilization rates about 30% greater than controls. Methimazole, a thyroid antagonist, delayed the rise in transport activity. Cerebral blood flow, as estimated by $^{3}H_2O$ appearance in the brain after peritoneal injection, may have been somewhat faster in thyroxine treated infants.

AUDIOGENIC EXTINCTION. A SIGN OF DEFENSE IN FUNCTIONAL DEAFNESS AND AUDITORY INCOMPETENCE. Frederick Richardson. Univ. of Miami School of Med.

Many test responses to threshold sound stimuli give little information as to auditory competence. A study commenced at Johns Hopkins in 1962 of 200 children with aphasia, deafness or language disorders revealed two major groups: 1.Those responding consistently to sounds and words who developed speech spontaneously. 2.Those without speech whose responses were variable or absent. Follow up revealed that many children in Group 2 had "normal" responses to audiometric tests but in the enviroment had variable or no response to sound. Children who had responded to sound when younger appear to have extinguished responses to sound when permitted to do so. A hierarchy of serial testsof auditory competence from basic reflexes such as 1.Alerting and 2. Orienting to perceptual-cognitive coding and organization of auditory material in memory by 3.Discrimination 4.Association 5.Categorization and 6.Recognition, has been developed and can be used by the pediatrician. The sign of audiogenic extinction is disease, age and personality dependent. Auditory competence in middle ear deafness or mental retardation is relatively unaffected unless profound, or an associated language disorder is present.

HYPERPYREXIA AS A DRAMATIC SIGN OF INTRAVENTRICULAR HEMORRHAGE IN THE NEONATE. Jeffrey J. Pomerance and C. Joan Richardson. Univ. of Cal., San Diego Sch. of Med., Dept. Ped., Div. Perinatal Med., La Jolla. (Intr. by Louis Gluck).

Temperature elevation above 102°F is a rare finding in the immediate neonatal period. Previously there have been only 18 cases reported of intraventricular hemorrhage with associated hyperpyrexia in the neonatal period. In these reports, the excessively high temperatures usually were terminal events. Three additional cases of hyperpyrexia in the newborn are reported here.

The elevated temperatures were seen in infants of 992, 2842, and 4380 grams birth weight. The temperatures ranged between 102.4 and 107.2°F. Only one of these infants survived. The diagnosis of intraventricular hemorrhage was established by appropriate diagnostic procedures, including ventriculography. In the two infants who expired, one death was a direct result of the hemorrhage, and the other death was due to meningitis following a ventriculoperitoneal shunt. The surviving infant is developmentally normal at 18 months of age.

In each of these cases hyperpyrexia was an early sign and underscores the need for careful temperature monitoring of newborn infants in intensive care units. This dramatic finding may enable early recognition of intraventricular hemorrhage. Vigorous therapy may enable some of these infants to survive intact.

HYDROCEPHALUS EX VACUO DUE TO HEXACHLOROPHENE (HCP) TOXICITY IN IMMATURE RATS, Arthur L. Rose, Wendy Cammer and Henry K. Wisniewski. (Intr. by Henry Barnett), Albert Einstein Coll. of Med., Dept. of Neurol. and Ped., Bronx, N. Y.

The evolution of neuropathological lesions due to HCP toxicity, and the process of recovery, was studied in immature rats. Sprague-Dawley female rats with nursing litters were fed a diet containing HCP 500 p.p.m. (31 mgs/kg/day) starting on 9th day after delivery (day 0). The baby rats developed HCP blood levels of 2.0-2.5 uG/ml. during the first week of feeding. They were sampled on days 1,2,4,7,9 and 15. Nine babies were weaned and continued to receive HCP diet, resulting in HCP blood levels of 1.5-4.0 uG/ml. Six of these rats were killed on days 25,35,45,64,73 and 86. Three rats were taken off the HCP diet on day 45 and were killed 60-120 days later.

All animals were completely asymptomatic. The brains were examined by light and electron microscopy. Spongy degeneration due to intralamellar vacuolation of myelin sheaths was present in the optic and olfactory tracts on day 4, and in most other areas of white matter on day 7. Hydrocephalus was present after day 15 and E.M. showed axonal degeneration. 60-120 days after discontinuation of HCP diet the hydrocephalus was more severe and the optic nerves were atrophic.

These findings show that irreversible loss of neural tissue, resulting in hydrocephalus ex vacuo, occurs in the developing nervous system exposed to HCP. This is in contrast to the previous reports of almost complete morphological recovery in adult rats. (Supported by NINDS 1 R01 NS 09064-01).

FUNDAL ABNORMALITIES IN NEPHROPATHIC CYSTINOSIS. John E. Read, Morton F. Goldberg, Gerald Fishman, Ira M. Rosenthal. Abraham Lincoln Sch. of Med. of the Univ. of Illinois Col. of Med., Depts. of Ped. and Ophthalmology, Chicago.

The presence of cystine crystals in the cornea and conjunctiva of patients with nephropathic cystinosis has been recognized for some years. It has recently been appreciated that there may be abnormalities as well in the fundus of these patients. The changes principally described include patchy depigmentation of the retinal pigment epithelium. A 5-year-old child with nephropathic cystinosis has recently been studied intensively from an ophthalmological standpoint. In addition to depigmentation of the retinal pigment epithelium, refractile bodies, presumably cystine crystals, were seen in the fundus. Additional crystals were observed floating freely in the aqueous of the anterior chamber. An abnormal electroretinogram was recorded. Despite these changes, visual acuity was normal. With the development of renal transplantation, patients with nephropathic cystinosis may live longer. Progressive observation of such patients is required to determine if the ocular abnormalities described will eventually disturb visual function.

ASSOCIATED DISORDERS IN 100 CONSECUTIVELY STUDIED CHILDREN WITH SCOLIOSIS. A. David Rothner, Abe Chutorian, Hugo Keim (Intr. by R. E. Behrman). Col. Physicians & Surgeons, Columbia University, New York.

Previous studies of children with scoliosis have been retrospective and have failed to clearly document the incidence and character of associated occult, neuromuscular, congenital and other disorders. The authors therefore consecutively studied 100 patients referred for evaluation of scoliosis. Results: 72 children had "idiopathic" (genetic) scoliosis and 28 children "symptomatic" scoliosis. Eleven of the latter had one or more congenital anomalies of the bony spine. Four of these had associated diastematomyelia. Five children had occult neuromuscular disease, including: myopathy (2), polyneuropathy, hydromyelia, and anterior horn cell variant. All were asymptomatic! An additional 12 children had miscellaneous abnormalities including: multiple congenital anomalies, neurofibromatosis, congenital heart disease, metabolic bone disease, post-traumatic denervation, osteoid osteoma, and the "Epidermal Nevus Syndrome."

Children with symptomatic scoliosis had a statistically significant increase in operative morbidity ($p. < 0.01$).

It is concluded that all children with scoliosis should have careful neurologic and pediatric evaluation. Associated anomalies and neurogenic disorders must be identified early in order to plan appropriate management, identify those at increased risk of operative complications, and identify those expected to undergo progressive neurologic impairment.

DIAGNOSIS AND TREATMENT OF REYES SYNDROME. Frederick J. Samaha, Edward Blau and John L. Berardinelli (Intr. by Thomas K. Oliver, Jr.). Univ. of Pittsburgh, Children's Hospital of Pittsburgh, Department of Pediatrics, Pittsburgh, Penna.

Twenty two cases of Reyes syndrome were studied to place the clinical diagnosis on firmer ground and to explore the possible usefulness of peritoneal dialysis as a mode of therapy. About 7 days after an apparent viral infection, these patients exhibited repeated vomiting followed by cortical excitation. Abnormal blood prothrombin time, SGPT and SGOT were found in almost every case. Mixtures of metabolic acidosis and respiratory alkalosis were a constant feature. In the 12 cases where blood ammonia levels were determined, it was elevated from 1.6 to 20 times normal. These criteria along with a relatively normal spinal fluid and an absence of evidence for drug intoxication formed the basis for the diagnosis of the Reyes syndrome. Examination of liver histology in 13 cases did not conflict with these criteria. A study of the literature also showed a close clinico-pathological correlation between our criteria and the liver histopathology. Thirteen consecutive control cases were treated with the usual hepatic coma regimen while the next 9 children were treated with the hepatic coma regimen and peritoneal dialysis. The two groups did not differ with regard to clinical and laboratory parameters. Two of the 13 control cases (15%) survived while 7 of the 9 dialyzed cases (78%) survived. The results between these 2 groups are statistically significant ($p < .025$).

FAMILIAL MYOPATHY AND ENCEPHALOPATHY WITH LACTIC ACIDEMIA PARTIALLY RESPONSIVE TO CORTICOSTEROIDS Yehuda Shapira, Stephen D. Cederbaum, Barbara M. Lippe and M. Anthony Verity. (Spon. Richard J. Schain) U.C.L.A. School of Medicine, Dept. Ped., Psych. and Path., Los Angeles

The one surviving sibling of two affected with a syndrome of growth retardation, muscular wasting, exercise intolerance, seizures, intellectual deterioration and early death demonstrated elevated resting blood lactate levels of 3.9 mM which rose to 12.2 mM with minimal exercise. The lactate:pyruvate ratio was 40:1. Cardiac output and oxygen consumption at rest were both elevated. Muscle biopsy revealed preferential Type I fiber atrophy, a "ragged red" appearance with Gomori trichrome stain and subsarcolemmal excess of mitochondrial oxidative reactions. The mitochondria of unaffected fibers appeared normal on electron microscopy.

Large doses of corticosteroids reversed an episode of acute CNS deterioration and increased exercise tolerance dramatically. Resting blood lactate fell to 2.3 mM and rose little in response to minimal exercise. Moderate exercise on a treadmill resulted in lactate levels of only 6.6 mM.

Preliminary experiments reveal that her skin fibroblasts in culture may oxidize pyruvate [2-C^{14}] to $C^{14}O_2$ at a rate somewhat below that of controls.

These data suggest that an inborn error of mitochondrial function is present in this family and may be partially responsive to steroids by a mechanism as yet undetermined.

REYE'S SYNDROME: ACID-BASE PARAMETERS. Daniel C. Shannon, Barry Bercu, Thomas Glick, I.David Todres, John T. Herrin. Harvard Medical School, Massachusetts General Hospital, Children's Service, Boston, Massachusetts.

Six children with clinical and hepatic pathological diagnosis of Reye's syndrome were subjects. Lumbar CSF and blood were drawn anaerobically from arterial (a) and internal jugular (IJV) vein catheters at intervals and analyzed immediately for P_{O_2}, P_{CO_2}, pH, lactic (Lact) and pyruvic acids and ammonia content. Pa_{O_2} was greater than 95mmHg while $PaCO_2$ varied from 36 to 18mmHg and pH was above 7.38 in all. $[NH_3]_a$ varied from 150 to 540 ug%, $[NH_3]_{IJV}$ from 0 to 460 ug% and $[NH_3]_{CSF}$ from 50 to 150 ug%. $[Lact]_a$ varied from 0.5 to 6.0 mM/L and $[Lact]_{IJV}$ from 0.5 to 9.0 mM/L. $[NH_3]_a$ was related to $PaCO_2$ ($r=0.74$) and was not directly related to $[NH_3]_{IJV}$. In 2 non-survivors $[NH_3]_{IJV}$ was below $[NH_3]_a$ by as much as 495 ug% initially and was associated with $[Lact]_{IJV}$ of 6.5 and 9.0 mM/L. In 2 survivors $[NH_3]_{IJV}$ was above $[NH_3]_a$ by as much as 320 ug% and was associated with $[Lact]_{IJV}$ less than 2.65 mM/L. Hyperventilation can be related to increasing NH_3 suggesting that NH_3 is a respiratory stimulant. This may be related to cerebral lactic acidosis observed in these patients.

RETINAL CHANGES IN THE PIGLET PRODUCED BY OXYGEN AND LIGHT. T.R.C. Sisson, S.C. Glauser, N. Chan, E.M. Glauser, and G. Chan. Depts. Pediatrics, Ophthalmology, and Pharmacology, Temple Univ. School of Medicine, Philadelphia, (intr. by Angelo Di George).

