7 RESPONSE OF HUMAN AND RAT SMALL INTESTINE TO ORAL ADMINISTRATION OF SACCHAROMYCES BOULARDII (SB).

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To assess the response of the small intestine to SB, a yeast widely used as adjuvant drug to antimicrobial therapy, 7 healthy volunteers were treated with high doses of SB (250 mg 4 times per day) for 2 weeks. A jejunal biopsy was performed on day 0 and 15. Histology of the post-trial biopsy revealed no morphological alteration but at day 15, sucrase, lactase and maltase were increased by 75-82% ($p < 0.05$) over the basal enzyme activities measured on day 0. Similar changes were found in the jejunum of adult rats treated with SB for 5 days. In vitro assays on suspensions of SB cells (10^8 cells/ml) evidenced a high activity for sucrase ($\bar{X} \pm SE$: 8364 ± 1280 U.g.prot^{-1}) but no maltase, lactase or acid β-galactosidase activity. To determine whether treatment with SB influences the incorporation of neutral lactase into brush border membrane (BBM), 14-day-old sucklings treated with either saline or SB, were given IP 20 μCi D-(1-^{14}C) glucosamine 3h before sacrifice. Expressed per mg of BBM lactase protein, the incorporation rate of the label was similar between treated rats (8.167 ± 1622) and controls (9602 ± 1803 dpm.mg prot^{-1}). In conclusion : oral treatment of human volunteers and rats with SB causes a marked increase in the activity of disaccharidases without enhancement of enzyme incorporation into the BBM.

8 PREFERENTIAL USE OF LIPOSOMAL-CHOLESTEROL FOR THE FORMATION OF MURICHOLIC ACID IN THE RAT

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Cholesterol (C) needed for bile acid synthesis is recruited from de novo synthesized C as well as lipoprotein C. It is not known whether C used for the primary bile acids and biliary C originates from one or more hepatic C-pools. By using different C vehicles, i.e. lipoproteins and liposomes, we tried to discriminate between metabolic pathways for the formation of bile acids in the liver. Studies were undertaken in rats with a permanent biliary drainage. Bile acids were analyzed by HPLC and capillary GC. (3H-)C was administered intravenously in three vehicles: lipoproteins (LP), multilamellar vesicles (MLV) and small unilamellar vesicles (SUV). For all vehicles over 75% of C was excreted in bile as bile acids after 120 hrs. Initial biliary excretion was highest for LP (5.7% at 1 h after injection), followed by MLV and SUV (1.3 and 0.9% resp.). No differences were detectable between the specific activities of the excreted bile acids 12 h after injection, but at 1 h the specific activity of murocholic acids was markedly increased in experiments with SUV-C, but not with LMV- or LP-C. Also the gly/tau conjugation ratio was increased for SUV-C at 1 h.

We conclude that more than one C pool in the hepatocytes exists from which C is recruited for bile acid synthesis, with SUV-C preferentially used for muricholic acids synthesis. Zonal heterogeneity might be responsible for the observed differences.

9 INTESTINAL ABSORPTION OF SULFATED LITHOCHOLIC ACID CONJUGATES: EFFECT OF CALCIUM.

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Sulfated glycolithocholic acid (SGLC) causes intrahepatic cholestasis in experimental animals. In 2 patients with intermittent cholestasis during symptom-free periods a marked postprandial rise in serum SGLC was observed, which could be prevented by adding cholestyramine to the testmeal. This suggests an intestinal origin of SGLC. We therefore studied the absorption characteristics of (S)LC conjugates in vivo. Rats were provided with permanent catheters in the bile duct and the duodenum. Intraduodenal infusions of ^{14}C-labeled gly and tau conjugated LC and their 3α-sulfated esters were performed after 4 days of bile drainage. Excretion of ^{14}C-labeled bile acids in bile, urine and faeces was measured over 24h. At a dose of 125 nmol/min GLC, TLC, SGLC and STLC were efficiently absorbed. In contrast to their unsulfated precursors, SGLC and STLC were not metabolized in the liver. Replacement of NaCl in the infusate by $CaCl_2$ reduced the absorption of SGLC and STLC with 63% and 52% resp., that of GLC and TLC with 19% and 22%. Absorption of taurocholic acid (TC) was not affected by $CaCl_2$.
Absorption of SGLC was dose-dependent, and not affected by co-infusion of rat bile or an equimolar TC solution.
SGLC and STLC may undergo enterohepatic circulation, thereby continuously exposing the liver to their hepatotoxic actions. In presence of calcium, however, their absorption is impaired.

