Gastroduodenal F197–F207

F197

H PYLORI INFECTION AND DYSPEPSIA WITHIN THE GENERAL POPULATION.
E. El-Omar, S. Banerjee, A. Wiz, K.E.L. McColl. University Department of Medicine and Therapeutics, Western Infirmary, Glasgow, Scotland.

Dyspeptic symptoms are common in the general population. 30–40% of the population experience some dyspepsia but most never seek medical advice. In order to determine the possible role of H pylori infection in this, we have prospectively studied a random group of healthy subjects who had never consulted the medical profession on account of dyspepsia.

Subjects and Methods: Eighty subjects were selected randomly from the general population. They were interviewed by one investigator who scored their dyspeptic symptoms using the Glasgow Dyspepsia Severity Score which had been developed and validated in this Unit. It measured dyspeptic symptoms experienced over the preceding six month period. Following this, all subjects had a C-urea breath test to determine their H pylori status. In those found to be H pylori-negative, the investigators measured gastric acid secretion, using a recently developed radiolabelled peptide (40pmol/kg/h) measured the investigator performing the acid secretory test was blind to the H pylori status of the subjects.

Results: Of the 80 subjects, 52 were found to be H pylori negative (36% females, mean age = 31; range: 20-52) and 28 were H pylori positive (37% females, mean age = 33; range: 19-49). The mean dyspepsia score in the H pylori negative group was 1.2 (range: 0-7) and in the H pylori positive group 2.5 (range: 0-8) (p<0.05). Sixty-three percent of those H pylori negative had experienced no dyspepsia over the preceding six months (dyspepsia score = 0) whereas the corresponding figure for those H pylori positive was only 72%. The median acid output in the H pylori negative group was 7.7mmol/h (range: 5.1-18.8) and in the H pylori positive group 16.9mmol/h (range: 1.8-37.9) (p<0.005). Linear regression analysis showed that acid output was positively correlated with dyspeptic score within the H pylori positive subject group (r=0.43, p<0.01).

Conclusions: (1) The prevalence of dyspeptic symptoms in the general population with H pylori infection is more than twice that in H pylori negative subjects. (2) The severity of dyspeptic symptoms in those with the infection is positively correlated with the degree of H pylori induced acid hypersecretion.

F199

CHANGES IN THE EXPRESSION OF SOMATOSTATIN AND GASTRIN mRNA IN RESPONSE TO GASTRIN RELEASING PEPTIDE ARE ALTERED BY H.PYLORI INFECTION.
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Introduction: The increased gastric acid secretion seen in H pylori (Hp) infection probably occurs because the inhibitory feedback mechanism involving somatostatin (SST) is defective. Gastrin releasing peptide (GRP), an SST receptor agonist, stimulates pancreozymin, a protein which induces acid hypersecretion in Hp positive patients. Therefore we compared the changes in expression of gastric SST and gastrin mRNA seen with GRP infusion in Hp+ and Hp- patients.

Methods: Dyspeptic non-ulcer patients, 9 Hp+ and 8 Hp-, received a 3 hour intravenous infusion of GRP (14-27), 200 pmol/kg/h or vehicle alone on separate occasions. At the end of each infusion endoscopy was performed and biopsies taken from the gastric corpus and antrum for RNA extraction. SST and gastrin mRNA levels were determined on blots of total RNA using [3H]-labelled cDNA probes for human SST and gastrin and the signals quantified using phosphor imaging. To correct for RNA loading and transfer variations, GAPDH mRNA levels were measured.

Results: Infusion of GRP produced a significant rise in antral gastrin mRNA in the Hp+ patients, but a significant fall in the Hp- group. Plasma gastrin rose in the Hp+ group but not appreciably in the Hp- patients. GRP stimulated a significant rise in corpus SST mRNA in the Hp+ group, but not in the Hp- group. SST mRNA showed a similar trend but the rise in the infected group was not statistically significant.

F198


Introduction: Reactive oxygen species (ROS), many of which are free radicals, are increased in H pylori gastritis (HPG). ROS have been implicated in carcinogenesis, and HP may predispose to gastric cancer through ROS-mediated mechanisms. Ascorbic acid may protect against gastric cancer by scavenging free radicals, producing the AR in the process. We have used electron spin resonance (ESR) to quantify the AR in gastric mucosa and have related this to histology and ROS activity as assessed by luminol-enhanced chemiluminescence (CL).

Methods: 70 patients were recruited from those undergoing endoscopy for dyspepsia. Biopsies were taken for measurement of ESR, CL and for histology.

Results: Medians and interquartile ranges

<table>
<thead>
<tr>
<th>AR (signal strength/μg)</th>
<th>Normal (n=21)</th>
<th>HPG (n=39)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15</td>
<td>0.27</td>
<td>&lt;0.01</td>
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<tr>
<td>0.31 - 0.62</td>
<td>0.16 - 0.45</td>
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<tr>
<td>CL (cpm/μg)</td>
<td>1249 (433-8626)</td>
<td>16681 (3697-47864)</td>
<td>&lt;0.05</td>
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* Mann-Whitney Test

Discussion: We have demonstrated the AR in gastric antral mucosa for the first time, and find it is significantly higher in HPG compared with normal histology, and to correlate with CL (R=0.25, P=0.01). These results suggest that ascorbate does have a free radical scavenging role in gastric mucosa, and may protect against gastric cancer in HPG in this way.

F200

RECURRANCE OF DUODENAL ULCER AFTER HELICOBACTER PYLORI ERADICATION IS RELATED TO HIGH GASTRIC ACID OUTPUT.
AW Harris. PA Guccione, PS Philly, MR Jacyna, JJ Misiewicz, JH Baron. Parkside Helicobacter Study Group, Central Middlesex and St Mary’s Hospitals, London and *Gastroenterology Unit, Northwick Park Hospital, Harrow, England.

Eradication of H pylori (Hp) reduces recurrence of duodenal ulcer (DU) to <2% per annum. It is not clear why DU recurs rarely in the absence of Hp reactivation or NSAID. Baso (BASO), gastrin releasing peptide (GRP) and pentagastrin (PG) stimulated peak acid outputs (PAO24, -PAO1, PAO20) are increased in Hp+ DU, and return to the range of Hp-controls after eradication. Does acid output in patients with recurrent DU after Hp eradication also return to the control values? We studied 50 men (mean age 40, range 28-58) with symptoms of endoscopic recurrence of DU+ six months (mean 18 months) after eradication, and compared them with 10 Hp- controls with normal endoscopy (4 men, mean age 33, range 24-40), and with Hp+ non-recurrent DU before (n=8, 7 men, mean age 37, range 22-58) and six months after eradication.

None had taken NSAID. Hp status was determined by antral and body histology and culture and by the C-urea breath test, and classified as Hp+ on any acid -ve and Hp- on all three tests -ve. After an overnight fast, a NG tube was passed, the stomach emptied, and one 30 min basal aspirate collected. GRP (40 pmol/kg/h) was then infused for 45 min. After a 30 min washout, Pg (6μg/kg) was injected i.m. Three 15 min aspirates were collected after each stimulus.

Peak outputs were expressed as mmol/l and normalised to 70kg body weight. BASO, PAO20, and PAO20 were significantly (p<0.05, Mann Whitney test) higher in Hp+ DU than Hp+ controls, with median (range) BASO 7 (2-17), 2 (3-6), PAO20,20 (3.6-64) vs 10 (1-25) and 10 (1-25) and 25 (12-40). Six months after eradication, median (range) BASO, PAO20 in DU were 3 (1-11), 14 (1-45) and 29 (6-60), respectively; all significantly (p<0.05, Wilcoxon signed rank test) lower than before eradication and within the range of controls. In Hp- recurrent DU median (range) BASO, PAO20 and PAO20 were 5 (0-15), 17 (2-24) and 33 (27-49), respectively; PAO20 was significantly (p<0.05) higher than Hp+ controls and within the Hp- DU range.

In DU recurrent after Hp eradication PAO20 remains within the Hp- DU range. By contrast in non-recurrent DU PAO20 fell to the control range after Hp eradication. Our findings suggest that there may be a subset of DU who retain an abnormally high response to pentagastrin.

AWH is supported by a grant from Lederle Laboratories, UK.
PATHOGENESIS OF GASTRIC METAPLASIA IN DUODENAL ULCER DISEASE.

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Introduction: Duodenal ulcers (DU) are associated with gastric metaplasia (GM) of the duodenum. The pathogenesis of GM is unclear. We aimed to determine the roles of acid, H. pylori infection and of ulceration, by assessing the effect of each singly and in combination.

Methods: In a double blind placebo controlled trial 97 consecutive subjects with H. pylori positive DU were randomised to: amoxicillin, metronidazole and colloidal bismuth for two weeks plus omeprazole for six months (AMBO, n=32); or amoxicillin, metronidazole and colloidal bismuth for two weeks plus omeprazole placebo for six months (AMBP, n=12); or two weeks of antibiotic placebo plus omeprazole for six months (PO, n=33). Three duodenal bulb biopsies were obtained per patient before and after six months treatment. Biopsy sections were stained with the haematoxylin and eosin stain of GM in each biopsy section was determined as a % of the entire sections epithelial lining. The severity of duodenal inflammation was assessed in the same sections. Gastric antral biopsies were also assessed for the severity of inflammation. An ulcer at follow-up or incomplete compliance with treatment resulted in the exclusion of 8 and 13 subjects respectively.

Results: AMBO produced an 85% H. pylori eradication rate. AMBP produced a rate of only 63%. A 4% eradication rate was obtained with PO. Ulcer healing alone without H. pylori eradication and in the absence of acid suppression produced no change in GM or in the severity of duodenal or gastric inflammation. Ulcer healing together with H. pylori eradication produced a 42% reduction in GM (p<0.002). Eradication of H. pylori also resulted in reduction of antral and duodenal inflammation. Ulcer healing alone resulted in a reduction in GM by 43% (p<0.002), but without significant change in antral or duodenal inflammation, indicating that acid has an effect on the formation of GM which is independent of ulceration. Ulcer healing plus a combination of H. pylori eradication and acid suppression produced an additive effect with 66% reduction in gastric metaplasia (p<0.002).

Conclusion: The extent of GM in the duodenal bulb of DU subjects is unrelated to presence or absence of ulceration but is in part due H. pylori and in part due to acid.

VALIDATION OF A RAPID WHOLE BLOOD TEST FOR THE DIAGNOSIS OF HELICOBACTER PYLORI INFECTION


Introduction: The non-invasive methods of diagnosing H pylori infection include breath tests and serology. The results for both these methods can take several days to return. Recently a rapid whole blood test has become available (Helisaltm) which may be simple to perform and gives a diagnosis of H pylori infection within 10 minutes. The accuracy of this test needs independent validation before it can be recommended for use in routine clinical practice.

Methods: Patient’s H pylori status was evaluated by histology (2 antral and 2 corpus biopsies), culture (1 antral biopsy), rapid urease test (1 antral biopsy) and carbon urea breath test. The patient was defined as H pylori positive if two or more tests gave a positive result and negative if all tests gave a negative result. The H pylori status was considered indeterminate if only one test was positive.

Results: The results of the rapid blood test (RBT) were compared with the gold standard.

106 patients took part in the study (median age 47, range 21-71 years, 55 males). 51% of patients gave indeterminate results. Of the 54 gold standard H pylori positive patients the RBT gave 5 false negative results (sensitivity 91% CI 95% 80-97%). In 50 gold standard H pylori negative patients the RBT gave 3 false positive results (specificity 96% CI 95% 84-99%).

Conclusion: The RBT diagnoses H pylori infection with good accuracy. As this test gives a diagnosis within 10 minutes it should prove useful in clinical practice especially in primary care.

THE ONTOGENIC ROLE OF EGF IN THE DEVELOPING HUMAN STOMACH

EJ Kelly, SJ Newell & KG Brownlie (introduced by G Davie). Department of Paediatrics, St. James University Hospital, Leeds, UK.

Epidermal Growth Factor (EGF), a small polypeptide shown to facilitate growth of gastric and small intestinal mucosa, and is present in high concentrations in breast milk and liquor. The source of production in neonates is not known. EGF shares a receptor with Transforming Growth Factor alpha (TGF α) and both may play a role in the stomach. We have examined fetal and infant stomachs for the expression of EGF and TGF α, for the expression of their receptor Scpeomin were further examined for Progesterone Cell Nuclear Antigen (PCNA), which stains cells in G2 of the cell cycle as a measure of mitotic activity. Finally we wished to determine the concentrations of EGF in fetal urine and liquor to determine the site of EGF production.

Sections from 15 fetal and 5 infant stomachs were stained with antibodies to EGF, TGF α, EGF receptor and PCNA using standard immunohistochemical techniques. Liquor, fetal urine and cord blood samples were collected from 10 term infants born by caesarean section and analysed using a radioimmunoassay to determine EGF concentration.

In none of the stomachs was EGF immunoactivity detected. TGF α and EGF receptors were detected from 18 weeks gestation in mucous cells in the gastric glands. TGF α positive cells were located on the surface cells of the gastric mucosa whereas the EGF receptor positive cells were located towards the base of the gastric pits. PCNA immunoactivity was detected in all the stomachs, being located in cells that stained positive for the EGF receptor. Mean (Standard Deviation) concentrations of EGF were significantly higher in liquor (30 ±10 pg/ml) than fetal urine (16 ±6 pg/ml). We have confirmed the high concentration of EGF in liquor, and our data support the hypothesis that it originates from the anionic membranes rather than the fetal kidneys. The presence of the EGF receptor on PCNA positive cells located on the luminal surface of the stomach is consistent with an important oncogenic role for EGF in liquor swallowed by the fetus, and in breast milk in the newborn infant.
LACK OF A SIMPLE EXPLANATION FOR GASTRIC TUMOURS INDUCED BY H2 ANTAGONISTS IN RATS A Garner Department of Pharmacology, Faculty of Medicine, UAE University, Al Ain, UAE.

Gastric carcinoids appearing in rats after long-term administration of proton pump inhibitors at supratherapeutic doses represent a quasi-physiological response to prolonged achlorhydria. Interpretation of the mechanism(s) underlying pathological changes in the rodent stomach following chronic administration of potent, long-acting H2 antagonists is far more complex. Tiotidine is a competitive H2 antagonist which caused gastric adenocarcinoma in 17 of 828 rats after dosing for up to 2 yr. This compound is a structural chimera of cimetidine and famotidine and intermediate in potency between these two drugs, neither of which caused gastric tumours. An entirely different lesion (nodular hyperkeratosis) affecting squamous cell in the forestomach, which does not express gastrin receptors, occurred in rats receiving the non-competitive H2 antagonist SKF 92479. ICI 162846 which also displays non-competitive kinetics, is the most potent H2 receptor antagonist to be described (IC-50 3.9 kg/l i.v. in the rat). Like omeprazole, this compound caused neuroendocrine cell tumours but these occurred in mice as well as rats and did not appear to be directly related to hypergastrinaemia. Thus in groups of rats receiving ICI 162846 p.o. daily for 24 months, the incidence of carcinoid tumours increased with dose; 2 of 24 (20 mg/kg), 4 of 30 (100 mg/kg) and 8 of 38 (500 mg/kg). Mean serum gastrin also displayed a dose-related rise from 17 pmol/l in controls to 39 (20 mg/kg), 54 (100 mg/kg) and 79 (500 mg/kg) in treated rats. However there was no relationship between tumour incidence and gastrin levels amongst individual animals. Furthermore, since all three doses of ICI 162846 induced total achlorhydria, the dose-related rise in serum gastrin must have occurred by a mechanism other than simple elevation of intragastric pH. These data demonstrate that tumourogenic changes in the stomach after prolonged administration of very high doses of H2 antagonists affect cells other than gastrin-responsive neuroendocrine cells. In addition to the lack of correlation with hypergastrinaemia, pathological changes appear unrelated to chemical structure, the mechanism of receptor interaction, or the antiserotin potency of histamine H2 receptor antagonists.

ANTRAL DENERVATION BY BENZALKONIUM CHLORIDE LEADS TO GASTRIC RETENTION OF SOLIDS IN THE RAT. A. D. Higham, D. G. Thompson, G. J. Dockray.

Physiological Laboratory, University of Liverpool, and Department of Medicine, University of Manchester, Hope Hospital, Salford.

Background and Aim: The present studies were undertaken to develop an animal model of antral denervation in order to examine the role of the antral innervation in the control of gastric motility and secretion.

Methods: Antral denervation was induced by localisation of 0.5% (w/v) benzalkonium chloride (BAC); controls received vehicle (0.9% NaCl). After 9 or 23 days, rats were fasted for 48h, or fasted and refed for 30min; the dry weight of gastric content and plasma gastrin were determined, and the antrum was extracted for assay of the neuropeptides substance P (SP) and gastrin releasing peptide (GRP).

Results: The dry weight (g) of gastric content in fasted controls (11 d, 0.09 ± 0.02, n=7; 25 d, 0.21 ± 0.05, n=6) was significantly less than in BAC-treated rats (1.5 ± 0.4, n=5; 2.1 ± 0.7, n=5, respectively; p<0.05 for both). Fasting plasma gastrin (pM) was 8.5 ± 2.1 (day 11) and 7.7 ± 0.7 (day 25) in controls, and was significantly elevated in BAC-treated rats (32 ± 9.5, and 28.4 ± 7.4, respectively; p<0.05 for both). Antral GRP (pmol/g) was 19.6 ± 1.5 (day 11) and 21.8 ± 1.9 (day 25) in controls and was reduced to undetectable concentrations (p<0.005) after BAC-treatment. Similar results were found for SP. In both control and BAC-treated rats the dry weight of gastric contents was increased by refeding, and plasma gastrin rose 3.4-fold (controls, 26.8 ± 5.1; BAC-treated, 85.6 ± 23; p<0.05 for both).

Conclusion: 1. Antral denervation by BAC leads to gastric retention of solids in the rat. 2. The gastrin response to refeeding after BAC treatment persists in the absence of antral GRP-containing neurons. 3. Antral denervation by BAC may provide a model for study of the adaptive neuroendocrine responses of the gut to chronic gastric retention.

USE OF ECHO PLANAR IMAGING (EPI) TO ASSESS EFFECT OF POSTURE ON INTRAGASTRIC DISTRIBUTION OF LIPID AND GASTRIC EMPTYING

P实景, F Bowland, V Adams & R C Spiller. Dept. of Physics & Dept. of Medicine, University of Nottingham, Nottingham NG7 2UH

Previous scintigraphic studies have suggested that fat layers on the top of the squamous phase of mixed fat/water meals. Fat and water signals can be easily distinguished using the ultra fast MR imaging variant ‘EPI’. We have used EPI to assess the intragastric distribution of oil and water with subjects lying either right side down (RSD) or right side up (RSU). Gastric emptying was assessed by serially measuring gastric volumes for up to 90 minutes.

