148 J.Tyson Tildon*,Pinar Ozand*,Alp Karahasan*and Marvin Cornblath,(Intro.by F.A. Hommes),Laboratory of Developmental Biochemistry,Department of Pediatrics, University of Groningen,Groningen.The Netherlands and Department of Pediatrics,University of Maryland,Baltimore,Maryland 21201,USA.

Biochemical Studies of Glycerol Neurotoxicity.

Metabolic studies of a 4 year old child that revealed a neurotoxicity to glycerol (MacLaren et al J.Ped.86,43-49.1975) have been extended to include measurements of serum dopamine ß hydroxylase (DBH) as an index of sympathetic nervous system activity.Within an hour after the oral administration of glycerol (1.0 gm/kg),DBH activity decreased to approximately 50% of the initial value in each of six volunteers.In the child,the rate of glycerol disappearance was different,and the DBH decreased to less than 20% when he was given a similar glycerol load.To assess the metabolic fate of glycerol,enzyme measurements and in vitro oxidation rates were determined in rat brains.Using sucrose density gradients,glycerol kinase activity was found predominantly in the mitochondrial fraction, and the rate of glycerol oxidation by this fraction was 3 times higher than the synaptosomal or myelin fractions.The kinetics of the oxidation indicated both a "high" and a "low" affinity for glycerol.The results suggest that this oxidation provides the brain with a detoxification mechanism that prevents the accumulation of glycerol,in situ,which may affect norepinephrine metabolism.It is hypothesized that glycerol neurotoxicity might result from an alteration of this process.(Supported by grants HD -03959,HD-06291 and AM-18127 from the NIH and the John A.Hartford Foundation.J.T.T. is a Josiah Macy Jr. Foundation faculty Scholar).

149 J. Schrijver* and F.A. Hommes,Laboratory of Developmental Biochemistry, Department of Paediatrics,University of Groningen,The Netherlands

Biotin deficiency in the rat as a model for Leigh's syndrome.

Leigh's syndrome is characterized by symmetrical lesions in the brain,especially in the stem.Reported biochemical abnormalities are liver pyruvate carboxylase (PC) deficiency and thiamine triphosphate (ThTP) deficiency in the brain. We are using rats put from the time of conception on a biotin-poor, avidin-rich diet as a possible animal model for Leigh's syndrome. In rats fed a normal diet liver PC activity rises steep after birth with a maximum between 2 and 3 days.From day 3 on the activity decreases to a minimum reached at day 9,followed by an increases to the adult level which is reached at day 20. PC activity of the liver of biotin deficient young rats is lowered for more than 80%.PC in the brain of normal rats, shows a highest activity in the brainstem.After birth the activity of PC of total brain rises slowly to a maximum which is reached between 30 and 32 days,contrary to the activity in the brainstem, which shows maxima during the periods 20-22 and 36-38 days.PC activity in the brain of biotin deficient rats is inactivated from 60 to 70 %. Thiamine concentrations in liver and brain of biotin deficient rats hardly differs from normal rats.There is no deficiency of ThTP in the brain or in the liver of biotin deficient rats,which show,especially after fasting, a marked lactic acidosis and hypoglycemia.

150 A.J. STECK*, R.SCHAEFER*, P.SIEGRIST* and N.HERSCHKOWITZ. Department of Pediatrics, University of Bern, Bern and Department of Structure Biology, Biozentrum, University of Basel, Basel, Switzerland. Phosphorylation of myelin basic protein by vaccinia virus cores.

In the postinfectious encephalomyelitis associated with vaccinia virus, both direct viral effects and immunological factors have been invoked as causes of this disorder. In the present study we explored the possibility that the protein kinase associated with vaccinia virus could directly modify host proteins. Purified basic myelin protein or human myelin membranes were incubated in the presence of [γ-^{32}P] ATP with vaccinia virus cores. The phosphorylated products were analysed on SDS polyacrylamide gels. Our experiments demonstrate that purified basic myelin protein is both a substrate and an activator for the viral protein kinase. Unlike most mammalian protein kinases, cyclic AMP does not stimulate the viral enzyme. Of the myelin polypeptides only the basic protein is phosphorylated. These results indicate that the virus core associated protein kinase phosphorylates myelin basic protein in vitro and suggest that vaccinia virus has the potential of directly modifying a cellular membrane.