This study was designed to investigate the effect of light at 420-470 nm. and O_2 at 10, 20, 40, and 100% conc. upon the retinas of newborn piglets. In environmental chambers, 32 piglets were exposed to these various conc. of O_2 under blue light (2.9 w/cm^2) and in the dark for 24 hrs. at 3 days of age. Indirect ophthalmoscopy, ERG, and retinal photography were done just prior to exposure to these environments and 24 hours and 21 days after. The eyes were enucleated at 24 days of age and processed for histologic examination.

Vascular changes were seen following exposure to 40% and 100% O_2 in the dark. Changes in fundal background homogeneity were noted after exposure to light with and without elevated O_2 conc. ERG's revealed flattening of wave-form response. Histologic sections showed narrowing of retinal vessels after O_2 exposure, as did retinal photos. Degeneration of visual receptors and ganglion layer nuclei, and alterations of pigment epithelium were seen in eyes exposed to blue light. Generalised edema of retinal layers was observed. ERG recordings established loss of visual reception after light exposure regardless of oxygen concentration.

These results indicate that pathologic changes in the retina of the piglet are increased when exposure to visible light is added to elevation of oxygen concentration in the atmosphere. Reduced oxygen (10%) did not produce the vascular changes of higher concentration, with or without light.

HYPERVENTILATION AND HYPERAMMONEMIA. Daniel C. Shannon, Joste Wichser and Homayoun Kazemi. Harvard Medical School, Pulmonary Unit, Massachusetts General Hospital, Boston, Massachusetts.

Buffered ammonium chloride was given to dogs anesthetized lightly by constant infusion to determine its effects on $\dot{V}_E$, and on acid-base balance and on $[NH_3]$ in cisternal CSF and in arterial and sagittal venous blood. Brain NH_3 content was measured at the end of each experiment. NH_3 was given by 1) IV infusion 2) lateral ventricle infusion and 3) cisternal injection. $\dot{V}_E$ increased linearly with CSF $[NH_3]$ following IV and ventricular infusion and did not change after cisternal injection. Cisternal CSF $[NH_3]$ increased after all three infusion methods while arterial $[NH_3]$ rose only after IV infusion. The slope of $\dot{V}_E$/CSF $[NH_3]$ was two-fold greater than that during ventricular infusion. Thus brain $[NH_3]$ appears to be the primary determinant of $\dot{V}_E$ rather than NH_3 in arterial blood or CSF.

Ammonia acts as a respiratory stimulant on the brain and may account for the respiratory alkalosis seen during various hyperammonemic syndromes such as Reye's syndrome. The effect may be mediated through cerebral lactic acidosis as observed in patients with Reye's syndrome or perhaps through depletion of high energy phosphates.

SCALP HAIR PATTERNING: ITS ORIGIN, SIGNIFICANCE, AND RELATIONSHIP TO EARLY BRAIN DEVELOPMENT. David W. Smith and Bradley T. Gong, Dept. of Ped., Univ. of Washington School of Med., Seattle, Washington

Studies of hair follicle development and scalp hair patterning in normals and those with disorders of early brain development were indicative of the following hypothesis: hair directional slope is secondary to the plane of stretch exerted on the skin by the growth of underlying tissues during the period of downgrowth of the hair follicles, around 10 to 12 gestational weeks. The posterior parietal hair whorl was interpreted as the epicenter from which the growth stretch is exerted by the domelike outgrowth of the brain during the time of hair follicle development. Anomalies such as encephalocele and dicephaly, which must have antedated hair follicle development, showed anticipated aberrations in scalp patterning. Among patients with primary microcephaly 85% had altered scalp hair patterning, indicating an early onset of the problem in brain development. This included 25% with no parietal whorl, a finding previously noted only in non-human primates. Aberrant scalp patterning was also found to be a frequent finding in 5 established syndromes, including Down's syndrome, in each case being compatible with a problem in early brain development.

Thus, aberrant scalp hair patterning may be utilized as an indicator of altered size and/or shape of the brain prior to 12 weeks of gestation.

DEVELOPMENTAL AND NEUROLOGIC OUTCOME OF INFANTS WITH BIRTH WEIGHT UNDER 1500 GRAMS. Annabel J. Teberg, Paul Y.K. Wu, Joan E. Hodgman. (Intr. by Paul F. Wehrle) Los Angeles County-USC Medical Center, Los Angeles.

186 premature infants born at LAC-USC Medical Center from 1964-1970, were followed for a minimum of 9 months to 7 years of age. In 40 infants with birth weight <1000 Gm, 8 (20%) had evidence of significant neurologic and/or developmental abnormality. Five (12.5%) infants had questionable abnormality. Twenty-seven (67.5%) infants were normal neurologically and developmentally. In 146 infants weighing 1001-1500 Gm, 36 (24.7%) showed significant neurologic and/or developmental abnormality. Six (4.1%) had evidence of questionable abnormality. One hundred and four (71.2%) of the group were normal both neurologically and developmentally. Results indicate the more recently born infants (1969-1970) have fewer and milder neurologic and developmental problems than do those born earlier. Of the 37 infants born 1964-1965, 8 (21.6%) were found significantly abnormal neurologically and/or developmentally. In contrast, of the 60 infants born 1969-1970, 2 (3.3%) showed evidence of significant neurologic and/or developmental abnormality. Not only are more infants surviving in the later period, but their outlook for normal growth and development is improved.

MYOPATHY WITH LIPID ACCUMULATION. David H. VanDyke and Robert C. Griggs. Dept. of Ped. and Neurology, Univ. of Rochester, Sch. of Med., Rochester, N.Y., (Intr. by Robert J. Haggerty).

Myopathy with accumulation of lipid droplets in Type I (red) muscle fibers is an unusual cause of weakness in childhood. When an 8 year old male with slowly progressive weakness was found to have this disorder, detailed study of the patient and his parents was performed. Prominent muscle weakness had been observed since age 18 mos. On examination, there was weakness and atrophy greater proximally than distally. Tendon reflexes were diminished but sensory exam was normal. Creatine phosphokinase (CPK), LDH and SGOT were elevated and electromyography (EMG) confirmed myopathy. Although formalin-fixed sections of muscle showed non-specific changes, frozen sections demonstrated abnormal spaces in many muscle fibers which were positive with lipid stains (oil red O), negative for glycogen and positive with oxidative enzyme stains. Asymptomatic cardiac disease was present with right ventricular hypertrophy by ECG, vectorcardiography, echocardiography and chest x-ray. Although study of family members detected no weakness and CPK and EMG of both parents were normal, the father's biopsy showed myopathic features.

Treatment with prednisone (80 mg qod) reversed the EMG and enzyme abnormalities and modestly improved strength. This striking histologic picture in myopathy may have important prognostic and therapeutic implications.

CARBOHYDRATE METABOLISM IN TERM FETAL AND EARLY POSTNATAL RAT BRAIN, CSF AND BLOOD. R.C. Vannucci* and T.E. Duffy*, Dept. of Neurology, Cornell Univ. Med. Col., New York, N.Y. 10021 (Intr. by W.W. McCrory)

The process of birth is accompanied by major physiologic & biochemical adjustments, but its effects upon brain metabolism are largely unknown. Concentrations of high energy phosphates, lactate and pyruvate were measured in term fetal and neonatal rats aged 1,10 & 60 min; 8 & 24 hrs. Vaginally delivered rats were compared with C-sectioned controls. Fetuses were frozen in amnio in liq. N_2<15 sec after maternal decapitation & laparotomy. Fetal brain substrate levels(mM/Kg) were: 2.66(ATP), 1.74(P-Cr), 2.59(lac) & 0.20(pyr). In vaginally delivered rats frozen 1 min postnatally, brain ATP & P-Cr fell to 72% & 28%, respectively, of fetal control, with near total recovery by 10 min, & attained 1-day old values(2.6 & 3.0mM) by 60 min. At 1 min, brain lactate was 9.51mM, while pyruvate was unchanged, resulting in an elevated L/P ratio(54). At 10 min, lactate was 10.59mM, but pyruvate had risen 150%, reducing the L/P ratio to 23. Both substrates then fell to adult levels(1.0 & 0.09mM) by 24 hrs. In C-sectioned rats, brain P-Cr fell to 1.17mM at 1 min, with complete recovery by 10 min; ATP was unchanged. Brain lactate & pyruvate rose to 3.42 & 0.26mM at 1 min, but the L/P ratio was the same as fetal control. These changes suggest transient cerebral hypoxia-acidosis in vaginally delivered rats. Cisternal CSF & cardiac blood lactate, pyruvate & L/P ratios, while parallel in fetal & vaginally delivered rats at 1 min, did not accurately reflect the subsequent postnatal cerebral metabolic state. (Supported by USPHS Grant #NS-03346)

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LUNG GROWTH AND FUNCTION FOLLOWING REPAIR OF CONGENITAL DIAPHRAGMATIC HERNIA. Mary Ellen B. Wohl, N. Thorne Griscom, Samuel R. Schuster, Robert G. Zwerdling and Denise Strieder. Harvard Med. Sch. Children's Hosp. Med. Ctr., Dept of Cardiology, Radiology, Surgery and Medicine, Boston, Mass. Introd. by Charles A. Janeway

Anatomical studies of the lung of infants with congenital diaphragmatic hernia have shown a decrease in the number of airway generations, pulmonary vessels and alveoli (Brit. J. Surg. 58:342, 1971). To determine whether this hypoplasia at birth affects subsequent development of the lung, 17 patients, repaired before the age of one year, were studied at age 6 to 19 years. Total lung capacity, vital capacity and forced expiratory volume were normal. Maximum expiratory flow volume curves showed the same variability as that present in normal children of the same age. In 7 patients, Xenon radiospirometry was used to determine the volume, perfusion and ventilation of the right and left lungs. In our technique the lungs are scanned posteriorly and volume, ventilation and perfusion are normally equal on each side. The volumes of the two sides were equal which confirmed the radiographic impression. However, perfusion to the side of the hernia was decreased in all 7 and perfusion/volume indices averaged 0.86 instead of the predicted 1.0. In the 4 patients with indices below 0.87 there was demonstrable radiographic evidence of diminished blood flow. The vascular abnormality on the side of the hernia suggests that pulmonary hypoplasia persists into late childhood. (Supported by NIH grant #HDO 1392)

HYPERSENSITIVITY LUNG DISEASE IN CHILDHOOD: AN EXPERIMENT OF NATURE. Donald N. Gillespie, Edward C. Rosenow III, and Edward J. O'Connell (Intr. by Gunnar B. Stickler). Mayo Clinic and Mayo Fndn., Rochester, Minnesota.

Four brothers living on a Minnesota farm developed respiratory symptoms every September. Thoracic roentgenograms revealed diffuse pulmonary infiltrates bilaterally, suggesting viral or Mycoplasma pneumonia. The four boys had been exposed to much organic dust, including 10 species of airborne fungi, during the fall harvest. Later the oldest boy (age 15) was found to have hypersensitivity pneumonitis with fibrosis. Open-lung biopsy confirmed the diagnosis, and Aspergillus versicolor and M. faeni were cultured from the specimen. Then the clinical histories of the brothers aroused suspicion. They were less severely affected but all had abnormal pulmonary function. All four had serum precipitins positive to Aspergillus fumigatus and excess serum IgE. Two gave positive immediate and 6-hour responses to skin tests with Aspergillus fumigatus. The family was removed from the farm and, for the first time in 5 years, remained free of pulmonary symptoms during the fall. Two children, challenged by a brief return to the farm under supervision, developed clinical and roentgenographic pneumonitis within 4 days. Their two brothers remained well. The evidence strongly suggests that an environmental inhalant caused the pneumonia. The natural history of hypersensitivity pneumonitis and the importance of identifying and avoiding the precipitating factors were demonstrated by this experiment of nature. If unrecognized, the disease may be devastating in children, and it is not often diagnosed.