Methods: 8 healthy volunteers underwent 4 gastric emptying studies after consuming either test meals A or B in either RSD or RSU positions. The meals were 400 ml of beef consomme soup plus either 100 ml water (meal A), or 100 ml olive oil (meal B).

Results: Gastric emptying of meal A was marginally faster with the Right Side Down, time to 50% gastric emptying T(50) being 2744 versus 3344 Right Side Up, p<0.05. Meal B emptied significantly slower than A in the Right Side Down position. T(50) being 4332 min, p<0.05. By contrast after meal B in the RSD position, gastric emptying was markedly delayed and gastric volumes actually rose over the 90 min of study, p<0.01. After ingestion of meal B oil was clearly observed layering above the water, filling the duodenal cap in the RSD position and the fundus in the RSD position.

Conclusion: Gastric emptying of a low calorie meal is marginally faster when the pylorus is dependent. Adding fat inhibits gastric emptying, an effect which is much larger in the right side up position. We conclude that layering of fat influences gastric emptying which is strongly inhibited when fat fills the duodenum.

Small bowel F208–F216

OATS CEREAL IS NOT TOXIC IN COELIAC DISEASE


Depts. of Immunology, Histopathology* and Gastroenterology*, St. James’s Hospital and St. Vincent’s Hospital*, Dublin and Trinity College Dublin*, Ireland.

Coeliac disease is characterised by a small bowel lesion which occurs in response to the ingestion of dietary proteins contained in cereal grains. At present coeliac patients are instructed to exclude wheat, barley, rye and oats from their diet but definite toxicity has been proven only for the former two cereals while the toxicity of oats remains controversial. To address this issue, we challenged 9 coeliac patients in disease remission with 50g of oats daily for a 3 month period. The patients were evaluated regularly and laboratory, histological and immunological markers of coeliac disease activation were measured. All patients remained asymptomatic and laboratory indices were normal for the duration of the challenge period; no gross morphological damage was seen on routine histological examination of post-challenge duodenal biopsies. Furthermore, oats did not cause immunological activation since no rise in antibodies (alpha gliadin, endomysial) or in MHC class II staining of enterocytes was evident. This information suggests that oats cereal is neither toxic nor immunogenic in coeliac disease. The inclusion of oats in the coeliac diet provides a valuable source of fibre, vitamins and minerals. In addition, our understanding of the mechanisms of cereal toxicity underlying coeliac disease is furthered by these findings as oats does not appear to contain the putative toxic peptide sequence contained in the 3 other cereals.

Small bowel F208–F216
Differential binding of gluten peptides to HLA class II molecules (DQ2) correlates with in vitro and in vivo toxicity in coeliac disease.

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Coeliac disease is a gluten-sensitive enteropathy characterised by villous atrophy, crypt hyperplasia, and lymphocytic infiltration and is associated with the HLA-DQ2 allele. The condition is thought to be a cell-mediated, class II-restricted process with antigen(s) which occurs only in the presence of gluten. Immunogenetic studies suggest a tight HLA association between coeliac disease and HLA DQ2 (a*0501, b*0201). Recent work has demonstrated in vitro and in vivo organ and epitope toxicity of a novel oligopeptide (peptide a) corresponding to amino acids 31-49 of a- gliadin (Shidrawi, et al. Scand J Gastroenterol, in press, and Stertss, et al. Lancet 1994; 343:758). Peptide B (amino acids 202-220 of a-gliadin) derives from the same a sequence but begins one glutamine residue earlier. Peptide C (amino acids 3-21 of a-gliadin) has PSQQ and QQP tetra-peptide motifs and b reverse turns, both implicated in toxicity. Neither peptide B nor peptide C induced toxic histological changes.

 Aim & Methods: To compare the relative binding of these gluten-derived peptides to HLA class II molecules, we used lymphoblastic B-cell lines derived from the peripheral blood of a patient with coeliac disease homozygous for HLA DQ2 in a competitive inhibition assay using an avidin-antibody/avidin amplification technique to detect binding of a biotinylated indicator peptide (peptide MB) derived from a 65KDa Mycobacterium bovis heat-shock protein and shown to bind selectively to affinity-purified HLA DQ2 (Johnson, et al. Int Immunol 1994; 6:491).

 Results: A 20-fold signal/noise ratio was achieved in our flow cytometric assay using a final concentration of 250 mM of peptide MB. Significantly, inhibition of binding was observed with Peptide MB and Fraction III, a peptic-tryptic digest of gluten with known coeliac toxicity (76% inhibition at a 1:1 molar ratio), and peptide A (78% inhibition at a 1:1.5 molar ratio), but not with peptides B or C or ovalbumin over the same range of molar ratios.

 Conclusions: These findings support the hypothesis that peptide binding to HLA class II molecules (DQ2) and subsequent CD4+ T-cell activation is important in initiating the coeliac lesion and provide a new technique to dissect the molecular pathogenesis of coeliac disease.

In vivo activity of peptides 31-43, 44-55, 56-68 of a-gliadin in gluten sensitive enteropathy (GSE).

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Despite the use of various in vitro methods, few in-depth studies of the in vivo activity of short, synthetic oligopeptides of a-gliadin in relationship to GSE have been reported. This study concerned the intestinal response of 12-16 mers of a-gliadin spanning positions 31-68. METHODS: One peptide was synthesised by F-moc and adjacent >96% pure by HPLC and FAB-mass spectroscopy. Each peptide was infused directly into jejunum of treated GSE patients (two patients per peptide). Mucosal biopsies were analysed in terms of (i) CD3+ and CD8+ epithelial infiltrates (surface + crypt combined) (ii) mucosal morphology in terms of volumes of villus and crypt epithelium, per 10μm2 test area of muscularis mucosae (iii) lamina propria inflammation, as evidenced by CD3+ infiltrates, edema (as measured increases in volume) and production of PGE2/LTC4, and (iv) brush border membrane hydrolase (BBMH) levels to monitor enterocyte damage. Fithly, serum IL-1α and IL-2R levels were measured, by ELISA, in each challenge. Finally, the structure of each peptide was analyzed by physical methods (NMR; circular dichroism (CD) and computerized modeling). RESULTS: Peptides 31-43 and 44-55 were active in GSE based on rises in CD3+ and CD8+IEL; evidence of lamina propria swelling associated with lymphoid infiltration, generation of PGE2, and LTC4 and impairment of enterocyte viability as occurred by fall in BMMH activity. Architectural change resulted in infiltrates associated with crypts, but no major changes of surface epithelial volume. Transient rises in serum IL-1α and IL-2R were documented during challenge. Thus peptides 31-43 and 44-55 are active, while sequence 56-68 is clearly inactive. Both active peptides contain the amino motif P.S.I.P (NMR, and circular dichroism spectroscopy) failed to reveal any common conformation in either peptide studied, indicating that α-helix or 8 reverse turn is unnecessary for recognition. CONCLUSIONS: 1. Wide assay of mucose revealed that peptides 31-43, 44-55 of a-gliadin are "active" regarding GSE. 2. Each peptide contains the motif P.S.I.Q.QP. 3. Physical analysis revealed no conformational relationship to bio-activity.

Detection of endomyosal and reticulin antibodies in coeliac disease using a single substrate: human umbilical cord.


Introduction Anti-endomyosal antibody (EmA) is an effective serological marker for coeliac disease, though its use has been limited by the high cost of kits which require sections of primate oesophagus (PO). A recent report has suggested the alternative use of human umbilical cord. EmA was detected in the smooth muscle of the umbilical arteries. We performed experiments to assess the efficiency of this new substrate, and to characterise the antibody that is detected.

Methods Cross-stated sections (5μm) of PO and UC were made and examined by indirect immunofluorescence, using FITC-labelled anti-human IgA as the secondary antibody. Sera were tested, blind, from patients with untreated coeliac disease (n=20) and controls (n=13). Positive sera produced fluorescence of connective tissue surrounding smooth muscle cells, but in UC we also noticed antibody binding to reticular stromal connective tissue unassociated with muscular elements, i.e. human anti-reticulin antibody (ARA). Quenching experiments were performed by adsorption of a positive serum with homogenised UC, primate intestine, or mouse liver. Rodent anti-reticulin antibody was assayed before and after adsorption with mouse liver, to confirm the effectiveness of the process in quenching rodent ARA. Anti-gliadin antibodies, measured pre- and post-adsorption, were used to assess dilutional effects.

Results Sensitivity of the assay using PO was 90% (18/20), and using UC was 95% (19/20). Both assays had a specificity of 100%. Adsorption with mouse liver did not affect antibody detection on PO, UC endomyosal, or UC reticulin, but did result in loss of rodent antireticulin antibody. Adsorption with either primate intestine or UC caused loss of antibody detection by PO, UC endomyosal, and UC reticulin.

Conclusion Our findings support the proposal that UC is a suitable and readily available substitute for the section of EmA in coeliac disease. Additionally, we have found that this substrate detects antibody on both vascular (endomyosal) and stromal (reticulin) connective tissue, and that these antibodies have similar binding specificities.

The small intestine in microscopic colitis: mucosal permeability and secretory immunity to gliadin.


Background: There is a recognised association between microscopic colitis and coeliac disease. There are a variety of subtle small intestinal changes in patients with "latent" gluten sensitivity, namely, high jejunal epithelial lymphocyte (IEL) counts, abnormal mucosal permeability, and high levels of secretory IgA and IgM antibody to gliadin. These changes have hitherto not been investigated in microscopic colitis.

Patients: We investigated 9 patients with microscopic colitis (4 collagenous colitis, 5 lymphocytic colitis). These patients had normal villous architecture.

Methods: Small intestinal biopsies were obtained by Crosby capsule; small intestinal fluid was aspirated via the capsule. IEL counts were expressed per 100 epithelial cells and intestinal IGA and IgM anti-gliadin antibody levels were measured by ELISA. Small intestinal permeability was measured by the lactulose/mannitol differential sugar permeability test.

Results: There were 7 females and 2 males; median age 52 (22-76). IEL counts were normal in all cases: median 17 (7-30). Intestinal anti-gliadin antibodies were measured in 8 cases and were abnormal in 2 and borderline in one. These abnormalities did not overlap; 4 of the 9 patients had evidence of abnormal small intestinal function.

Conclusion: Subclinical small intestinal disease is common in microscopic colitis.
T-CELL ACTIVATION IN THE SMALL INTESTINAL MUCOSA OF AFRICAN AIDS PATIENTS WITH CHRONIC DIARRHOEA. A.M. Yatco, M.F. Kelly, N. Luo, T.M. Pobe, L.S. Penney, and M.I.G. Farthing. 1Digestive Diseases Research Centre, and 2Department of Paediatric Gastroenterology, Medical College of St. Bartholomew’s Hospital, London, and 3University of Zambia, Lusaka.

T cell activation has been implicated as a cause of villous atrophy. To examine its role in the enteropathy of AIDS and cryptosporidiosis we studied 20 Zambian patients with AIDS and chronic diarrhea, 10 of which with intestinal cryptosporidiosis and 10 without intestinal infection. 9 patients without AIDS were studied as controls.

Distal duodenal villous height and crypt depth was measured by computerised image analysis of parafin-processed biopsies. Snap-frozen duodenal biopsies were cryostat-cut and subjected to double immunofluorescence staining for CD4/CD3, CD8/CD3, CD25/CD3, CD69/CD3, HLA-DR/CD3, and H-1/CD3, allowing a quantitative assessment of T cell expression of the above antigens.

Mean percentage CD4/CD3 was lower in the AIDS patients compared with controls (11.4 vs 48.6, p<0.001). Cryptosporidiosis was not more prevalent in those with the lowest mucosal CD4 counts. Mean percentage H-1/CD3 was greater in the AIDS patients compared to controls (89.1 vs 81.8, p=0.049). There was no difference in H-1/CD3 expression between the cryptosporidiosis and pathogen-negative AIDS patients, and no difference in expression of CD25, CD69 or HLA-DR between any of the study groups. T cell expression of the above antigens was not related to villous height or crypt depth.

H-1 expression, reflecting activation of T cells of intestinal origin, was increased in AIDS patients. This suggests that T cell activation may be involved in the pathogenesis of HIV-induced enteropathy.

PERIPHERAL CELL MEDIATED IMMUNE RESPONSE TO MYCOBACTERIA IN CROHN’S DISEASE AND CONTROLS. D.S. Rowbotham, L.K. Trejosdiewicz, P. Quirke*, P.D. Howdle. Academic Unit of Medicine, St. James’s University Hospital and Academic Unit of Pathological Sciences*, The General Infirmary at Leeds, The University of Leeds, LS2 9LT.

The aetiology of Crohn’s disease remains unclear. Recent attention has focused on mycobacteria, and in particular Mycobacterium paratuberculosis, as having a potential aetiological role in this disease. However culture, serological and DNA amplification techniques have produced conflicting results. We have investigated peripheral blood lymphocyte (PBL) cell-mediated immune responses towards mycobacterial antigens in vitro.

PBL isolated from 13 patients with histologically-confirmed Crohn’s disease, 17 with histologically-confirmed ulcerative colitis (UC) and 17 non-inflammatory bowel disease controls (NIBD) were cultured for 8 days with mycobacterial antigens prepared as purified protein derivatives of M. tuberculosis (Tuberculin) and M. paratuberculosis (Johnin). PBL responses were evaluated by flow cytometry to assess lymphocyte activation (expression of CD69 and CD25) and by HML-1/CD3 incorporation to assess cellular proliferation (stimulation indices).

Results showed that PBL activation and proliferation were closely related. Subjects in all three patient groups showed wide variation in PBL stimulation indices with both Johnin and Tuberculin. There was no evidence of increased antimycobacterial (Tuberculin) cell mediated immunity in patients with Crohn’s disease when compared to patients with UC and NIBD controls. Nor was there any evidence of increased specific cell mediated immunity towards Johnin in patients with Crohn’s disease compared to UC and NIBD controls, or when compared to Crohn’s PBL responses towards Tuberculin. Indeed, in all three patient groups, there was very close correlation between PBL responses towards PPD of different mycobacterial species (r²=0.91).

These results do not support an aetiological role for mycobacteria or M. paratuberculosis in Crohn’s disease.
Infection and inflammation F217–F228

F217

ANTI-CRYPTOSPORIDIUM PARVUM ANTIBODY RESPONSE IN ZAMBIAN PATIENTS WITH HIV-RELATED DIARRHOEA. AM Cevallos, P Kelly, B Ngwenya, N Loo, JOM Pobe, MG Farthing. Digestive Diseases Research Centre, Medical College of St. Bartholomew's Hospital, London ECI 6Q0.

In immunodeficient patients particularly those with AIDS, C. parvum may cause chronic and life-threatening diarrhoea. We investigated the prevalence of cryptosporidiosis in 71 Zambian patients with HIV infection and chronic diarrhoea (mean duration 9 months) and determined the value of serum antibody responses in the diagnosis of active infection. In all cases, the diagnosis of cryptosporidiosis was established or excluded by examination of small bowel biopsies (light and electron microscopy and PCR amplification) and faeces. Ten non-AIDS patients without diarrhoea undergoing routine gastrointestinal endoscopy were used as controls. IgG, IgM and IgA serum antibody responses were determined by ELISA using sonicated C. parvum oocytes as antigens.

Of the 71 patients with chronic diarrhoea and HIV infection, 18 had cryptosporidiosis (25%). Infection was present in small bowel biopsies in 8 patients (proximal cryptosporidiosis) and only in faeces (distal cryptosporidiosis) in 10 patients. Patients with HIV infection and cryptosporidiosis had increased specific Anti-Cryptosporidium IgG and IgA antibody responses (geometric mean 0.79 [interquartile range 0.59-1.19] (p<0.001) and 0.58 [0.40-0.72] (p<0.001) respectively) when compared to patients with HIV infection with no evidence of cryptosporidiosis (IgG: 0.57 [0.39-0.81]; IgA: 0.27 [0.17-0.46]) and to control subjects (IgG: 0.38 [0.26-0.44]; IgA: 0.13 [0.10-0.26]). Anti-Cryptosporidium IgM antibodies were not increased in any disease group (p=0.3). There was no difference in antibody response between patients with proximal and distal cryptosporidiosis. The most discriminating test was IgA which had a sensitivity and specificity of 83% and 74%, respectively with positive and negative predictive values of 46% and 87%. The lack of IgG response in these patients may be due to the prolonged duration of the infection. Increased anti-Cryptosporidium IgG and IgA antibodies in a patient with chronic diarrhoea and HIV infection suggests active cryptosporidiosis.

F219

Bromelain protease inhibits intestinal secretion caused by Escherichia coli heat labile (LT) and heat stable (STa) toxins and Vibrio cholerae cholera toxin (CT). TRACEY L. MYNOTT, STEFANIO GUJANDALI* AND ALESSIO FASANO. Centre for Vaccine Development and Division of Paediatric Gastroenterology and Nutrition, University of Maryland, Baltimore, MD, USA, and *Paediatric Unit, University of RC, School of Medicine, Catanzaro, Italy.

Diarrhoea is still one of the most important health problems in developing countries and in travellers to those areas. Despite much knowledge about disease causing pathogens and their enterotoxins, safe and effective therapy against diarrhoea is not available. In this study we investigated the antisecretory properties of bromelain, a proteolytic extract obtained from the pineapple plant (Ananasa comosus). Rabbit ileum mounted in Ussing chambers was treated with bromelain (15 μg/ml) or PBS (control) for 30 minutes prior to the addition of enterotoxicigen Escherichia coli LT (2.5 μg/ml), STa (30 mouse units) or Vibrio cholerae CT (1 μg/ml). Short circuit current (Isc) and potential difference (PD) were monitored as indicators of net anion transport and secretion. Bromelain was effective in inhibiting ISc and PD changes caused by all three bacterial enterotoxins (p<0.001). Bromelain was also effective in inhibiting net secretory changes caused by theophylline (5 mM) (p<0.04), prostaglandin E2 (10 μM) (p<0.02), and Ca-ionophore A23187 (1 μM) (p<0.02). The anti-secretory effect of bromelain occurs distal to the formation of cyclic nucleotides as bromelain inhibited net secretion induced by the cyclic nucleotide analogs 8-Br cAMP and 8-Br cGMP (0.2 mM) (p<0.001). The well characterised 28 kda cysteine protease, designated stem bromelain, was purified from the crude mixture and found to have antisecretory effects against 8-Br cAMP and 8-Br cGMP indistinguishable to the crude extract. We conclude that stem bromelain inhibits intestinal fluid secretion mediated by secretogogues that act through cAMP, cGMP and calcium-dependent signalling pathways and is therefore a candidate anti-diarrhoeal drug.

