The British Society of Gastroenterology

A 5 YEAR AUDIT OF SPHINCTER REPAIR (1988 - 93)
N. Nikiteas, M R B Keighley, Queen Elizabeth Hospital, Birmingham

We decided to audit the results of sphincter repair because of a result of a change of surgical policy: All patients had a complete sphincter defect and all patients had been followed up for more than 6 months.

Sphincter repair was performed in 6 men: 3 posterior defects associated with fistula operations and 3 anterior defects from perineal trauma associated with urethral injury and pelvic fractures. Only one of the 3 posterior repairs was associated with any residual minor incontinence, which was cured by graciloplasty. All of the anterior defects had required a stoma at the time of injury; despite this and complete reconstruction of the anterior rectum, only one patient is continent.

Eighteen women had sphincter repairs, one was a lateral repair from previous fistula surgery with a good result. The remaining 17 defects were anterior reconstructions and included anterior levatorplasty, Z-plasty as well as sphincter repair. Only 2 had a covering stoma (both delayed for 48 hours). Seven patients had persistent incontinence (major 3, minor 4) and two have had successful repeat repairs. Factors in this female group associated with persistent incontinence were age over 50 years (3 of 4), obesity (2 of 3), post-operative sepsis (3 of 4) and gross perineal descent (5 of 8).

These results raise the role of a stoma, particularly in obese and elderly women having sphincter repair.

APPENDICECTOMY, TONSILLECTOMY AND INFLAMMATORY BOWEL DISEASE
J E Smithson, G Radford-Smith and DP Jewell
Department of Gastroenterology, Radcliffe Infirmary, Oxford

Aims. Recent studies have indicated an inverse relationship between appendicectomy and ulcerative colitis (UC). The explanation for this is not clear but it has been suggested that removal of the appendix with its associated lymphoid tissue may protect against subsequent development of UC. However we hypothesized that factors which promote appendicitis may be protective rather than appendicectomy per se. Therefore the aim of the present study was to determine the frequency of primary appendicectomy in Oxfordshire patients with inflammatory bowel disease (IBD). The frequency of tonsillectomy was also examined.

Methods. A prospective questionnaire-based survey of 197 consecutive patients with UC (mean age 50.4) and 117 patients with Crohn's Disease (mean age 41.3) was carried out. Primary appendicectomy was defined as operation for 'suspected appendicitis'. Two hundred and forty three unsellected dermatology outpatients (mean age 43.3) at a neighbouring hospital acted as a control population.

Results. Primary appendicectomy was significantly less common amongst UC patients than controls (age/sex adjusted odds ratio 0.20, confidence limits 0.07-0.53, p=0.0005, Mantel-Haenszel test) but not reduced in patients with Crohn's disease (CD). Of the 7 UC patients who had had a primary appendicectomy, the operation was carried out in 5 before the onset of their disease. The frequency of tonsillectomy in both groups of IBD patients was no different from the controls. However tonsillectomy was significantly more common in CD patients who had undergone colorectectomy (7/10) compared to those who had had other resections or no surgery at all (23/107, p=0.005, Fisher's exact test, two-tailed).

Conclusions. These results are consistent with the hypothesis that factors which promote appendicitis may protect against UC. Alternatively the failure to develop appendicitis may confer later susceptibility to UC. For patients with CD, prior tonsillectomy may be a risk factor for subsequent coclectomy.

INHERITED COAGULOPATHIES PROTECT AGAINST INFLAMMATORY BOWEL DISEASE
NP Thompson, AJ Wakefield, RE Pounder
Inflammatory Bowel Disease Study Group, Royal Free Hospital School of Medicine, Rowland Hill St, London NW3

Introduction: Only 3 patients with ulcerative colitis and 1 patient with Crohn's disease are reported occurring in association with haemophilia. Histological studies have revealed mesenteric vessel microthrombs in both Crohn's and ulcerative colitis, and there is haematological evidence of a persistent activation of coagulation in those with inflammatory bowel disease (IBD). Heparin has been reported as being beneficial in ulcerative colitis. This study tested the hypothesis that concurrent IBD and an inherited disorder of coagulation is a rare event.

Methods: 9,562 patients with haemophilia A or B, or von Willebrand's disease, are managed by 129 UK Haemophilia Directors. All Directors were contacted by questionnaire or telephone to determine if they were either caring for or had cared for a patient with IBD and haemophilia or von Willebrand's. Those responding positively were sent a second questionnaire to ascertain further details. An expected number of IBD in this population was determined using data from all 5 UK studies with age-specific data. Significance between observed and expected numbers was tested using Chi-squared with Yates correction.

Results: 13 patients with IBD were identified. 4 had Crohn's disease compared with an expected 1.5 (95% CI 0.2-5.6, p=0.12). 9 had ulcerative colitis compared with an expected 19.43 (95% CI 0.025) (p<0.005). 7 had IBD in the first degree relative. The difference was significant only in Crohn's disease (p<0.02). No patient had haemoglobinopathy.

Discussion: These results are consistent with an increased frequency of IBD in patients with haemophilia or von Willebrand's disease. The increased risk is greatest in those with Crohn's disease. Conclusion: An increased frequency of IBD in those with haemophilia or von Willebrand's disease is not observed.

Patients were identified from the UK Crohn's disease and IBD registry (3500 cases) by questionnaire.

CONCLUSION: The reported cases are too few to be conclusive but the hypothesis that patients with IBD and haemophilia or von Willebrand's have a higher frequency of IBD is supported by this study.

Inflammatory bowel disease

T110

APPENDICECTOMY, TONSILLECTOMY AND INFLAMMATORY BOWEL DISEASE

J E Smithson, G Radford-Smith and DP Jewell
Department of Gastroenterology, Radcliffe Infirmary, Oxford

Aims. Recent studies have indicated an inverse relationship between appendicectomy and ulcerative colitis (UC). The explanation for this is not clear but it has been suggested that removal of the appendix with its associated lymphoid tissue may protect against subsequent development of UC. However we hypothesized that factors which promote appendicitis may be protective rather than appendicectomy per se. Therefore the aim of the present study was to determine the frequency of primary appendicectomy in Oxfordshire patients with inflammatory bowel disease (IBD). The frequency of tonsillectomy was also examined.

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Conclusions. These results are consistent with the hypothesis that factors which promote appendicitis may protect against UC. Alternatively the failure to develop appendicitis may confer later susceptibility to UC. For patients with CD, prior tonsillectomy may be a risk factor for subsequent coclectomy.

GI Science Research Unit, The Royal London Hospital and *Department of Gastroenterology, St Mark's Hospital, London.

Thromboxane A2 may be an important inflammatory mediator in Crohn's disease (CD). Antiplatelet agents may play a pathogenic role in multifocal microvascular infarction in CD. Picotamide (Sandoz, Italy), a thromboxane receptor antagonist / synthesis inhibitor, is widely used in Italy as an antiplatelet agent, in prophylaxis of ischaemic heart and peripheral vascular disease. The aim of this study was to assess the therapeutic effect of picotamide in active CD.

Methods: 9 outpatients with active CD (CDAI 150-300) who had not received steroids or changes in other medication during the previous 4 weeks, were treated with oral picotamide (600 mg bd) for 6 weeks. Progress was assessed by clinical and laboratory indices.

RESULTS: (given as medians (IQR), P values derived from paired t tests for week 0 vs week 6 scores or values): score / value week 0 week 6 P n 9 9 8 CDAI 231(203-293) 189(112-229) 172(173-181) 136(61-187) 0.001 stools / week 31(26-36) 26(33-30) 18(20-22) 16(26-29) 0.01 abdominal pain 116(12) 52(9) 20(5) 10(5) 0.006 well-being 97(14) 63(10) 21(6) 20(5) 0.002 platelet count 299(239-415) 346(266-397) 323(248-399) 334(231-401) 0.1 NR CRP ng/ml 23(35-38) 15(5-23) 24(35-9) 21(5-34) 0.01 albumin g/dl 42(38-42) 41(38-43) 43(41-43) 41(39-43) 0.1

1 patient was withdrawn at week 4 with no improvement in symptoms and a fall in serum albumin. 5 patients entered remission (CDAI<150) during treatment and 1 further patient had done so by follow-up 6 weeks after treatment. 5 of these 6 patients remained well after 7 months (4-9) follow-up. 2/8 patients who completed treatment later required surgical intervention (obscure drainage, right hemicolectomy for ileal stricture).

No adverse events occurred.

CONCLUSION: Picotamide safely ameliorates symptoms in ambulant patients with active CD; this effect is maintained after cessation of therapy. A controlled study is warranted.
AN OPEN TRIAL OF ANTIOXIDANT NUTRIENT THERAPY IN ACTIVE ULCERATIVE COLITIS

AD Miller, DR Blake*, DS Rampton. GI Science and Bone and Joint Research Units, The London Hospital Medical College, Whitechapel, London E1.

Ulcerative colitis (UC) is associated with excessive mucosal production of damaging reactive oxygen species (ROS). Antioxidant therapy may lessen disease activity by reducing excess ROS production.

Method: Seven patients with active UC were given oral antioxidants (β-carotene (1500 units), ascorbate (540mg), α-tocopherol (270 units), selenium (100μg) and methionine (500mg)) daily for two weeks. 47 patients were on aminosalicylates at entry, 1/7 on additional azathioprine and 2/7 on no treatment. Patients were assessed using clinical, histological and laboratory criteria. Five continued antioxidants for 4-8 weeks. Rectal mucosal ROS production was assessed by chemiluminescence (CL).

Results: Shown as median and interquartile range:

<table>
<thead>
<tr>
<th>Score/Value</th>
<th>Week 0</th>
<th>Week 1</th>
<th>Week 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid/soft stool (av/day)</td>
<td>6.2(2-8.7)</td>
<td>3.9(0.8-8.6)*</td>
<td>2.3(3.3-2.2)*</td>
</tr>
<tr>
<td>Stool frequency (av/day)</td>
<td>6.9(3.1-10.8)</td>
<td>5.9(2.9-8.9)</td>
<td>3.7(2.7-6.3)</td>
</tr>
<tr>
<td>Bloody stools (av/day)</td>
<td>6.4(1.2-8.9)</td>
<td>1.6(1.3-5.1)</td>
<td>1.1(0.6-3.5)*</td>
</tr>
<tr>
<td>Sigmoidoscopic score</td>
<td>1.0(2-2)</td>
<td>1.0(1-2)</td>
<td>1.0(2-2)</td>
</tr>
<tr>
<td>ESR</td>
<td>1.0(2-9)</td>
<td>20(14-25)</td>
<td>24(14-29)</td>
</tr>
<tr>
<td>Histological score</td>
<td>6.5(6-9)</td>
<td>-</td>
<td>9.5(7-10)</td>
</tr>
<tr>
<td>CL (Kphotons/mg/min)</td>
<td>53(10-113)</td>
<td>-</td>
<td>43(28-192)</td>
</tr>
</tbody>
</table>

* P < 0.05 = Week 0 by t test + P < 0.05 by ANOVA. Haemoglobin and albumin were unchanged. Two patients achieved remission after two weeks' antioxidant therapy. Four patients continuing antioxidants maintained clinical improvement after 4-8 weeks but did not achieve full remission.

Conclusions: (1) Antioxidant nutrient therapy for two weeks in active UC improves stool consistency, rectal bleeding and sigmoidoscopic score, but in this pilot study no benefit was seen in histology nor mucosal ROS production. (2) The clinical response to antioxidants suggests that ROS may be pathogenic in UC. (3) Controlled studies of antioxidant therapy in active UC are needed.

A REALLY USEFUL SIMPLE COLITIS ACTIVITY INDEX.

R S Walmley, R Ayres, R N Allan. Gastroenterology Unit, Queen Elizabeth Hospital, Birmingham.

Numerous Activity Indices for Ulcerative Colitis including sigmoidoscopy, blood tests and clinical questionnaires (based on the initial Powell-Tuck index in 1978) have been developed but are often inappropriate for the busy clinician. Biochemical and haematological markers correlate well with clinical signs and symptoms, but are not instantly available to the clinician. Sigmoidoscopic appearances correlate less well being prone to interobserver error. We have therefore assessed a new simple activity index of clinical assessment alone using the Powell-Tuck index as the gold standard.

