Abstracts

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1 IN VIVO ASSESSMENT OF DYSPLASTIC RENAL TISSUE'S ABILITY TO PRODUCE DIHYDROXYLATED METABOLITES OF VITAMIN D. B.M.Mogilner,A.Alkalay,F.Nissim*and S.Edelstein,** Neonatal Intensive Care Unit and *Department of Pathology, Kaplan Hospital, Rehovot and **Biochemistry Department, Weizmann Institute of Science, Rehovot, Israel.

Scant information has been hitherto obtained in humans regarding the ability of dysplastic renal tissue to produce the dihydroxylated metabolites of vitamin D and to respond to high doses of the vitamin. A full-term neonate who was born with hypoplastic lungs as a result of kidney malformation, and with serum creatinine level of 12.5 mg%, allowed us to study the metabolism of vitamin D. At the age of two weeks, blood samples were obtained at 0,24,36 and 72 hrs following i.m. injection of 100,000 I.U. of vitamin D_2 (Detamine). The serum samples were subjected to lipid extraction and to the analysis of 25(OH)D, 24,25$(OH)_2$D and 1.25$(OH)_2$D. The infant died at the age of 26 days due to end-stage renal failure. The post-mortem examination revealed single dysplastic-hypoplastic kidney weighing 1.5 gr. The levels of the dihydroxylated metabolites of vitamin D were normal in spite of the small renal mass, only 7.5% of the normal. While the concentration of 25(OH)D was low, a marked increase in circulating levels of 1.25$(OH)_2$D and of 24,25$(OH)_2$D were noted at 24 hrs following the injection. At 36,72 hrs the changes varied but with no definite trend. The present study suggests that during neonatal period a very small renal mass is sufficient to maintain optimal circulating levels of the dihydroxylated metabolites of vitamin D.

2 CROSS-REACTION OF 24,25-$(OH)_2$-VITAMIN D_3 WITH COMPETITIVE PROTEIN BINDING ASSAY FOR 25-OH-VITAMIN D.

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When the CPB assay to measure 25-OH-vitamin D was developed preparative chromatography was used to separate vitamin D and its hormonally active metabolites which all react with the binding protein. No precise data, however, have been published concerning possible interaction of 24,25-$(OH)_2$-vitamin D_3 with the CPB assay to measure 25-OH-vitamin D. In this study 24,25-$(OH)_2$-vitamin D_3 was assayed in a CPB assay developed to measure 25-OH-vitamin D (25-OH-vitamin D_3 [H-3], Bühlmann Laboratories, Basle, Switzerland). A cross-reaction of 20-36 % was found when 24,25-$(OH)_2$-vitamin D_3 was assayed purely at several concentrations. No cross-reaction was found in experiments measuring samples which contained 25 ng/ml 24,25-$(OH)_2$-vitamin D_3 and various concentrations of 25-OH-vitamin D_3 (5.0 - 80 ng/ml). Samples containing 50 ng/ml 24,25-$(OH)_2$-vitamin D_3 showed no cross-reaction in the presence of 80 ng/ml 25-OH-vitamin D_3 but a cross-reaction rate up to 54 % was observed when concentration of 25-OH-vitamin D_3 was reduced step-wise to 2.5 ng/ml. Conclusion: 25-OH-vitamin D in human serum can be reliably measured in the used assay without preparative chromatography because no cross-reaction of 24,25-$(OH)_2$-vitamin D_3 was observed up to concentration of 25 ng/ml which is far above that found in human serum (4.3 ± 1.6 ng/ml).

3 PSEUDOHYPOPARATHYROIDISM (PHP) SECONDARY TO VITAMIN D DEFICIENCY RICKETS.

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Vitamin D deficiency rickets was discovered in two Pakistani adolescents with hypocalcemia and increased serum alkaline phosphatase. They had a radiological appearance of rickets but their serum inorganic phosphorus was abnormally high. Serum parathyroid hormone levels were markedly increased and vitamin D metabolites, 25OH D and 1,25$(OH)_2$D strongly decreased (respectively 285 uU/ml, 1,7 ng/ml and 16,5 pg/ml for the boy, and 615 uU/ml, 1,2 ng/ml and 22,3 pg/ml for the girl). The was no cAMP response nor decrease in renal reabsorption of phosphate (TRP) after PTE injection in the girl. In the brother, the kidney responsiveness was completely absent regarding to TRP and markedly depressed for cAMP. Vitamin D therapy given at very low dosis (2000IU/day) led to a clinical healing of rickets. The kidney responsiveness to PTE was restored at a normal level or subnormal level when serum 1,25 $(OH)_2$D had reached a normal value ; at that time, serum PTH and 25OH vit D were still low. Therefore these two children presented with clinical features of pseudohypoparathyroidism (PHP). These features represent probably a rare consequence of vitamin D deficiency. The restoration of kidney responsiveness by vitamin D treatment seems to demonstrate that 1,25$(OH)_2$D has a key role as determinant of cAMP response to PTE. The relationships between the renal responsiveness to parathyroid extract (PTE) and vitamin D metabolism, are still not elucidated. In 2 ricketic children presenting with PHP, the urinary cAMP response to PTE could be clearly correlated with 1,25(OH)2 D serum levels.

4 EFFECT OF LACTOSE IN AN ARTIFICIAL MILK ON CALCIUM ABSORPTION BY PRETERM INFANTS.
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Lactose specifically promotes calcium (Ca) uptake by small bowel mucosa in vitro, but its role in neonatal mineral metabolism is uncertain. Two otherwise identical soy-protein artificial milks containing either lactose (L) or a sucrose/corn syrup mixture (S) as the carbohydrate component were fed to groups of preterm infants of similar age, maturity, and weight: Net natural Ca absorption was determined by means of 3-day metabolic balance. True Ca absorption was measured after a single 2 mg. dose of the stable nuclide 48-Ca by neutron activation analysis of faecal aliquots. S-milk fed infants (n=7, mean weight 1.98 kg) retained 15 ± 37.2 mg/kg/d (mean ± 1.S.D., range (-)43.1 to (+)60.3) (11.8 ± 29.3% intake) natural Ca compared with L-milk fed infants (n=10, mean weight 2.08 kg) 15.1 ± 29.3 mg/kg/d (range (-)17.4 to (+) 76.4) (10.8 ± 20.9% intake). 48-Ca retentions for L and S were 41.0 ± 15.9 (range 18.9 to 69.0) and 45.4 ± 7.6 (range 32.4 to 54.7)% intake respectively.

Conclusion: The data for L and S did not differ significantly, therefore lactose neither promoted absorption nor impaired faecal endogenous excretion of calcium by artificially fed preterm infants.

5 NEONATAL HYPERCALCEMIA IN PRETERM INFANTS.

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Hypocalcemia is a common problem in neonatology but hypercalcemia (HRCa) was been seldom mentionned in preterm neonates. A plasma concentration higher than 2.76 mmol/l (2.77-3) was detected and investigated in 8 infants (bw = 1250-1800 g ; gest. age : 31-35). It occurred at the age of 6 to 34 days with no seasonal preponderance. Plasma phosphorus (P) concentration was between 0.77-1.7 mmol/l (median 1.39). Plasma magnesium was normal. All infants were fed exclusively with breast milk (BM) (130-190 ml/kg/24 hr). Urinary Ca excretion (0.12-0.42 mmol/kg/24 hr) represented 13 to 39 % of Ca intake and urinary P excretion (0.009-0.09 mmol/kg/24 hr) less than 3 % of P intake (18-24 mg/kg/24 hr) in all infants but one. All infants received daily 2200 IU of vitamin D from the 10th day of life. Plasma 25-OH-vit. D was normal (45-78 nom/l) and serum immunoreactive parathyroid hormone was also normal (25-60 µlEq/ml). No acidosis was found and X-Ray of the bones was normal. HRCa disappeared spontaneously in 2 infants with a humanized formula in two infants and with supplementation of BM with P : 15 mg/kg/24 hr. Plasma P and urinary P increased in all infants without any change in serum PTH levels. These data suggest that HRCa in breast-fed preterm neonates is related to P depletion.

6 FETAL HEMOGLOBIN (HbF_1) AS AN INDEX OF INTRAUTERINE NUTRITION.

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Glycosylation of haemoglobin provides an index of the glucose environment during the red cell's life. It has been shown to be low in adults with chronically low blood glucose (insuloma), and we are investigating its possible use in the assessment of intrauterine malnutrition. Fetal glycosylated and acetylated haemoglobin cannot be separated and therefore the two components have been measured together (HbF_1). Cord blood haemoglobin was separated by narrow range isoelectric focussing followed by elution of the bands for spectrophotometric quantitation. HbF_1 has been estimated in the cord blood from 48 infants with gestational age from 36-42 weeks (mean 39.5). Results are shown in the table. Birth weight is expressed as a percentage of the median weight for gestational age, and HbF_1 as a percentage of total HbF (HbA excluded).

N	% Wt	%HbF1 (mean ± se)	t	p
3	<80%	7.86±0.68	2.28	<0.05
27	80+-100%	9.93±0.59		
18	100+-120%	10.3 ±0.73		

The trend for the lowest levels of HbF_1 to be found in the lightest, and presumably least well nourished, neonates together with the statistical evaluation suggest that this measure may be of use in distinguishing babies suffering from intrauterine malnutrition from those who may be small for other reasons.

7 INSULIN RECEPTOR INTERACTION IN CORD BLOOD ERYTHROCYTES AS AN INDEX OF FETAL GROWTH.

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Decreased insulin receptors in placentas from S.G.A. infants together with less insulin contents in their B cells, suggest that their intrauterine growth retardation could be partly due to a decreased insulin action.
Human erythrocyte insulin receptors can be considered as representative of insulin receptors in the cells of other body tissues. Specific insulin binding to erythrocytes was studied from 21 samples of term cord blood, 12 pre-term and 6 corresponding to S.G.A. infants. A modified R.R.A. was made in duplicate in all samples. Data obtained from the binding assay, were analysed and plotted by the Scatchard method.
Sites per cell in S.G.A. erythrocytes were 76 ± 10, significantly less ($p < 0.05$), than pre-term and full-term groups : 134 ± 12 and 104 ± 9 respectively. No differences in affinity constants were observed.
According to "up regulation" of insulin receptor binding in fetal life, we assume that the decreased insulin receptors in S.G.A. infants was related to their fetal growth retardation.
The data confirm previous reports of increased insulin binding to fetal cells and the positive correlation between insulin binding and red cell age, measured by reticulocyte concentration in all samples.

8 COMPOSITION OF WEIGHT GAIN IN PRETERM INFANTS FED THEIR OWN MOTHERS MILK (OMM).

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The macronutrient adequacy of OMM for feeding very low birthweight preterm infants was evaluated using nutrient balance, open-circuit indirect calorimetry & anthropometry. 15 studies were performed in 11 growing infants (M ± SE: gest age 30 ± 0.4 wks; birthweight 1.16 ± 0.04kg; study age 21 ± 2d; study wt 1.27 ± 0.06kg). The infants were gaining weight (15.2g/kg.d), length (0.98cm/wk) & head circ (0.76 cm/wk) at approximately intrauterine growth rates. The infants received 172 ± 4ml/kg.d of OMM containing 64.8 ± 7 Kcal/dl.

RESULTS: (M ± SE)	Energy (Kcal/kg.d)	Protein (g/kg.d)	Fat (g/kg.d)	CHO (g/kg.d)
Intake	111 ± 4	3.02 ± 0.1	4.74 ± 0.3	12.6 ± 0.4
Losses	11 ± 2	0.37 ± 0.01	0.86 ± 0.2	0.2 ± 0.02
Oxidation	56 ± 1	0.68 ± 0.07	1.63 ± 0.3	9.5 ± 0.7
Storage	44 ± 4	1.97 ± 0.1	2.25 ± 0.5	2.9 ± 0.9

The mean accretion rates of protein & fat, & the proportional composition of the daily weight gain (fat 16.6%, protein 13.4%) were comparable to those reported for the 3rd trimester fetus, suggesting that 172ml/kg.d of OMM provided a source of energy & macronutrients sufficient to promote growth in the preterm infant of similar quality to that of the fetus.

9 HUMAN MILK IMPROVES FAT ABSORPTION (FA) AND BILE ACID (BA) METABOLISM IN PRETERM INFANTS (PTI).

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PTI (n=66) were fed either pooled, expressed human milk partly supplemented with the mother's milk (PHM), or adapted formula, or such formula supplemented with taurine or taurine plus cholesterol. Fat intake in each group was 6.75 g/kg/day. Fat from PHM was absorbed more efficiently (93%) than that from formulas (82%, $P<0.001$). FA did not correlate with age in the PHM-group but correlated with postnatal ($r=0.31$, $P<0.05$) and postconceptional age ($r=0.41$, $P<0.01$) in the formula-fed infants. No differences were found among the infants fed various formulas. There was a linear correlation with total and all individual BA and FA when the conc of BA was expressed logarithmically (for total BA $r=0.63$, $P<0.001$). The formula-fed infants had FA <80% when the fasting intraduodenal BA conc fell below the median conc. This conc was for total BA, 3.63 mM; taurocholate, 1.26 mM; glycocholate, 0.96 mM; taurochenodeoxycholate, 0.64 mM; and glycochenodeoxycholate, 0.50 mM. When BA conc was greater than the median most infants had FA >80%. In the PHM-fed group FA was never <80%, and the intraluminal BA conc was mostly greater than the median for the formula-group. Thus, FA from PHM is aided by the larger BA conc and pool size, as well as by other intrinsic milk factors, e.g. bile salt-stimulated lipase.

10 DIAZEPAM METABOLITE EFFECTS IN PRETERM INFANTS.

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High dosage of diazepam administered to the mother just prior to delivery may affect the newborn infant. We have observed well known side effects like muscular hypotonia, hypothermia in neonates starting immediately after birth, related to high plasma concentrations of diazepam. In preterm infants whos mothers received 80-160 mg of diazepam within 16 hours prior to delivery, we have in some observed these symptoms after a free interval of several days. In 4 of these preterm infants blood samples were taken frequently from birth and up to 22 days of life for other reasons, and diazepam and N-desmethyldiazepam were also determined in plasma by gas liquid cromatography. We observed high plasma concentrations of diazepam during the first days of life (100 ng/ml to several 1000 ng/ml, T/2 : 60-100 hours) without any clinical symptoms. N-desmethyldiazepam was not detectable in plasma before one to several days after birth, reached high plasma concentrations (above 1000 ng/ml) after several days and disappeared slower than did diazepam (T/2: 70-220 hours). The clinical symptoms were most severe after a free interval with high plasma diazepam concentrations and were related in time to high plasma concentrations of the main metabolite with low concentrations of diazepam in plasma. Our results may indicate a difference in pharmacological activity between diazepam and N-desmethyldiazepam, and one might speculate if this difference reflects a developmental process in benzodiazepine receptors.

