71 INTESTINAL LACTULOSE PERMEABILITY REFLECTS EXOCRINE PANCREATIC DYSFUNCTION.

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Our previous work suggested that increased intestinal permeability was related to the degree of exocrine pancreatic dysfunction in Cystic Fibrosis (CF). Others have suggested this finding represents a specific abnormality of CF. To further evaluate this question we studied CF (n=31) and non-CF (n=10) patients (Shwachman Syndrome) with variable degrees of exocrine pancreatic dysfunction They were categorized into pancreatic insufficient (PI) and pancreatic sufficient (PS) groups based on fecal fat balance studies. After an overnight fast, patients were fed 20 grams of lactulose and urine was collected for 8 hours. Urinary lactulose excretion (% intake) was determined by thin-layer chromatography. Mean urinary lactulose excretion was no different between CF and non-CF patients with either PI (2.10±1.21 and 1.92±0.76) or PS (0.63±0.49 and 0.61±0.34). There was a significant difference in lactulose excretion between PI and PS patients in CF and non-CF groups ($p<0.0001$ and $p<0.013$, respectively). This relationship was explained by comparing pancreatic function (trypsin output, units/kg/hour) with urinary lactulose excretion in 26 patients. A non-linear inverse relationship was observed between the two parameters. A plot of the log(x) transformation of duodenal trypsin output against urinary lactulose excretion revealed a highly significant linear relationship ($r=0.778$, $p=0.0001$). These data confirm a direct relationship between intestinal lactulose permeability and the degree of exocrine pancreatic dysfunction, unrelated to etiology.

72 THE COLON PLAYS A SIGNIFICANT ROLE IN E.COLI HEAT STABLE ENTEROTOXIN (ST_a)-MEDIATED DIARRHEAL DISEASE.

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In order to determine the potential contribution of the colon to E.coli enterotoxigenic diarrheal disease, we compared ST_a binding, guanylate cyclase activation (GCA), and ST_a-induced water flux in the ileum and colon of adult male rats. Receptor binding characteristics were determined by Scatchard analysis of ^{125}I-ST_a binding to isolated ileocytes and colonocytes. ST_a stimulated GCA was determined in crude membrane homogenates prepared from ileum and proximal colon. Water flux was measured in segments of ileum and colon continuously perfused with a non-absorbable marker containing solution, with and without ST_a. Scatchard analysis suggested a single class of ST_a receptors with a K_a of $\approx 10^9$ L/M in both ileocytes and colonocytes, however maximum binding was 3.5 fold greater ($p=.02$) in colonocytes (8.32×10^5 receptors/cell) than ileocytes (2.33×10^5). ST_a stimulated GCA in an identical dose dependant manner in ileal and proximal colonic membranes. ST_a inhibited net water flux to a similar degree in both ileum (-48 ul/cm/hr) and colon (-38 ul/cm/hr). In the ileum, this change induced net secretion. In the colon, because of a higher baseline of absorption, absorption continued, but at a diminished level. Conclusion: The colon may contribute to the pathophysiology of ST_a-mediated diarrheal disease by a decreased absorptive capacity in the face of increased small intestinal fluid secretion.

73 IDENTIFICATION OF THE HUMAN INTESTINAL RECEPTOR FOR E. COLI HEAT-STABLE ENTEROTOXIN.

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Enterotoxigenic E. coli that elaborate heat-stable enterotoxin (ST_a) are a world-wide cause of diarrheal disease. We have previously demonstrated that ST_a, a polypeptide able to induce intestinal secretion, binds to a specific receptor in human intestine. In order to characterize this receptor, we used a calcium precipitation technique to prepare brush border membranes (BBM) from human small intestine. BBM were incubated with radiolabeled ST_a (4-Tyr-^{125}I-ST_a) in the presence and absence of excess native ST_a. Radiolabeled ST_a was crosslinked to BBM using the photoaffinity agent N-hydroxysuccinimidyl-4-azidobenzoate. These BBM, with the ST_a receptor covalently crosslinked to radiolabeled ST_a, were subjected to SDS-PAGE and autoradiography. Two proteins of M_r 66,000 and 98,000 were highly radiolabeled by this technique. Crosslinking of radiolabeled ST_a to these proteins was markedly diminished in the presence of unlabeled ST_a. These same proteins were specifically crosslinked in specimens from 25 different patients as well as in multiple specimens obtained throughout the jejunum and ileum of 2 different patients. We conclude that these proteins are probably major binding components of the human intestinal ST_a receptor.

