58 G. PUTET*, G. VERELLEN*, T. HEIM, P. SWYER* and J. SMITH*. Department of Pediatrics, University of Toronto and Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada.

The protein sparing of exogenous lipid during i.v. alimentation.

The prerequisites of a rational nutritional design for premature receiving total parenteral nutrition (TPN) are the:

(a) estimation of coverage of the energy cost of basal metabolism muscular activity, chemical thermogenesis and growth by the metabolizable energy (ME) intake.

(b) estimation of the desirable protein and fat deposition for optimal growth and development.

Using nutrient balance, indirect calorimetry (IC) and urinary nitrogen output (UN), the coverage of energy metabolism by carbohydrate (CHO), protein (P) and fat (F) were determined by studying 36 infants during (TPN). The majority of patients were 2-3 day postoperative surgical; mean gestational age 36 weeks, birth weight 2600 g, postnatal age 16 days. During initial hypocaloric infusion (18-35 Kcal/kg/day) of glucose (GL) the nitrogen balance was negative (-70 mg/kg/day). GL + amino acid (AA) infusion resulted in a positive nitrogen balance and correlated significantly with the daily protein intake ($y = 0.61 X + 0.17$; $r = 0.54$; $p < 0.01$; n=19) which varied between 0.5 - 3.5 g/kg/day. By increasing the caloric intake with Intralipid (IL) at a fixed protein intake nitrogen retention was increased (51 mg/kg/day), resulting in 0.3 g/kg/day extra protein deposition. Over 50-60% of the IL was oxidized. These results suggest that this protein sparing is attributable to the preferential utilization of fat for energy metabolism.

59 L. SANN, M. MATHIEU*, Y. LASNE*, A. RUITTON*. Hopital Debrousse, Lyon, France. Hyperglycemic effect of an oral load of triglycerides in preterm neonates.

A nasogastric load of 2,7 g/kg of lipids with 67 % of medium chain triglycerides and 30 % of grape seeds oil was given at the age of 48 hours to 20 preterm neonates (mean bw = 2164 g ; gest. age : 35 weeks). Its effect on the concentrations of plasma glucose free fatty acids (FFA), serum B-OH-butyrate (BOHB) and lactate was compared to their evolution in 11 control preterm infants (mean bw 2102 g ; gest. age : 35 weeks). The basal levels were similar in both groups. In the supplemented group, plasma glucose increased from (mean ± SD) 57 ± 7 to 74 ± 11 mg/dl at 30 min. ($p < 0.001$) and to 80 ± 11 mg/dl at 60 min. ($p < 0.001$) while no significant change was recorded in the control group. At 60 min., serum BOHB had increased in the supplemented group from 339 ± 165 to 631 ± 230 µmol/l ($p < 0.01$) and it was higher than in the control group : 439 ± 200 µmol/l ($p < 0.05$). Plasma FFA decreased from 303 ± 178 to 199 ± 90 µlEq/l ($p < 0.05$) in 60 min. in the control group while no significant change was observed in the supplemented group. Serum lactate decreasedin 30 min. from 1777 ± 809 to 1257 ± 375 µmol/l in the supplemented group ($p < 0.05$) while the change (from 1820 ± 600 to 1581 ± 438 µmol/l) was not significant in the control group. In 7 supplemented infants, there was no change of serum insulin or glucagon concentration and no change of serum glycerol levels in 6 other supplemented infants.

These data suggest that in preterm infants 1)this load of triglycerides can provide glucose and ketone bodies which could be available for the neonatal brain 2)this hyperglycemic effect independant of hormonal influence is probably achieved through gluconeogenesis.

60 A. NIEDERWIESER*, J. BIERI*, B. BLEHOVA*, H.-CH. CURTIUS*, A. MATASOVIC*, F. REY*, B. SCHIRCKS*, M. VISCONTINI* (Intr. by A. Prader) Dep. Pediatrics and Org. Chemistry, University of Zurich; Detska Klinika, Prague; Hôp. des Enfants Malades, Paris. Urinary pterins in atypical phenylketonuria.

