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Tyrosine aminotransferase in human liver; development, properties and substrate induction in organ culture.

Hypertyrosinemia is often seen in low-birth weight newborn infants and is related to both quantity and quality of protein intake. Tyrosine catabolism may be insufficient due to low activity or incomplete development of tyrosine aminotransferase (EC. 2.6.1.5, TAT). TAT was present in 12 to 17-week-old fetal human liver, the activity being 2.25% of adult human liver (45 and 2000 nmoles/g/min respectively). Liver activities in pre-term and full-term infants of various ages will be presented. The Km for tyrosine was 1.6×10^{-3}M and for α-ketoglutarate 3.5×10^{-4}M in fetal human liver. The coenzyme pyridoxal-phosphate seemed to be completely dissociable in both fetal and adult enzyme preparations. Isoelectric focusing showed that the fetal enzyme preparation had an activity profile different from that of the adult. The fetal enzyme showed only one major peak whereas the adult had three peaks indicating different subforms of the enzyme. TAT activity could be increased by its substrate, tyrosine (0.5 mM) in fetal liver explants maintained in organ culture. Studies on the perinatal regulation of enzyme activities and levels in human tissues are of importance in light of the clinical attempts to enhance enzyme differentiation in functionally immature infants.

38 Changes of microsomal membrane compositions modulate microsomal enzyme activity; N. Herschkowitz, H.P.Siegrist, H. Jutzi, U. Wiesmann. Department of Pediatrics, University of Berne.

We have shown previously, that the activity of cerebrosulfotransferase (CST) a microsomal enzyme involved in myelin synthesis is modulated in vitro by the cholesterol-phospholipid ratio (C/P) in microsomal membranes. To further investigate this mechanism, we studied two different models: 1.) Removal of cholesterol by phospholipid micelles and 2.) Inhibition of cholesterol synthesis in cultivated brain cells. 1. We incubated mouse brain microsomes from 14 day old animals with phospholipid micelles, which caused a decrease of C/P from 0.60 to 0.53 (Aequivalent age of 18 days). Under these conditions CST activity rose from 4'900 dpm to 9'800 dpm, aequivalent the activity of 18 day animals. Concomitant electrospinresonance measurements showed an increase in membrane fluidity. 2. In C_6 mouse glioblastoma cells cholesterol synthesis was temporarely inhibited by adding estradiol to the medium. This caused a decrease in C/P from 0.32 $\pm$0.02 to 0.22$\pm$0.03 from 19 to 24 hours after the addition of the inhibitor. During this time CST activity rose from 3200$\pm$340 dpm to 6'300$\pm$500 dpm. After this period C/P and CST activity returned to normal. We therefore speculate that microsomal CST activity is modulated by changes in membrane fluidity, which is caused by changes in the molar-ratio of cholesterol to phospholipid in microsomes.

39 Elevation of erythrocyte galactose-1-Phosphate (Gal-1-P) in children with double heterozygosity for Duarte variant and galactosemia.

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In 1977 a partial deficiency of Gal-1-P uridyl transferase (Gal-1-PUT) was found in 0.015% of 73691 Beutler tests performed by Swiss laboratories for neonatal screening. Some of these cases with marked reduction of Gal-1-PUT activity (mean 18% of normal) were identified as double heterozygotes for Duarte variant and galactosemia (2-0). Fasting plasma galactose concentrations did not exceed 2.2 mg/dl in 19 (2-0) patients and 1.4 mg/dl in age-matched controls, erythrocyte (Ec) Gal-1-P varied between 0-1.6 mg/dl in both groups. Oral galactose tolerance tests (1g/kg b.w.) were done in all (2-0) patients: 6 neonates (A), 6 infants after 1 year of galactose-free diet (B) and 7 patients (age 1.8-18.6 years) who had never received a diet (C). Peak plasma galactose levels were reached at 60 or 90 min. in all subjects. Gal-1-P reached 6.0-22.7 mg/dl Ec in A, 3.7-31.3 mg/dl in B and 6.0-11.7 mg/dl in C at 60 min. By contrast, in controls, maximum Gal-1-P levels never exceeded 2.7 mg/dl. As Gal-1-P is the incriminated toxic agent in galactosemia, this finding may be of pathophysiological importance.

