●602 ISOLATION OF THE ACTIVATOR (A) OF BILIRUBIN GLUCURONYL TRANSFERASE (BGt) FROM NORMAL (JJ) AND HOMOZYGOUS (jj) GUNN RATS. Gerard B. Odell, William Mogilevsky, Univ of Wis., School of Medicine, Dept. of Peds., Madison, WI.

Lubrol solubilized microsomes of liver from JJ and jj rats were salt fractionated and the 60% $(NH_4)_2$ SO_4 precipitate was redissolved, dialyzed and further fractionated by DEAE cellulose chromatography (DE-52) . After initial protein elution with 10 mM PO_4 buffer, pH8.0, a linear salt gradient from 0-0.5M KCl was begun. Three separate elution fractions (F1, F2, F3) were collected with BGt activity for bilirubin (B). The DE-52, F3 fractions from both JJ and jj rats activated the BGt activity of the F1 fraction of JJ rats 5-10 fold (n=20). The A was separated from the BGt in F3 by Sephacryl-300 (S-300) sieving into two molecular weight fractions BGt≅230 kD and A≅60 kD. Further purification of the BGt from F1 and F3 of JJ liver was performed by affinity chromatography. After adsorption on UDP-hexanolamine Sepharose 4-B, the purified BGt was eluted by 5mM UDPGA which showed by polyacrylamide gel electophoresis (PAGE) a subunit couplet of 52 and 54 kD. This purified BGt was activitated 5-8 fold by the A from the S-300 eluate of both JJ and jj microsomes. The subunit size of A by PAGE is similar to its size after S-300 sieving (≅60 kD). It is heat labile, has no BGt activity, is precipitated by TCA, inactivated by alkylation but not by trypsin or iodoacetamide. The A has no effect on the purified transferase (Gt) for p-nitrophenol. Thus in addition to separate Gts in microsomes additional cofactors appear important in determining their substrate specificity. The A increases the formation of both the mono- and diglucuronides of B by BGt.

●603 REGULATION OF INTESTINAL EPITHELIAL CELL ATTACHMENT AND GROWTH BY EXTRACELLULAR MATRIX (ECM). Allan Olson, Hannah Blau, Debra Danna, Robert Bienkowski, Murray Davidson. Sch Med, SUNY Stony Brook; Schneider Children's Hospital, LIJMC, Dept Peds, New Hyde Park NY

Adherence of the intestinal epithelial cell (IEC) to its basement membrane may critically effect migration and differentiation of the IEC. We have quantitated attachment of IEC-6 cells, an established rat IEC line, to various ECM macromolecules. Cells ($6.0x10^5$/well) were added to wells coated with type I collagen (Col I), type IV collagen (Col IV), laminin (Ln), fibronectin (Fn), gel matrix derived from EHS sarcoma, which has been shown to support culture of differentiated epithelial cell (GM), or albumin (Al) and incubated for 4 h. The attached cells were washed, lysed and the nuclei were counted. After 4 h, the number of cells attached to GM ($2.6x10^5$) was 50-100% greater than for the other substrates ($p < .001$, ANOVA). GM: $2.7\pm0.1x10^5$; Col_5 IV: $1.95\pm0.4x10^5$; Col I: $1.3\pm0.3x10^5$; Fn: $1.4\pm0.2x10^5$; Ln: $1.9\pm0.1x10^5$; Al: $1.0\pm0.3x10^5$. Phase contrast microscopy showed that the substrate had a pronounced effect on cell morphology: after 24 h cells on GM formed cylindrical arrays which penetrated the gel, whereas cells on all other substrates were polygonal and showed little aggregation. Culture of IEC on GM may be a useful model for intestinal development.

604 EFFECT OF MALNUTRITION ON THE ADHERENCE OF SALMONELLA TYPHIMURIUM (ST) TO RAT ENTEROCYTE. Irekpono Omoike, Bo Lindquist, Ping-Cheung Lee, Emanuel Lebenthal. Children's Hospital of Buffalo, International Institute and State University of New York, Department of Pediatrics, Buffalo.

The adherence of bacteria to intestinal mucosa is an important initial event in the pathogenesis of enteric infections. Such infections occur frequently in association with undernutrition. We investigated the adherence of two strains of ST to enterocytes from ad-libitum fed and nutrient restricted rats in order to study the effects of nutrient restriction on the adherence of ST to enterocytes. An animal model of malnutrition was produced by feeding young male adult rats 50% of the daily intake in control ad-libitum fed rats. Enterocytes were isolated from proximal and distal small intestinal segments by agitation in isolation medium. Adherence was studied using fimbriated (S7471F) and non-fimbriated (S7471N) strains of ST labelled with 3H-adenine. Labelled bacteria were incubated with freshly isolated enterocytes for 30 min. at 37 C. and the free bacteria were separated from those bound to enterocytes by membrane filtration and washing. The bound bacteria was quantitated by liquid scintillation. Adherence was significantly higher with S7471F both with proximal and distal intestinal enterocytes. There was no significant difference between the malnourished and control rats with respect to degree of adherence despite significant differences in body and organ weights and serum albumin. Prolongation of the period of nutrient restriction (to 12 wks) did not produce any change in pattern of adherence. We conclude that the adherence patterns of these strains of ST to enterocytes are not significantly altered by malnutrition.

605 DIARRHEA INDUCED BY HYPERTONIC MANNITOL AFFECTS CARBOHYDRATE HYDROLYSIS BUT NOT TRANSPORT. Maria Palacios, Hugo Madariaga, Leo Heitlinger, Ping-Cheung Lee, Emanuel Lebenthal. Children's Hospital of Buffalo, International Institute and State University of New York, Department of Pediatrics, Buffalo.