Three further patients with atypical PKU caused by tetrahydrobiopterin deficiency were investigated: D.K. (B. Blehová), A.C. and T.Y. (F. Rey). Pterin analyses in urine were performed by two-dimensional high-voltage electrophoresis/paper chromatography as well as by gas chromatography-mass spectrometry. The trimethylsilyl derivatives were analyzed by gas chromatography on a 20 m OV-1 glass capillary column and detected by a nitrogen detector as well as by mass fragmentography at m/e 409. Mass spectra were identical with those of the pure reference compounds. All three patients excreted high amounts of neopterin and smaller quantities of dihydroxanthopterin in urine but no biopterin or dihydrobiopterin. This pterin pattern was the same as in the first two patients (M.K. and Z.Y.) shown to suffer from dihydrobiopterin synthetase deficiency (A. Niederwieser et al. Lancet I: 131, 1979; H.-Ch. Curtius et al. Clin. Chim. Acta 93: 251, 1979). Neopterin was shown to be of D-erythro and not of threo configuration. Furthermore, the patients' elevated serum phenylalanine level was normalized by oral administration of L-erythro-tetrahydrobiopterin bishydrochloride, 2.5 mg/kg body weight. Conclusion: Analysis of urinary pterins is of value for the early detection of atypical PKU and - in combination with pterin administration tests - for the localization of the corresponding enzyme defect.

61 DHONDT JL*, FARRIAUX JP.*, DAUTREVAUX M*, ARDOUIN P*, (Intr. by Corbeel L.) - Service de Génétique et Maladies Héréditaires du Métabolisme - LILLE (F) -

Reduction of serum phenylalanine levels in PKU rats with intestinal absorption inhibitors of phenylalanine (PHE).- It has been often suggested that inhibition of the intestinal absorption of PHE would be a new approach for PKU treatment. We have previously shown that a PKU model for dietetic studies is obtained by means of the synergistic inhibition of Phenylalanine-Hydroxylase with p-chlorophenylalanine + cotrimoxazole (daily intraperitoneal injection of the 2 inhibitors leads to hyperphenylalaninemia without need for a phenylalanine load). Addition of beta-2-thienylalanine (THI) or phenylalaninol (PHE-ol) to the diet (1g%) significantly reduced phenylalaninemia: PHE: 1.30 ± 0.30, + THI : 0.70 ± 0.27, + PHE-ol:0.87 + 0.29 mmol/l. However, the effectiveness of THI or PHE-ol was mainly dependent on diet quality: with 5 isonitrogenous-isoosmotic diets (amino acids, lactalbumin & its hydrolysate, casein & its hydrolysate), decrease of serum PHE with THI or PHE-ol only occurred with high PHE diets (inhibitor/PHE molar ratio 1.4), without significant difference between results with intact or hydrolysed protein diets. It has been claimed that amino acid (AA) changes in serum, liver and brain, induced by hyperPHE, could explain disturbed protein synthesis in PKU; we confirm such changes in our PKU model (mainly: essential AA decrease in serum, liver and brain), however AA imbalance did not disappear on THI or PHE-ol supplemented diets. These data suggest that PHE analogues (such THI or PHE-ol)used in diets to decrease intestinal absorption of PHE cannot ensure serum PHE reduction comparable to that obtained with PHE restricted diets.

62 D.MICHALK*, H.SCHMIDT*, G.MITTERMAIER*, P.WONG*, P.LUTZ: University Children's Hospital, Heidelberg, FRG. Homocystinuria due to 5,10-methylenetetrahydrofolate (THF) reductase deficiency: clinical presentation and treatment.

In 5,10-THF-reductase deficiency, first described by Mudd et al. in 1972, homocystinuria with normal plasma methionine is clinically associated with mental retardation, behavioural abnormalities and proximal muscular weakness. We observed a family with 7 children. Two of them, a 17 yrs old boy and a 19 yrs old girl, presented with mental retardation from early childhood and progressive muscular rigidy, mainly of extrapyramidal origin with onset at school age. Two other siblings with similar symptoms suddenly had died prior to our investigation at the age of 13 and 21, respectively. A marked homocystinuria (3 mmol/d) was found in our patients. Plasma homocystine ranged between 100 and 200 µmol/l; methionine, however, was subnormal. The determination of the 5,10-THF-reductase in lymphocytes and fibroblasts revealed a very low activity. (15-30% in LC and 0-7% in fibroblasts)

A therapeutic trial with leucovorine (5-formyl-THF) resulted in a prompt reduction of urinary homocystine excretion accompanied by an improvement of the neurological disturbances.

63 L.R. GARIBALDI*, I. REZVANI*, H.G. ARTMAN*, C.S. PHILLIPS*, and A.M. DIGEORGE*(Intr. by P. Durand). Temple Univ. Sch. Med., Dept of Ped.,St. Christopher's Hospital for Children, Philadelphia, Pa., U.S.A. Excessive suppression of serum dehydroepiandrosterone sulfate (DHEAS) in patients treated for congenital adrenal hyperplasia (CAH).

