Pediat. Res. *3:* 89–98 (1969)

ABSTRACTS

Meeting of The European Club for Pediatric Research

Vienna, Austria, August 27–29, 1968

1 *Relative Blood Flow of Various Organs Prenatally and in the Postnatal Period in Normal and Hypoxic Conditions Studied by Isotopic Method in Newborn Rabbits.* D. Boda, M. Bélay*, E. Eck* and L. Csernay*, Department of Paediatrics, University of Szeged, Hungary.

Knowing of the disturbance of pulmonary circulation in the respiratory distress syndrome of the newborn and the sensitivity to oxygen tension of the blood vessels of the fetal or newborn organisms, we have investigated in rabbits
at the end of pregnancy *in utero* and
in newborn age under normal and
hypoxic conditions by Sapirstein's ^{86}Rb isotope 'indicator fractionation' method the blood flow in the brain, intestine, liver, kidney and muscle, as related to total body blood flow.
In utero, blood flow is high in the brain, liver and it is low in the intestine and kidney.
After birth the distribution of blood flow in the organism changes quickly. The blood flow of the brain and of the liver decreases and that of the intestine and kidney becomes higher.
In response to hypoxia the distribution of blood becomes again similar to the *in utero* situation, but there is no increase in cerebral flow.

While the blood flow properties observed in single organs may be considered as being one of the preconditions of intrauterine life, the same will lead in extrauterine life to catastrophic consequences.

2 *The Effect of Starvation at Different Environmental Temperatures on Heat Production in the Newborn Rabbit.* T. Heim* and Agnes Cser*, Dept. of Pediatrics, Univ. of Pécs, Hungary (introduced by J. Mestyan).

Brown adipose tissue is an important site of thermoregulatory heat production. In the newborn rabbit most of the extra oxygen is consumed by brown adipose tissue in response to noradrenaline or cold exposure. During starvation the fat disappears from brown adipose tissue in the newborn rabbit only in a cold environment, but not in a thermoneutral environment.

In the present experiments newborn rabbits were starved 48–72 h at 35–36° (thermoneutral) and at 20–23° (cold), respectively. During the last 3 h of starvation oxygen consumption, colonic and brown fat temperatures were measured in the thermoneutral and the cold environment. Well-fed littermates served as controls.

The basal metabolic rate was equally reduced in rabbits starved at both ambient temperatures. Thermoregulatory heat production was severely impaired in rabbits starved in the cold environment and less severely in those starved in the thermoneutral one in spite that the latter had lost a higher percentage of their birth weight and had endured starvation longer. Quantitative differences could be observed also in the magnitude of the metabolic response to cold between well-fed controls and the animals starving at ambient temperature of 35–36°. In the starved group metabolic rates (O_2 ml/kg/min) were lower, and hypothermia ensued by the end of the 80 minutes of cold exposure.

After each experiment the animals were sacrificed and the fat content of their brown adipose tissue, blood sugar and liver glycogen content were determined. Fat practically disappeared from brown adipose tissue of rabbits starved in the cold environment but was full of fat in both the well-fed controls and in the animals starving at thermoneutrality. Hypoglycaemia and the greatly diminished liver glycogen content were similar in both starving groups.

These results indicate that thermoregulatory changes during starvation cannot be simply explained by the lack of substrates and also imply that the fat content of brown adipose tissue is not the only determining factor. The *in vitro* noradrenaline sensitivity of brown adipose tissue of well-fed and starving newborn rabbits have shown a decrease of the calorigenic response to both noradrenaline and noradrenaline+insulin in starved rabbits independently of the environmental temperature at which they had suffered starvation.

3 *Die Bedeutung pränatal verabreichter Medikamente für Früh- und Neugeborene.* W. Tulzer*, Landesfrauenklinik und Landeskinderkrankenhaus Linz, Österreich (eingeführt von G. Weippl).

Diazepam (7-chloro-1, 3,-dihydro-1-methyl-5-phenyl-2H-1, 4,-benzodiazepin-2-one), ein Medikament, das in zunehmendem Maße auch in der Geburtshilfe Eingang gefunden hat, wurde im Hinblick auf seine möglichen Auswirkungen auf das Früh- und Neugeborene untersucht. Es wurden 118 Neugeborene und 36 Frühgeborene, deren Mütter kurz vor oder während der Entbindung Diazepam in einer Dosis von 20 mg i.v. oder i.m. erhalten hatten, einer entsprechenden Kontrollgruppe von Kindern unbehandelter Mütter gegenübergestellt.

* By invitation

Während sich im APGAR-score als Folge der direkten Medikamentwirkung eine deutliche Auswirkung zeigt (Neugeborene 9,4+9,8, Frühgeborene 7,3:8,2), die vor allem in einer auffälligen Muskelhypotonie begründet ist, zeigen Blutgasanalysen und Serumbilirubinkontrollen während der ersten Lebenswoche keine signifikanten Unterschiede zu Kindern unbehandelter Mütter.

Es darf also geschlossen werden, daß Diazepam zwar einen kurzzeitigen Depressionszustand beim Neugeborenen bewirken kann, daß es aber anscheinend keinen nachhaltigen schädlichen Effekt auf das Kind ausübt.

4 *Blood Glucose of the Intact Human Fetus* in utero. KARI O. RAIVIO* and KARI TERAMO*, Children's Hospital and Department I of Obstetrics and Gynecology, University of Helsinki, Finland (introduced by N.O.B. Hallman).

The intrauterine blood glucose levels of human fetuses were studied at a time when labor had not yet commenced. The samples were taken by dilating the intact uterine cervix sufficiently to admit an amnioscope through which the fetal scalp was pricked and capillary blood collected. The procedure involved discision of the membranes and resulted in induction of labor, which was faciliated by an intravenous infusion of oxytocin in saline, when necessary. Further samples were taken during labor, and from the umbilical artery and vein at delivery. Maternal blood glucose levels were measured simultaneously with the fetal determinations.

In preliminary studies it was established that the glucose level in fetal scalp capillary blood represents closely the level in fetal arterial system, the mean difference between these two being 7.6 percent (nine cases).

