606 CHARACTERIZATION OF THE COLLAGEN TYPES IN NORMAL AND INFLAMMED HUMAN INTESTINE. M. F. Graham, C.O. Elson, R. F. Diegelmann, R. Gay, S. Gay (Spon. by H. Maurer) MCV/VCU, Dept. Pediatrics, Children's Medical Center, Medicine, and Surgery, Richmond, Va., U. of Alabama, Dept. Medicine, Birmingham.

We have studied the connective tissue changes in the bowel wall of patients with Crohn's disease. Surgically resected specimens from normal margins and inflammed areas were compared. The various collagen types were identified by specific purification schemes followed by slab gel electrophoresis, immunoblotting, and by specific staining with antibodies directed against collagen types IV & V. Results. Histological sections from inflammed areas demonstrated expansion of the muscularis propria and the muscularis mucosa, and vascular proliferation in the submucosa. Collagen had accumulated in the latter two areas. Collagen isolated from inflammed bowel and separated by electrophoresis contained in addition to the type I found in normal margins, bands that comigrated with a type V standard. The presence of type V was confirmed by immunoblotting. Immunohistochemistry of sections from inflammed areas revealed marked accumulation of types IV and V in the submucosa. Conclusion. In Crohn's disease, chronic inflammation of the intestine leads to the accumulation of abnormal amounts of collagen types IV & V. As these collagen types are synthesized by smooth muscle cells, we postulate that these cells may play a role in the stricture formation characteristic of Crohn's disease. (Supported by a grant from the National Foundation for Ileitis and Colitis.)

609 ENTERIC PROTEIN LOSS, MEASURED BY FECAL ALPHA-1-ANTITRYPSIN (AT) CLEARANCE, IN THE ASSESSMENT OF CROHN'S DISEASE ACTIVITY. A.M. Griffiths, S. Soldin, G. Hewitt & R. Hamilton, Depts. Pediatrics & Clin. Biochemistry, Hosp. Sick Children & University of Toronto, Toronto, Ontario.

In 63 patients with Crohn's disease, less than 19 yr. of age, we compared conventional measures of disease activity against 24 hr. fecal alpha-1-AT clearance. Clearance was significantly increased in patients with abnormal ESR (>15 mM/hr) but not in those with abnormal Crohn's disease activity index, CDAI, (>150) or increased platelet count (>400,000). As expected, in those with diminished serum albumin levels (<35 g/L), fecal alpha-1-AT clearance was significantly increased.

Fecal alpha-1-AT clearance - ml/24 hr (normal >22)

	CDAI		Plat. count (10^3/mm^3)		ESR-mM/hr)		Albumin (g/L)	
	≤150	>150	≤400	>400	≤15	>15	≤35	35
m	79	111	64	87	47	107	124	57
SD	80	78	70	68	46	93	94	71
p	NS		NS		.005		.005	

Further analyses did not demonstrate significant correlations between alpha-1-AT clearance and CDAI (r=0.09), platelet count (r=0.13), or ESR (r=0.17). We conclude that severity of enteric protein loss is not closely related to degree of abnormality of commonly used measures of disease activity in young patients with Crohn's disease. However, since available indices of Crohn's activity are of questionable validity, and since an objective index of activity is badly needed for therapeutic trials, further evaluations of fecal alpha-1-AT clearance are warranted in this disease.

●607 THE EFFECTS OF MATERNAL ULTRAVIOLET-B IRRADIATION ON THE VITAMIN D CONTENT OF HUMAN MILK. Frank R. Greer, Bruce W. Hollis and Reginald C. Tsang. Univ. of Wisconsin, Madison, WI, Case Western Reserve Univ., Cleveland, OH Univ. of Cincinnati, Cincinnati, OH.

We studied the effects of a known amount of total-body ultraviolet-B light irradiation (1.5 M.E.D. equivalent to 30' of sunshine at noon on a clear summer day in fair-skinned women) on both serum and breast milk concentrations of vit D_3, vit D_2, 25-OH vit D_3, 25-OH vit D_2 and 1,25(OH)$_2$ vit D in 5 lactating Caucasian women. By 48 hrs post UV-B exposure, serum vit D_3 increased from 1.0±0.2 (SEM) ng/ml to 22.9±5.4 ng/ml ($p<0.01$, paired t test), remaining increased up to 14 days (2.30±0.12 ng/ml, $p<0.01$). Simultaneously, breast milk vit D_3 rose from 6 to 71 IU/L ($p<0.02$, range 34-148 IU/L) within 48 hr. of irradiation, remaining elevated up to 14 days (12 IU/L, $p<0.05$). Breast milk vit D_3 correlated with serum vit D_3 (r=0.91). 48 hrs post irradiation serum 25-OH vit D_3 increased from 13.9±2.7 to 17.6±3.3 ng/ml ($p<0.05$), peaking at 7 days (20.5±3.0 ng/ml, $p<0.02$) and remaining elevated at 14 days (20.3±3.2 ng/ml, $p<0.05$). There was no change in 25-OH vit D_3 in breast milk post irradiation. There was no change in serum or milk vit D_2, 25-OH vit D_2, or 1,25(OH)$_2$ vit D following irradiation. There was no correlation between breast milk fat (mean 3.4±2.4 [SD] g/dl) and milk vit D_3 or its metabolites. We conclude that maternal UVB irradiation (and thus sunshine exposure) significantly increases vit D_3 in human milk. Despite a rise in serum 25-OH vit D_3, vit D_3 is the only vitamin form which is readily transferred into human milk from maternal serum following maternal irradiation.