11 PLASMA STEROIDS IN PREMATURE INFANTS (PI) AT BIRTH AND DURING THE NEONATAL PERIOD AFTER ANTENATAL BETAMETHASONE (BM) THERAPY *

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The value of prenatal corticoid administration for the prevention of respiratory distress syndrome(RDS) has been amply documented,but little detailed information is available on the effects of such treatment on adrenocortical function of the neonate. We therefore measured plasma levels of aldosterone(Aldo),corticosterone(B),11-deoxycorticosterone(DOC),progesterone(P),17-hydroxyprogesterone(17-OHP),11-deoxycortisol(S),cortisol(F) and cortisone (E) simultaneously in 5 BM-treated PI (Gest.Age 32-35 wks; 2x8 mg BM, 48 and 24 h prior to delivery). Multi-steroid analysis was done by specific RIAs after extraction and automated LH-20 chromatography of a 100 µl sample from umbilical vein(UV) and artery(UA),and at 2,12,24 h,4,7,10 and 14 days after birth. Compared to 8 Gest.Age matched controls,fetal(UA) levels of the active (B,F) and inactive (E,S) glucocorticoids were significantly ($p<0.01$) suppressed by 55%,82%,45%,44%,respectively,whereas mineralocorticoid (Aldo,DOC) and progestin (P,17-OHP) levels were unaffected.However in the neonate,beginning with 2 h of age,we found no suppression of all the 8 steroids determined.B-levels were even higher (2,24 h;$p<0.05$) in the BM-treated group.The data suggest,that antenatal BM-treatment will neither cause gluco-,nor mineralocorticoid insufficiency in the immediate newborn period.

* study approved by the hospitals' Ethical Committees

12 AETIOLOGY AND FUNCTIONAL ABNORMALITIES IN ASTHMATIC CHILDREN. RESPONSIVENESS TO BRONCHODILATING DRUGS.

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The clinical findings, allergo-immunological results and lung function data of 208 asthmatic children (aged 4-18y), free of clinical symptoms were investigated. In respect of the clinical findings the patients were divided into 5 aetio-pathogenetic groups: Group A comprising patients with "infectious asthma" 6%, group B with "seasonal asthma" 30%, group C with "exogen perennial asthma" 55%, group D with "intrinsic asthma" 4% and group E patients with "exercise-induced asthma" 5%. All groups presented with a decreased specific airway conductance (sGaw < 85% pred.). On functional basis, group C was subdivided into C1: predominant overinflated (thoracic gas volume (TGV) 142% pred), C2: intermediate type (TGV 134%, sGaw 50% pred.) and C3: predominant obstructed (sGaw 61%). The influence of a sympathomimetic (Salbutamol) by inhalation was studied on both, the overinflation and bronchial constriction. In A,B,D and E the range of normal values was reached. In subgroups C1-C3 significantly different efficacy to sympathomimetics was found. Overinflation in C1 and C2 was only partially reversible. In addition bronchoconstriction was not completely removed. It is suggested that C1 is potentially susceptible to develop functional emphysema and seems to have a more serious prognosis.

13 VENTILATION DURING SLEEP IN OBESE ADOLESCENTS.

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Obesity has been implicated as a cause of upper airway obstruction during sleep. In order to establish whether respiration is compromised during sleep in obese subjects, we studied 10 obese adolescents (183 ± 23% of ideal weight (m ± SD)) overnight in a sleep laboratory. Mean age was 14 ± 2 years. Hemoglobin oxygen saturation was measured with an ear oximeter. Ventilation, as well as the relative contribution of abdomen and ribcage to tidal volume was monitoredwi th a respiratory inductive plethysmograph. During quiet sleep there was a complete reversal in the relative contributions to tidal volume, the ribcage contribution being predominant. We observed an average of 2.6 apneas per subject per night which is similar to that in normal adolescents. The average duration of the apneas was 12 ± 2 seconds occurring mainly during quiet sleep. During sleep we observed a mean maximum fall in saturation of 3 ± 2%. The falls in saturation occurred exclusively during periods of apnea and hypopnea. The mean minimal saturation during sleep (91 ± 2.5%) was significantly lower than in normal adolescents (96 ± 1%. $p<0.01$). We conclude that in adolescents obesity per se does not increase the incidence of sleep apnea, however, the increased ribcage contribution suggests that there is an increase in the work of breathing during quiet sleep, probably due to increased upper airway resistance.

14 The Different Responses of Asthmatic Children to Exercise and Isocapnic Hyperventilation.

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Fifteen asthmatics each exercised while breathing either cold and dry air or warm fully saturated air. Each subject performed 4 tests arranged in pairs. Test pair A consisted of 2 cold dry exercise tests and test pair B consisted of a warm humid exercise followed by a cold dry exercise. 9 of the patients repeated the study following the same protocol except that they performed isocapnic hyperventilation instead of exercise. In test pair A all subjects were rendered refractory by the first cold dry maneuver as shown by a significant attenuation of their bronchoconstriction following the second cold dry test (ΔFEV =38±4% SE and 16±4% for exercise, and ΔFEV =39±5% and 21±5% for hyperventilation). The warm humid maneuver caused neither exercise- nor hyperventilation-induced asthma. However, 12 of 15 patients were rendered refractory after the warm humid exercise while none were refractory following the warm humid hyperventilation. These experiments suggest that while bronchoconstriction renders all subjects refractory, in the majority of them exercise per-se may cause refractoriness without airway cooling or bronchoconstriction.

15 Perinatal Risk, Persistent Sleep Apnea and SIDS

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On the basis of a perinatal non-optimal score 27 preterm infants were classified as either high risk (n = 15) or medium risk (n = 12) and compared with normal fullterm controls (low risk) as to the number, duration and type of sleep apnea at 40, 52, and 64 weeks conceptional age. Factor one was assigned to the different parameters for respiratory pauses in the low risk infants. Thus, the corresponding factors for high and medium risk infants are indicating a significant and persistent lack of respiratory drive.

	low risk			medium risk			high risk		
conc. age:	40	52	64	40	52	64	40	52	64
periodic breathing	1	1	1	2,1	0,4	0,7	4,4	1,5	4,6
apnea density	1	1	1	2,6	0,4	1,3	1,4	1,9	1,1
apnea duration	1	1	1	2,3	0,3	2,2	1,8	0,6	2,5

Furthermore, obstructive apnea seems to be a striking persistent abnormality in high risk infants. In our polygraphic study it so happened that the same data became available from 2 infants prior to a sudden infant death and from 2 other infants prior to a Near Miss for Sudden Infant Death event. Their apnea factors fell into or even above the range of the risk groups. Thus, persistent sleep apnea can probably be considered as a risk factor for SIDS.

16 Partial Expiratory Flow-Volume Curves in Infants.

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Until recently it was impossible to study the function of the small airways in infants because of the inability of the infant to cooperate with the forced expiratory flow-volume maneuver. We have developed a technique of pneumatic thoraco-abdominal compression using an inflatable cuff which enables us to obtain forced partial expiratory flow-volume curves in infants. We have used this technique breathing air or a helium/oxygen mixture combined with measurements of lung volume and airway resistance in an infant whole body plethysmograph. Studies in 11 normal full term infants have established that the normal maximal expiratory flow at FRC is 186±17(SE) ml/sec or 1.91±0.18(SE) FRC's/sec, which increases by 13.5±4.2(SE) % after breathing helium/oxygen. We have also studied 13 infants with a variety of lung problems including 5 with wheezy bronchitis and 8 with cystic fibrosis. It was found that small airways obstruction as shown by changes in forced expiratory flow and the response to breathing helium/oxygen was a major component in many infants.

17 HUMAN SURFACTANT SUBSTITUTION. M. Hallman, H. Schneider, T.A. Merritt, and L. Gluck, Children's Hospital, Univ. Helsinki, and Univ. California, San Diego, Department of Pediatrics.

Surfactant substitution is complicated by lack of effective synthetic surfactant and possible immunological side effects of foreign surfactant protein. To decrease these problems biologically active human surfactant (HS) was isolated from amniotic fluid (Pediatr. Res. 15, 663, 1981). We report the first cases of substitution. Diagnosis of RDS was based on clinical and radiographic findings, and analysis of lung effluent phospholipids. 70 mg/kg of HS was given to endotracheal tube in 3.5 ml saline. The immediate response was as follows:

No	Birth weight	Gest. age	Age at HS	FiO_2 before HS	ΔPaO_2 5min after HS	FiO_2 1 hr after HS
1	960 g	27 w	4.7 h	1.0	185 torr	0.4
2	920 g	28 w	4.8 h	1.0	225 torr	0.6
3	900 g	27 w	10.0 h	0.8	130 torr	0.4

After HS, cases 1 & 3 required less oxygen and less mean airway pressures seen in RDS of similar size and gestational age. However, patient 2 was on pre-treatment respirator settings and FiO_2 by 3 h after treatment. Leucocyte elastase activity in tracheal aspirate from cases 1 & 3 was low, whereas elastase was 5-20-fold higher in tiny babies with RDS and no HS. HS has positive, although variable effect on severe RDS in tiny babies. Further controlled trial using HS is indicated, to study the role of surfactant substitution in management of RDS.

18 HYPOXANTHINE (HX) AND O_2 INDUCED LUNG DAMAGE. A BASIC MECHANISME MEDIATED BY FREE RADICALS? Ola D. Saugstad Mikko Hallman, Jerrold L. Abraham, Charles Cochrane, Louis Gluck. Univ. California, San Diego, UCSD Med. Ctr., Dept. Ped.&Path., & Scripps Clinic & Research Foundation, La Jolla, CA.

Metabolism of HX may generate O_2 radicals, which, if elevated may be a major factor in lung damage during hyperoxia. Study rats were treated with 100% O_2 and continuous i.v. HX for 48 h (Group 1). Controls: Untreated (Group 2). HX + room air (Group 3). O_2 + glucose i.v. (Group 4). Group 1 showed significantly more lung hemorrhage and alveolar edema ($p < 01$) than the others and had elevated levels of a_1-antitrypsin (a_1-PI) in lung lavage, suggesting acute inflammation. No elastase was found.

	group 1	group 2	group 3	group 4
a_1-PI (ug/mg)	16.0±8.5	3.3±2.0	7.6±5.6	4.8±5.4
p (vs group 1)		<0.005	<0.005	<0.01

The distribution of phospholipids in lung lavage from group 1 did not differ from the controls. However, the surface activity in lavage from group 1 was inhibited, as evidenced by a higher minimum surface tension and a faster surface collaps rate than in the other groups. The protein-rich fraction of lavage from group 1 and 4 increased minimum surface tension of lung surfactant. Conclusion: HX and O_2 in combination are destructive to lung tissue, possibly due to increased free radicals. HX accumulates in neonatal hypoxia, and free radicals can be produced during resuscitation with O_2. The present study contributes to the understanding of the pathophysiological mechanism of hypoxic-hyperoxic insult. Supported by HL-14169, and NIH grant HD-10622.

19 HEMODYNAMIC EFFECTS OF NITROPRUSSIDE IN CHRONICALLY INSTRUMENTED LAMBS WITH A LEFT-TO-RIGHT SHUNT. J.R.G.Kuipers, G.P.Toorop, R.Hardjowijono, J.H.Koers, H.Bavinck and C.R.H.Wildevuur (intr. by J. Fernandes), Depts of Pediatrics, Thoracic and Experimental Surgery, University Hospital of Groningen, Groningen, The Netherlands.

To evaluate the effects of afterload reduction, we infused 5 and 10 $mg.kg^{-1}.min^{-1}$ nitroprusside (NP) for 1 and 2 hr resp. into 9 lambs with a left-to-right shunt. A Goretex^R graft was inserted between the aorta (Ao) and pulmonary artery (PA). Electromagnetic flow probes were placed around ascending Ao and PA to measure pulmonary (q_p) and systemic (q_s) blood flows, resp. In addition, catheters were inserted to measure Ao, left (LA) and right atrial and PA pressures. Another 9 lambs were instrumented in a similar way but without a shunt, to serve as a control group. Measurements were made 3-4 days postoperatively. Pre-infusion levels of q_p and LA pressure were markedly higher in the lambs with than in those without a shunt. q_p/q_s averaged 2/1. q_s was almost the same in both groups. Upon infusion of NP the changes in both groups were about the same and dose dependent. The blood flows initially decreased, after which they returned towards their pre-infusion values and becoming stable from 30 min on. Heart rate(f) and systemic vascular resistance(Rs), however, remained higher and lower, resp.

We conclude that afterload reduction by NP in case of a left-to-right shunt and a normal R_s is contra-indicated as q_p and q_s do not change while f increases substantially. This increase of f could affect myocardial oxygen consumption unfavourably despite a decrease in peak systolic Ao pressure.

20 Importance of arginine vasopressin for blood pressure control in infant rats. APERIA, A., HERIN, P., St.Göran's Children's Hospital, S-112 81 Stockholm, Sweden.

Birth preceeded by labour is one of the most potent stimulates to arginin vasopressin (AVP) release. Since the kidney can not respond adequately to the antidiuretic effect of AVP we have postulated that AVP is a mainly vasoactive hormone in the perinatal period. We have therefore performed a descriptive study of the vasoactive effect of AVP in infant(i). As a reference we have studied adult(a) rats. To reveal the physiological effect of AVP we have used Brattleboro rats that can not synthesize AVP. The rats were studied under control conditions and under hemorrhagic hypotension. We found:

1) Infant rats were more susceptible to hemorrhagic hypotension than a-rats. 0.5 % bw in i-rats and 1 % bw in a-rats caused an equivalent(60 %)fall in mean arterial pressure(MAP).

2) Hemorrhagic hypotension could be prevented in both i-rats and a-rats by continuous infusion of low doses AVP(2000 $pg/100g^{-1} \times min^{-1}$.

3) The effect of AVP on MAP was linearly related to the MAP before AVP was given. At low pressure, AVP increased MAP and at high pressures AVP decreased MAP. We conclude that AVP has a dual effect on vasomotortonus.

4) When MAP ranged between 90 and 100 in i-rats and 140-160 in a-rats AVP neither increased nor decreased MAP. We conclude that AVP balances MAP at a lower level in i-rats than in a-rats.

21 **INCIDENCE, SEVERITY AND TIMING OF SUBEPENDYMAL (SEH) AND INTRAVENTRICULAR HAEMORRHAGE (IVH) IN LOW BIRTHWEIGHT INFANTS BORN IN A PERINATAL CENTER. Dolfin T, Skidmore M, Fong K, Hoskins E, Shennan A.** (Intr. by: Prof.S.Levin) **Regional Perinatal Unit, Women's College Hospital, Canada.**

95 infants of less than 32 weeks gestation or less than 1500 grams birthweight were studied prospectively with serial echoencephalograms from soon after birth.

The incidence of severe IVH, Papile Grades III & IV, was 13%. SEH/IVH occurred in 33%. 5 infants developed post-haemorrhagic ventriculomegaly, but none required treatment. Mortality in the group of infants with SEH/IVH was only 9.4%. 75% of SEH/IVH occurred in infants of less than 29 weeks gestation. Only 1 infant of more than 30 weeks sustained an IVH. All the haemorrhages occurred in the first 72 hours of life, 25% in the first 2 hours of age. 60% of IVH in infants of less than 29 weeks occurred in the first 24 hours. No correlation was found between development of IVH and birth asphyxia, pneumothorax or PDA.

It is shown that the incidence and severity of IVH in low birthweight infants can be decreased by delivery in a perinatal setting, and those infants especially at risk are infants of 29 weeks gestation or less, in the first 72 hours of life. Events during labour and delivery, may be of etiological significance because 25% of SEH/IVH occurs in the first 2 hours of life.

22 EFFECT OF CARNITINE ON LIPID METABOLISM IN THE PREMATURE NEWBORN. A.Orzali, F.Donzelli, *G.Enzi, and F.F.Rubaltelli, Depts. of Pediatrics and *Medicine, University of Padova, School of Medicine, Padova, Italy.