A total of 38 cases were then studied, and these were classified as normal (12 cases), toxemia (8 cases), Rh-immunization (8 cases) or prolonged pregnancy (10 cases). The mean value of all determinations performed prior to the onset of labor was 61.5 mg/100 ml, and the differences between the normal and abnormal groups were insignificant. The mean materno-fetal gradient in the normal group was 25 mg/100 ml and in the abnormal groups between 18 and 30 mg/100 ml. During labor an approximately parallel increase was observed in fetal and maternal values, again with no essential differences between the normal and abnormal cases. A mean value of 68 mg/100 ml was measured in umbilical artery and 78 mg/100 ml in umbilical vein blood.

The results are discussed with reference to earlier suggestions of low intrauterine blood glucose levels in man, and to the placental transfer of glucose.

5 *The Effect of Glucose on the Serum Levels and on the Excretion of Acetoacetate in Newborn Infants.* W. SCHRÖTER* and G. VOGLER*, Universitäts-Kinderklinik Hamburg, Germany (introduced by W. Lenz).

The levels of acetoacetate increase sixfold in the serum of fullterm newborn infants during the first three days after birth if feeding with breast milk starts at the second day after birth. In infants receiving additional 9 calories/kg body weight/24 hours orally as glucose during the first two days after birth, the postnatal increase of the acetoacetate levels is the same. Intravenously infused glucose (7 calories/kg body weight/ 24 hours, diminishes the increase of acetoacetate slightly. Only the infusion of a 10 percent solution of glucose (25 calories/kg body weight/24 hours) suppresses the increase of acetoacetate almost completely. In the first group, starved for 24 hours, the renal excretion of acetoacetate is high. At the second and third day of life approximately 4 nmoles acetoacetate/min has been excreted in this group. Low doses of glucose, administered together with 70 ml water/kg body weight/24 hours, which did not decrease the acetoacetate concentration in the serum, had no significant effect on the renal excretion of acetoacetate, though the water load increase the urinary output threefold. In the last group, which received 25 calories of glucose/kg body weight/24 hours, the excretion of acetoacetate is diminished to approximately 2 nmoles/min. These results support the hypothesis that the cause of the postnatal increased lipolysis with concomitant increase of the levels of acetoacetate and other ketone bodies is a deficiency of glucose.

6 *The Measurement of the Bilirubin Binding Capacity of Serumalbumin and its Clinical Significance.* HENRIK HERTZ*, The Protein Laboratory, University of Copenhagen, The University Clinic of Pediatrics, Rigshopitalet, Copenhagen, Denmark (introduced by B. Friis-Hansen).

The determination of the concentration of total serumbilirubin is not sufficient in order to evaluate the indication for exchange transfusion.

Since the toxicity of bilirubin seems to be intimately dependent of the binding sites of serumalbumin available for unconjugated bilirubin, the concentration of albumin is of great importance. However, several other substances in serum are transported by albumin on the same binding sites as bilirubin. The concentration of these substances may vary considerably.

A new rapid micromethod is introduced, which determines the Albumin Reserve Binding Capacity (ARBC) for unconjugated bilirubin by means of competitive colourbinding with bromphenolblue and differential spectrophotometry. The theoretical basis and problems of the method are discussed and the clinical value is judged.

382 sera taken from No. 1 exchange transfusion have been investigated during the last 3 ½ years. In a selected part of this material consisting of 19 sera (i.e. 5 %) and characterized by having the lowest ARBC, one will find 5 out of 6 infants, in whom kernicterus was found by autopsy. At the time of the exchange transfusion the mean serumbilirubin concentration in these 5 infants was 140 mg/l.

The conclusion is that the method probably is as valuable as the concentration of serumbilirubin in the evaluation of the indication of exchange transfusion.

Finally a preliminary note is made of the finding of another possible criterion for increased risk in neonatal hyperbilirubinemia. It deals with an observation of binding bilirubin to the highest molecular weight serumproteins in the cases mentioned above.

7 *Capillary Permeability to Albumin in Children.* P.A. KRASILNIKOFF*, Department of Pediatrics and Department of Clinical Physiology, Glostrup Hospital, Copenhagen, Denmark (introduced by J. Vesterdal).

In a previous study of albumin metabolism in children I found the distribution ratio (= the intravascular mass/the total mass of albumin) to be lower in prematures and infants than in bigger children and adults.

The distribution of a plasma protein between the intravascular (I) and the extravascular (E) pool is

determined by the relationship between the transcapillary escape rate (k_1) and the extravascular return rate (k_2) of the protein, according to the equation: $I \times k_1 = E \times k_2$. The transcapillary escape rate reflects the total capillary permeability to the protein.

In order to determine the transcapillary escape rate I have investigated 12 prematures and newborns (age 4–47 days, weight 1100–4400 g) and 12 bigger children (age 4 months to 12 years, weight 7–45 kg). ^{131}I-labelled human albumin was injected intravenously. Plasma samples were collected frequently during the first hour after the injection. The albumin concentration was determined immunochemically in each sample. The specific activity (counts per min/albumin concentration) decreased monoexponentially during the period of investigation. The slope of the curve was taken as an expression of the transcapillary escape rate.

In the small children an average of 27 percent of the intravascular mass escaped through the capillary wall per hour. This was significantly higher than the 12 percent per hour found in the bigger children. A highly significant negative correlation was found between the transcapillary escape rate and the distribution ratio ($r = -0.93$, $p < 0.001$).

Thus, the higher transcapillary escape rate in prematures and newborns might explain the reduced distribution ratio of albumin found in this group.

8 *Calcium Utilization in Healthy Infants Fed with Diets of Different Fat Contents During the First Three Months of Life.* HELGA STOLLEY*, W. DROESE and D. KUNZE*, Forschungsinstitut für Kinderernährung, Dortmund, Germany.

An investigation of daily balances of calcium and fatty acids carried out on 43 infants from birth up to three months of age is being presented.

It was the goal of our investigation to find out whether and to which extent Ca utilization depends on the age of the infants, the Ca and fat concentrations of the diets, and the kind of fats, butter or cotton seed or corn oil added to the diets.

As could be demonstrated there is an improved Ca utilization in the course of the first 3 months of life.

