178 J.P.OSBORNE*,S.M.TUCKER* and J.W.SCOPES.Departments of Paediatrics and Renal Transplantation,St.Thomas' Hospital,London. "Urinary Enzymes in Neonates after Asphyxia and during Gentamycin therapy".

Two β-Glycosidases,N-Acetyl-β-Glucosaminidase (N.A.G.),and β-Galactosidase (G.A.L.),and Acid Phosphatase (A.P.) have been estimated fluorimetrically in the urine of neonates to detect renal damage and the results expressed per m.mole urinary creatinine. Bag urines were collected, but to minimise contamination were discarded if faeces were passed simultaneously. A.P. is less affected by contamination than are G.A.L. or N.A.G.. There is no sex difference in the excretion of A.P. N.A.G. and G.A.L. can be stored at 4°C before estimation for more than one month;A.P. should be estimated within four days. N.A.G. levels were raised in three cases of perinatal asphyxia and normal in one. Acute asphyxia in two cases did not cause any elevated enzyme levels. G.A.L. was raised in only one of the three cases while A.P. was raised once only in the same case. These results support the theory that acute asphyxia does not damage the renal tubules, while more prolonged asphyxia may. Two neonates have been studied during treatment with Gentamycin. N.A.G. levels are markedly raised during treatment - in one case eight times higher than pre-treatment levels within 24 hours. G.A.L. was intermittently raised and A.P. was not raised during treatment. The consistently raised levels of N.A.G. during Gentamycin therapy in neonates is suggestive of renal tubular cell dysfunction.

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179 F. O. ADEBONOJO*, P. M. COATES* and J. A. CORTNER*, (Introduced by A.M. Bongiovanni)Children's Hosp.Phila. Relation between Lysosomal acid lipase and Neutral lipase in cultured human adipocytes.

Earlier we described some of the enzymatic changes which occur in cultured human adipocytes as they gradually assume a fibroblast like appearance. Using a newly developed fluorometric assay we are studying the relationship between lysosomal acid lipase (LAL) and neutral lipase (NL) in human adipocytes as they adapt to culture. In freshly isolated adipocytes the ratio of NL to LAL is considerably less than one. With time, in culture, the specific activity of LAL sharply increased while that of NL decreased. The ratio of LAL to NL ultimately resembled that seen in cultured skin fibroblast. The cultured adipocyte, however, can be distinguished from skin fibroblast by the presence of large amounts of intracellular lipid droplets which are absent from skin fibroblast cultured under the same conditions.

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Introduced by A. PRADER

A HEREDITARY DEFECT OF THE TRANSFORMATION OF P-HYDROXYPHENYLPYRUVATE INTO HOMOGENTISIC ACID.

Previously, two of us (D.M.D. and P.T.) have described a new form of prolonged transient tyrosinemia in a young baby biochemically characterized by a severe metabolic acidosis and striking p-hydroxyphenyl-lactic and p-hydroxyphenylpyruvic aciduria with only mild tyrosinemia. (Acta Paediatr. Scand. 64:209,1975). In addition, the patient and her mother excreted an unknown compound in urine.

This compound was isolated from the mother's urine and identified by gas chromatography - mass spectrometry, chemical degradation, and comparison with the synthesized reference compound as 2-(S-cysteinyl)-1-carboxymethyl-1,4-dihydroxy-cyclohexen-5. It is a reduced cysteine adduct of 2-(1-hydroxy-4-oxo-2,5-cyclohexadien-1-yl)-acetic acid, a quinol postulated as an intermediate in the oxidation of p-hydroxyphenylpyruvic acid. The nature of this intermediate metabolite together with further biochemical studies probably will help to clarify the still obscure mechanism of enzymatic p-hydroxyphenylpyruvate hydroxylation.

181 J.P.G.M.VAN BIERVLIET*, S.K.WADMAN*, University Children's Hospital, Utrecht and L.A.H.MONNENS*, J.L.WILLEMS*, University Children's Hospital, Nijmegen. (introduced by Prof.Dr.H.K.A.Visser)

A patient with pyruvate carboxylase deficiency.