Sixty patients (47 out patients and 13 in patient) have been assessed. The clinical questionnaires and sigmoidoscopic assessment were carried out by one observer (RSW). The index was calculated from frequency of bowel motion by day (1-3 = 0, 4-6 = 1, 7-9 = 2, > 9 = 3) and at night (1-3 = 0, 4-6 = 2, urgency (hurry) = 1, immediately = 2, incontinence = 3), blood in stool (trace = 1, occasionally frank = 2, usually frank = 3), general well being (very well = 0, slightly below par = 1, poor = 2, very poor = 3), terrible = 3), extracolonic manifestations (score one for each).

The correlation between this clinical index and the Powell-Tuck index, which includes sigmoidoscopy, temperature and examination, is highly significant (R = 0.915, P < 0.001) (Fig 1) and suggests that the new index is a Really Useful Simple Colitis Activity Index requiring symptomatic assessment only.

PREDICTING OUTCOME IN SEVERE ULCERATIVE COLITIS.

Travis SW, Farrant JM, Hayllar K*, Kettlewell MGW, Mortensen NM, Jewell DP. John Radcliffe Hospital, Oxford and King's College Hospital, London.

Identifying at an early stage which patients with severe ulcerative colitis (UC) will respond to treatment or come to colectomy remains difficult. To assess whether changes in clinical and biochemical variables predicted the failure of medical treatment, we prospectively measured stool frequency, pulse, temperature, Hb, WBC, albumin, ESR, CRP, omissions, albumin and potassium for 5 days in all patients with severe UC (Trueolle and Witts, 1955) admitted Mar 1992 - Sep 1993. All 51 episodes in 49 patients were treated with intravenous and rectal steroids. 21 (42%) responded completely, 15 (29%) came to colectomy and 15 (29%) with an incomplete clinical response were given cyclosporin. On admission, elevation of the CRP and serum omissions were significantly associated with colectomy in 18 days (P=0.028 and P=0.050 respectively). This was consistent with more severe inflammation and the presence of rectal ulceration at sigmoidoscopy in the colectomy group (93% vs 39%, P=0.002). During 5 days of intravenous therapy, repeated measures analysis of variance showed significant differences between groups in pulse rate (P=0.016), stool frequency (P=0.002), Hb (P=0.044), CRP (P=0.012), and omissions (P=0.0004). Against expectations, other factors including albumin and platelet count had no diagnostic significance. On day 3, the presence of mucosal islands on plain x-ray discriminated between the colectomy group and responders (50% vs 10%, P=0.013). Furthermore on day 3, all complete responders had a score < 6/day with Hb > 11.5 g/dl and CRP < 6ng/L. The number of patients studied was insufficient to produce a stable prognostic model using multivariate analysis. However, we anticipate that this will be possible with the recruitment of further patients.
GENETIC PREDISPOSITION TO ULCERATIVE COLITIS: A DETAILED STUDY OF THE HLA REGION BY LINKAGE ANALYSIS. LSNauma1, JC Lee2, DFord3, D.Eaton3, SVH Hodgson1, J Lennard-Jones1, CGMathew1, 1Div Med and Mol Genetics, UMDS Guy's Hospital, London; 2St. Mark's Hospital, London; and 3Section of Epidemiology, Institute of Cancer Research, Surrey, UK

Distinct HLA class II genes, which are located within the major histocompatibility complex (MHC), have been reported to be associated with ulcerative colitis (UC), making them logical candidate genes to investigate for genetic predisposition in UC.

To test the hypothesis that a major gene within the MHC determines susceptibility to UC, a comprehensive study for evidence of possible genetic linkage between the MHC and UC was undertaken.

DNA was obtained from 23 families with multiple members affected with UC. Of these, 17 families had only UC affected members and 6 had family members with UC and Crohn's disease (CD). Two of the 23 families had 4 kindred affected with UC; 16 families had 3, and 5 families had 2. In order to maximise any potential positive LOD score (> 3 = evidence of linkage), 5 highly polymorphic markers (D3A, LHI, TNBF, D6S105 and APMZ02) within and around the MHC were used. Statistical analysis of the data was based on different genetic models, including both dominant and recessive inheritance with varying penetrance, and haplotype sharing amongst affected siblings.

The LOD scores obtained under all genetic models tested did not achieve significant values. Using a genetic model of a dominant gene with a high frequency but low penetrance, the maximum LOD score obtained was 0.82 at a recombination fraction (θ) of 0.1 for the marker LHI. If the gene was rare but had a higher penetrance, the maximum LOD score achieved was 0.95 at a θ of 0.2 for the marker D6S105. Sib-pair analysis showed that haplotype sharing observed in the affected siblings was similar to the numbers expected if no linkage was present. When UC and CD were regarded as a single disease, LOD scores were also not significant.

No evidence of genetic linkage between the MHC and UC has been provided in this study. The major susceptibility gene(s) in UC are unlikely to be situated within the MHC locus.

METABOLITES OF NITRIC OXIDE IN SEVERE ACUTE ULCERATIVE COLITIS.

DC Rees1, JSazzani, SPL Travis, JWhit2, PL Corneliussen and DP Jewell. Institute of Molecular Medicine, John Radcliffe Hospital; Gastroenterology Unit, Radcliffe Infirmary; and Department of Immunology, Churchill Hospital, Oxford, UK

Introduction: Nitric Oxide (NO) has been implicated as an important mediator of mucosal inflammation in acute ulcerative colitis (Lancet 1993; 342: 338-340). NO has a short half-life, and is rapidly oxidized to stable metabolites, nitrites and nitrates (NOx). Serum NOx concentrations reflect NO synthase activity, and may provide a direct marker of colonic inflammation in acute UC.

Methods: Twenty-six patients (16 male, mean age 45.5 years) with acute severe UC were studied: all required hospital admission (on day 1) for parenteral steroid therapy. Serum concentrations of NOx and C-reactive protein (CRP) were measured daily. Standard indices of disease activity were also closely monitored. NOx concentrations in deproteinized sera from patients and 24 healthy subjects were measured spectrophotometrically using the modified Griess reaction.

Results: 13 patients (CR group) made a complete clinical response after 5 days' parenteral steroid treatment, 13 (IR group) made an incomplete recovery, and needed further treatment (7 colectomy, 6 cyclosporin A). On day 1, mean serum NOx concentrations for all patients was significantly elevated compared to the control group (mean ± SEM 87 μmol ± 15 vs 33 μmol ± 1.7, p = 0.001). There was no difference in mean NOx concentrations between CR and IR groups (84 ± 16 μmol). Mean CRP concentrations was higher for IR than CR group, but did not attain statistical significance (4.2 ± 2.7 v 6.2 ± 2.9, p = 0.08). Mean serum NOx concentrations fell from day 1 to day 3 (mean fall 41 ± 12 μmol, p = 0.003); only three individuals (all IR) did not show this fall. Mean serum NOx concentrations fell significantly (5.5μmol/dl ± 1.2, p <0.001). On day 3, mean serum concentrations of NOx and CRP were lower in CR group than IR group. Only the differences in CRP between the groups were significant (2.34mg/dl ± 0.45 v 4.8mg/dl ± 0.93, p = 0.02). No correlation between NOx and CRP concentrations was present.

Conclusions: Circulating concentrations of NO metabolites are increased in active UC, and fall during steroid therapy; however, concentrations do not reliably predict clinical response.

UPREGULATION OF INTERLEUKIN 1 (IL) 4 mRNA IN INFAMED INTESTINAL MUCOSA: IS THIS INCREASE REAL OR APPARENT?

G Breedon-Smith, MMcGowan and DP Jewell. Gastroenterology Unit, Radcliffe Infirmary, Oxford

Introduction: Interleukin 4 (IL-4) plays a fundamental role in development of humoral immunity in man. It promotes B cell proliferation, production of IgM, IgG, and IgE antibodies and increases CD23 and HLA-DR expression. Conversely, it suppresses cell-mediated immunity by inhibition of production of pro-inflammatory cytokines such as IL1 and IL6 by activated monocytes.

Detection of IL4 in human intestinal mucosa has been notoriously difficult. The aim of this study was to use a nested polymerase chain reaction (PCR) for detection of IL4 mRNA, and to compare levels in patients with inflammatory bowel disease (IBD) and healthy controls.

Methods: Intestinal biopsies were obtained from 10 control patients, 10 with active ulcerative colitis (UC), 10 with inactive Crohn's (ACD), 10 with inactive UC (AUC) and 6 with inactive Crohn's (AUCD). After nested PCR reaction using RNA20, 5 micrograms of total RNA was reverse-transcribed to cDNA, and signal strength assessed for each patient using the internal standard β-actin, and the T cell marker, CD38. Samples were then tested for IL4 using conventional and nested PCR.

Results: Although signal strength for β-actin did not vary significantly between groups, CD23 expression was significantly higher in the AUC and ACD groups compared to controls (p<0.05, Mann-Whitney U test). After one round of PCR, IL4 was only detected in the AUC (6/10) and ACD (5/10) groups. After nested PCR, signals were found in 6/10 controls compared to 10/10 and 9/10 in the AUC and ACD respectively. The inactive disease groups showed variability.

Conclusions: Interleukin 4 mRNA production is upregulated in the inflamed mucosa of patients with AUC and ACD. This may reflect the number of infiltrating T cells, as shown by the increase in mRNA for CD38, rather than an increase of IL4 per cell. The use of similar "physiological" internal standards may be applied to any mRNA, quantitative work may be done on heterogeneous cell populations found in biopsy material.

Anorectal physiology and disease T120–T125

REPRESENTATION OF THE ANAL SPHINCTER ON THE HUMAN CEREBRAL CORTEX.

G.K. Turnbull, QAiziz, SHandy, JBarlow, **K.Singh, S.Alani, D.G.Thompson. Dept. of Medicine & Neurophysiology, Hope Hospital; Manchester, UK; *Dept. of Surgery, University; Halifax, Canada; **Dept. of Vision Sciences, Aston, UK.

Aim: To locate the site of the cortical centre for control of anal sphincter and pelvic floor (PF) motor function in intact man and their representation on the 2 hemispheres. Method: Five right-handed male volunteers, mean age 35 years (range 27-45) were studied. Focal stimulation of the cerebral cortex was conducted using magnetic stimulation with a double coil stimulator at 2.0±0.1 Tesla. A 2x2cm grid was positioned over the cranium 2cm anterior and 6cm lateral to the vertex and 6cm lateral to the midline over each cerebral hemisphere to guide the stimulation. Anal sphincter EMG response was recorded using a surface plug electrode positioned in the anal canal; a rectal electrode was used for PF EMG responses. At each grid point stimulation was repeated 3 times and the EMG responses were averaged. Results: Both anal and PF responses were recorded in all subjects. The ratio of responses left:right (L:R) from the anal EMG were 2.3 (range 1.1-3.7). Seven subjects had a ratio > 2. The ratio of responses L:R from the PF EMG were 1.5 (range 0.9-2.1). Only one subject had a PF ratio > 2. The latencies of the anal EMG were similar (left: 23.1±1.7ms and right: 24.5±4.7ms) as were the PF latencies (left: 21.4±2.1ms and right: 22.3±2.4ms). Superimposition of the magnetic stimulation mapping data onto MRI scans of subjects showed localization of anal and PF centres to the left of the midline over the precentral gyrus. Cerebral hemisphere localization of anal and PF loci differed, with the anal loci lateralized to the left hemisphere while the PF motor loci showed more bilateral representation. Conclusion: Different areas represent the anus and pelvic floor on the cerebral cortex, their unilateral distribution (i) refutes standard teaching that the perineum has dual innervation centrally and (ii) explains the variable effect that central neurologic disease or stroke has on anal and pelvic floor function.
DO RECTAL MYENTERIC NITRIC OXIDE SYNTHASE NEURONES INNERVATE INTERNAL ANAL SPHINCTER? RETROGRADE NEURONAL TRACING IN A GUINEA PIG MODEL
J F Stebbing, A F Brading and N J McC Mortensen
University Department of Pharmacology and Department of Colorectal Surgery, John Radcliffe Hospital, Oxford.

