47 N- and O-GLYCOSYLATION OF HUMAN INTESTINAL SUCRASE-ISOMALTASE: DIFFERENTIAL O-GLYCOSYLATION OF THE SUCRASE SUBUNIT CORRELATES WITH ITS POSITION WITH IN THE ENZYME COMPLEX.

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Sucrase-isomaltase (SI) is a major microvillar glycoprotein of the small intestine. It is synthesized as a single polypeptide chain, which is extracellularly cleaved by pancreatic secretions to its two enzymatically active subunits sucrase (S) and isomaltase (I). We have investigated the biosynthesis and maturation of this complex with particular emphasis on the glycosylation events. Enzymic and chemical deglycosylations of SI with endo-β-N-acetylglucosaminidase F (endo F) and trifluoromethanesulfonic acid (TFMS) as well as probing for the binding capacity of SI to Helix pomatia lectin demonstrated that pro-SI, I and S are N- and O-glycosylated. Furthermore, the results were indicative of a post-translational O-glycosylation of pro-SI, since (i) the earliest detectable precursor form, pro-SI_h, did not bind to H. pomatia lectin and (ii) its deglycosylation products with both endo-β-N-acetylglucosaminidase H (endo H) and TFMS were identical. Both the S and I subunits contain eight N-linked glycan units, at least one of which is of the high mannose type and found on S. Finally, S, but not I, was shown to display at least four populations varying in their content of O-linked glycans. The heterogeneous O-glycosylation pattern of S could be correlated with the distal position of this subunit (and its O-glycosylation sites) within the pro-SI molecule thus affecting the extent of O-linked oligosaccharide processing and their subsequent presentation on the mature molecule.

48 DIFFERENTIATION OF HT-29 CELLS: EFFECT ON THE BIOSYNTHESIS AND GLYCOSYLATION OF SUCRASE-ISOMALTASE AND DIPEPTIDYL PEPTIDASE IV.

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The expression and mode of glycosylation of sucrase-isomaltase (SI) and dipeptidyl peptidase IV (DPP IV) have been studied in HT-29 cells grown with glucose (undifferentiated) or inosine (differentiated) as carbon-source. 10-day-confluent cells were labelled with ^{35}S-methionine, immunoprecipitated with monoclonal antibodies and analysed by SDS-PAGE. The mode of glycosylation was determined by treatment of immunoisolated proteins with endo H, endo F and trifluormethane sulfonic acid (TFMS). In differentiated HT-29 cells biosynthesis and processing of these two markers was as in normal enterocytes, although the level of expression was lower. Undifferentiated HT-29 cells showed a very low level of expression of SI whereas the amount of DPP IV expressed was not appreciably altered. There was however a significant increase in molecular weight of the mature DPP IV in differentiated cells. This size-difference has been shown to be partially due to increased O-linked glycosylation of DPP IV in differentiated HT-29 cells.
Conclusion: The changes observed in the expression and the post-translational processing of SI and DPP IV thus represent molecular key-events in the differentiation of HT-29 cells and provide us with a working model to study exogenous factors that influence differentiation-state of small intestinal epithelial cells.

49 ABNORMAL SPECIES OF SUCRASE-ISOMALTASE (S-I) ARE ASSOCIATED WITH VARIATIONS OF LUMINAL PANCREATIC ENZYME CONTENT IN RAT INTESTINE.

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The S-I complex is synthesized as a single chain precursor P. Once it reaches the brush-border surface, P is split by pancreatic proteases into S and I. Recently, we have documented in vitro this cleavage. Among the proteases tested: trypsin (T), elastase (E) and chymotrypsin (C), T was the only one involved in producing normal S-I. But, depending on the protease and its concentration, abnormal forms of S and I were observed. Thus, to document those observations, modifications of pancreatic enzyme balances in intestinal lumen were induced in rats either physiologically (i.e. by diet changes), either as a result of surgical transfert of pancreatic ducts. By electrophoretic analysis of S-I immunoprecipitates: 1) when T was increased over E and C, an abnormal form of I (I'; mw<I) was found S was inchanged; 2) when E was increased over T or C, two species, E1 and E2, larger than normal S and I, were only observed; 3) when T, E and C, all together were hightly increased, I' and a new specie, S (mw<<S) were the only forms characterized. By both S and I enzyme determinations: 1) all complexes, S-I', E1-E2 and S'-I', indicated a significant decrease in the S/I activity ratio; 2) only the S' species showed an increase of the normal S Km value (24.8 v.s 19.1, p 0.001). Conclusions: without alterations of the intracellular biosynthetic process, modified S-I complex can be found depending on the luminal environment in pancreatic proteases. Moreover, the observed S'-I' complex, suggests that abnormal function of the exocrine pancreas may induce carbohydrate malabsorption.