F218

P-FIMBRIATED ESCHERICHIA COLI ADHERE TO DIFFERENTIATED INTESTINAL EPITHELIAL CELLS (Caco-2) AND STIMULATE PRODUCTION OF INTERLEUKIN-8 (IL-8). H. Embaye, 1 R.M. Odedra, 2 C.A. Hart, 2 B. Getty, 2 J.R. Saunders, 3 J.N. Fletcher, 1 and R.M. Bait. 1 Department of Small Animal Medicine and Surgery, 1 Royal Veterinary College, University of London, and Departments of Medical Microbiology, 2 and Microbiology and Genetics, 3 University of Liverpool, UK.

The gastrointestinal tract is thought to be the source of P-fimbriated E.coli which are a major cause of non-obstructive acute pylonephritis. However, it is not known whether adherence and other properties that convey uropathogenicity of PFEc might be relevant to the intestinal mucosa. This has been investigated by comparing the ability of PFEc and enteropathogenic bacteria to adhere to intestinal epithelial cells and stimulate production of the pro-inflammatory cytokine IL-8.

Differentially adherent bacteria were infected with 10^9 cfu/ml of PFEc, enteropathogenic E.coli (EPEC), enteroinvasive E.coli (EIEC) or Salmonella typhimurium and examined after 4 hours. Adherence and invasion assays showed that all four strains of bacteria were adherent, whereas only EIEC and Salmonella typhimurium exhibited invasion. Assay of cell supernatants for IL-8 (mean±SEM pg/ml) by ELISA showed anticipated increases in production following infection with the invasive organisms EIEC (556±59; n=5; P<0.05), and Salmonella typhimurium (1020±170; n=9; P<0.01), compared to EPEC (44.4±7.5; n=6) which had no invasive capacity. However, although no invasion was apparent, PFEc-Caco-2 cells resulted in a significant stimulation of IL-8 production (480±51; n=7; P<0.05) compared to EPEC.

These findings indicate that PFEc can adhere to intestinal epithelial cells and evoke a production of IL-8 comparable to that achieved by invasive enteric pathogens. This introduces the possibility that IL-8 production is a consequence of PFEc binding to specific surface receptors which can then elicit an inflammatory response in the intestinal mucosa.

F220

PERSISTENT MEASLES VIRUS INFECTION IN CROHN'S DISEASE: CONFIRMATION BY IMMUNOGOLD ELECTRON MICROSCOPY. Daspak P, Lewin J, Dhillon AP, Purcell M, Pitillo RM, Pounder RE, Wakefield AJ.

Inflammatory Bowel Disease Study Group, Royal Free Hospital, London and Kingston University, Surrey, UK.

Aim: To investigate persistent measles virus infection of the intestine and its specificity for the Crohn's granuloma.

Methods: A novel protocol for immunogold electron microscopy was developed using a polyclonal MV nucleoprotein antibody on reprocessed, formalin-fixed paraffin embedded tissue sections. Antibody binding was detected using (i) immunoperoxidase and light microscopy on tissue sections and (ii) 10nm conjugated secondary antibody by electron microscopy on ultrathin sections. Techniques were validated using both measles infected Vero cells and human tissues with established MV infection -brain affected by subcortical spongyencephalitis and acute AVM appendicitis. The technique was applied to tissues from Crohn's disease (N=13), ulcerative colitis (N=5), normal intestine (N=5) and ileocecal tuberculosis (N=2). Mumps primary antibody - applied to either uninfected or MV infected mumps infected MV Vero cells and studied by immunoperoxidase, and MV antibody on mumps infected cells studied by immunoperoxidase and immunogold - we used specificity controls: the primary antibodies identified their respective target antigens and there was no antibody cross-reactivity. Results: MV nucleocapsids labelled with gold conjugated-antibody. Both MV infected cells and tissues, including 8/9 foci of granulomatous infiltration in Crohn's disease but 0/4 foci of non-specific inflammation 1/5 UC cases, 0/4 non-inflammatory controls, and 1/2 TB cases with a very low level of signal. Labelling adopted a characteristic pattern in infected cells and tissues, strengthening the specificity of the observation. Conclusion: This study confirms persistent measles virus infection of the intestine, which was a consistent observation in the Crohn's granuloma. This study provides further support of a role for measles virus in Crohn's disease.
A56

**F219**

**DESULFOVIBRIO spp.: An Aetiological Agent Common to Ulcerative Colitis and Porcine Proliferative Enteropathy?** M.C. Pitcher, M. Goddard, S. McNab, J.H. Cummings. MRC Dunn Clinical Nutrition Centre, Cambridge, CB2 2DH; Addenbrooke's Hospital, Cambridge; Royal (Dick) School of Veterinary Studies, University of Edinburgh.

95% of patients with active ulcerative colitis (UC) carry sulphate-reducing bacteria (SRB) in their faeces compared to only 55% of those in a state of disease remission and 46% of patients with Crohn's disease (CD) without evidence of pouchitis. Over 90% of UC-associated SRB belong to the genus Desulfovibrio and produce high concentrations of hydrogen sulphide within the colonic lumen. Such reducing sulphur compounds are highly toxic, causing inhibition of N-butylate oxidation within colonocytes predominantly of distal gut origin. Proliferative enteropathy (PE) is an inflammatory intestinal disorder of pigs, ferrets and hamsters associated with the presence of an intracellular organism, IS10, in affected mucosa. These are morphologically similar but SRB and 16s rDNA sequence analysis has shown a 91% homology with the genome to Desulfovibrio desulfuricans. Since intramural bacteria have been demonstrated to be both inflamed UC tissue and in ileo-anal pouch mucosa a novel histochemical study was conducted to determine whether PE, UC and pouchitis share a common bacterial aetiology.

10 rectal biopsies from UC patients (12 active, 7 remission) and 8 biopsies from ileo-anal pouches (5 pouchitis, 3 mild acute inflammatory changes only) were used. Sections were stained with rabbit anti-mouse FITC and observed for fluorescence within antigen within the mucosa. Intestinal tissue from a pig with PE and a healthy pig were used as positive and negative controls respectively. Fluorescing bacilli were observed within colonocytes of enteroctyes in the positive control specimen but in none of the colonic or ileal pouch biopsy specimens taken from UC patients. It is concluded that although Desulfovibrio spp. are present within the tissues of UC and ileo-anal pouch patients, monoclonal antibodies directed against porcine PE could not demonstrate evidence of mucosal invasion in PE patients. A strong histiocytogenic reaction between human and porcine bacterial strains limiting antibody specificity may be an explanation for these findings. The development of specific monoclonal antibodies may prove useful in future studies specifically relating bacteria to disease being clearly identified in similar lesions in animals. It is postulated that SRB exert toxic effects on the intestinal mucosa by luminal production of hydrogen sulphide. An invasive strain cannot be excluded (Acknowledgements to NACC and Peol Medical Research Trust).

**F220**

**HIGH EXPRESSION OF INOS IN COLONIC MUCOSA IN ULCERATIVE COLITIS**

P.D. Reynolds, S.J. Middleton, J.O. Hunter, P. Facer, A. Bishop, T. Evans, J.M. Polak. Department of Gastroenterology, Addenbrooke's Hospital, Cambridge; CB2 2QD and Department of Histochemistry, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12

Increased nitric oxide synthase (NOS) activity has been demonstrated in colonic biopsies in ulcerative colitis (UC) by an increase in citrulline concentration.1 However, this method does not determine the cellular site of the NOS.

Colonic tissue was obtained from eight patients with active UC, prior to treatment. Specimens were prepared either from biopsies taken at colonoscopy or from colon which had been removed surgically and had been frozen in liquid nitrogen. Eight control biopsies were taken from patients at normal colonoscopy surveillance for polypos or in the investigation of idiopathic diarrhoea. Tissues were fixed in Zamboni's fluid or 1% paraformaldehyde. Frozen tissue was post fixed in formalin.

NOS activity was assessed by immunostaining with a rabbit antiserum to a peptide fragment of human iNOS using the ABC method, and (2) by in situ hybridisation with an NOS riborope which was transcribed with isotopic labelling (35P), from a linearised 200 base pair human iNOS cDNA. High expression of iNOS mRNA and translated iNOS protein localised to the surface epithelium and crypts in the UC mucosa. The in situ hybridisation signal was of high intensity with the antiserum iNOS riboprobe only in the UC specimens. This was patchy in distribution through the mucosa. Immunostaining was also demonstrated in inflammatory cells in the mucosa, although this needs to be further clarified.

We conclude that the high activity of iNOS in UC, originates predominantly from the colonic epithelium.

**F221**

**USE OF A NEW ENZYME LINKED PCR ASSAY TO SHOW INCREASED INDUCTIBLE NITRIC OXIDE SYNTHASE AND INTERLEUKIN 6 mRNA IN ULCERATIVE COLITIS**

J. McLoughlin, R. Sibb, A. Roberts, G. Vastics, B. Scott, D. Jenkinson. 1 Depts Gastroenterology, 2 Histopathology and 3 Immunology, University Hospital, Nottingham and 4 Lincoln County Hospital, UK

**INTRODUCTION:** We have previously shown increased activity of inducible nitric oxide synthase (iNOS) and interleukin (IL) 6 in active ulcerative colitis (UC), but not in Crohn’s disease (CD). Using a sensitive new method to quantitate RT-PCR products, we now show similar changes in mRNA.

**METHODS:** Total RNA was extracted from whole mucosal biopsy samples, reverse transcribed and amplified by PCR. The number of cycles used for iNOS (28), IL 8 (23), and glyceraldehyde 3 phosphate dehydrogenase (GAPDH, 23) mRNA corresponded to the linear part of the amplification curve. Products were dot blot onto nylon membrane and hybridised to specific oligonucleotide probes labelled with alkaline phosphatase. After incubation with Lumigen™PPD, chemiluniscent product was quantified by scintillation counting. Levels of active (neutrophil) inflammation were quantified blindly on a 10 point scale.

**RESULTS:** RT-PCR product as a ratio of GAPDH RT-PCR product is shown in median (QQR).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Normal</th>
<th>0.23 (0.15-0.20)</th>
<th>0.6 (0.02-1.13)</th>
<th>14</th>
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</thead>
<tbody>
<tr>
<td>iNOS</td>
<td>9</td>
<td>0.15 (0.03-0.68)</td>
<td>0.95 (0.01-0.45)</td>
<td>18</td>
</tr>
<tr>
<td>IL 8</td>
<td>0.46 (0.31-1.25)</td>
<td>0.05 (0.29-1.08)</td>
<td>8</td>
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</tr>
<tr>
<td>GAPDH</td>
<td>1.27 (0.55-1.90)**</td>
<td>0.27 (0.29-1.90)*</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>CD</td>
<td>0.50 (0.13-0.99)</td>
<td>0.15 (0.02-2.10)</td>
<td>24</td>
<td></td>
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</table>

**p<0.01, *p<0.05 vs normal. Inducible NOS (r=0.532 p<0.01) and IL 8 (r=0.521 p<0.01) correlated with the neutrophil score.**

**CONCLUSION:** In active ulcerative colitis, but not other conditions, both iNOS and IL 8 mRNA are increased.

**BILIARY LACTOFERRIN CONCENTRATIONS ARE INCREASED IN ACTIVE INFLAMMATORY BOWEL DISEASE (IBD): A FACTOR IN THE PATHOGENESIS OF PRIMARY SCLEROSING CHolangitis?**

P. Peevers, J.M. Rhodes*. J. Han* **K. Kumar* G. M. Murphy, B.H. Dowling. Gastroenterology Unit, Guy’s Hospital Campus, UMDS, London, *Dept of Medicine, University of Liverpool, Liverpool, and **Academic Dept of Surgery, Queen Elizabeth Hospital, Birmingham, U.K.

Primary sclerosing cholangitis (PSC) affects 3-10% of patients with IBD. Although the pathogenesis of PSC is unknown, histological theory suggests that an enterohepatic circulation of chcmotactic factors or autoantigens, such as bacterial N-formyl peptides or lactoferrin (LF), stored in secondary granules of neutrophils, may play a role in the initiation of PSC. Normally, plasma LF concs are low but in active IBD, high circulating and faecal LF concs are found. Moreover, serum anti-neutrophil cytoplasmic antibodies (ANCA) against LF are present in 22-79% of patients with IBD, and in 50-86% of patients with PSC. If the theory of an enterohepatic circulation of LF is correct, then resection of the diseased bowel should reduce biliary LF concentrations in IBD. Methods: To study this, we obtained gallbladder bile at laparotomy from 42 patients with ulcerative colitis (14 active colitis, 17 post- colectomy, 11 pouchitis) and 21 patients with Crohn’s disease (7 active colitis, 5 post-colectomy with no ileal disease, 9 active ileitis or ileitis necrosis) – none of whom had clinical or biochemical evidence of PSC. Biliary LF was separated from high molecular weight glycoproteins by gel filtration, and quantitated by ELISA. Cross-reactivity of the assay with other biliary proteins was excluded by SDS-PAGE and Western blotting. To determine whether or not LF is released selectively in IBD, myeloperoxidase (MPO) (normally present in neutrophils) was also determined by ELISA (Biosynex, France). Results: In active ulcerative colitis, the mean (sSEM) LF conc in gallbladder bile was 8.6±0.40 mg/l (range 1.25-5.8 mg/l) – significantly higher than that post-colectomy (mean 1.3±0.15 mg/l, range 0.3-3.2 mg/l, p=0.001). In those with pouchitis, the mean LF conc was 8.6±0.14 mg/l (range 5.9-11.5 mg/l) – intermediate between that of the other two groups (p=0.05). In patients with clinically active Crohn’s colitis, the mean LF conc was 3.7±0.6 mg/l, compared with 1.1±0.24 mg/l in the post-colectomy group (p=0.05) and 0.9±0.18 mg/l in those with clinical ileitis or ileitis necrosis (p=0.06 v pre-colectomy value). In contrast, biliary myeloperoxidase concs were low and comparable in all groups, with a mean conc in the 63 patients of 0.2±0.2 μg/l (range 0.2-0.4 μg/l). Summary/Interpretation: In active ulcerative colitis and Crohn’s disease, biliary LF concs are increased, but fall with colectomy/disease remission. These findings are consistent with the hypothesis that bacterial chcmotactic peptides (which selectively induce LF release from neutrophils) and/or LF itself, undergo an enterohepatic circulation, and that LF may play a role in the pathogenesis of PSC – although prospective studies of biliary LF concs in IBD patients ± PSC are needed to test the theory that LF may initiate PSC.
A PHARMACOKINETIC STUDY OF SINGLE AND DIVIDED DOSE DELAYED-RELEASE MESALAZINE. Hussain P, Trudgil N, Riley S. Dept of Gastroenterology, Northern General Hospital, Sheffield S5 7AU.

Poor compliance with maintenance medication is a common problem in patients with inflammatory bowel disease. Since once daily dosing may improve compliance we have compared the pharmacokinetics of delayed-release mesalazine in single and divided doses. Six healthy volunteers (5 male, age 18-33) were given delayed-release mesalazine 400 mg tds for 8 days. On the 8th day plasma samples were drawn and 24 hour urine and stools were collected. Following a 7 day wash out period, the protocol was repeated with 1200 mg once daily. Serum 5-aminosalicylic acid (5-ASA) and N-acetylsalicylic acid (NA-5ASA) were measured by HPLC.

There were no significant differences in the mean 24 hour urinary or faecal excretion of 5-ASA and NA-5ASA between the two dosage regimens. Urine: 400 mg tds - 5-ASA 28.5(2-160.5) mg, NA-5ASA 261.5(56.8-841.6) mg; 1200 mg o.d. - 5-ASA 35.5(2.7-177.6), NA-5ASA 362(133.8-825.4) ng/mL. Faeces: 400 mg tds - 5ASA 190.5(47.6-399.9), NA-5ASA 256.2(97.1-402.5); 1200 mg o.d. - 5ASA 280.6(261.2-543.5), NA-5ASA 324.1(181.4-369.2). Mean plasma Cmax values (400mg tds - 5ASA 478.4, NG/mL, NA-5ASA 1230.3 ng/mL; 1200mg o.d. - 5ASA 1200, NA-5ASA 1398.7) and plasma AUCs were not significantly different. The disposition of delayed-release mesalazine 1200 mg o.d and 400 mg tds is similar. Clinical trials utilising once daily dosing are indicated.

BACTERICIDAL/PERMEABILITY-INCREASING PROTEIN (BPI): A NOVEL TARGET ANTIGEN FOR P-ANCA IN INFLAMMATORY BOWEL DISEASE. Hamilton M., Law MH, Chapman P, Jayne D., Pounder R, Wakefield AJ, Lockwood CM. Inflammatory Bowel Disease Study Group, Royal Free Hospital, London & Department of Medicine, Addenbrooke's Hospital, Cambridge.

**Aim** To examine the frequency, immunofluorescence pattern and BPI-specificity of ANCA in a group of patients with IBD.

**Introduction** BPI is the target antigen for a subset of ANCA in systemic vasculitides where samples show positive indirect immunofluorescence (IIF), yet recognised neither proteinase 3 (PR3) or myeloperoxidase (MPO). BPI is a highly cationic, 55kD membrane associated, cytotoxic protein found in cells of myeloid lineage. It has target specificity for, and is neutralized by Gram-negative bacterial lipopolysaccharide (LPS).

Coded sera from 32 patients with Crohn's disease (CD) and 26 patients with ulcerative colitis (UC) were studied. ANCA positivity was assessed by IIF, using alcohol-fixed human neutrophils. Immunoreactivity with PR3 and MPO was examined. BPI specific IIF was confirmed by fluid-phase inhibition assay incubating purified BPI with the test sera, and in addition a solid-phase BPI-ELISA was also performed. No serum reacted with either PR3 or MPO. Anti-BPI antibodies were found in 46 normal control sera.

ANCA were found in 30% of patients with CD and colonic involvement, and in 8.3% of patients with small bowel involvement. The association of ANCA with Crohn's colitis is significant (p=0.02 - Fisher's exact test)

**Conclusions** BPI appears to be a target antigen for ANCA in a subset of patients with IBD. It is possible that auto-antibodies to BPI block bacteraicidal and LPS-neutralizing properties of BPI in IBD; these non-neutralised products may induce intestinal inflammation.

CYCLOSPORINE FOR THE TREATMENT OF ACUTE STEROID RESISTANT ULCERATIVE COLITIS. WA Stack and CJ Hawkey. Division of Gastroenterology, University Hospital, Queens Medical Centre, Nottingham NG7 2UH.

**INTRODUCTION** Recent studies suggest that Cyclosporine (CyA) may be of therapeutic benefit in refractory ulcerative colitis (UC) although the overall number of UC patients treated with CyA is relatively limited. The effect of CyA in active acute UC was therefore tested in patients with steroid resistant UC who would otherwise have probably undergone colectomy.