Serum levels of DHEAS and 17-hydroxyprogesterone (17OHP) were measured in 20 females (2.5-22 yrs) and 11 males (2.5-16.5 yrs) with CAH due to 21-hydroxylase deficiency treated with oral hydrocortisone (HC) (non-salt losers, n=12), or HC and Florinef (salt losers, n=19). Adequacy of therapy was judged by clinical findings, growth rate, bone age, serum 17OHP, urinary 17 ketosteroids and pregnanetriol. These allowed separation of patients into 3 groups: A- poor control (n=11), B- good control (n=12), and C- mildly overtreated (n=8). The groups were comparable for sex ratio but mean age in group C (5.5±1.9 yrs) was lower than group A (13.7±2.1 yrs) or group B (11.4±6 yrs). In "optimally" treated patients (group B), whose serum levels of 17 OHP were consistent with adequate treatment (250±189 ng/dl), mean serum level of DHEAS was significantly lower than that in age matched controls (23±20 vs 82±42 µg/dl, P <0.01). Serum levels of DHEAS in group C were all below the sensitivity of the assay (<5 µg/dl). Serum levels of 17OHP were also suppressed in this group (12±5 ng/dl). In poorly controlled patients (group A), mean serum level of DHEAS was slightly but not significantly higher than controls (121±80 vs 92±39 µg/dl) while serum 17OHP levels were abnormally high (>1000 ng/dl). These data indicate that doses of HC necessary for "optimal" treatment of CAH result in oversuppression of serum levels of DHEAS. This supports the hypothesis that the secretion of DHEAS may be controlled by a hormone other than ACTH.

64 R. OLEGÅRD+, K.G. SABEL+, M. ÅRONSSON+, M. KYLLERMAN+, E. KARLBERG+, P.R. JOHANSSON+, B. SANDIN+, K. ADRIAN+, T. HRBEK and K. IVERSEN+ (Intr. by P. Karlberg). Departments of Pediatrics, University of Göteborg, Göteborg, Sweden. Fetal alcohol syndrome. Controlled and prospective studies.

An earlier retrospective study of children to alcoholic mothers showed correlation between size at birth and later mental performance. In a study of 23 children born by 15 presently alcoholic women (study group) with matched controls (sex, birthweight, gest. age, city area) the study group had slower growth after birth. Study children scored lower than controls in tests for intelligence (Griffith and WISC mean IQ 93.8 and 112.7 respectively, $p<0.001$), perception (Frostig test, $p<0.001$), gross motor age (score 91 and 105 resp., $p<0.005$) and fine motor age (score 89 and 105 resp., $p<0.005$).

Prospective tracing of alcoholic pregnancies was done in some areas of Göteborg. 28 alcoholic pregnancies were traced from May -77 to Nov -78. 5 legal abortions were done. 2 infants were still-

born, 21 were born alive. Treatment and support aiming at sobriety could be started at different stages of pregnancy and with varying response. Early sobriety resulted in average size at birth, sobriety from mid-pregnancy gave a mean suppression of size at birth of 1 SD from the mean Swedish growth charts, and abuse throughout pregnancy a mean suppression of 1.7 SD. Pathological evoked response electroencephalograms (ER) were found in all three groups at birth. Only 1 of 14 investigated infants had a normal ER.

The incidence of the complete alcohol syndrome was 1/600 deliveries. In children born 1975-77 in Göteborg every sixth case of cerebral palsy was associated with an alcoholic pregnancy.

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THEOPHYLLINE (T) METABOLISM IN THE PREMATURE NEONATE.

The recent use of methylxanthines in the treatment of apnea of prematurity and their possible efficacy in the prevention of hyaline membrane disease has underlined the need for detailed investigation of their disposition in the premature neonate. The metabolism of T was studied in 10 premature newborns(g.a. 27-35 wks) during the first month of life and in 3 adult volunteers for comparison. T was injected i.v. and blood and urine assayed for T, Caffeine (C) and their metabolites by HPLC. T was found to be methylated to C only in the preterm group. 1 Methyluric (1-MU) and 1-3 Dimethyluric (1,3-DMU) acids were the major metabolites of T in the newborn. Demethylation to 3 Methylxanthine (3-MX) was seen only in the adults. The molar ratios of the metabolites in urine are reported in the table:

	3-MX	1-MU	1,3-DMU	T	C
Premature neonates	-	.11	.40	.43	.04
Adults	.19	.28	.41	.12	-

This study confirms the possibility of a methylative pathway from T to C in the newborn, but not in the adult. Hydroxylation accounted for about 90% of T metabolism in the premature neonate, while N-demethylation was absent at birth. This finding is in contrast to what seen for other drugs (e.g. diazepam).