We have compared the effect of parenteral L-carnitine administration on blood plasma levels of triglycerides, glycerol, FFA and ketone bodies in 12 preterm (34 to 36 weeks of gestational age) and 20 full-term neonates who received an intravenous lipid infusion (1 gr/kg b.w./4hrs). Of these, 5 premature and 7 full-term neonates also received the contemporaneous infusion of carnitine (100 mg/kg b.w./6 hrs). While no significant differences on triglyceride concentration were found between the two groups of full-term newborns (treated or not with carnitine), the triglyceride levels at 2, 4 and 6 hrs after the start of the infusion (380.7±54.1; 551.1 ±14.9; 233.2±34.7 mg/dl respectively) were found to be higher in the group of premature without carnitine supplementation. Preterm infants infused with carnitine showed a triglyceride pattern (before infusion: 67.4±6.2; 2nd hr: 301.9±92.9; 4th hr: 414.4±93.5; 6th hr: 143.6±38.2 mg/dl) similar to that of full-term newborns, suggesting the possibility that carnitine enhances the activity of plasma lipoprotein lipase. We found a more pronounced effect of carnitine on lipolysis (increased glycerol plasma concentration) in premature than in full-term newborns (2nd, 4th, 6th hr. respectively: 0.56±0.10; 0.60±0.12; 0.18±0.04 vs. 0.40±0.06; 0.37± 0.06; 0.17±0.03 mM/l). On the contrary, the ketogenetic effect of carnitine seems to be lower in preterm than in full-term infants (B-OH: 0.40 ±0.06; 0.62±0.07; 0.34±0.10 vs. 0.63±0.13; 0.78±0.17; 0.54±0.17 mM/l.A.A.: 0.26±0.04; 0.32±0.06; 0.18±0.11 vs. 0.38±0.07; 0.40±0.10; 0.34±0.14 mM/l).

23 EFFECT OF ORAL VIT. E ADMINISTRATION TO PREMATURES ON ERYTHROCYTE-MEMBRANE-VIT.E AND HYDROGEN PEROXIDE INDUCED HEMOLYSIS.

P.Tuchschmid, E.Meyer, F.Rieser, G.Duc. Dept. Pediatrics, University of Zurich, Switzerland.

Lack of vit.E as seen in premature causes hemolytic anemia and may be a factor inducing retinopathy and bronchopulmonary dysplasia. Controversy exists on the effect of oral vit.E on its plasma levels and hydrogen peroxide induced hemolysis (H_2O_2-test). This study investigates the effect of oral vit.E in prematures on erythrocyte-membrane vit.E (EC-vit.E) and on a modified H_2O_2-test where EC-catalase was blocked to prevent undesired, enzymatic cleavage of H_2O_2. 24 premature infants (GA:33±2 w; BW: 1800±400 g) were studied at birth, at 2 and 4 weeks of age. Vit.E (7.5 mg/d) was administered from day 2. Normal values were obtained from 13 adults and 18 term infants. Results: In premature and term infants low plasma vit.E (34±15% of adult values) corresponded to low EC-vit.E (0-50% of adult values). Within 2 weeks of vit.E administration plasma levels rose 3-fold but EC-vit.E rose insign. from 40 to 60% of adult values. After additional 2 weeks plasma vit.E remained unchanged and EC-vit.E rose more than 2-fold to 180% of adult values. This is reflected by the modified H_2O_2-test which remained elevated when EC-vit.E was low (0-2 weeks) and was halved when EC-vit.E was doubled (2-4 weeks). Iron administration (1.5 mg/d) did not prevent rise in plasma or EC-vit.E. Conclusion: Oral substitution with vit. E leads to rapid increase of plasma-levels but incorporation into EC-membranes and decrease in H_2O_2-induced hemolysis occurs with a lag of at least 2 weeks.

24 RETINOPATHIA PRAEMATURORUM: FACTORS POTENTIATING THE ETIOLOGIC EFFECT OF O_2.

E. Bossi and F. Koerner, Dept. of Pediatrics and Dept. of Ophthalmology, University of Berne, Switzerland. The following questions were asked: 1. Is the potentiating effect of blood transfusions on the O_2-induction of ROP due to the adult Hb administered or to volume load? 2. Does the extent of i.v. fluid therapy facilitate the development of ROP? 3. Do low paO_2-values also correlate with ROP? These questions had remained open after we had shown that $paO_2 > 100$ torr, $paCO_2 > 50$ torr, $paH < 7.25$, number of blood transfusions and volume of transfused packed rbc correlated significantly with the development of ROP (Bossi et al. Retinopathy of Prematurity Conference, Syllabus p. 536 (1981), Ross Laboratories, Publ.). Procedure: The following mean values were elaborated from the charts of the same study population (53 ROP-infants vs. 53 controls) for the first 14 days of life by using the same approach with matched pairs statistics. Results:

Factors investigated	ROP	Controls	p
1. tot. volume of oncotic substances administered, (rbc+plasma+alb.) ml/kg/d	2.6	2.5	ns
2. total i.v. fluid, ml/kg/d	70.2	62.2	ns
3. number of paO_2-values < 50 torr (as long as determined)	2.9	1.2	ns

Conclusions: Factors leading to augmented O_2-delivery to the retinal tissue (elevated paO_2 and $paCO_2$, acidosis, administration of HbA) increase the risk of developing ROP. Volume of oncotic substances administered, i.v. fluid, and low paO_2-levels play no role.

25 Apnea of Prematurity Treated by Aminophylline and Doxapram.

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A clinical trial was undertaken to evaluate the efficacy of doxapram (chlorhydrate d'Ethyl-1-(morpholinyl-4)-2 ethyl-4 diphenyl-3-3 pyrrolidinone - 2 monohydrate; supplier: A.H.Robins, Richmond, Virginia) in preventing apnea of prematurity. Sixteen infants who developed idiopathic apneic attacks received either aminophylline or doxapram in a double blind fashion. Treatment was continued for 48 hours during which the frequency of attacks was recorded. If the initial treatment failed or apneic attacks recurred after cessation, the other drug was given. Over half the patients responded to either drug with complete cessation of apneic attacks and there was no statistically significant advantage of one drug over the other. In a further double blind study, the efficacy of doxapram was compared with placebo in 10 infants in whom apneic attacks were still present despite complete therapeutic levels of theophylline. Eight of these infants responded fully to doxapram while none responded to placebo. Doxapram has been shown to be valuable in the treatment of idiopathic apnea of prematurity. It has an additive effect when combined with aminophylline and is especially recommended in theophyllinised patients in whom apneic attacks are still present and who would otherwise require respiratory support.

26 PURINE METABOLITES AND LACTATE AS PARAMETERS OF HYPOXIA IN THE NEWBORN.

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To look for better criteria of tissue oxygenation than blood gases, we took arterial samples from 29 sick neonates at 6-hr intervals for lactate (Lac) and blood gas analyses, as well as HPLC assays of purine metabolites in plasma; only hypoxanthine (Hx) and uric acid (UA) are considered. The state of the infant at each sampling was classified as severe, moderate, or mild (to no) hypoxia on the basis of clinical course, $tcpO_2$, pH, pO_2, and FiO_2. Elevated values for Hx, UA, and Lac were defined as ≥2SD above the mean for infants in mild or no hypoxia (pH ≥7.35, pO_2 ≥50, FiO_2 ≤0.40). Of the total of 288 samples, 209 were in the mild, 49 in the moderate, and 30 in the severe category (pH ≤7.25, pO_2 ≤40, FiO_2 ≥0.80). Hx value was high (≥36 µM) in 4.3% of mild and 16.7% of severe cases, while the corresponding incidences for UA (≥460-800 µM, age-dependent) were 10.5% and 30%, and for Lac (≥3.8 mM) 6.2% and 50%. Conversely, when Hx values were elevated (N=21), severe hypoxia was present in 24% and mild in 43% of the instances, while the corresponding incidences for elevated UA (N=45) were 20% and 49%, and for Lac (N=38) 43% and 37%.

We conclude that neither purine catabolite nor Lac levels in intermittent samples of arterial blood are reliable indicators of tissue oxygenation in the newborn.

27 PLASMA CATECHOLAMINES AND HYPOXANTHINE (HX) LEVELS IN NORMAL AND DISTRESSED NEWBORN BABIES.

Ola D. Saugstad, M. Ziegler, Bruce Kessel, Brian Saunders, Louis Gluck. Univ. of Calif. San Diego, Depts. of Pediatrics, Medicine, Reproductive Medicine, La Jolla, Calif; Kaiser Foundation Hospital Dept. of Pediatrics.

Plasma catecholamines and HX levels were determined by radioenzymatic assay and a P_{O_2} method respectively in hypoxic and normal neonates. Norepinephrine (NE) was elevated 4,7 time ($p < 0.0025$), epinephrine (E) 2.0 times ($p < 0.05$), and HX 2.2 times ($p < 0.005$) in hypoxic vs. non-hypoxic term neonates. E is released in response to hypoxia as demonstrated by the positive correlation of E and HX: $LgE = 0.0197 \times HX + 1.98$, $r = 0.45$ ($p < 0.05$). HX level was elevated and dopamine (DA) synthesis diminished in hypoxia illustrated by the negative correlation of DA and HX: $LgDA = 2.34 - 0.041 \times HX$, $r = 0.65$ ($p < 0.01$); this synthesis reduction is probably because the rate limiting enzyme tyrosine hydroxylase is O_2 dependent.

Prematures with RDS had NE and E values similar to those in venous cord plasma of normal term infants, but DA was lower, near the limit of detection of the assay ($p < 0.005$). Infants given continuous DA infusion had DA levels 35-400 times greater than those found in venous umbilical cord blood but NE and E were not elevated. This illustrates that DA does not metabolize further to NE and E under such treatment.

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28 STUDIES ON THE MECHANISM OF PHOTOTHERAPY: EFFECTS OF BLUE LIGHT IRRADIATION ON LIVER CYTOCHROME P450 AND b5.

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Visible light can penetrate abdominal wall of Wistar adult rats at a rate that will permit photochemical reactions to occur in vivo. Experimental evidence suggests that visible light can influence liver enzymatic activities and the effects of phototherapy may be due, to some extent, to such a mechanism(s). Five heterozygous (Jj) and 5 homozygous (jj) female Gunn rats (weight: 160-248 g) were exposed for 24 hrs. to a blue light source (wavelength: 425-475; irradiance: 20 µW/sq cm/nm); 5 Jj and 5 jj Gunn rats (weight: 170-245) were kept as controls. Livers were excised and the microsome fractions obtained by differential centrifugation. Cytochrome P450 and b5 were determined according to Omura and Sato (1964).

* $p < 0.02$

	P450 (nMoles/mg of proteins)			b5 (nMoles/mg of proteins)	
	controls		exposed	controls	exposed
jj Gunn rats	0.107±0.056	*	0.179±0.052	0.239±0.044	0.290±0.039
Jj Gunn rats	0.211±0.018	*	0.287±0.051	0.286±0.021	0.304±0.030

These results agree with previous work from our laboratory in which it was possible to document an increased activity of the monooxygenase system after blue light irradiation. Furthermore, they strongly support the evidence that visible light can influence enzymatic activities located on liver microsomes, even if it is not possible to explain its molecular mechanism.

29 THE BEHAVIOUR OF JAUNDICED INFANTS TREATED WITH PHOTOTHERAPY

R.Paludetto, C.Corchia, G.Mansi, P.Rinaldi, M.De Curtis, L.Orfeo, F.Ciccimarra (Introd. by A.Rubino) Neonat. Unit 2°, School of Medicine, University Naples, Italy.

This study was performed in order to evaluate possible changes in behaviour in jaundiced infants without perinatal complications other than hyperbilirubinemia, treated with phototherapy. 30 jaundiced infants ($\bar{x}$ bilir. 13.3 mg/100ml, range 8.4-17.5) born spontaneously at term and undergoing phototherapy for 6 hours or more, and 30 controls, comparable for sex, b. w, g, a, and Apgar scores were examined during the 3rd day of life according to Brazelton Behavioural Neonatal Assessment Scale. For 6 of 26 items we found higher values for controls (Wilcoxon Test): inanimate visual, median (m) 5vs3 ($p<0.05$): animate visual, m5vs3 ($p<0.01$): visual and auditory, m5vs4 ($p<0.005$) pull-to-sit, m6vs5 ($p<0.01$): cuddliness, m5vs4 ($p<0.01$). Orientation responses were the most compromised. On the 4th day of life 14 infants terminated phototherapy, and were compared with their controls, and the same significantly poorer performances, mainly in orientation, were found. At one month of age 12 of the infants treated with phototherapy still showed significantly poorer performances in 2 items of orientation: inanimate visual, m5vs4 ($p<0.05$), and visual and auditory, m5vs4 ($p<0.05$). Whether these results depend on the jaundice or on the phototherapy remains to be established.

30 RENAL AND AUDITORY FUNCTION IN NEONATES TREATED WITH AMIKACIN (A).

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Methodological difficulties account for the limited information on aminoglycoside (AG) safety in neonates. We studied auditory and renal function of 35 neonates treated with A 7.4mg/kg bid from the first day of life for 6-12 days (GA from 29 to 41 wks, birth weight .9-4 kg). Brain Stem Response Audiometry (BSRA) allowed objective hearing evaluation of the young uncollaborative infant. Latency and amplitude of specific potentials (V wave) evoked by auditory stimuli (clicks) were analyzed. During and after therapy serum creatinine (SCr) and urinary excretion of N-acetyl glucosaminidase (NAG) an early sign of tubular damage in adults were determined. A serum concentrations were monitored. 41 matched neonates untreated with AG were similarly followed. A serum peak and trough concentrations exceeded recommended values for adults in 20% of the neonates, hypoxia and prematurity being aggravating factors. BSRA at 7-9 months of age revealed minimal damage in a single A treated infant. The parameters of the V wave did not differ between the groups. Postnatal decrease of SCr was similar in both groups, while NAG excretion was : (uM/h/mg urinary creat. mean(SD)—significant differences according to Tuckey's test for non-confounded means at $p<.01$)

Days of life	1-2	3-5	6-8	20-22
A treated	2.1(1.7)	2.0(1.0)	3.0(1.5)	1.2(.5)
Controls	1.4(.8)	1.4(1.0)	1.5(1.0)	1.7(1.1)

Conclusions : NAG excretion and BSRA are useful to reveal minor and reversible AG damages in neonates.

31 CONGENITAL FOLATE MALABSORPTION WITH MENTAL RETARDATION AND CEREBRAL CALCIFICATIONS.

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A boy was seen at the age of 7 months with a 3 month-history of convulsions, hemiplegia, severe diarrhea, fever, failure to thrive and recurrent infections. Megaloblastic anaemia, leucopenia, thrombocytopenia and hypogammaglobulinaemia were also present. Normal blood levels of vit. B12 and transcobalamine II, low plasma folate (< 0.9 ng/ml; nl: 3-15 ng/ml) and increased urinary excretion of figlu and orotic acid prompted administration of oral folic acid (1 mg twice weekly). This treatment cured the anaemia, diarrhea and infections, but failed to prevent convulsions and the appearance of mental retardation and cerebral calcifications. Further investigations revealed that oral administration of 5 to 10 mg of folate provoked a slow increase in plasma folate to 2-15 ng/ml. Oral or parenteral administration of 3-6 mg of 5-formyltetrahydrofolate (leucovorin) resulted in a plasma folate of about 15 ng/ml. CSF folate, below 0.9 ng/ml before therapy (nl: 15-40 ng/ml), was barely modified by folate treatment, but could be increased to around 5 ng/ml with oral leucovorin. Treatment with leucovorin, vit. B12 and methionine supplements improved the neurologic condition. It is postulated that the congenital defect of folate absorption and transport to the central nervous system could be explained by an abnormal folate binding protein.