ADENYLATE CYCLASE (AC) ACTIVITY IN FETAL RABBIT LUNGS AND ITS RESPONSE TO EPINEPHRINE (E), NaF, CORTISOL (C) AND GLUCAGON (G). Cynthia T. Barrett, Alex Sevanian and Solomon A. Kaplan Department of Pediatrics, UCLA School of Med., Los Angeles

AC activity in lung homogenates of fetal rabbits is 10-20 times greater than that found in fetal or post-natal liver. We measured AC activity at 21, 24, 27 and 30 days of gestation, on the day of birth and in adult rabbits. Theophylline was added to each assay to inhibit phosphodiesterase activity. AC activity was measured as picomoles of ATP converted to cAMP/ mg. of tissue protein.

Mean AC activity (4 animals in each group)

Age	21	24	27	30	NB
Control	438	472	457	432	390
E	498	615	610	649	713
NaF	363	405	608	646	503
C	421	407	446	536	393
G	423	450	483	462	429

Control AC activity did not change during the period tested although it was higher than the mean adult level of 152. E stimulated AC at all ages studied, NaF first at 27 and C first at 30 days ($p<.05$). After delivery, AC activity decreased slightly as did its stimulation by NaF and C, although E increased AC activity by almost two-fold. At no age tested did G have a significant effect. Serial activation of AC activity may represent differential maturational rates of cell membranes and of receptors for the hormones tested.

THE EFFECTS OF POSITIVE END EXPIRATORY PRESSURE (PEEP) ON PULMONARY HEMODYNAMICS. J. R. Hessler, R. D. Garrison, D. V. Eitzman and S. Cassin, Depts. of Comp. Med., Pediatrics and Physiology, College of Medicine, Univ. of Fla., Gainesville.

The effect of PEEP on the pulmonary vasculature was studied in 6 postnatal goats. A Starling Resistor (SR) model was used to analyze pulmonary vascular resistance. Use of this model permits the calculation of resistances proximal (R_p) and distal (R_d) to the vessels behaving as SRs as well as the SR surrounding pressure (P_S). R_p, R_d, and P_S were calculated at PEEPS of 0,5,10,15 and 20 cm H_2O. Data from these experiments indicate that (1) P_S closely parallels PEEP; (2) R_p is unchanged or decreased when PEEP rises up to 15 cm H_2O; when PEEP is 20 cm H_2O R_p increases and systemic pressure falls precipitously, and (3) all increments of PEEP increased R_d. From these results we conclude that: alveolar vessels near the transition to prealveolar vessels act as SRs; prealveolar vessels are primarily responsible for R_p; and at a PEEP of 20 cm H_2O, the SR site moves downstream as indicated by the rise in R_p. In the small animal or child with minimal hydrostatic pressure influences, blood flow to the lung is a function of the difference between pulmonary artery pressure and P_S divided by R_p, and R_d does not relate to flow. Therefore, pulmonary hypertension produced by PEEP is due to an increase in P_S alone until the SR site is moved downstream. When the shift in SR site occurs in response to high PEEP, both R_p and P_S contrubute to produce pulmonary hypertension which eventually becomes lethal. (Supported by NIH grants HL 13749, RR 05003, HL 5979, and RR 00421.)

LUNG ELASTIC RECOIL IN CYSTIC FIBROSIS. Anthony Mansell*, A. Charles Bryan*, and Henry Levison. Dept. of Peds., Research Inst., Hosp. for Sick Children, Univ. of Toronto.

The pulmonary involvement of cystic fibrosis is most commonly assessed by measurements of maximum expiratory flow and residual volume. Elastic recoil is an important determinant of maximum expiratory flow, residual volume and "closing volume" (CV) in the normal lung and we have examined these relationships in cystic fibrosis. We measured static elastic recoil [Pst(L)], maximum expiratory flow curves, static lung volumes and closing volume in 23 patients (aged 8-31 years) with cystic fibrosis and compared the results to those in 46 normal subjects (aged 6-69 years). In the normal subjects high closing volumes were associated with low values for elastic recoil. In the patients residual volumes were high [mean 41.3% of total lung capacity (TLC)] and closing volumes were often above the range of tidal breathing but the same relationship between closure and recoil held [CV/TLC in % = 70.9 - 4.58 x Pst(L) at 60% TLC, r= .82] as in normal subjects. Loss of elastic recoil thus accounted for the closure at high lung volume and recoil was markedly reduced (Pst(L) at 60% TLC more than 2SD below normal for age) in 11 of the 23 patients. However this loss of recoil per se did not account for the patients' low maximum expiratory flow rates since "upstream resistance" was high. We conclude that flow reduction in cystic fibrosis is primarily a manifestation of small airways disease but that the severe gas trapping is explained by loss of lung elastic recoil.

INDUCTION OF THE PULMONARY SURFACTANT IN THE FETAL PRIMATE BY THE INTRAUTERINE ADMINISTRATION OF CORTICOSTEROIDS. R.A. de Lemos* and G.W. McLaughlin*. Dept. of Pedicatrics, Wilford Hall USAF Medical Center and University of Texas Medical School, San Antonio, Texas. Intr. by M.J. Sweeney.

Twenty-one pregnant Rhesus monkeys between 80 and 101 days (gestation 155 +/- 3 days) were selected for study. Methylprednisolone 10 mg, dexamethasone 1.3 mg or normal saline was administered transabdominally into the fetal abdominal wall or peritoneum in three injections over a seven day period. The fetus was allowed to mature for an additional week and then delivered by hysterectomy. All fetal organs were weighed and fixed for pathologic examination. The right lung was analyzed for surface tension on a Wilhelmy balance. Adrenal weights were significantly less when related to body weight on all of the steroid treated animals (control 0.0007, methylprednisolone 0.0004, dexamethasone 0.0004). No gross or microscopic abnormalities were found in other organs except in two of the methylprednisolone treated fetuses where renal changes were detected. Pulmonary surface tensions were similar on the six animals under 100 days gestation. In those over 100 days surface tension was lowest in the dexamethasone treated monkeys (mean 12.4 dynes/cm^2), next lowest in the methylprednisolone treated animals (mean 17.2 dynes/cm^2) and highest in the controls (21.8 dynes/cm^2).

The administration of glucocorticoids to the fetal primate results in premature appearance of the pulmonary surfactant. There may be specificity for certain steroid configurations on eliciting this effect.

EVIDENCE OF LOWER AIRWAY OBSTRUCTION IN CHILDREN WITH HEART DISEASE, Etsuro K. Motoyama, Hiroshi Goto, Bernard Wu, Natalie de Leuchtenberg, (Intr. by C.D. Cook), Depts. Ped., Anesth. and Lung Research Ctr., Yale Sch. of Med., New Haven.

A total of 30 children (6-18 years) with heart disease were studied for evidence of airway obstruction. Using a body plethysmograph, measurements were made of vital capacity (VC), total lung capacity (TLC), functional residual capacity (FRC), FRC/TLC ratio, 1-sec. forced expiratory volume (FEV_1), and maximum expiratory flow rate ($\dot{V}max$) at 50% TLC. Previous studies (Pediatrics, 48:64, 1971) showed that $\dot{V}max$ is a highly sensitive test for detecting lower airway obstruction in children. In 15 children with increased pulmonary blood flow, $\dot{V}max$ was significantly decreased (i.e.> 2 SD below predicted values) in 3 and FEV_1, in 5. FRC/TLC was increased in 3 while VC and TLC were within normal limits in all. In 6 patients with mitral or aortic stenosis with increased left atrial pressure (mean: 21.1 mm Hg), $\dot{V}max$ was significantly decreased in all, FEV_1, in 5, VC and TLC, in 2. FRC/TLC was increased in 4. On the other hand, all of 9 children with limited pulmonary flow showed normal $\dot{V}max$, FEV_1 and FRC/TLC. There was a significant inverse correlation ($p< 0.05$) between $\dot{V}max$ and left atrial pressure. Lower airway obstruction often exists without clinical symptoms in children with pulmonary congestion and/or increased pulmonary flow. (Supported in part by NIH grants: USPHS HD-03119, HL14179).

ALTERATIONS OF PULMONARY PRESSURE-VOLUME RELATIONSHIPS IN THE PERINATAL PERIOD: PHYSIOLOGIC EVIDENCE FOR RELEASE OF SURFACTANT AT BIRTH. W.Taeusch,Jr., I.Wyszogrodski and M.E.Avery, McGill University-Montreal Children's Hospital Research Inst. & Dept. Physiol., McGill University, Montreal, Canada.

Gluck et al.,(Ped.Res. 1:237,1967) have reported biochemical data indicating increased amounts of surface active material in alveolar washes from newborn rabbits within 30 min of birth. Using pressure-volume relationships as an indicator of intra-alveolar surfactant,we have carried out quasistatic deflation pressure-volume curves on 46 rabbits of 28 days gestation after 30 minutes of life and compared these data with those of Kotas & Avery (J.Appl.Physiol.30:358,1971) who studied 18 fetal rabbits of identical gestational age prior to air breathing. At similar maximum inflation pressures, lungs of the animals that breathed were significantly more distensible, i.e. contained more air per gm of lung tissue. These changes were associated with a 19% decrease in lung water as measured by lung weight/body weight. Mean stability on deflation (volume expressed as a percentage of total lung capacity at a transthoracic pressure of 7 cm H_2O) was 3X greater in the animals that breathed for 30 minutes. In a separate series of 12 experiments, this measure of stability was found to be independent of changes in lung water.These data support the hypothesis that surfactant release into the alveolar space occurs within minutes of birth. Therefore disturbances of mechanisms controlling release as well as synthesis of surfactant may affect the respiratory status of newborn infants.

PULMONARY INACTIVATION OF PROSTAGLANDINS E_1 IN THE FETAL AND NEWBORN LAMB. Peter M. Olley, Flavio Coceani, and Geraldine Kent (Intr. by Dr. A. Sass-Kortsak).
The Hospital for Sick Children and The Research Institute. Toronto, Canada.

Inactivation of Prostaglandins E_1 (PGE_1) during passage through the pulmonary vascular bed was determined in 4 lambs and 6 fetal lambs (126-142 days gestation) with an intact placental circulation using the systemic vasodilator response technique. Graded doses of PGE_1 were infused proximally and distally to the pulmonary vascular bed for two minutes through catheters in the pulmonary artery (fetuses) or right atrium (lambs) and in the ascending aorta. In newborn and fetal lambs the ductus arteriosus was ligated prior to the test to prevent shunting. The fall in systolic pressure for each infusion was plotted against the log of the dose and a dose response curve obtained for each injection route. From the dose required to produce the same fall in systolic pressure through each route the percentage pulmonary inactivation was calculated. Inactivation varied between 56 and 98% (mean 77%). These values in the fetuses did not differ significantly from those obtained in the lambs under comparable experimental conditions. These experiments suggest that pulmonary inactivation of PGE_1 is well developed in the near term lamb and is uninfluenced by the low fetal PO_2.

POSITIVE END-EXPIRATORY PRESSURE BREATHING IN INDUCED HYALINE MEMBRANE DISEASE. Lars Victorin, Dan Lindstrom, Hakan Sundell, Alex Tsiantos, A.B. Brill, Mildred Stahlman. Depts. Pediatrics and Radiology, Vanderbilt Univ. Sch. Med., Nashville.