**Patients** Eighteen consecutive patients with histologically diagnosed acute on chronic UC who had exhibited a poor response to at least three weeks of conventional therapy, including high dose steroids, were given CyA 4mg/Kg IV for four days and then oral treatment. Patients with toxic megacolon were excluded. trough CyA levels were maintained at the range 100-300ng/mL. The study included 11 males and 7 females ranging from 27 to 80 years (Median 47). Eleven patients had pan colitis and 7 had distal disease. Three of the 18 patients had pyoderma gangrenosum all with active colitis when started on CyA.

**RESULTS** Sixteen out of the 18 patients (88%) responded initially, only two requiring emergency colectomy on their initial stay in hospital. Four other patients relapsed within two months whilst on oral CyA and 1required colectomy. Of the 12 patients not requiring surgery post treatment (66%), 8 have stopped steroids and 8 have been weaned off CyA (6 by transfer to azathioprine) with a mean CyA treatment period of 3.5 months (range 1 to 9). Of the 4 patients still on CyA, 2 have stopped steroids. For patients both requiring and avoiding surgery, mean haemoglobin and albumin levels increased and ESR and CRP levels were reduced while on CyA with no significant difference between the groups. Six (33%) patients experienced side effects whilst on CyA; 3 with headaches, 2 with back pain and 1 with hirsutism but none were severe enough to stop treatment.

**Conclusion** CyA appears to be an effective alternative to surgery in refractory steroid resistant colitis with relatively few side effects. Although one third of patients with severe UC treated with CyA will subsequently require surgery, a third of patients will also be successfully weaned off both CyA and steroids.

NOVEL IgA ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES (ANCA) AGAINST PHOSPHOLIPID TARGETS IN INFLAMMATORY BOWEL DISEASE. Hamilton M, Ardies P, Chapman P, Jayne D, Pounder R, Wakefield AJ and Lockwood CM. Inflammatory Bowel Disease Study Group, Royal Free Hospital, London, NW3 and Department of Medicine, Addenbrooke's Hospital Cambridge.

**Aim** To test the hypothesis that phospholipids are targets for ANCA in IBD. **Introduction** In a subset of patients with systemic vasculitides, phospholipid targets for ANCA have been identified. Methods In a prospective study, coded sera from patients with Crohn's disease (CD) (n=32) and ulcerative colitis (UC) (n=26) were analysed for ANCA. ANCA were detected using an indirect immunofluorescence (IIF) with alcohol-fixed human neutrophils, and an ELISA using an ethanol extract (EE) of human neutrophils. Idiotype reactivity was determined. Positive control sera were also analysed. Serum reactivity was then determined against a panel of commercial phospholipid extracts. Sera from 59 healthy blood donors were used as controls. Fisher's exact test was used to compare results. Results: no serum showed reactivity with either proteinase3 or myeloperoxidase. Of samples showing phospholipid immureactivity, the target antigen was inositol. There was a correlation of 0.8 between anti-EE and anti-inositol antibodies. Significantly more patients with IBD had anti-phospholipid IgA antibodies compared with controls (p<0.001).

**Conclusions** We have identified a novel class of IgA anti-EE antibodies in both CD and UC. Incomplete correlation between anti-EE and inositol implies the presence of a further unidentified phospholipid antigen. Discordance between the IIF and anti-EE may reflect loss of antibodies by EE. IBD exhibits parallels with other microthrombotic phospholipid syndromes.
NSAIDs F229–F237

F229

THE ROLE OF GASTRIC MUCOSAL MICROCIRCULATION IN ULCER FORMATION N.Kaas, N.J.Brown, S.Jacob, M.W.R.Reed*, K.D.Bardhan, Departments of Biological Science and Surgery*, University of Sheffield, S16 2TN; Rothamsted Experimental Station, Harpenden, SG4 9TP

Background/Aims: The focal nature of gastric ulcers raises the possibility of underlining regional disturbances in the gastric mucosal microcirculation. Also, previous studies have implicated a central role for leukocytes in gastric ulcer formation (Kvietys et al, 1990). We investigated whether lesion formation, induced by administration of alcohol to the rat gastric mucosa, involved the microcirculation and leukocyte adhesion. Fluorescent in vivo microscopy was used.

Methods: Fluorescein isothiocyanate-bovine serum albumin (FITC-BSA;0.2ml 100g/kg) was applied for quantitating macrovascular leakage (n=8), or acridine red (0.1ml 100g/kg) a marker of leukocytes and platelet thrombi (n=3) was administered intra-arterially to anaesthetised rats. At laparotomy, the stomach was opened and three regions were selected randomly for study. Ethanol (60%) was administered topically to the entire mucosal surface for 5 mins and the areas of interest observed for two hours. Intestinal fluorescence changes due to macrovascular leak of FITC-BSA were quantified using computerised image analysis and the frequency of adherent and flowing leukocytes determined. Controls were given water topically.

Results: Three distinct patterns of response to ethanol were observed; i) lesion formation within 5 mins with vessels demonstrating persistent blood flow stasis, a rapid increase (P<0.05) in macrovascular leakage and no observable leukocyte adhesion; ii) periphery of the lesion demonstrated initial blood flow stasis with resumption of flow within 5 mins, a further and sustained increase in macrovascular leakage (P<0.05) and adherent leukocytes and platelet thrombi present after 15 mins; iii) distant sites from the lesion also showed initial flow stasis with resumption within 5 mins, transient macrovascular leakage and leukocyte adherence but with decreased velcrocytosis.

Conclusions: Only regional microcirculatory disturbances were observed despite uniform application of alcohol. Lesion formation was always associated with such microcirculatory change. The periphery of lesions demonstrated typical inflammatory responses: sustained vessel permeability and leukocyte adherence. Stasis of blood flow, and hence lesion formation, occurred before leukocyte adhesion. In conclusion, this model, mucosal damage is associated with microcirculatory change and is independent of leukocyte adherence.

Kvietys et al, 1990 Gastroenterology, 88: 905-920

F230

INDOMETHACIN-INDUCED JEJUNAL VILLOUS CONTRACTION AND MICROVASCULAR OCCLUSION: A DETAILED NON-INVASIVE EXPERIMENTAL STUDY A.Antonio, AP Dhillon, C Thrasivoulou, RE Pounder, AJ Wakefield, Inflammatory Bowel Disease Study Group, Royal Free Hospital School of Medicine, London NW3, UK.

Background: In histological studies of indomethacin (IN)-induced jejunal ulceration in the rat, villi undergo both early microvascular injury and villous shortening that may involve contraction of villous smooth muscle.

Aim: We examined these early pre-inflammatorv changes using 3-dimensional imaging techniques.

Methods: At 2 and 6 h after oral IN 15mg/kg or vehicle, groups of rats (n=5) were killed and the vasculature of the small intestine was visualised by both carbon ink perfusion/confocal microscopy and injection casting. The mucosa was also examined for lesions by dissection microscopy and scanning electron microscopy.

Results: IN caused shallow jejunal haemorrhages at 6 h but not at 2 h. By scanning electron microscopy and histology, the mucosa, at 2 h, showed shortening of the villi and wrinkling of the surface epithelium; at 6 h the mucosa was flattened and villi were ‘ contracted’. Confocal microscopy allowed 3-dimensional visualisation of villi that showed both significant reductions of mucosal height and villous inter-capillary distance (VICD) in IN-dosed rats (table), with reduced filling of surface capillaries at 2 and 6 h. Scanning electron microscopy of injection casts at 2 and 6 h indicated similar changes. All control (CO) gastric tissues were normal. (a = P < 0.05; b = P < 0.001. n = 5 rats/group).

Mucosal height (μm) VICD (μm)

IN 2h 300±2 17±2
Co 2h 494±9 30±4
IN 6h 234±3 15±2
Co 6h 498±10 31±3

Conclusions: Histology, confocal microscopy and scanning electron microscopy support the proposal that villous shortening and disruption of surface capillary architecture are early changes in INA. induced ulcerative enteropathy. (Funded by Glaxo Research & Dev.UK).

F231

COMBINED INTESTINAL TREFOIL FACTOR AND EPIDERMAL GROWTH FACTOR IS PROPHYLACTIC AGAINST INDOMETHACIN-INDUCED GASTRIC DAMAGE IN THE RAT.

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Introduction A great deal of interest has been focused on the potential use of recombinant epidermal growth factor (EGF) to stimulate healing of mucosal ulceration. However, because of its potent mitogenic activity, this is also used for strategies which reduce the dosage required. Intestinal Trefoil Factor (ITF) stimulates mucosal healing without increasing proliferation. This study investigated the effect of co-administration of EGF with rat (r)ITF on mucosal healing using in vitro and in vivo modeling studies.

Methods Wounds in vitro were created in confluent layers of the colonic epithelial cell line, HT-29. Cells were maintained in a serum-free medium in the presence (10μM) or absence of recombinant EGF and/or rITF. Restitution was quantified as the rate of epithelial cell migration into the wound. In the in vivo model of gastric ulceration, fasted male Wistar rats received subcutaneous injections of saline, EGF (1μg/kg/hr), rITF (150μg/kg/hr), or EGF and rITF 30 minutes before the induction of ulceration (indomethacin: 20mg/kg i.c.) and 3 hours thereafter. Animals were sacrificed and the extent of gastric damage was assessed using a dissecting microscope.

Results The in vitro study of epithelial restitution demonstrated a marked synergistic effect on the rate of migration of the wound edge when rITF was used in combination with EGF. There was no increase in cellular proliferation due to the addition of ITF to the cells when given alone or to the stimulatory effect of cells treated with EGF. In the rat model of ulceration, the presence of both peptides protected against the development of indomethacin-induced gastric damage (saline: 303±32mm2; (7); 1μgEGF/kg/hr 254±44mm2 (6); 150μgITF/kg/hr 263±32mm2 (6); 150μgITF/1μgEGF/kg/hr 32±32mm2 (6)).

Conclusion Our findings suggest that combination therapy of ITF with EGF is likely to increase ulcer healing properties of EGF whilst also improving its safety profile.

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ACUTE GASTRIC DAMAGE DEPENDS ON MUCOSAL CONTACT WITH NSAIDS.

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Topical application of NSAIDs causes acute gastric mucosal damage and a fall in antral mucosal blood flow (MBF). The aim of this 28 day study was to compare changes in gastric morphology and blood flow during oral (dispersable, uncoated and enteric coated tablets) and rectal administration of the NSAIDs, naproxen and diclofenac.

Forty eight healthy, H. pylori negative volunteers were given either naproxen 500mg bd (uncoated tablet or suppository) or diclofenac 50mg bd (effervescent or enteric coated) and gastroscopied before, 1, 7 and 28 days during treatment. Acute gastric glandular damage was assessed using a modified Lanza score (0=normal, 4=severe), biopsies taken for microscopic assessment of damage (0=negative, 4=severe) and mucosal blood flow measured using laser doppler flowmetry in the corpus and antrum.

Mean gastric damage (median grade + IQR) occurred after 24 hours of uncoated naproxen (2.0 IQR 1.0-3.0 p<0.01) and effervescent diclofenac (1.5 IQR 1.0-2.0 p<0.01) and was associated with a fall in antral MBF (mean ± SEM) from 58±3.3 to 46.6±4.1 (p<0.01) and 50.9±8.3 to 39.1±7.7 arbitrary units (p=0.05) respectively. Only half the subjects on enteric coated naproxen (1.0 IQR 1.0-2.0 p=0.06) and in those antral MBF decreased from 57.7±12.1 to 46.9±12.0 arbitrary units (p=0.01). No macroscopic damage or significant changes in antral MBF were observed during rectal administration and none of the NSAIDs altered corpus MBF or produced mucosal damage. With continued NSAID administration any gastric damage or reduced antral MBF resolved by day 28.

These observations suggest that NSAID induced acute mucosal damage and changes in MBF mainly depend on topical rather than systemic actions.
**ANTRAL AND CORPUS INDOMETHACIN-INDUCED GASTRIC CONTRACTILE CELLS**

A Anthony, AP Dhillon, R Sim, RE Pounder, AJ Wakefield.
Inflammatory Bowel Disease Study Group, Royal Free Hospital School of Medicine, London, UK.

**Background/Aim:** In indomethacin (IN)-induced jejunal ulceration, villi undergo early pre-ulcerative shortening that may involve smooth muscle contraction. We compared gastric mucosal contraction in the rat and correlated mucosal contractile cells within the corpus and antrum of other species with the reported site susceptibility to indomethacin-induced ulceration. **Methods:** Two groups of fed rats (n=6) received IN 35mg/kg po. 3 and 48 h later the stomachs were examined grossly and microscopically for antral (A) and corpus (C) mucosal changes. Two control groups of rats received vehicle only. Mucosal thickness (μm) was measured in histologically abnormal (Ab) and adjacent normal mucosa (N). Rat sections, and archive control sections from other species were stained, immunohistochemically, for actin to identify potentially contractile cells. **Results:** In the C only, IN caused erosions by 3 h, and deeper inflammatory ulcers by 48 h. Many of the erosions showed an intact but thinner mucosa with clumping of contractile cells (IN; 3h, Ab/N = 0.7 ± 0.1; 48 h, 0.27 ± 0.07; controls; 3 and 48 h, Ab/N = 1.0). Reported site-specific susceptibility in other species coincided with the presence of mucosal contractile cells (Table: actin positive cells in A and C were scored 1+ to 6+).

**Species**

<table>
<thead>
<tr>
<th>A</th>
<th>C</th>
<th>Ulcer</th>
<th>Species</th>
<th>A</th>
<th>C</th>
<th>Ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>1+</td>
<td>3</td>
<td>Mouse</td>
<td>3+</td>
<td>3+</td>
<td>A</td>
</tr>
<tr>
<td>Dog</td>
<td>5+</td>
<td>3+</td>
<td>Rabbit</td>
<td>6+</td>
<td>3+</td>
<td>A</td>
</tr>
</tbody>
</table>

**Conclusions:** Indomethacin causes erosions in the rat corpus that show mucosal thinning and clumping of actin-positive cells. The distribution of contractile cells correlates well with the reported sites of IN-induced gastric injury in various species (Supported by Glaxo Research & Development, UK).

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**HIGH DOSE FAMOTIDINE FOR HEALING AND SUBSEQUENT MAINTENANCE IN NON-STERoidal ANTI-INFLAMMATORY DRUG-ASSOCIATED GASTRODUODENAL ULCERATION**

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**Introduction:** We previously reported that high dose famotidine (40mg bd) was effective primary prophylaxis against non-steroidal anti-inflammatory drug (NSAID) ulcers. We therefore assessed whether this dose of famotidine can heal and maintain remission in patients who have already developed NSAID-associated ulcers.

**Methods:** One hundred and four of 389 unselected NSAID users with peptic ulceration or bleeding were found to have gastric (75%), duodenal (40%) ulceration (13% both) at endoscopy. Sixteen stopped their NSAID, whilst the rest continued. All were treated with famotidine 40mg bd for 4 or 12 weeks. After ulcer healing patients were randomised to receive placebo or famotidine 40mg bd for 6 months or until endoscopic relapse after 1, 3 or 6 months.

**Results:** Ulcer healing was 100% for patients who stopped vs 89% for those continuing NSAIDs (NS). During the maintenance phase of 78 patients who continued their NSAID the cumulative relapse was 53.5% (95% CI, 36.6%-70.3%) for those taking placebo. This was reduced (p=0.011) to 26.0% (12.1%-39.9%) in patients using famotidine 40mg bd. There was a reduction in gastric ulceration from 41.4% (24.0%-58.7%) to 19.1% (6.3%-31.9%, p=0.026). Five patients developed duodenal ulcers on placebo vs 3 on famotidine (NS). The cumulative ulcer incidence on placebo was higher than the 28.3% (18.7%-38.0%) previously reported for comparable primary prophylaxis conditions.

**Conclusion:** Patients who have developed NSAID-associated ulcers have a high subsequent relapse rate. This, the first study to investigate such patients, shows famotidine 40mg bd healed NSAID-associated ulcers effectively and reduced later relapse.

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**ACUTE GASTRIC TOLERABILITY OF S(+) I BUPROFEN COMPARED TO RACEMIC IBUROFEN**

Dj Cullen, N Hudson, JC Atherton, B Filipowicz, CJ Hawkey, Div Gastroenterology, University Hospital, Nottingham

**INTRODUCTION:** The S(+), isomer of ibuprofen is a more potent analgesic and anti-inflammatory agent than standard racemic ibuprofen. Poor safety assessment reported gastric tolerability endoscopically in 16 healthy volunteers.

**METHODS:** Sixteen healthy volunteers, aged 19-41, all received on separate occasions (order randomised) under double blind conditions, 10 day courses of comparable doses of racemic ibuprofen (600mg tds) and S(+) ibuprofen (400mg tds). Subjects were endoscoped before and after 5 and 10 days of each treatment, when ulcers, erosions and intramucosal haemorrhages were counted for oesophagus, body, antrum, duodenal bulb and second part of duodenum. Ex vivo prostaglandin (PG)E2 synthesis was stimulated by vasoconstricting and assayed by radiommunoassay.

**RESULTS:** Both forms of ibuprofen were similarly effective in suppressing gastric mucosal PGF2a, from 30.5 (median, interquartile range 12.0-35.0)pg/mg to 2.1 (0.9-3.0)pg/mg for racemic and from 20.6 (7.7-41.6)pg/mg to 1.5 (0.9-2.0)pg/mg for S(+) ibuprofen (Day 10) with (p<0.001). No subject had gastric erosions at baseline. Four had 1-9 erosions at Day 5, and 8 had 2-16 erosions at Day 10 on racemic ibuprofen. Six had 1-4 erosions at Day 5 and 5 had 1-2 erosions at Day 10 on S(+) ibuprofen (p=0.22 for drug comparisons). There was a small treatment by day interaction (p=0.04) for haemorrhagic erosions which rose from 1-9 in 5 subjects (Day 5) to 2-16 in 7 (Day 10) on racemic ibuprofen, but fell from 1-4 in 6 to 1-2 in 2 subjects on S(+) ibuprofen.

**CONCLUSIONS:** At doses suitable for use over the recommended duration for self-medication both treatments caused a marked suppression of prostaglandin synthesis, but showed little gastrointestinal toxicity. Whether adaptive changes occur more readily with S(+) ibuprofen would require further evaluation.