32 ANIMAL STUDIES OF POSTNATAL BRAIN DEVELOPMENT IN FETAL ALCOHOL SYNDROME.

Spohr, H.L., Stoltenburg-Didinger, G. and I.Chahoud (Intr. by F.Hanefeld), Kinderklinik, DRK-Rittberg-Krh. Institute of Neuropathology and Embryonal-Pharmakology, Free University Berlin (FRG).

Symptoms in Fetal Alcohol Syndrome include microcephaly, craniofacial dysplasia, ataxia and mental retardation. The brain development of young Wistar-rats was studied under different conditions. One group was fed "Stardit"-diet plus ethanol (35% of the caloric intake) only during pregnancy, whilst in the other this "alcoholic" diet was continued over the period of lactation. The daily caloric intake was equal in both groups (65-70Kcal/d).

The bodyweight of the offsprings of the alcoholic treated animals was significantly decreased compared to controls of the same litter size. After decapitation at day 1, 4, 8, 12, 16 and 21 a selective decrease of brain weight in the experimental pups was found in comparison to their bodyweight at birth and postnatally.

These results could be confirmed histologically and by ultrastructural investigations in which no irreversible cell damage due to alcohol was found, but major disturbed neuronal migration and a retarded synaptogenesis were evident.

The latter could specially be proven by Golgi impregnation method in the synapses of Purkinje cells, pyramidal cells and the neurons of the hippocampus.

33 ONTOGENY OF ANDROGEN RECEPTORS IN HUMAN FETAL EXTERNAL GENITALIA :

Ch. Sultan, C. Devillier, P. Mares, B. Descomps (intr. by R. Jean) INSERM U.58, Montpellier, France.

We have recently shown (Pediat. Res. 1980, 13, 67) that as early as the 10th week of gestation, dihydrotestosterone (DHT) receptor activity was detected in genital skin fibroblasts. This study was disigned to establish the ontogeny of DHT-receptors in human fetal skin fibroblasts and to find out whether there is any variation of androgen receptor levels in relation to sexual differentiation. Specific receptor bound-DHT in the cell (Bmax) was measured by a charcoal adsorption method and expressed as fentomoles/mg of DNA. Physico-chemical properties of the binding component were studied by Sephadex G25 column chromatography, sucrose density gradient ultracentrifugation and by polyacrylamide gel electrophoresis.

Age (weeks)	8	10	12	15	17	18
Sex	M	M	M	M	M	M
Bmax	492	608	560	696	412	488

In fetal genitalia, the mean number of binding sites ($\bar{m}$ = 543 ± 92 fmoles/mg DNA) was found in the range of that of newborn foreskins ($\bar{m}$ = 689 ± 381 fmoles/mg DNA). The plasma testosterone pattern is not correlated by any variation in sex skin DHT receptor content. These data do not support that fetal androgen receptor level is modulated by testosterone and indicate that androgen receptor are present in genital area before the fetal differentiation of external genitalia.

34 FURTHER PURIFICATION OF HUMAN PITUITARY FRACTIONS STIMULATING GLUCAGON AND INSULIN RELEASE IN RABBITS.

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Human growth hormone and a human pituitary lipid-mobilizing factor (LMF) increase plasma levels of glucagon in rabbits (Ped.Res.(1980)14:174). After gel filtration (Biogel P2 and Sephadex G25 in 0.5N acetic acid), the LMF preparation gave three active fractions. These have been further purified by gel filtration, reversed phase separation and absorption chromatography. Doses, corresponding to 100 nmol amino acids after alkaline hydrolysis, were injected into fasted rabbits, and glucagon and insulin measured in peripheral plasma samples:

	Glucagon (pg/ml) basal level	peak level	p<0.05 after	(n)	Insulin (μU/ml) basal level	peak level	p<0.05 after	(n)
LMF 1	173±14	673±144	15min.	(3)	17±3	55±12	45min.	(3)
LMF 2	130±13	370± 77	5min.	(3)	10±2	21± 2	30min.	(3)
LMF 3	110± 8	313± 58	5min.	(4)	12±3	53±16	4min.	(4)

As these LMF fractions probably have molecular weights from 2000 (LMF 3) to 4000 (LMF 1), the doses employed may be within the physiological range. The purity of the fractions is being studied, as well as their relationship to known pituitary polypeptides.

35 FETO-MATERNAL TRANSFER OF PARATHYROID HORMONE IN AN IN VITRO HUMAN PLACENTAL PERFUSION MODEL.

F. Pohlandt, S. Balabanova*, I. Henrichs* and W. M. Teller. Center of Paediatrics, University of Ulm, Federal Republic of Germany.

Clinical improvement during pregnancy was reported in two cases of maternal hypoparathyreoidism. Diaplacental passage of fetal PTH may have caused the improvement. In order to study the basis of this hypothesis we investigated diaplacental passage of PTH in an in vitro model of human placental perfusion.

Bovine parathyroid extract was infused into the fetal artery during 20 minutes. Perfusate samples from fetal vein and maternal vein were taken at intervals of 3 and 5 minutes over a period of 40 minutes. The concentrations of PTH were measured by PTH-C-Terminal RIA. To validate the assay for use in the presence of perfusate a typical standard curve for assay of beef PTH was obtained in parallel with assay tubes containing added perfusate. Complete separation of maternal and fetal side was proved by dextran-blue.

Results: minutes following start of perfusion

0	3	6	9	12	15	20	23	30	35	40
Fetal vein					PTH (ng/ml)					
1	15	17	25	28	25	33	1.2	0.5	0.5	0.5
Maternal vein										
0.5	0.5	0.9	3.5	2.8	4.0	3.4	2.3	1.5	0.8	0.8

The appearance of PTH in maternal venous blood showed permeability of placenta to fetal PTH. This finding supports hypothesis of clinical improvement of maternal hypoparathyroidism by fetal PTH.

36 TESTICULAR ESTRADIOL RESPONSE TO GONADOTROPINS DURING PUBERTY : A MATURATION PROCESS ?

Ch. Sultan, B. Descomps, N. Bressot, P. Garandau, R. Jean. Dept. of Pediatrics, & INSERM U.58, Montpellier, France.

The response of testicular androgens to gonadotropins (H.C.G.) is well documented in boys, but few data are available concerning simultaneous estradiol (E_2) variations during these tests. This study was designed to determine if E_2 response to H.C.G. does exist before puberty or at what pubertal stage its develops. 50 normal subjects : 25 prepubertal and 25 pubertal boys (pubertal stages from P_1 to P_5) received 5000 I.U. H.C.G./days during 3 days. Plasma E_2 was measured by radioimmunoassay before and after each injection. Informed consent was given. Results are expressed as mean plasma E_2 (pg.ml) $\pm$ SEM. The maximum E_2 response was obtained 24 hours after the first H.C.G. injection (Day 1).

	P1	P2	P3	P4	P5
Basal	$\frac{6.1}{2.8}$	$\frac{9.2}{3.0}$	$\frac{20.2}{4.8}$	$\frac{28.8}{6.8}$	$\frac{37.2}{6.8}$
Day 1	$\frac{6.2}{3.1}$	$\frac{22.1}{6.1}$	$\frac{87.2}{13.6}$	$\frac{99.3}{55.5}$	$\frac{99.3}{31.0}$

These data show that 1) there is no E_2 testicular response to H.C.G. before puberty ; 2) sub-maximal E_2 response occurs only at P_3 ; 3) from P_3 to P_5 the E_2 peak remains constant (in spite of the increase of T response). These results suggest that the induction of testicular aromatization by gonadotropins appears to be an accurate component of pubertal maturation.

37 CORTEX AND MEDULLA OF HUMAN KIDNEY DIFFER IN THEIR α-GLUCOSIDASE ISOENZYME PATTERNS:

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In two infants who died from typical glycogenosis II, α-glucosidase was active at acid pH in kidney cortex but not in medulla. Therefore, structure and kinetic properties of α-glucosidases of normal human kidney medulla and cortex were probed and compared with those of liver using the following criteria: heat stability, electrophoretic mobility and sensitivity to the inhibitor turanose. Activity was measured at pH 4.0 (4.5), 6.0 and 8.0 (8.5). The properties of enzymes active at pH 4.0 (4.5) were comparable. Upon electrophoresis, 4 to 5 neutral forms (pH 6.0, 8.5) were identified in the various tissues; medulla had one band, cortex and liver had 3 to 4 with the same mobility but different catalytic staining intensity. Neutral cortex α-glucosidase was more heat stable and more sensitive to turanose than that of medulla and liver. It was the major band in cortex and had the lowest mobility. This enzyme form of cortex was not expressed in medulla or liver and may be identical with the "kidney enzyme" reported by others. It is responsible for the α-glucosidase activity present at low pH in kidneys of patients with glycogenosis II. The disorder can not be diagnosed by standard activity measurements on kidney unless they are done on medulla which is carefully freed of cortex.

38 NEW OBSERVATIONS IN PATIENTS WITH GENERALIZED LIPODYSTROPHY (SEIP-LAWRENCE).

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In 1977, we reported preliminary results of fenfluramine therapy in a patient with lipodystrophic diabetes. Consequently 3 more patients with congenital generalized lipodystrophy and diabetes have been treated. In all a marked improvement of glucose tolerance, a reduced insulin resistance, and a reduction of the hyperlipidemia have occurred. In the most seriously affected (retinopathy, nephropathy) patient hypophysectomy followed by fenfluramine therapy were necessary to achieve good diabetic control. In 6 patients with the congenital and 1 with the acquired form of generalized lipodystrophy remarkable low serum testosterone binding globulin levels (TeBG) and correspondingly low testosterone levels were found. In 1 female a marked increase of TeBG followed estrogen therapy. One- and twodimensional echocardiography demonstrated hypertrophic cardiomyopathy in all patients, but only once obstructive cardiomyopathy was discovered. The septum was thickened, mean 15 mm, the posterior wall 11 mm. 2 patients had asymmetrical septal hypertrophy. Echocardiography showed signs of deterioration of cardiac function, which may become an additional determinant prognostic factor. CT scan of the enlarged liver showed massive infiltration of fat.

39 DIHYDROBIOPTERIN DEFICIENCY: MONOTHERAPY WITH TETRAHYDROBIOPTERIN (BH_4) AND DIACETYL BH_4.

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Considering side-effects in the treatment of dihydrobiopterin deficiency a better therapy would be desirable. In rats only 1% of BH_4 present in the periphery enters the brain. In the patients it may be possible to provide the brain with an adequate amount of BH_4 cofactors when treated with excessive doses of BH_4. Therefore, a 2 year old patient with defective dihydrobiopterin synthesis was treated only with BH_4; L-DOPA, 5-hydroxytryptophan and Carbidopa were withdrawn and the BH_4-dose was increased from 3.4 mg/kg b.w. daily to 20-40 mg/kg every second day. This monotherapy also reduced ataxia and rigor, however, slight truncal hypotonia persisted probably due to the low dopamine production. Urinary serotonin and phenylalanine remained normal and neopterin was slightly elevated. Similar results were obtained after a trial with 1',2'-diacetyl BH_4, 20 mg/kg. It is probable that in this patient BH_4 as well as diacetyl BH_4 do not effectively cross the blood-brain barrier. Until more suitable derivatives of BH_4 are available, we decided to reintroduce the therapy with neurotransmitter precursors and Carbidopa.

40 TREATMENT OF ALANINE INDUCED HYPERAMMONEMIA WITH BENZOATE OR PHENYLACETATE IN LYSINURIC PROTEIN INTOLERANCE (LPI).

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To quantitate the influence of sodium benzoate (B) and sodium phenylacetate (PA) on alanine induced hyperammonemia and orotic aciduria, we gave an L-alanine load, 6.6 mmol/kg as a 5 % solution in 90 min, either alone or with 2.0 mmol/kg or B or PA to 5 patients with LPI. Plasma samples were drawn before and at 120, 180, and 270 min after the beginning of the load. Urine was collected for 4 consecutive 6 hr periods. Alanine caused moderate hyperammonemia and massive orotic aciduria in all. Both changes were significantly diminished by addition of B or PA to the infused solution. Most plasma and urinary amino acids increased after alanine. B and PA additions changed these responses only slightly. Massive excretion of waste nitrogen as hippurate (benzoylglycine) after B and as phenylacetylglutamine after PA adequately explain the improved tolerance to the alanine load. Thus, both B and PA partly prevent hyperammonemia after an acute nitrogen load, but are less effective in LPI than the urea cycle intermediates ornithine, arginine and citrulline which totally abolish the alanine induced hyperammonemia.

41 RENAL LOSS OF BIOTIN: A CAUSE OF BIOTIN-RESPONSIVE MULTIPLE CARBOXYLASE DEFICIENCY.

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2 unrelated infants with metabolic acidosis, neurol. symptoms, skin rash and organic aciduria typical of deficiency of the 3 biotin-dependent carboxylases (propionyl-CoA-(PCC), 3-methylcrotonyl-CoA-(MCC) and pyruvate carbox. (PC)) showed prompt clinical and biochemical response to oral biotin (10mg/day). In cultured fibroblasts PCC, MCC and PC activities (act.) did not differ from controls even when cultured in low biotin medium (150pmol/l). After withholding biotin supply, plasma biotin conc. (P-bi) fell to subnormal levels, carbox. act. in lymphocytes decreased to 20% of controls and clinical symptoms and abnormal metabolites reappeared. Administration of an extremely low oral dose of biotin (1.5 µg/kg) was sufficient to normalize P-bi and carbox. act. in lymphocytes within 1 hr. This ruled out a defect of intestinal absorption and of transport of biotin into the cell. However, during biotin treatment urinary excretion of biotin was increased to 669 µmol/mol creatinine in the patient (5 controls at similar P-bi: 183±39 µmol/mol creat.). After cessation of biotin, P-bi fell from 22 to 0.45nmol/l (normal: 1-2.5nmol/l) within 3 days. These findings suggest renal loss of biotin in the patient. Biotin clearance in 5 controls at normal P-bi was 44±9 ml/min/1.73m^2, consistent with tubular reabsorption of biotin. We thus speculate that our patients suffer from a defect in renal handling of biotin.

42 PLASMA CARNITINE IN DIABETIC KETOSIS

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Carnitine is an essential cofactor in ketogenesis. Plasma total-,free- and acylcarnitine levels were determined in 4 ketotic, but not acidotic (K) and 6 ketoacidotic (KA) diabetic children as well as in controls (C).There was no significant difference in the total carnitine level of the three groups. Acylcarnitine, however, was markedly increased, free carnitine decreased (as expressed as acyl/total ratio) in parallel with the accumulation of ketones and the increase of the BHB/AcAc ratio.

	Total ketones/mM	BHB/AcAc	Total carnitine/µM	Acyl/Total
C	0.07±0.01	1.7±0.1	58.5±10.9	0.11±0.01
K	3.06±0.3^x	6.1±0.3^x	40.9±3.2	0.41±0.03^x
KA	6.96±0.8^x	8.6±1.4^x	49.0±4.5	0.57±0.03^x

x significantly different from control

This redistribution of free- and acylcarnitine was reversed by insulin treatment. These data are consistent with the postulated role of carnitine in ketogenesis.