Because of interest in the direct effects of positive end-expiratory pressure (PEEP) on pulmonary ventilation ($\dot{V}$) and perfusion ($\dot{Q}$), the regional distribution of these functions in lambs with induced hyaline membrane disease (HMD) was studied. While premature lambs born by C-section from previously hypotensive ewes were being treated with PEEP, scintillation images of the lungs were recorded during ^{133}Xe washout. The relative $\dot{V}$ of each element of a 32 x 32 matrix of the image was computed yielding a distribution of $\dot{V}$ coefficients with a mean of 1.0 and a standard deviation proportional to the amount of regional variation of $\dot{V}$. Similarly, the regional variation of $\dot{Q}$ was determined from images of the lungs following injection of ^{99m}Tc labelled albumin microspheres into the RV. In 7 lambs, 2-5 complete studies were done with different pressures of 2-12 cm H_2O, 30 min apart. Despite progression of their HMD during the studies, the lambs' PO_2 levels rose and fell after airway pressures were raised and lowered, demonstrating effective application of the PEEP therapy. Regional variation of $\dot{V}$ decreased with increasing PEEP from 2 to 7 cm H_2O, but did not change with a further increase to 12 cm H_2O. Regional variation of $\dot{Q}$ also decreased with increasing airway pressure and this effect occurred over the entire range of pressures. These findings indicate that one effect of PEEP breathing in HMD is to reduce the regional variation of pulmonary $\dot{V}$ and $\dot{Q}$.

PULMONOLOGY

Read by Title

LUNG MECHANICS IN CONGENITAL HEART DISEASE WITH INCREASED AND DECREASED PULMONARY BLOOD FLOW. Eduardo H. Bancalari,* Otto L. Garcia,* Mary J. Jesse,* and Henry Gelband.*(Intr. by William W. Cleveland) Dept. of Pediatrics, Univ. of Miami, Sch. of Med., Miami, Florida.

In an attempt to determine the effect of pulmonary blood flow (PBF) on lung mechanics, we measured respiratory rate (RR) tidal volume (V_T), functional residual capacity (FRC), lung compliance (C_L) and specific C_L in 20 infants who underwent cardiac catheterization for congenital heart disease. 13 had cardiac lesions with increased PBF (group A) and 7 with decreased PBF (group B). All were less than 9 months of age and had the pulmonary studies performed within 24 hours of their cardiac catheterization. Mean values for pulmonary mechanics for group A infants were;RR 64/min, V_T 8.7 ml/kg, FRC 26 ml/kg, C_L 4.9 ml/cm H_2O, specific CL 45.6 ml/cm H_2O/L-FRC, while group B values were RR45/min, V_T 10 ml/kg, FRC 31 ml/kg, C_L 8 ml/cm H_2O and specific C_L 71 ml/cm H_2O/L-FRC. Mean pulmonary to systemic flow ratio was 3.0/1 in group A as compared to 0.76/1 in group B. Pulmonary hypertension was present in 11/12 group A patients. No correlation was found between C_L and left atrial pressure. In group A patients the decrease in specific C_L correlated with pulmonary artery pressure (r=-0.91) but not with PBF. The fact that the specific C_L is decreased in group A indicates that there are changes in the elastic properties of the lung and not a reduction in lung volume. Serial measurements of specific C_L may be a helpful non invasive technique to evaluate increasing pulmonary hypertension.

CYTOLOGICAL STAGING WITH OXYGEN THERAPY & ASSISTED VENTILATION Lorayne Barton, Gerrit D'Ablaing III and Betty Bernard. (Intr. by Paul F. Wehrle). USC School of Medicine, L.A. Calif.

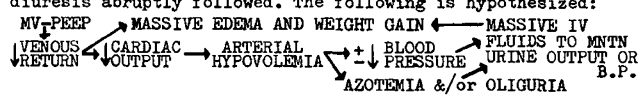
Twenty infants, 28-37 wks. gestation, requiring O_2 therapy & assisted ventilation, had daily tracheo-bronchial washings obtained for cytologic staging of broncho-pulmonary dysplasia as described by Northway & Rosan (Hosp.Prac., Jan.,1969). The infants ranged from 2 hrs. to 4 mos. when cytologic specimens were obtained. Initial indications for respiratory assistance were hyaline membrane disease (HMD) 14 pts., pneumonia & apnea with patent ductus arteriosus. Duration of O_2 & respirator care ranged from 1 day to 3 mos. Stage I was characterized by exfoliation of ciliated columnar epithelial cells of good morphology. Stage II revealed decreasing numbers of columnar cells exhibiting loss of cilia and nuclear degeneration (pyknosis). Stage III disclosed an inflammatory hyperplasia of parabasal cell morphology dominated by nuclei exhibiting marked vesiculation, prominent nucleoli & increased parachromatin. Stage IV of metaplasia to inflammatory atypia (dysplasia) was characterized by increased coarsening of nuclear chromatin & condensation of nuclear membrane. Twelve of 20 infants had <48 hrs. exposure to >60% O_2. All exhibited Stage I & II cytologically. Of these 12, 5 expired by the 4th day with severe HMD. 8 infants had >60% O_2 for 64-500 hrs. One (highest O_2 <80%) exhibited severe Stage III by 3 days of age reverting to Stage I by 5 days when respirator was discontinued. Upon reinstitution of respirator care for pneumonia on day 21, cytology was "normal". During next 4 days, Stage I-III developed again.

FURTHER IN VIVO MUCUS PENETRATION STUDIES OF INTRAMUSCULAR (I.M.) 7-CHLOROLINCOMYCIN (7-CLM) IN CYSTIC FIBROSIS (C/F). Robert A. Campbell, Julia L. Grach, Joan M.Crosby,Ann Marie Dolney,& Mary D.Willis, Dept.of Peds., Univ.of Oreg.Med.Sch., Portland. (Intr. by Richard L. Olmsted).

Type 1 Staphylococcus aureus (SA-I) are coagulase positive, strongly hemolytic and bright yellow. SA-I C/F chest burden was 10^7-10^8 per ml sputum in 12/14 patients. Eight days IM 7-CLM therapy resulted in elimination of SA-I organisms quantitatively determined in 4/12 SA-I strain sensitive patients. A decline of at least 2 orders of magnitude was seen in 10/12. Type II Staphylococcus aureus (SA-II) weakly hemolytic, light yellow and occasionally slow coagulase positive, chest burden SA-II 10^6-10^8, when found (7/14) could not be detected at end of 7-CLM Rx. in $10_7$of 14 patients, as expected. Hemophilus influenzae (HI) 10^1-10^9 per ml sputum showed little change with Rx. Pseudomonas aeruginosa (PA) smooth (S), rough (R) or mucoid (M) strains in a given case did not show an inverse relationship to the SA-I or SA-II changes. One patient in whom SA-I and SA-II were no longer detectable by day 4 of IM Rx. and in whose admitting sputum no PA were detected, S,R, & M strains of PA were demonstrated by day 8. Two weeks prior to hospitalization PA had been noted in his sputum. Two other patients who "developed" pseudomonas a. during Rx., upon review, had similar pseudomonas kinetics historically. In 7-CLM profoundly depresses or eliminates the Staphylococcal burden in the C/F chest. The IM route is well tolerated. No study patients deteriorated clinically with Rx.

CLINICAL EVIDENCE FOR HEMODYNAMIC TOXICITY OF PEEP. Morton L. Cohen, Univ. of California, San Diego, Dept. of Ped. (Intr. by William L. Nyhan)

In 1971, 49 neonates with the respiratory distress syndrome (RDS) were treated with mechanical ventilation with positive end-expiratory pressure (MV-PEEP). 29% survived. Among the 19 who died after 48 hours of age, 16 had marked edema with an average weight gain of 60 grams/day. There is evidence to suggest that MV-PEEP caused the fluid retention: (1) Weight gain and edema often correlated directly with the mean pressure applied to the airway. (2) In a few cases weight gain did not begin until lung compliance improved at 5-7 days of age. (3) All of the edematous babies required massive infusions of water and colloid to maintain urine output &/or blood pressure, and some developed azotemia &/or oliguria. 2 were thought to have primary renal disease because of enlarged kidneys and poor function on IVP, but both had normal kidneys at autopsy. (4) In the few cases in which PEEP was discontinued, massive diuresis abruptly followed. The following is hypothesized:



Since the recognition of this syndrome, we have successfully corrected renal failure and edema in 1 patient by removing the PEEP. He is the only baby who was taken off PEEP because of suspected hemodynamic toxicity, and the only baby with clinical evidence of hemodynamic toxicity who survived.

We conclude that MV-PEEP should be considered only in the RDS baby who has inadequate spontaneous respirations and who cannot be oxygenated without PEEP despite 100% O2 and maximum respirator settings.

CO2 RETENTION DURING CONTINUOUS POSITIVE AIRWAY PRESSURE. Morton L. Cohen, Dept. of Ped., Univ. of California, San Diego. (Intr. by William L. Nyhan)

In order to avoid using high oxygen concentrations and prevent atelectasis in the respiratory distress syndrome (RDS), continuous positive airway pressure (CPAP) was applied to neonates with mild hypoxemia (pO2≤40-50 in 40-50% O2). Of 60 RDS babies in 1971, 31 were tried on CPAP. Though oxygenation was often improved, most infants developed CO2 retention (mean pH 7.28→7.15; mean pCO2 43→62) on CPAP. Because the respiratory acidosis was felt to indicate worsening RDS rather than possible CPAP toxicity, 24 of the 31 babies progressed to mechanical ventilation with positive end-expiratory pressure (MV-PEEP) and only 4 were successfully treated with CPAP. In 3 cases, removing the CPAP and returning the baby to O2 corrected the respiratory acidosis. The overall survival rate on MV-PEEP was only 29%.

It is suggested that CO2 retention occurs in inverse proportion to the degree of hypoxemia, or, more likely, to the degree of loss of lung compliance. Thus CPAP should not be employed as an alternative to O2, nor as a method of saving O2, but rather is indicated only when the baby can no longer oxygenate despite maximum ambient O2 concentrations. In such patients subsequent CO2 retention probably means improving lungs rather than worsening of the disease, and the pCO2 can be used as a guide in determining when to lower or discontinue the CPAP. If oxygen is restricted and CPAP is applied too early, many babies may be unnecessarily subjected to mechanical ventilation. In this study, MV-PEEP could not be considered a reasonable alternative to CPAP since the survival rate was low.

EXERCISE-INDUCED PULMONARY HYPERINFLATION IN ASTHMATICS AND ITS REDUCTION BY ISOPROTERENOL AND CROMOGLYCATE.
Gerd J.A. Cropp. National Asthma Center. Denver, Colorado.

Many asthmatics develop exercise-induced bronchospasm; it is not recognized that they also often develop acute hyperinflation, which may lead to marked reductions in Vital Capacity (VC). We exercised 18 asthmatic children on a bicycle to heart rates above 170/min. and measured exercise-induced changes in Specific Airway Conductance (SG), Thoracic Gas Volume (TGV) and VC. Changes were considered insignificant when SG and VC were ≥75% and TGV≤125%, moderate when SG and VC were 50-74% and TGV 126-200% and marked when SG and VC were <50% and TGV>200% of pre-exercise values. The % of patients showing various changes in SG, TGV and VC are listed:

		No Δ	Moderate Δ	Marked Δ
SG	Reg. Treat. Only	33%	6%	61%
	Reg. Treat. + Isop.	34	33	33
	Reg. Treat. + Cromo.	23	29	47
TGV	Reg. Treat. Only	44	17	39
	Reg. Treat. + Isop.	44	56	0
	Reg. Treat. + Cromo.	47	41	12
VC	Reg. Treat. Only	44	34	22
	Reg. Treat. + Isop.	83	17	0
	Reg. Treat. + Cromo.	59	35	6

The results show that in asthmatics exercise frequently triggers bronchospasm, leading to hyperinflation and decreases in VC.

ACCURATE PO_2 VALUES OBTAINED FROM ARTERIALIZED EARLOBE BLOOD
by Richard H. Davis, Anthony V. Beran, and Stanley P. Galant (Intr. by Thos. L. Nelson) Dept. of Pediatrics, Univ. of California at Irvine.

The purpose of this study is to compare earlobe capillary blood gases in asthmatic patients with arterial values after the earlobe is made hyperemic with thurfyl nicotinate (Trafuril), a vasodilator derivative of nicotinic acid. Capillary blood gases have been investigated in the past and it is thought that poor peripheral perfusion may decrease their accuracy. However, they have not been evaluated in status asthmaticus. In our study Trafuril is applied to the earlobe approximately 15 minutes prior to sampling and the capillary blood is then compared with an arterial specimen drawn simultaneously. PO_2 values in our group of 10 patients with lower respiratory disease (primarily asthma) have a +0.97 correlation. CO_2 and pH correlations were also good. We have found this technique to be a safe and useful procedure particularly in small children. In the future it might be performed by nurses or technicians to provide the clinician with crucial information which is often not obtained because of the difficulty in getting an arterial specimen from the young patient.