50 CORRELATION BETWEEN THE ONTOGENY OF SMALL INTESTINAL MUCOSAL LYMPHOCYTES AND SUCRASE IN THE PREWEANED RAT.

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Immunoreactive sucrase in villous enterocytes of rat jejunum appears by 21 days of age. We attempted to correlate the ontogeny and type of intraepithelial and lamina propria lymphocytes during the same period. Suckled Sprague-Dawley rat pups (0 - 24 days) were sacrificed at regular intervals. The distal jejunum was removed and 4- to 5-micron frozen sections were cut, fixed in absolute methanol, and stained with mouse monoclonal antibodies BBC 1/35 (sucrase), MRC OX-4 (B-cell), MRC OX-8 (T-suppressor), W3/25 (T-helper), MRC OX-19 (pan T-cell) and W3/13HLK (pan T-cell). The sections were counterstained with goat anti-mouse IgG-FITC conjugated antiserum. The sections were then stained with Evans blue for morphologic definition and evaluated for type, relative numbers and location of lymphocytes. T-suppressor cells were present in the intraepithelial and lamina propria areas up to age 12 days and were absent between 12 and 17 days. From 17 to 24 days, T-suppressor cell levels increased and approached adult levels. B-cells were present at all ages in the lamina propria of villi and crypts. T-helper cells were found irregularly; pan T-cell levels increased from 21 days onward. Our observations confirm and extend those of Lyscom and Brueton (*Clin Exp Immunol* 54:158-162, 1983) in which the number of T-suppressor intraepithelial cells increased after 21 days of life. We report that this increase coincides with the expression of sucrase by villous enterocytes.

51 CHOLERA TOXIN STIMULATES NA^+-GLUCOSE COTRANSPORT IN A HUMAN EPITHELIAL SECRETORY CELL LINE.

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Cholera toxin (CT) stimulates chloride secretion in the crypt cells with preservation of the Na^+-glucose cotransport in the villus cells. The role of CT on the relationship between Cl secretion and glucose absorption was studied in an intestinal epithelial cell line HRT-18. Cells were studied after confluence on HAHY Milliporte filters that were mounted in Ussing chambers. Addition of 3.5 µg/ml CT to the mucosal side raised short circuit current (Isc) from 0.05 ± 0.008 to 0.32 ± 0.05 $\mu eq.hr^{-1}.cm^{-2}$ after 60 min which was accompanied by JCl net secretion (-0.04 in the Ringer, to -0.33 $\mu eq.hr^{-1}.cm^{-2}$). Intracellular cAMP content increased from 8.98 ± 1.66 pmole/filter in Ringer solution to 19.59 ± 4.19 after CT. Addition of 10^{-4}M glucose after CT raised the Isc further to 0.70 ± 0.08 $\mu eq.hr^{-1}.cm^{-2}$ by stimulating JNa net from -0.06 to +0.58 $\mu eq.hr^{-1}.cm^{-2}$. This additional augmentation of Isc was reversed by 0.5 mM phlorizin and was mimicked by 3.0 methyl glucose and was absent in chloride free solution. When filters were stimulated by cAMP for 15 min, Isc was also enhanced by addition of glucose. In untreated filters Isc, JNa net and JCl net did not differ significantly before and after addition of glucose. These data indicate that the cells which secrete under the action of cholera toxin are able to express their Na^+-glucose absorptive capacity. This may implicate the possibility of recruitment of an additional reserve of Na^+-glucose transporters in cholera.

52 INTESTINAL Na UPTAKE DECREASES AFTER HIGH SALT DIET (HS) IN YOUNG BUT NOT IN ADULT RAT

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Net sodium absorption in the jejunum was determined in 20, 40 and 80 day-old rats. The jejunum was perfused in vivo (10 ml/100 g BW/h) with an isotonic electrolyte solution containing C^{14} PEG 4000 (MW range 2500-6000) 5 g/l, which was used as a marker of water absorption. In 20 d controls (C) there was a significantly higher net sodium absorption (48.3±2.45 µEq/min/g dry weight) than in 40 d (27.3±2.57) $p<0.01$ and in 80 d (32.8±2.53) $p<0.01$. HS was accomplished by replacing the drinking water with isotonic saline for 4 days prior to study. In 20 d HS rats net sodium absorption was significantly decreased compared to 20 d C (34.6±0.9) $p<0.01$, but there were no differences compared to controls in 40 d HS (33.1±2.79) or in 80 d HS (34.1±2.91). In the kidney locally produced dopamine DA will contribute to the natriuretic response to salt load (Kidney Int 1987, 31: 258). When 20 d HS were fed benserazide, an inhibitor of L-dopa decarboxylase, prior to study, the net sodium absorption significantly increased compared to untreated 20 d HS to the same magnitude as in controls (49.9±1.81) $p<0.01$. There was no effect of benserazide in 40 d HS and 80 d HS.
Conclusion: Basal jejunal Na uptake is higher and changes in jejunal Na uptake play a more important role for the adaptation to HS intake in young than in adult rats. Dopamine contributes to the response of the young jejunum to HS.