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**RISK OF UPPER GI COMPLICATIONS IS CONSTANT WITH CONTINUOUS NSAID USE: A RECORD-LINKAGE STUDY**

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The profile of risk during and after treatment with NSAIDs is poorly defined. The AIM of this study was to characterise this profile. **METHOD:** We prospectively constructed a record-linkage population based database (n=400,000) containing (a) data on all dispensed NSAIDs and (b) all validated upper GI haemorrhage and perforations (GHP). We studied all patients >50y who received one or more NSAID prescriptions from Jan89-Dec91. NSAID exposure was defined as the 45 day period following a prescription. Risk profiles from the start of continuous NSAID exposure and non-exposure were constructed. Odds ratios (OR) with 95% CI were estimated by Poisson regression relative to event rates prior to NSAID exposure per 1,000 patient yrs (PTY). **RESULTS:** 52,382 patients received 297,574 NSAID prescriptions totalling 29,703 patient yrs of NSAID exposure and 125,954 patient yrs of non-exposure. There were 430 GHP events (208 exposed). The event rate in patients with a history of an upper GI event was high regardless of NSAID exposure (111 per TPY exposed v 90 non-exposed, OR 1.5 (0.6-3.6)), but these 0.4% of patients accounted for only 7.7% of events. In patients without prior history the GHP rate was much lower (1.3-2.2 per 1000 patient years of NSAID exposure, OR 1.8 off NSAID), but with a higher OR of 7.3 (5.2-10.1). This risk was constant during continuous exposure (7.0 per TPY) and remained significantly higher for 1 month following end of exposure, OR 4.8 (3.1-7.4). GHP risk was related to dose of NSAID (low v high OR 1.5 (1.1-2.2)) and age (80-89y v 50-69y OR 6.5 (3.1-8.1)) but not with sex. The dose-years rank order of toxicity was azapropazone > piroxicam > flurbiprofen > ketoprofen > diclofenac SR > diclofenac > naproxen > mefenamic acid > ibuprofen > indomethacin > nabumetone > fenbufen. **Summary:** The risk of GHP was constant with continuous NSAID treatment, with a carry over period of at least one month following exposure. This study confirms the rank order of NSAID toxicity previously reported and also the increased risk with increasing age or NSAID dose. Prior UGIH is independently the most important risk factor for GHP.
**HELICOBACTER PYLORI DOES NOT INFLUENCE GASTRIC MUCOSAL ADAPTATION TO NSAIDS IN MAN.**

G.B. Lipscomb, N. Wallis, G. Armstrong, M.J. Goodman, W.D.W. Rees. Departments of Medicine and Histopathology, University of Manchester School of Medicine, Hope Hospital, Salford, and Bury General Hospital.

Topical application of NSAIDs results in acute gastric mucosal injury and a fall in antral mucosal blood flow (MBF). This flow is followed by adaptation when gastric damage resolves and MBF returns to normal despite continued NSAID intake. The influence of H.pylori on these responses has not been investigated previously.

Twelve healthy volunteers, H.pylori positive by gastric biopsy, CLO test or C13 urea breath test, were given a 28 day course of either diclofenac 50mg bd or naproxen 500mg bd and gastroscoped before, 1, 7 and 28 days during treatment. Macroscopic gastric mucosal damage was assessed using a modified Lansa score (0=normal, 4=severe) and mucosal blood flow measured by laser doppler flowmetry in the corpus and antrum. Twelve age, sex and drug matched healthy H.pylori negative volunteers underwent the same protocol.

Maximal gastric mucosal damage (median grade 1 - IQR) occurred during the first 24 hours in both groups and was of the same magnitude (H. pylori -ve: 2.0, 1.0-3.0, H. pylori -ve: 2.0, 1.0-3.0). This damage was associated with a fall in antral MBF (mean ± SEM) from 53.0 ± 4.1 to 0.3 ± 3 arbitrary units in the H.pylori -ve group (p=0.05) and from 57.2 ± 3.2 to 36.8 ± 4.6 arbitrary units in the H. pylori -ve group (p=0.005). There was no significant difference in antral MBF changes between the 2 groups. Corpus MBF was not significantly affected by NSAID intake in either group. With continued NSAID administration gastric damage resolved in both groups and MBF returned to baseline (H.pylori -ve: 53.2 ± 2.9, H.pylori -ve: 49.2 ± 3.7 arbitrary units). These adaptive responses were not influenced by H. pylori.

These observations suggest that H. pylori has no influence on either acute gastric mucosal damage or subsequent adaptation to NSAIDs.

**Liver** F238–F248


Hepatitis C virus (HCV) is predominantly spread by parenteral routes, but some sources have suggested a significant intrafamilial (vertical) transmission rate (1).

**Aim:** we surveyed 60 women who had been infected with HCV after receiving contaminated anti-D immunoglobulin.

These 60 women were infected in 1977 (6), 1979 (2), 1982 (1), 1987 (2), 1990 (2), 1999 (3), 1993 (4) and 1994 (1). All were positive for HCV antibodies by ELISA (Ortho, Murex) and RIBA3 (Ortho) and were viramic by PCR for HCV-RNA (Roche). Liver biopsies were performed which revealed mild to moderate chronic liver disease in 55 and severe chronic liver disease in 5. All were in stable longerm relationships. Thirty partners and 109 children were tested for HCV antibodies by ELISA (Ortho, Murex). No other risk factors were elicited in the contacts.

**Results:** No male partners or children tested positive for HCV antibodies indicating no previous exposure over a combined time period of 544 years for partners and 1575 years for children.

**Conclusion:** This study suggests a zero female to male sexual transmission rate of HCV and a zero vertical transmission rate in anti-D associated HCV infection. This contrasts with previous studies and may possibly be explained by the HCV genotype, low inoculum at infection, the overall mild hepatic insult and other factors such as genetic and geographic variables. The rest of the partners (30) and children (99) are presently undergoing further testing.


**Introduction** TIPS are now accepted therapy for variceal bleeding. Increasing numbers of TIPS’ed patients are coming to subsequent transplantation (OLT) or surgical shunting. We report our experience with this subgroup of patients.

**Methods** Over a 28 month period 8 patients who had TIPS for intractable variceal bleeding had subsequent OLT (N=4) or surgical shunt (N=4). The latter for TIPS occlusion. Surgery was performed a mean of 10 months after TIPS placement. Only 2 patients (subsequently transplanted) did not have any balloon dilation of TIPS or repeat TIPS.

**Results** The TIPS led to surgical problems in all 8. In the 4 who had shunts the distal end of the stent was well into the portal vein, limiting the length of vessel available for anasotomosis. In one of these patients, the stent was at the confluence of the splenic vein (SV) and superior mesenteric vein (SMV). Portal vein rigidity due to the TIPS led to difficulty in ligating the hepatic portion of the portal vein in all 4, and necessitated the use of orthopaedic crushing clamps in 2 cases. In the 4 patients transplanted, 2 TIPS extended into the upper caval clamp, and another had penetrated through the wall of the portal vein. In 2, the TIPS extended close to the confluence of SV and SMV requiring pancreatic mobilisation, shortening the recipient portal vein available for anastomosis. Pulling the metal stent from the portal vein denuded the endothelium. A feature seen in all of the long term TIPS was marked peri-portal vein fibrosis, complicating vessel dissection, and 3 of the 4 patients subsequently received anticoagulation. TIPS did not influence blood transfusion requirements, and in all the operations were successful.

**Conclusion** Whilst TIPS is a life saving procedure, the stents present the surgeon with unique complications, principally due to the accuracy of placement, and this is particularly relevant in liver transplantation. Because of these potential difficulties, TIPS deployment should remain confined to major liver units.

**REPEAT HLA MISMATCHES IMPROVE SURVIVAL AFTER SECOND LIVER TRANSPLANTATION** T. Wong, F. Donaldson, J. Devlin, Roger Williams. Institute of Liver Studies, King’s College Hospital, London SE5 9RS.

The role of HLA (Human Leukocyte Antigen) matching in liver transplantation is controversial, and its effect in retransplantation has not been studied. If the same HLA locus mismatch from the primary transplant is repeated in a second kidney graft there is an increased risk of immunological graft failure.

78 patients who received second liver transplants in the King’s College programme between 1984 and 1994 were studied. Follow up time ranged from 33 to 3653 days (median 1025). HLA typing was performed using serology for Class I antigens, and a combination of molecular and serological methods for Class II.

Analysis of results from the whole group showed that patients with the same B locus mismatch in the second graft as for the first graft (ie repeat B mismatch) had a survival at follow up of 79% compared to 4% in those without (p<0.02). A similar effect was noted for repeat DR mismatches (67% vs 47%, p=0.06). Graft survival was also improved in those patients who were retransplanted with a repeat B or DR mismatch (65% vs 40% p=0.08, 59% vs 41% p=0.09 respectively). In the subgroup of patients who were retransplanted for rejection, 90% of patients retransplanted with a repeat B mismatch were alive at follow up compared with 46% without B mismatches (p=0.02). The improvement in survival is seen within two months post-retransplant. There was a reduced incidence of rejection in surviving patients with repeat DR mismatching (54% vs 25%, p=0.08), but not with a repeat B mismatch.

Repeat HLA B, and DR loci mismatching in liver retransplantation improves patient and graft survival. This clinically important finding, which is contrary to experimental theory and clinical observation in renal retransplants, is further evidence of the unique immunological characteristics of liver allografts.
Mesoaoval shunt versus radiological intervention in the treatment of budo-chiari syndrome.

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The Liver Unit and Hepatobiliary Unit, Queen Elizabeth Hospital, Birmingham, U.K.

Mesoaoval shunt (MCS) and radiological intervention methods (RIM) in the form of balloon angioplasty or stent placement, have been reported to successfully treat manifestations of portal hypertension in Budd-Chiari disease (BCS). To compare between these two treatment modalities, we have analyzed the data of 25 patients with BCS admitted between 1984 & 1994. All data were collected at the time of presentation. 11 patients were treated with a RIM (mean age 40.1 years, range 21-65) and 14 patients were treated with a MCS (mean age 33.8 years, range 17-63) using TIPS var. (RIM vs MCS); were: AST 152 v 286, Bilirubin 50.7 v 77.5, Alkaline phosphatase 364 v 563 & Albumin 31 v 32. Clinical presentations, in particular, evidence of encephalopathy were comparable in both groups. Etiologically there was higher incidence of Myeloproliferative in the MCS group. The following table summarizes the outcome in both groups.

<table>
<thead>
<tr>
<th>RIM (%)</th>
<th>MCS (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>CLINICAL &amp; BIOCHEMICAL</td>
<td>9/11(62%)</td>
</tr>
<tr>
<td>ONE YEAR MORTALITY</td>
<td>2/11(18%)</td>
</tr>
<tr>
<td>COMPLICATIONS</td>
<td>1/11(9%)</td>
</tr>
<tr>
<td>FOLLOW UP (MEAN)</td>
<td>2.3 years</td>
</tr>
</tbody>
</table>

CONCLUSION: The outcome of radiological intervention is comparable to MCS in the treatment of BCS. Because it is the most physiological treatment, we believe it should be the first line treatment in patients with BCS, MCS and liver transplantation should be only used if RIM is unsuccessful.

Differential actions of propranolol and nitrates in cirrhosis - a rationale for combination therapy to prevent variceal haemorrhage.

Forest EH, Jalan R, Redhead D, Hayes PC.
Departments of Medicine and Radiology, The Royal Infirmary, Edinburgh.

Propranolol [Prop] and Isosorbide-5-Mononitrate [Is-5-Mn] are being used either separately or in combination to reduce the risk of variceal haemorrhage in patients with cirrhosis. The action of these drugs on portal haemodynamics is disputed. Patients with TIPS access to the portal vein to study this in more detail.

Methods: We studied 16 patients with cirrhosis who had a TIPS sin. Portal vein flow (PVF), direct reverse thermodilution method, pressure [PVP], right atrial pressure [RAP], heart rate [HR] and mean arterial pressure [MAP] were measured at the time of routine portography. Eight patients received oral Prop 80mg, 8 oral Is-5-Mn 20mg.

Results: Haemodynamic changes with treatment:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>HR (bpm)</th>
<th>PVP (mmHg)</th>
<th>PPG (mmHg)</th>
<th>PVF (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>80.0 (3.2)</td>
<td>14.0 (1.0)</td>
<td>10.8 (1.2)</td>
<td>814 (186)</td>
</tr>
<tr>
<td>60</td>
<td>83.0 (3.4)*</td>
<td>10.9 (0.5)#</td>
<td>9.2 (1.0)*</td>
<td>921 (21)</td>
</tr>
<tr>
<td>160</td>
<td>77.2 (2.2)</td>
<td>13.8 (2.0)</td>
<td>7.7 (2.3)</td>
<td>925 (123)</td>
</tr>
</tbody>
</table>

Mean (SEM), PVP=PVP-RAP, *p<0.05, **p<0.01, #p<0.005 compared to baseline.

In the Prop treated group there was a correlation between the fall in PVF and HR [r=0.794, p<0.02]. MAP fell significantly only in the Is-5-Mn group from 91.1 (3.0) to 85.0 (1.1).

Conclusions: Prop caused a fall in PVF and PPG consistent with a reduction in splanchic hyperaemia. In contrast Is-5-Mn tended to increase PVF whilst still lowering PPG - consistent with a further reduction in intrahepatic resistance. The differing actions of these drugs implies combination therapy may be of benefit.
SCREENING FOR HEMOCHROMATOSIS IN A DIABETIC CLINIC

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Diabetes melitus is a common clinical manifestation of hereditary hemochromatosis (HH). We have investigated the prevalence of HH in a diabetic population. Methods: Serum transferrin saturation (TS) was measured on blood taken from patients attending a diabetic clinic if TS > 55%, fasting transferrin saturation (FTS) and serum ferritin (SF) were measured. Diabetic patients with FTS >55% and elevated SF underwent liver biopsy. Exclusion load was assessed histologically and biochemically.

Results: 1194 diabetic patients were studied, 857 males (median age 57, range 18-88) and 337 females (median age 56, range 20-86) had diabetics diagnosed before 40 years and 890 after 40 years of age. 21 patients had TS >55% and 10 of these had FTS >55%. Of the 10 patients with FTS >55%, 2 had previously been diagnosed as having HH and 2 had normal SF. 6 patients had both FTS >55% and elevated SF and all consented to liver biopsy. A candidate for the Wilson’s disease was also found to have HH, the iron load having been removed by multiple venesections. Liver biopsy confirmed the diagnosis of HH in 3 patients, 2 patients had haemochromatosis but insufficient iron overload to confirm hemochromatosis and one patient had alcoholic cirrhosis. Family screening using HLA typing, TS and SF has so far revealed 4 relatives with HH, 3 of whom are asymptomatic and 1 symptomatic with established cirrhosis. Of the 6 patients with definite HH, 3 had abnormal liver function tests and 3 had established cirrhosis. The positive predictive value of FTS > 55% in our study was between 50% and 70% depending on the eventual diagnosis of the 2 cases with FTS > 55% but normal SF. The prevalence of HH in this population is 0.8% - 0.7% (0.7% - 0.8% in maturity onset diabetics).

Conclusions: A raised fasting transferrin saturation is a simple biochemical test which could be used routinely to screen patients attending diabetic clinics for HH.

ISOLATION OF COPPER-BINDING PROTEINS IN BILE CANULAR Membranes: A POSSIBLE LINK TO COPPER EXCRETION AND WILSON’S DISEASE

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We have recently shown that normal bile contains a different molecular form of caeruloplasmin (125kD), a copper-transporting protein, from the usual form predominant in plasma (132kD). Furthermore, this biliary caeruloplasmin is absent in genetic copper toxicity (Wilson’s disease) even when the plasma form (132kD) is present. Since body copper homeostasis is regulated uniquely at the level of biliary excretion, this finding suggests that caeruloplasmin is involved in biliary excretion and could explain in part the resulting hepatic accumulation of copper and is likely to be related to the Wilson’s disease gene product. The Wilson’s disease gene product has now been identified which predicts an abnormal P-type ATPase copper transporter which has yet to be characterised. Our current hypothesis is that copper is excreted in bile bound to caeruloplasmin and that this process may involve a specific caeruloplasmin receptor or transport protein situated in the bile canicular membrane which either must be the Wilson’s disease gene product or associated with it. We have previously identified in purified human bile canicular membranes by Western blotting, a 200kD protein that possesses caeruloplasmin-like activity when probed with an antibody specific to caeruloplasmin and which is also copper-sensitive. In this present study using affinity chromatography (metal chelating gel) and identification of copper-binding proteins by SDS gel electrophoresis, we have also identified a number of copper-binding proteins in crude bile canicular membrane preparations. Two of these protein bands have been identified as the known molecular forms of caeruloplasmin (132kD and 125kD), but the predominant copper-binding protein, not cross-reacting with caeruloplasmin antibody, has a molecular weight of 53kD and can be visualised by staining with Coomassie blue. These findings are relevant to an understanding of the normal pathway for biliary copper excretion, and also characterise in part the Wilson’s disease defect.

IRON OVERLOAD IN LONG TERM SURVIVORS OF BONE MARROW TRANSPLANTATION (BMT)

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Liver dysfunction is common in long term (10 year) survivors of BMT. Transfusional iron overload is one possible cause of such dysfunction. We reviewed transfusional iron overload in 890 survivors of BMT at our centre who had a serum ferritin 550ug/l suggesting iron overload. 35/67 with elevated serum ferritin had normal liver function tests (LFT) and 22/67 were hepatitis C +ve. We have studied the degree of iron overload in 19 of these 67 patients in more detail by performing liver biopsy. The biopsies were assessed histologically using a modified Scheuer method and biochemically by measuring dry liver iron weight (DLIW). 11/19 patients had previously been veneseeted to try and reduce iron overload.

In all 8 patients not previously veneseeted, DLIW (normal range 0.17-1.4mg/g) was elevated (mean 8.8mg/g, range 1.9-23.2mg/g). In 8/8 cases, hepatocyte iron staining grade was > reticuloendothelial staining grade. DLIW correlated poorly with transfusional iron load (r=0.48), 7/11 veneseeted patients still had elevated DLIW (mean 7.0mg/g, range 2.8-17.5mg/g). DLIW correlated poorly with serum ferritin measured at time of biopsy (r=0.31). 2 patients had established cirrhosis (1 hepatitis C+ve, 1 hepatitis C-ve), 4 patients had fibrosis on liver biopsy (2 hepatitis C+ve, 2 hepatitis C-ve) and 1 patient had iron overload above the threshold known to cause fibrosis (DLIW > 25mg/g).

Our results demonstrate marked liver iron overload in patients post BMT with the potential to cause fibrosis and cirrhosis. Neither serum ferritin nor transfusional iron load accurately predict the degree of liver iron overload, therefore other factors must have a role. We recommend that any patient post BMT with abnormal LFT or elevated serum ferritin should have iron overload assessed by liver biopsy.
Gut 1995; 36 (suppl 1)

EORTHERAPY, INFECTION AND COLITIS F249–F258

F249

JEJUNAL BACTERIA IN ARTHRITIS: IMPORTANT IN PATHOGENESIS OF DISEASE OR DEVELOPMENT OF NSAID ENTEROPATHY?

Small bowel microflora may be important triggering factors in rheumatoid arthritis (RA) and ankylosing spondylitis (AS). It has been proposed that bacteria are involved in the pathogenesis of small bowel ulceration by non-steroidal anti-inflammatory drugs (NSAID). Push enteroscopy and jejunal aspiration allow simultaneous endoscopic examination and bacterial sampling of the jejunum.