EFFECT OF INCREASED OXYGEN-HEMOGLOBIN AFFINITY ON OXYGEN CONSUMPTION (V_{O2}) AND CARDIAC OUTPUT OF PIGLETS AND LAMBS FOLLOWING INHALATION OF CARBON MONOXIDE (CO).
M. Delivoria-Papadopoulos, J. H. Chen, J. M. Senior, F. A. Oski and R. E. Forster. University of Pennsylvania, School of Medicine, Philadelphia, and Upstate Medical Center, Syracuse, New York.

Previous studies have shown that increased blood carboxy hemoglobin levels of newborn infants shift the oxyhemoglobin equilibrium curve to the left. The present studies were designed to investigate the effect of this increased oxygen affinity in lambs and piglets. Measurements of (HbO_2), (HbCO) saturation, P_{50} (PO_2 for 50% HbO_2 saturation at pH 7.40), blood gases, and (V_{O2}) were obtained before and after blood HbCO was raised 10-15% by breathing a gas containing a known amount of CO. Arteriovenous oxygen content difference (AVD) and cardiac output were calculated. In 18 studies in lambs and 7 studies in piglets mean P_{50} decreased by 4 to 6 mm Hg., AVD decreased by 1.5-2.2 ml. O_2/100 ml. of blood, while total body V_{O2} increased by 1.4 to 2.2 ml.O_2/kg./min. respectively. Cardiac output increased from a control value of 170 to 294 ml./min./kg. in the lambs and 142 to 222 ml./min./kg. in the piglets. These studies inciate that a decreased P_{50} *in vivo* results in a lower mixed venous PO_2, and an increase in cardiac output; the latter presumably designed to raise mixed venous and therefore tissue PO_2 in compensation for the decreased P_{50}.

THE EFFECT OF INTRAVENOUS LIPIDS ON THE SWEAT SODIUM CONCENTRATION IN CYSTIC FIBROSIS.
R. B. Elliott (Intr. by D. O'Brien), School of Medicine, University of Auckland, Department of Paediatrics, Auckland, New Zealand.

Lung disease in essential fatty acid deficient chicks resembles that found in cystic fibrosis, and serum lipid changes of essential fatty acid deficiency have been previously described in cystic fibrosis.

Intravenous lipid emulsions containing soya oil and egg lecithin (1 g lipid/Kg body weight) given to seven children with cystic fibrosis lowered the sweat sodium concentration at a standardized sweat rate of 1.5 g/m^2/min. by a mean of 30% one week after the infusion. The effect appeared maximal 3 days after infusion and lasted for more than 30 days.

Prostaglandin $F_{2\alpha}$ administered by iontophoresis produced a similar but smaller effect, whereas prostaglandin E_2 produced variable results. It is tempting to hypothesize that provision of extra substrate for P.G. synthesis has effected the changes noted after lipid infusions.

No clinical alteration in the course of the disease was noted in an 8 year old cystic fibrosis patient given repeated lipid infusions, but three younger children have shown marked weight gains.

EFFECTS OF VITAMIN E DEFICIENCY IN PATIENTS WITH CYSTIC FIBROSIS (CF).
Philip M. Farrell, Robert E. Wood, and John G. Bieri (Intr. by Paul A. di Sant'Agnese), NIH, Bethesda, Md.

Protean manifestations of tocopherol deficiency occur in animals but man has not been shown to develop clinical symptoms. Patients with CF provide the best available models of chronic vit. E deficiency in humans. To evaluate the efficacy of vit. E therapy in CF, we have studied two disturbances found in animals: RBC hemolysis and muscle degeneration.

Plasma α-tocopherol (α-T), determined after thin layer chromatography, was uniformly decreased in 20 CF patients with pancreatic achylia (mean = 170 µg/100 ml, range = 23-410; control mean = 730 µg/100 ml, range = 550-960). Oral supplementation with water-miscible α-T, 3-5 I.U./kg/day, resulted in normal plasma levels, and in one case at autopsy normal liver and muscle concentrations of α-T. Vit. E deficiency was invariably accompanied by abnormal RBC hemolysis *in vitro* with H_2O_2. *In vivo*, however, ^{51}Cr-RBC survival determinations have shown no significant change from normal in 4 severely deficient patients (mean half-life 25 days). Creatinuria has been found in 5 of 8 patients thus far examined and correction of this abnormality occurs following α-T therapy. However, elevations in muscle-derived enzymes (e.g. serum creatine kinase), which are sensitive indicators of myopathy in animals, were absent in 25 tocopherol deficient patients.

Studies are continuing but preliminary conclusions are: 1) no major clinical effects of vit. E deficiency occur in CF, 2) the presence of creatinuria suggests that α-T supplementation is desirable.

THE EFFECT OF POSITION ON THE DISTRIBUTION OF PULMONARY BLOOD FLOW IN THE NEWBORN BABOON.
David E. Fisher, John B. Paton and Rosario F. Esperanza. (Intr. by Samuel P. Gotoff.) Abraham Lincoln Sch., Univ. of Ill. Coll. of Med., Dept. of Ped., Chicago.

Studies on adult animals and man indicate a significant positional effect on the distribution of pulmonary blood flow. Since ventilation/perfusion abnormalities are frequent in the neonatal period the effect of prone vs supine position on pulmonary blood flow was measured in 6 baboons in the first week of life to determine if one position provided more uniform perfusion of lung tissue.

Each animal was studied both prone and supine; 3 were initially studied prone and 3 were initially studied supine. Pulmonary and systemic distributions of blood flow were simultaneously determined in each position using 2 different radiolabelled 50µ microsphere injections each time.

The proportions of pulmonary blood flow were determined on divisions of lung tissue of approximately 1 cubic centimeter; the data from total lung were then reconstituted in three planes for analysis: apex to base, lateral to medial and anterior to posterior.

Analysis of these data showed no effect of position or the microsphere injections on acid-base, oxygenation status, cardiac output, % ductus arteriosus shunt in either direction or systemic distribution of blood flow. No positional difference of total pulmonary blood flow or its distribution in the planes described was demonstrated in this group of animals.

BLOOD GASES IN T AND A PATIENTS. Richard L. Fowler and Ravi Midha. Louisiana State University School of Medicine, New Orleans, Louisiana.

Cor pulmonale and pulmonary hypertension are known to result from chronic upper airway obstruction severe enough to produce hypoxia and respiratory acidosis. Since hypertrophy of adenoids and tonsils is a relatively common cause of such obstruction, prospective assessment of the incidence and severity of blood gas disturbances in children hospitalized for "routine" adenotonsillectomy was carried out in 52 children aged 19 months to 13 years. Pre and postoperative studies of arterial pH, pCO_2, and pO_2 were obtained together with chest x-rays and EKG's.

Arterial pH and pCO_2 showed no abnormalities and were unchanged by surgery. 13/52 (25%) had preoperative pO_2 values below 80 mm Hg. A rise of 20 mm Hg followed surgery in these children. Clinical evidence of pulmonary hypertension was not recognized in any of the patients although one child had a smaller heart, less evidence of RVH on EKG, and lower pCO_2 postoperatively.

	pH	pCO_2	pO_2	Axis	C/T
Preop	7.38-7.53(7.44)	26-42(35.5)	60-101(84.5)	+57.6°	48.2%
Postop	7.35-7.48(7.44)	29-45(35.5)	66-113(89.7)	+52.4°	47.5%

pO_2 below 80:
Preop 60-78(69); Postop 78-98(89)

Arterial blood gas studies may be helpful in selecting patients who will be benefitted by adenotonsillectomy.

CONTINUOUS POSITIVE AIRWAY PRESSURE APPLIED BY NOSEPIECE Lee I. Hoffman, A.G.M. Campbell, Etsuro K. Motoyama, Marcello M. Orzalesi (Intr. by Charles D. Cook), Dept. of Ped., Yale Univ. Sch. of Med., New Haven

Continuous positive airway pressure (CPAP) is an effective method of increasing PaO_2 in the respiratory distress syndrome (RDS) and various techniques for applying this pressure have been described. These techniques have certain disadvantages including those of endotracheal intubation, headbox noise and difficulty in maintaining pressure. An alternative method of delivering CPAP through a nosepiece was evaluated. Seven infants with clinical and radiographic evidence of RDS who met the criteria for nosepiece CPAP ($F_IO_2 > 0.60$ to maintain PaO_2 50-70 mm Hg) were treated. 4/7 infants (mean B.W. 1928 g., G.A. 33 wks.) showed a good response to nosepiece CPAP begun at a mean age of 22 hrs. They had a mean initial PaO_2 increase of 33 mm Hg and received CPAP for a mean duration of 49 hours. 3/7 infants (mean B.W. 2535 g., G.A. 35 wks.) in whom CPAP was begun at a mean age of 35 hrs. showed no significant improvement; two of these were subsequently treated with CPAP by endotracheal tube, one of whom required mechanical ventilation and was the only death. Nosepiece CPAP may be most effective when used early in the course of RDS and in the smaller infants in whom adequate positive pressure can be more easily maintained. Thus, CPAP by nosepiece can be used effectively without the hazards of the headbox and endotracheal intubation. It can also avoid or shorten the use of mechanical ventilation and function as an adjunct to the weaning process.

HYPOXIC APNEA AS THE CAUSE OF CRIB DEATH. Warren G. Guntheroth Univ of Wash Sch of Med, Dept of Ped, Seattle.

Although mild hypoxia stimulates respiration, moderate hypoxia (PaO2 below 50mm) has been shown to depress ventilation. If the PaO2 falls sufficiently, a positive feedback loop would be entered, with decreasing ventilation causing decreasing PaO2, etc. Since the infant normally functions dangerously close to this breakpoint, the cumulative effects of deep sleep and increased airway resistance with a respiratory infection might result in struggle-free apnea. In animal experiments on adult, anesthetized rats, dogs, and monkeys, we have shown by temporary airway occlusion the existance of an intermediate level of hypoxia which maintains apnea in a manner similar to the normal fetal state (fetal PaO2 ca 25mm). At very low levels of PaO2, gasping commences. With fiber optic oximetry, a single gasp can be shown to raise the oxygen saturation by 50% or more; if gasping continues at sufficient frequency and duration, regular respirations may resume, if the damage from hypoxia to the CNS is not too great. The neonate during the first month of life is well-equipped to survive anerobically, which may account for the curious absence of sudden infant death for the first month. In animals with increased intracranial pressure, gasping was found to be distinct from regular respirations, and appeared to be an integral part of the CNS ischemic pressor response. We suggest that this is the mechanism of the first breath after birth.

THE MOST RECENT SURVIVALSHIP OF PATIENTS WITH CYSTIC FIBROSIS (CF). Nancy N. Huang, Lourdes Laraya-Cuasay, Shirley Braverman, Judy Palmer, Clarito Panganiban. Temple Univ. Sch. of Med., St. Christopher's Hosp. for Children, Dept. of Ped., Philadelphia, Pa.

An earlier publication presented the 5-year cumulative survival experience of patients with CF admitted during three 5-year periods from July 1952 to June 1967.(Amer J Dis Child 120: 289, 1970). Since 1967, in addition to the usual emphasis on bronchial drainage, antibiotic therapy and supervised home care program, more agressive measures have been instituted in the management of respiratory infections and respiratory insufficiency. These consist of intensive antibiotic therapy by parenteral routes, tracheo-bronchial lavage and ventilatory assistance (the latter for infants and young children only). These measures have markedly reduced infant mortality and prolonged the life expectancy of older children. Survival rates of patients admitted in July 1967 - June 1972 are now available. The 5-year cumulative survival rates showed 35.0% $\pm$ 8.7% for 1952-1957 (I), 63.6% $\pm$ 6.4% for 1957-1962 (II), 76.6% $\pm$ 7.0% for 1962-1967 (III), and 91.6% $\pm$ 3.0% for 1967-1972 (IV). The difference between period III and period IV was significant on statistical analysis. Thus, the outlook for patients with CF is improving.

