23 SUCRASE-ISOMALTASE (SI) LOCALISATION AND BIOGENESIS IS NORMAL IN MICROVILLOUS ATROPHY

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In microvillous atrophy PAS positive secretory granules accumulate in the apical cytoplasm of intestinal epithelium and brush border (BB) PAS staining is reduced. This suggests that protein transport to the BB may be disrupted in the disease. We have studied SI localisation and metabolism in order to investigate this hypothesis using organ culture, radioiodination with immunoprecipitation, immunocytochemistry, and immunogold EM. Disaccharidase activities were decreased in the cases. Immunoperoxidase (n=6) and immuno EM (n=3) demonstrated SI antigen in the BB apical membrane and not in the secretory granules. Radioiodination and immunoprecipitation (n=3) showed initial transport to, and processing in, the Golgi complex to be normal; no cleaved subunits could be detected, although proenzyme did not accumulate. Organ culture (n=1) showed normal synthesis and processing of SI. Thus SI synthesis and transport appear to be normal in microvillous atrophy and the nature of the secretory granules is elusive, but may hold the key to this disease. A second exocytotic pathway may exist in man which is associated with intractable diarrhoea when disrupted.

24 PROTEIN TURNOVER INCREASES IN PARENTERALLY FED PREMATURE INFANTS - A STUDY WITH [13C]LEUCINE

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There are few studies of protein turnover in premature infants (PMI). Most have used [15N]glycine which may be criticised for two reasons: the high concentration of urea in breast milk and the possibility that glycine is essential for PMI. Both lead to tracer dilution and overestimation of flux. We have used an intravenous (iv) infusion of [13C]leucine to measure whole body leucine flux in PMI. Leucine oxidation was derived from urinary nitrogen excretion, enabling protein synthesis and degradation to be calculated. Enrichment of plasma leucine was measured by gas chromatography/mass spectrometry. Baseline data were obtained for protein turnover in PMI on iv glucose and the studies repeated if total parenteral nutrition (TPN) was introduced. Fifteen PMI, gestational ages 27-33 weeks and mean weight 1.42 +/- 0.29 kg, were studied on the 2nd, 3rd, or 4th postnatal days while receiving iv glucose: mean rates (+/- SD) of protein synthesis and breakdown were 141 +/- 42 µmoles/kg/hr and 150 +/- 40 µmoles/kg/hr respectively (NS). Five infants started TPN and were studied on 5 consecutive days: there was a mean increase in protein synthesis between day 1 and day 5 of 152 +/- 28 µmoles/kg/hr ($p = 0.005$) and a simultaneous rise in breakdown of 54 +/- 23 µmoles/kg/hr ($p = 0.03$). Synthesis rose significantly more than breakdown ($p = 0.003$). In conclusion, TPN results in a greater increase in protein synthesis than in breakdown with a consequent improvement in protein retention.

25 CONSTANT INCREASE OF GAMMA/DELTA+ T CELLS IN COELIACS

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We studied the numbers of T cell receptor-alpha/beta and gamma/delta bearing lymphocytes in 27 jejunal specimens from 19 coeliac patients and in 14 control specimens. Monoclonal antibodies and a three-layer peroxidase staining method were used. In the lamina propria and epithelium of a normal jejunum only low numbers of gamma/delta+ cells were seen. In the lamina propria of coeliac patients, the mean number of gamma/delta+ cells was significantly higher than in the controls before treatment ($p<0.001$), during gluten free diet ($p<0.05$) and after the gluten challenge ($p<0.001$). In the jejunal surface and crypt epithelium of coeliac patients, the number of gamma/delta+ cells was elevated before and during gluten elimination and after the challenge test (in all comparisons for surface epithelium $p<0.0001$; for crypt epithelium before treatment and during gluten free diet $p<0.05$, after the gluten challenge $p<0.001$). In the epithelium, the absolute number of these cells remained constant during gluten elimination and provocation.

We infer that the constantly elevated population of gamma/delta+ T cells in the epithelium of coeliac patients may play an important role in the pathogenesis of coeliac disease.

