17 KINETICS OF HUMAN SMALL INTESTINAL BRUSH BORDER MEMBRANE D-GLUCOSE TRANSPORT: JEJUNUM AND ILEUM COMPARED

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Translocation of glucose across the small intestinal brush border membrane is of fundamental physiological and clinical importance, yet little is known about the kinetics of this transport process in man. Using a recently developed miniaturised technique employing human brush border membrane vesicle (BBMV), we have studied the initial 3 sec uptake of glucose by BBMV under Na^+ gradient (out:100mM; in:0) and non-Na^+ gradient (out and in:0) conditions, over a range of substrate concentrations (extra-vesicular glucose:0-3.2mM). BBMV were derived from small (150-175 mg), full thickness specimens of jejunum (n=8) or ileum (n=12), obtained from children undergoing elective intestinal resection. Eadie-Hofstee transformation of the data showed that in the jejunum, glucose was transported by 3 different mechanisms:
(i) a diffusional process and/or a low affinity, Na^+-independant carrier
(ii) a high affinity, low capacity, Na^+-dependant saturable system (approximately Km 0.3mM; Vmax 13nmol/mg/min).
(iii) a Na^+-dependant diffusional process and/or a very low affinity, high capacity saturable carrier.
In contrast, it was not possible to consistently demonstrate saturable glucose uptake by ileal BBMV. These data provide a basis for an explanation of the heterogeneity seen in glucose-galactose malabsorption, and suggest that in the ileum, transcellular glucose transport may be relatively unimportant.

18 CHARACTERIZATION OF DIFFERENT PHENOTYPES OF CONGENITAL SUCRASE-ISOMALTASE DEFICIENCY (CSID) IN MAN.

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Congenital sucrase-isomaltase deficiency (CSID) is an autosomal recessive malabsorptive disease of the human small intestine. The absence of SI in patients with this disease results in failure of digestion of dietary sucrose. We have investigated CSID at the protein and cell level in biopsy specimens from 8 patients with this disease. SI was immunoprecipitated from ^{35}S-methionine-labelled organ cultures and from ^{125}I-labelled mucosal homogenates. Further, immunoelectron microscopic visualization of SI by the protein A-gold labelling technique was undertaken. The data accumulated from these studies revealed at least three phenotypes of CSID: one in which sucrase-isomaltase accumulates intracellularly likely in the endoplasmic reticulum, as a high mannose precursor protein (SI-h), one in which the intracellular transport is blocked in the Golgi and finally, one which is transported to the cell surface as catalytically inactive enzyme. All patients expressed SI-h with indistinguisable (6 cases) or slightly elevated apparent molecular weight (2 cases). The data suggest that different mutations in the sucrase-isomaltase gene lead to the synthesis of transport incompetent or enzymatically inactive molecule which results in CSID.

19 EFFECT OF INSULIN ON INTESTINAL MATURATION OF VILLUS AND CRYPT CELL FUNCTIONS IN SUCKLING RATS

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To study the influence of insulin on the postnatal maturation of the rat small intestine, 9-day-old sucklings received a daily injection of insulin (12.5mU.g body wt^{-1}) during 4 days. The hormone had no effect on the mucosal mass parameters determined in the jejunum ileum and colon but a premature appearance of sucrase was noted in jejunal enterocytes. The level of activity reached by the enzyme was dependent of the amount of insulin given. After a single injection of insulin (12.5mU), sucrase activity was already detected by 6 hours in all the cell fractions along the villus-crypt unit. In villus cells of insulin-treated-rats, lactase, maltase and aminopeptidase activities were markedly increased (+50%, +201%, +207% respectively vs controls, $p < 0.001$) whereas the concentration of the secretory component of p-IgA was enhanced by +83% in crypt cells ($p < 0.01$ vs controls). Administration of Actinomycin D (0.1µg.g body wt^{-1}) to sucklings 1 hour after insulin injection completely inhibited the intestinal adaptive response to the hormone. Our data indicate that (1) insulin accelerates the intestinal maturation of both villus and crypt cell functions (2) the response to the hormone occurs rapidly over the entire villus-crypt unit (3) the effect of insulin appears to be regulated at the level of transcription.