43 INFLUENCE OF INTRAUTERINE GROWTH RETARDATION ON ENERGY AND MACRONUTRIENT METABOLISM.

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We have evaluated the utilization and storage of energy and macronutrients, and the growth pattern of intrauterine growth retarded (SGA) very low birthweight (<1300g) infants, by the combination of nutrient balances, open-circuit indirect calorimetry and anthropometry. 14 studies in 6 SGA infants (M±SE: gest. age 33.1±0.3 wks; birthwt. 1.12±0.03 kg; study age 26±3 days; study wt. 1.55±0.05 kg) were compared with 22 studies in 13 normally grown (AGA) infants (gest. age 29.3±0.4 wks; birthwt. 1.15±0.04 kg; study age 21±2 days; study wt. 1.27±0.06 kg).

The energy and macronutrient intakes were similar in the two groups. The SGA infants had decreased absorptions of fat and protein and hence an increased energy loss in excreta (30±2.8 vs 18.2±1.5 Kcal/kg.d; p<0.001). The higher energy expenditure of SGA infants (67.4±1.3 vs 62.6±0.8 Kcal/kg.d; p<0.005), was associated with increased fat oxidation. Despite lower energy storage (58.2±3.7 vs 67.8±3 Kcal/kg.d; p<0.05), SGA infants were gaining weight, length and head circ. at higher rates that the AGA group. The energy storage/gram of weight gain was lower in the SGA group (3.0±0.14 vs 4.26±0.26 Kcal/g.wt. gain; p<0.001) reflecting the lower fat (22±2% vs 33±2.5%; p<0.001), and lower protein (8±0.6% vs 12.5±1%; p<0.001) composition of weight gain in the SGA group.

44 POSTNATAL DEVELOPMENT OF ADIPOSE TISSUE IN INFANTS OF DIABETIC AND OBESE MOTHERS.

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The relations between adipose tissue development at birth and later expansion of fat mass, and the behaviour of fat mass and fat cell growth from birth to 12 months of age have been studied in 42 infants on strictly controlled calorie intake. Body fat mass (BFM) was calculated by anthropometric measurements, according to Dauncey (Arch Dis Childh 52: 223, 1977). Fat cell weight (FCW)was evaluated on microsamples of adipose tissue from the gluteal region, using butterfly needle, having obtained written consent from both parents. The averages of the neonatal values were: BW 3249+69 g, BL 49.2+0.3 cm, BFM 439+19 g, sum of the skinfold thickness (ΣST) 19.4+0.7 mm, and FCW 0.22+0.02 µg. In the first 3 months of life a marked increase of fatty tissue from 13.4+0.4 to 20.3+0.8 % of total body mass was observed. Subsequently, a sharp decrease in the relative amount of BFM occurs, probably related to an increased energy expenditure. No sex related differences in BW, BFM, ΣST or FCW were found throughout the study. No significant difference in BFM, ΣST, and BFM as % of BW was observed at birth and at 3 or 6 months of age in infants of obese or diabetic mothers in comparison to infants of normal mothers, and no significant correlation was found between maternal adiposity or glucose tolerance and adipose tissue development in the first 6 months of life. Thus, in infants on strictly controlled calorie intake, obesity or diabetes in the mother do not relate to the rate of fat accumulation.

45 INFLUENCE OF GESTATIONAL AGE (GA) ON AMIKACIN (A) KINETICS IN THE NEONATE.

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The effect of GA on A kinetics was studied in 29 neonates(29-41 wks GA;0.9-4.0kg birth weight).A was given at 7.5mg/kg for 6-12 days.After last dose,the plasma and urinary concentrations were measured for 200hrs,the data fitted by nonlinear analysis,and parameters of a compartmental model calculated.(Initial(a)and terminal(b)half life in hrs;central compartment(c)and steady state (d)volumes of distribution,l/kg;body clearance(e),ml/min/kg).Linear regression analysis of the parameters vs GA was performed. Results:

	a)$T_{½}$in	b)$T_{½}$ ter	c) Vc	d)Vdss	e) Cl
mean (±SD)	6.8(2.8)	63(33)	.5(.2)	.8(.2)	.8(.3)
r	.76*	.13**	.63*	.6*	.2**

r = linear regression coefficient, *=p<.01, **=ns.
Though not linearly correlated to GA,Cl was lower in small preterm infants with GA<34wks(0.7+0.2SD vs 1.0+0.4,p<.05)This accounts for the higher serum accumulation of A found in small prematures.However the ratio Vc/Vdss was inversely related to GA(r=.55,p <.05)indicating that A penetration into tissues increases with GA. Thus the effects of maturation on serum and tissue accumulation of A are opposite.Long term urinary recovery showed that 5% of the total administered A is retained in the body and slowly released. Though small,this amount may be relevant for the potential nephrotoxicity of the aminoglycoside antibiotics.

46 PLASMA PROLACTIN LEVELS AND SODIUM BALANCE IN PRETERM INFANTS

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The role of prolactin(PRL) in the adaptation of premature infants to the alterations of sodium balance was investigated by measuring plasma PRL levels serially in a group of low-birthweight premature infants with(group S) and without(group NS) NaCl supplementation.The study was performed on the 7th day and weekly intervals thereafter until the 5th week of life. NaCl supplementation was given in a dose of 3-5 mEq/kg/day and 1,5-2,5 mEq/kg/day for 8-21 days and 22-35 days,respectively. It was demonstrated that before NaCl supplementation plasma PRL concentration was similary elevated in the two groups(5980,6±668,2 mU/l in group S versus 5859,3±739,7 mU/l in group NS) and without supplementation it remained at about the same level throughout the study.When supplemental sodium was given plasma PRL level declined with age at a steady rate to the mean value of 3276,6±681,5 mU/l by the end of 5th week.In the 3-4th weeks it proved to be significantly higher in the NS than in the S group. It is concluded that physiological sodium depletion may account for the prolonged hyperprolactinaemia and PRL might have some importance in the control of sodium homeostasis in low-birthweights infants.

47 PLASMA IMMUNOREACTIVE SOMATOSTATIN (IS) CONCENTRATION IN THE PRETERM NEONATE.

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The volume density of pancreatic somatostatin cells is 20 times higher than in the neonate than in adults. IS is detected early in hypothalamus and in the digestive tract of human fetuses. Since these findings suggest an increased production of S, we measured plasma IS by a radioimmunoassay which detects S-14 and S-28 and does not cross react with the other gastrointestinal peptides. The recovery of S-14 and S-28 was greater than 75 %. The postnatal evolution was studied in 18 infants (gestational age : 33-37 weeks, birthweight 1700-2140 g). At 2-8 hours of age mean (+SEM) IS concentration was 21±2 pmol/l vs 11±1 in 30 control adults. There was no significant change on the second day : 24±2 pmol/ and at the age of 8 days : 25±2 pmol/l. No correlation was found between IS concentration and gestational age. The effect of oral feeding (20 ml pasteurized breast milk) was studied at the age of 2 days in 8 infants (GA = 30-39 weeks ; bw : 1500-2180 g). Basal IS concentration was 21±4 pmol/l. No change was found at 5 minutes (22±4) and 15 minutes (19±1)but a decrease was found at 30 minutes 16±2 and 60 minutes 15±2 pmol/l ($p < 0.05$).

These data suggest a hyperproduction of IS in the newborn infant which persists throughout the first week of life. The stimulatory effect of feeding on S secretion in adults is not found in preterm neonates. Therefore S could play a particular role in the functions of the neonatal pancreas liver and digestive tract.

48 The Ontogeny of Posterior Pituitary Hormones in the Human Foetus.

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Posterior pituitary polypeptides, Arginine-vasopressin (AVP), Oxytocin (OT) and Arginine Vasotocin (AVT) were extracted and measured by highly specific and sensitive radioimmunoassay on 42 human foetus 10-24 weeks gestation, and 19 fresh still births or neonatal deaths 26-40 weeks gestation. At 10-14 weeks gestation the mean content of AVP is 2.06ng/pituitary and the mean content of oxytocin is 0.54ng/pituitary, giving a ratio of 3.8. Between then and term, the AVP content increases over 100 fold and the oxytocin nearly 1000 fold. The molar ratio of AVP : OT is fairly constant until 10 weeks prior to term, hence it decreases to unity at term. AVT, a polypeptide not found in mammals after birth, increases from 0.43ng at 10-14 weeks to 14ng at 20-24 weeks but is not demonstrated in more mature human pituitaries. It is possible that this hormone is important to the foetus in its fluid environment in utero.

49 AGAROSE GEL ISOELECROFOCUSING OF RED CELL GALACTOSE-1-PHOSPHATE URIDYLTRANSFERASE. A NEW MUNICH VARIANT AND TWO FORMS OF THE DUARTE VARIANT.

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Isozymes of uridyltransferase (EC 2.7.7.12) were studied by isoelectrofocusing on thin-layer agarose gels (AGIF) with the pH range 4-7. Separation of the isozymes was better than on polyacrylamide gels. The Duarte variant was divided into two types (D1 and D2) according to the enzyme activity. The activity in D1 was 20-30% higher than in D2 and the intensity of AGIF bands was also correspondingly stronger in D1. The enzyme activity (µmol/hr/g Hb) and AGIF patterns of various genotypes are summarized in the following table:

genotype	activity	(n)	IF band	IF points(pHs)
N-N	29.7±2.3	(18)	4	5.40-5.60
D1-N	30.2±2.1	(16)	6	5.20-5.60
D2-N	22.3±1.8	(18)	5-6	5.25-5.60
D1-D1	27.3	(1)	5	5.20-5.50
D2-D2	12.5,12.9	(2)	4	5.25-5.50
LA-N	33.5±0.7	(3)	6-7	5.10-5.60
Bern-N	23.2	(1)	6	5.40-5.80
Munich-N	37.7	(1)	5	5.30-5.60

These results show the polymorphism of uridyltransferase and indicate the existence of several variants.

50 OXIDANT EXPOSURE OF NORMAL AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT RBC:EFFECTS ON PHAGOCYTOSIS, INTRAERYTHROCYTIC AND MEMBRANE PARAMETERS.

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Glucose-6-phosphate dehydrogenase(G6PD)deficiency affects large pediatric populationsin the world.Increased hemolysis occurrs mostly after exposure to oxidants,can be intravascular or by phagocytosis.The objcetives of this study were to determine phagocytosis by microscopy and Cr^{51}labelling of normal and G6PD deficient RBC exposed in vitro to oxidants,and relate them to metabolic and membrane changes.Exposure of cells to oxidants affected both No. of RBC phagocytosed and percentage of phagocytes involved: 3mM paraminophenol resulted in an eightfold increased in phagocytosis and a fifty percent involvement of phagocytes,both in normal and G6PD deficient RBC,in a decrease of GSH to 1%,of free SH membrane protein to 31% of normal,the appearance of membrane protein and hemoglobin aggregates and methemoglobin formation of 55%.ATP concentration decreased to 80%.RBC filtrability time increased 3x normal.In contrast RBC exposure to β naphtol did not affect phagocytosis or oxidise SH groups,no aggregates were formed yet methemoglobin increased to 10%,ATP concentrations decreased to 55% and filtrability time increased 2,8x preincubation level.Exposure to phenylhydrazine,hydroxylamine and hydroquinone showed intermediate results.It is thus evident that different oxidants caused markedly different RBC metabolic and membrane changes which were reflected in their phagocytosis.The type of change produced may determine the locus and nature of RBC destruction.

51 β-THROMBOGLOBULIN(βTG)RELEASE BY HUMAN BLOOD PLATELETS AFTER IMMUNOLOGICAL STIMULI.

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Little is known about molecular events involved in the human platelet activation and secretion of α granule proteins during inflammation.We have studied the βTG release(as a marker of α granule release)by radioimmunoassay,after a 20 min incubation of platelets with opsonized-zymosan at 37°C.Controls were unstimulated or zymosan-stimulated platelets,which released ng 172/$5x10^8$ platelets(SE 53,n=6).The release was completely abolished by inhibitors of respiratory chain and prostaglandin synthesis.Opsonized-zymosan induced a greater release of βTG(ng 1680±121,n=6;$p<0.001$).The inhibitors reduced the release(ng 1086±53;$p<0.01$).By increasing the H_2O_2 platelet production with the addition of NADH(1mM) a decrease of βTG was shown(ng 530±36;$p<0.01$);the addition of catalase increased it(ng 1880±121;$p<0.01$).In the presence of atropine(1mM)no release occurred,also after the addition of NADH or catalase.Thus platelet cGMP and H_2O_2 generating system modulate α granule release by immunological stimulus.

52 EPSTEIN-BARR VIRUS SPECIFIC CELL-MEDIATED IMMUNE REACTIONS IN INFECTIOUS MONONUCLEOSIS,CERTAIN IMMUNODEFICIENCIES AND DURING IMMUNOSUPPRESSION

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Previously we found that crude extracts of Epstein-Barr virus(EBV)-genome carrying cells,as well as purified EBV nuclear antigen(EBNA) cause leukocyte migration inhibition(LMI) in healthy seropositive(SP)but not seronegative donors. Enrichment of extracts in early antigen(EA) and virus capsid antigen(VCA) did not change the LMI of SPs.Patients in the acute phase of infectious mononucleosis(IM) (within 1 month after initial symptoms) and those in chronic mononucleosis (persistent clinical symptoms after 1 year),as well as some children with ataxia-teleangiectasia,Hodgkin- or non-Hodgkin lymphoma with elevated antibody titers to EBV antigens showed only EA/VCA directed LMI without EBNA sensitization. In IM,the appearence of LMI to the different EBV antigens corresponded to that of antibodies.Emergence of suppressor T cells (OKT4/OKT8 ratio < 1) and absence of EBV specific memory T cells in the outgrowth inhibition assay were found in most patients with "mono-type" LMI pattern.In addition,lymphocytes derived from IM patients in the acute phase suppressed EBNA- but not EA/VCA directed LMI in healthy SPs.Furthermore,we found that EBNA directed LMI is exclusively due to T cells and recuires the presence of macrophages or interleukin-1.

53 CELL MEDIATED IMMUNITY, SERUM AND LYMPHOCYTE ZINC LEVELS IN HODGKINS DISEASE

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Serum, lymphocyte zinc levels and cellular immunity was assessed by atomic absorption spectrophotometry and conventional methods in 22 childrens with Hodgkins disease (age between 3 and 18 years) and in 13 healthy age matched children. The effect of zinc on the in vitro response to phytohemagglutinin (PHA) was also evaluated. The serum and lymphocyte zinc levels, total and T lymphocyte counts, lymphocyte proliferative response to PHA and zinc were found to be depressed in all patients, being more striking in advanced stages of the disease ($P<0.01$). Only PPD skin test response was impaired in patients. There was a correlation between zinc levels and various parameters of cellular immunity. In vitro addition of zinc to cultures improved the patients proliferative responses to PHA. These results suggest that zinc may play an important role in the cellular immune defects observed in patients with Hodgkins disease.

