2 A. A. VON RUECKER*, P. RAHM*, B. HAAS*, and J. SCHAUB

University Children's Hospital, Munich, F.R.G.

A new possibility for the prenatal diagnosis of metabolic diseases. Example: Pompe's Disease.

Antibodies were made against acid α -glucosidase (α -glu) from human liver. This enzyme is missing in patients with Pompe's disease (PD). Fibroblasts from patients with PD and controls were labelled in culture with 3 H-leucine for a 24 hour period. Afterwards the fibroblasts were harvested and immunoprecipitation was carried out employing the mentioned antibody and Protein A - Sepharose Cl-4B gel. The resultant immunoprecipitates were analysed by SDS gel electrophoresis. It could be demonstrated that α -glu deficient fibroblasts form polypeptides which can be precipitated with antibodies against α -glu. These polypeptides show the same electrophoretic mobility as subunits of α -glu in controls. "Chase experiments" (overdose of unlabelled leucine after labelling with 3 H-leucine) showed that these polypeptides disappear rapidly in fibroblasts of patients with PD. By multiplying the percentage of 3 H-leucine incorporated in α -glu with the specific enzyme activity, a value is formed which seems characteristic for the variant forms of PD. It may be speculated that with the help of 1. specific antibodies, 2. Protein A - Sepharose Cl-4B gel and 3. radioactive labelled cells many enzyme defects cannot only be diagnosed more correctly but also characterized and localized within the cell.

3 H. WICK and R. BAUMGARTNER, University Children's Hospital, Basel, Switzerland. Thiamine (Th) dependent pyruvate dehydrogenase (PDH) deficiency.

The biochemical abnormalities in vitamin dependencies are poorly understood. Defects of coenzyme synthesis or transport improved by mass action, compensation of decreased affinity of the apoenzyme for its coenzyme and stabilization of a mutant apoenzyme by high concentrations of coenzyme are some of the mechanisms discussed. - Fibroblast cultures from two children suffering from congenital lactic acidosis responding well to very high doses of Th (50 - 100 mg/kg/d) were studied. Reduced CO_2 -production from $1,^{14}\text{C}$ -pyruvate and deficient activity of PDH-complex were found. Culture of these fibroblasts in the presence of high concentrations of Th (0.4 $\mu\text{g/ml}$) resulted in stimulation (20 - 100 %) of pyruvate decarboxylation in both systems. This effect could not be prevented by puromycin (50 $\mu\text{g/ml}$), an inhibitor of protein synthesis. Surprisingly the same result was also obtained in normal cell lines. Furthermore the same effect could also be demonstrated for 2-oxoglutaric acid dehydrogenase and for 2-oxoisocaproic acid dehydrogenase. - The type of activation mechanism involved is not yet clear. In the case of PDH activation might be due to the conversion of the enzyme complex to the dephosphorylated (i.e. activated) form by high concentrations of thiaminepyrophosphate. The clinically relevant conclusion is that in any patient with a partial deficiency of one of these dehydrogenases a therapeutic trial with very high doses of Th is warranted.

4 G. Mc KHANN*, B. FRANCOIS*, P. EVRARD* (intr. by G. Van den Bergh) Neurolog. Dept., Johns Hopkins Med. Sch., Baltimore, USA and Neuropediat. Dept., Université de Louvain, Brussels, Belgium. Long term use of low doses of dichloroacetate in a child with congenital lactic acidosis.