Increasing the calcium uptake with food is associated with an improved calcium utilization, whereas increasing the fat concentration in the diet does reduce it. The utilization of calcium is reduced by adding butter to the diets, whereas equicaloric amounts of corn oil or cotton seed oil do not appear to cause the same effect.

High doses of vit. D (200,000 to 400,000 I.U. Vit. D_3 = 'Vigantolstoss', according to the health laws of Bavaria) administered between the third and fifth day of life do not show any visible influence on the utilization of calcium in the course of the first 14 days of life. Adding lactose instead of saccharose substrates does not improve calcium utilization.

9 *Studies on Heterozygous Carries of Mucopolysaccharidoses.* W. M. TELLER, Department of Pediatrics, University of Heidelberg, Germany.

Some years ago attempts at the identification of healthy, heterozygous carries of mucopolysaccharidoses (MP) revealed qualitative abnormalities of the AMPS material in the urine [TELLER *et al.*, 1961]. The colorimetric methods employed were fairly crude and subsequent authors were unable to confirm our results [LINKER and TERRY, 1963; MAYES and HANSEN, 1966]. Investigating cellular metachromasia of skin fibroblasts in cell culture DANES and BEARN [1967] als suggested differences between heterozygotes and normal persons.

In the present report urinary AMPS patterns of homozygous and heterozygous carries are reevaluated by a more refined and specific method. Total 24 h urine specimens were examined from 5 patients with MP type I (Hurler), 4 patients with MP type II (Hunter), 6 patients with MP Type III (Sanfilippo), their respective fathers, mothers, and some siblings. The following method was employed [TELLER and ZIEMANN, 1966]: Dialysis of the total 24-h urine against normal saline, precipitation with cetyltrimethylammonium bromide (cetavlon), washing of the precipitate with 95 % ethanol, column chromatography on Dowex 1×2, colorimetric determination of hexuronic acid (carbazole) and/or neutral sugar (anthrone) contents of each fraction. The different peaks were pooled and their values expressed in percentages of total carbazole material eluted from the column. The results were as follows: Parents of patients with MP type I revealed abnormal urinary AMPS patterns with increase of percentages of heparitin sulfate (HS) and decrease of chondroitin sulfate A/C (CSA/C), although the total AMPS excretion was clearly within normal ranges. The three siblings examined showed normal AMPS patterns, both qualitatively and quantitatively. The mothers of patients with MP type II again excreted more HS than CSA/C. The percentages of CSB varied from normal (1) to pathologically increased values (2). One father excreted more HS than would be considered normal. Two siblings had normal AMPS excretion. The most striking findings were revealed by parents of patients with MP type III. Both mothers and fathers had a predominant HS excretion with reduction of CSA and CSB. The patterns conformed closely to those of their diseased children, however, total AMPS excretion was normal. The AMPS excretion of four healthy siblings did not differ from agematched controls.

10 *Detection of Heterozygotes in Phenylketonuria.* S. RAMPINI*, P. W. ANDERS*, H.-CH. CURTIS* and TH. MARTHALER*, Dept. of Pediatrics and Dental Institute, University of Zürich, Switzerland (introduced by W. Hitzig).

An oral phenylalanine load (100 mg/kg) was performed in 26 controls and in 19 parents of patients with phenylketonuria. Phenylalanine and tyrosine in plasma were determined by column chromatography before and 1, 2, 3 and 4 hours after phenylalanine administration. The values of 2 controls (C21 and C25) and of 2 parents (P4 and P6) were unusually low or high. In the two groups the following calculations of the values after load were made (the 4 unusual cases omitted):

(1) Mean and s of phenylalanine at each time: in spite of a significant difference between the two groups, there is a slight overlap;

(2) Sum of the phenylalanine values at 1, 2, 3 and 4 hours: significant difference, no overlap;

(3) Sum of phenylalanine/tyrosine-ratios at 1, 2, 3 and 4 hours: significant difference, no overlap.

Using these criteria C21 and C25 have to be considered heterozygotes. The values of P4 were within the range of the control group: paternity was excluded by blood group analysis. The unusually high values of P6 cannot be explained.

For discriminatory analysis C21, C25 and P4 were excluded, but calculation of the coefficients was made with and without P6. To compare the different criteria

of evaluation the index of Penrose (discriminating power = D/s) was calculated. A better separation was obtained with discriminatory analysis than with the sum of the phenylalanine values or of the phenylalanine/tyrosine ratios.

11 *Properties and Developmental Changes of p-Hydroxyphenylpyruvate-Specific Enzymes in Human Liver.* N. CONSTANTSAS*, D. NICOLOPOULOS, A. AGATHOPOULOS*, N. MATSANIOTIS*, Pediatric Clinic, University of Athens, Greece.

Tautomerase, tyrosine transaminase, and p-hydroxyphenylpyruvate hydroxylase activities were measured in *post-mortem* liver specimens from 13 full term newborns; from bigger prematures weighing more than 1.5 kg; from 7 prematures weighing less than 1 kg who died within 6 days after delivery; from 3 full term stillborns; and from 14 adults who died at age of 41 to 90 years.

Tautomerase activity in the very small prematures and in the adults was significantly higher than in the full term and in bigger premature infants. The infant and adult tautomerases are different.

About 80 % of total liver p-hydroxyphenylpyruvate hydroxylase in the newborn is inactive, whereas in adults 90 % of the enzyme exists in a 'natively' active form. The total enzyme activity was 6-fold higher in adults than in newborns. The properties of both active and inactive enzymes are affected during activation *in vitro*.

Observed differences in tyrosine transaminase among the various age groups were not significant statistically. About 50 % of the enzyme after homogenization is conjugated with coenzyme. The enzyme appears in a form completely saturated by coenzyme when livers are homogenized with pyruvate, oxaloacetate, 2-oxoglutarate, or citrate. This treatment also raises the total enzyme activity.

Possible kinship between these enzymes acting on a common substrate, and the probable role of tricarboxylic cycle acids in regulating intracellular tyrosine pools available for catechol amine synthesis through activation of tyrosine transaminase are discussed. (Supported by US Public Health Service, NIH Grant 1 RO5 TW00277-01)

12 *Abnormal Patterns of Amino Acids in Serum and Urine in Methylmalonic Acidemia.* S. HALVORSEN*, O. STOKKE* and L. ELDJARN*, Pediatric Research Institute and Institute of Clinical Biochemistry, Rikshospitalet, Oslo, Norway (introduced by M. Seip).