610 EVALUATION OF IgG4 IN CHRONIC DIARRHEA IN INFANTS. Bruce B. Grill, Hong D. Bui, and Raj Juahar (Spon. by James J. Quilligan), Loma Linda University School of Medicine, Department of Pediatrics, Loma Linda, CA

To evaluate the role of IgG4 in infants with gastrointestinal (GI) disease, we measured serum IgG4, total IgG, and IgA in seven patients with milk allergic enterocolitis (MAE), confirmed by milk challenge after stabilization on milk restriction, 10 patients with nonallergic chronic diarrhea ≤ 24 months old (ND), and randomly chosen normoalbuminemic control infants without GI disease. IgG4 was measured using a monoclonal anti-IgG4 radioimmunoassay. Lower limit of sensitivity was 0.01 µg/ml. IgG and IgA were measured by nephelometry. The ratio of IgG4 to total IgG was calculated. Differences between groups were evaluated by the Wilcoxon rank sum test.

Group	N	Mean Age (Months)	IgG4(µg/ml) (X̄ ± S.E.M.)	IgG4/IgG(%) (X̄ ± S.E.M.)
MAE	7	6.4	5.8±2.0*	0.10±0.05+
ND	10	12.9	14.4±2.3	0.30±0.08
Control	11	8.5	56.9±7.4*	0.48±0.10+

*p<0.01 +p<0.05

No patient was IgA deficient. Three patients with ND and IgG4 ≤ 10 µg/ml had enteric pathogens (1 Giardia, 2 Salmonella). We conclude that in infants with chronic diarrhea, low IgG4 and IgG4/IgG are associated with allergic enterocolitis and enteric pathogens. We speculate that IgG4 may function as a GI mucosal protective antibody, and that its measurement may be useful in predicting allergic or infectious causes of chronic diarrhea.

608 CHANGES IN FAT CONCENTRATION OF HUMAN MILK WITH CONTINUOUS MECHANICAL PUMP INFUSION VERSUS INTERMITTENT BOLUS DELIVERY. Frank R. Greer, Ann McCormick, Jeff Loker. Univ. of Wisconsin, Madison, WI (Spon. by R.W. Chesney)

We studied changes in fat conc. and fat loss in human milk during 8 hr continuous infusion with 2 mechanical pumps (Harvard syringe [HARV] and Holter roller [HOLT] with Buretrol) and for comparison intermittent bolus delivery. We varied rate of pump infusion (1,4 or 7 ml/hr), state of homogenization and syringe position (HARV, tip vert or horiz). For each set of variables, infusions were repeated x4. Hourly samples were analyzed for fat. Infusion apparatus held only enough milk for an 8 hr. infusion, milk in the tubing being recovered by air infusion at the same rate. At 1 ml/hr with HARV and HOLT, 26-39% of fat was lost regardless of conditions. Fat conc. during infusion decreased to 32% of baseline for HARV and to 2% for HOLT, regardless of syringe position (HARV) or homogenization. During the 8th hr of infusion a large fat bolus was delivered (240±74% HARV & 435±131% HOLT > baseline fat conc). At 4 and 7 ml/hr fat losses for HARV and HOLT were 1-23% & 5-31% respectively and fat conc. decreased to 30-70% of baseline for HARV and 5-40% for HOLT. A large fat bolus was delivered during the 8th hr of infusion (144-333% HARV, 277-567% HOLT > baseline fat conc). Homogenization made no difference. For HARV changes in fat conc. could be decreased 12-22% by keeping syringe tip vert. With intermittent bolus delivery of milk (1,4,7 ml) no loss of fat, change in fat conc., or fat bolus occurred. Thus, for NG delivery of human milk to LBW infants, intermittent bolus is preferred. Continuous NG pump infusion should not be used because of large changes in fat conc. and fat loss.

611 BONE MINERALIZATION IN PRETERM INFANTS FED HUMAN MILK Steven J. Gross. Boston University School of Medicine Boston City Hospital, Dept. of Pediatrics, Boston.

Bone mineral status was assessed in 20 healthy, appropriately grown infants <1600g birthweight assigned randomly to one of 2 diets: 1. Preterm human milk (PT milk) pooled by postpartum week and fed corresponding to infants postnatal age, providing calcium 22-26 mg/dl, phosphorus 13-17 mg/dl. 2. Supplemented PT milk, an equal mixture of premature infant formula and PT milk providing calcium 72-74 mg/dl, phosphorus 37-38 mg/dl. All infants were fed by gavage at 180 ml/kg/day and received 400 IU Vitamin D daily. Infants fed PT milk had significantly lower serum phosphorus levels at 4 and 5 wk (4.6±0.4 and 4.1±0.4 mg/dl) than infants fed supplemented PT milk (6.1±0.2 and 6.0±0.2 mg/dl), $p<.005$. They also had significantly higher serum alkaline phosphatase levels at 4 and 5 wk (569±81 and 824±127 IU/l) than the supplemented group (340±40 and 347±49 IU/l), $p<.02$. The serum differences were reflected in lower bone mineral content (BMC) of humerus by photon densitometry at 5 wk (PT milk, .084±.005 vs supplemented PT milk .107±.007 g/cm, $p<.05$). At 35-37 postconceptual wk, infants in both groups were discharged on standard infant formula (calcium 51 mg/dl, phosphorus 39 mg/dl). At 44 postconceptual weeks, infants initially fed either PT milk or supplemented PT milk, had similar serum phosphorus (6.7±0.2 and 7.2±0.2 mg/dl) and alkaline phosphatase (301±33 and 285±23 IU/l; BMC in both groups (.252±.021 and .246±.014 g/cm) was comparable to that of healthy term infants (.223±.006). Preterm infants fed unsupplemented PT milk show bone demineralization. BMC increases significantly after routine formula feeding irrespective of PT milk supplementation.