26 EFFECT OF ORAL OR PARENTERAL SENSITIZATION TO COW'S MILK ON MUCOSAL PERMEABILITY IN GUINEA-PIGS.

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The systemic and local immune responses and intestinal barrier function were examined in orally or parenterally milk-sensitized guinea-pigs. Both types of sensitization led to positive passive cutaneous anaphylactic responses and high IgG titers against β-lactoglobulin (β-lg) especially in parenterally immunized animals. In Ussing chambers, sensitized jejunum had higher short-circuit current (Isc) than control jejunum, with and without β-lactoglobulin challenge. The further increase in Isc induced by serosal β-lg treatment was higher in parenterally (23.2 ± 3.4 µA/cm²) than orally (10.9 ± 2.9 µA/cm²) sensitized animals. Barrier function was tested as the intestinal transport and degradation of Horseradish peroxidase (HRP) in the presence and absence of β-lg. There was a five-fold increase in degraded HRP transport in sensitized (39.4 ± 6.6 pmoles/h.cm²) versus control (7.37 ± 2.51 pmoles/h.cm²) animals, with and without β-lg challenge. Serosally applied β-lg enhanced transport of intact HRP in sensitized but not in control animals. These results indicate that sensitization of guinea pigs to cow's milk permanently increases endocytic and electrogenic activities. The challenge with β-lg induced a further transient rise in Isc and increased intact HRP transport.

27 DEPLETION OF PROSTAGLANDINS E_2 FROM MATERNAL MILK PREVENTS DROPS OF GASTRIC ACID SECRETION IN THE NEWBORN RAT.

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We have previously demonstrated that in the rat, gastric acid secretion (AS) declines after birth and severely drops on day 12 of life. In the current study, we investigate the role of prostaglandins E_2 (PGE_2) from maternal milk in this inhibition ; PGE_2 content was first measured in milk of untreated dams at 0, 1, 5, 10, 12, 15 and 18 days after parturition. PGE_2 levels were high in the first 5 days (range 123.5-200.5 pg/ml), then declined significantly ($p < 0.05$) between 10 and 15 days (range 56.6-85.4 pg/ml) to finally reach 18.4 pg/ml on day 18. The effect of a milk depleted from PGE_2 on inhibition of AS was then studied on suckling rats of 12 days of age. Indomethacin (IM ; 5 mg/kg) injected to lactating dams significantly reduced (65 %) milk PGE_2 content of untreated dams. In pups from IM-treated dams, AS was no longer inhibited. On the contrary, in vivo, basal and histamine-induced AS was markedly increased (80 and 120 %, respectively) when compared with controls, and in vitro, net movements of ^{36}Cl and ^{22}Na measured in Ussing-type chambers indicated that active secretion of chloride has resumed. Finally, no significant role was attributed to mucosal PGE_2 since administration of IM to pups from untreated dams did not significantly modified AS on day 12.

These data indicate that depletion of PGE_2 from maternal milk prevents the drop of gastric AS previously observed in the 12-days old pup. They suggest a physiological role for maternal PGE_2 in the regulation of AS in the infant rat.

28 PARAMETERS OF THE ANTIOXIDANT PROTECTIVE SYSTEM IN PATIENTS WITH CYSTIC FIBROSIS (CF)

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Selenium (Se) dependent glutathione peroxidase (GPH-Px) and α-tocopherol (α-T), as part of the antioxidative system, synergistically protect membrane lipids from free radical damage. GPH-Px activity has been shown to be a more reliable indicator of Se bioavailability than blood Se levels. In CF patients, vitamin E deficiency is well known; in contrast, there are only a few data on impaired GPH-Px activity in these patients.

Patients and methods: In 41 CF patients aged 0.8-27.6 yrs, GPH-Px activity and α-T in red blood cell (RBC) membranes, plasma α-T and plasma α-T/cholesterol ratio were determined. Correlations with parameters of nutritional status (NS), nutrient intake, fat absorption and liver disease (LD) were studied.

Results: GPH-Px activity was decreased in 34 patients, ranging from 3% to 79% ($\bar{x}$=44.12, SEM=3.49) below the lower limit of the normal range. In 17 patients, RBC α-T was 2% to 82% ($\bar{x}$=29.12, SEM=5.67) below the cut-off point, in spite of oral α-T supplements (100-300 mg/d). In addition, only 4 patients had normal values both for GPH-Px and RBC α-T, and 14 were deficient for both. Severe GPH-Px deficiency tended to be associated with worse NS. In patients with LD (n=7), α-T levels were lower than in the others. No correlation with the other parameters studied was found.

Conclusions: The combined deficiency of both GPH-Px and α-T in 34% of CF patients may be particularly deleterious due to their synergism in preventing lipid peroxidation. α-T supplementation has been recommended for GPH-Px deficiency; yet, in our CF patients, high dose oral α-T was not always effective in correcting vitamin E deficiency and will therefore obviously not compensate for the lack of antioxidative capacity due to GPH-Px deficiency.