Dichloroacetate (DCA) has been shown to reduce plasma lactate levels in healthy and diabetic adults as well as in a child with congenital lactic acidosis (CLA). (F. Coude et al. New Engl. J. Med. 299: 1365, 1978). In a 12 year-old boy with CLA, DCA was administered orally during 12 months. The underlying biochemical defect in this patient is still unknown. In cultured fibroblasts, enzymatic activities of pyruvate dehydrogenase (PDH), pyruvate carboxylase, phosphoenolpyruvate carboxykinase were within the normal range. DCA therapy given at a dose as low as 15 mg/kg b.d. every second day, was effective in lowering plasma lactate (7.6 mMol. to 0.9 mMol). In addition growth rate was stimulated (7 cm/year). Routine serial analysis were made throughout the study including blood levels of glucose, lipids, ketones, uric acid, oxalic aciduria, and the search of renal, hepatic, ocular and neural dysfunction. No adverse physical or biological effects associated with DCA therapy were observed. However DCA was not more effective on the gradually deteriorating neurological condition of this child than previous therapeutic attempts with thiamine, biotin, lipoic acid and ketonemetic diet. We conclude that the return to normal of lactate levels was probably secondary to the activation of muscular PDH; it is not clear whether the neurological disorder is related to abnormal levels of a metabolite in the plasma.

5 P. ROSSI*, I. QUINZI*, E. GALLI*, F. AIUTI*, L. BUSINCO. Dpt. of Pediatrics I^o and Dpt. of Internal Medicine III^o, University of Rome, ITALY.

THYMOSIN (TPI) THERAPY IN PATIENTS WITH SEVERE VIRAL INFECTIONS AND T-CELL DEFECT.

Recent experimental and clinical researches suggest that thymic humoral factors may be helpful in restoring immune balance in patients with T-cell defect. A calf thymic extract (TPI Sero In-

stitute) induces the maturation of pre-T-cells and increases the capacity of mature T-cells to respond to antigen. Our previous investigations have shown a maturation of pre-T-cells in vitro, a clinical and immunological reconstitution in primary T-cell defect after TPI therapy. Now we have treated 10 children suffering from T-cell defect associated with severe viral infections, with TPI 75 mg/m²/day for a week and at the same dose twice a week for 3 months. Our results showed an increase of E-rosette forming cells after incubation of peripheral blood lymphocytes with TPI in vitro and after 1 month of treatment in vivo (fig). Furthermore, during the therapy, a complete recovery of viral infections was observed in all subjects.

6 J. VOSSEN, R. LANGLOIS VAN DEN BERGH*, J.P.M. GERAEDTS*, E. VAN LOGHEM* and L.J. DOOREN. Depts. of Pediatrics and Human Genetics, University Hospital, Leiden, and Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands. Different pattern of chimerism of lymphoid cells after bone marrow transplantation.

A direct immunofluorescence staining of either T or B lymphocyte membrane markers was combined with a quinacrine staining for the Y-chromatin in the interphase nuclei of these cells. In this way it was possible to identify the origin of both lymphocytic populations in the peripheral blood after a successful bone marrow transplantation from a donor of the opposite sex in 2 patients with severe combined immunodeficiency (SCID) and in 8 patients with aplastic anemia (AA). The findings were correlated with the results of karyotyping following PHA stimulation of (T) lymphocytes and with the serum Ig allotypes. In the 2 SCID patients T cells of donor origin were found together with B cells of recipient origin. In one of these patients both donor (G3m (c3)(c5)) and recipient (G2m (n)) Ig allotypes were produced by the recipient following immunological reconstitution, indicating the presence of both donor and recipient B cell populations. In the 8 patients with AA both T and B cell lineages were of donor origin except in 2 patients in whom the combined staining technique an indication was found for partial T cell chimerism. One of them had different Ig allotypes as compared with the donor, but still produced recipient Ig type (G1m (a)(x)(z), G3m (g)) at more than one year after transplantation indicating also the presence of the donors' and the patients' B cell population.

