53 LOSS OF AN AMILORIDE SENSITIVE cAMP DEPENDENT Na ABSORPTIVE PATHWAY DURING ONTOGENY OF RAT COLON

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We studied the ontogeny of Na and water transport in the rat colon by using in vivo perfusion technique with PEG as an indicator of water absorption. There is in growing rats an exponential decrease in net colonic sodium and water absorption. The most rapid decrease occurs between the age of 10 to 20 days. The decrease is delayed if weaning, which normally occurs between day 16 and day 20, is delayed.

In young rats amiloride treatment results in a significant decrease in net sodium and water absorption. Amiloride has little effect on net sodium and water absorption in adult rats.

In adult rats cAMP given intravenously causes as an expected decrease in net sodium and water absorption. In young rats (10 to 20 day old) cAMP causes a paradoxical increase in net sodium and water absorption. This increase is abolished if the rats are simultaneously treated with amiloride. Our results in adult rats agree with those previously reported. The amiloride sensitive sodium uptake has been estimated to be approximately 2 % of the total sodium uptake in the adult colon. cAMP stimulates sodium secretion. In the young rats we found that the major portion of sodium uptake occurred through amiloride sensitive pathways. This amiloride sensitive sodium pathway could be stimulated with cAMP. cAMP therefore has a paradoxical effect on sodium and water transport in the immature colon.

54 BILE SALTS (BS) EFFECTS ON SMALL INTESTINAL TRANSPORT.

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Scanty data are available on the effects of BS in the small intestine. Previous evidence,obtained only in in vivo systems, suggested an antiabsorptive effect on water and nutrient absorption, and an increase in tissue permeability. We studied the effects of Chenodeoxycholate (CDCA) and Ursodeoxycholate (UDCA) in the rabbit stripped small intestinal mucosa mounted in Ussing chambers, aiming at defining and characterizing such effects in an in vitro system. Results: 1.Both CDCA and UDCA (1mM) induced an increase in the rate of transmural Lactulose transfer in jejunum and ileum. 2.In the ileum such effect by CDCA was dose-dependent (being half maximal at 0.1mM). 3.The enhancement in Lactulose permeability by 1mM CDCA was reversible, subsiding both in jejunum and ileum after BS removal. 4.Both BS, when added to the mucosal side of ileum, provoked a non additive, dose-dependent increase in short circuit current (Isc); on the contrary, no effect was seen in the jejunum. 5.CDCA 1mM added to the mucosal side of rabbit stripped ileal mucosa mounted in Ussing chambers, provoked an increase in net transephitelial Na absorption (probably due to the BS-Na cotransport) and a shift toward secretion in Cl transport. 6.Preliminary data show that 1mM CDCA markedly inhibited the influx rates from 2mM Glucose, 2mM L-Phenylalanine and 5mM L-Glutamic acid.

CONCLUSIONS: in the small intestine in vitro, BS: 1.have a direct inhibitory effect on nutrient uptake; 2. increase tissue paracellular permeability and 3. in the ileum, affect electrolyte transport by promoting Na absorption and anion secretion. As also suggested by their reversibility, such effects are likely to play a role in the modulation of postprandial water and solutes transport.

55 PROTEIN ENDOCYTOSIS AND PROCESSING BY VILLUS AND CRYPT CELLS OF THE MOUSE SMALL INTESTINE.

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The transport of proteins across the epithelial cells of small intestine is increased after rotavirus infection but it remains unaltered in secretory diarrhea. In addition, the cell turnover is increased in rotavirus but not in secretory diarrhea. The protein uptake and processing was therefore compared in villus and crypt cells isolated from the small intestine of healthy adult mice. In each fraction, lysosomal cathepsin activities and cell binding, uptake and degradation of Horseradish peroxidase (HRP) were measured. The following results were observed : (1) mice showed a decreasing gradient of cathepsins B and D from crypts to villi following the gradient of thymidine kinase, the reverse was observed for sucrase activity. (2) Binding, and internalization of HRP was twofold increased in crypt cells compared to villus cells indicating a double rate of endocytosis (binding : 12.4 ± 1.7 versus 20.3 ± 3.4 ng/mgP and internalization 60.0 ± 11.2 versus 142.8 ± 29.1 ng/mgP for intact HRP in villus and crypt cells respectively). (3) The percentage of degraded HRP into the cell remained similar in both cell types suggesting that the lysosomal degradation was not the limiting step of endocytosis. Chloroquine and ammonia but not monensin increased intracellular intact-HRP. These results suggest that intestinal villus and crypt cells do not transport proteins at the same rate. This may have important immunological consequences in pathological states in which the crypt/villus ratio is greatly increased.