ACUTE BRONCHIAL ASTHMA AND POSSIBLE INAPPROPRIATE SECRETION OF ANTIDIURETIC HORMONE. Ralph W. Higer and Malcolm A. Holliday. Kauikeolani Children's Hospital, Dept. of Ped., Honolulu, and Univ. of California, San Francisco, Dept. of Ped.

The frequency of hyponatremia in asthmatic children was determined by comparing the serum and urine osmolality of 25 children seen in the emergency room with acute bronchial asthma with another group of 25 children who presented for routine physical examination or for admittance for elective surgery. Five of the 25 children with asthma had Na_S < 133 meq/L and were excreting concentrated urine (U/P_{osm} > 1.5) compared with none for the controls. Both groups had children excreting concentrated urine with Na_S < 138 meq/L. Considering these findings, it is thought that asthmatic children may excrete antidiuretic hormone independent of water ingestion and hypoosmolality. The evidence is sufficient to alert physicians to the advisability of monitoring Na_S in children with acute bronchial asthma and the need of limiting the intake of water in those patients with hyponatremia.

CONTINUOUS MASS SPECTROMETER ANALYSIS OF PULMONARY FUNCTION IN NEONATES, Carl E. Hunt, Sadi Matalon, O. Douglas Wangensteen, Arnold S. Leonard, (Int. by Russell V. Lucas, Jr.) Dept. Ped., Physiol. and Surg., Univ. of Minnesota Hosp., Minneapolis.

A pulmonary function analyzer (PFA-5) has been developed for analysis of respiratory and blood gases. The PFA-5 includes a quadrupole mass spectrometer, flow meter, analog computer for calculation of O_2 consumption and CO_2 production, and has been interfaced with a Honeywell D.D.P. 516 Digital Computer. The PFA-5 allows for simultaneous, continuous measurement of alveolar-arterial gradients (AaD) of any 5 gases in patients of any age or clinical condition and of flow measurements in anyone intubated or cooperative enough to accept a mouthpiece for 1-3 minutes. Ventilation-perfusion ($\dot{V}A/\dot{Q}$) inequalities have been assessed by serial measurements of $AaDCO_2$ and $AaDO_2$ in 100 neonates with pulmonary disease, including 70 with RDS, 40 of whom required continuous positive pressure ventilation (PEEP). $AaDCO_2$ values have ranged from 0-27 mm Hg in non-ventilated RDS and from 0-47 in patients requiring PEEP. Infants dying of progressive bronchopulmonary dysplasia have had $AaDCO_2$ levels up to 60 mm Hg. Initial $AaDO_2$ levels were as high as 330 mm Hg in RDS patients not requiring assisted ventilation and up to 608 mm Hg in those requiring PEEP. For each level of assisted ventilation, $AaDCO_2$ and $AaDO_2$ provide an objective measurement of severity of the clinical disease. Sequential measurements of $\dot{V}A/\dot{Q}$ ($AaDCO_2$, $AaDO_2$, and $AaDN_2$) in RDS are helpful in defining effect of the various therapeutic interventions and the potential for pulmonary survival.

EFFECT OF PROLONGED OXYGEN EXPOSURE ON PULMONARY SURFACTANT. W.N. Keidel, L. Gluck, M.V. Kulovich, M.J. Westberg. 97th Gen. Hosp. (Germany) & Univ. of Cal., San Diego, La Jolla.

Pathogen free rats exposed to 90% O_2 at one atmosphere for 75 hours had no respiratory distress until day 3 of exposure. Shown below, lungs of study rats were 2.3 x heavier than controls with significant increase in total lipid of lung tissue and alveolar wash (AW). Phospholipids (PL) comprised 69% of AW lipids in control rats and 34.5% of O_2-exposed rats. Significant changes occurred in composition of AW PL but lung tissue PL were relatively unchanged. Prolonged O_2 exposure produced major changes in lipid composition of pulmonary surfactant. Further study is needed to determine if these changes are produced by disordered lipid metabolism and/or pulmonary edema and cell death.

	Body Wt (gm)	Lung Wt (gm)	AW Lipids (mg)	Tissue Lipids (mg)
Study	286.5 ± 14.9	3.7 ± 0.6	4.8 ± 1.2	77.4 ± 7.5
Control	292.0 ± 6.0	1.6 ± .3	1.9 ± .5	47.9 ± 3.7
	AW PL (mg)	% Lec	% Sph	% PDME	% PE
Study	1.6 ± .5	59 ± 14	7 ± 5	21 ± 5	13 ± 6
Control	1.4 ± .2	93 ± 4	6 ± 5	tr	tr
	Tissue PL(mg)	% Lec	% Sph	% PDME	% PE
Study	59.5 ± 11.8	65 ± 4	11 ± 3	tr	23 ± 6
Control	33.2 ± 3.6	49 ± 4	23 ± 3	tr	38 ± 5

Supported by SCOR grant USPHS HL-14169

PILOCARPINE INDUCED PULMONARY ALVEOLAR HEMORRHAGE. Robert V. Kotas and Elizabeth J. Trainor, W. K. Warren Medical Research Center and Children's Memorial Hospital, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma. (Introduced by Harris D. Riley, Jr.)

Intra-alveolar hemorrhage followed bronchoconstriction produced by treating neonatal rabbits with pilocarpine (PC). Within three hours of birth, twelve weight-matched littermate rabbit pairs (LMP) were acclimatized for two hours in a warmed chamber (38°C) maintained at 80% oxygen concentration with 15 liters/minute gas flow through the chamber. After two hours of exposure, one newborn of each LMP was given 5 mg pilocarpine nitrate subcutaneously while the other member of the LMP was given an equal volume of saline. Two hours later, the animals were sacrificed, the lungs excised and weighed, and the left upper lobes fixed immediately in 2% paraformaldehyde. The rest of the lungs were dried in a lyophilizer. The lungs of the pilocarpine treated neonates (PC) were significantly heavier than controls (LC); dry weight x 1000 body/weight (DW/BW) $\bar{m}$ = 2.8 ± 0.18 S.D., LC $\bar{m}$ = 3.27 ± 0.57, P < 0.001. There were no significant differences in water content; PC $\bar{m}$ = 82.95 ± 0.9, LC $\bar{m}$ = 83.33 ± 1.25. Large areas of hemorrhage were seen in the PC on gross inspection. On light microscopy, PC had between 42% and 65% of their alveoli filled with red blood cells (RBC) compared to none in LC. We suggest that the absence of collateral ventilation in the newborn predisposes to pulmonary hemorrhage if the infant develops bronchoconstriction while breathing elevated concentrations of oxygen.

Bronchoscopy and Bronchial Lavage (BBL) Relieving Respiratory Distress in Cystic Fibrosis (C/F) Patients. L.Kulczycki, P. Altman, J. McClenathan, J. Randolph (Intr. by S. Leikin). George Washington & Georgetown Univ., and Children's Hosp.DC

During 1965-66, BBL was introduced at this hospital as an emergency measure in the treatment of C/F patients, who had advanced stages of the pulmonary disease not responding to usual measures of therapy. Blood gases indicated progressive hypoxia and hypercarbnia with alarming clinical cyanosis, dyspnea and fatal outlook. Treated patients were catagorized as emergency or elective cases. Below is a summary of BBL: (done 1965-1972)

	TOTALS		EMERGENCY		ELECTIVE	
AGE GROUP	No.Pts.	No.BBL	No.Pts.	No.BBL	No.Pts.	No.BBL
Below 1 yr.	20	20	15	19	7	11
1-5 yr.	29	45	9	20	21	25
6-10 yr.	51	68	9	12	44	56
11-15 yr.	34	52	9	17	25	35
16-30 yr.	28	42	10	14	22	28
TOTALS	162	237	52	82	119	155

In the emergency group, 27 patients improved, and 25 patients expired after a period of several weeks to eight years. In the elective group, majority of patients have had multiple BBL, and all improved. The above results suggest that BBL, when skillfully done promptly relieves respiratory distress in C/F patients. It's effect is greater in the younger age group and depends on the degree of pathology already existing in the lung, and additional supportive measures used prudently on a long term basis.

A METHOD FOR MEASUREMENT OF CONTINUOUS OXYGEN CONSUMPTION, CARBON DIOXIDE PRODUCTION AND RESPIRATORY EXCHANGE RATIO. George Lister, Jr.* and Julien I. E. Hoffman, Dept. of Ped. at Yale Univ. Sch. of Med. New Haven, Conn. and Univ. of California, San Francisco, Cal.

Oxygen consumption ($\dot{V}O_2$) is useful clinically for assessment of metabolic status and determination of blood flow by the Fick method. Because it is difficult to measure, particularly in infants, $\dot{V}O_2$ is often estimated from tables. This may lead to large errors in flow calculations, especially in sick children. An apparatus based on the flow-through principle was designed to measure $\dot{V}O_2$ continuously in infants and children. A paramagnetic analyzer was used to determine oxygen concentrations. Mixed expired gas was also sampled to obtain carbon dioxide concentration, thereby giving carbon dioxide production, respiratory exchange ratio, and corrected $\dot{V}O_2$. Ethyl alcohol tests (r=.987) and expired gas collections with Douglas Bag (r=.933) were performed to evaluate the accuracy and reproducibility of the system. Standard deviation for measurements of a known $\dot{V}O_2$ was 4.2% of the mean. $\dot{V}O_2$ in 15 infants between 2 days and 8 months measured at the time of cardiac catheterization was 150±25 ml/min/M^2. This system has proven to be simple, inexpensive, portable, and stable, permitting $\dot{V}O_2$ to be easily obtained during both steady and unsteady states.

THE MECHANICAL EFFECTS OF BRONCHIAL PROVOCATION IN ASTHMATIC CHILDREN. Anthony Mansell*, Chagai Dubrawsky*, Henry Levison, Howard Langer*, A. Charles Bryan*, and Robert Orange*. Dept. of Peds., Research Inst., Hosp. for Sick Children, Univ. of Toronto.

Attacks of asthma in some children progress rapidly to a stage which is refractory to bronchodilators and it is probable that mechanisms other than bronchoconstriction are important in the gas trapping and flow reduction of acute asthma. To investigate these mechanisms we studied lung mechanics during induced attacks of asthma in 11 children. In each child we used a nebulized allergen which had previously provoked asthma and measured static elastic recoil, flow-volume relationships, airways resistance and static lung volumes. There were two basic patterns of reaction. One group had marked losses of static compliance with apparent gas trapping at high lung volumes. The other had equivalent increase in airways resistance but no losses of static compliance.

Number of Patients	5	6
% Change in Static Compliance	-33±11	- 2± 5
% Change in Conductance	-73±15	-52±18
% Change in Flow Rate at 50% Vital Capacity	-67±15	-38±15

Gas trapping is difficult to explain on the basis of bronchoconstriction alone and we suggest rapid formation of mucosal edema and transudation of fluid as an additional mechanism.

DESQUAMATIVE INTERSTITIAL PNEUMONIA IN CHILDREN: A RETROSPECTIVE STUDY. Donald G. Norris, Edward C. Rosenow III, Edgar G. Harrison, Jr., and Edward J. O'Connell (Intr. by Gunnar B. Stickler). Mayo Clinic and Mayo Fndn., Rochester, Minnesota.