66 F.F.RUBALTELLI,E.ROSSI* and G.JORI*. Dept. Pediatrics and CNR Center for Physiology and Biochemistry of Hemocyanins. Univ. of Padova, Padova, Italy. Evidence for visible light-induced covalent binding between bilirubin and serum albumin "in vitro" and "in vivo".

Acetone-induced precipitation of the 1:1 serum albumin-bilirubin complex, followed by treatment with 7M guanidine, allows complete removal of bilirubin from albumin by gel filtration. After illumination in the 440-470nm wavelength region (300 µW/sq.cm/nm), bilirubin or its photoproduct(s) cannot be removed any more from albumin. This was also confirmed by gel filtration on Sephadex G-75 (30% acetic acid as eluant) of BrCN-treated albumin, after 30 min. irradiation. This suggests the formation of a covalent binding which was detected also "in vivo" in the sera of jaundiced newborns. After 7 hrs.phototherapy with 4 F20T12/BB Westinghouse lamps (irradiation intensity at the infant level = 22 µW/sq.cm/nm, in the range of 440-470 nm), the covalent adduct began to appear. The quantity of the adduct was dependent upon the amount of light energy received during treatment. The adduct disappeared at 15 to 20 days after treatment.

67 H.T.VERSMOLD, G.LISTER*, P.R.DALLMAN*, A.M.RUDOLPH*. Department of Pediatrics, Univ.of Munich, Germany, and the Cardiovascular Research Institute, Univ.of California, San Francisco, U.S.A. Limits of O_2 Delivery during Propanolol-Induced Reduction of Cardiac Output in Lambs after Birth: A Longitudinal Study.

To test the ability to increase O_2 extraction when cardiac output is decreased, we assessed total body O_2 delivery before and after ß adrenergic blockade during the early postnatal period. 8 lambs were studied chronically over the first 2 month of life. Cardiac output (CO), heart rate (HR), hemoglobin (Hb), Hb O_2 affinity and 2,3-DPG, oxygen consumption (VO_2), systemic O_2 transport (SOT), and arteriovenous O_2 content difference were measured while the lambs were resting and unsedated, and again after intravenous propanolol (PROP), 1mg/kg. After birth CO, HR and VO_2 at rest decreased steadily while fractional O_2 extraction (VO_2/SOT) remained constant. After PROP CO was consistently reduced by 1o - 2o %, and there were no significant differences with age. Most of the decrease in CO was explained by the reduction in HR.Upon this decline in CO there were significant decreases in VO_2 after PROP during the first postnatal week, and at 4 weeks. This was when O_2Hb affinity was greatest (≤1 wk) or Hb was lowest (3-4 wk). Moreover, only when resting mixed venous Po_2 was less than 29 torr was VO_2 consistently and predictably decreased after PROP.

Compared to the lamb in human infants P_{50} after birth is lower, and Hb is decreasing relatively more. We conclude that in the human infant like in the lamb there is very little reserve to increase O_2 extraction or arterialvenous O_2 content difference early in infancy when P_{50} or Hb is low.

68 M.S. TANNER *, B. PORTMANN *, and C.F. MILLS *, (Intro by Prof. J.K. Lloyd) Department of Child Health, St George's Hospital Medical School, London; Liver Unit, King's College Hospital, London; and the Rowett Institute, Aberdeen, U.K.

Raised hepatic copper in Indian Childhood Cirrhosis.

Percutaneous liver biopsies from 19 children with liver disease were studied. Indian Childhood Cirrhosis was diagnosed in 5, and in these orcein staining demonstrated widespread granular deposits within hepatocytes The hepatic copper content in these 5 cases was greatly increased at 1389 ± 525 µg/g dry weight (range 1045 - 2303 µg/g, normal range 15 - 55 µg/g) The other 14 children had various hepatic disorders. None had widespread granular orcein staining Hepatic copper concentrations were normal in 12, and slightly elevated in 2 (170 and 292 µg/g). The absence of prolonged jaundice, histological cholestasis, or serum lipoprotein X in the cases of ICC indicated that hepatic copper accumulation was not secondary to cholestasis Serum ceruloplasmin concentrations were greater than 20 mg/dl in all 19 cases. A survey failed to detect any cases of ICC in Asian children born in the UK, so implicating environmental factors. We suggest that copper is implicated in the aetiology of ICC, that early treatment with penicillamine may be effective,and that reduction of copper intake may be preventative.