A patient suffering from pyruvate carboxylase (PC) deficiency was observed during more than 2 years. Psychomotor retardation, muscular hypotonia and abundant subcutaneous fat were present. The neurologic picture was not suggestive for Leigh's disease. An extreme hyperlactacidemia (ranging from 4-20 mM) was observed. Prolonged fasting induced hypoglycemia, which was absent during a similar experiment 6 months later. A severe 'diabetic' keto acidosis was observed after the first fasting experiment. Low insulin levels were assessed. Besides high urinary concentrations of lactic acid, increased concentrations of malic acid, fumaric, succinic, and α-ketoglutaric acid were observed. PC-activity, (determined immediately), was shown to be extremely decreased in liver tissue. Pyruvate dehydrogenase activity (liver, leucocytes), PEP-carboxykinase- (liver)and fructose-1,6-diphosphatase - (liver) were normal. Administration of the coenzymes of pyruvate carboxylase and the pyruvate dehydrogenase complex, of supplements of Mg^{++}, of aspartic acid, glutamic acid combined with pyridoxin did not improve the hyperlactacidemia.

A chronic granulocytopenia was observed. Severe bacterial infections have not occurred. Normal granulocyte concentrations were observed during bacterial infections, stress or epinephrine administration.

183 St. DI DONATO*, U.N. WIESMANN and N. HERSCHKOWITZ. Dept. of Pediatrics, University of Berne, Berne, Switzerland

Membrane adsorption and internalisation of ^{14}C-chloroquine by cultured human fibroblasts.

Chloroquine was previously shown by us to interfere with the binding of lysosomal enzymes at the membranes of cultured fibroblasts (BBRC 66: 1338, 1975). Therefore the uptake of ^{14}C-chloroquine into human fibroblasts was studied. ^{14}C-chloroquine, added in concentrations between 1-10 μmol to the medium of normal cultured fibroblasts, is rapidly adsorbed and taken up by the cells. About 15% of the total uptake can be removed by washing the cells with Hank's solution, up to 34% can be detached from the surface by trypsin and about 50% appears to be intracellular. At an extracellular pH of 7.4 the adsorption of chloroquine to the cell surface is the highest, it approached saturation at concentrations in the medium exceeding 10 μmol.Both at pH 6.7 and 8.1 less chloroquine is adsorbed. On the other hand the apparent intracellular uptake of chloroquine rises with increasing pH. Incubation at 6° C greatly reduces both surface adsorption and even more intracellular uptake. The data suggest energy dependent binding and accumulation of chloroquine at the cell membrane before entering the cells.

184 F. AMONN*, U. BAUMANN*, U. WIESMANN, K. HOFMANN*, N. HERSCHKOWITZ. Department of Pediatrics, University of Berne, CH-3010-Berne, Switzerland. Toxic effects of antibiotics routinely used in cell culture on primary cultures of dissociated mouse brain cells.

100-300 IU Penicillin and 50 ug Streptomycin per ml medium are widely used for maintaining sterility in cell cultures. No adverse effects of these antibiotics on growth and differentiation of these cultured cells have been reported. We have investigated whether Penicillin, Streptomycin or Gentamicin interfere with total protein, DNA and the synthesis of sulfatide (a component of myelin) in brain cell cultures of newborn mice after 6, 9, 12 days in culture. The combination of 200 IU Penicillin and 50 ug Streptomycin per ml as well as 50 ug Streptomycin per ml alone depressed total protein, DNA and sulfatide synthesis. Up to 300 IU Penicillin per ml or 50 ug Gentamicin per ml showed no effect on protein and DNA while there was a dose-dependent interference with sulfatide synthesis. Less than 100 IU Penicillin or 6 ug Gentamicin per ml did not even affect sulfatide synthesis. Since the antimicrobial activity is preserved even at lower concentrations, the recommended doses should be reduced to non-toxic levels. Dissociated brain cell cultures could possibly be used to study the effect of antibiotics or other drugs on growth and differentiation of brain cells in vitro. This might help to understand drug action and side effects in vivo.