The first reported series of desquamative interstitial pneumonia (DIP) included one adolescent, age 16 (Liebow, 1965); and since then 11 cases in children less than 15 years old have been reported. DIP is characterized histologically by intra-alveolar desquamation of pneumocytes with minimal fibrosis, and therapeutically by clinical improvement with corticosteroid therapy. It has been suggested that prognosis in this type of interstitial pneumonia is better than in others. Records of more than 2,500 children (aged less than 15 years) with pneumonia were reviewed to learn how many had had histologic evidence of DIP and to evaluate their response to therapy. Eight cases were found. Four of the patients were less than 1 year of age at diagnosis and four were between 4 and 11 years. Presenting complaints were pulmonary symptoms in four cases, congestive failure in two, and growth failure and glomerulonephritis in one each. All received therapeutic doses of steroid, but survival varied. Four patients are alive, 2, 3, 12, and 16 years after diagnosis. One is still receiving steroids on alternate days, 2 years after diagnosis; and one is taking prednisone and cyclophosphamide (Cytoxan) 3 years after diagnosis. The other four patients died 3 weeks to 8 months after the start of treatment. These results suggest doubt of the overall benefit of steroid therapy alone.

EFFECTS OF STEROIDS AND OXYGEN IN EXPERIMENTAL KEROSENE INGESTION. Denise M. O'Connell, Edward B. Lewin, and Gary L. Huber (Intr. by Jerome O. Klein). Channing and Thorndike Mem. Lab., Harvard Med. Unit, Boston City Hospital, Boston.

Kerosene ingestion is a common cause of accidental poisoning in children. Kerosene ingestion (10ml/kg) depressed the intrapulmonary inactivation of an aerosolized challenge of radiolabeled (^{32}P) S. aureus. Four hours after exposure to the bacteria, control mice inactivated 86.3% of the staphylococcal challenge. Kerosene ingestion resulted in depression of host defenses, with 72.4% inactivation at 4 hours and bacterial replication exceeding inactivation at 24 hours after ingestion. Administration of methylprednisolone succinate (1mg or 5mg/kg) had no beneficial effect on bacterial inactivation, fluid accumulation or histologic appearance of the lungs. Administration of massive doses of steroid (50mg/kg) accentuated the impairment of host defenses, and rendered animals more susceptible to infection. Administration of 100% oxygen had no significant beneficial or adverse effect on kerosene treated animals. Pulmonary histology and wet and dry lung weights revealed a chemical pneumonitis, with alveolar hemorrhage, bronchial necrosis, and pulmonary edema. These studies demonstrate a substantial and progressive depression of pulmonary antibacterial defenses following ingestion of kerosene, with no beneficial effect demonstrable with pharmacologic doses of steroids or 100% oxygen. Administration of massive doses of steroids enhanced the toxicity of kerosene.

CONTINUOUS PRESSURE BREATHING IN RDS: COMPARATIVE TRIAL OF 3 METHODS. Anthony Olinsky, S. Brock MacMurray, Paul R. Swyer Dept. of Paediatrics, Univ. of Toronto and the Research Inst., The Hospital for Sick Children, Toronto, Canada.

The efficacy was compared of 3 modes of continuous pressure breathing (CPB) in RDS, continuous positive airway pressure by naso-tracheal tube (NTT-CPAP), by face mask (mask CPAP) and continuous negative pressure by body box (CNP).

	Mask CPAP	NTT-CPAP	CNP
No.	45	33	20
Mean birth weight	1.86	1.54	1.87
Mortality %	31	33	30

50% of infants in each group subsequently required IPPV. Increase in P_aO_2 was similar in the 3 groups (mean 32 mmHg). The duration of CPB in the non ventilated survivors was longer in the NTT-CPAP group (100±50 hrs.) than mask (45±25 hrs.) $P < .01$ or CNP (52 ± 22 hrs.) $P < .02$. The increased duration could be related to the lower mean birth weight, but it was not associated with increased mortality, lower mean age on admission (7 hrs.) or an increased duration of $F_{IO_2} > .6$ (mean 22 hrs.) which were similar in the 3 groups. Complications were similar in each group. Pneumothorax 9%, intracranial haemorrhage 23%, pulmonary haemorrhage 10%, sepsis 9% and PDA 10%. The use of CPB has resulted in a 10% reduction in mortality. The duration of $F_{IO_2} > .6$ has been reduced from a mean of 84 hours in 1968 using IPPV alone to 22 hours and there has been a decrease in the incidence of bronchopulmonary dysplasia.

EARLY TREATMENT IN CYSTIC FIBROSIS: A SIBSHIP STUDY David M. Orenstein, Thomas F. Boat, Edward L. Charnock, Carl F. Doershuk, Robert C. Stern, Arthur S. Tucker, LeRoy W. Matthews. C.W.R.U. School of Medicine, Department of Pediatrics, Cleveland, Ohio.

It has been assumed but not well documented that the improved prognosis in cystic fibrosis (CF) is related to earlier diagnosis and vigorous therapy. Therefore, we have compared the effects of early versus delayed institution of a standard therapeutic regimen on the course of 25 pairs of CF siblings. Sibling pairs were studied to minimize variability in genetics, environment and home therapy. All younger siblings had started on therapy before 1 year of age and older siblings at an average age of 2 8/12 years. When compared at age 7 years, the younger siblings did significantly better than their older siblings with regard to the following: chest xray score ($p<.005$), total clinical score ($p<.005$), residual volume/total lung capacity ratio (RV/TLC) ($p<.05$), and weight percentile ($p<.05$). The younger siblings also had lower residual volume (RV) and higher height percentile but p values were only <.1 and <.15, respectively. There was no significant difference for other pulmonary tests. Of the younger siblings, 72% had a higher total score than their older sibling, 72% had a higher chest xray score, 70% had a lower RV/TLC ratio, 67% had a higher vital capacity (VC), 68% were in a higher weight percentile and 76% were in a higher height percentile. These data indicate that early institution of treatment significantly reduces morbidity at age 7 years in patients with CF.

LONG TERM PROGNOSIS FOR SURVIVORS OF THE WILSON-MIKITY SYNDROME. Eugene W. Outerbridge, Barry D. Fletcher and Leo Stern. McGill Univ.-Montreal Children's Hosp. Research Inst., Montreal, Quebec.

Of 29 infants diagnosed in the neonatal period as having the Wilson-Mikity Syndrome, 1 has subsequently died at 3 months of age of his pulmonary disease, and 2 others have died of unrelated causes. Follow-up information is available on 24 of the 26 survivors from 6 months to 8 years of age. Six children had clinical findings in the neonatal period of a patent ductus arteriosus, confirmed in 4 either by catheterization, dye curves or at time of surgical intervention in 2. Thirteen (54%) have had clinically and radiographically documented subsequent lower respiratory tract illness of a bronchiolitic type, requiring hospitalization in 10. Radiographic evaluation shows persistent abnormalities in 16 (66%) which were present even in 4 of the 5 children older than 7 years of age. These consisted of linear streaking, usually in the upper lobes. One child is a spastic diplegic, another a spastic quadriplegic severely developmentally retarded and both have been institutionalized. These latter neurological sequelae are more likely to be a consequence of the severe degree of immaturity rather than the pulmonary disease itself. Three children are hyperactive. One child has a seizure disorder, another is deaf. Two children have cicatricial retrolental fibroplasia. Fifteen (62%) have normal neurological examinations. The mean I.Q. for the 6 who are over 5 years of age is 100.6 (range 84-132).

ALPHA-1-ANTITRYPSIN DEFICIENCY IN THE WILSON-MIKITY SYNDROME. Eugene W. Outerbridge, George Chan, Ruth Russell and Leo Stern. McGill Univ.-Montreal Children's Hosp. Research Inst., Montreal, Quebec.

The Wilson-Mikity Syndrome is characterized by pulmonary hyperaeration, focal initially and then diffuse in distribution. Of 24 children who have survived the syndrome and been seen in follow-up, 10 have had subsequent admissions to hospital for lower respiratory tract illness, most frequently clinically of a bronchiolitic type. To determine if deficiency of alpha-1-antitrypsin might play an etiologic role in either the Wilson-Mikity Syndrome itself or in the later respiratory illness, we have measured the serum trypsin inhibitor capacity (TIC) of 12 of these children. The following results were obtained.

Patient	TIC	Patient	TIC	Patient	TIC	Patient	TIC
1	1.09	4	0.94	7	0.85	10	0.46
2	1.07	5	0.94	8	0.84	11	0.58
3	1.09	6	0.98	9	0.67	12	0.67

Values for TIC obtained in 15 normal controls ranged between 0.85 and 1.36 (mean 1.07) mg. trypsin to inhibit 1 ml. serum. The mean value for TIC in the 12 patients is 84.8 mg. Four children fall into the heterozygous range of 0.4 to 0.8. While none are homozygous deficient, an increased predisposition to obstructive lung disease has been reported among heterozygotes. It is suggested that this relative deficiency may play an etiologic role in the syndrome, or in the later respiratory illness.

PERIODIC BREATHING AND HYPOXIA: FURTHER EVIDENCE THAT LOW ARTERIAL PO2 UNDERLIES RESPIRATORY PERIODICITY IN THE PRETERM INFANTS. Henrique Rigatto, June Brady, Fe Dumpit. (Intr. by Victor Chernick). Dept. of Pediat., Univ. of Manitoba and Cardiovasc. Res. Inst., Univ. of California, San Francisco.

We tested the hypothesis that low arterial PO2 (PaO2) underlies periodic breathing in preterm infants. Fifteen babies (b.w. 1-2 kg) were studied during the first 30 days of life. They were given 21, 15 then 21% O2 to breathe for 5 min each (n=30) or air then 100% O2 for 5 min each (n=65). We related the incidence of periodic breathing to arterialized PO2 during air breathing and used the magnitude of the immediate changes in ventilation during 15% or 100% O2 breathing to test peripheral chemoreceptor function. No baby breathed periodically in the first 4 days of life. During this period PaO2 was similar in babies who later had periodic breathing & in those who did not ($p>.4$). After the 4th day of life PaO2 was always lower in babies breathing periodically ($p<0.01$) and the lowest PaO2 coincided with the highest incidence of periodic breathing. When 100% O2 was substituted for air, the immediate decrease in ventilation was greater ($p<.05$) in babies breathing periodically. When breathing 15% O2 the immediate increase in ventilation was greater at 1 min ($p<.05$) during periodic breathing. These findings suggest that low PaO2 underlies periodic breathing in preterm infants. The ventilatory response to high and low O2 suggests greater contribution of peripheral chemoreceptors to respiration in these infants.

ACID-BASE ALTERATIONS ASSOCIATED WITH PNEUMONIA IN INFANCY AND CHILDHOOD. Shyamal K. Sanyal, A.M. Berry and S. Madhavan (Intr. by Donald Pinkel). Dept. of Pediatrics, Safdar-Jung Hospital, New Delhi, India, and St. Jude Children's Research Hosp., Memphis, Tenn.

There is little data published regarding acid-base alterations during pneumonia in infancy and childhood. This prospective study was done to analyze the acid-base profile in 33 children who had unequivocal clinical and radiological evidence of pneumonia. The age range of the subjects varied between 1 month and 10 years. An arterial blood sample was obtained anerobically prior to start of therapy and analyzed for pH, pCO_2, pO_2 and bicarbonate. The tests were repeated at 24- and 72-hour intervals and compared to data from normal children.

In patients with pneumonia, the initial mean values for pH, pCO_2 and bicarbonate were 7.31 ± 0.072, 42.6 ± 6.74 and 21.86 ± 4.17 as compared to 7.42 ± 0.004, 31.5 ± 3.66 and 26.13 ± 1.14 in the controls ($p < 0.001$). Acidosis was observed in 27 (81%). The acidosis was of the respiratory type in 16 (uncompensated 9, compensated 7), metabolic in 6 and of mixed variety in 6. At the end of 72 hours, when the patients were clinically improved, the acid-base profile remained significantly abnormal ($p < 0.001$). Acidosis persisted in 19 (67%), metabolic in 1 and respiratory in 18 (uncompensated 6, and compensated 12). Three patients expired, each with progressive acidosis, respiratory and/or metabolic.

We conclude that pneumonia in infancy and childhood results in significant acid-base alterations which may persist in the face of clinical improvement. Early recognition and treatment may be important in preventing a fatal outcome.