56 SCANNING PHOTOMETRY IN MICROVILLOUS ATROPHY (MVA): THREE DIFFERENT AND INCOMPLETE PERIODIC ACID SCHIFF (PAS) STAINING ABNORMALITIES

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PAS staining of small intestinal mucosa in MVA demonstrates cytoplasmic PAS staining in the epithelium. Scanning photometry was applied to this abnormality in order to determine objectively: 1) its extent and specificity, 2) its origin on the crypt/villus axis, and 3) any variability within the disease. Small bowel was studied from histologically normal (n=4) and abnormal (n=5) controls and from patients with MVA (n=8). Each was divided into low, mid, and upper mucosal regions in which 5 epithelial cells were scanned photometrically (520nm filter) under computer control for PAS stain and the results meaned. All controls showed only a brush border peak of PAS stain in all 3 regions. 5 cases of congenital MVA showed only a brush border peak in the low crypt region, mainly cytoplasmic staining with a brush border peak in the upper crypt region, and cytoplasmic staining alone in surface/villous cells. 2 patients with late onset MVA showed relatively reduced cytoplasmic staining in upper crypt cells plus a brush border peak in the surface/villous region. 1 child with congenital MVA showed only cytoplasmic staining in crypt epithelium and a variable, but more normal, appearance in villous cells.

Thus the PAS abnormality in MVA appears specific but is incomplete, and 3 patterns and 2 sites of origin can be defined.

57 CHILDHOOD ACUTE DIARRHOEA IN ITALY

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During 1985-86, a nation-wide survey was conducted to assess: 1. Prevalence of acute diarrhoea in 1-3 years old children; 2. Etiology and clinical characteristics of acute-onset diarrhoea in children of 1 month to 14 years of age. METHODS: 1. A cohort of 457 healthy children of 1-24 months of age from 3 towns (Milano, Napoli, Messina) was selected accordingly to criteria that made them representative of the town population and perspectively followed at by-weekly intervals for 1 year. 2. 736 children of 1 month to 14 years of age with acute-onset diarrhoea and presenting for care (567 as inpatients) at the 6 participating pediatric Centers (Milano, Mantova, Roma, Napoli, Messina and Palermo) were enrolled and their stools were looked for rotaviral infection (ELISA test), routine microbiology and osmolality and electrolytes. RESULTS: 1) 266 of the 457 healthy young children (58.2%) showed at least one episode of diarrhea during a 12 months follow-up. The number of episodes significantly differed among different towns, and correlated with the social status. 2) Rotavirus was the most common pathogen (30.8%) followed by Salmonella (10.1%) and Campylobacter (6.0%). In 50 of 241 patients in which both stool osmolality and electrolytes were measured (20.8%), diarrhoea was found to be of secretory type; in the remaining, an osmolar-type diarrhoea was present. Mean duration of diarrhoea was 4.1 days. In 25 cases out of 736 (3.4%) it became protracted (>14 days): previous or early use of antibiotics, food intolerances and Rotavirus infections were all found to be significantly more frequent in patients running a protracted course.

58 ADHERENCE PROPERTIES OF ENTEROTOXIGENIC E. COLI (ETEC) PRODUCING COLI SURFACE ANTIGENS 4, 5 & 6 (CS4,CS5,CS6)

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ETEC are a major cause of diarrhoeal disease in young infants in less-developed countries. Mucosal adherence of ETEC, an essential early event in intestinal colonisation, is promoted by serologically distinct bacterial surface 'Colonisation Factor Antigens' (CFAs). CFA/I, CFA/II and CFA/III have been characterised in some ETEC but even in some commonly isolated ETEC serotypes CFAs still remain to be identified. 3 coli surface antigens (CS4, CS5 & CS6) were recently described in ETEC serogroups 025, 027, 092, 0115, 0148, 0159 and 0167 but a role in mucosal adhesion has not been confirmed. We have therefore examined a collection of 20 ETEC isolates producing CS4, CS5 and CS6 for their ability to adhere to the brush border (BB) of human small intestinal enterocytes and to cultured human intestinal mucosa. 025 and 0167 serogroup ETEC producing CS4,CS6 and CS5,CS6 showed good BB adhesion (2.3 and 4.2 bacteria/BB); complementary ultrastructural studies of colonised mucosa confirmed CS4 and CS5 as rod-like fimbrial structures which promote BB adhesion of these strains. Good adhesion was also observed with CS6 producing ETEC of serogroups 027, 0148 and 0159 (1.8, 2.3, and 3.0 bacteria/BB) but electron microscopy of these strains revealed, in addition to CS6, fimbrial or fibrillar structures which probably represent previously unidentified CFAs since ETEC producing only CS6 were nonadherent in our assay (0.01 bacteria/BB). This study, therefore, has confirmed CS4 and CS5 as fimbrial CFAs and identified 4 putative CFAs in ETEC serogroups 027, 0148 and 0159 but, as yet, we have been unable to confirm CS6 as a human ETEC CFA.