54 SYSTEMIC MASTOCYTOSIS SUCCESSFULLY TREATED WITH SODIUM CROMOGLYCATE.

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Systemic mastocytosis (SM) is a disorder characterized by mast cell proliferation in various organs of the body. Signs and symptoms are attributed to histamine release. We report here a 4-year-old boy, who since age 4 months suffered from generalized skin lesions, vomiting, diarrhea, abdominal pain, flushing, transient blindness, hypotension, tachycardia, and somnolence. The diagnosis of SM was done, and oral sodium cromoglycate (SCG) (100 mg/Kg/day) in 4 divided doses or placebo were blindly administered. The child was studied for 21 months during which time he received 3 courses of SCG and 3 courses of placebo. Symptoms and signs were evaluated and recorded daily by the mother, and histaminemia was determined during each course. During active treatment signs and symptoms markedly improved, and histaminemia decreased, whereas they recurred during the placebo periods, and histaminemia increased.
In conclusion, as SCG inhibits mast cell degranulation, and because it is minimally absorbed from the gastrointestinal tract, the results reported here suggest that the clinical manifestations of SM may be due to histamine release from intestinal mast cells.

55 IN VITRO LYMPHOCYTE REACTIVITY TO CANDIDA ANTIGEN (CA) AFTER KETOCONAZOLE (KC) AND TRANSFER FACTOR (TF) TREATMENT IN CHRONIC GRANULOMATOUS CANDIDIASIS.

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A girl, 13 months of age, presented with generalized granulomatous skin, hair and mucosal candidiasis. Serum immunoglobulins, complement components, granulocyte functions (phagocytosis and fungicidal power), T cell subsets, mitogenic and allogeneic lymphocyte stimulation, natural killer cell activity and immune interferon production were all found to be normal. No circulating immune complexes were detected. However, the patients' lymphocytes failed to respond in vitro to CA; the intradermal test with CA was also negative. Ketoconazole, an antimycotic drug, 5 mg/kg twice daily for two months, spectacularly cleared all lesions. Afterwards, four monthly injections with TF were given. Intradermal reactivity to CA was observed after the second TF injection. The lymphocyte responsiveness to CA in vitro increased progressively and was strongly positive 3 months after the last injection. The level of CA precipitins in serum, which was very high (11 lines) before treatment, decreased to 4 lines. No serum inhibitor of lymphocyte proliferation to CA could be demonstrated in the patient's serum before or after treatment. The child remained free of lesions until the last control examination. Specific unresponsiveness against CA could be due to suppressor cell induction by excessive antigen load, to the suppressive activity of high antibody levels on T cell function or by a specific defect in antigen reactivity that was restored by TF injection.

56 THE EFFECT OF KIDNEY TRANSPLANTATION (TP) ON RENAL ANEMIA IN CHILDHOOD.

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Anemia in children with terminal renal failure is characterized by insufficient hematologic compensation with low serum erythropoietin (EPO) levels, inadequately reduced oxygen (O_2) affinity of hemoglobin (Hb), and iron overload induced by blood transfusions. The influence of TP on these alterations has not been investigated in children so far. We studied 15 pediatric patients (mean age 12.6 ± 2.8 yrs) by serial measurements of Hb (Coulter Counter S), half saturation pressure of Hb (p50, by Hemoscan), EPO (fetal mouse liver cell assay) and serum ferritin (SF, by immunoradiometric assay). Mean Hb increased from 57 g/l pre TP to 117 g/l seven weeks after TP and subsequently was inversely correlated with serum creatinine ($r=-0.70$). 24 months after TP mean EPO (39.5 U/l) and p50 (25.9 mmHg) were in the range of normal children. Mean SF dropped from a pre TP level of 559 µg/l gradually to 77 µg/l 20 months after TP. Decreased SF values (<40 µg/l) indicating iron deficiency were detected in 38% of patients. Our results indicate that from the third month after TP Hb is dependent mainly on GFR. The insufficient compensation of anemia by increased p50 before TP is normalized after grafting. Intact endocrine activity of the graft is documented by normal EPO. Successful TP contributes to the reduction of iron overload induced on prolonged dialysis.

57 INVESTIGATIONS ON THE RELATIVE CHOLESTEROL CONTENTS OF LIPOPROTEINS IN CHILDREN.

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Quantitative lipoprotein (LP) electrophoreses is a very useful method for the examination of cholesterol metabolism in children[1]. It is essential to know the differences in the relative cholesterol contents of LP of children and adults and to study the influence of food intake in cholesterol contents. Therefore pure LP-fractions of children and adults were isolated before and after a test breakfast. After dialyzation, lyophilization and further intensive drying cholesterol and protein contents were determined. It could be shown for the first time by t-test for paired data and by comparison of regression lines that there is no statistically significant difference in LDL- and HDL-fractions neither between children or adults nor before and after a test breakfast. It can be concluded that fasting is not necessary for the determination of LDL- and HDL-cholesterol and that the calculation factors derived from adults may be used for children.

1. K. Dörner et al.: Clin.Chim.Acta 119, 99 (1982).

58 CONTROL OF BREATHING DURING EXERCISE IN HEALTHY CHILDREN:

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Occlusion pressure (P.1) and mean inspiratory flow (Vt/Ti) are thought to be indices of respiratory drive, while inspiratory time as fraction of total respiratory time (Ti/Ttot) represents an index of respiratory timing. In order to study the relationship between respiratory pattern (P.1, Vt/Ti, Ti/Ttot) and ventilation (VE), heart rate, O2-uptake (VO2), CO2-output (VCO2), respiratory quotient (RQ) and basal metabolic rate (BMR), measurements at rest and during submaximal exercise have been investigated. In 22 healthy children, aged 7-17 years bicycle ergo-oxymetry up to a working capacity at heart rate of 170/min (0.5-2.5 watt/kg lean body mass (LBM)) has been performed. The steady state measurements show a linear increase of P.1 (374 Pa/watt/kg LBM, age-dependent) and of Vt/Ti of 327+39.1 l/s/watt/kg LBM, while Ti/Ttot remains unchanged (0.45+0.044). A similar increase was found in VE, VO2 (11 +0.8 ml/watt/kg LBM) and VCO2 (10.1+0.7 ml/watt/kg LBM), both age-independent. The ratios P.1/VCO2 and P.1/VE are in wide ranges slightly increasing during exercise. It is concluded that respiratory timing is not affected by exercise but respiratory drive increases curvilinear (calculated per unit metabolic load) with increasing working load. The increasing P.1/VE ratio demonstrates the inverse relationship to the effectiveness of thoracopulmonary system to "convert" neuromuscular signals of ventilation.

59 CLINICAL AND BIOCHEMICAL FINDINGS IN THREE UNRELATED FAMILIES WITH FABRY DISEASE.

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Three obligate and one suspected heterozygotes were discriminated from controls (n=15) by the low ratio of α-galactosidase/ ß-hexosaminidase activity in serum (0.9-1.5) and leucocytes (2.5-4.9),using p-N-phenyl-α-D-galactopyranoside as substrate (controls 2.4-4.8 and 9.0-25.5, respectively). All obligate heterozygotes showed cornea verticillata and alterations of conjunctival vessels, two additionally slight cutaneous angiectasias.
Routine laboratory data, ECG, echocardiography, chest X-ray, EMG, NCV and EEG were normal in all heterozygotes (14 - 45 years). Interestingly cornea verticillata disappeared half a year after wearing soft or hard contact lenses.
One of the hemizygote patients (n=3) showed relative high residual α-galactosidase activity in serum and leucocytes. In one healthy brother of an affected hemizygote serum α-galactosidase activity was found to be 5-10 % of that of controls by repeated determinations, whereas activity in leucocytes was normal as tested with the p-N-phenylsubstrate.

60 PLATELETS IN PATIENTS WITH REDUCED SELENIUM STATE

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Selenium deficiency was observed in chinese children with endemic cardiomyopathy of unknown pathogenesis. We measured similar low selenium values in blood and hair of dietetically treated patients with phenylketonuria. Selenium was estimated by instrumental neutron activation analysis and glutathione peroxidase with t-butyl hydroperoxide or hydrogen peroxide as acceptor substrates. Glutathione S-transferase was measured with 1-chloro-2,4-dinitrobenzene. Platelets are very rich in selenium. In dietetically treated PKU patients the platelet selenium content decreases to less than 5o% of the control values. The platelet glutathione peroxidase activity is reduced to 2.12-o.92 $U/10^{11}$, versus 8.3-1.15 $U/10^{11}$(t-BOOH) in healthy children. The activity is equally reduced with t-butyl hydroperoxide and hydrogen peroxide, thus indicating that the glutathione S-transferase measured with 1 chloro-2,4-dinitrobenzene has no peroxidatic activity. After selenium supplementation the glutathione peroxidase activity normalises within 2 weeks. The patients do not show clinical signs of impaired platelet functions.

61 GRANULOPOIESIS IN PREMATURE NEWBORNS: POSSIBLE ROLE OF A PLACENTAL HUMORAL FACTOR.

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Newborn infants have marked neutrophila, "shift to the left" and monocytosis during the first 4 days of life, along with a significant rise in their urine and serum Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) levels (1). In the present study, the same parameters were evaluated serially in a group of 40 premature infants. Also, the capacity of the placenta to produce GM-CSF was determined in 20 premature and 10 fulterm neonates. Urinary and leukocyte GM-CSF were assayed by the soft agar culture technique, and the production of placental GM-CSF was determined in the supernatant of 7-days placental cultures. Significant reduction in both neutrophilia and urinary GM-CSF levels were found in the premature newborns, as compared with the findings in fullterm neonates. Placental GM-CSF production was marked in full term newborns,slightly decreased (by 20%) in "large" prematures (>1500g) and significantly decreased (by 40-60%) in small prematures (<1500g). These results suggest that the maturity of the placenta is important in establishing the neutrophilia of the newborn. It appears that the relative reduction in neutrophil counts in the premature neonates is related to the reduced levels of GM-CSF produced by their immature placenta.

1. Barak Y. et al. Ped. Res. 14: 1026, 1980.

62 AGGREGATION OF RED CELLS FROM FULL-TERM AND PREMATURE NEWBORN INFANTS:

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Red blood cells (RBC) aggregate in standing or slowly flowing blood in vitro and in vivo. Moreover, RBC aggregation causes flow stagnation and is therefore an important determinant of blood flow. RBC aggregation was studied in 10 normal adults, 10 full-term and 10 premature infants (placental blood) using a Rheoscope and light transmission, zeta sedimentation and microscopic evaluation. RBC were studied at a hematocrit of 0.45 1/1.

The following results were obtained: 1) RBC in autologous plasma: Strong RBC aggregation in adults; little aggregation in full-term infants; and hardly any aggregation in premature infants. 2) RBC in 1% dextran (mol wt 500,000): Strong and not significantly different RBC aggregation in the 3 groups. 3) Adult RBC in neonatal plasma: RBC from one adult donor (blood group 0) in the neonatal plasma samples showed the same aggregation pattern as neonatal RBC in neonatal plasma. 4) The results of the 3 aggregation methods showed significant correlations to each other as well as positive relationships to the plasma viscosity and fibrinogen concentration. These data indicate that RBC aggregation in neonates is decreased as a result of different plasma composition while neonatal RBC are able to aggregate as strong as adult RBC. Low RBC aggregation might explain why thromboembolic complications in neonates are rare despite physiologically high hematocrit.

63 BREASTFEEDING, COW'S MILK-FEEDING AND LEUKOCYTE COUNTS DURING THE FIRST YEAR OF LIFE

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The significance of nutritional factors on the leukopoiesis has been uncertain. The total leukocyte (TLC) and differential (DC) counts were therefore studied longitudinally in 45 infants. Of these, 35 were breastfed for 6 months or more and 10 fed cow's milk mixtures from their first months of life.- The TLC were significantly higher in the cow's milk-fed infants as compared to the breastfed infants from 4 to 6 months of age ($p \leq 0.003$). The TLC ($x10^9/l$) in the cow's milk-fed and breastfed infants were 11.7±0.87 and 7.8±0.52, 11.6±1.01 and 7.0±0.52, and 13.2±1.27 and 7.9±0.58 at 4, 5, and 6 months of age, respectively (mean value ± 1 SEM). The absolute number of eosinophil and neutrophil granulocytes, and lymphocytes were also significantly higher than those observed in breastfed infants from 3 to 4, 4 to 6, and 4 to 6 months of age, respectively, while the monocyte counts were significantly higher at 9 months of age.- The study demonstrates the different leukocyte responses in infants on different dietary regimens, and the need for a reevaluation of the reference values for the TLC and DC during the first year of life. The mechanisms involved have to be evaluated.

64 PLASMA AND URINARY C-PEPTIDE MEASUREMENT AS AN INDEX OF BETA CELL FUNCTION IN HEALTH AND DISEASE.

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C-peptide (CP) was determined by RIA in the plasma and/or urine of several groups of children & adolescents aged 4-20 yrs. In part of them plasma CP was determined also after OGTT or iv glucagon (0.3 mg/kg). The results for the individual groups were:

Diagnosis	n	C-Peptide Plasma (pmol/ml) Basal	Peak (iv gluc.)	Urine (pmol/mg creat^{-1}24h^{-1})
Control	17	0.20-0.80	0.8 -1.0	16.70 - 39.8
Obesity	4			8.34 - 27.7
IDDM - At Dg.	82	0.08-0.47	0.095-0.75	
0-1 y	25	0.05-0.18	0.050-0.20	2.20 - 16.0
1-3 y	57	0.03-0.11	0.010-0.14	0.50 - 1.7
3-7 y	85	0.08-0.33		
OGTT	12	0.08-0.29	0.085-0.60	
Insulinoma	2	0.09-0.36	0.145-0.18	8.0 - 10.0
Hypoglycemia	3	0.06-0.11	0.115-0.18	

In hGH deficiency urinary CP was low (9-14 pmol/mg creat^{-1}24h^{-1}). We conclude that CP measurement, especially urinary, is a very useful aid in evaluation of beta cell function in normal and pathological states in which changes in insulin secretion are suspected.

65 PROINSULIN, INSULIN AND C-PEPTIDE IN AMNIOTIC FLUID DURING THE LAST TRIMESTER OF DIABETIC PREGNANCY.

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Proinsulin,insulin and C-peptide and insulin-binding antibody and glucose were determined in amniotic fluid(AF)samples from 43 insulin treated diabetics and 17 nondiabetic controls during the last trimester of pregnancy. Only 3 diabetic patients had insulin-binding antibodies in AF although antibodies in maternal serum were found in 22 of the mothers. There was no relation between insulin-binding antibodies and C-peptide in AF. The average AF-level of proinsulin, 0.07 nmol, insulin 0.08 nmol/l,C-peptide 1.17 nmol/l and glucose 2.09 mmol/l were markedly elevated(<0.001) in diabetics as compared to controls.C-peptide was related to both insulin($r=0.69$)and proinsulin($r=0.35$)in the diabetic group only. Infant birth weight was significantly correlated in both groups with AF C-peptide. Diabetic pregnancies associated with neonatal morbidity,transient tachypnea,polycythemia,hypoglycemia, hypocalcemia,hyperbilirubinemia and feeding problems had significant higher mean AF-concentrations of both insulin and C-peptide than pregnancies without neonatal morbidity. C-peptide and insulin in these two subgroups were overlapping. Though it can be concluded that fetal betacell function is exaggerated in diabetic pregnancy our data do not suggest that individual AF C-peptide values can serve to predict outcome in the individual case.