Nitric oxide (NO) has been implicated as the inhibitory neurotransmitter which mediates neurogenic relaxation of the internal anal sphincter (IAS) during the rectoanal inhibitory reflex. NO synthase (NOS) neurones are numerous in the rectal myenteric ganglia but cell bodies are sparse or absent in the sphincter itself. It is therefore important to establish with certainty whether NO-containing neurones in the rectum project into the IAS.

Methods
Eight adult guinea pigs, each weighing 500-600g, were anaesthetised using Hypnorm® (0.5ml/kg, i.m.) and midazolam (0.5ml/kg). Injections (10µl) of 3% wheatgerm agglutinin conjugated to horseradish peroxidase (WGA-HRP, Sigma, n=6) or distilled water (n=2) were made into IAS using a Hamilton syringe. Animals were sacrificed after a survival time of 24 hours. Anatomical localisation of the injection site and transported tracer was determined in both cryostat sections and whole mount preparations by revealing tracer using a tetramethylbenzidine/molybdate (TMB) method. NOS neurones were identified in the same sections using NADPH-diaphorase histochemistry in both control and experimental tissue.

Results
Injection sites were revealed reliably using TMB and localised to the IAS and inter-sphincteric space. In whole mount preparations, tracer was identified in several rectal myenteric neurones of which all subsequently stained positive for NOS. Labelled neurones could be readily distinguished from efferent neurones stained with TMB reaction product. Identification of transported tracer in cryostat sections was difficult due to the presence of many cells containing endogenous peroxidase in the submucosa.

Conclusion
These early results suggest that the retrograde neural tracer WGA-HRP is taken up from IAS and transported to NO-containing neurones in the rectal myenteric plexus. This provides evidence for the presence of a descending recto-anal neuronal pathway.

NEURAL MEDIATION OF THE HUMAN RECTOCOLONIC INHIBITORY REFLEX
J S Warren, M G Lord, J Rogers and N S Williams
Surgical Units, The Royal London & Newham General Hospitals

We have recently described the human rectocolonic inhibitory reflex whereby distension proximal colonic contractile activity. Furthermore, we have shown that this reflex exists in both normal and constipated patients and postulated that chronic rectal distension is thus potentially capable of evoking this reflex, exacerbating the constipation. However, the mechanism through which this reflex acts is unknown.

We assessed the reflex twice in 4 patients who had an end colostomy and rectal stump. Spontaneous colonic intraluminal pressure activity recorded per-stoma was compared to a subsequent period of rectal balloon distension, using a computer derived motility index.

<table>
<thead>
<tr>
<th>Spontaneous activity</th>
<th>Rectal distension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonic motility index</td>
<td>4.5 (1.3 - 6.4)</td>
</tr>
<tr>
<td>Rectal motility index</td>
<td>3.1 (0.1 - 6.1)</td>
</tr>
<tr>
<td>median (interquartile range)</td>
<td>4.7 (14.7 - 46)</td>
</tr>
</tbody>
</table>

* p < 0.05 Wilcoxon Ranked Sum

There is a statistically significant reduction in colonic contractile activity when the rectal stump is distended (as well as a local rectal motility increase). Therefore, the presence of this rectocolonic inhibitory reflex does not depend on an intact intrinsic neural pathway, and its rapid onset suggests a hormonal mechanism is not involved. The remaining intact extrinsic neuronal colonic innervation is thus a stong candidate to mediate this reflex, as has been demonstrated in vitro in animal preparations with the similar colocolonic reflex.

SENSORY EFFECTS OF TOTAL ANORECTAL RECONSTRUCTION
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Surgical Unit, Royal London Hospital, London. E1 1BB

Little is known about anorectal sensory function in patients undergoing total anorectal reconstruction (TAR) following abdominoperineal excision of the rectum. Neorectal reports suggest that some patients retain the ability to detect the presence of faeces and even gas in their neorectum. The aim of this study was objectively to measure anorectal sensory function in a group of patients following TAR. 6 patients (mean age 61; range 30 to 71) were seen between 3 and 30 months after TAR were studied.

Neorectal sensation was measured by distension of a party balloon with water at 37°C at a rate of 1.3mls/sec. No patient reported a feeling similar to normal rectal filling. The sensory threshold volume was recorded. Neosalon sensation was measured, 0.5, 1 and 1.5 cm from the neocanal margin, using a standard mucosal electrosensitivity technique.

Electrosensitivity (mV)

<table>
<thead>
<tr>
<th>Patient Sensation</th>
<th>Vol (mV)</th>
<th>1 cm</th>
<th>0.5 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Abdominal pain</td>
<td>72</td>
<td>25</td>
<td>&gt;25</td>
</tr>
<tr>
<td>2 Abdominal pain</td>
<td>305</td>
<td>25</td>
<td>&gt;25</td>
</tr>
<tr>
<td>3 Perineal pain</td>
<td>114</td>
<td>25</td>
<td>&gt;25</td>
</tr>
<tr>
<td>4 Abdominal pain</td>
<td>26</td>
<td>25</td>
<td>&gt;25</td>
</tr>
<tr>
<td>5 Perineal pain</td>
<td>107</td>
<td>25</td>
<td>&gt;25</td>
</tr>
<tr>
<td>6 Perineal pain</td>
<td>135</td>
<td>25</td>
<td>&gt;25</td>
</tr>
</tbody>
</table>

No patient perceived balloon distension of the neorectum as the presence of flatus or stool. The only sensations reported were either abdominal or perineal pain. None of these patients report "normal" rectal perception under normal daily conditions. Anal sensation was absent or impaired in two patients in whom some mucosa was preserved.

TAR following anorectal excision results in complete loss of anorectal sensation. This is likely to result in incontinence or faecal impaction even if patients have undergone successful construction of a neoanocinet.

PRESERVATION OF COMPLETE ANAL SPHINCTERIC PROPRIOCEPTION IN THE COURSE OF RESTORATIVE PROCTOCOETOPLASTY (RP) FOR ULCERATIVE COLITIS.
A S Miller, W G Lewis, M R Williamson, P M Sagar, P J Boldrwork, D J Cagnostics
Academic Unit of Surgery, The General Infirmary, Great George Street, Leeds, LS1 EEX.

The hypothesis tested in this study was that provided the anal sphincter is preserved intact during RP, even without mucosectomy or endo-anal anastomosis, then normal anal proprioception might also be preserved. Thirty consecutive patients of median age 31 (14-62) years (10 male, 20 female) underwent paired tests of anorectal function before, and a median of 6 (2-12) months after RP (17 J, 13 W reservoir) with stapled ileal anastomosis.

Before operation distension of the rectum with 50 ml of air produced a median (IQR) pressure increase of 21.4 (13.7-29.1) cm H2O in the rectum, and reflex inhibition in the upper anal sphincter to a pressure of 18 (4-40) cm H2O. Near RP distension with 50 ml of air produced a pressure increase of only 4.4 (3.8-7.8) cm H2O (P<0.001 compared with before RP). Maximal reflex inhibition of the upper anal sphincter to a pressure of 26 (3-35) cm H2O occurred at a pressure increase in the pouch of 16.4 (11.6-22.3) cm H2O. Significantly greater inflation volumes, 100 (100-150) ml were necessary to produce this pressure increase, (P<0.01 compared with before RP). After RP, all patients were continent and 25 patients could discriminate with confidence between flatus and faeces.

Thus, reflex function in the sensitive upper anal sphincter was preserved in response to pressure change, thus ensuring that the subtle aspects of anal continence might be preserved.
BIOFEEDBACK TRAINING FOR FECAL INCONTINENCE: FUNCTIONAL IMPLICATIONS OF VECTOR VOLUME ANALYSIS.

J.D. Barlow, N.A. Scott, D.G. Thompson, M.H. Irving.
University of Manchester, Hope Hospital, Salford M6 8HD

Background: Fecal incontinence is a distressing condition which is prevalent in women particularly following traumatic vaginal delivery and in both men and women following anal dilatation. Biofeedback training (BF) consisting of exercise regimes to improve both resting tone and squeeze pressure in the anal canal is commonly utilised as a first line therapy. Aim: To evaluate vector volume analysis (VVA) (3 dimensional profiling of anal pressure data) as a means of providing more detailed assessment of the internal and external anal sphincter function pre and post biofeedback. Method: Thirty patients underwent station and rapid pull through manometry pre and post BF training using the Gasstronsterech Lower G.I. ano-rectal and biofeedback software (Synetics Medical Inc.(Sweden)). Pressure data from an 8 lumen PVC catheter positioned in the high pressure zone (HPZ) of the anal canal was displayed as colour columns on a VDU and recorded for further analysis. The exercise regime consisted of repeated squeezes of increasing duration with incremental rises in the rectal balloon volume whilst the patient observed the visual display. This was repeated at weekly intervals and the patient continued with the exercise regime at home and a log sheet of incontinence/urgency episodes was kept.

Results: 16 patients underwent BF, 13 had pre and post VVA which allowed asymmetry scoring to be made. All 16 patients had improved squeeze pressures, 14 also had improved resting tone. 9 of 13 had increased squeeze pressures which 5 had also increased resting VVA. 10 of 13 had reduced asymmetry of the pressure profile. 6 had improved symmetry for both rest and squeeze pressures, 2 had improved resting and 2 had improved squeeze symmetry with 3 having no change in symmetry. Incidence episodes were reduced or absent in all patients with restoration of normal activity. Group mean data showed 56% increase in resting tone, 36% increase in squeeze pressure, 52% increase in squeeze VVA, 23% improvement in rest and 26% improvement in squeeze symmetry. Conclusion: Visual feedback of radial pressure data allows preferential strengthening of areas of asymmetry. VVA and asymmetry scoring provide detailed assessment of the functional integrity of the anal canal which enables better evaluation of biofeedback training.

Colorectal cancer screening

AN EMPLOYEE TARGETED APPROACH TO BOWEL CANCER PREVENTION. A.I. Stern, M.G. Korman, J. Hanksy and C. McGregor (Introduced by M.A. Kann). Gastroenterology Unit, Monash Medical Centre, Clayton, Victoria, Australia.

The polyp cancer sequence in colorectal cancer (CRC) seems well established. It is believed that most CRC can be prevented by surveillance colonoscopy and polypectomy. Whilst screening those at average risk in the community may not be cost-effective, the identification of those at high risk for CRC (eg. family history) is desirable. However, few organized programs exist. We used a questionnaire to establish CRC risk and targeted employees (age > 40) of a large organization. Methods: Employees received 2 letters from the Director of the organization together with educational material regarding bowel cancer. An explanatory letter from us was then sent together with 2 questionnaires, one for the employee and one for a spouse/partner. Results: 3568 Questionnaires were sent and 1395 responses were received (39%). A high risk was suggested in 190 subjects (13.6%) with a first degree relative with CRC and in 138 with rectal bleeding (9.9%); age >50 years was a relative risk factor in 218 subjects (15.6%). Flexible sigmoidoscopy was offered to people with rectal bleeding or age >50; colonoscopy to those with a family or past history of CRC. Of 546 tests offered, 354 were performed in our program. Polyps were found in 25%; of these 58% were hyperplastic and 42% adenomas. One cancer (Duke's A) was found. Conclusion: This novel program was well accepted in the workplace and achieved a higher response rate than an expert run

INTERVAL CANCERS IN A COLORECTAL CANCER SCREENING PROGRAMME.

P.H. Bennett, C.M. Lysham, H.T. Lang, J.D. Hardcastle: Department of Surgery, University Hospital, Nottingham

Interval cancers, defined as those cancers which occur in the screened population but are not detected by the screening test, were examined in a randomised prospective trial of colorectal cancer screening by faecal occult blood testing at 2-yearly intervals. The interval cancer presentation rate was 4.9/10,000 screened for each year post-screening. The mean duration of follow up of the population offered screening is 6.95 years. Tumour stage and grade were compared with screen-detected cancers and the results are shown below.

