13 THE PRENATAL DIAGNOSIS OF 3-HYDROXY-3-METHYLGLUTARIC ACIDURIA BY GC-MS AND ENZYMOLOGY ON CULTURED AMNIOCYTES AND ON CHORIONIC VILLI.

R.A. Chalmers, B.M. Tracey, J. Mistry, T.E. Stacey, I.R. McFadyen & M.J. Madigan. Perinatal & Child Health & Clinical Mass Spectrometry, MRC Clinical Research Centre, Harrow, Middlesex, UK.

3-Hydroxy-3-methylglutaric (HMG) aciduria is a disorder of L-leucine metabolism and ketone body biosynthesis characterised by recurrent acute life-threatening episodes resembling Reye's syndrome. Prenatal diagnosis was requested by the parents of a child with this condition and was carried out by amniocentesis at 16 weeks' gestation. Amniotic fluid concentrations (capillary GC and GC-MS) (µmole/L) were for HMG 34.6 (N=1.21+0.38), 3-hydroxyisovalerate (HIV) 33.9 (N=4.78+1.91), 3-methylglutaconate (3MΔG) 31.7 (N=0.62-0.90) and 3-methylglutarate(3MG) 1.23 (N=0.12-0.26) consistent with an affected fetus. Termination was carried out on the basis of these data. The diagnosis was confirmed immediately by analysis of fetal plasma for organic acids:concentrations of HMG, HIV, 3MΔG and 3MG were 67.3, 36.9, 31.3 and 11.3µmoles/L respectively. Subsequent enzymology on cultured amniocytes gave mean results for isovalerate incorporation into protein (pmol/h/mg protein) of 48 compared to simultaneous controls of 252; direct HMG CoA lyase activity (nmoles HMG CoA removed/min/mg protein) was 0.05 compared to simultaneous controls of 9.16. Direct HMG CoA lyase measurements on chorionic villus tissue obtained at termination gave mean activities of 0.87 compared to controls of 8.04. These results provide the first prenatal diagnosis of HMG aciduria, demonstrate the rapid, accurate and unambiguous diagnosis at 16 weeks using direct GC-MS analysis of amniotic fluid and indicate the suitability of chorionic villus biopsy for future earlier (9 weeks) prenatal diagnoses of this condition.

14 ORGANIC ACIDURIA IN PATIENTS WITH GLYCOGENOSIS TYPE I.

J Fernandes, R Berger, SA Stoker-De Vries and I Mulder
Department of Pediatrics, University Hospital, 59 Oostersingel, 9713 EZ Groningen, The Netherlands.

Intrigued by the persistent growth retardation of some glucose-6-phosphatase-deficient patients, we investigated the urinary excretion of lactate, 2-oxoglutarate and citrate, reflecting organic acid overproduction, and glycerol, reflecting impaired gluconeogenesis, in 17 patients with type IA and 1 patient with type IB glycogenosis. The urine was collected during 8-10 successive days twice daily.

The mean lactate-creatinine ratio (mM/mM) ranged from 0.27 to 11.17 (normal 0.010 to 0.058, N=36) in 5 patients with a height < P3. The lactate excretion in these patients varied considerably during successive days and less during gastric drip feeding at night. The mean lactate-creatinine ratio in the other patients was more constant and ranged from 0.04 to 0.34. The mean 2-oxoglutarate-creatinine ratio (mM/mM) ranged from 0.17 to 0.84 (normal 0 to 0.05, N=15) in 4 of 5 growth retarded patients. In the other patients it ranged from 0.01 to 0.43. The 2-oxoglutarate excretion per patient varied little. The mean citrate-creatinine ratio (mM/mM) ranged from 0.03 to 0.84 in all patients (normal 0.12 to 0.73, N=15). The mean glycerol-creatinine ratio (mM/mM) ranged from 0.01 to 0.11 (normal 0.005 to 0.03, n=15).

We conclude that the lactate-creatinine ratio reflects both the adequacy of the dietary treatment and the severity of the disease, whereas the 2-oxoglutarate-creatinine ratio reflects mainly the latter. The citrate-creatinine ratio and the glycerol-creatinine ratio do not give important information.

15 METABOLITE PATTERNS AND CLINICAL EXPRESSIONS OF URIDINE DIPHOSPHOGALACTOSE EPIMERASE DEFICIENCY

Y.S. Shin, W. Endres, M. Rieth, Univ. of Munich, F.R.G., K. Kruse, Univ. of Würzburg, F.R.G., C. Jakobs, Free Univ. of Amsterdam, The Netherlands.

Two forms for the UDP-galactose-epimerase (EP) deficiency have been reported, one benign and the other severe. Through the routine screening of galactosemia recently we found three patients who had a deficient EP activity in erythrocytes. The EP activity assayed with a two-step radioisotopic method (Clin. Chem. 28, 2322, 1982) was 9.3, 11.5 and 7.9 umol/h/g Hb respectively (normal range 18-35, n=55). The former patients who showed no apparent clinical symptoms had a mild elevation in galactose-1-phosphate (GP) in erythrocytes (21 and 18 µmol/L) and a normal galactitol (GL) in plasma. GP was determined enzymatically (Clin. Chim. Acta 127, 77, 1982) with the normal range of 1-12, and GL by gas chromatography-mass spectrometry (Pediatr. Res. 18, 714, 1984) with the normal range of 0.08-0.91 µmol/L. However, after a short treatment with galactose-free diets GP was normalized in these patients. The third patient whose clinical pictures were similar to those of classical galacosemia with congenital cataracts revealed an extremely high level of GP (310.0) and GL (99.1). The EP acivity in the liver biopsy of the first patient was completely normal suggesting that the former patients have a partial deficiency possibly due to the EP isoenzymes.