PATHOPHYSIOLOGY OF MEDIASTINAL SHIFT IN INFANTS. Daniel C. Shannon, I.David Todres. Harvard Medical School.Massachusetts General Hospital,Children's Service, Boston, Massachusetts.

Regional lung function was measured in 8 infants to determine the pathophysiology of mediastinal shift. Regional ventilation ($\dot{V}$) and blood flow ($\dot{Q}$) were measured following intravenous bolus injection of 0.3 millicurie Xenon-133 using a 4-detector system. Normal $\dot{V}$=90% washout in 30 sec after peak activity and normal $\dot{Q}$=25± 5% per region. In 2 newborns, $\dot{Q}$ to one lung was 16% with normal $\dot{V}$ indicating primary increase in regional pulmonary vascular resistance and suggesting pulmonary artery hypoplasia. In 6, $\dot{V}$ was 35% washout in 30 secs.with only mildly reduced $\dot{Q}$ indicating either primary increase in airway resistance or decrease in compliance. In 4 of these 6, the radiographic diagnosis was lobar emphysema which was treated without surgery. The physiologic deficit resolved over several days. In one, poor $\dot{V}$ in the entire R lung lead to bronchoscopy and removal of a peanut. In one, poor $\dot{V}$ in the entire lung failed to respond to therapy.

This technique provides rapid, safe, bedside quantitation of regional lung function in infants and can help differentiate the causes of mediastinal shift and guide subsequent therapy.

ASTHMA IN CHILDREN IN THE BRONX: INCREASED INCIDENCE AND DEMOGRAPHIC CHARACTERISTICS. Alan Schmerler and Mark Abramowicz, Albert Einstein College of Medicine, Bronx Municipal Hospital Center, Department of Pediatrics, New York.

The number of children admitted to the Bronx Municipal Hospital Center with the diagnosis of bronchial asthma has risen sharply over the last 10 years:

62-63	63-64	64-65	65-66	66-67	67-68	68-69	69-70	70-71	71-72
15	23	30	44	61	117	151	177	228	228

This 15 fold increase in asthma admissions occurred in a period when the number of pediatric admissions fluctuated between 2300 and 3300 per year. Although there was a marked increase in the number of very young children (less than 2 years) with severe asthma, the relative age distribution of pediatric asthma admissions did not change over the 10 year period.

Fifty patients with asthma seen in the emergency room were compared with controls (the next patient seen by the same doctor). Asthmatic and control populations were different in sex distribution (3:1 males among asthmatics) and average age (asthmatic mean 8.1 years compared to control mean of 4.9 years). They were alike in ethnic composition: there were no more Puerto Ricans or black Americans in the asthmatic population than there were among controls. Asthmatics and controls were also alike in birth order, family size, and socioeconomic status (measured by dividing the number of rooms in these dwellings by the number of people living in them.

IN VIVO CONTINUOUS MONITORING OF INTRA-ARTERIAL OXYGEN. Arnold W. Strauss, Marilyn B. Escobedo, David Goldring and Richard E. Marshall. Washington University Medical School, Department of Pediatrics, St. Louis Children's Hospital, St. Louis, Missouri.

The accuracy and complication rate of a disposable indwelling umbilical artery (UA) electrode for continuous monitoring of pO_2 were evaluated. Alternate infants requiring PaO_2 monitoring and UA catheters were assigned to a test group (13) and a control group UA catheters only (10). Over 800 hours of electrode use with 196 measurements of PaO_2 were compared with the bench PaO_2 values and showed a standard error of 17 mm Hg. No significant differences were observed between the two groups in 42 blood cultures, 83 blood pressures (Doppler) and mean UA pressure by manometer and 15 pull-out aortograms. No morbidity related to catheters occurred in either group. Four electrodes were unreliable (results not included) and poor engineering design made the use of 4 electrodes impossible. The continuous monitoring electrode demonstrated its safety and utility; its reliability in measuring pO_2; the reduction of blood loss (no sampling); decreased handling of infants; and instantaneous knowledge of changes in PaO_2 because of changing cardiorespiratory status or by institution of therapy.

OBSERVATIONS RE: TYPE OF VENTILATORY SUPPORT FOR RESPIRATORY FAILURE (RF) IN RESPIRATORY DISTRESS SYNDROME (RDS). Mohamad R. Sedaghatian, Braden E. Griffin, Iraj Farzaneh, Montgomery C. Hart, Belton P. Meyer, and William J. Daily. Good Samaritan and St. Joseph's Hosps., Phoenix.

Gregory et al.(APS,1970) and Chernick et al.(APS,1971) reported favorable early experience with continuous pressure ventilatory techniques in neonates with RDS and RF. The role of different ventilatory techniques in RDS with RF has not been defined re: birth weight (BW), age at which ventilation is required (AV) and survival. Since 1970, all 190 infants with RDS and RF meeting the criteria of Cave et al.(APS,1968) admitted to two regional newborn centers have received ventilatory support. Experience with these 190 infants re: type of ventilatory support required, BW, AV, and survival is presented below.

	Vent by Method		Alive		BW <1500 gms		AV <12 hrs.	
Method	#	%	#	%	# Vent	#Alive	#Vent	# Alive
CPAP Only	28/190	15	23/28	82	8/70	3/8	16/90	12/16
CPAP+IPPV <12 hrs.	24/190	13	23/24	96	3/70	3/3	6/90	6/6
CPAP+IPPV >12 hrs.	90/190	47	59/88	67	35/70	15/35	33/90	20/33
IPPV Only	48/190	25	2/50	4	24/70	0/24	35/90	2/35

107/190 (56%) of the infants survived. None of 14 infants, BW<1000 gms, AV<24 hrs survived. Our data suggest that CPAP is a valuable adjunct to ventilatory support for RF with RDS. However, only 15% of allinfants ventilated and 11% of infants BW<1500 gms could be maintained using this technique alone.

VENTILATION AND LUNG PERFUSION AFTER INTRALIPID INJECTION IN NEWBORN LAMBS. Lars Victorin, Dan Lindstrom, Albert Otten, A.B. Brill, Mildred Stahlman, H.C. Meng. Depts. Pediatrics, Radiology and Physiology, Vanderbilt Univ. Sch. Med.,Nashville.

Infusion of Intralipid in adult man has been shown to decrease pulmonary diffusion capacity. Because of interest in using Intralipid as a source of calories in prematures, especially those chronically on respirators, the effects of a bolus injection of 0.5 gm Intralipid/kg was studied in 9 lambs of <1 week. Triglycerides, phospholipids, glucose, pH, PCO_2, PO_2 and aortic and RV pressures were monitored. Scintillation images of the lungs were recorded during ^{133}Xe washout and following the injection of ^{99m}Tc labelled albumin microspheres into the RV. From the ^{133}Xe washout phase, the relative ventilation ($\dot{V}$) of each element of a 32 x 32 cm matrix of the image was computed yielding a distribution of $\dot{V}$ coefficients with a mean of 1.0 and a standard deviation proportional to the amount of regional variation of $\dot{V}$. Similarly, the regional variation of perfusion ($\dot{Q}$) was determined from images recorded following the injection of microspheres. Total lipids peaked immediately after injection with a half life of 20 min. No change in RV or aortic pressure, glucose, pH or PCO_2 was noted after injection. However, PO_2 dropped a mean of 10 mm Hg at 15 min (range 0-23 mm Hg), returning after 30 min. No change in regional variation of $\dot{V}$ or $\dot{Q}$ was noted. It is suggested that the drop in arterial PO_2 corresponding to high level of total serum lipids may reflect either diffusion disturbance or increased peripheral oxygen utilization during metabolism of the fat injected.

A COMPUTER PROGRAM WHICH INTERPRETS ARTERIAL BLOOD GAS VALUES
by Daniel H. Wiseman (Intr. by Paul F. Wehrle) Dept. of Ped. Los Angeles County-Univ. of Southern California Medical Center

I have been building a computer program which accepts raw blood gas analysis data, performs all necessary corrections and calculations, and prints a report which includes a written description of the abnormalities found. The logic of this program is based on three premises: (1) that four tests reflect four key body functions or conditions, (2) that these same four tests are essential, on a one-to-one basis, to guide four important therapeutic modalities, and (3) that the more sophisticated blood gas analysis concepts require these four tests and other tests be considered in groups of two or more.

TEST VALUE	FUNCTION OR CONDITION	THERAPEUTIC MODALITY
Arterial PCO2	Alveolar Ventilation	Assisted Ventilation
Arterial PO2	Arterial Oxygenation	Oxygen Therapy
Blood HCO3	Body Buffer Status	Infusions of Buffers
Hemoglobin	Oxygen Carrying Capacity	Infusions of Red Cells

Both the report of test values and the printed comments are divided into five horizontal sections, one for each of the four basic tests and the fifth for the pH. The names of the tests, the normal values, the measured values, and the calculated values are displayed in vertical columns. Values which differ by more than two standard deviations from their normals are noted in a fifth column. The pH value is used to estimate the chronicity of alterations of PCO2 or HCO3. Warnings of certain changes in pH or PO2 which migh result from sudden changes in ventilation (PCO2) are printed.

DEXAMETHASONE IN THE MANAGEMENT OF KEROSENE PNEUMONIA.
J. Wolfsdorf and H. Kündig (Intr. by M.E. Avery).
Godfrey Huggins Med.Sch., Harari Central Hosp., Dept. of Ped. & Child Health, Salisbury, Rhodesia and Univ. of the Witwatersrand Med.Sch. Dept. of Clinical & Experimental Pharmacology, Johannesburg, South Africa.

The efficacy of steroids in the treatment of acute aspiration pneumonia, following intratracheal kerosene instillation in primates, was examined. Forty Chacma baboons were divided into 3 groups; Group I (normal animals), Group II (animals receiving .3 ml/Kg intratracheal kerosene alone - control group) and Group III (animals receiving .3 ml/Kg intratracheal kerosene plus pre- and post-treatment with 5 mg/Kg IMI dexamethasone). The lungs from all animals were examined macroscopically and microscopically and their lung wet weight/dry weight and lung weight/body weight ratios determined. On all parameters utilized, the lungs from Groups II and III were different from Group I. No statistical difference, however, could be detected between values obtained from Groups II and III. This data tends to support the contention that steroids, even when given early, and in large doses, in the course of kerosene pneumonitis, do not alter the acute inflammatory process that occurs.

PHOSPHORYLCHOLINE CYTIDYLTRANSFERASE OF HUMAN NEWBORN LUNG.
Richard D. Zachman and Michael S. Thom (introduced by Stanley N. Graven), Univ. of Wis., Dept. of Peds., Madison, Wis.

Lecithin biosynthesis and its relationship to respiratory distress syndrome is being investigated by assaying the pertinent enzyme activities in human newborn lung. Isolation and properties of phosphorylcholine cytidyltransferase (PCyT), the enzyme responsible for the second step of the CDP-choline pathway, are reported. PCyT activity was assayed by determining the amount of cytidine disphosphoryl choline-C^{14} synthesized from phosphorylcholine-C^{14} in the presence of cytosine triphosphate (CTP) and $MgCl_2$.

Lung tissue was removed 1-10 hours after death. The enzyme was stable under such conditions, and retained activity for several weeks frozen at -12°C. Product synthesis was linearly dependent upon the amount of homogenate protein, was linear up to 20 minutes, and maximal activity was attained at pH 6.5 and a temperature of 40-50°C. Oxygen had no effect on activity. PCyT catalyzes a bisubstrate reaction and so required kinetic studies at variable concentrations of each substrate. The apparent K_m for CTP (2.0×10^{-3}M) was similar to the K_m' for CTP (2.5×10^{-3}M). However, the apparent Km of phosphorylcholine (1×10^{-3}M) differed from the actual K_m' (0.25×10^{-3}M), suggesting enhanced binding of phosphorylcholine in the presence of CTP. Lysolecithin (0.4 μMole) stimulated human neonatal lung PCyT activity, suggesting a possible feedback control of lecithin biosynthesis in lung. Several newborn lung and liver samples were analyzed.