<table>
<thead>
<tr>
<th>DUKES' STAGE</th>
<th>DIFFERENTIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Int 1</td>
<td>14</td>
</tr>
<tr>
<td>Int 2</td>
<td>10</td>
</tr>
<tr>
<td>Int U</td>
<td>5</td>
</tr>
<tr>
<td>Screen</td>
<td>104</td>
</tr>
</tbody>
</table>

Int 1=interval cancer 1-2 months post screening
Int 2=interval cancer 13-24 months post screening
Int U=interval cancer >24 months post screening

The results show that there are significantly more Dukes's stage A and B tumours (p<0.001; Chi-squared test) detected by screening and significantly more Dukes's stage C and D cancers presenting as interval cancers (p<0.001; Chi-squared test). There was no statistical difference in the grade of tumour detected by screening or presenting in the interim. This suggests that interval cancers may not be biologically more aggressive but are simply not detected by the screening test and therefore present at a more advanced stage.


Normal rigid sigmoidoscopy is associated with decreased risk of rectal cancer, however, no such data is yet available for flexible sigmoidoscopy. We have studied 1683 patients with bowel symptoms who underwent flexible sigmoidoscopy up to 60 cm from 1979 and 1989. Nine hundred and seventy five patients above the age of 45 years were identified who had no mucosal abnormality seen. Patients with neoplasia, or any other mucosal abnormality were excluded. They were compared with a near ideal control group of 2,098 age and sex matched patients identified from the general population. Current status of individuals was assessed by checking hospital, pathology, G.P. and OCPS records.

Median follow up of flexible sigmoidoscopy group (FS) was 109 months (range 60-196) compared with 87 months (range 60-158) in controls. Seven patients developed colorectal cancer in the FS group compared with 37 patients in control group (X^2=4.4, p=0.035). Risk of colorectal cancer in study group was 0.8/1000 patient years. With 2.4/1000 patient years in the control group.

A negative flexible sigmoidoscopy indicates a low risk of subsequent development of colorectal cancer.
HAEMOCOULT SCREENING IMPROVES STAGE-SPECIFIC SURVIVAL FROM COLORECTAL CANCER (CRC)
D H Bennett, C N Mangham, M M Lang & J D Hardcastle: Department of Surgery, University Hospital, Nottingham NG7 2UH

Between August 1982 and April 1989 almost 130,000 persons were randomised to either 2-yearly screening by Haemocault or a control group. Positive tests were investigated by colonoscopy or flexible sigmoidoscopy and barium enema. The results of at least 5-year follow-up (mean 9.2 years) of 332 persons with either screen-detected or symptomatic CRC is shown below.

<table>
<thead>
<tr>
<th>SCREEN DETECTED:</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>U</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>alive</td>
<td>41</td>
<td>11</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>59</td>
</tr>
<tr>
<td>died from CRC</td>
<td>7</td>
<td>12</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>died other cause</td>
<td>13</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
</tbody>
</table>

**unstaged tumours.**

The results show screen detected Dukes' stage A+B tumours have a significantly lower mortality from CRC than stage-equivalent control group tumours (11% vs 30%, p<0.001: Chi-squared test). Overall 5-year survival is also significantly better in the screened group than in the control group (58% vs 30%, p<0.001: Chi-squared test).

Paediatrics T130–T133

DIARRHOEA, PROTEIN LOSING ENTEROPATHY (PLE) AND TRACE ELEMENT DEFICIENCY IN CHILDREN FOLLOWING BONE MARROW TRANSPLANTATION (BMT)
A Papadopoulou, DR Lloyd, *S Basu, *MD Williams, *PJ Darbyshire, KA Nathavitharana, IW Booth Institute of Child Health, University of Birmingham and *BMT Unit, Birmingham

Diarrhoea, malnutrition and hypoalbuminaemia are common following BMT, but the aetiology is uncertain. To define these disorders further, we carried out serial analyses in 17 consecutive children (median age 9.4 years, 12 males) undergoing BMT. 13 diarrhoeal episodes developed in 11 children at a median of 7 days (range 1-50) after BMT. 85% of diarrhoeal episodes were associated with a PLE (mean stool α-1-antitrypsin 11.5 μg/dl; controls <2 μg/dl). PLE was more severe (mean α-1-antitrypsin 21.8 versus 5.1 μg/dl; p<0.005) and more persistent (median duration 58 versus 10 days; p=0.03) in graft versus host disease (GVHD) and CMV infection compared with other causes. The onset of PLE was associated with a later and substantial fall in serum albumin (median change -6.5 g/L; p=0.0002). Significant correlations were found between the fall in serum albumin and both the increase in stool α-1-antitrypsin (r=0.85; p=0.001) and the duration of PLE (r=0.73; p=0.05). Relative pancreatic insufficiency (defined by stool chymotrypsin <120 μg/dl wet stool) developed in 3, but steatorrhoea was mild. Secretory diarrhoea was present in 8 children: median stool (mmol/L) Na+ 77, K+ 38. Zinc deficiency associated with hypoalbuminaemia was more common in diarrhoeal children (p<0.05), hypomagnesaemia was common during diarrhoea, while biochemical selenium deficiency was almost invariable, irrespective of diarrhoea. The nutritional and gastrointestinal insult following BMT is complex and the nutritional management challenging. The onset of a severe PLE suggests GVHD or CMV infection.

PAEDIATRIC TPM AND HYPERMANGANASAEMIA
A R Reynolds, N Meadows, P Mills Great Ormond Street Hospital for Children NHS Trust, London WC1N 3JH

Long term total parenteral nutrition (TPN) in paediatrics is commonly associated with the development of cholestasis. Current commercial trace element formulations are stated to be based on balance studies performed over twenty years ago resulting in guidelines published by the American Medical Association.

Following a report by Ejima et al (2) hypermanganasaemia in a long term TPN adult presenting with Parkinsonian movements, we investigated an infant 17 months of age (KS) on long term TPN since birth, due to a short gut, who had exhibited an abnormal MRI compatible with deposition of a paramagnetic trace element (12 months of age) and now exhibited abnormal dystonic movement of both arms. Whole blood manganese (Mn) was found to be 8.3 x the upper limit of the reference range (740 μmol/L) range 73-210 μmol/L). Subsequent investigations of seventeen infants aged 10 Kg receiving Pedial® all exhibited hypermanganasaemia with toxic levels (>360 μmol/L) in all those with evidences of cholestasis.

The removal of manganese from TPN solutions has in many cases resulted in the resolution of this condition, supporting the hypothesis that Mn is a contributory factor.

Commercial multi-element solutions were subsequently found to contain 5-25 μmol/L. Given this finding, together with the fact that Mn is excreted primarily via the biliary route (<1% in urine), it would suggest that multi-element solutions for paediatric TPN should contain no more than 40-200 μmol/Kg and that currently available solutions containing in excess of these recommendations should not be used.
MONITORING PANCREATIC ENZYME SUPPLEMENTS IN CHILDREN WITH CYSTIC FIBROSIS USING A MIXED TRIGLYCERIDE BREATH TEST S Anbrig, M Harding*, WA Coward*, TJ Evans*, JY Paton* and LT Weaver. Dept of Human Nutrition and *Dept of Child Health, Royal Institute of Sick Children, Glasgow University, and *MRC Dunn Nutrition Centre, University of Cambridge

Children with cystic fibrosis (CF) have variable degrees of exocrine pancreatic insufficiency which, if untreated, is the main cause of fat malabsorption. Pancreatic enzyme supplements are given to improve energy absorption, mainly from fat. Efficacy is usually assessed by fat balance but this lacks sensitivity. Direct pancreatic function tests are invasive and not suitable for repeated use in children. Assessment is therefore usually based on clinical symptoms and detection of fat in single stool samples.

We measured the effect of pancreatic enzyme supplements on the digestion and absorption of fat in 13 children with CF (mean [SD] age 8.6 [3.0] yr; 7 females and 6 males) using a non-invasive test of intraluminal lipolysis. 1.3-dioctanoyl-2(14C)octanoyl glycerol was given by mouth and 14CO2 enrichment in the breath was used as an indirect measure of fat digestion. After an overnight fast 2 baseline breath samples were taken and children then ingested the 14C-labelled triglyceride (10 mg/kg <30 kg or 5 mg/kg > 30 kg body weight) mixed with a long chain triglyceride emulsion: 1.4 ml/kg to a maximum of 30 ml. Breath samples were obtained every 30 min for 6 h thereafter. 20 ml samples of expired air were analysed for 14CO2 enrichment by isotope ratio mass spectrometry. CO2 production rate was calculated by indirect calorimetry. The test was performed twice, with and without the usual dose of enteric-coated microspheres.

A median [range] total 14CO2 cumulative percentage dose recovered (PDR) of children without enzymes was 2.3 [0-28.4] %, and with enzymes 19.5 [0-34.9] % (P=0.007). Six children had negligible 14CO2 recovery in breath without enzymes (3 with wandering baselines), 5 of whom showed marked rises with enzymes. There was considerable intersubject variation in PDR, reflecting wide degree of pancreatic insufficiency. There was no significant relation between units of lipase ingested and 14CO2 recovery in the breath, nor between the results and age and nutritional status of the children. The mixed triglyceride breath test offers a simple, non-invasive and repeatable way of assessing the need for pancreatic enzyme supplementation in children with CF.

PANCREATIC FUNCTION:

For Sick Children, University Hospital, Pancreatic Nutrition Centre, University of Cambridge

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T135

DAY CASE TRANSFER OF IN-PATIENTS FOR DIAGNOSTIC AND THERAPEUTIC ERCP: EFFICACY AND SAFETY C.A. Kyriakides, Y. Service, D.E.F. Tweedle, D.F. Martin Departments of Radiology & Surgery, South Manchester University Hospitals NHS Trust, Manchester M20 8LR

In order to use facilities flexibly and efficiently, we have offered a diagnostic and therapeutic ERCP service to in-patients from referring hospitals. After telephone or fax referral, the patient was transferred by ambulance, with a nurse escort, returning to the parent hospital after a short period of recovery. 158 patients (57M 101F mean age 63.7, range 22 - 94Y) were referred, 44 by physicians, 114 by surgeons, from 18 hospitals, up to 100 miles distant. 88 (56%) had jaundice, 11 (7%) acute cholangitis and 6 (4%) acute pancreatitis. All arrived between 8.30a.m. and mid day, 94% between 9.30 and 11.00a.m., all but 1 with an escort. 106 travelled less than 10 miles. The mean time spent in South Manchester was 6.1h (range 3.5 to 9.5). The mean time spent away from the parent hospital by patient and escort was 8.1h (5.5 - 11). Cannulation failed in 9 (6%), 2 with duodenal tumour invasion. Spincterotomony was performed in 62 and stents inserted for failed duct clearance in 7. 44 stents were inserted for malignancy. 9 were admitted, 3 for a percutaneous stent insertion after failed ERCP, 3 for abdominal pain (1 pancreatitis). 2 patients with sepsis and malignancy died after admission. Follow-up revealed only 1 further complication (acute pancreatitis). These findings show that short stay ERCP can be undertaken on transferred in-patients safely and effectively without adverse effect on success or complication rates.

PANCREATIC FUNCTION:

For Sick Children, University Hospital, Pancreatic Nutrition Centre, University of Cambridge

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INCREASED RATE OF CHOLECYSTECTOMY IN SCOTLAND FOLLOWING INTRODUCTION OF LAPAROSCOPIC CHOLECYSTECTOMY C M Lam, P A Murray, A Cuschieri. Departments of Surgery and Clinical Pharmacology, Ninewells Hospital and Medical School, University of Dundee, Dundee, DD1 9SY

The total cholecystectomy rate in some countries has increased following the introduction of laparoscopic cholecystectomy. The aim of this study was to examine the trends in cholecystectomy rate in Scotland with the advent of laparoscopic cholecystectomy.

Methods: The rates of cholecystectomy were obtained from the Scottish Health Statistics and the Information and Statistics Division of the National Health Service in Scotland. The Office of Population Censuses and Surveys (OPCS) 3 & 4, Classification of Surgical Operations codes for open cholecystectomy (522,518) and laparoscopic cholecystectomy (J08.8 or Y05.8) were examined.