Methylmalonic acidemia (MMAemia) is a recently described inborn error of metabolism, clinically characterized by acidosis, lethargy and dehydration in the neonatal period or later in infancy or childhood [OBERHOLZER *et al.*: Arch. Dis. Childh. *42:* 492 (1967); STOKKE *et al.*: Scand. J. clin. Lab. Invest. *20:* 313 (1967)]. Biochemically the disease is characterized by a marked accumulation and urinary excretion of large amounts of MMA. The accumulation is most probably due to a block in one of the two enzyme systems which are involved in the conversion of MMA-CoA to succinyl-CoA.

Studies on the amino acid metabolism in our patient with MMAemia have revealed an almost generalized aminoacidemia which was clearly related to the protein intake. On moderate protein intake the serum amino acid levels were only slightly elevated, while high protein intake was correlated with elevation particularly of glycine, serine, threonine, lysine, alanine, tyrosine, phenylalanine, ornithine, arginine, valine and leucine. The urinary excretion of amino acids, in particular the excretion of glycine and lysine, was also markedly increased on high protein intake.

The clinical picture of hyperglycinemia is identical to that of MMAemia. The amino acids valine, isoleucine, threonine, and probably methionine, are precursors of MMA-CoA. Of these, valine is most likely the most important quantitatively. In hyperglycinemia valine, isoleucine, threonine, methionine and leucine are known to give rise to ketoacidosis. These similarities both in the clinical picture and in the biochemical findings indicate that some of the patients diagnosed as suffering from hyperglycinemia may actually have the same basic defect as in MMAemia, i.e. a block in the conversion of MMA-CoA to succinyl-CoA [HALVORSEN *et al.*: Lancet *1:* 756 (1968)].

13 *Dietetic Experiments in Cystinosis and Homocystinuria.* P. LUTZ*, Univ.-Kinderklinik, Heidelberg, Germany (introduced by H. Bickel).

In patients with cystinosis a deposition of crystalline cystine in several tissues is the most characteristic finding. We assumed as a working hypothesis that the tubular insufficiency and the slowly developing renal failure is caused by a metabolic defect in cystine degradation. In three patients we therefore restricted the daily intake of cystine and that of its metabolic precursor methionine to minimal requirement. In contrast to earlier authors we substituted two thirds of the protein intake by a mixture of pure L-amino acids. Even when lowering the daily methionine intake to 25 mg/kg body weight, no nutritional deficiency developed during 11 months of treatment: The nitrogen balance remained positive, the plasma albumin and the methionine levels which were assayed with the STEIN and MOORE technique, were about normal. The children started to thrive soon after beginning this regimen. It is too early to judge whether renal insufficiency will improve and whether this fatal complication may be altogether prevented by starting the diet as early in life as possible.

In two children with homocystinuria, a boy and a girl, we introduced as therapy a diet poor in methionine. They showed the typical clinical picture with subluxation of lens, mental retardation, and skeletal deformities. – The methionine reduction was achieved by reducing the daily protein intake to 1.25 g/kg body weight. Furthermore, pure L-cystine was added (0.15 g/day and kg body weight). The elevated methionine level in plasma became normal, homocystine disappeared from plasma and urine. The same results were obtained during normal protein intake but with pyridoxine (0.6 g per day) added.

14 *Pyridoxine-dependent Homocystinuria: Clinical, Metabolic and Enzymatic studies.* G.E. GAULL*, D.K. RASSIN* and J. STURMAN*, Department of Pediatric Research, N.Y. State Institute for Basic Research in Mental Retardation, Staten Island, N.Y., and Department of Pediatrics, Mount Sinai Hospital School of Medicine, New York, N.Y. (introduced by W. Swoboda).

Patients with homocystinuria have been studied untreated and while on massive doses of pyridoxine. During treatment, while on an unrestricted diet, hypermethioninemia and homocystinemia disappeared in

one pair of siblings. Another pair of siblings could not be so controlled until methionine intake was limited to 75 mg/kg/day. When pyridoxine was discontinued, hypermethioninemia and homocystinemia reappeared, regardless of diet.

When loads of L-methionine (100 mg/kg, p.o.) were administered while untreated, methionine failed to return to resting levels after 24 hours and homocystinemia was unchanged. During pyridoxine treatment, methionine started at normal levels but remained elevated at 24 hours, and homocystine, absent at zero time, gradually increased to untreated levels.

Trans-sulphuration enzymes of liver were studied in nine patients. When untreated, activity of cystathionine synthase is apparently absent, whereas methionine-activating enzyme and cystathionase are present in normal amounts. Two patients treated only by dietary restriction of methionine showed normal activity of cystathionase, but methionine-activating enzyme was reduced and cystathionine synthase remained absent. With biochemically successful pyridoxine therapy, there is an apparent increase in cystathionine synthase activity, as measured by our method. However, it is not clear that the product being measured in the presence of large amounts of pyridoxine is cystathionine, since these assays were performed on needle biopsies and there was not sufficient material to identify the product. A compound with similar chromatographic behavior can be produced chemically by homocysteine in the presence of substrate quantities of pyridoxal phosphate. The possible significance of this compound in relation to the non-enzymatic catalytic effects of pyridoxal phosphate and pyridoxine 'dependency' are discussed briefly.

These biochemical results should be interpreted cautiously: (1) The role of hypermethioninemia and homocystinemia in the pathogenesis of homocystinuria is not clear. (2) The experience with untreated homocystinuria and pyridoxine therapy is small and uncontrolled. (3) It is uncertain whether all patients with homocystinuria will respond.

15 *Relationship Between Plasma and Red Cell Lipids.* JUNE K. LLOYD*, MAUREEN M. SHACKLADY* and E.M. DJARDJOURAS*, Institute of Child Health, University of London, England (introduced by O. Wolff).