66 METABOLIC RESPONSE TO MAXIMUM EXERCISE IN DIABETIC AND NON-DIABETIC CHILDREN.

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An incremental work load test, using a bicycle ergometer, was performed on 7 diabetic (age 11.4-15.4 yrs) and 9 non-diabetic children (age 9.9-14.9 yrs). During the test to maximum work capacity, direct measurement of oxygen consumption (VO_2) was obtained together with blood sampling for analysis of blood glucose, lactate, pyruvate and ketone bodies; and plasma insulin, growth hormone and cortisol concentrations.

There was a significant difference in the maximum VO_2 between the two groups (diabetic mean $\pm$ SD 33.0 $\pm$ 5.5 ml/kg/min; non-diabetic mean $\pm$ SD 42.5 $\pm$ 8.9 ml/kg/min, $p<0.05$). There was also a significant difference in the change in the blood glucose with all diabetics showing a marked fall (diabetics mean change $\pm$ SD 3.55 $\pm$ 2.28 mmol/l; non-diabetics 0.23 $\pm$ 0.16 mmol/l, $p<0.03$). The original blood glucose at the start of the exercise was different between the two groups; diabetic 10.5 mmol/l (range 3.3-19.2 mmol/l), non-diabetic 5.2 mmol/l (range 4.7-5.6 mmol/l). All the diabetic children are C-peptide negative. C-peptide in the non-diabetic group showed a fall during the exercise (mean change $\pm$ SD 244 $\pm$ 220 pmol/l). Plasma free insulin in one diabetic child rose two-fold, and fell to 50% in one non-diabetic child. No significant differences between the two groups were seen in the response to blood lactate, pyruvate and ketone bodies to maximum exercise.

67 ALBUMIN EXCRETION INDUCED BY EXERCISE IN DIABETIC CHILDREN-Effect of metabolic control(m.c.) and duration of diabetes. Dahlquist G,. Aperia A., Carlsson L., Linné T., Persson B., Thorén C., Wilton P., Dep. of Pediatr. St. Göran's Hospital, Stockholm, Sweden. - Children with diabetes for 3.5-16.8 yrs(n=19) without signs of nephropathy were examined by standardized exercise test. Albumin excretion rate during rest, exercise,GFR,FF,C_{PAH} were studied a) when children were in poor m.c.($HbA_{1c} \geq 12\%$), b) after 6 wks of improved m.c., i.e. normalized blood glucose profiles and sign. decreased HbA_{1c},c) in 8 healthy controls. Metabolic improvement decreased the provoked albumin excretion rate in diabetics($p<0.025$). In improved m.c. albuminuria during exercise correlated to duration of diabetes ($p<0.05$ Spearman's, $r=0.44$).Albuminuria was unrelated to blood glucose, diuresis or heart rate, but correlated to systolic blood pressure($p<0.05$). GFR and FF were increased in all diabetics and did not change with duration of diabetes.

Conclusions: 1) Albumin excretion rate provoked by exercise is partly dependent on m.c. in diabetic children i.e. reflects a reversible functional effect of diabetes on the kidney. 2) Mean albumin excretion rate in poor and improved m.c. correlates with duration of diabetes indicating that part of the provoked albumin excretion is dependent on the progressive irreversible morphological changes in diabetic nephropathy.

GFR = glomerular filtration rate, FF = filtration fraction, C_{PAH} = clearance PAH.

68 ENZYME REPLACEMENT THERAPY IN β-GLUCURONIDASE DEFICIENT MICE BY BONE MARROW TRANSPLANTATION.

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Enzyme replacement therapy was successfully accomplished in β-glucuronidase - deficient C3H/HeJ mice following transplantation of bone marrow cells obtained from normal BALB/c donors. Marrow recipients were prepared for transplantation by fractionated total lymphoid irradiation (TLI). Enzyme activity increased from 20.5±7.0 to 180.0±30.2 nmoles/mg protein/hr in the liver ($p<0.001$) and from 8.2±2.0 to 17.5±5.0 nmoles/ml/hr in the plasma ($p<0.05$) at 50 days post marrow infusion. β-Glucuronidase activities in normal BALB/c donors were 148.0±35.0 nmoles/mg protein/hr in the liver and 20.0±5.0 nmoles/mg protein/hr in the plasma. Normal enzyme activity was maintained in treated mice for at least 100 days post marrow transplantation as documented by repeated liver biopsies and examination of plasma samples. The marrow donors and the recipients were fully histoincompatible. Both immunologic rejection of the marrow allograft and graft versus host disease (GVHD) were prevented due to the prior conditioning of the recipients with TLI, resulting in bilateral transplantation tolerance of host versus graft and graft versus host. The data suggest that allogeneic bone marrow transplantation may provide a possible therapeutic approach for certain enzyme deficiency syndromes.

69 CYSTINOSIS-DEFECTIVE LYSOSOMAL TRANSPORT SYSTEM FOR CYSTINE. WA Gahl, N Bashan, F Tietze, R Steinherz, JD Schulman,(Spons. M Nitzan). NICHD & NIADDK NIH, Bethesda, Md. USA

The lysosomes of human leucocytes can be loaded with cystine to supracystinotic levels by exposure of the cells to cystine dimethyl ester, which penetrates into the lysosomes and is hydrolyzed there to the free amino acid. The mean clearance rate of cystine from loaded normal leucocytes is much faster than from cystinotics with heterozygotes intermediate (all p less than 0.01) This method can improve heterozygote detection of cystinosis. There is negligible efflux of cystine from preloaded isolated lysosome rich cystinotic leucocyte granular fractions (GF) compared to normal (mean half time 46 min.) In normals loss of GF cystine is quantitatively accounted for by cystine recovered in the efflux medium. Efflux of cysteine, leucine, tryptophan or methionine from isolated cystinotic lysosomes is not substantially impaired. Normal lysosomal cystine transport demonstrates saturation kinetics with half normal V max in heterozygotes and negligible rates in homozygotes. Normal transport is ATP,pH and temperature dependent bidirectional, with stimulation of influx by increased intralysosomal cystine concentrations, stimulated by valinomycin and inhibited by ouabain. Efflux of cystine is resistant to n-ethyl maleimide and does not involve prior reduction to cysteine. Elucidation of the etiology of cystinosis had led to the discovery of a previously unidentified carrier mediated lysosomal cystine transport system.

70 CNS MALFORMATIONS IN KRAKOW REGION-PREVALENCE,SEASONAL VARIATION AND CYTOGENETIC EXAMINATION.J.J.Pietrzyk,Dept.of Medical Genetics Institute of Pediatrics,Kraków,Poland.

From 1979 till 1981; 46,818 live- and stillborn neonates were prospectively ascertained in Kraków region, what constituted 99% of all deliveries in this area. The overall prevalence (‰) of CNS malformations was 1.35.The frequency of anencephaly was 0.23,spina bifida and encephalocele:0.73,isolated hydrocephaly: 0.26, and other CNS malformations: 0.13.The observed rates were below the medium European level.The striking female preponderance was observed for all CNS malformations except hydrocephaly.Among 39 newborns who have had cytogenetic examination 38 revealed normal karyotype,however two children demonstrated small structural variants.One newborn with abnormal karyotype showed trisomy 18.The seasonal trend of the probands' birthdate and the date of the last menstrual period of mothers was tested by the Edwards method for estimation of cyclic trends.The peak incidence of CNS anomalies was observed in Spring and in Fall.The test for seasonal trend revealed no significant cyclic variation of the birthdate ($\chi^2=.750$ $p>.6$).The highest numbers of conceptions were observed for June,August and December and the lowest for October,however the trend was also nonsignificant ($\chi^2=.327$ $p>.8$).

71 MYOPATHIC FEATURES IN GLYCOGEN STORAGE DISEASE TYPE 3

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Glycogen storage disease type 3 expresses genetic heterogeneity among tissues. In our case material, 16 out of 17 cases showed, in addition to hepatic features, muscular amylo-1,6-glucosidase deficiency. This included all 9 North African cases examined. The clinical spectrum varied from a severe neuro-myopathic disease leading to complete paralysis, through slight muscular weaknesses on exertion, to no clinical evidence of myopathy. Yet all cases examined had myopathic EMG changes and elevated serum enzymes. Some had, in addition, neurological involvement. Electron microscopically, destruction of myofibrils and segmental demyelinisation of the sural nerve was found in the most severely affected case. ECG showed biventricular hypertrophy in 6 cases. Echocardiography was less revealing. A family with three affected brothers permitted us to study the progressive myoneuropathic features commencing after 20 years of age. A striking but temporary remission of symptoms was attained by continous high amino acid administration. The exact metabolic abnormalities leading to this progressive neuromyopathic disease, at an age when the liver usually ceases to cause serious metabolic problem, remain to be determined.

72 Effect of Thymic Humoral Factor (THF) on T-cell subsets.

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THF is a peptide hormone from calf thymus which induces differentiation of immature T-cells in conditions of immunodeficiency and immunosuppression. The use of anti-human T-cell monoclonal antibodies of the OK-T series has permitted the evaluation of THF's effect on the maturation of T-cell subpopulations in 6 immunosuppressed pediatric patients with neoplastic diseases. OK-T3 measures % of mature T-cells, OK-T4 the helper subset, and OK-T8 the suppressor/cytotoxic subpopulation. The patients included: 4 with lymphoproliferative disorders 2 of whom with severe Herpes simplex infection, one with Histiocytosis X and one with Neuroblastoma and Varicella pneumonitis. All were treated with THF during 2-4 weeks by daily I.M. injections. The classical parameters of cell-mediated immunity were reconstituted, OK-T3, -T4 and T8 subsets returned to normal in all patients, except the one with Neuroblastoma, where OK-T4 remained depressed. Further evidence of immunoregulatory effect of THF was the normalisation of the T4/T8 ratio. All recovered from their secondary infections.

73 INTERACTION BETWEEN THYMOCYTES AND MACROPHAGES (MØ): INFLUENCE ON THYMOCYTE VIABILITY AND FUNCTION BY FORMATION OF THYMOCYTE-MACROPHAGE-ROSETTES.

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The rosette-formation of Ia-negative MØ and thymocytes was investigated in different species (human, mouse, rabbit). This interaction was antigen-independent and not MHC-restricted. Immature thymocytes were involved in rosette-formation with more than 80% of the MØ, while more mature thymocytes (i.e., PNA-negative or steroid-resistent cells) showed only 5% involvement. A strong suppressive effect on rosetted thymocytes was observed: after 60 hours of co-cultivation, thymocyte viability was less than 30% in comparison to controls without MØ; mitogen-induced proliferation was impaired more than 90% by addition of MØ. Inhibition of rosette-formation by Cytochalasin B abolished the suppressive effect, while supernatants from MØ cultures had no suppressive effect on thymocytes. Therefore, cell-cell-contact such as rosette-formation is apparently necessary for thymocyte inhibition. Suppression, however, seems to depend on the functional state of the MØ: ingestion of carbon-particles significantly diminished the inhibitory effect on thymocytes despite further presence of rosettes. The results suggest that this MØ-thymocyte-interaction might be involved in induction of the known physiological intrathymic death of thymocytes. It appears that Ia-positive MØ promote thymocyte differentiation and proliferation, as described by others, while Ia-negative MØ may control and limit the number of thymocytes released by the thymus.

74 Histamine-driven suppressor activity: role of monocytes and PGs.

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T suppressor cells express specific Histamine(H) membrane recognition sites and produce Histamine Suppressor Factor(HSF). In the present investigation we showed, using a singeneic co-colture system, that T lymphocytes triggered by H were able to suppress in a dose dependent manner T-T cell cooperation, evaluated by PHA, ConA response, using H_2 depleted population as responder cells, with a % suppression of thymidine uptake varying from 15% to 35%. This suppressor activity was monocyte dependent and was completely abolished by the addition of PGs inhibitors. On the basis of these data we showed that supernatants derived from H-activated T cells stimulate release of PGE, $PGF_{2\alpha}$, and Tromboxane(TxB_2) from monocytes in a dose dependent fashion.

	Control sups. ng/ml	HSF sups. ng/ml
TxB_2	19.3± 2.3	28.8± 2.3
PGE_2	5.5± 1.2	8.4± 1
$PGF_{2\alpha}$	1.6± 0.2	3.3± 1

These data suggest that PGs released by monocytes in response to HSF may be the final negative feed-back regulators of H-driven suppressor activity and support the notion that PGs play an important role in fine-tuning immunocompetent cells.

75 CHARACTERIZATION OF T LYMPHOCYTE SUBPOPULATIONS IN THE NEWBORN WITH SPECIFIC MONOCLONAL ANTIBODIES.

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The development of new reagents: monoclonal antibodies to T lymphocyte cell surface antigens (CSA), combined with new techniques for isolation and quantitation of T subsets by the Fluorescence Activated Cell Sorter (FACS), enables exact quantitation of those subsets. We have developed recently, a monoclonal antibody to human Thy-1 antigen (Seeger and Danon, J. Immun, Feb. 1982). We have used it in addition to monoclonal antibodies to T4 and T8 antigens expressing inducer and suppressor subsets respectively. T cell subpopulations were studied in 12 normal full term newborns aged 24 hours. Mononuclear cells were separated and stained with anti Thy-1, T4 and T8 antigens monoclonal antibodies and fluorescinated goat-anti-mouse IgG for evaluation in a FACS System. We could not detect any Thy-1 responsive cells in neonatal peripheral lymphocytes. The proportion of T8 (Suppressor/cytotoxic) cells was reduced in newborns-18.3 ± 9.6%, compared to 25.0 ± 5.8% for normal controls. T4 positive cells (helper/inducer) comprised 29.2 ± 21.1% of peripheral lymphocyte population vs 46.4 ± 8.3% for normal adults. Further studies are needed to clarify whether these T cell subsets act on B cell differentiation and on macrophages in the same manner as in adults.

76 RECONSTITUTION OF SERUM IMMUNOGLOBULIN LEVELS IN NUDE MICE BY T-CELL DEPLETED THYMUS EPITHELIUM.

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In some patients with severe combined immunodeficiency (SCID) grafted with cultured thymus tissue obtained from patients undergoing heart surgery, reconstitution of the serum immunoglobulin levels was observed prior to T-cell reconstitution. To investigate if polyclonal B-cell reconstitution without requirement of T-cells is possible nude mice (BALB/c, $H\text{-}2^d$) were transplanted with allogeneic (CBA, $H\text{-}2^K$; C57Bl/6, $H\text{-}2^b$) or human thymus that were longterm precultured or pretreated in vitro with Carrageenan for three days. None of the thymus tissue transplants showed lymphatic repopulation 9 weeks after transplantation. Histological investigation of the peripheral lymphatic tissue did not reveal any change in the thymus-dependent area. On the other hand, plasma cells and germinal centers could be found in significantly increased numbers. In addition, a normalization of the serum immunoglobulin concentrations could be found, as no specific antibodies against thymus-dependent antigens were present after immunization and T-cell function did not improve. Similar results were obtained 9 weeks after injection of irradiated thymocyte suspensions or of peritoneal macrophages from immunocompetent donors. It is concluded that thymus epithelial cells could act via macrophages on the polyclonal maturation and differntiation of B-cells without involvement of T-cells.