Results: The rate of open cholecystectomy had declined gradually from its peak in 1977 until 1990, when it declined abruptly by 62.4% from 1990 to 1993. The percentage of laparoscopic cholecystectomy increased from 2% in 1990 to 64% of total in 1993. The overall cholecystectomy rate increased by 28% in 1993 compared with 1991 (see graph). The increase in cholecystectomy rate was mainly observed in the 65-74 age group in both sexes.

Conclusion: As reported in other countries, the introduction of laparoscopic cholecystectomy has led to an overall increase in the performance of cholecystectomy. The increased performance in elderly patients suggests a lowered threshold in elderly patients.
A DOUBLE BLIND PLACEBO CONTROLLED STUDY OF BB-882 (A POTENT PAF ANTAGONIST) IN HUMAN ACUTE PANCREATITIS.
SW Galloway, L Formella, AN Kingsnorth et al.
Department of Surgery, University of Liverpool PO Box 147, Liverpool L69 3BX.

Platelet activating factor is a key inflammatory mediator implicated in the pathogenesis of experimental acute pancreatitis (AP). The aim of this clinical study was to evaluate the effects of 3 days intravenous BB-882 on the clinical course and serum markers of inflammation in human AP. Eighty three patients with a diagnosis of AP were randomised to receive either placebo (P) or active (A) medication in a double blind study. Patients were monitored daily by APACHE II scoring and measurement of serum parameters, polymorphonuclear (PMN) E-selectin and interleukin-6 (IL-6). The two groups were found to be evenly matched at baseline.

Endoscopy TI40–T144

TI40

EXAMINING THE UNPREPARED BOWEL WITH THE 60cm FIBRESCOPE – OUR FLEXIBLE FRIEND IN ALL PATIENTS.
K Kapur, D Clements, M Sebastian, M C Allison
Medical Unit, Royal Otwnt Hospital, Newport.

We have conducted a six month prospective audit of the feasibility, diagnostic yield and patient tolerance of examining the 60cm fibre to achieve a specific diagnosis without prior bowel preparation on patients presenting to our gastroenterology clinic with colorectal symptoms. A total of 390 patients, 187 with colorectal symptoms were recruited. Out of 192 patients presenting to our gastroenterology clinic 74 patients had undergone digital examination, which was subsequently excluded from the study. The indications in the 200 patients were altered bowel habit (107), colitis, colonic polyp or diverticular disease (54), anal bleeding (41) and other (25). An Olympus QSP fibrescope was used and the median insertion depth was 35cm (range 10–60cm). The consultant achieved a median 55cm. Insertion was unimpeded in 77 patients (38%) but was limited by fesses in 77 (38%), anatomy in 21 (10%) and pain in 35 (18%). Luminal contents were: empty in 70 (35%), formed stool in 79 (39%), pelleted stool in 24 (12%), loose stool in 9 (5%), other in 13 (6.5%) (steatorrhoea, blood, mucopus, iron-laden stool or melaena). Eighty six (44%) patients were excluded in 25 cases. Results were normal in 125 (63%), ulcerative colitis or proctitis proximal extent negotiated in 36 (18%), colitis with limited view in 13 (7%), Crohn's colitis in 6 (3%), polyps in 6 (3%), cancer in 5 (2.5%) and other in 19 (10%). Five patients (2.5%) found the procedure intolerable and would not consent to a repeat unless given premedication. We conclude that fibrescopiography without preparation or sedation is feasible and mostly acceptable to gastroenterology outpatients. Examination of luminal contents may help in assessing colorectal symptomatology. There are also savings in nursing time and toilet facilities.
BLEEDING PEPTIC ULcer: INJECTION OF ADRENAline OR TISSUE ADHESIVE (Histoacryl).

C.P. Choudri & K. R. Palmer

G.I. Unit, Western General Hospital, Edinburgh.

Permanent haemostasis is achieved by endoscopic injection with dilute adrenaline in most patients who present with peptic ulcer bleeding. Failed haemostasis occurs in 15% of cases, presumably because the bleeding artery remains patent. In experimental animals neither adrenaline nor a range of sclerosants caused arterial thrombosis and in a clinical trial combination injection therapy with adrenaline and ethanolamine was no more effective than adrenaline alone. The tissue adhesive histoacryl solidifies instantly in blood and causes arterial thrombosis; it appears to be an ideal haemostatic agent for bleeding peptic ulcer injection therapy.

Methods: 249 patients underwent emergency endoscopy for acute gastrointestinal haemorrhage. Sixty eight of these presented with arterial peptic ulcer bleeding or a non-bleeding visible vessel within an ulcer and were randomised to endoscopic injection with 3-10 ml 1:100,000 adrenaline (36 patients) or 0.5 ml of a mixture of histoacryl and lipiodol (5:8). Results: The two groups were well matched. 3 patients in the adrenaline group and 2 in the histoacryl group rebled. Operation rates (2 and 1 patient, median transfusion requirements (4.0 and 3.5 units) and median hospital stay (9 and 7 days) were similar. No acute complications occurred.

Conclusions: Both adrenaline and histoacryl are effective agents for bleeding ulcer. Adrenaline remains the treatment of choice because of greater simplicity and lower cost.

SUBLINGUAL GTN SPRAY IMPROVES SUCCESS AT ERCp CANNULATION

I. Donnellan, M Hallisy, C Nwokolo, D Loft, I Fraser

Depts of Surgery & Gastroenterology.

Walsgrave Hospital, Coventry.

Anecdotal reports have suggested that the smooth muscle relaxant Glyceryl trinitrate (GTN) may facilitate cannulation of the ampulla, and so avoid the need for pre-cut papillotomy in purely diagnostic ERCP procedures. We have undertaken a randomised, controlled study to assess whether the routine use of sublingual GTN improved the success rate of ERCP cannulation.

251 patients were randomly allocated into two groups. The GTN group received 2-4 puffs of Nitroglycerin spray immediately prior to the ERCP and again on sight of the ampulla. Both groups received Pethidine 50mg i.v. and tetrades of Midaclomazol, and Butylscopolamine. The ERCPs were performed by 2 Consultants and 2 Senior registrars. The time taken to achieve cannulation was measured, and whether deep cannulation was achieved or required. Reasons for failure were noted. The statistical significance of the results was calculated using the y² test.

There were 124 patients in the control group and 127 in the GTN group. Of the 27 failed cannulations (overall failure rate 10.8%) 19 were in the control group (15% and 8 in the GTN group (6%). This difference was not achieved in 26 controls (21%) and 19 of the GTN group (15%), against p<0.05. This effect of GTN was seen in both the Consultant and Senior registrar results. There was no significant difference in the time taken for the procedure, and no complications arose relating to the use of GTN.

Sublingual GTN offers a safe inexpensive method of improving success at ERCP cannulation for both trainee and experienced endoscopists and we recommend its routine use.

LESSONS FROM AGGRESSIVE COLONOSCOPIC FOLLOW UP OF LARGE BOWEL CANCER.

J. G Williams, D G Borman, A Donovos, J P Neoptolemos

Department of Surgery, City Hospital NHS Trust, Dudley Road, Birmingham. B18 7QN.

A patient with large bowel cancer is at increased risk of developing a further neoplastic tumour following resection. Regular colonoscopy has been suggested as a way of identifying metachronous lesions at an early stage.

One hundred and six patients, who underwent resection of large bowel cancer (rectum 65, left colon 21, right colon 20) were followed for a median of 36 months (range 5-137 months). 303 colonoscopic examinations were performed, of which 289 (95%) were complete examinations of the remaining colon. 30 patients underwent a single examination, the remaining patients underwent between 2 and 9 examinations. Three metachronous cancers (3%) were discovered at 12, 48 and 79 months. In two patients (1 Dukes A, 1 Dukes C) tumours were first detected during colonoscopy. The third (caecum, Dukes C) was not detected, despite 9 previous colonoscopies, 4 of which revealed polyps. 41 adenomatous polyps were removed during ERCPs in 19 patients (18%). 5 patients had polyps removed on more than one occasion. 15 patients had polyps removed on their first examination. Only 4 out of 65 patients (6%) who had a clear first examination had a polyp detected on any subsequent examination.

All patients undergoing resection of large bowel cancer should undergo colonoscopy within 6 months of surgery. Frequent, repeat examinations are justified only in patients who have colonic polyps.

PUSH ENTEROSCOPY AND HEATER PROBE THERAPY FOR SMALL BOWEL BLEEDING

A J Morris, M Mokhasali, J F MacKenzie

Gastroenterology Unit, Glasgow Royal Infirmary.

Aim: To assess the efficacy of push enteroscopy and heater probe therapy in the treatment of patients with small bowel bleeding due to angiodysplasia. We have studied the effect of treatment on the following and points: 1) Ablation of small bowel lesions 2) Resolution of blood loss 3) Resolution of anaemia.

Patients/Methods: We have studied eleven patients 9M:2F aged 43-85 years. All had small bowel angiodysplasia and ten had required blood transfusion before the study. Duration of anaemia pre enteroscopy was 24 (8-108) months. We used a 1.7m push enteroscope (Olympus- SIOL) and heater probe unit (olymus HP1) for diagnosis and treatment. Ablation of lesions was carried out at diagnostic enteroscopy. If bleeding persisted or anaemia failed to resolve repeat enteroscopy was carried out until no further lesions were found. Patients were followed up for 9 (6-30) months after therapy which was a minimum of six months from last treatment. During the study colonoscopy was carried out to look for alternative sources of blood loss. 8/11 patients had simultaneous angiography of upper or lower gastrointestinal tract.

Results: Patients required 1 (1-5) enteroscopy examinations to treat lesions. All identifiable lesions were treated in 6/11 patients of whom 1/6 remained POB+ve but was not anaemic. In the remaining 5/11 patients treatment was incomplete and 4/5 were POB+ve. Only two of these patients remain transfusion dependant. Thus in 9/11 patients heater probe ablation of small bowel angiodysplasia resulted in significant reduction in bleeding. Hemoglobin at entry was 8.5 (5.3-10.6) g/dl and after therapy 13.5 (7.6-15.6) g/dl (p<0.01) * results: median (range)

Conclusion: Push enteroscopy and heater probe ablation offers a potential therapy for bleeding from small bowel angiodysplasia. Following therapy there is a reduction in blood transfusion requirement and improvement in anaemia. *Wilcoxon matched pairs test signed rank test.
INHIBITION OF HELICOBACTER PYLORI CYTOXIN BY OMEPRAZOLE. OR Zhang, IM Nakshabendi, CG Gemmell, RI Russell. Departments of Gastroenterology and Bacteriology, Royal Infirmary, Glasgow G12 9ER

Recent studies suggest that Helicobacter pylori (Hp) cytotoxin may play an important role in chronic active gastritis and peptic ulcer disease. Omeprazole can suppress Hp-related gastric mucosal inflammation and is effective in treating Hp infection together with an antibiotic. We have shown that omeprazole can inhibit Hp cytotoxic activity, but it is not known if it is also effective against Hp cytotoxin.

**Aim**: To investigate if omeprazole can inhibit purified Hp cytotoxin as measured by cell vacuolation in vitro.

**Methods**: Gel and ion exchange chromatography were used to purify cytotoxin from culture supernatants of Hp. Doses of purified toxin 5μg/ml caused cell vacuolation. A range of concentrations of ulcer-healing drug solutions including cimetidine, famotidine, misoprostol, omeprazole, ranitidine, sucralfate and triptadin dicitratobismuthate were prepared in saline. Purified toxin was incubated with drug solutions for 1h at 37°C and tested on Vero cells. Cell vacuolation was assessed by light microscopy and verified by assaying neutral red uptake.

**Results**: Only omeprazole inhibited cell vacuolation induced by purified Hp cytotoxin. At concentrations 50μg/ml, omeprazole abolished (95%) the toxicity (p<0.001) and at 25μg/ml inhibited the toxicity by 55% (p<0.05). Over the range 5-50μg/ml a linear dose-response curve was observed. Other ulcer healing drugs had no effect on Hp cytotoxin.

**Conclusion**: Omeprazole inhibits the activity of Hp cytotoxin. This action by omeprazole might be beneficial in reducing gastric mucosal inflammation and in treating peptic ulcer disease.