151 A. FREIBURGHAUS*, B. HADORN, J. SCHMITZ, M.J. TARLOW, H.W. ROTTHAUWE, P. KUITUNEN, K. LAUNIALA,S. CADRANEL, H. LOEB (Dept. of Pediatrics, University of Berne, Berne, Switzerland): Composition of brush border membranes in several congenital intestinal enzyme deficiencies investigated by use of SDS-polyacrylamide-gel-electrophoresis.

1. In 3 children with congenital lactose malabsorption and 2 adults with specific hypolactasia a reduction of the glycoprotein band corresponding to brush border lactase was found. The protein band was reduced in approximately the same proportion as the activity. In one child with congenital lactose malabsorption no band was detected on the gel. 2. In one patient with sucrose-isomaltose malabsorption an abnormal glycoprotein band together with a complete deficiency of the normal sucrase-isomaltase band was found. In addition this patient had a high residual isomaltase activity which migrated on the gel in the position of maltase-glucamylase. The finding of an abnormal glycoprotein band differentiates this patient from those described by Schmitz et al. and by Preiser et al. 3. Enterokinase deficiency (n=2): Two more cases were investigated and no visible alteration of the band corresponding to enterokinase activity was found. This confirms an earlier observation by Schmitz et al. Three different mechanisms must be postulated to be responsible for the reduced activity in 1, 2 and 3. In sucrase-isomaltase deficiency differencies in the mechanism leading to low enzymatic activity may exist from one patient to the other.

152 S.GUANDALINI*, G.MAGAZZU'*, A.CONTI*, and A.RUBINO. Depts. of Child Health and Pediatrics, 2nd School of Medicine, Univ. of Naples and 2nd Dept. of Pediatrics, Univ. of Messina, Italy.

A dual mechanism for transport of amino acid residues of dipeptides across the intestinal brush border.

When chemically defined diets are required to provide optimal nutritional care in children, the choice of the most appropriate formula should be based on more detailed knowledge on absorption of protein hydrolytic products. Dipeptides can cross intact the intestinal brush border (b.b.)by way of selective transport systems; they can also be split at the b.b.membrane. While some peptides unhydrolyzed in the b.b. only follow the first pathway, for those which can be split in the b.b. the relative relevance of the two mechanisms is unknown.We have studied, in rabbit's intestinal mucosa, the uptake of Glycyl-Phenylalanine (GP) which both has affinity for the dipeptide transport process and is split at the b. b. GP(14CPhe) uptake has been measured in presence of large excesses either of Glycyl-Proline (Gly-Pro) or of Leucine, which are selective inhibitors of the peptide and the Phenylalanine transport systems, respectively. Under both conditions GP uptake follows Michaelis-Menten kinetics; in presence of Gly-Pro, GP(14CPhe) uptake shows kinetic constants similar to those reported for Phe influx, while in presence of Leucine the kinetic constants are similar to those reported for Gly-Pro influx. On the basis of the two sets of constants, it is concluded that GP is in part translocated by the peptide transport system,but at the concentrations at which it may occur in the intestinal lumen,the pathway 'hydrolysis + amino acid transport'is prevailing. In the latter pathway, the amino acid transport step seems to be the rate limiting one.

153 E.EGGERMONT,H.EYSSEN,L.CORBEEL,R.DE VOS, F. GARMYN, D. DE WOLF-PEETERS,H.WILLEKENS and R.EECKELS. Kindergeneeskunde, K.U.Leuven, Belgium.

Intrahepatic cholestasis and excessive formation of bile acid intermediates.

The patient, a boy and first infant of healthy unrelated parents,was first seen at the age of 2 months because of jaundice. The hyperbilirubinaemia had appeared at the age of 1 month and persisted up to the age of 3 1/2 months when the child died from pulmonary infection.At the age of 2 months serum values were: bilirubine total 16 mg% and direct 12mg%,transaminases GOT 410 IU/l and GPT 170 IU/l and normal alkaline phosphatases. The serum level of bile acids was 67 ug/ml but cholic and chenodeoxycholic acid only made up 15 percent of it. In addition trihydroxycoprostanic acid, dihydroxycoprostanic acid and probably cholestane-tetrol and cholestane-pentol could be detected.At laparotomy, complete opacification of the extrahepatic biliary system was seen. Optical microscopy of the liver showed evidence neither of hepatitis nor of intrahepatic biliary atresia but of cholestasis at the site of the liver cell. Electron microscopy revealed interruptions of the bile canalicular membrane, granular material within the canaliculi and an excessive amount of filaments in the pericanalicular ectoplasm of the hepatocyte.

The disturbance of bile acid metabolism could be explained by a block of the 24-hydroxylation of coprostanic acid.