Patients/Materials and Methods: We examined 92 patients with RA, 17 with AS and 7 controls. History of NSAID and second line therapy was noted for each patient. 29 patients were on long term NSAID and 10 patients were not. Patients with previous gastric or small bowel surgery receiving antibiotics or drugs affecting gastric acid secretion were excluded. Patients had enteroscopy, jejunal biopsy and aspiration and penta-gastrin stimulation to assess gastric acid production. Results: Jejunal organisms were isolated in 6 (38%) of RA patients, in 10 (58%) AS patients and 4 (57%) controls. Staphylococcus was isolated in 5 (31%) organisms contained in 4 (18%) RA, 6 (35%) AS and 3 (42%) controls. Enterobacteria were isolated in 2 (9%) RA, 5 (29%) AS and 1 (14%) controls. Candida were isolated in 3 (14%) RA, 7 (41%) AS and 2 (38%) controls. No difference in frequency of culture was noted in patients treated with NSAID or sulphasalazine. There was no correlation between the numbers of microflora NSAID enteropathy and small bowel ulcers.

Conclusion: I) No primary abnormality of small bowel flora was found in AS or RA. II) An increased isolation of both enterobacteria and Candida was found in AS compared to RA patients (p<0.05, Fishers exact test). III) Jejunal bacterial colonisation is not required for NSAID enteropathy in man.

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THE Beta-ADRENERGIC AgONIST CL316243 PREVENTS INHOMOGENEOUS JENULAL ILEAL ULCERATION IN THE RAT A Amatucci, AK Bahl², AP Dhillon, IG Oakley¹, C Fiascetti, CF Spragg³, MA Trevethick⁴, RE Founder, AJ Wakefield. Inflammatory Bowel Disease Study Group, Royal Free Hospital School of Medicine, London, UK and *Glanz Research and Development Ltd, Ware, UK

Background and aim: In a rat model of indomethacin (IN)-induced jejunal ulceration, will undergo early shortening that may be due to contraction of villous smooth muscle. We investigated the effects of CL316243, a highly selective β₂-adrenoceptor agonist and smooth muscle relaxant, on this rat model.

Methods: Groups of rats received oral IN 15mg/kg preceded 0.5 h by oral CL316243 (doses = 0 and 0.01 to 10mg/kg). A control group received CL316243 10mg/kg alone. Jejunal ulceration was assessed 48 h after IN. Further groups of rats received CL316243 1mg/kg either 6 h prior to, or 3 or 6 h after, IN. Plasma IN levels were determined in groups of rats with and without prior CL316243 1mg/kg at 1, 2, 3, 6 and 48 h after IN. Early IN-induced histological changes (villous shortening) were determined at 2.3 and 6 h after IN with and without prior CL316243 1mg/kg.

Results: CL316243 (0.01 to 10mg/kg po) showed potent dose-dependent inhibition of IN-induced jejunal ulceration (more than 98% inhibition at doses of 0.1mg/kg; ED50 = 0.025mg/kg). CL316243 1mg/kg afforded highly significant protection (>90%) when given 6 h prior to and 3 h after IN but not when given 6 h after IN. CL316243 1mg/kg both significantly inhibited and completely reversed early shortening of villi by 6 h. CL316243 1mg/kg did not affect IN plasma levels at any of the selected times (40mg/kg 10mg/kg 1mg/kg 0.1mg/kg) but IN caused marked villous shortening that may not cause either gross or histological injury to the jejunal mucosa.

Conclusion: The β₂-adrenoceptor agonist CL316243 is a potent inhibitor of indomethacin-induced jejunal ulceration in the rat. It does not effect the bioavailability of indomethacin and the mechanism of action of this novel protectant appears to involve reversal of villous shortening which is an early, pre-ulcerative phase of indomethacin-induced injury.

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VALIDITY OF α-1 ANTITRYPSIN AS A TEST OF ENTERIC PROTEIN LOSS - UPPER GASTRO-INTESTINAL ORIGINS MORAN A, PANCHOZ M*, JONES AP*

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Background: Faecal α-1-antitrypsin (A1AT) is used as a measure of enteral protein loss as a screening test for colorectal cancer. This study evaluates the derivation of faecal A1AT from the upper gastro-intestinal tract.

METHODS AND RESULTS. A1AT was measured by radial immunodiffusion, and samples were not haptoglobin. Patients with an uncomplicated duodenal ulcer (DU) (n=16), gastric ulcer (GU) (n=11) or a normal gastro-duodenal mucosa (n=2) provided single faecal sample 6-8 days after endoscopy. Faecal A1AT concentrations the DU group (median, range [mg/g wet faecal weight]) 0.32, 0.05-0.89) and in the GU group (0.05-0.74). Only the DU group results were significantly greater (p=0.02) than faecal A1AT results from an asymptomatic control group (p=0.91) (0.01-0.69). 4 of the DU group had results greater than the 97.5% limit of the control group (0.58mg/g).

A1AT was substantially degraded in acidic solutions of pH 1.4 (73% of expected concentration after 4h at pH 1) and so A1AT was detected after 30min incubation in a solution at pH 2 containing 1mg/mL porcine pepsin. Samples of bile were obtained at ERCP if the bile duct was cannulated early and were obtained from 8 patients, some of whom had common bile duct stones. Median A1AT was 22mg/l (range 7.9-97mg/L), which represents a faecal A1AT concentration of 0.009mg/g. Faecal A1AT concentration (mg/mL) had a linear flow of 600ml/d, daily wet faecal weight of 150g and a 100% extraction of homogenised stool.

CONCLUSIONS. A1AT would normally be expected to be completely degraded in the stomach except at meal-times. Faecal A1AT concentrations are infrequently increased by the presence of uncomplicated gastric or duodenal ulcers. Bilary A1AT is unlikely to provide a major contribution to faecal A1AT in normal individuals.

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INCIDENCE AND ACTIVITIES OF SULPHATE-REDUCING BACTERIA IN PATIENTS WITH ULCERATIVE COLITIS. M. Pitcher, E.R. Beatty, G.R. Gibson, J.H. Cummings. MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge, CB2 2DH

Sulphate-reducing bacteria (SRB) are a major producer of hydrogen sulphide within the colonic environment. SRB may cause mucosal damage possibly through anaerobic sulphate reduction or sulphate toxicity. SRB are found in the colonic environment. This mechanism is thought to be characteristic of the colonising epithelial cell in ulcerative colitis (UC) and it has been postulated that SRB may be important in the pathogenesis of mucosal damage.

Anaerobic faecal slurry was prepared from 29 UC patients (20 active, 9 remission). 13 were neither SRB carriers nor had any faecal SRB and 10 healthy controls from which SRB were cultivated. SRB enrichment media. Sulphate was measured spectrophotometrically, sulphate by anion exchange chromatography with conductivity detection and sulphate-reduction by radiotracer estimation. Disease activity was graded clinically using a symptom score index and histologically from rectal and pouch biopsy material. Results were statistically assessed by analysis of variance. The incidence of SRB carriage in the active and remission UC groups was 95% and 55% respectively with mean log₁₀ countings wet weight faeces (SE) of 8.15 (0.7) and 5.02 (1.6) (p=0.041). All controls harboured SRB with counts of 7.9 (0.7) but these were not significantly different (p=0.61). 46% of the pouch group carried SRB but in lower counts of 3.64 (1.28) (p=0.011). Mean sulphate-reduction rates mmol/L (SE) in the UC groups were not significantly different from controls (0.13 (2.5)) as were median faecal sulphates mmol/L (SE) (0.24 (0.08)). Median faecal sulphate was significantly lower in pouches (0.09 (0.01)) compared to controls (0.81 (0.09)) (p=0.011). The ratio of total/mean faecal sulphate was higher in the UC group (0.75 (0.09) (p=0.009) and pouch group (1.03 (0.19) compared to controls (0.81 (0.09)) (p=0.011). It is concluded that SRB are most prevalent in patients with active UC although bacterial activities do not appear to differ from controls. The higher ratios of total/mean faecal sulphate in UC and pouch patients may reflect a decreased incorporation of sulphate into methanogenic metabolism and the availability of free sulphate for oxidative growth of SRB. The resultant luminal production of sulphide could trigger colonic inflammation in individuals with genetically determined defects in sulphide detoxification. (Acknowledgements to NACC, Peel Medical Research Trust & Addenbrooke’s NHS Trust Endowment Fund for financial support.)
COMPARISON OF 99M TECHNETIUM-HMPAO LABELED LEUCOCYTE WITH 111-INIIMUM LABELLED GRANULOCYTE SCANNING AND ULTRASOUND IN THE DIAGNOSIS OF INTRA-ABDOMINAL ABSCESSES.

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Fifty patients with suspected intra-abdominal abscesses were investigated prospectively with ultrasound and isotope scanning using 99mTc-HMPAO mixed leucocytes and 111-Indium granulocytes. 25 patients had inflammatory bowel disease; 21 had Crohn's disease (16 with a Crohn's mass) and 4 ulcerative colitis. The remainder were 9 patients with post operative fever and 16 with fever of unknown origin. An abscess was diagnosed when increasing focal activity on serial 111-Indium images at 1hr, 3hrs and 24hrs resulted in activity at least greater than liver activity at 24hrs. Using these criteria, 13 abscesses were diagnosed. 99mTc-HMPAO detected all 13 with 100% specificity. All 3 serial images were required. If the 1hr scan was negative, no later scans were needed. Bowel inflammation was easily distinguished from abscess using both isopetopes on serial images. Eight abscesses were corroborated by aspiration and four clinically; the remaining patient was later found to have a segment of necrotic liquefied small bowel. No additional abscesses were detected by ultrasound. 8 of the 13 abscesses were detected by ultrasound, (62% sensitivity, specificity 86%). Four cases of non-pyogenic intrabepic abscesses were not detected by white cell scanning while seen on ultrasound. 99mTc-HMPAO scanning is more sensitive and specific than ultrasound for abdominal abscess detection. It is as accurate as 111-Indium scanning, and has several advantages including cost, convenience, simplicity and superior image quality. Liver abscesses are best detected using ultrasound or by colloid subtraction scanning if white cell scanning is used.

HUMAN TERMINAL ILEAL MUCOSA EXPRESS NOVEL ANTIMICROBIAL ACTIVITY.

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There is a large resident population of bacteria in the normal human colon (estimated 10^{14} organisms) but the small intestine contains more than a million fold fewer microorganisms. Expression of antimicrobial activity by the terminal ileal mucosa may explain this difference.

Samples of normal terminal ileal mucosa were obtained from operation resection sections (8) and brain-dead organ donors (4). Aqueous acetic acid extracts of whole mucosa or EDTA-isolated epithelial cells were studied. The extracts were tested for antimicrobial activity before and after ultrafiltration (using 1 kD cut-off membranes), digestion with trypsin and in the presence of lysozyme. Radial diffusion and microtitre well antimicrobial assays were performed using a phyA mutant of Salmonella typhimurium and two strains of E. coli (ATCC 25922 & 29648).

Extracts from all the mucosal specimens demonstrated potent antimicrobial activity [mean(±SD) reduction in bacterial OD: 88.6(±13)%]. Following ultrafiltration, antimicrobial activity was present in the filtrate (<10 kD in size), but not retentate (>10 kD), of the mucosal extracts. The antimicrobial activity of the filtrates persisted after extensive dialysis (using 1 kD cut-off membranes) and was enhanced in the presence of lysozyme. Ultrafiltrates (>10 kD) of extracts of EDTA- isolated villus as well as crypt epithelial cells also demonstrated antimicrobial activity which was lost after proteolytic degradation with trypsin.

In conclusion, potent antimicrobial activity has been demonstrated in the human terminal ileal mucosa. It is likely to be mediated by peptides of 1-10 kD in size and localised to epithelial cells which may secrete them into the lumen. The mucosal antimicrobial activity has also been shown to be enhanced by lysozyme which is normally produced by Paneth cells.

IS TUBERCULOUS ENTERITIS IMPORTANT IN AFRICAN SLIM DISEASE?

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As HIV infection spreads across Africa, TB notifications have risen dramatically. Chronic diarrhoea has also become a major problem, so it is important to establish whether M. tuberculosis (MTB) is autochthonically involved. M. avium complex (MAC) in the gut is common in Western AIDS, but rarely reported in Africa.

We set out to assess the frequency of enteric carriage of mycobacteria in 69 Zambian AIDS patients with chronic diarrhoea. 120 stool cultures were studied for 6 months in Kirchner broth and on Lowenstein-Jensen medium after decontamination. 6 (8.7%) stools from 6 patients were positive: 2 for MAC and 4 for MTB. 2 of these patients (1 MAC, 1 MTB) were diagnosed as having active pulmonary TB, but neither were on treatment. Biopsies from distal duodenum were examined with Zielh-Neelsen stain and biopsies from patients with stool mycobacteria were cultured. One biopsy had a low number of recognisable acid-fast organisms, and the same biopsy grew MAC. Patients with intestinal mycobacterial carriage did not have more severe malnutrition than other patients with HIV related diarrhoea, and none had subclinical vilious atrophy.

In a parallel study performed in London, 10 (12%) cases of mycobacterial carriage were found in 81 HIV infected patients, but only 36% had diarrhoea. 7 were MAC, 1 MTB, and two environmental spp. (M. malmoense and a scotochromogen).

We conclude that mycobacterial carriage in AIDS related diarrhoea in Zambia occurred in 9% of patients; four cultures were MTB, and two MAC. Severe infiltrative MAC infection of the small bowel does not occur commonly in Africa despite its presence in the environment. The significance of intestinal MTB in 6% of cases is uncertain, but may well represent shedding of organisms from pulmonary infection.

THE EFFECTS OF CLOSTRIDIUM DIFFICILE TOXINS A AND B ON THE ACCUMULATION OF THE SECOND MESSENGER INOSITOL 1,4,5-TRISPHOSPHATE IN MEMBRANES PREPARED FROM HUMAN DUODENAL BIOPHES. *L Smith, #S Hyde, ##P Borriello, *R G Long. *Medical Research Centre, City Hospital Nottingham, UK; #Institute of Infection, Immunity and Division of Microbiology, Queens Medical Centre, Nottingham, UK.

The molecular events across a membrane immediately after toxin binding are unknown and are the subject of this study.

METHODS: Filtrates of media, which do not toxigenic (NDT) or toxigenic (B-1 and VPI) strains of C. difficile were grown, or purified toxin A (TxA) or B (TxB) were tested for their ability to affect the accumulation of inositol 1,4,5-trisphosphate (IP3) in membrane preparations, from human duodenal biopsies. IP3 was assayed by radioreceptor assay and the results expressed as nM IP3/mg membrane protein/assay. RESULTS: The filtrate from the toxigenic strains of C. difficile produced greater IP3 accumulation than the non-toxigenic strain, B-1 and VPI generated 338±57.1 (p=0.0018) and 344±52±73 (p=0.0097) respectively compared to 269±5±64.6 generated by M-1 (n=11). C. difficile toxins A and B, appear to have opposing effects on IP3 accumulation. When incubated at 37°C for 10mins 25μg/ml TxA increased the accumulation of IP3 by 42% (n=4). In contrast, an equivalent concentration of TxB reduced the accumulation of IP3 by 16.5% (n=8). This represents a net stimulation of 25.5% (42% stimulation-16.5% inhibition). This figure is consistent with the filtrate data which also shows a net stimulation of approximately 25%. Heat treatment of either toxin did not alter their effect on IP3 accumulation. TxA increased the accumulation of IP3 in a temperature, time and concentration dependent manner. The presence of 50μg/ml TxA, maximal IP3 accumulation was reached at 30mins at 37°C (125±5±54±6.0; 30±20.9; 0±5; n=5) and by 90mins at 4°C (9=179±9±8.0; 0=132±36±33.1; n=3). Thus greater stimulation above the respective zero time points was obtained at 4°C (693%) than at 37°C (50%). This is consistent with the known thermal binding characteristics of TxA to its receptor. CONCLUSIONS: These data demonstrate a significant effect of C. difficile toxins on IP3 production and to our knowledge represents the first example of enteric bacterial toxins subverting host phosphatidylinositol signal transduction mechanisms.
RECTAL CHALLENGE WITH HIGHLY PURIFIED ω-GLIADIN IDENTIFIES THE REPEAT OCTAPEPTIDE PQQPFFQQ AS AN IMMUNOPATHOLOGICAL MOIETY IN GLUTEN SENSITIZED (GS) SUBJECTS. M.N. Marsh*, A. Ensari*, C.M. Moore, R.J. Fido, A.S.Tatham. University Dept. Medicine, Hope Hospital, Salford, and 1ACR-Long Ashton Research Station, University of Bristol, UK.

For the few, previously reported studies concerning the in vivo activity of gluten antigens, comparative methodologies have involved ion-exchange chromatography (IEC), purity being judged by electrophoretic mobility. We re-investigated the immunopathogenicity of ω-gliadin using a fraction developed containing α-, β-, γ-gliadin. METHODS: White flour (cv. Merst) was defatted and albumins/globulins removed with 1M salt. Mixed gliadins were extracted with 70% ethanol and separated by carboxymethyl cellulose IEC. The ω-gliadin fraction was re-chromatographed by IEC, dialysed, freeze dried and passed over an activated thiol-Sepharose column to remove residual contaminating cysteine-containing α-, β-, γ-gliadins, which were later eluted with the addition of reducing agents. The highly pure ω-gliadin was dialysed and freeze dried. Rectal challenges were performed on two treated GS patients, each receiving 1.5g ω-gliadin in 30ml saline. Mucosal biopsies were obtained pre- and at 2, 5, 8 and 12 hours post-challenge. Frozen sections were reacted with either anti-CD3, or anti-γδ, monoclonal antibodies. Absolute populations of each cell type were quantitated per specimen, per 10⁵ μm² muscularis mucosa. RESULTS: Acid-PAGE analysis of the ω-gliadin fraction confirmed the absence of contaminating α-, β-, γ-gliadins. Secondly, monoclonal antibodies (Mabs) specific for sulfur (cystine)-containing gliadins failed to react to the ω-gliadin preparation, indicating that it was highly pure. The Mabs did recognize protein eluted from the thiol column with reducing agent, indicating that after two chromatography steps, ω-gliadin fractions are still contaminated with other gliadin species. After challenge, both subjects developed CD4(+), CD8(+) T-cell infiltrates into surface and crypt epithelium peaking at 8hr post-challenge, representing 100-150% rises on pre-challenge values. The slower γδ(+) 1EL2 responses peaked at 12hr post-challenge, comprising γδ(+) 300% increment on pre-challenge values. The γδ/CD3 ratio increased progressively throughout each experimental challenge. CONCLUSIONS: 1. Thiol-Sepharose chromatography yields highly purified ω-gliadins. 2. This highly-purified ω-fraction is immunogenic for GS mucosa. 3. ω-type prolamins are polymers of the consensus repeat PQQPFFQQ, suggesting that this motif embodies an immune-active epitope that is relevant to disease (GS) pathogenesis.