Serial analyses of red cell membrane lipids have been made in a child with hyperlipoproteinaemia due to liver disease. Initially plasma total cholesterol (374 mg/100 ml) concentrations were high, and both beta- and alphalipoproteins were increased. In the red cell membrane, cholesterol was increased (1.68×10^{-10} mg/cell; normal $1{,}15 \times 10^{-10}$ mg/cell) but total phospholipid was normal. The distribution of individual phospholipids, however, was altered with an increase in the ratio of phosphatidyl choline to sphingomyelin (3.5; normal 1.3). When the plasma alphalipoprotein was reduced by high carbohydrate feeding, the plasma total cholesterol remained high (408 mg/100 ml) and little change occurred in the red cell membrane lipids. However, when a diet rich in polyunsaturated fat was given, both beta- and alphalipoproteins were reduced and the plasma cholesterol fell to 265 mg/100 ml. On this regime the red cell cholesterol concentration became normal (1.12×10^{-10} mg/cell) and the phospholipid distribution nearly normal (phosphatidyl choline/sphingomyelin ratio 1.0).

Studies on five patients with inherited lipoprotein deficiency states (4 children with abetalipoproteinaemia and one adult with Tangier Disease) showed normal concentrations of cholesterol and total phospholipid in the red cell membrane in spite of very low plasma levels, but in both conditions the distribution of phospholipids was abnormal. In abetalipoproteinaemia the ratio of phosphatidyl choline to sphingomyelin was low (0.66–0.78) and in Tangier disease it was increased (2.3). Similar changes in phospholipid distribution were present in the plasma, and the fatty acid pattern of the individual phospholipids also reflected that of the plasma.

The results show that changes in red cell membrane structure occur not only in genetically determined disorders of plasma lipoproteins but also in acquired conditions, and that the abnormalities are potentially reversible.

16 *Etude des lipides totaux, des phospholipides et de la clarification du sérum chez l'enfant néphrotique au cours de l'épreuve d'hyperlipémie provoquée par voie intraveineuse.* R. JEAN, A. CRASTES DE PAULET, P. CRASTES DE PAULET et M. ASTRUC, Clinique des Maladies des Enfants, Monpellier, France.

Après perfusion à débit continu pendant quatre heures d'une émulsion de triglycérides à la dose de 2 g/kg, l'étude comparative des résultats obtenus chez des enfants normaux (14 cas) et des enfants à la phase d'état d'un syndrome néphrotique (20 cas) montre les faits suivants.

– La flèche d'hyperlipémie, indépendante du taux de départ est en moyenne à peine plus élevée chez les néphrotiques que chez les enfants normaux.

– Le retour au taux de départ de la lipémie est obtenu dans un délai de temps comparable pour les deux groupes d'enfants.

– Les modifications des phospholipides totaux, bien que le chiffre de départ soit plus élevé chez les néphrotiques, sont comparables pour les deux groupes d'enfants.

– Les valeurs des pourcentages des différentes fractions phospholipidiques (lysolécithines, lécithines, sphingomyélines) subissent des variations comparables chez les deux groupes d'enfants.

– Au décours de la perfusion d'émulsion lipidique, apparaissent chez les néphrotiques comme chez les enfants normaux des pré-bêta-lipoprotéines.

Il n'existe donc pas de différence notable dans les processus de clarification entre l'enfant normal et l'enfant néphrotique, malgré la présence d'une forte hyperlipidémie de départ chez ce dernier.

L'hyperlipémie de base de la néphrose lipoïdique ne peut donc s'expliquer ni par un défaut de captation périphérique des lipides, ni par un trouble de la synthèse par le foie des lipoprotéines.

Ces constatations sont en faveur de la conception d'une hyperlipémie par hyperproduction des protéines A et B des lipoprotéines par le foie. La protéine A de faible poids moléculaire est éliminée par les reins, la protéine B de poids moléculaire élevé retenue dans le sang fixerait des lipides qui seraient ainsi maintenus en excès dans le plasma.

17 *New Methods for the Determination of Calcium Levels in Biological Fluids.* P. ROYER, Clinique de Génetique Médicale – Unité de Recherche sur les Maladies du Métabolisme chez l'Enfant, Paris, France.

Calcium metabolism studies in infants and children are still hindered by technical difficulties in the measurement of the different physicochemical forms of calcium in biological fluids. Three potentially useful methods are in progress in this field.

1. Total Calcium

An important advance in the determination of total calcium was the application of atomic absorption spectrophotometry to this measurement. The main advantages of this methods are: (1) the necessity for only small quantities of biological fluids; (2) the possibility for complete automation; (3) the concomitant measurement of magnesium; and (4) the concomitant measurements in plasma, urine as well as in feces or tissue extracts. One must emphasize two points: (a) this technique detects calcium as low as 1 ppm; (b) the plasma values are slightly lower than those obtained utilizing classic complexometric methods. The details of this technic are well known so that it is unnecessary to describe them further.

2. Ultrafiltrable Calcium

At the present time there is no acceptable method for the determination of ultrafiltrable, non protein bound, calcium on very small volumes of plasma or serum. BALSAN, SACHS, JACQUES and MALICHECQ have been working in our laboratory for two years to solve this technical problem. Practical difficulties arise from the necessity for complete collection of an ultrafiltrate obtained over a short period of time from an initially small volume of fluid. In addition the results appeared to be affected by variation of temperature and pH and by the Donan effect.

Several of these problems have been partially resolved:

(1) Reduction in the size of the ultrafiltration chamber permitting the analysis of 0.5 to 2 ml of fluid.

(2) Continuous mixing with a turbine driven through a specially constructed gas-tight connection.

(3) Temperature control of the ultrafiltration chamber.

(4) Collection of ultrafiltrate trapped between the membrane and its support by a system of counterpression.

(5) Reduction of ultrafiltration time by use of a highly permeable membrane.

(6) Maintenance of constant pCO_2 in the gas mixture ($CO_2 + N_2$) used for ultrafiltration.

Further work is in progress to solve the remaining difficulties.

3. Ionized Calcium

The potential applicability has been greatly broadened through the use of a divalent cation electrode. SACHS, BOURDEAU et BALSAN, in our laboratory, have studied and improved this technique.

The measurement is made directly with a liquid membrane electrode. The membrane is a porous support saturated with an organic-phosphoric, hydrophobic ion-exchange resin specially selective for calcium in the pH range of 5.5 to 11. The original electrode was adapted for the measurement of calcium in protein-containing solutions employing a perfusion device in order to prevent clogging of the membrane by proteins. The technique does not require more than 0.5 ml of fluid. The absolute error is between 1 and 2 mg.

