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 pyruvatedehydrogenase (PDH) deficiency.

The biochemical abnormalities in vitamine 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}C-pyruvate and deficient activity of PDH-complex were found. Culture of these fibroblasts in the presence of high concentrations of Th (0.4 µg/ml) resulted in stimulation (20 - 100 %) of pyruvate decarboxylation in both systems. This effect could not be prevented by puromycin (50 µg/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-oxoglutaricaciddehydrogenase and for 2-oxo-isocaproicaciddehydrogenase. - 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.

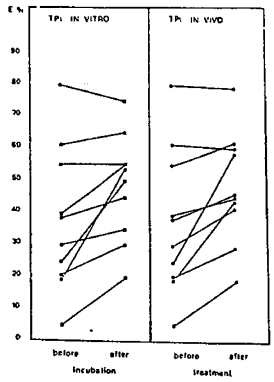
4 G.Mc KHANN*, B.FRANCOIS*, P.EVRARD* (intr. by G. Van den Berghe) Neurolog.Dept., Johns Hopkins Med. Sch., Baltimore, USA and Neuropediat.Dept., Université de Louvain, Brussels, Belgium. Long term use of low doses of dichloroacetate in a child with congenital lactic acidosis.

Dichloroacetate(DCA) has been shown to reduce plasma lactate levels in healthy and diabetic adults as well as in a child with congenital lactate 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 unkown. 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, biotine, lipoic acid and ketonemic 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.QUINTI*, E.GALLI*, F.AIUTI*, L.BUSINCO. Dpt. of Pediatrics I° and Dpt. of Internal Medicine III°, University of ROME, ITALY.

THYMOSIN (TP1) 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 (TP1 Serono Institute) 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 recostitution in primary T-cell defect after TP1 therapy.Now we have treated 10 children suffering from T-cell defect associated with severe viral infections,with TP1 75 mg/m^2/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 TP1 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 by 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. Gladtke) Children's Hospital Cologne, FRG

THE CELLULAR BASIS OF OXYGEN TOXICITY

The exposure of Guniea 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 an 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.

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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 hyperimmune anti-A serum were used as target cells.Haemolysis was quantitated by release of radioactivity from RBC prelabelled with ^{51}Cr-chromate.Comparison with the cytotoxicity elicited by similar or even lower monocyte numbers taken from