COELIAC DISEASE: MEASUREMENT OF A TOXIC WHEAT PEPTIDE IN GLUTEN-FREE FOODS.
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Coeliac disease is treated with a gluten-free diet that avoids wheat, barley and oat diets.

A sensitive, specific assay is required to detect trace quantities of gliadin present in "gluten-free" foods. Lack of knowledge of the amino acid sequence of the "coeliac-toxic" epitope has impeded the development of such an assay.

A recent study has identified a nineteen amino acid peptide which causes a release in the morphology of the small intestine of coeliac patients in remission. This peptide comprises nineteen amino acids corresponding to residues 31-49 of A-gliadin.

This synthetic peptide was used to raise murine monoclonal antibodies. Monoclonal antibody PN3 exhibits high titre to the prolamin fractions of wheat, rye, barley and oats and low molecular weight (LMW) gliutenins. There was no cross reactivity with prolamins from coeliac non-toxic rice, maize, millet and sorghum.

The monoclonal antibody PN3, was used to develop a double sandwich ELISA for measurement of gliadin in foods, including those nominally gluten-free foods that are based on wheat starch. The assay has a sensitivity of 1ng of wheat gliadin and can detect any gliadins were detected by the assay, but at lower sensitivity. The gliadin fractions of 16 varieties of wheat cross-reacted in the assay, suggesting that the epitope is widely distributed.

A synthetic peptide known to exacerbate CD has been used to produce monoclonal antibodies for use in a sensitive and specific assay for measurement of gluten in foods. The monoclonal antibody PN3, which was raised against a toxic gliadin peptide, cross reacts with LMW gliutenins. This suggests that the latter peptides may also be toxic to patients with coeliac disease.

OESOPHAGEAL MOTILITY DISORDERS SHOULD BE CLASSIFIED ON THE BASIS OF ABNORMAL NEUROPHYSIOLOGY RATHER THAN MANOMETRY.

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Introduction. The present classification of oesophageal motility disorders is largely descriptive and has little relevance to management.

Aim. To investigate neurophysiological abnormalities in patients with oesophageal motility disorders.

Methods. 12 healthy volunteers and 23 patients with manometric and radiological evidence of primary oesophageal motility disorders underwent graded intraoesophageal balloon distension (IOBD), cerebral evoked potential (CEP) measurement following oesophageal electrical stimulation and measurement of oesophageal electromyography following transcranial magnetic stimulation of the cortex i.e. the motor evoked potential (MEP).

Results. The following groups of abnormalities were found in symptomatic patients:
1. 6 patients (nonspecific motility disorder (NSMD)) - Proximal or distal spasm (ie spastic IOBD, normal CEP and MEP (intrinsic neuropathy)
2. 2 patients [ diffuse oesophageal spasm (DOS), myopathy] - Unresponsive to IOBD, normal CEP and MEP (intrinsic neuropathy or myopathy)
3. 4 patients (3 DOS, 1 NSMD)- Spastic IOBD, abnormal CEP and normal MEP (intrinsic neuropathy + possible afferent neuropathy)
4. 5 patients [achalasia] - Unresponsive to IOBD, abnormal CEP and MEP ( intrinsic extrinsic neuropathy)
5. 2 patients [NSMD] - Normal CEP, abnormal abnormal and MEP (extrinsic neuropathy)
6. 4 patients [3 NSMD, 1 nutcracker] - Normal IOBD and MEP but altered CEP threshold (Tnabnormal central processing).

Conclusion. Neurophysiological techniques demonstrating definite neuropathology in patients with oesophageal motility disorders. This may facilitate rational management.
AUTONOMIC FUNCTION TESTS IN FUNCTIONAL BOWEL DISORDERS. Hebben IM, Wilkinson SP. Gloucester Royal Hospital, Gloucester.

Previous work has demonstrated abnormal vagal function in reflux oesophagitis. Symptomatic reflux is common in patients with irritable bowel syndrome (IBS), and vagal dysfunction has also been demonstrated in this group. This study was designed to investigate autonomic function in patients with gastro-oesophageal reflux (GOR), IBS, and non-ulcer dyspepsia (NUD), or a combination, and to compare this with age and sex matched controls.

Method. Thirty patients with either endoscopically confirmed reflux oesophagitis, IBS as defined by the Rome criteria, NUD based on the typical symptom complex in the context of a negative endoscopy, or a combination, were recruited from the outpatient department. Five standard cardiovascular autonomic function tests were applied, and on the basis of the results patients were classified as having normal (N), early (E), definite (D), severe (S), or atypical (A) autonomic function. Thirty age and sex matched controls were assessed for comparison.

Results. Autonomic dysfunction was evident in all groups, including controls. Five of the 9 patients with GOR showed evidence of autonomic impairment (5E, 2D), as did 4 of the 21 patients with functional disorders (3E, 1D). Impaired vagal function was also evident in 7 of the control subjects (6E, 1D). There was no significant difference in the incidence of autonomic dysfunction between those with functional disorders 4/21 (19%) and controls 7/30 (23%) [p=0.76]. Although over half (5/9) of the GOR group had evidence of autonomic dysfunction, a statistically significant difference was not achieved [p=0.19].

Conclusions. Although there is a high incidence of vagal dysfunction in GOR disease, it occurs no more commonly in functional gut disorders than in the general population.

CAN MAGNETIC STIMULATION OF TRIGEMINAL AND VAGAL NERVE AFFERENTS BE USED TO ASSESS BRAINSTEM AND CORTICAL SWALLOWING PATHWAYS? S. Handy, Q. Aziz, A. Hobson, J. Barlow. Department of Medicine, Hope Hospital, University of Manchester.

Background: Cortical swallowing pathway characteristics have previously been described in healthy volunteers following magnetic stimulation of the motor cortex. Swallowing responses in animals have also been elicited by Trigeminal (T) or Vagal (V) afferent stimulation. In humans, however, the interactions between cortical and brainstem swallowing pathways following cranial nerve afferent stimulation are unexplored. Aims: To determine i), whether magnetic stimulation of T and V afferents excite brainstem swallowing pathways, ii), whether activation of these afferents modifies cortically evoked responses. Methods: 8 healthy volunteers were studied. Surface electrodes were placed on each mylohyoid muscle (M), and bipolar ring electrodes were positioned in the pharynx (P) and oesophagus (O). Magnetic stimulation was then applied at suprathreshold intensities to motor cortex, supraorbital nerve (T) and vagus nerve at the angle of the jaw (V), and the EMG responses from each of the three muscle groups recorded.

Protocol 1: Stimulation of either T or V afferents alone. Protocol 2: Cortical stimulation alone or following T or V stimulation at intervals varying from 5-500ms between the two stimuli. Results: Protocol 1: Two distinct responses were recorded in each of the three muscle groups: an early response, latency range 21.72-28.13ms: and a late response, latency range 50.08-93.9ms. Protocol 2: Cortical stimulation alone evoked EMG responses in the M, P, and O muscles, mean latencies 8.94, 9.11, and 10.16ms respectively. After either T or V afferent stimulation, the latencies of the cortically evoked responses shortened, the effect being maximal at an interval of 50ms, to 6.25, 7.21, and 8.26ms [p<0.05] respectively. Conclusions: Stimulation of cranial nerve afferents initiates early and late brainstem pathways to the oropharynx and oesophagus and when excited, facilitates the cortical pathways to these muscles. Magnetic stimulation can be used to non invasively assess the brain pathways to swallowing musculature and may provide a means for studying dysphagia secondary to neuromuscular disease.

ROLE OF GALLBLADDER (GB) MOTILITY DEFECTS IN PATHOGENESIS OF GALLSTONE RECURRENCE P Pazzi, RP Jazrawi, ML Petroni, S Gullini, TC Northfield Dept. of Medicine, St. George's Hospital Medical School London UK, and Dept. of Clinical Medicine, Ospedali S. Anna, Ferrara Italy.

Ultrasonography (US), and y-camera scintigraphy are not capable of fully assessing GB motility as the former measures net changes in GB volume (emptying-refilling), whilst the latter measures isotope ejection from GB (independent of refilling). We combined US and scintigraphy to determine new GB motor function such as postprandial refilling and turnover index which are both markedly impaired in gallstone patients. (RP Jazrawi et al., Gastroenterology, 1995: in press). Our aim was to assess these in patients with and without gallstone recurrence. We studied 11 patients with (R) and 11 without (NR) gallstone recurrence at least 24 months after complete gallstone dissolution and withdrawal of treatment, and 11 healthy controls (C). GB counts for tertHIDA and GB volumes were measured fasting and following a standard meal at 10 min intervals for 90 min. We calculated % refilling, turnover of bile and turnover index. Patients with recurrence (R) had larger [p<0.01] fasting GB volume (282±4 ml) than NR (192±3 ml) and C (161±1 ml). They also had reduced GB emptying [p<0.05] by scintigraphy but not US: reduced refilling [p<0.05], reduced bile turnover (p<0.05) and turnover index (1.72±1.4; p<0.01) compared to NR (3.12±1.5) and C (3.50±0.3) respectively. The NR group had less marked defects compared to C. One NR patient with a low turnover at study time developed recurrence 6 months later. We conclude that gallbladder motility abnormalities persist after gallstone dissolution and are more marked in those with recurrence: and that the turnover index may be valuable in determining those likely to develop recurrence.
Gut 1995; 36 (suppl 1) A67

SEEPAGE OF FAEces is a MARKer of INTERNAL ANAL SPHINCTER DYSFUNCTION.
A. S. Gee and P. Durdy. University Department of Surgery, Bristol Royal Infirmary, Bristol BS2 8BW

Inseensible seepage of faeces is distressing to incontinent patients. This study aimed to establish the physiological basis of seepage. Seventy-one consecutive patients with faecal incontinence were prospectively assessed by means of standard interview, physical examination and anorectal physiology. Forty-four (62%) patients complained of seepage. Both groups were similar with respect to age (62 (47-72) vs 51 (38-65) years, p>0.05*), sex (8 (36:6% vs 5:22 males, p=0.11), duration of symptoms (2.5 (1.5-5) vs 4 (2-7) years, p=0.3*), severity of incontinence (Cleveland Clinic Score 13 (10-16) vs. 15 (12-18), p>0.2*), and vaginal deliveries (2 (0-3) vs. 2 (0-4), p>0.3*). Those with seepage had lower resting anal canal pressures (26.1 (15-46.2) vs 37.6 (20.3-66.7) mmHg, p=0.3*). There was no difference between the groups with respect to anal electromanifedity (8 (6-15) vs. 8.5 (4.5-11) mV, p=0.3*), pudendal nerve terminal motor latency (2.3 (2.1-2.7) vs. 2.4 (2-2.7) msec, p>0.7*) or first sensation of rectal filling (50 (30-100) vs. 50 (40-60) ml, p>0.8*).

These data indicate that inseensible faecal loss is due to lower resting anal canal pressure secondary to impairment of internal anal sphincter function, and has no relation to impairment of rectal or anal sensation.

Figures are medians (interquartile range)

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ADEQUACY OF INFORMATION AND CONCERNs OF PATIENTS WITH JUVENILE ONSET IBD. HE Drummond, S Ghosh, A Ferguson, Department of Medicine, University of Edinburgh, Edinburgh EH4 2XU.

We have previously reported high morbidity in children with IBD. Little is known, however, about their perception of the information given to them and their concerns. Though concerns of adult IBD patients have been well investigated, there is paucity of similar studies in children with IBD. We explored these issues in a cohort of 35 juvenile onset IBD patients (23 Crohn's disease, 12 UC) diagnosed between 1984-88 derived from the Scottish Hospital Inpatient Statistics. The median age at onset was 12y (range 1-18y). Each patient was interviewed and answered a structured questionnaire. At the time of interview, the median age of the boys (16, CD, 5 UC) was 21y and the girls (4, CD, 7 UC) 17y (range 10y-31y).

Although 83% of patients felt well informed about their disease, 37% felt inadequately informed about their medications and side-effects and 47% of those operated upon felt inadequately informed about the nature of surgery. Only 39% were members of the NACC. Only 34% and 18% of patients considered that the nursing staff and GP respectively had contributed to their education. Five specific areas of concern were graded 1-5 by the patients and the grades of all patients were added (maximum possible score=175) to give the following cumulative scores for each area of concern: (a) possibility of their own children being affected=53, (b) economic/job prospects=52, (c) body image/self-esteem, (d) being treated as different to other children and (e) relationships=46. Twenty percent of patients did not admit to any specific area of concern.

In conclusion, juvenile onset IBD patients, though well informed about their disease, often feel that information about medications and surgery is inadequate. Previous surveys among NACC members showed that a trained nurse dedicated to the counselling and education of patients is considered useful. That only about a quarter of all patients had educational input from nursing staff highlights the general lack of availability of such trained personnel. Even at such a young age concerns about body image and personal relationships rank lower than economic/job prospects and the possibility of their own children being affected.

PSYCHOLOGICAL MORBIDITY IN INFLAMMATORY BOWEL DISEASE (IBD) - A CASE FOR PSYCHOLOGICAL COUNSELLING?
J D Smith, O Rose, S R MacRae

Quality of life is adversely affected by IBD. In previous studies we reported that physical disability and severe anxiety are commonly present but rarely reported by patients to their doctors. The extent of psychological morbidity and psychological coping mechanisms have not yet been adequately examined.

Fifty ulcerative colitis (UC) patients (aged 17-60 years, 25 females), fifty out-patients suffering from Crohn's disease (CD aged 16-84, 33 females and fifty healthy volunteers (aged 17-81, 27 females)undertook structured interviews and completed a range of questionnaires. 45% of CD and 32% of UC patients had active disease on presentation, 40% and 30% respectively felt that their social and occupational lifestyle had been disrupted by their disease.

Hospital Anxiety and Depression (HAD) scores recorded by IBD sufferers indicated significant anxiety levels, but not depression.42% of CD patients recorded case level anxiety as opposed to 36% of the UC group and only 16% in the volunteer group (p=0.5).

Significant psychological morbidity in IBD was directly linked to life events and to disease activity in many cases.

Using the "Attitudes and preference" questionnaire to examine the role of personality in dealing with stress, IBD sufferers had a tendency to over rehearse and to ruminate over emotionally distressing events. The "Styles and strategy" questionnaire demonstrates maladaptive coping mechanisms in dealing with the psychological stress related to their disease, a third of IBD patients showed some adaptive techniques and displayed emotional inhibition when dealing with their problems-recording scores of fifteen and above on the maladaptive strategies scale compared to the lower scores recorded by their healthy counterparts. There was no significant relationship between HAD scores recorded and abnormal coping mechanisms.

Psychological counselling and the use of stress management techniques increase the repertoire of behaviour available to patients with high level anxiety in IBD and may lead to an improvement in their overall quality of life.
Efficacy of percutaneous endoscopic gastrostomy versus nasogastric tube in enteral nutrition - a randomised trial


Introduction. We performed a randomised prospective trial comparing nasogastric tube feeding (NGT) versus percutaneous endoscopic gastrostomy (PEG) in order to evaluate complication rate and convenience of care of both methods in a general hospital population of patients needing enteral nutrition.

Methods. 109 patients who needed enteral nutrition were stratified in a neurological (n=50), ENT (n=50), and surgical group (n=50). In these three strata the patients were randomised, which resulted in 55 NGT and 54 PEG patients. All patients were visited each day. The complications were given a score of severity, and nurses and patients were asked to score for convenience of care and acceptability (1 = very good, 5 = very bad).

Results. In 7.3% of the NGT and 5.6% of the PEG group it was impossible to place the tube or the gastrostomy. Pulmonary aspiration was found in 8% of the NGT and 6% of the PEG group. In the NGT group, nasal decubitus and swallowing problems occurred in 14% and 24% respectively. In the PEG group, intraperitoneal bleeding and minor abdominal complaints were seen in 4% and 12% respectively. Fixation of the patient in order to achieve enteral nutrition was necessary in 10% of the PEG and 20% of the NGT group. The cumulative scoring of all complications was significantly lower in the PEG group (65) than the NGT group (86) (p<0.01). The convenience scores assessed by the nursing staff as well as by the patients were significantly better for PEG feeding versus NGT (1.9 vs. 2.4 and 1.9 vs. 2.3 respectively, both p<0.01).

Conclusion. In enteral nutrition, percutaneous endoscopic gastrostomy is preferable over nasogastric tube feeding concerning aspects of safety and convenience of care for both nursing staff and patients.

The basilic is the vein of choice for peripheral infusion of intravenous nutrition


Previous studies have focused on the role of the infusion, and the cannula, on the development of thrombophlebitis. Little emphasis has been placed on the choice of vein. Sixty-five patients received a standard complete feed via fine-bore catheters inserted into the most prominent patent vein in the antecubital fossa (ACF). Thirteen patients underwent ultrasonic measurement of the arm veins before catheterisation. The diameter of the venous lumen was measured in the ACF and 15 cm proximally (PROX).

Four catheters were removed inadvertently. One occluded with no clinical evidence of thrombophlebitis. Eight of 37 basilic vein catheters were associated with thrombophlebitis compared to 15 of 23 cephalic vein catheters (p = 0.0008, Fisher’s Exact Test). Lifetable analysis showed that basilic vein catheter survival was at all times greater than that of cephalic vein catheters (r = 11.33, p = 0.0001). The diameters (mm) of the venous lumens were:

<table>
<thead>
<tr>
<th>ACF</th>
<th>PROX</th>
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<tbody>
<tr>
<td>Cephalic 3.9 ± 1.3</td>
<td><em>3.1 ± 1.3</em></td>
</tr>
<tr>
<td>Basilic 4.7 ± 1.2</td>
<td><em>4.1 ± 1.2</em></td>
</tr>
<tr>
<td>p = 0.148</td>
<td>p = 0.006</td>
</tr>
</tbody>
</table>

(mean ± standard deviation, Student’s paired t tests)

The data suggest that the basilic is the peripheral vein of choice for infusion of intravenous nutrition via fine-bore catheters.

NUTRITIONAL INTAKE IN PATIENTS ADMITTED WITH ACUTE STROKE

Hussain, G. DOig, J. C. Cox. Wansbeck General Hospital, Woodhorn Lane, Ashington, Northumberland, NE63 9JJ

Patients with acute stroke have a reduced nutritional intake because of dysphagia (usually 30% at 24 hours). An increased basal metabolic rate with additional requirements for nutrition compounds the problem. Moreover, elderly patients are more likely than younger to be malnourished on admission to hospital.