The measurement of ionized calcium in blood is facilitated by the relative constancy of pH and ionic force as well as the high degree of selectivity of the resin for the competitive cations in this biological medium: magnesium, sodium, potassium.

It appeared to us, in the course of our study, that it was necessary to use serum obtained by immediate centrifugation of blood collected anaerobically, without an anticoagulant or tourniquet. Usual anticoagulants chelate calcium whereas heparin has a weak but definite chelating effect (1–2 mg/l). pH does not interfere with the measurement itself, but modifies the equilibrium of the different physico-chemical forms of calcium: that is why it is necessary to collect blood in vacuum tubes.

Under these circumstances, ionized calcium level has been found to be 40 mg/l in normal adult, and 45 mg/l in children in our to-date results.

The measurement of ionized calcium in urine presented severe difficulties due to the following factors: (1) The ionic force of the urine is very variable and raises the problem of the use of standards of different ionic forces; (2) the electrode responds to H^+ (and not Ca^{++}) below pH 5.5; (3) *in vitro* studies have demonstrated that the electrode is sensitive to NH_4 ions.

When these techniques will work, programs can be easily worked out in physiology, experimental and clinical pathology, as well as for *in vitro* systems.

18 Hydrochlorothiazide and Calcium Absorption. A. DONATH*, S. NORDIO, F. MACAGNO*, R. GATTI* and E. ROSSI, Children's Hospital, University of Berne, Switzerland, and Dept. of Pediatrics, University of Genova, Italy.

It is known that in idiopathic hypercalciuria, hydrochlorothiazide reduces urinary excretion of calcium. But in one of our cases under prolonged treatment no therapeutic effect on either osteoporosis or body growth was observed. For this reason the hypothesis of an action of hydrochlorothiazide at different levels (kidney, gut, etc.) in human subjects was put forward. Assays *in vitro* showed that thiazide drugs reduce sodium and water transfer in intestinal mucosa.

Considering the above-mentioned data, we have first shown that the administration of hydrochlorothiazide *per os* increases calciuria when a 3 hours calcium perfusion is performed under standard conditions and under hydrochlorothiazide. Balance studies showed again the increase of calciuria, but also of faecal calcium when the drug is administered. In order to study its influence on calcium absorption from the intestine, we have measured the absorption of Sr^{85} first under normal conditions and then under hydrochlorothiazide (2 mg/kg/day, maximum 50 mg/day). One to three μCi of Sr^{85} were given by mouth to a group of 17 normal children, without carrier, after a 12 hours fast, and the child did not eat anything for the next 2 hours. Stools were collected for the following 5 days and checked for radioactivity after homogenization. We have found a significant fall in calcium absorption under hydrochlorothiazide, as compared to the values without the drug. About $^1/_3$ more of the radio-strontium came out in the stool.

In order to be sure that this was not due to an increase of calcium secretion into the gut, we gave in 2 cases Ca^{47} intravenously under normal conditions and under hydrochlorothiazide administration. We did not find a significant change in Ca^{47} secretion into the intestine, but confirmed the previously already mentioned effect of hydrochlorothiazide to reduce calciuria. The results of our investigation confirm the

effect of thiazide at intestinal level, as previously demonstrated by experimental research *in vitro:* The drug has a definite lowering effect on calcium absorption.

19 *Hypomagnesemic Tetany with Magnesium-Dependent Hypocalcemia-Hyperphosphoremia.* S. NORDIO, A. DONATH, R. GATTI and F. MACAGNO, Department of Pediatrics, University of Genova, Italy, and Children Hospital, University of Berne, Switzerland.

A 7-month-old baby boy entered the hospital for convulsions, tetany and irritability, with hypocalcemia, hyperphosphoremia and hypomagnesemia. Psychomotor retardation and irritability were observed since the age of 4 months.

Clinical manifestations completely disappeared and calcium-phosphorus blood levels normalized when a diet-supplementation with high doses of Mg-glycerophosphate was given. The patient was followed from February 1967 to August 1968 and each time Mg-supplementation was stopped more or less severe clinical manifestations and biochemical alterations reappeared. Magnesemia practically never attained complete normal levels, either on normal diet or on Mg-supplementation. Many studies on the transport of stable Ca, PO_4 and Mg and of radioisotope Mg, Sr, K and Na were performed.

An intestinal Mg-malabsorption and high concentration of Mg in sweat were demonstrated. Sr^{85} was also malabsorbed in intestine. Stable-Ca balance was positive and its intestinal absorption did not significantly differ from normal children. Sr^{85} intestinal absorption lowered in a control boy fed with prolonged poor-Mg diet. Intracellular potassium and exchangeable K/Na ratio were also lowered, in the patient with chronic primitive hypomagnesemia. Intracellular Mg-deficiency was not as important as to determine an impairment of Mg-dependent metabolic activities (glycolysis) in the patient. Intestinal Mg-dependent ATP-ase, histochemically determined in the presence of Mg-sulfate, was normal. Mitochondria of epithelial cells of intestinal mucosa were swollen as in common and vitamin D-resistant rickets. Either in primitive or in secondary Mg-deficiency the sensitivity to parathormone and vitamin D was reduced.

In conclusion primitive hypomagnesemia with secondary hypocalcemia is a new disease with primitive alteration of Mg-transport and secondary impairment of the transport of other cations. Intestinal Mg-malabsorption represents a fundamental pathogenetic factor. The influence of Mg-deficiency on Ca-P homeostasis deserves further researches.

20 *Microscopic Aspects of Vitamin D-Resistant Rickets and Skeletal Fluorosis. A Common Lesion.* R. STEENDIJK, Departments of Paediatrics and Histology, University of Amsterdam, Holland.

In previously reported studies of compact bone from patients with vitamin D-resistant rickets (familial or essential hypophosphataemia) microradiographic examination of undecalcified bone sections revealed a lack of mineral around osteocyte lacunae and their canaliculi. On histological examination of decalcified sections a lesion of the bone matrix was observed at these sites. This lesion was characterized by an abnormally strong affinity for azure II, an interruption of the normal lamellar pattern and a globular appearance of the matrix. In bones from several patients with other types of rickets and osteomalacia these abnormalities could not be found; the question arose therefore whether the findings were specific for primary vitamin D-resistant rickets.