7 M. RISTER* (introduced by E. Gladtko) Children's Hospital Cologne, FRG THE CELLULAR BASIS OF OXYGEN TOXICITY

The exposure of Guinea pigs to 85 % oxygen decreased various host defense mechanism in Polymorphonuclear leukocyte (PMNs) and Alveolar Macrophages (AMs). These leukocyte functions are dependent on an intact cytoskeleton, consistent of the microtubulus and microfilament system, which can be assayed from the mobility of fluorescence labelled Concanavallin A (Con A) receptor complexes on the cell surface. Con A shows a uniform surface distribution on cells with an intact microtubulus system, whereas its disruption causes a Con A cap formation. The alteration of the microtubulus and microfilament systems induces a patchy Con A distribution. To study the effect of hyperoxia on the cytoskeletal elements, the Con A distribution was observed in PMNs and AMs obtained from guinea pigs exposed to 85 % oxygen. By 90 hrs. 23 % PMNs exhibited a capped and a 69 % PMNs a patchy Con A distribution, compared to 11 % and 18 % controls, respectively. The number of spontaneous capped AMs increased two-fold during the oxygen exposure, too. Again, there was an increase from 14 % to 58 % AMs exhibiting a patchy Con A distribution by 90 hrs. This study demonstrates that hyperoxia alters the cytoskeleton of PMNs. Supported by DFG, Ri 275/2

8 E. MANDYLA-SFAGGOU*, M. XANTHOU, C. MARAVELIAS*, E. CARSE*, J.D. BAUM and N. MATSANIOTIS. First Department of Pediatrics of Athens University and John Radcliffe Hospital, University of Oxford. Leucocytes and their function in breast milk.

The leucocytes in the breast milk of 14 mothers 2-5 days following delivery were studied. Total white cell counts ranged from 16,000/ml to 2,400,000/ml. The proportion of cell types varied in the different milk samples: Macrophages (monocytes) made up 30-85%, polymorphonuclear neutrophils 10-70% and lymphocytes 5-15%. In order to test milk-leucocytes function an assay of antibody-dependent cellular cytotoxicity which depends exclusively upon monocytes and polymorphonuclears as the effector cells was applied. Blood group A erythrocytes (RBC) from healthy human donors treated with hyper-immune anti-A serum were used as target cells. Haemolysis was quantitated by ⁵¹Cr-chromate. Comparison with the cytotoxicity elicited by similar or even lower monocyte numbers taken from

the peripheral blood of adults or neonates showed that of milk monocytes to be significantly lower. Since phagocytosis has been shown to be important ($p < 0.001$) in the lysis of sensitized A, red cells perhaps milk monocytes are not so active because they are loaded with fat granules.

9 KINMONTH, A-L*, and Baum, J.D. University Department of Paediatrics, John Radcliffe Hospital, Oxford. THE EFFECT OF ALTERING THE TIMING OF PRE-BREAKFAST INSULIN INJECTION ON POST PRANDIAL METABOLIC CONTROL OF DIABETIC CHILDREN.

A randomised cross over study was performed on children aged 10-16, at home, to evaluate the effect of varying the time of their morning insulin injection on the post breakfast glycaemic peak. Ethical approval was obtained and consent given by parents and children. After optimisation of diabetic control 9 children injected their insulin 30 minutes (early = 'E') or 5 minutes (late = 'L') before breakfast on two consecutive Saturdays. Blood was sampled over 3.5 hours at 30 minute intervals for estimation of glucose, ketones, pyruvate, lactate, alanine, free insulin and C peptide. Diet, insulin dose and exercise were kept constant on test days. The mean blood glucoses at breakfast time were 10.98 mmol/l (E) and 10.55 mmol/l (L). Subsequent blood glucoses were all lower after the early injection, the mean peak values being 16.75 (E) and 19.35 (L) at 90 minutes after breakfast. Insulin levels rose from 7 mU/l to 11.1 mU/l at breakfast time after the early injection while they were still at 7.6 mU/l after the late injection. C-peptide levels were 1/10 of those expected in normal subjects and changed in parallel with the glucose levels. Total ketones were high and rising before breakfast to peak at 0.1 mmol/l (E) and 0.4 mmol/l (L). By 3 hrs after breakfast they had fallen to normal values of 0.05 mmol/l (E) and 0.07 mmol/l (L). Other metabolites were normal and similar on the two regimes. This study suggests that giving the morning insulin injection before rather than after dressing can significantly improve post prandial metabolic control.

10 F.A.VAN ASSCHE*, L.AERTS* (Intr. by L.CORBEEL) - Dep. Obst.Gynaec.University of Leuven - Is gestational diabetes an acquired condition?