**ENTEROAGGREGATIVE ESCHERICHIA COLI INFECTION IN VIVO.** Higham RP, Walker-Smith JA, Knutton SP, Shaw R, Phillips AD. Academic Dept of Paediatric Gastroenterology, Queen Elizabeth Hospital for Children, Hackney Road, London, E1 8RG. Institute of Child Health, Birmingham, B16 9TY.

Enterohaemorrhagic E.coli [EAggEC] are a recently recognised category of E.coli which are associated with acute and chronic diarrhoea in childhood, and with traveller's diarrhoea. Their pathogenic mechanisms are unclear, although in vitro adhesion to distal intestine and toxin production have been reported. No in vivo cases of EAggEC infection have been described.

Here we report 3 children (1f, 29; aged 5, 10, & 13 months) undergoing proximal small intestinal biopsy for chronic diarrhoea (n=2) and vomiting with failure to thrive (n=1) who all had EAggEC identified in the stool at the time of biopsy. Stool E.coli were identified as EAggEC by their adherence to cultured HEp-2 cells in an aggregative manner and were not identifiable by routine microbiology.

All cases showed an enteropathy (mild crypt hyperplastic villous atrophy with acute & chronic inflammation), two demonstrated bacteria adhering to the epithelial surface and to mucus overlying the mucosa. The bacteria showed aggregation in 'attacked-brick' and 'chain' patterns in section, which is a characteristic property of EAggEC in vitro; there was no evidence of an attaching-effacing lesion or of discrete microvillous damage at sites of attachment, although microvillous height was generally reduced.

These in vivo cases demonstrate that: (1) EAggEC adhere to the proximal small intestine in childhood, and thus may cause or enable extensive colonisation along the gut, and (2) EAggEC are associated with an enteropathy.

**IDENTIFICATION OF HIV-1 RNA IN INTESTINAL TISSUE.** McGowan I1, Roka S1,2, Alyiss U1, Koterl DP1, Jewell DP1. 1Department of Gastroenterology, Radcliffe Infirmary, Oxford, UK, 2Department of GI Immunology, St Luke's Hospital, New York, USA, 3Department of Virology, Middlesex Hospital, London, UK

Introduction: HIV RNA has previously been identified in large intestinal tissue using in situ hybridisation and HIV DNA using the polymerase chain reaction (PCR). The aims of this study were firstly to assess the sensitivity of reverse transcription nested PCR in the identification of mucosal HIV RNA in both small (SI) and large (L) intestinal tissue and secondly to localise viral RNA using in situ hybridisation.

Methods: Nested PCR was performed using primers specific for the HIV pol gene. cDNA was examined from 16 control and 32 HIV (14 CDC stage II and 18 CDC stage IV) positive patients. The HIV plasmid HB2 was used to assess PCR sensitivity and to act as a positive control. In situ hybridisation was carried out on formalin fixed paraffin embedded intestinal tissue obtained from 12 HIV positive (5 CDC stage II and 7 CDC stage IV) and 12 control patients using 35S sense and antisense riboprobes. 8E5, a T cell line transfected with a defective HIV strain, was used as a positive control.

Results: Nested PCR was able to detect 104-107 copies of the HIV plasmid. Positive signals were seen in 18 of 24 SI and 8 of 8 L samples from the HIV patients and 0 of 16 control samples. Positive in situ signals were identified in 4 of 12 (2 SI and 2 L) of the HIV positive patients and none of the controls. Signals were either in the lamina propria just beneath the epithelial surface or in association with lymphoid aggregates within the mucosa. No epithelial signals were noted.

Conclusions: Using a sensitive PCR technique we were able to detect HIV RNA in 75-100% of intestinal tissue obtained from HIV seropositive patients. RNA was localised to the lamina propria. The demonstration of viral RNA in both SI and L at all stages of HIV infection implies that active viral replication is occurring within the intestinal mucosa throughout the natural history of HIV infection.

**POLYMERASE CHAIN REACTION DETECTION OF CRYPTOSPORIDIUM sp. IN HUMAN SMALL INTESTINE.** Kelly P, Carnaby S, Ngwenya B, Farthing MJG. Digestive Diseases Research Centre, Medical College of St Bartholomew's Hospital, London; University of Zambia School of Medicine, Lusaka.

Identification of specific small intestinal pathogens in AIDS-related diarrhoea can have a major impact on management. Faecal diagnosis and small bowel light (LM) and electron microscopy (EM) require special expertise and are labour intensive.

We have therefore developed a polymerase chain reaction (PCR) technique, using published primers, to detect Cryptosporidium sp. in distal duodenal biopsies. The PCR amplifies a Cryptosporidium sp. specific 452 bp sequence of unknown function. The performance of the PCR has been assessed in nine African patients with HIV-related chronic diarrhoea. These patients underwent thorough parasitological diagnosis using wet stool smears and modified Ziehl-Nelsen and immunofluorescent stains, and endoscopic distal duodenal biopsy for histology and electron microscopy. Duodenal biopsies were snap frozen and then processed to extract genomic DNA. The PCR reaction products were electrophoresed and visualised with ethidium bromide.

The PCR detected parasite DNA in all biopsies from the four patients with duodenal cryptosporidiosis on LM or EM. One additional case was detected in which conventional methods had failed to identify the parasite. The PCR is sensitive and genus-specific, and its use may offer insights into the distribution of the parasite and its contribution to diarrhoeal disease, in addition to its diagnostic potential.
CELLULAR RESPONSES OF INTESTINAL EPITHELIAL CELLS TO TOXIN A OF C. DIFFICILE.

Y.R. Mahind, S.Makh, S.Hyde, T.Gray & S.P.Borriello. Div of Gastroenterology, Dept of Pathology & Microbiology & Institute of Infectious & Immunity, University Hospital, Nottingham NG7 2UH.

Clostridium difficile is the aetiological agent of pseudomembranous colitis and animal studies suggest the essential role of secreted toxin A in inducing disease. We have examined responses of the cells first encountered in the colon by the toxin, the epithelial cells.

Toxin A was purified by tryptophol affinity and anion exchange chromatography. Responses of confluent human colonic epithelial cell lines, Caco2, HT29 and T84 and of primary epithelial cells in organ cultures of colonic biopsies were examined by phase contrast microscopy, electron microscopy and MTT assay [which reflects mitochondrial dehydrogenase (MD) activity].

At conc. of 1 & 10ng/ml of toxin A, there was loss of domes in the Caco2 cells. At higher conc. (100 & 1000ng/ml) cell rounding was seen in all three cell lines. By 24h the Caco2 monolayer was beginning to detach but cell death (as assessed by trypan blue uptake) occurred over the subsequent 24-72h. For all cell lines, even after three days of exposure to the toxin, a majority of the cells were viable as shown by trypan blue exclusion. MTT assays demonstrated MD activity over this period, supporting the findings with trypan blue. In organ culture, the effect on the epithelial cells was seen at conc. of 100 & 1000ng/ml of toxin A, over 1 to 24h. This effect was characterised by morphological changes in the epithelial cells with detachment from the basement membrane, presence of intracellular vacuolar structures but preservation of subcellular structures such as mitochondria and endoplasmic reticulum.

These studies suggest that toxin A of C.difficle initially causes epithelial cell detachment (by affecting the cell cytoskeleton) which is then followed by cell death. Responses of the injured epithelial cells and of the exposed lamina propria cells may initiate the subsequent inflammatory response.

Gastrointestinal cancer  T150–T157

EXPRESSION OF PLASMINOGEN ACTIVATORS AND THEIR INHIBITORS IN OESOPHAGEAL CARCINOMA

DF Hevin, MN Vipond, D Alderson. Department of Surgery, Bristol Royal Infirmary, Bristol BS2 8HW

Local proteolysis of the extracellular matrix is an important step in the invasion of malignant cells. The plasminogen activator system is thought to play a major role in this aspect of tumour cell invasion. There have been no studies of the role of urokinase (uPA), tissue plasminogen activator (tPA) or their specific inhibitors, PAI-1 and PAI-2, in oesophageal carcinoma. This study investigates the expression of these components of the plasminogen activator system in oesophageal carcinoma.

Tumour and normal mucosa were obtained from five resected adenocarcinomas (AC) and five resected squamous cell carcinomas (SCC). Antigen levels of uPA, tPA, PAI-1 and PAI-2 were determined in whole cell homogenates of each sample by ELISA and expressed as ng antigen per mg protein.

uPA levels were significantly higher in tumour than in matched normal mucosa (AC: median uPA 6.2 ng/mg vs. 1.5 ng/mg, p<0.01; SCC: median uPA 20.7 ng/mg vs. 0.5 ng/mg, p<0.01).

There was no significant difference in uPA between AC and SCC. tPA levels were not significantly different in tumour or normal mucosa.

PAI-1 could not be detected in either tumour or normal mucosa. PAI-2 levels were significantly higher in normal tissue matched to AC: (median PAI-2 2.4 ng/mg vs. 30.8 ng/mg, p<0.05; SCC: 8.2 ng/mg vs. 105.2 ng/mg p=ns).

This study shows that there is increased expression of uPA and decreased expression of PAI-2 in oesophageal carcinomas compared with normal mucosa. Expression of tPA is unaltered and PAI-1 is not detected in either normal or malignant tissue. These data support a role for these enzymes in the invasive behaviour of oesophageal carcinoma whereby the physiological inhibition of proteolysis in normal tissue is lost as a result of induction of uPA and suppression of PAI-2.

p53 MUTATION IN MIXED HEPATOCELLULAR CHOLANGIO-CARCINOMA (HCC-CC).

PM Rizzi, SD Ryder, B Portmann, JK Ramage, N Naoumov and Roger Williams. Institute of Liver Studies, King's College Hospital, London SE5 9RS.

Background. Mixed HCC-CC is a rare primary liver cancer showing features of both hepatocellular and biliary epithelial differentiation. It has been suggested that this cancer originates from a stem cell from which hepatocytes and biliary cells epithelium are derived. Immunohistochemical staining for intracellular components such as alphafetoprotein and keratin have been used to distinguish cell type with hepatocellular phenotype. Mutations of the p53 tumour suppressor gene have not been studied in HCC-CC.

Patients and Methods. We studied 3 patients with mixed HCC-CC. Immunohistochemistry for mutant p53 was performed in paraffin embedded tissue using as primary p53-antibody D01 and an avidin-biotin method, following microwave treatment.

Results. In all patients mutant p53 was demonstrated in the cholangiocarcinoma (CC) area but not in the hepatocellular carcinoma (HCC) zone. In 2/3 patients more than 80% of the nuclei showed strong positive staining. Mutant p53 was also demonstrated in the CC tissue from distant metastases in two cases, in the peritoneum and in the lung respectively.

Conclusions. This is the first observation of p53 mutations in mixed HCC-CC. Mutant p53 is present selectively in malignant cells showing biliary epithelial differentiation and associated with distant metastases. Staining for p53 protein may represent an additional diagnostic criteria in distinguishing the tumour cells showing biliary epithelial differentiation from those with hepatocellular phenotype.

IDENTIFICATION OF KIRSTEN-RAS MUTATIONS IN BILE OF PATIENTS WITH PANCREATIC AND BILIARY CARCINOMAS.

S O'Mahony, M Longfellow, MJ McMahon, ATR Axon, P Quirk. Centre for Digestive Diseases, The General Infirmary at Leeds, UK

Obtaining a positive tissue diagnosis is frequently difficult in patients with pancreatic carcinoma (PC). Tissues from patients with PC have been found to have a high prevalence of Kirsten (Ki)-ras mutations; the mutant gene occurs less commonly in patients with cholangiocarcinoma (CC) and ampullary carcinoma (AC). We have collected bile endoscopically from patients referred for endoscopic retrograde cholangiopancreatography (ERCP); these included 10 patients with PC; 4 with CC, 3 with AC, and 19 controls (normals and patients with bile duct stones). DNA was extracted from the aspirated bile, and Ki-ras codon 12 mutations were detected by polymerase chain reaction (PCR), DNA amplification and restriction enzyme digestion. Ki-ras mutations were detected in 6 of 10 patients with PC, in one of 4 with CC, in one of 3 with AC, and in none of the 19 controls. Ki-ras mutations are therefore specific to malignancy, and are detectable in bile in 60% of patients with pancreatic cancer. Detection of bile Ki-ras mutations may prove to be a valuable diagnostic tool in the management of PC.
IL-8 AND IL-4 ARE PRODUCED CONSTITUTIVELY BY HUMAN PANCREATIC CANCER CELL LINES.