20 MECHANISMS OF TRANSPORT OF SODIUM AND CHLORIDE AND THE EFFECTS OF INFLAMMATION IN THE HUMAN INFANT COLON

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A major function of the colon is the conservation of salt and water and we have previously shown (Jenkins et al GUT 27; 1283, 1986) that in the human infant it plays a major homeostatic role. Colitis may therefore assume greater importance than in later life. We have carried out a detailed study of transport in normal and inflammed isolated human infant colon using an Ussing Chamber and a voltage clamp procedure. Stripped colonic mucosa histologically normal n=6 pairs and inflamed from patients with non-clostridial Hirschsprung's enterocolitis, n=5 pairs was mounted and bathed in Krebs$_2$ solution. Under short-circuit conditions Na^+ (3.45±1.53 umol/hr/cm mean ± ISD) and Cl^-(0.63±3.61) were absorbed and a residual ion flux consistent with HCO_3^- secretion approximated Cl^- absorption. Short circuit current (I^{SC}) (3.8±0.28) was very similar to net Na^+ movement and was markedly reduced by mucosal 10^{-4} M amiloride (0.61±0.7 $p<0.01$). Inflammed mucosa generated a lower potential difference (2.44±0.1 vs 6.5±1.0 m V $p<0.01$) I^{SC} (1.62± 0.8 vs 3.87±0.5 $p<0.02$) and was of lower resistance (67.2±12 vs 104±10 Ohm $p<0.01$). Na^+ was secreted and anion exchange reversed due to large increases of serosal to mucosal fluxes (Na^+ 5.63±0.1 to 15.7±1.2 Cl^- 13.86±1.64 to 20.9±3) with no change in mucosal to serosal fluxes. These data show that Na^+ is absorbed electrogenically and Cl^- electroneutrally in exchange for HCO_3^-. In the presence of inflammation the electrical and flux changes suggest a decrease in resistance of shunt pathways which dissipate absorbed Na^+ and Cl^-. Inflammation of the infant colon therefore seriously impairs its ability to conserve salt and water, and would thus make an important contribution to a dehydrating diarrhoeal disease.

21 DEFECTIVE REGULATION OF INTESTINAL CHLORIDE CHANNELS IN CYSTIC FIBROSIS (CF).

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The possible manifestation of a Cl^--channel defect in intestinal epithelium of CF patients was investigated by Ussing Chamber measurements of the electrical response of stripped ileal mucosa from CF (n=4) and control (N) patients (n=4) undergoing take down of stomas following meconium impaction (CF) or intestinal resection for enterocolitis or atresias (N). Endogenous prostaglandin release was inhibited by serosal (S) and mucosal (M) indomethacin (10^{-5} M). The short circuit current (SCC) respons (uA/cm^2) to glucose (10^{-2}M, M), secretagogues acting through Ca^{2+} (carbachol 10^{-4} M, S; histamine 5.10^{-5}M,S; bradykinin 10^{-6} M,S,) through cGMP (8-Br-cGMP 10^{-4}M, S), or through cAMP (8-Br-cAMP10^{-3}M,S) is shown below (±S.E.M.).

	N	CF		N	CF		N	CF
Glucose	+35±10	+38±12	Histamine	+20±9	-8±4	8-Br-cGMP	+22± 7	0±0
Carbachol	+49±17	-24± 5	Bradykinin	+15±6	-5±2	8-Br-cAMP	+61±19	-10±8

The negative SCC respons in CF to all secretagogues (except cGMP) was inhibitable by Ba^{2+} (5.10^{-3}M,M), bumetamide (10^{-5}M,S), DIDS (10^{-4}M,S) and Cl^--free conditions and presumably reflects the opening of apical K^+ and basolateral Cl-channels by cAMP or Ca^{2+}. It is concluded that: 1.Na^+ coupled glucose uptake is unmodified in CF. 2.Apical Cl^--channels in CF enterocytes are either absent or completely insensitive to activating signals (cAMP, cGMP and Ca^{2+}). 3.CF enterocytes contain cAMP and Ca^{2+} (but not cGMP) sensitive apical K^+ channels and basolateral Cl^--channels which are absent or latent in control tissue. 4.The Cl^--channel defect in CF intestine may offer protection against secretory diarrhoea.

22 PHYSIOLOGICAL ROLE OF PREGASTRIC LIPASE

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Three lipases are involved in the digestion of milk triglycerides in breast-fed infants. Neither colipase-dependent lipase secreted from the pancreas, nor bile salt-stimulated lipase provided by the milk could by themselves, even in the presence of their respective cofactors, initiate triglyceride digestion. This was a unique functional property of pregastric (lingual) lipase, the lipase operating in the stomach. A major quantitative function of this lipase is, however, unlikely since it was inhibited by a fatty acid concentration corresponding to hydrolysis of only a few percent of the triglycerides. Interestingly, this low degree of hydrolysis had dramatic effects. The remaining globule triglycerides were easily hydrolyzed by the two other lipases. This qualitative function of pregastric lipase was mediated by the fatty acids released; addition of free fatty acid to a corresponding concentration was as effective. Marked differencies between various fatty acids were observed regarding both inhibition of pancreatic lipase and initiation of hydrolysis by pancreatic lipase. Interestingly, in both respects long-chain unsaturated fatty acids were most effective. The reason why intact milk fat globule triglycerides were resistant to hydrolysis by pancreatic lipase was an inability of the lipase, even in the presence of its cofactor colipase, to bind to the globule surface. The effects of fatty acids was to mediate this binding. We conclude that generation of a low amount of fatty acid already in the stomach is the physiological role of pregastric lipase.