10 CHARACTERISATION OF A TRANSPORT SYSTEM FOR CYSTINE IN LEUCOCYTES LYSOSOMAL MEMBRANES.

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Normal leucocyte lysosomal membranes are able to transport cystine by a saturable process exhibiting efflux (T) and Counter-transport (CT). Leucocyte lysosomes from cystinotic patients lack this carrier mediated transport and thus store cystine. In order to establish whether T and CT are mediated by the same site, the effect of proteolytic enzymes, ATP, different H^+ concentrations and N-Ethyl-Maleimid (NEM) on these two activities were examined. T and CT were measured in lysosome rich granular fractions extracted from normal PMN leucocytes loaded with cystine. Protease inhibited CT of cystine by 80% but did not influence the rate of cystine efflux. Trypsin and Chymotrypsin had no effect on both T and CT. ATP (2mM) and NEM (10mM) inhibited CT by 35% and 50% respectively. Cystine efflux was enhanced by 50% by ATP and not effected by NEM. Lowering the proton gradient across the membrane by incubating the lysosomes in an acidic medium (PH 5.5) caused an inhibition of both T and CT of cystine.
The observation that the two processes have different characteristics, yet both of them lack in cystinosis suggests that cystine T and CT occur at two different sites on a single protein carrier of the leucocyte lysosomal membrane.

11 *HAPLOTYPE ANALYSIS OF THE PHENYLALANINE HYDROXYLASE AND PRENATAL DIAGNOSIS OF PKU IN GERMANY*

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Using a full-length cDNA clone for the human phenylalanine hydroxylase (PAH), haplotype analysis of the PAH genes was performed in (20) German PKU-families by determination of the restriction fragment length polymorphism (RFLP). As previously described for another population, an association between normal and mutant PAH-genes and certain haplotypes was observed. The analysis of 8 polymorphic sites detected by the restriction endonucleases revealed that 80% of the normal PAH genes were represented by 5 haplotypes among a possible total of 256 haplotypes. Four of these 5 haplotypes also accounted for greater than 90% of the PKU-genes studied. If each RFLP haplotype is associated with a specific mutation in the PAH gene that causes PKU, the data would suggest that PKU is a heterogeneous disease, and that a great majority of the PKU patients contain only a limited number of different mutant PAH genes in the German population. Indeed, specific RFLP haplotypes appear to be associated with particular disease phenotypes in a limited number of patients studied to date, such that it may be possible to predict the prognosis of disease from the haplotype data of the PKU-genes in the patients. Of 40 obligate carriers studied to-date, a RFLP heterozygosity of 87.5% was observed. Since the restriction analysis can be readily performed using chromosomal DNA isolated from amniotic cells or chorionic villi, the data suggest that in 87.5% of the cases, prenatal diagnosis and carrier detection of PKU by gene analysis will be possible in Germany.

12 CHANGES IN CARNITINE AND METHYLMALONATE FOLLOWING WITHDRAWAL OF VITAMIN B_{12} IN B_{12} RESPONSIVE METHYLMALONIC ACIDURIA (MMA).

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We have suggested that carnitine modulates the acyl CoA:CoA ratio in the organic acidurias. Temporary withdrawal (at parental request) of vitamin B_{12} in a 7 year old developmentally normal boy with MMA provided an opportunity to test this. Free carnitine (fc) and acylcarnitines (ac) were measured by a radioenzymatic method and organic acids by gas chromatography. Initially plasma carnitines were (mean ± 1SD, n=4): fc 17.6 (2.5), ac 10.7 (3.8) μmol/l; normals 5-12 years), (mean ± 1SD n=20): fc 37.7 (7.7) (sig. diff $p < .001$), ac 7.1 (5.3) μmol/l). 24 hour carnitine excretion was : fc 4.3 μmol, ac 141.1μmol (mainly propionyl), ac:fc 32.6 (normal < 10). Plasma methylmalonate (ma.) was 142 μmol/l and 24 hour excretion was 22.3 mmol (normally undetectable). Reduction in B_{12} caused a fall in plasma fc to 2.5 and a rise in ac to 19.4 μmol/l. Plasma ma. rose to 686 μmol/l and urinary excretion to 126.2 mmol/24 hrs with associated clinical deterioration. B_{12} was recommended. Plasma fc rose to 16.3 μmol/l. Plasma ma. fell to 165 μmol/l and urinary excretion to 6.9 μmol/24 hours with clinical improvement. Redistribution of plasma carnitine between free and acylcarnitines reflected intramitochondrial depletion of Coenzyme A which followed from accumulation of abnormal metabolites as their CoA esters on stopping B_{12}. These changes reversed when cofactor was recommended. This provides further evidence for a modulating effect of carnitine on acyl CoA:CoA.