74 LACTASE mRNA APPEARS IN FETAL RAT INTESTINE SIMULTANEOUSLY WITH DEVELOPMENT OF COLUMNAR EPITHELIAL CELLS

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In order to elucidate the relation between the morphogenesis of the small intestine and the initiation of lactase gene expression, our previously characterized rat lactase cDNA was used as a probe for lactase mRNA. Detection of lactase mRNA by Northern blotting was correlated with intestinal development as assessed by microscopic examination of samples from the same fetus and by enzyme assay. Both mRNA and enzyme activity were detectable as soon as the stratified epithelium of the early intestine had been transformed into a single layer of columnar epithelium lining villi at about 18 days of gestation. Although PAS staining was positive for the brush border, electron microscopy demonstrated that the epithelial cells were still immature, with sparse microvilli, endoplasmic reticulum, and Golgi apparatus, and a relatively homogeneous cytoplasm. Significant levels of lactase mRNA were present in these cells, thus indicating that lactase expression begins as soon as the definitive intestinal epithelial cell develops. During the period of transformation when the columnar epithelium and villi were forming, no lactase mRNA was detectable in the immature distal third of the fetal intestine. CONCLUSIONS: 1) Initiation of lactase expression occurs concurrently with the morphogenesis of columnar epithelial cells. 2) Lactase expression does not require the formation of mature brush border structure. 3) Lactase enzyme activity and mRNA appear simultaneously during development of rat intestine. Supported by NIH grant DK 32658 and the Whitaker Health Sciences Fund.

75 PROTEIN TYROSINE KINASE SUBSTRATES IN RAT INTESTINAL MICROVILLUS MEMBRANES (MVM).

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Many growth factor receptors and proto-oncogenes regulate cellular proliferentiation and differentiation by an intrinsic tyrosine kinase which phosphorylates specific cellular substrates. These phosphotyrosyl proteins (p-tyr) are likely to be second messengers which transduce growth factor responses. To characterize these potential mediators of intestinal growth, we present the first identification of p-tyr in rat intestinal MVM, which we have previously shown to exhibit tyrosine kinase activity. (Gastroenterol 94:A460,1988).

MVM, purified from small intestine of fetal and adult rats by the calcium precipitation technique, or their corresponding intestinal homogenate, were incubated for 10 min at 20°C in vitro in Hepes/$MnCl_2$/ATP/Na orthovanadate. P-tyr were identified by either immunoprecipitation or Western blot using a highly specific anti-phosphotyrosine monoclonal or polyclonal antibody, respectively. A 68 kDa protein was the most abundant p-tyr identified in MVM (fetal > adult) using either technique. Immunoprecipitation demonstrated a 36 kDa p-tyr in fetal MVM and a 33 kDa p-tyr in adult MVM. A few other p-tyr were identified by Western blot in fetal MVM but only the 68 kDa was present in adult MVM. These p-tyr were significantly enriched in MVM compared to homogenates.

To determine if these p-tyr are present in vivo and to study their developmental expression, Western blots were performed on MVM prepared from fetal, suckling and adult rat intestine in the presence of vanadate and NaF. The 68 kDa p-tyr was present in all ages studied and in MVM of adult jejunum and ileum. 80, 90 and 175 kDa p-tyr, previously identified in vitro, demonstrated variable expression during development.

The epidermal growth factor receptor (EGFR), a known phosphotyrosyl protein, was immunoprecipitated from fetal MVM with an anti-EGFR antibody, incubated with ^{32}P-ATP and identified as a single band of MW 175 kDa by autoradiography, suggesting that the 175 kDa p-tyr expressed in MVM is the EGFR.

CONCLUSIONS: 1) MVM tyrosine kinase(s) phosphorylate p-tyr in vitro and in vivo. 2) A 68 kDa protein is the most adundant p-tyr present in fetal and adult MVM. 3) The 175 kDa p-tyr identified in MVM in vivo appears to be the EGFR which is autophosphorylated.

76 ENISOPROST (EN) IMPROVES FAT ABSORPTION IN CYSTIC FIBROSIS (CF) PATIENTS (PTS).

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Chloride (Cl) transport, deficient in CF, may be prostaglandin (PG) sensitive. Furthermore, exogenous pancreatic enzymes (PEs) are acid labile. The PGE1 analog EN increases gut pH within minutes of oral administration. We report a double-blind, placebo (P) controlled crossover study of EN, 4-8 ug/kg/day, in 20 CF Pts who required PEs to control steatorrhea. Ten Pts (Gp I, ages 5-23) were treated for 3 days and ten (Gp II, ages 5-14) for 13 days. We examined: stool (ST) fat, sodium (Na), potassium (K) and chloride (Cl); ST weight (Wt), frequency (F) and osmolality; sweat (SW) Cl, Na, K; essential fatty acids (EFA). Results (mean ± SEM):

		Fat (%)	STNa (%)	SWCl (mEq/L)	STWT (g/day)	STF (no./day)
GpI	EN	43±10	30±6	124±5	280±58	2.4±0.4
	P	41±8	21±4	122±5	280±53	2.0±0.2
GPII	EN	7±2*	15±4	122±3	77±17*	1.0±0.2
	P	17±4	15±4	124±3	117±22	1.2±0.2

*$p<0.04$

There were no significant differences in ST or SW electrolytes, or EFA between the two treatments for GpI or GpII. Conclusions: EN increases fat absorption in CF Pts when treated for 13 days but not when treated for 3 days. Long term adaptive responses to PGs, rather than enzyme sparing from short term changes in luminal pH, likely underlie our findings.