40 L. COICADAN*, M. HEYMAN*, E. GRASSET* and J.F. DESJEUX. INSERM U 83 and Département de Pédiatrie (Univ. Paris VII), Hôpital Hérold, 75935, Paris, France. Cystinuria : absence of lysine permeability in the brush border membrane of intestinal cells.

The mechanism of impaired transport of cystine and dibasic amino acids in the jejunum and the kidney of cystinuric patients is poorly understood. Na-dependent L-lysine intestinal transport has been assessed, in vitro, by measurement of intracellular accumulation and of unidirectional influx across the brush border membrane in per ora biopsies performed for diagnostic purpose on 27 "control" children and 2 cystinuric patients, in the presence of 3 mM lysine in the incubation solution. The project was accepted by the Committee on Ethics of the Department of Pediatrics of the University Paris VII. In cystinuria 1) the Na-dependent lysine intracellular accumulation is abolished, 2) the Na-independent accumulation persists (the accumulation ratio is 1.69 $\pm$ 0.34), 3) the lysine influx in µmol/h cm2 at the luminal membrane is hardly measurable both in the presence (0.17 $\pm$ 0.03) and in the absence of Na (0.22 $\pm$ 0.09). Control values : 0.95 $\pm$ 0.14 in the presence of Na and 0.57 $\pm$ 0.08 in the absence of Na. These results suggest a specific loss of the Na-dependent L-lysine transport at the luminal membrane of the enterocytes of the intestine in the 2 patients with cystinuria. The lysine permeability at the baso-lateral membrane is probably present.

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Lysinuric protein intolerance (LPI): treatment with citrulline and lysine.

LPI is an autosomal recessive defect of diamino acid transport in renal tubular and intestinal epithelium, and hepatocytes. This leads to deficiency of ornithine and other urea cycle intermediates, and inadequate function of the cycle which results in hyperammonemia after protein intake, and protein aversion. Lysine shortage may contribute to the growth failure of the patients.

Our patients have earlier been treated with an arginine supplement. We have now shown that, while the intestinal absorption is very poor for arginine, it is intact for citrulline, another urea cycle intermediate. Since autumn 1976 our patients have been given 1.5 g/kg of dietary protein daily supplemented with 2-3 g citrulline and 1-2 g lysine. The protein aversion decreased in 13/21 patients, growth accelerated in 12/21, and urinary orotic acid excretion rate normalized in 9/11 as a sign of improved nitrogen tolerance. 4 patients had very fragile and sparse hair; this normalized during the therapy. Gastrointestinal complaints appeared during the treatment in 6/21; these disappeared when lysine supplementation was stopped. Hepato- and splenomegalia, increased plasma lactate dehydrogenase, leukocytopenia and thrombocytopenia, and fasting plasma and urinary amino acids remained unchanged. They may depend on lysine deficiency. The severe intestinal transport defect of lysine seems to invalidate the oral route for lysine supplementation.

We suggest that citrulline, possibly with lysine, should replace arginine in the treatment of LPI.

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Hereditary tyrosinemia - fumarylacetoacetase deficiency.

Succinylacetone which has been isolated from urine from patients with hereditary tyrosinemia inhibits porphobilinogen synthase (EC 4.2.1.24) both in liver and erythrocytes. It accumulates together with succinylacetoacetate secondary to a low activity of fumarylacetoacetase (EC 3.7.1.2) in liver (0.09-2.3 U/g protein, n=9, ref. values 5-17 U/g protein, n=5). We suggest that this is the primary enzyme defect and that the severe liver and kidney damage arise secondary to accumulation of maleylacetoacetate and/or fumarylacetoacetase. This is supported by the following facts (1) maleylacetoacetate and fumarylacetoacetate react with SH-groups and are therefore potentially toxic metabolites, (2) injection of glutathione decreases S-succinylacetoacetate and S-succinylacetone probably by reacting with maleylacetoacetate and fumarylacetoacetate, (3) injection of homogentisate increases the tubular proteinuria, (4) among nine patients those with a more benign form of the disease had the lowest activity of 4-hydroxyphenylpyruvate dioxygenase in liver, i.e. accumulate less of maleylacetoacetate and fumarylacetoacetate, (5) 4-hydroxyphenulpyruvate dioxygenase (EC 1.13.11.27) is present only in the tissues seriously damaged in hereditary tyrosinemia. Oral treatment with SH-reagents like penicillamine and N-acetylcysteine has been evaluated in clinical trials in combination with dietary restriction of phenylalanine and tyrosine intake.