Intravenous injection of 30mg of streptozotocin per Kg. body weight induces a mild diabetes in pregnant rats (first generation); the non-fasting blood glucose is increased and the percentage of endocrine tissue and also the percentage of granulated B cells do not increase. The fetuses of these mildly diabetic pregnant rats have an increased percentage of pancreatic endocrine tissue and there is B-cell degranulation. The modifications of the endocrine pancreas during intrauterine life causes persistent changes in later adult life (second generation), which are not perceptible in basal conditions but become apparent in situations stressing the B cell activity, such as an intravenous glucose load or pregnancy. During pregnancy in the second generation rats have increased non-fasting blood glucose and no adaptation of the B cells is seen. This inadequate adaptation to pregnancy causes changes in the foetal endocrine pancreas of the fetuses of the third generation. From these experiments it may be concluded that gestational diabetes is an acquired condition.

11 GY.SOLTÉSZ*, L.KLUJBER*, D.MOLNÁR*, M.KARDOS*, V.JASZAI* and J.MESTYÁN /Intr. by J.MESTYÁN/ Department of Paediatrics, University Medical School, Pécs, Hungary. Changes in total-HDL-cholesterol, N-acetyl-β-glucosaminidase in relation to the quality of metabolic control judged by HbA_{1c} in diabetic children.

28 diabetic children were studied. In the fasting blood samples the following substances were measured: glucose, total-cholesterol, HDL-cholesterol, triglyceride, N-acetyl-β-glucosaminidase and HbA_{1c}. The results obtained were the following: 1. Both fasting blood glucose and urinary glucose excretion were found to be positively related to HbA_{1c} / $r=0.77$, $p<0.001$; $r=0.58$, $p<0.001$, respectively/. 2. The positive correlation between HbA_{1c} and total as well as HDL-cholesterol were highly significant / $r=0.51$, $p<0.01$; $r=0.68$, $p<0.001$, respectively/. This relationship demonstrates that poor control of juvenile diabetes is associated with an increased HDL-cholesterol concentration. 3. N-acetyl-β-glucosaminidase, a lysosomal hydrolase was found to be increased in diabetic children and directly related to the percentage of HbA_{1c}, fasting blood glucose and HDL-cholesterol. The present examinations extend the influence of juvenile diabetes on HDL-cholesterol concentration by the observation that the poorer the metabolic control the higher the HDL-cholesterol content in the plasma.

12 I. DESCHAMPS* and J. HORS* (Intr. by H. Lestrade) Groupe de Recherche sur le Diabète et la Nutrition

chez l'Enfant, Hôpital Hérold 75935 Paris, France. HLA GENOTYPING AND RISK OF DIABETES.

Five different HLA-loci were tested in 53 families with one or more diabetic children. The alleles B15, B18, Cw3, Cw5, B*F1, DRw3, DRw4 were increased in diabetics, and significantly higher frequencies were found for several haplotypes with stronger lin-

kage disequilibrium than in the control population. The strongest associations occurred with the DRw3 and DRw4 alleles, suggesting the existence of several "high risk axes" carrying the diabetogenic gene (s) closely linked to the DR-locus. The excess of HLA-identity in multiple affected siblings of 12 families is in agreement with a recessive transmission. Yet the lack of homozygosity does not support a single-gene hypothesis. Moreover, the highly significant excess heterozygosity DRw3/DRw4 in diabetics (32 %, $p < 0.001$) provides evidence for a pseudorecessive mechanism, two complementary acting genes being linked to each of the DR-alleles. In conclusion, the low frequency of DRw3/DRw4 heterozygotes in healthy siblings of diabetics (10 %) and their absence in controls, attribute the highest risk of diabetes to related as well as unrelated carriers of this combination. (Relative risk = 5.3 and 46.6, respectively)

13 J.L. CHAUSSAIN, P. GEORGES*, D. GENDREL*, A. BRIJAWI* and J.C. JOB. Hôpital Saint-Vincent de Paul, Paris, France.

Interest of the measure of serum branched-chain amino-acids (BCA) in the diagnosis of hyperinsulinism in infancy.