77 SURFACE MARKERS AND FUNCTIONAL PROPERTIES OF CSF LYMPHOCYTES IN CHILDREN WITH MUMPS MENINGITIS

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Mumps meningitis is accompanied by a marked pleocytosis of the cerebrospinal fluid (CSF). The nature and functional properties of the exudate cells are largely unknown. In this study, cryopreserved and thawed lymphocytes from 10 children with serologically proven mumps meningitis were analysed 1) by monoclonal antibodies against T cell subsets and 2) by a 5-hr ^{51}Cr release assay against mumps virus-infected target cells with known HLA specificities. The frequency of OKT8-positive cells (bearing suppressor/cytotoxic T cell markers) was significantly increased in CSF as compared to peripheral blood. (49.6 ± 7.3 % versus 24.6 ± 7.9 %). CSF lymphocytes displayed high cytolytic activity against mumps virus-infected target cells. Cytotoxicity was specific for the infecting virus and restricted by "self" HLA antigens. The results show that virus-specific cytotoxic T cells (CTL) are locally enriched in the CNS of patients with mumps meningitis. It is proposed that CTL in mumps might substantially contribute to the severity of the course of disease.

78 COW'S MILK PROTEIN HYPERSENSITIVITY (CMPH): A DEFECT IN IMMUNOREGULATION?

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CMPH is a well-known, transient, sensitivity state affecting 0.5% of infants in the first two years of life. The diagnosis is based on clinical features, and on certain immunological parameters such as the production of MIF on incubating lymphocytes with beta-lactoglobulin (BLG). In this investigation we employed the blastogenic effect of BLG on peripheral blood lymphocytes in the diagnosis of CMPH. We found good correlation between the clinical diagnosis of CMPH and the blastogenic effect of BLG in 22 patients aged 1-20 months. BLG produced a significantly higher ($p<0.00003$) blastogenic response in lymphocytes of patients with CMPH (mean stimulation index 7.7 ± SEM 0.7) than in 26 age matched controls (2.7 ± 0.4). This response was most marked in infants up to 5 months of age. Using the clinical features and the immunological parameters outlined above, we investigated the non-specific concanavalin A induced suppressor cell activity in our patient population and compared it with a group of age matched controls. The mean suppressor cell activity was 28.8 ± 4.7 in the patients 1-5 months old as compared to 55 ± 5.7 in the control group ($p<0.05$). The difference between patients and normals persisted in 5-20 months old infants. It is suggested that the development of CMPH may be due to delayed maturation of the suppressor cell population.

79 NUTRITIONAL HAZARDS OF FOOD ALLERGEN ELIMINATION IN SEVERE ECZEMA.

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21 children, aged 6m to 13yr (median 2yr), with severe intractible eczema unresponsive to conventional dermatological treatment, were treated by dietary allergen avoidance. Suspect foods were identified by history, skin testing, specific IgE antibodies, and in 6 cases by use of an elemental diet followed by introduction of single foods. In each case a dietician advised the parents how to exclude the suspect foods, and milk substitutes provided. More than 7 foods were avoided in half the cases. Once established on the diet, a 5 day diet record was completed in 19 cases, and the intake of calories, protein, calcium, iron, folate and vitamins A,B1,B2,B3,C and D were compared with the recommended dietary intake. Deficient intakes were found in 11 cases and comprised: calories 2 cases protein 3, iron 5, calcium 7, vit.A 1, folate 9, vit.D 11. All deficiencies were then corrected by further dietary advice, with the exception of vitamin D "deficiency" which was disregarded in all but one case. We conclude that children on restrictive diets for multiple food allergy must be supervised by a dietician, not only at the onset of the diet but also when the diet is in operation.

80 CHOLELITHIASIS IN CHILDREN WITH IMMUNOGLOBULIN A DEFICIENCY - A NEW GASTROENTEROLOGIC SYNDROME

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Immunoglobulin A(IgA) is the major immunoglobulin appearing in bile in the form of biliglobulin, responsible for the local humoral defense of the host. In view of this important role of biliglobulin and the possibility of recurrent gastrointestinal infections in children lacking IgA, a sonographic survey was performed in 13 children lacking serum IgA: 8 males and 5 females with age span between 1 year and 18 years. Range of serum IgA was 0-2 mgr/dl, IgA was tested by a nephelometric system and electroimmune diffusion. Children with other immunoglobulin deficiencies were excluded. Most of the patients were referred due to recurrent mucosal infections. All patients were examined by ultrasonography. Five patients had normal sonograms, in 8 pathologic sonograms were detected,in four of them a presence of choleliths was diagnosed and in the other four a billiary sludge pattern was demonstrated.

This finding adds a new gastrointestinal anomaly to those known to occur in IgA deficiency.

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81 STUDIES ON THE EFFECT OF INTRALIPID ON HUMAN MONOCYTE FUNCTION IN VITRO.

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Intralipid (IL) a soybean emulsion is widely used in parenteral alimentation. Patients, who have received large amounts of IL, have characteristic pigment deposition within the reticuloendothelial system. We examined the effect of IL on several in vitro functions of human monocyte cultures. IL (0.9-9.1 mg/ml) was added by pulse to the medium and washed away by the end of incubation period of up to twenty hours. Basal secretion of Prostaglandin E_2 or lysozyme was not altered, nor was there an increase in glucose consumption from the medium. Prior phagocytosis of IL by monocytes did not impair the production of superoxide following "activation" by concanavalin A and phosphomyrebil acetate. The rate of phagocytosis of labelled zymosan particles by monocytes was increased in a dose dependent manner (increase of 28±8%; 42±4.5%; 59±22% by 0.91; 4.55 and 9.1 mg IL/ml medium). A similar effect was observed in macrophages (derived from blood monocytes in culture). Morphological studies of these cultures showed that the lipid particles were phagocytised and remained in situ for at least 21 days during culture. In conclusion, our in vitro system showed no change in monocyte functions due to IL ingestion, except for an increase of phagocytosis.

82 POSSIBLE PATHOGENICITY MECHANISM FOR MYCOPLASMA PNEUMONIAE INFECTIONS.

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Mycoplasma pneumoniae pathogenicity in human respiratory tract infections is still unclear. H_2O_2 produced by Mycoplasma pneumoniae as its respiratory end product has been incriminated as a factor in the pathogenicity of this organism. However, the existence of tissue cell catalase questioned its effectiveness in causing injury to the host cell. We postulated that host cell catalase activity may be inhibited by M. pneumoniae, enabling H_2O_2 toxic effect to be expressed. Catalase activity of M. pneumoniae infected cells was studied using human skin fibroblasts, human epithelial cells in culture, and human erythrocytes. Catalase activity of the cells was measured with an oxygen electrode following H_2O_2 dependent O_2 production. Our results show that after 20 hours of exposure to viable virulent M. pneumoniae catalase activity of erythrocytes, and cells in culture, decreased to 40% and 20-25% respectively. It is suggested that M. pneumoniae pathogenicity is mediated through inhibition of host cell catalase thus contributing to the toxic effect of H_2O_2.

83 A NEW DISORDER OF LIPID TRANSPORT: CHYLOMICRON RETENTION DISEASE

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Seven children aged 1½ to 19 years developed fat malabsorption during the first year of life and were recently studied. Chylomicron formation was absent in that after a fat load of 50 g/1.73 m^2 triglyceride levels (X+SD) of 74.9 + 38.5 mg/dl did not change at 2 hr (72.0 + 20.3), 3 hr (88.3 + 36.1) and 5 hr (66.6 + 19.7). Total cholesterol (mg/dl) was reduced (65.4 + 10.2) due to a marked decrease in both HDL-C (14.3 + 3.9) and LDL-C (20.7 + 11.4). Fasting serum levels (mg/dl) of apo A-I (90.1 + 12.1) and of apo A-IV (8.9 + 3.0) measured by electroimmunoassay were reduced by 30% and 40% respectively and did not change after the fat load. Small bowel biopsies showed vacuolated enterocytes loaded with fat but both abeta and hypobetalipoproteinemia were excluded since LDL apo B measured by a radial immunodiffusion method was neither absent nor reduced. Values of 61.4 + 8.6 mg/dl did not differ from controls (80 + 15 mg/dl). Immunoperoxidase staining showed increased apo B in intestinal enterocytes. Although the pathophysiological defect remains to be determined, the failure to secrete chylomicrons could perhaps be explained by a failure of intestinal synthesis or secretion of one or several apo B species.

84 REDUCTION OF DIARRHEA WITH CHOLESTYRAMINE IN CONGENITAL CHLORIDE DIARRHEA (CCD).

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CCD is an autosomal recessively inherited disease caused by impairment of active Cl^-/HCO_3^- exchange in the distal intestine. This results in loss of Cl^-, water, Na^+ and K^+ in acid stools. Unrecognized, most patients die. Some may survive with retarded growth and development, and renal damage. Replacement of the fecal losses of NaCl, KCl and water results in normal growth and development, but the diarrhea persists. Though most patients get toilet trained at a normal age, some patients have soiling problems. Thus, numerous therapeutic trials have been made to reduce the diarrhea, all without success.

We have tried cholestyramine treatment in five patients with soiling problems. Fluid and electrolyte status, fecal volume, electrolytes and bile acid excretion were recorded prior to and during treatment. Electrolyte composition of the watery stools remained unchanged but there was a 30-40 % reduction in fecal volume. This was accompanied by a clear reduction of soiling habits.

We propose that bile acids are flushed into the colon by increased intestinal volume in CCD. In the colon they cause secretory diarrhea which adds to the primary chloride diarrhea. This secretory component can be eliminated by cholestyramine which may thus be used to help CCD patients with soiling problems.

85 EXCRETORY PATTERN OF BILE DURING PHOTOTHERAPY

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We studied the acute effects of phototherapy (PT) on bile flow (BF), and on biliary excretion of bilirubin (BB) and of bile salts (BS), in male homozygous Gunn rats (120-150 Gm). PT was given to 13 rats; 10 rats remained in the dark (C). Bile was collected in hourly aliquots by cannulation of the common bile duct, from one hour prior to PT and until 4 hours after starting lights. We measured plasma bilirubin, BF, and the output of biliary BB and BS. Results: Prior to PT all values were similar in both groups. After 4 hours of treatment, plasma bilirubin had fallen from 145.3 to 99.2 µmol/L in the PT rats but did not change in C rats. During the "lights on" period, the rats receiving PT had a significantly higher hourly excretion of bile, BB and BS than the C rats. Over the total 4-hour PT period, when compared with C rats, the light-treated rats had a higher mean output of bile (1.48±.32 vs .94±.18 ml; p<.01), of BB (.15±.008 vs .08±.01 µmol; p<.005) and of BS (55.2±14.8 vs 35.1±4.6 µmol; p<.01). To the jaundiced neonate under PT, an increased bile and bile salt excretion may signify an extra bile salt load reaching the gut. Due to the inherent immaturity of distal ileal function in the newborn, such added bile salt load might gain access to the colon and cause green watery stools.

86 SERUM APO B, APO AI, APO E, AND LP(a) DURING THE FIRST WEEK OF LIFE

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During the first days of life VLDL and LDL concentrations increase markedly whereas HDL rise moderately. So far, there is only one report describing the associated change in serum apolipoporteins B and A-I. The present study gives the first results concerning Apo E levels during the first week and establishes the presence of Lp(a) in neonatal serum.

We measured Apo B, Apo AI, Apo E and Lp(a) concentrations in cord serum and in capillary serum obtained at the age of 5 days in healthy term newborns by electroimmunoassay in a longitudinal study.

Apo B ($\bar{x}$ ± SD) in cord serum was 26 ± 10 mg/dl (n=25), ApoAI 51 ± 10 mg/dl. The mean value for Apo E was 16 ± 4 mg/dl (n=7). During the first week serum Apo B and Apo AI rose significantly to 53 ± 22 mg/dl and 76 ± 27 mg/dl, respectively. Serum Apo B levels were not significantly different in newborns fed breast milk from those mainly fed infant's formula. Apo E showed a moderate increase during the first 5 days. Low concentrations of Lp(a) (2,6 ± 1,1 mg/dl) could be detected in 5 of 9 cord sera. Lp(a) levels remained low during the first week.

The results of our study describe the dynamic changes of various apolipoproteins during the first week of life reflecting the evolution of serum lipid transport system of the neonate.

87 IMPROVED N-RETENTION DURING L-CARNITINE SUPPLEMENTED TOTAL PARENTERAL NUTRITION (TPN).

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The influence of i.v.-L-carnitine on oxydation of intravenously administered lipids was studied during TPN of male mini pigs(n=7; $\bar{x}$ weight 4150 g).The infusion protocol was divided into three 48 hour periods of isocaloric infusions of different compositions.Amino acids(3g/kg/d) were administered throughout all 3 periods.140 Cal/kg/d were given as non-protein calories,consisting only of glucose during period 1.During periods 2+3 an amount of glucose calorically equivalent to 4g fat/kg/d was substituted with a 20% lipid emulsion. In period 3, L-carnitine(1.5 mg/kg/d) was added. During the entire regime key parameters of fat and nitrogen metabolism were determined in serum and urine and N-balances were calculated. During all 3 periods indirect calorimetry was performed and the $\Delta CO_2/\Delta O_2$-ratio calculated.The major results were:

	Period 1	(p)	Period 2	(p)	Period 3
NEFA (meq/l)	0.048 ± 0.02	++	0.20 ± 0.06	++	0.08 ± 0.04
Glycerol (mg/dl)	0.064 " 0.30	-	0.99 " 0.60	+	1.70 " 1.50
ß-OH-Butyrate(mg/dl)	0.20 " 0.07	-	0.18 " 0.08	++	0.32 " 0.06
Free Carnitine (µM)	8.50 " 1.3	++	13.1 " 0.4	++	19.2 " 2.3
Acyl Carnitine (µM)	39.9 " 8.6	++	57.6 " 7.8	++	69.5 " 2.3
$\Delta CO_2/\Delta O_2$	0.97 " 0.05	++	0.82 " 0.01	++	0.76 " 0.03
N-Balance (g/d)	+0.31 " 0.08	-	+0.23" 0.14	++	+1.05" 0.50

The data suggest that L-carnitine supplementation during TPN improves the energy gain from administered lipid emulsions which is reflected by improved N-retention.(-ns.;+ $p < 0.05$;++ $p < 0.01$).

88 ONTOGENY OF COLONIC DNA SYNTHESIS (CDS) IN RATS: EFFECT OF GLUCOCORTICOIDS.

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The developmental pattern of CDS was determined in 8 groups of Wistar rats killed at 5, 10, 14, 20, 30, 40, 70 and 100 days of age. The colon was removed from the caecum to the anus and devided into 4 segments (S_1, S_2, S_3, S_4). The growth in colonic weight per cm was greater before than after weaning. The incorporation rate of (3H)-thymidine was expressed in dpm per mg dry Wt tissue or per µg of DNA content. In each group of rats, CDS was higher in the mid segments (S_2, S_3) than in S_1 or S_4. For each colonic segment, CDS was increased during the suckling period with a peak at day 14 postpartum (49-119 % increase, $p < 0.01$ vs adult values). At the 3rd week, the values decreased abruptly and remained unchanged up to 100 days of age. The decrease in CDS rates was concomitant with an upsurge in plasma total corticosterone. The steroid levels averaged 0.5 ± 0.1 µg/dl between day 5 and 14 and reached 18 ± 0.8 µg/dl at day 17. Treatment of 10-day old sucklings with physiological doses of hydrocortisone for 4 consecutive days produced significant decreases in colonic DNA content (-36 %; $p < 0.001$ vs controls) and DNA synthesis rates (-37 % dpm/µg DNA; $p < 0.02$).

Conclusion : CDS which is very active during the suckling period of the rat, declines to adult levels during the 3rd postnatal week. These maturative changes could be under dependence of the adrenal cortical hormones.