69 B. STEINMANN*, L. TUDERMAN*, G.R. MARTIN* AND D.J. PROCKOP* (intr. by R. GITZELMANN). Dept. Pediatrics, University of Zurich, CH-8032 Switzerland.

FIRST DEMONSTRATION OF A STRUCTURAL MUTATION OF PROCOLLAGEN IN A PATIENT WITH EHLERS-DANLOS SYNDROME (EDS).

Three patients with severe hypermobility of the joints but only mild hyperelasticity of the skin were previously reported (Science *182*,298-300,1973) to have partial deficiency of procollagen N-protease, one of the two enzymes necessary for the conversion of procollagen to collagen. One 9 year old patient was reinvestigated. In a biopsy sample of her skin, collagen was more extractable in neutral 0.5 M NaCl containing protease inhibitors. In addition to the normally occurring α1- and α2-chains, α2-precursor chains (pNα2), but no pNα1 were detected by SDS-PAGE. Digestion of the collagen with animal collagenase generated the three N-terminal fragments $\alpha1^A$, $\alpha2^A$ and $pN\alpha2^A$ but only the two normally occurring C-terminal fragments $\alpha1^B$ and $\alpha2^B$. Digestion of the extracts with purified procollagen N-protease did not remove the N-propeptide from the pNα2 chains. This excluded the possibility of incomplete conversion of pN-collagen owing to partial procollagen N-protease deficiency. The findings were corroborated by the study of radioactive procollagen produced by cultured skin fibroblasts. The latter had normal N-protease. Results suggested that the proα2-chains had a structural defect near the N-protease cleavage site preventing the enzymatic removal of the N-propeptide. Since equal amounts of pNα2- and α2-chains were produced, gene dosage was evidenced in this sporadic case of EDS probably caused by a new mutation.

70 I. SIPILÄ*, O. SIMELL* and P. ARJOMAA* (Intr. by K. Raivio). Children's Hospital, University of Helsinki, Finland. Guanidinoacetate (GAA) excretion and plasma amino acids after an arginine load in patients with hyperornithinemia and gyrate atrophy of the choroid and retina (GA).

GA is an autosomal recessive disease characterized by progressive atrophy of the choroid and retina starting by age 5-9 years, and atrophy and formation of tubular aggregates in type I muscle fibers. Plasma ornithine concentration is 10-20 times increased. To quantitate in GA the efficiency of the first reaction in creatine production from arginine, we gave a 5 min i.v. load of arginine-HCl, 1.1 mmoles/kg, to 7 patients and 4 controls. Urinary excretion of GAA was measured in 15-60 min collections for 6 hours, and plasma amino acids at 15-60 min intervals. The plasma arginine values increased similarly in patients and controls. Plasma ornithine concentrations at 0 and 30 min (peak value) were 757±134 µM (mean ±SD) and 1600±279 µM in the patients and 43±9 µM and 209±64 µM in the controls, respectively. In the patients, only the 15 min arginine value exceeded that of ornithine; the controls always had higher arginine. The basal GAA excretion by the patients was only 10% of that of the controls. In the patients, the excretion was increased for the first 30 min after the load, but returned then to the low levels. In the controls, excretion was increased throughout the collections. Thus, the high ornithine concentration may inhibit GAA formation and subsequently creatine and phosphocreatine synthesis; the resulting lack of high energy phosphagens may be a mediator in the muscle and eye atrophies in GA.

71 H.P. SCHWARZ*, K. ZUPPINGER, T. SCHAEFER*, H.P. SIEGRIST*, U. WIESMANN and N. HERSCHKOWITZ. Department of Pediatrics, University of Bern, Switzerland. Galactose enhances sulfatide synthesis in glucose-deprived cultured mouse brain cells.

Glucose-deprived brain cell cultures have a markedly reduced sulfatide synthesis (SfS). As SfS is glucose dependent, galactose (gal) was studied to delineate its role as a substitute for glucose (glu). SfS is a mandatory step in the formation of myelin. Gal is necessary to form cerebroside which is converted to sulfatide by incorporation of sulfate. Neonatal mouse brains were dissociated and cultured for 10 days. Cell cultures