Data was collected for 18 consecutive patients admitted with acute stroke (M7, F11; mean age 77). Twelve had dysphagia at presentation, all of which were kept nil by mouth and received intravenous fluids. Eight of these 12 went on to receive Percutaneous Endoscopic Gastrostomy (PEG) feeding at 2-3 weeks (mean 17 days), swallowing having recovered in the other four by the end of the first week. The average energy intake in the 8 patients who had IV fluids prior to PEG feeding was 400 Kcal, those four able to eat normally received 900 Kcal. This compared with 2000 Kcal in patients with PEG feeding (estimated average requirements for energy in that age group, 1800-2100 Kcal).

Conclusions: i) Not a single patient had adequate nutrition by 17 days even if swallowing had recovered, despite the catabolic nature of the illness, ii) Enteral feeding was introduced via PEG late after stroke. At two weeks there were negative energy balances of 2200-3000 Kcal, iii) PEG feeding could have been considered for short term nutritional support for those whose swallowing did not recover, or supplementation for those patients whose swallowing had recovered, iv) There is a need for a structured approach to provide nutritional support to stroke patients who cannot swallow.
AUDIT OF THE PROVISION OF ARTIFICIAL NUTRITION SUPPORT (ANS). J.P. McWhirter, K Hillj, J Richards2, CR Pennington. Departments of Clinical Pharmacology, Dietetics1, and, Pharmacy2, Ninewells Hospital, Dundee.

The Nutrition Advisory Group (NAG) compiled guidelines which were used as the standard against which to audit the extent goals were set and achieved and to record complications of ANS.

Adults receiving ANS in hospital and at home were studied over a 6 month period. Nutritional status was determined at the start of the feeding period, and at the end in patients (pts) supported for ≥7 days. The aim of NS was defined for each pt. Retrospectively calculated energy requirements were compared to the prescription and delivery of ANS. Outcomes and complications were documented.

167 pts were recorded. 108 received EN, 84 in hospital (mean 18.3 days, range 1-80), 24 at home (20 not available for follow up study). 49 received PN in hospital (mean 17.3 days, range 1-60). 10 were supported at home throughout. Nutritional status was determined in 118/147 pts (80%). The aim of ANS was to maintain the nutritional status of 56% (55 EN, 27 PN) and to improve that of 44% (34 EN, 31 PN). 91(62%) were recommenced at the end of the feeding period. EN achieved the aim of ANS in 37/88 (42%), failed in 13 (15%). The outcome was unknown in 38 (43%) because of death, transfer or early cessation. With PN, the aim was achieved in 28/59 (48%), failed in 13 (22%) and unknown in 18 (30%).

The mean % of requirements prescribed, % of prescriptions delivered, and % requirements delivered for EN and PN were 91, 95: 87, 85 and 84, 80%. Complications occurred in 69/147 (47%) pts resulting in loss of 2.3% feeding time, 92 hours tube related (22 pts); 48 hours gastrointestinal intoleration (11 pts); and 376 hours due to delivery problems (16 pts). PN complications resulted in 747 lost hours by 19/53 pts (mean 39). Catheter related sepsis (5 pts); delivery problems (3 pts); 2 metabolic (48 hours lost).

This study suggests a need for an interventional role of the NAG in the form of a nutrition support team.

JP McWhirter is supported by Clinitec Nutrition Ltd.

CHANGE IN USER'S PERSPECTIVE OF PERCUTANEOUS ENDOSCOPIC GASTROSTOMY (PEG) AFTER ADOPTION OF A TEAM APPROACH. S. Ghosh, A. Sawyer, F. Phillips, R. Yoll, N. Eastwood. GI Unit, Western General Hospital, Edinburgh EH4 2XU.

An audit of 162 patients receiving PEG feeding compared with nonanastomotic (NS) feeding identified leakage and infections as major problems of PEG feeding. The majority of respondents needed further training on PEG feeding compared with NG feeding. To rectify these problems, a team approach was adopted. The team consisting of gastroenterologists, dietitians, speech therapists, nursing staff and endoscopists met monthly to discuss the problems identified with PEG feeding.

The patient/carers' perception of PEG and NG feeding was assessed by a questionnaire containing 10 items: tolerance, interruptions, cosmetic aspects, time for care, need for professional help, leakage, severity and site of leakage, degree of problems with infection, pain and leakage and overall contribution to enteral nutrition.

The inclusion criteria for the audit was a minimum of 3 weeks of NG feeding followed by at least a month of PEG feeding. Only patients cared at home were considered for the audit and hospital/nursing home patients were excluded. The initial audit performed in 1992 included 18 patients who had PEG inserted in 1991-92. The PEG nutrition team became operational in 1993 and a further audit was performed in 1994 which included 9 patients who had PEG inserted in 1993-94. The reason for fewer patients in the recent audit was earlier referral for PEG insertion so that few had enough experience of NG feeding for adequate comparison. In the 1992 audit 81% of respondents considered leakage a problem with NG compared with PEG; however, in the 1994 audit, this proportion had dropped to 46%. 15/16 patients in the 1992 audit had leakage problems compared with 3/9 in the 1994 audit (p<0.01). In the 1992 audit, 81% of respondents had problems with infections at the PEG insertion site; in contrast, in the 1994 audit, only 44% had infective complications. Only 20% of patients needed professional assistance more frequently with PEG feeding compared with NG feeding in the 1992 audit; this proportion too had dropped to 22% in the 1994 audit. The improvement could not be attributed to use of any particular type of PEG tube. Both audits yielded similar response to the other items in the questionnaire.

In conclusion, this small audit lends support to the usefulness of a PEG nutrition team in improving the quality of service as assessed by the users.


EFFECT OF ENTERAL FEEDING ON POST NATAL SPLENIC HAEMODYNAMICS IN HUMAN PRETERM NEONATES. A.J.P. Lane*, R.C. Coons**, D.H. Evans– and R.J. Levin†. Departments of Neonatology* and Biomedical Science†, University of Sheffield and Department of Medical Physics*, University of Leicester.

We have used pulsed Doppler ultrasound to examine the effect of enteral feeding on human preterm splanchic blood flow velocities over the first 10 postnatal days. Velocity recordings were made from the coeliac axis (CA) and superior mesenteric artery (SMA) of 46 preterm neonates (birthweight 1.50±0.5kg; gestational age 31±2.9 weeks). In two groups matched for birthweight and gestational age, 15 babies received total parenteral nutrition (TPN) and 31 babies were fed enterally from birth (day 0) to day 10.

Babies receiving enteral feeds had significantly higher CA peak systolic velocity (PSV, cm sec-1) than SMA PSV on the first 3 postnatal days after which there was no significant difference, supporting previous observations (Lane et al. J. Physiol. 479 P 25P). The TPN babies showed the same pattern but with different values. The CA PSV is significantly (p<0.001) higher than the SMA PSV on days 0 (85±2 vs. 30±2), 1 (85±4 vs. 47±6), 2 (83±11 vs. 44±8) and 3 (76±9 vs. 42±5), but in this group this difference remains significant (p<0.05) over days 4–10. This is due to both an increase in CA PSV and a decrease in SMA PSV in TPN babies over days 0–10 compared to those fed enterally.

The ratio of PSV between the two vessels is an index of relative downstream vascular resistance in the SMA. In TPN babies the ratio decreases from birth to day 1 and remains higher than babies fed enterally. These results suggest that the postnatal changes in peak systolic velocity in the two splanchnic vessels occur irrespective of the method of feeding but are greater in enterally fed babies. This implies that other factors, as well as enteral feeding are responsible for these changes.

This research was funded in part by Milupa.

SMALL INTESTINAL WATER MOVEMENT IN BASAL AND CHOLELITHA TOXIN INDUCED SECRETORY STATES AFTER 5-HT DEPLETION. J. Turvill, F. Mourad, D. Perrett, M. G. Farthing. Digestive Diseases Research Centre, Medical College of St Bartholomew's Hospital, London, UK.

5-Hydroxytryptamine (5-HT) is a potent intestinal secretagogue. It is released from enterochromaffin cells and is implicated in cholera toxin (CT) induced secretion. para-Chlorophenylalanine (PCPA) selectively inhibits tryptophan hydroxylase, the enzyme acting at the rate limiting step of 5-HT synthesis. We sought to assess whether depletion of small intestinal 5-HT by pretreatment with PCPA would effect basal water movement and CT induced secretion.

Adult male Wistar rats (180-220g) were gavaged daily for 3 days with either 500mg/kg PCPA in 1ml water or water alone (control). 75µg CT or saline was then instilled into isolated whole small intestine. After 2h, in situ perfusion was performed with plasma electrolyte solution (Na 140, K 4, Cl 104, HCO3 40mmol/L) to assess net water and electrolyte movement. At the end of the experiment 5-HT levels were determined in freeze-clamped, full thickness segments of small intestine by high performance liquid chromatography with fluorometric detection.

Table 5-HT levels in controls (median 73pmol/mg dry weight [interquartile range 35.8 to 80], n=11) were significantly decreased by PCPA pretreatment (6 [4 to 7] vs 11 [0 to 11], p<0.01). CT reduced total tissue 5-HT levels in the controls (32 [25.5 to 38.5], n=12, p<0.01) but not in PCPA pretreated rats (4 [6 to 3.5], n=6). Net water absorption in controls (50 [41 to 61], n=8) was enhanced by PCPA (21µl/mm²/g [74.5 to 85], n=8, p<0.05). In addition, CT-induced net water secretion (-75.2 [-58 to -99], n=11) was reduced by pretreatment with PCPA (-38.3 [-34.3 to -42], n=6, p<0.05). Electrolyte movement paralleled water.

These findings support a role for 5-HT in mediating CT induced secretion. The failure of PCPA pretreatment to completely reverse secretion may be explained by the efficacy of sub-maximal levels of 5-HT in the secretory state. That 5-HT depletion enhances water absorption in normal intestine indicates a role for 5-HT in basal water transport.
INTESTINAL DYSMOTILITY IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) SEROPOSITIVE SUBJECTS WITH DIARRHEA. P.J. Neild, P J Evans*, F D Castello*, D L Williams*, B G Gazzard. HIV Unit, Chelsea and Westminster Hospital and *Gl Science Research Unit, London Hospital Medical College, London, UK.

Chronic diarrhoea is a common and debilitating problem in Human Immunodeficiency Virus seropositive (HIV+) individuals and its pathogenesis remains poorly understood. There is evidence of submucosal enteric nerve disruption and, in addition, HIV infection is associated with autonomic dysfunction. In this study we tested the hypothesis that, in HIV+ individuals with diarrhoea, gut motility is altered as a result of neurological dysfunction.

Methods: 8 HIV+ subjects with chronic diarrhoea and 17 healthy controls were studied by intraluminal ambulatory manometry. A triple sensor strain gauge catheter was introduced transanally and positioned fluoroscopically at the level of the Ligament of Treitz. Pressure was recorded for 24 hours using a miniature solid-state data logger. Data was downloaded to diskettes and we analysed, using validated software, the 8 hour nocturnal fasting period, and the response to meals.

Results: The number of migrating motor complexes (MMC's) during the nocturnal fasting period was increased in HIV+ subjects, compared with controls. The amplitude of MMC Phase II (P2) was decreased, though frequency and duration were the same. The duration of MMC Phase III (P3) was decreased in HIV+ subjects, but amplitude, frequency and propagation velocity were not affected. The time from meal to the first P3 was decreased in HIV subjects.

<table>
<thead>
<tr>
<th>Median (interquartile)</th>
<th>Control</th>
<th>HIV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>P3 amplitude (mmHg)</td>
<td>17 (15-18)</td>
<td>13 (13-13.5)**</td>
</tr>
<tr>
<td>Meal to P3/min</td>
<td>327 (276-375)</td>
<td>225 (87-300)**</td>
</tr>
<tr>
<td>No. of MMC/Ce/hr</td>
<td>4 (3-5)</td>
<td>7 (5-9)*</td>
</tr>
<tr>
<td>P3 duration (mins)</td>
<td>5.6 (3.7-7.2)</td>
<td>4.6 (4.5-4.7)*</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.005 (Mann-Whitney U Test)

Conclusion: There are significant differences in upper GI motility between HIV+ subjects with diarrhoea and healthy controls, both in the fasting and fed state. In neuropathy of the myenteric plexus, diminished incidence, propagation and slow migration of P3 are prominent. In contrast, P3 is relatively normal in HIV+ subjects, but the impaired response to food, similar to that reported in vagotomized patients, suggests an extrinsic autonomic neuropathy.

SMALL BOWEL TRANSIT IN HIV-SEROPOSITIVE INDIVIDUALS. D Sharpestone, P Neild, RS Crane*, IS Menzies*, H Bjarnason#, B Gazzard. Departments of HIV/GUM, Chelsea and Westminster Hospital, Chemical Pathology*, St Thomas's Hospital and Clinical Biochemistry#, Kings College Hospital.

Aim: Diarrhoea occurs in up to 90% of HIV-seropositive individuals, yet there are no studies examining small bowel transit.

Methods: 49 subjects were recruited (20 AIDS no diarrhoea, 19 cryptosporidiosis and/or microsporidiosis, 5 pathogen-negative diarrhoea and 5 with CMV colitis). 19 healthy HIV-negative volunteers acted as controls. Small bowel transit time was calculated by subtracting oro-cecal transit time, measured using salazopyrine suspension (3g), from gastric emptying, using 3-0-m-glucose (5g).

Results:

<table>
<thead>
<tr>
<th></th>
<th>Small bowel transit (mins) mean±sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>250 ± 54</td>
</tr>
<tr>
<td>AIDS-no diarrhoea</td>
<td>263 ± 71</td>
</tr>
<tr>
<td>AIDS-crypto/micro</td>
<td>156 ± 89*</td>
</tr>
<tr>
<td>AIDS-path-neg</td>
<td>144 ± 62*</td>
</tr>
<tr>
<td>AIDS-CMV colitis</td>
<td>276 ± 91</td>
</tr>
</tbody>
</table>

*p<0.05 compared to controls.

Conclusion: Small bowel transit time is markedly decreased in protozoal and pathogen-negative diarrhoea but is normal in AIDS patients free from diarrhoea and in CMV colitis.

BRUSH-CYTOLGY: A RELIABLE METHOD FOR DETECTING HELICOBACTER PYLORI GASTRIC COLONIZATION. M. Dalla Libera, P. Pasti, G. Carli, R. Scognamillo, S. Gamberini, A. Merighi, P. Rici, S. Gullini. Department of Gastroenterology, St Anna Hospital, Ferrara, Italy.

Helicobacter pylori (Hp) gastric colonization can be detected by a variety of methods. The most popular and practical diagnostic method is histology, but it is relatively expensive and time-consuming, and theotty distribution of Hp on the gastric mucosa is a possible source of error. Brush-cytology, involving a wider surface of mucosa, seems a more sensitive technique. During gastroscopy, specimens are taken by a re-usable cytology brush, from a wide area of antral mucosa (multiple rubbing from the pyloric ring up to incisura). Brushings are then smeared onto glass slides, air-dried, and, after staining with 1% Methylene blue, observed under optical microscope with 10x and then with 70-100x lens.

The overall procedure, from brushing to microscopic observation, requires no more than 15 min. We compared the effectiveness of brush cytology with histology in detecting Hp in 307 consecutive patients undergoing elective gastroscopy. Patients could be divided in 3 groups: A: 89 with active duodenal ulcer, without previous medical treatment; B: 115 with duodenal ulcer after treatment with Omeprazole (75) or Ranitidine (40); C: 103 with non-ulcer dyspepsia. In all patients, after brushing, were taken two biopsies from the antrum and two from the body. The overall frequency of Hp detected by brush-cytology was significantly higher compared with histology (76% vs 60%, p<0.001). Cytology and histology did not show any significant differences in sensitivity in group A (p=0.052), whereas cytology was more sensitive than histology in group C (p=0.01) and group B-patients treated with Omeprazole (75% vs 51%, p<0.01), but not in patients treated with Ranitidine. The overall inter-observer agreement in brush-cytology was 94% (kappa coefficient =0.86). Culture of brushing material from 26 consecutive patients confirmed the specificity of Hp morphological detection. In conclusion, the cytological brushing seems a simple, cheap, fast and practical test to aid diagnosis of Hp gastric colonization. It is more sensitive than histology, particularly when Hp gastric density is low (i.e. after treatment with drugs which inhibit Hp).

PRE-CUT PAPILLIOM: DANGEROUS DESIRE OR DEFINITIVE DEVICE. Dr. Derrick F. Martin. Department of Radiology, Withington Hospital, South Manchester University Hospitals NHS Trust, Manchester M20 2LR.

Pre-cut papillotomy is a technique used during ERCP to gain deep access to the bile duct when this cannot be achieved by simple cannulation of the papillary orifice. Many endoscopists find pre-cut a valuable technique, but some are ambivalent as there is a perception that its use may expose the patient to significant risk. This concern persists despite published results from this hospital and elsewhere, that the technique allows immediate access to the bile duct in 70% and delayed access in 70% of the remainder, with a complication rate similar to that of endoscopic sphincterotomy. In patients likely to have disease amenable to endoscopic therapy, pre-cut papillotomy usually allows success in the face of potential failure.

This short video describes the author's technique based on an understanding of the anatomy of the ampullary segment, illustrating this graphically and with a number of video demonstrations of the technique, including the equipment necessary for its performance. The success rate is recorded, as are complications and methods for their avoidance.
DILATATION OF BENIGN PEPTIC STRICTURES.
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The aim of this video is to demonstrate the technique of dilating benign oesophageal strictures using semi rigid dilators (KAD) after flexible OGD and the placement of a guidewire. The target audience for whom the video is produced would include trainee endoscopists, endoscopy nurse assistants and nurses on gastroenterology or day wards. However, the film is designed to have broad educational applications. The actual technique of dilatation is discussed and demonstrated in detail but emphasis is placed on patients safety and comfort. The close involvement of the endoscopy nurses before, during and after the procedure, their expertise and confidence is demonstrated. The video attempts to show that well practised team work is likely to guarantee high quality service.

CRYOTHERAPY AND RESECTION FOR ADVANCED LIVER CARCINOID TUMOUR
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The symptomatic control of patients with metastatic hepatic carcinoid often poses a difficult management problem. Although many patients benefit from somatostatin analogue therapy, a significant number of patients fail to respond to medical management and may benefit from liver surgery.

The video demonstrates the combination of surgical resection of a large carcinoid metastasis in the quadrate lobe of the liver with hepatic cryotherapy to a number of other smaller metastases elsewhere in both lobes. The patient originally underwent pneumonectomy for primary bronchogenic carcinoid and subsequently failed to respond to somatostatin therapy. Following resection, she was clinically and biochemically restored to normality. The video places great emphasis on the techniques of hepatic cryotherapy and liver resection.

In conclusion, we recommend this approach for the symptomatic control of patients who fail to respond to medical management.