It has been found by others however that the same pattern of perilacunar low mineral density is present in chronic skeletal fluorosis. Therefore bone from a patient with this disease was examined with the methods used in the above-mentioned study. It was found that the perilacunar lesions were identical both in undecalcified and in decalcified sections.

Although the gross skeletal changes in vitamin D-resistant rickets and in skeletal fluorosis are largely different, both diseases have a common aspect: impairment of the process of mineralization. It appears that the perilacunar lesion is a manifestation of this impairment, which may be caused by different mechanisms and which is not specific for vitamin D-resistant rickets. Probably it is only found in diseases in which mineralization is interfered with for long periods of time.

21 *Pseudo-Vitamin D Deficiency Rickets.* A. FANCONI and A. PRADER, Department of Paediatrics of the University of Zurich, Switzerland.

Two previously reported and three new cases of hereditary pseudo-vitamin D deficiency rickets demonstrate the clinical aspects of this disorder from birth to adult life.

Two sisters are discussed more in detail. The older one suffered from extremely severe rickets at the age of 16 months, in spite of previous vitamin D treatment. Growth and development were retarded. The main biochemical findings were hypocalcaemia, normal serum P, elevated alcaline phosphatase and hyperaminoaciduria. Vitamin D treatment with 10,000 U/day was inefficient, whereas 40,000 U/day cured rickets clinically, radiologically and biochemically, and normalized the aminoacid excretion. I.m. PTE had no effect on serum calcium until the rickets was completely cured. The younger sister was followed since birth. In spite of a regular daily supplement of 800 U vitamin D, she showed resistance to PTE and hyperaminoaciduria at the age of two months and developed mild rickets at the age of 5 months. The 3 other patients are 6, 8 and 27 years old. Their general health, growth, bone structure and serum chemistry are normal as long as the vitamin D treatment of about 1 mg per day is given.

From the literature and from personal communications we are aware of 23 other cases seen in recent years. Affected siblings and parental consanguinity are frequent. In most cases the transmission seems to be autosomal recessive.

The disorder can be explained by a decreased sensitivity of the intestinal mucosa and the bone to vitamin D ('vitamin D dependency'). The disease has to be distinguished from vitamin D deficiency rickets and from hypophosphataemic refractory rickets.

22 *Amino Acid Clearances in Vitamin D Resistant Rickets.* J. P. COLOMBO, A. DONATH and P. BACHMANN, Children's Hospital, Berne, Switzerland.

Vitamin D resistant rickets in contrast to vitamin D deficient rickets is not accompanied as a rule by an elevated excretion of amino acids in urine. We presumed that using column chromatographic technique quantitative differences in the clearances of amino acids in cases of vitamin D resistant rickets of the ordinary type compared to normals might become evident. The GFR was assessed using a single injection clear-

ance technique with Cr^{51}-EDTA. Amino acid clearances in 4 cases of vitamin D resistant rickets fell into the same range as the controls except those for glycine which was elevated in two cases.

In a second type of experiments the influence of calcium infusions on the tubular reabsorption of amino acids in normals and patients with vitamin D resistant rickets was studied. Amino acid clearances were done before and after a calcium infusion (1500 mg/m²) of 12 hours duration. A decrease of the fraction of filtered amino acid excreted was observed after calcium infusion in normals ($p < 0.05$) and in the cases of vitamin D resistant rickets ($p < 0.025$). In 4 instances the filtered load of amino acids was even greater after calcium infusion. Changes of the GFR after calcium infusion were variable.

Since amino acid clearances in patients with vitamin D resistant rickets lay in the normal range, a reabsorption capacity for amino acids similar to that in normal individuals exists, though it is not necessary working at full rate in both groups. This transport rate can be augmented by calcium infusions, for increased reabsorption of amino acid occurred in normals and in vitamin D resistant rickets.

The effect of calcium on amino acids in the kidney could be mediated by the action of parathyroid hormone, which might, through influencing the rate of amino acid transport, represent one of the physiological regulatory mechanism in tubular amino acid reabsorption.

23 *Bile Acid Excretion and Steatorrhea in Intrahepatic Cholestasis of Infancy (Neonatal Hepatitis).* A. Norman*, Birgitta Strandvik* and R. Zetterström, Department of Pediatrics, Karolinska Institutet, Stockholm, Sweden.

Bile acid excretion has been studied in four patients with intrahepatic cholestasis of infancy (neonatal hepatitis) after administration of cholic acid-24-C^{14}.

Bile acid excretion to the intestines was found to be highly impaired in intrahepatic cholestasis. The main route of bile acid excretion was via the urine. Practically all of the administered labeled cholic acid was conjugated prior to excretion. The main conjugates were glycocholic acid and taurocholic acid. In addition at least two more conjugates of cholic acid were recovered from the urine.

Analysis of bile obtained from three of the patients before operative cholangiography showed a very low concentration of bile acids, phospholipids and cholesterol. The bile was of normal colour due to the presence of bilirubin.

Severe steatorrhea and markedly impaired vitamin A absorption was demonstrated when the patients were jaundiced. The impairment of bile acid excretion to the gut and the degree of steatorrhea were well correlated. In some of the patients steatorrhea persisted after the disappearance of jaundice. In these patients the impairment of bile acid excretion to the gut was found to remain.

24 *Extensive Copper Storage in a Patient with Cirrhosis.* A. Sass-Kortsak*, I. B. Sardharwalla* and P. M. O'Sullivan*, Department of Pediatrics, University of Toronto, Hospital for Sick Children, Toronto, Canada (introduced by W. Swoboda).

A patient with slowly advancing idiopathic cirrhosis had normal serum copper and ceruloplasmin levels, but marked cupriuria. Oral administration of D-penicillamine caused urinary output of copper far in excess of normal.