In diarrheal diseases with accompanying carbohydrate malabsorption, the unabsorbed carbohydrates and products of bacterial fermentation results in osmotic diarrhea and alterations in transit time. It is unclear whether the loss of this nutrient is mediated by alterations in mucosal hydrolytic enzymes and/or the transport carriers. Adult rats were force-fed by hyperosmotic solution of 20% Mannitol (1300 Mosm.) at a dose of 5 ml/100g. for seven days; a similar group of control rats were treated similarly with plain water. After an overnight fast, the animals were anesthesized with Innovar (2.0cc/Kg.). All carbohydrates were infused by intraduodenal bolus at a dose of 0.5g/Kg. Portal venous blood glucose was assayed at intervals (0,30,60,90, and 120 min.). The areas under the glucose curves were used to compare groups; in the mannitol group the glucose absorption was diminished by 9%, maltose by 20% and glucose polymer (D.E. 24) by 12%, when they were compared with the control group. These data suggest that when the intestinal mucosa is exposed to hyperosmolar loads, digestion of carbohydrate is suppressed more than is its absorption. This correlates well with the decreased disaccharidase activity found in the animals treated with mannitol. The rise in portal venous blood in both groups was blocked by phlorizin (10^{-2}M) administration which demonstrates that the transport of glucose as measured is carrier mediated and not related to alterations in mucosal permeability in the mannitol group.

●606 COLONIC INTESTINAL ANAPHYLAXIS: IN VIVO AND IN VITRO TRANSPORT STUDIES. Mark K. Patrick, David Forbes, Mary H. Perdue, Andre Buret, D. Grant Gall. Intestinal Disease Research Unit, Univ. of Calgary, Alberta, Canada.

Hypersensitivity reactions to ingested proteins occur in different parts of the intestine, including the colon. Using an animal model of intestinal anaphylaxis we examined the effect of IgE-mediated reactions on colonic function. Electrolyte transport was assessed in the proximal colon by in vivo marker perfusion and in vitro in short-circuited chambers, before (Ag-F) and after antigen (Ag) challenge. Rats sensitized (S) to egg albumin were compared to sham-treated controls (C). In vivo: Net fluxes of H_2O, Na^+, K^+ and Cl^- were similar in S (n=8) and C (n=6) during the Ag-F period. Antigen challenge resulted in a significant ($p<0.05$) reduction in H_2O, Na^+, Cl^- absorption in S, but not C. In vitro: Ag challenge of stripped proximal colon from S produced a significant ($p<0.05$) rise in Isc (Ag-F, 56±9 µA vs Ag, 77±11) whereas tissue from C (47±8 vs 48±7) was unaffected. Na^+ and Cl^- flux studies demonstrated that the increase in Isc following antigen exposure in S was due to stimulation of Cl^- secretion (Jnet) secondary to an increase in Jsm Cl^- flux (Table, µ Eq $cm^{-2}h^{-1}$, *$p<0.05$).

			Jms	Jsm	Jnet
C	(5)	Ag-F	16.2±6.9	20.7±1.0	-2.4±8.4
		Ag	19.4±5.0	20.9±1.4	-1.5±6.2
S	(7)	Ag-F	17.1±5.9	16.7±2.3	0.4±6.2
		Ag	16.6±5.1	20.3±2.5	-3.8±4.9

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No changes in Na^+ fluxes were observed following Ag challenge. Conclusion: Food protein initiated IgE reactions in the proximal colon leads to abnormalities of H_2O and electrolyte transport which are secondary to Ag stimulated Cl^- secretion.

607 DIAGNOSIS AND TREATMENT OF CHRONIC ACTIVE HEPATITIS (CAH) IN PEDIATRIC PATIENTS-NEED FOR PROLONGED THERAPY. E Pehlivanoglu, JH Vargas, WE Berquist, and ME Ament. Department of Pediatrics, University of California, Los Angeles, CA.

13 children with CAH have been diagnosed by histopathologic, laboratory, and clinical criteria and managed at UCLA during the past 13 years. Purpose of the study is to show that treatment may be required indefinitely and growth may be normal if long term corticosteroid therapy is kept at low dosage with concurrent use of azathioprine. Mean age at diagnosis was 11 yrs 3 mos (range 3 yrs 8 mos-17 yrs). Mean duration of illness at time of diagnosis was 10 mos (range 2-22 mos). 2 of the pts presented as endstage liver disease and 2 were HBsAg positive. 25% had elevated titers of anti-MA, 33% were anti-SMA positive, 50% had elevated ANA titers. All cases were treated with prednisone (1-2 mg/kg/day) until remission was established, and tapered to a maintenance level of 0.15-1 mg/kg/day. Corticosteroids were decreased when azathioprine (1-2 mg/kg/day)was introduced after 2-6 weeks of therapy. The mean time of treatment for 12 pts was 6 yrs, 5 mos (range 22 mos-10 yrs, 1 mo). 1 pt died in the second month of treatment. Biochemical and clinical recurrences were observed in all cases at the first attempt to taper the prednisone. Biochemical and clinical remission was obtained with medical treatment in 12, but 9 cases relapsed when corticosteroids were tapered. 3 pts are in complete remission and are off treatment. 2 of 12 had growth failure. 10 had heights between the 25th-95th percentiles at last visit. HBsAg negative CAH pts need prolonged treatment. Early azathioprine treatment in addition to low-dose corticosteroids can reduce the inhibition of growth of children.