University Department of Surgery, Royal Infirmary of Edinburgh, Laurnston Place, Edinburgh, EH9 9YW, UK.

Pancreatic cancer is associated with profound changes in metabolism, including the cancer cachexia syndrome, which is thought to be mediated by pro-inflammatory cytokines. The origin of these cytokines however remains uncertain. This study examines the endogenous cytokine production by pancreatic tumour cells.

Methods: The pancreatic cancer cell lines, MIA PaCa-2, CF PAC and PANC-1 were cultured. Supernatants were collected at defined intervals and assayed for the presence of IL-8 and IL-6 by ELISA.

Results of cell culture:

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>IL-6 pg/ml</th>
<th>IL-8 pg/ml</th>
<th>CF PAC</th>
<th>PANC-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<tr>
<td>72</td>
<td>0</td>
<td>14350</td>
<td>$3200^*$</td>
<td>1625</td>
</tr>
</tbody>
</table>

*p <0.03  p<0.0006 (Student's 2-tailed t test)

**p <0.007  p<0.0005 (Student's 2-tailed t test)

Correction for cell count indicated that, although levels of cytokines in the culture media increased, cytokine production per cell remained relatively constant.

Conclusion: These results demonstrate the ability of unstimulated pancreatic cancer cell lines to produce the pro-inflammatory cytokines IL-8 and IL-6. The relatively constant cytokine production per cell implies the absence of an autoregulatory inhibitory or stimulatory loop mediated by either IL-8 or IL-6. It is possible that in vivo production of the pro-inflammatory cytokines IL-8 and IL-6 by tumours may contribute to the alterations in metabolism which are observed in patients with pancreatic cancer.

T154

FUNCTIONAL VITAMIN D3 RECEPTORS ARE PRESENT IN HUMAN COLORECTAL NEOPLASMS. KE Kane, NP Michell, MJS Langman, GR Williams. Department of Medicine, The University of Birmingham, Birmingham, B15 2TH.

1.25(OH) vitamin D3 (D3) has antiproliferative and differentiating effects in a number of tissues, including in vitro models of colon cancer. Epidemiological studies reveal a protective role for D3 in large bowel malignancy and D3 has been proposed as a therapeutic agent in colorectal carcinoma. Synthetic D3 analogues have been synthesised that have enhanced antiproliferative actions relative to their calcemic potency and are presently undergoing Phase I trials in advanced breast cancer. However, previous Scatchard analysis demonstrates high affinity, low capacity D3 binding sites in only 32% of colorectal tumours, implying that D3 would only have limited chemotherapeutic value in this malignancy.

D3 action is dependent on expression of the vitamin D receptor (VDR) in all responsive tissues. In addition VDR can heterodimerise with retinoid X receptors (RXRs) to modify D3 response. We have previously demonstrated that mRNAs encoding VDR and RXRα and γ are expressed in 100% of large bowel adenocarcinomas indicating that D3 may have greater potential in colon cancer treatment than formerly thought, provided the receptor is active.

The aim of this study was to determine whether the VDR expressed in colorectal tumours is functional. Western blot analysis in 16 paired samples from the same subjects (colonic tumours and normal mucosa) demonstrated a 52 kDa protein specific for VDR in all tissues examined. Short term primary cultures from 10 human colorectal carcinomas have been established and target gene responsiveness to D3 treatment was studied. Following 12 hours treatment with 10⁻¹⁰ M D3 the classical D3 responsive gene 24-hydroxylase was induced in all the tumours studied but was undetectable in vehicle treated controls.

These results suggest that functional VDR protein is present in all colorectal adenocarcinomas and imply that D3 analogues are potential therapeutic agents in the treatment and prevention of large bowel cancer.

T155

P53 OVEREXPRESSION IS CLOSELY CORRELATED WITH GRADE OF DUODENAL NEOPLASM IN FAMILIAL ADENOMATOUS POLYPOSIS (FAP)

Hiroshi Nakahasi, AD Spigelman, IC Talbot, RKS Phillips. St. Mark’s Hospital, Polyposis Registry & ICRF, London

Introduction: Colonic p53 overexpression occurs late in carcinogenesis, but there are some reports that p53 expression may occur earlier in the foregut. Given the evidence for a biliary factor in duodenal polyp formation in FAP, we tested duodenal polyps and cancers of varying histology for p53 overexpression. Methods: Samples from endoscopic biopsies of 147 duodenal polyps (patients=79) and from 15 surgical specimens (patients=7) were examined by routine histology and by immunohistochemistry, the latter following an Avidin-Biotin Complex method using mouse monoclonal antibody D07 and a microwave retrieval technique. Results: P53 was overexpressed in: no case of normal mucosa(0/29), 25% of tubular adenomas(27/110), 69% of tubulovillous/villous adenomas(11/16) and 100% of duodenal cancers(5/5); and in 28% of mildly dysplastic(32/113), 46% of moderately or severely dysplastic(6/13) and 100% of cancers. In Stage I duodenal polyps, p53 was overexpressed in 8%(1/12), in Stage II 21%(4/19), in Stage III 34%(10/29) and in Stage IV 50%(6/12).

Conclusion: P53 overexpression increases with all markers of more severe duodenal cancer risk and is present in 100% of cancers. These prospectively derived duodenal findings mirror Vogelstein’s view of the place for p53 overexpression in colorectal carcinogenesis and may help clinically in identifying those who need close scrutiny/intervention.

T156

A SURVEY OF PHENOTYPIC ABNORMALITIES IN JUVENILE POLYPOSIS. Desai DC, Neale K, Murday V, Mills PJ, Hodgson SV, Phillips RKS. St. Mark’s Hospital, London, EC1V 2PS and Great Ormond Street Hospital for Children, London WC1N 3JH.

Characteristic phenotypic abnormalities occur in two conditions associated with gastrointestinal polyps, familial adenomatous polyposis and Peutz-Jeghers syndrome but, although described, the extracolonic manifestations have not been studied in juvenile polyposis. Aim: To study unusual phenotypic features in patients with juvenile polyposis and determine any overlap with other genetic syndromes. Patients and Methods: 15 patients with established juvenile polyposis were studied; they underwent clinical examination, x-rays of skull, hands, chest and cardiac studies where indicated. Results: Phenotypic abnormalities were found in 9 (60%): skin and mucous membranes in 7 (freckles, macules, naevi, basal cell carcinoma, skin pits and telangiectasia); skeletal in 6 (macrocephaly, hypertelorism, broad thumbs, short metacarpals, bifid rib and bony cyst); mental retardation in 4; cardiac anomalies in 3 (VSD, PDA), clubbing in 3; undescended testes in 2; and gut abnormalities in 3. Two patients each should perhaps be reclassified as Bannayan Riley Ruvalcaba syndrome (Macrocephaly, mental retardation and genitai pigmentation) and Gorlin’s syndrome (basal cell naevi and carcinoma and skeletal abnormalities).

Conclusions: Important phenotypic abnormalities are common in juvenile polyposis, some having features of Bannayan Riley Ruvalcaba and Gorlin’s syndromes.
GERMINE APC MUTATION AT CODON 1061 IS ASSOCIATED WITH SEVERE DUODENAL POLYPSIS BUT NOT HIGH COLONIC POLYP COUNTS IN FAMILIAL ADENOMATOUS POLYPSIS (FAP).

H.S.Deb, N. Pack, S. Cottral, S.V. Hodgson, A. Spigelman, R.C. Philips, St. Mark's Hospital and Imperial Cancer Research Fund, London.

Site specific APC germline mutations may be related to variations in phenotype and associated cancer risk. A severe colonic phenotype related to mutation at codon 1309 has been previously described.

We have documented severe duodenal polyposis relating to a 5 base pair deletion at codon 1061 on exon 15. Thirteen patients (M=9, F=4) from 5 families with the 1061 mutation are currently participating in an endoscopy surveillance programme. The staging of duodenal disease is based on combined endoscopic and histological assessment.

Advanced staging was noted in this group (stage 0 n=6, stage 1 n=1 (8%), stage 2 n=2 (13%), stage 3 n=6 (46%), stage 4 n=2 (13%) compared to our 182 patients who have undergone initial surveillance endoscopy (stage 0 n=11 (6%), stage 1 n=43 (24%), stage 2 n=68 (37%), stage 3 n=50 (27%), stage 4 n=10 (5%) p = 0.1, N.S.3 patients with the 1061 mutation had tubulo-villous adenomas at their index endoscopy. No duodenal cancers have occurred during surveillance and there is no family history of duodenal cancer.

These patients with advanced disease are young (35.7 ± 9.5 yrs) and their median colonic polyph count documented at previous resection were only modest (720 range 300-1400).

Mutation at codon 1061 may be associated with advanced duodenal but not colonic polyposis.

GI infection T158-T165

The association of Hepatitis C viral infection (HCV) with Porphyria Cutanea Tarda (PCT) in the Lothian region of Scotland

Ukhbar Hassain, N.C. Hepburn*, Alison Jones, Kevin O' Rock**, P.C. Hayes

Hepatitis C viral infection may be associated with PCT. We have reviewed the prevalence of HCV infection in a series of patients with PCT in Lothian Scotland.

Porphyria cutanea tarda is believed to be due to reduced hepatic uroporphyrinogen decarboxylase activity associated with risk factors such as alcohol abuse, oral contraceptives and other drugs. Recently it has been suggested that hepatitis C viral infection may be associated with PCT. We have reviewed the prevalence of HCV infection in a series of patients with PCT in Lothian Scotland.

We identified 12 patients with PCT all of whom had abnormal LFT's. Liver histology revealed 6 with chronic active hepatitis, 4 with micronodular cirrhosis, one hepatocellular carcinoma and one normal (HIV positive). Out of 12 patients tested, 11 were positive for anti HCV antibodies by second generation Enzyme linked immunosorbent assay (ELISA II), Recombinant Immunoblot Assay (RIBA II) and confirmed by Polymerase chain reaction (PCR).

In a second group 14 patients with chronic HCV infection matched for age and sex with the PCT patients, urinary excretion of uroporphyrin was measured and was found to be normal in all.

Conclusions

We have demonstrated a strong association between HCV infection with PCT in Scotland, similar to that in Spain and Italy and much higher than observed in Ireland and Germany. Hepatitis C viral infection could explain the development of inflammatory changes in the liver and progression of liver disease in patients with PCT. Porphyrin metabolism however, appears normal in patients with chronic HCV infection without PCT.

STIMULATION OF GUT ORNITHINE DECARBOXYLASE ACTIVITY BY GIARDIA LAMBILA IN VITRO. A.M. Cevallos, Patchett SE, Alstead EM, M.J.G. Farthing. Digestive Diseases Research Centre, Medical College of St. Bartholomew's Hospital.

The pathogenesis of giardiasis is poorly understood. Diarrhoea and malabsorption may occur even in the absence of morphological abnormalities of the small bowel mucosa. We have previously demonstrated that ornithine decarboxylase (ODC), the rate limiting enzyme in cellular proliferation, is stimulated by Giardia lamblia in vitro. An increased proliferation rate with presence of immature enterocytes in the mucosal villi may contribute to the development of malabsorption in giardiasis. Therefore, it is important to investigate if ODC activity is also stimulated in vivo. Six day old Sprague Dawley (CD) rats were inoculated with 5 x 10^10 trophozoites strain VNB3. At 5 and 10 days post-inoculation (PI) animals were killed and a 2.5 cm segment of proximal jejunum excised, opened longitudinally, washed 4 times in cold PBS to remove attached trophozoites, homogenized and stored at -70°C until assayed for ODC activity. The number of trophozoites present in the rest of the bowel was assessed. Similar samples were obtained from age-matched non-infected animals. ODC activity was measured using a [14C]-orotidine bioassay and results are expressed in pmol/h/mg of protein (median [interquartile range]).