Following i.v. administration of a single dose of ^{64}Cu, there was no incorporation of ^{64}Cu into ceruloplasmin, fecal excretion of ^{64}Cu was normal, its urinary excretion increased. Aminoacidemia and aminoaciduria were absent. The unrelated parents were not heterozygotes of Wilson's Disease. Patient is now being treated with D-penicillamine. By these means large amounts of copper are being removed. It is proposed that the copper storage in this patient is secondary to a peculiar form of cirrhosis, or that he has a copper storage disease distinct from Wilson's disease with secondary cirrhosis. (Supported by The John A. Hartford Foundation of New York)

25 *The Effect of Unabsorbed Solutes on the Human Small Intestinal Motility and Absorption.* Kari Launiala*, Children's Hospital, University of Helsinki, Finland (introduced by J. K. Visakorpi).

As there is evidence to suggest that the osmotic effect of unabsorbed disaccharides is the principal mechanism of diarrhoea of disaccharide malabsorption, the effect of different concentrations of unabsorbable solute on the movement of water and electrolytes and on the mean transit time was studied by perfusing a 50 cm segment of proximal small intestine. Sucrose was the unabsorbable solute in a child with congenital sucrose-isomaltose malabsorption and mannitol in three normal children.

In both cases a steady state was approached in which the intestinal fluid was isotonic to the extracellular fluid and contained about 95 mEq/l of Na^+, and in which every millimole of the unabsorbable solute retained ca. one millimole of NaCl and 10 ml of water. Concentrations of the test substances exceeding 100 mmoles/l thus caused net secretion of water and electrolytes. This resulted in an accelerated flow in the distal part of the test segment. The flow rate was further increased by shortening of the mean transit time, which was proportional to the content of unabsorbable solute.

Secondly the absorption of arginine, xylose and palmitic acid was studied during normal and accelerated passage of a test solution through the segment. When the transit time was shortened by 50–70 %, the absorption of palmitic acid, xylose and arginine was reduced by an average of 42, 35 and 9.5 %, respectively.

The findings suggest that in disaccharide malabsorption the increase in intraintestinal volume caused by the osmotic effect of unabsorbable disaccharide and the relatively poor Na^+-concentrating capacity of the jejunal mucosa leads to accelerated transit through the small intestine, in proportion to the quantity of disaccharides ingested. The slight generalized malabsorption observed in this disease is evidently due to accelerated passage through the small intestine.

26 *Contribution to Chromosomal Breakage Study in Man.* D. Germain*, Ch. Requin* and N. Philippe*, Laboratoire d'hématologie – Hop. Ed. Herriot et Département de Cytogénétique, Université de Lyon, France (introduced by R. François).

1. The authors report the chromosomal abnormalities they observed in 6 children presenting Fanconi's anemia.

Peripheral blood and bone-marrow cultures show breaks and chromatidian gaps with chromosomal rearrangements.

– 10 to 94 percent mitoses for peripheral blood.
– 7.6 to 68 percent mitoses for bone-marrow.

2. They seek to specify the casual role of lymphocyte cultures and mitoses preparation conditions in the apparition of chromosomal breaks. For this purpose peripheral blood of a patient suffering from Fanconi's anemia and peripheral blood from a normal subject of opposite sex are cultivated in the same tube.

Upon metaphasic plates coming from these mixed cultures, the gonosomic equipment marks respective mitoses from both subjects; only patients' mitoses have a chromosomal abnormalities high rate. For example:
Patients: 14 abnormal mitoses out of 19
Control: 1 abnormal mitoses out of 18

3. Report and argue of the results of chromosomal examinations practised in:
4 cases of idiopathic bone-marrow aplasia
4 cases of Blackfan Diamond's anemia
1 case of amegacaryocytic thrombopenia.

4. Expound first results of a cytogenetic study practised on blood in 43 children suffering from chronic nephropathy and subjected to an immuno-depressive treatment by nitrogen mustard, azathioprine and chlorambucil.

27 *Clinical Significance of Precipitating* E. coli *Antibodies in Urinary Tract Infections.* U. JODAL*, J. HOLMGREN*, L.A. HANSON and J. WINBERG, Department of Paediatrics and Institute of Medical Microbiology, Department of Bacteriology, University of Göteborg, Sweden.

It is well known that attacks of pyelonephritis are followed by production of antibodies demonstrable by the passive haemagglutination technique using O-antigen from the infecting *E. coli* strain. This method, however, reflects mainly the amount of IgM-antibodies present, but IgG-antibodies are produced as well as shown by earlier studies. The latter type of antibody is better demonstrated using precipitation techniques such as immunodiffusion methods.

This study was initiated to test our preliminary findings that IgG antibodies are mainly formed during recurrent but not during the first attack of first-time pyelonephritis.

Sera from 25 children with acute pyelonephritis were collected. Ten of these children had definite recurrencies, while 15 were suffering from their first urinary tract infection as far as could be ascertained by clinical history and examination. The diagnosis of pyelonephritis was made by clinical signs and symptoms, quantitative bacterial cultures (>100.000 *E. coli* per ml of urine), pyuria and elevated titers of agglutinating *E. coli* antibodies. In addition sera from 4 patients with renal scars ('chronic' pyelonephritis), but without demonstrable actual bacteriuria were investigated.

Each serum sample was analysed with O antigen from the infecting strain (bacterial suspension boiled for 2 hours), as well as from type strain of the eight O groups most prevalent in cases of urinary tract infection (purified lipopolysaccharides).

A double-diffusion method was used in the micro modification of Wadsworth.

In all sera from patients with recurrent urinary tract infections precipitating antibodies against O antigen from their infecting *E. coli* strain could be demonstrated. In the sera from the 4 patients with renal scars, but without actual bacteriuria precipitating antibodies were also found using as antigen the eight different purified O antigens. On the other hand such antibodies could be detected in only three of the 15 sera from children with their first attack of pyelonephritis. One of these three patients had antibodies against three different O groups, indicating that also this patient had a recurrent infection. Precipitating *E. coli* antibodies could not be found in sera from 20 adult blood donors.

Thus precipitating antibodies against the infecting *E. coli* strain seem to be demonstrable in all children with recurrent or 'chronic' pyelonephritis, while such antibodies are rarely found in cases with probable first infections.

The diagnostic possibilities of this method especially for screening of recurrent or 'chronic' pyelonephritis and for prognostic evaluation will be further investigated.

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