Fasting values of BCA (valine, leucine and isoleucine) were measured by column chromatography in the sera of 27 normal children aged 2 days to 9 years, 14 children with ketotic hypoglycemia (KH) aged 1 to 7 years, and in 12 sera from 5 infants aged 15 days to 2 years with documented hyperinsulinism. In normal and KH children, each individual BCA and their sum were highly significantly negatively correlated with blood glucose (BG) values ranging between 0.11 and 0.92 g/l ($r = 0.61$, $p < 0.001$). In infants with hyperinsulinism BCA concentrations were significantly lower ($p < 0.001$) than in the other groups without correlation with BG values (ranging from 0.13 to 0.51 g/l), and with plasma insulin concentrations (ranging from 9 to 85 µU/ml). In all the children studied, the sum of BCA was highly significantly correlated with blood beta OH butyrate measured by enzymatic method in the same time ($r = 0.75$, $p < 0.001$). The association low BG-low BCA during fast seems characteristic of hyperinsulinic states, and the measurement of fasting BCA in these infants offers a simple way of diagnosis, avoiding the technical difficulties of beta OH butyrate determination.

14 G.DAHLQUIST*, (St.Görans's Childrens Hospital, S-112 81 Stockholm), J.GENTZ, L.HAGENFELDT*, A. LARSSON*, H.LÖW*, B.PERSSON, R.ZETTERSTRÖM, Stockholm. Ketotic hypoglycemia-a clinical trial of several unifying ethiological hypotheses.

After the informed consent by parents and children we have studied 15 children referred to our clinic because of suspected ketotic hypoglycemia. The patients were investigated according to a program designed to test several hypotheses - old and new postulated to explain the ethiology of ketotic hypoglycemia. The plasma levels of glucose, FFA, glycerol, β-HBA, alanine, cortisol and insulin as well as the urinary excretion of nitrogen, 3-methylhistidine and catecholamines and an i.v. glucose tolerance test were measured before and after a classical ketogenic provocation test. Out of the 15 children 6 will fill the criteria of ketotic hypoglycemia at the time of the study. The most remarkable finding in these 6 children in contrast to the other children studied was that they did not decrease their peripheral glucose utilization (measured as Kg) during starvation. These 6 children seemed to be more "advanced" in their adaptation to ketogenic diet in all other parameters studied. The children with ketotic hypoglycemia did not differ from the other children in plasma level of cortisol or urinary excretion of nitrogen, urea, 3-methylhistidine and catecholamines. - We favour the concept that the children with ketotic hypoglycemia represent the tail of the gaussian curve in the normal physiological development of the adaptation to starvation.

15 CORNILLIE F.*, LAUWERYS J.*, CORBEEL L., BOEL M.*, ECKELS R.* VAN DE WALLE J.* (Departments of Pathology, Pediatrics and Anesthesiology, University of Leuven). Acquired ultrastructural abnormalities of bronchial cilia in recurrent airway infections and bronchiectases as compared with the findings in Kartagener syndrome.

Electron microscopic studies revealing a lack of the dynein arms of the microtubular doublets of bronchial cilia and sperm tails suggest that Kartagener syndrome is a special manifestation of a hereditary disease, now referred to as the immotile cilia syndrome (Eliasson et al. N Engl J Med 297: 1, 1977). Anomalies of the bronchial cilia were observed in 5 patients with recurrent pulmonary infections. Patient 1 had Kartagener syndrome and an absence of the outer dynein arms in most ciliae, although occasional shortened and even normal arms were seen. Patient 2 and 3 had unilateral bronchiectases without family history of Kartagener syndrome. Serial studies of the bronchial epithelium showed a bilateral lack of the inner dynein arms and incomplete outer arms. These abnormalities persisted after recovery from acute pulmonary infection. In patients 4 and 5 with recurrent pulmonary infections without bronchiectases, many shortened outer dynein arms were observed, but these anomalies disappeared after recovery. In all 5 patients additional striking ciliary anomalies were found: megacilia, fused cilia, nude cilia and completely disorganized axo-