16 FAMILIAL SUDDEN CHILD DEATH IN A FAMILY WITH PSEUDO-DOMINANT DICARBOXYLIC ACIDURIA AND HYPOKETOTIC HYPOGLYCEMIA.

M Duran[1], M Hofkamp[2], JM Saudubray[3], WJ Rhead[4], SK Wadman[1]
1. Univ. Children's Hosp.'Het Wilhelmina Kinderziekenhuis', Utrecht, Netherlands, 2.Lukas Hosp., Apeldoorn, Netherlands, 3. Hôp. Necker Enfants Malades, Paris, France, 4.Dept. Pediatr. Univ. Iowa City, USA.

A family is described in which the father and three (and probably all four) children had a decreased capacity for the oxidation of medium chain fatty acids resulting in a defective ketogenesis. The 4th child died at the age of 16 months during an episode of hypoketotic hypoglycemia (plasma glucose 0.7 mmole/l; 3-hydroxybutyrate 1.7 mmoles/l). Previously the child had always been in good health. Medium-chain fatty acids accumulated in the patient's plasma; the urinary organic acid profile showed large amounts of dicarboxylic acids and fatty acid conjugates such as hexanoylglycine, octanoylcarnitine, and octanoylglucuronide. The activity of medium-chain acyl-CoA dehydrogenase in an autopsy liver sample was only 15% of the mean control. The first child had died at the age of 19 months under similar conditions. When subjected to a 17 h fast the father and the 2nd and 3rd child, but not the mother, accumulated octanoic, decanoic and cis-4-decenoic acids in their plasma.Their urinary organic acid profile resembled that of the deceased 4th child, but excretions were at a lower level. Measurement of [^{14}-C]-octanoate degradation in cultured fibroblasts revealed low values for the father and the two 'healthy' children as compared to the controls. It is suggested that a -partial- deficiency of medium-chain acyl-CoA dehydrogenase may lead to life-threatening illness when gastro-intestinal abnormalities eventually lead to a severe depletion of the glycogen stores. Careful monitoring of at-risk patients is indicated under these circumstances.

17 ARGININE-GLYCINE TRANSAMIDINASE IN BOVINE NEURORETINA AND PIGMENT EPITHELIUM

(Introduced by K Raivio) E Repo & I Sipilä, Children's Hospital, University of Helsinki, Helsinki, Finland

Gyrate atrophy (GA) patients exhibit chorioretinal degeneration and histologic muscle abnormalities. Due to autosomal recessive deficiency of ornithine aminotransferase they accumulate ornithine to levels, which are 10x normal and well above the K_m of arginine-glycine transamidinase of rat kidney. We have previously shown inadequate synthesis of guanidinoacetate and low creatine concentration in plasma, urine, erythrocytes, and muscle tissue of GA patients. Supplementary creatine corrected the atrophic muscle pathology but the chorioretinal atrophy continued. We alleged that this discrepancy was due to ineffective transport of creatine across the blood-eye barrier and sought for synthesis of guanidinoacetate in ocular tissues.

We developed a new assay for measuring the arginine-glycine transamidinase activity in bovine eye tissues with a new assay based on the detection of formed guanidinoacetate with alkaline ninhydrin reaction. We found transamidinase activity only in the neuroretina (NR) and retinal pigment epithelium (RPE). The pH optimum was 7.5 in NR and 7.9 in RPE and the apparent K_m for arginine was 1.10 in NR and 4.96 mM in RPE, and for glycine 1.03 and 0.95 mM, respectively. Our results indicate that a potential energy-rich phosphagen compound can be formed in eye tissues. Reduction of the ornithine concentration in ocular tissues can possibly enable the transamidination also in gyrate atrophy.

18 HETEROGENEITY IN ADENYLOSUCCINASE DEFICIENCY.

J. Jaeken, G. Van den Berghe. Department of Paediatrics, University of Leuven, and Laboratory of Physiological Chemistry, International Institute of Cellular and Molecular Pathology, Brussels, BELGIUM.

Adenylosuccinase deficiency was suspected in 3 mentally retarded, autistic children by the discovery of succinyladenosine and succinylaminoimidazole carboxamide riboside in their body fluids and confirmed by the finding of a deficient enzyme activity in the kidney and in the liver of patient A. (Jaeken and Van den Berghe. Lancet 2 : 1058, 1984). In patients B and C (brother and sister) follow-up showed a slowing of weight gain and, to a lesser degree, of length growth. In patient B, weight and length at the age of 3 9/12 y were at the 3rd percentile but at the age of 5 3/12 y they were 2 kg and 7 cm respectively below the 3rd percentile. In patient C, weight and length at the age of 1 6/12 y were at the 25th and at the 75th percentiles respectively, while at the age of 3 6/12 y weight was 1.5 kg below the 3rd percentile and length at the 25th percentile. Serum muscle enzymes, electromyography, nerve conduction velocity and muscle histology were normal in both patients. Patient A, in contrast, had a normal weight gain and growth. Adenylosuccinase activity (nmol/min mg protein vs means ± SEM in 8 controls) was deficient in skeletal muscle of patients B and C (0.18 and 0.66 vs 2.61 ± 0.34) but normal in patient A. No abnormalities of muscle nucleotide profiles could be detected. Conclusion : these findings indicate clinical and biochemical heterogeneity in adenylosuccinase deficiency. The presence of this deficiency in skeletal muscle of patients B and C only, suggests a causal relationship between this defect and their muscular wasting.