Results: Trophozoite load was 3.4±1.5 (1.1-5.0±1.5) at 5 days post inoculation and 7.1±1.5 (3.3-18±15) at 10 days ODC activity

<table>
<thead>
<tr>
<th>ODC activity</th>
<th>Control</th>
<th>Infected</th>
</tr>
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<tbody>
<tr>
<td>5 days PI</td>
<td>80.1 (33.2-113.7)</td>
<td>137.2 (110.5-228.8)</td>
</tr>
<tr>
<td>n=12</td>
<td>n=12</td>
<td></td>
</tr>
<tr>
<td>10 days PI</td>
<td>25.4 (11.9-54.9)</td>
<td>338 (182-1018)</td>
</tr>
<tr>
<td>n=10</td>
<td>n=10</td>
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ODC activity was increased 3.5 fold in animals infected with G. lamblia (p=0.0001), this effect was more evident at 10 days PI (1.7 fold at 5 days PI and 13.3 fold at 10 days PI). There was no correlation between trophozoite load in the rest of the small bowel and ODC activity (r=0.15). In summary, G. lamblia stimulates ODC activity in vivo. This stimulation may have a primary role in the pathogenesis of malabsorption in giardiasis.

We have previously demonstrated that ornithine decarboxylase (ODC), the rate limiting enzyme in cellular proliferation, is stimulated by *Giardia lamblia* in vitro. ODC activity of Caco-2 cells is stimulated by live, attached trophozoites but not by dead trophozoites or their membrane, cytoskeletal or cytosolic fractions. Live trophozoites, separated from the Caco-2 cells by a porous membrane, do not stimulate ODC activity. Our aim was to evaluate the role of the cytoskeleton in the stimulation of ODC activity by *G. lamblia* (GL). Monolayers of Caco-2 cells were co-incubated with 2×10⁵ trophozoites resuspended in HEPES media, with or without cytoskeletal inhibitors, for 1 h at 37°C. After incubation, plates were cooled at 4°C for 15 min to detach *Giardia* trophozoites, and washed 3 times with cold PBS. After washing, cells were homogenised and stored at -70°C until assayed. ODC activity was measured using a [³H]-ornithine bioassay. The cytoskeletal inhibitors used in these experiments were cytochalasin D (0.1–3 μg/ml) and colchicine (10⁻⁴ to 10⁻⁵ M). Basal ODC activity was not affected by cytochalasin D or colchicine at any of the concentrations used. *Giardia*-stimulated ODC activity 5.4 fold, from 69.4 pmol/h/mg (median) (9.1–170.3 [interquartile range]) to 378.6 (84.7–1532.6) pmol/h/mg (p<0.05). Cytochalasin D inhibited GL-stimulated ODC activity at all concentrations tested. Basal ODC activity in the presence of the same concentration of cytochalasin D failed to stimulate ODC activity (112.5 [61.4–291.0]; p=0.23). Colchicine inhibited GL-stimulated ODC activity at all concentrations tested. Basal activity for colchicine at a concentrations of 10⁻⁴ M was 28.6 (9.0–88.3) pmol/h/mg which did not increase after incubation with *Giardia* (27.8[23.2–107.2]; p=0.75). *Giardia lamblia* stimulates ODC activity in vitro. Inhibition of cytoskeletal function results in loss of stimulation of ODC activity. This data suggest that attachment is a pre-requisite for ODC stimulation.

CONFIRMATION OF PERSISTENT MEASLES VIRUS INFECTION OF INTESTINAL TISSUE BY IMMUNOGOLD ELECTRON MICROSCOPY. Wakefield AJ, Lewin J, Dhillon AP, Sim R, Pounder RE. *Inflammatory Bowel Disease Study Group*, Royal Free Hospital School of Medicine, London, UK.

**Aim:** To provide direct confirmation of persistent measles virus infection of intestinal tissue using immunogold electron microscopy.

**Methods:** Entirely novel strategies for immunogold detection of measles virus were developed and applied, initially, to measles infected and uninfected vero cells, and archival formalin-fixed paraffin processed tissues including subacute sclerosing panencephalitis and acute measlesappendicitis, and finally, to 5 cases of granulomatous Crohn’s disease. Sections were exposed to polyclonal anti-measles N-protein antibody linked to 10nm colloidal gold, and examined by transmission electron microscopy. Mumps (a related paramyxovirus) infected vero cells, were used to exclude antibody cross-reactivity. Negative control tissue sections were treated either by omission of the primary antibody, or by exposure to a mumps monoclonal antibody.

**Results:** Measles antibody co-localised with both intranuclear and intracytoplasmic nucleoplasids in vero cells, brain, and appendix, with established measles infection: an identical signal was observed in all five cases of Crohn’s disease, and localised to macrophages, endothelium and lymphocytes. The measles antibody did not cross-react with mumps in infected vero cells. No signal was observed in negative controls.

**Conclusion:** This preliminary study provides the first direct confirmation of persistent measles virus infection of intestinal tissue.

This study was supported by the National Association for Colitis and Crohn’s Disease.

ABSCESSES AFTER LIVER TRANSPLANTATION: A 10% YEAR STUDY. L. Sandoval, N Rolando, J. Wade* and Roger Williams. Institute of Liver Studies and *Department of Medical Microbiology, King’s College School of Medicine & Dentistry, London SE5 9RS, U.K.

Abscesses are a major complication following orthotopic liver transplantation (OLT) and a cause of morbidity and mortality. No study of this complication has been reported. We performed an analysis of 80 abscesses that developed in 60 patients undergoing 64 OLT during the period January 1984 to June 1994. The mean age was 43 years (22–33 55%) were male. PBC (21.6%) and PSC (10%) were the most common aetiologies for OLT. Abscesses were localized in: liver 25/80 (31.2%); abdomen 27/80 (33.8%); brain 9/80 (11.2%); wound 1/80 (15%); soft tissue 5/80 (6.2%) and lungs 2/80 (2.6%). 43 patients had a single abscess and 17 had multiple abscesses at different anatomical sites. There were 25 hepatic abscesses in 22 patients, 3 of these had recurrent abscesses, 10 had multiple abscesses and in 7 the graft was lost due to this complication and were retransplanted. 14/22 patients had hepatic artery thrombosis; 11/22 biliary problems and 8/22 both. Culture of 20/25 abscesses yielded a single microorganism (45% Gram positive, 25% Gram negative and 15% fungal), 25/30 were polymicrobial. 7/25 (27.5%) abdominal abscesses were related to biliary leaks. Of 9 brain abscesses, 5 were due to *Nocardia spp* in patients with disseminated disease; 1 was due to Aspergillus and in 3 cultures were negative. 12/12 wound abscesses required surgery in 88% of cases overall. After OLT, 31/60 (53%) patients died, 23/32 (71.8%) patients died within the first year post OLT and infection including abscesses was a major contributing factor. Despite the decrease through the years of this complication, abscesses remain an important cause of early mortality, morbidity and retransplantation.

NOVEL MOLECULAR APPROACHES IN GASTROENTEROLOGY: DIRECT IN SITU REVERSE TRANSCRIPTION/PCR (IN SITU RT-PCR) FOR DETECTION OF MEASLES VIRUS. Ray RA, Cooper PJ, Chadwick N, Sim R, Earle P, Dhillon AP, (Pounder RE), Wakefield AJ. *Inflammatory Bowel Disease Study Group*, Royal Free Hospital School of Medicine, London, UK. Queen’s University, Belfast.

**Aim:** To establish an in situ RT-PCR protocol for amplification and detection of measles virus for application to the study of persistent measles virus infection in the gut. **Methods:** Novel protocols for in situ RT-PCR were developed, initially, in measles virus infected vero cells, either in suspension or on slides, and subsequently in formalin-fixed paraffin processed sections of mixed populations of measles virus-infected and uninfected vero cells. Large volumes of novel reaction solutions were placed into a well, created by a heat-resistant gum, in order to minimise evaporation, and permit large sections to be evaluated. Both single round and nested in situ PCR were performed, with detection of amplimicants by direct incorporation of Digoxigenin-11-DUTP and immunocytochemistry. Results were compared with standard in situ hybridisation (ISH) and immunocytochemistry (ICC) for measles virus. Amplificant specificity was confirmed by cDNA extraction from cells and gel electrophoresis, Southern blotting and sequencing.

**Results:** Direct in situ RT-PCR detected measles virus in all infected cell preparations but not in uninfected cells. Controls, consisting of either no primers, irrelevant primers or no Taq polymerase, gave no signal. RNase pre-digestion greatly reduced signal. The signal pattern was identical to that obtained with ISH and ICC, and superior to the former. **Conclusion:** In situ RT-PCR can be applied successfully to routinely processed sections, and may be ideally suited for detecting number RNA viruses in infected tissues. This study was supported by NACC.
Inflammatory bowel disease T166-T176

COLONIC EPITHELIAL CELL NEUTRAL ENDOPEPTIDASE IS AN IMPORTANT INACTIVATOR OF FMET-LEU-PHE

Cole AT, Garlick NM, Galvin A, Robins AH, Hawkey CJ, Division of Gastroenterology and *Department of Immunology, University Hospital, Nottingham

Deficient inactivation of luminal bacterial proinflammatory peptides such as f-met-leu-phe (fMLP) is a potential mechanism underlying colitis, so understanding the inactivation of these peptides is important. We have previously shown that Caco2 cells, an immortal colonic epithelial cell line, are capable of neutralizing fMLP and have now tested this hypothesis with the cecot-enzyme neutral endopeptidase 24:11 (NEP) contributes to this inactivation.

Methods: Caco2 cells were grown to confluence (day 14-23), washed with HBSS/10% MOPS buffer, incubated with 10 ml fMLP (1x10⁶ M in HBSS/MOPS) for 4 hours and 1.2 ml aliquots removed at 1 h intervals and frozen at -70°C for subsequent bioassay. Inactivation of fMLP was inhibited by the specific endopeptidase inhibitors phosphoramidon (Po, 1 x 10⁶ M) and thiorphan (Th, 1 x 10⁶ M). Bioactivity of aliquots was subsequently assayed by using a flow cytometric methodology involving the use of an ELISA plate coated with MoAb against the proinflammatory peptide (Garlick NM, Clin Sci 1994 abstract in press) with excess NEP inhibitor present. Our bioassay for NEP dependent fMLP degradation was validated in experiments with purified rat kidney NEP (1:200 dilution, gift of Fisons PLC) and carboxypeptidase (0.5 μM/ml). Results are expressed as mean %NSC ± SD.

Results:

<table>
<thead>
<tr>
<th>No enzyme</th>
<th>NEP (10 min)</th>
<th>NEP (30 min)</th>
<th>NEP + Ti</th>
<th>NEP + Po</th>
</tr>
</thead>
<tbody>
<tr>
<td>blank fMLP</td>
<td>14(7)</td>
<td>895(5.8)</td>
<td>924(7.0)</td>
<td>94(3.5)</td>
</tr>
<tr>
<td>Po 1 x 10⁶ M</td>
<td>14(7)</td>
<td>240(5.6)</td>
<td>879(6)</td>
<td>924(4.5)</td>
</tr>
<tr>
<td>Th 1 x 10⁶ M</td>
<td>14(6.9)</td>
<td>15(6.1)</td>
<td>13(6.2)</td>
<td></td>
</tr>
<tr>
<td>Caco2 (n=4, b)</td>
<td>15(5.3)</td>
<td>22(10)</td>
<td>52(18.8)</td>
<td>61(10.4)</td>
</tr>
</tbody>
</table>

Conclusion: NEP is responsible for inactivation of fMLP by Caco2 cells, though not all the inactivation of fMLP is due to this enzyme. Alterations in expression of this peptidase by colonic epithelium may have important consequences for gut inflammation.

DIAGNOSTIC IMPLICATIONS OF RAISED ANTIBODY TITRES TO SACCHAROMYCETES CEREVEAII IN GASTRO-INTESTINAL DISEASES


The finding of raised antibody titres and description of clinical improvement on a yeast free diet have implicated Saccharomyces cerevisiae (Sc) (baker’s yeast) in the aetiopathogenesis of Crohn’s disease. We have therefore evaluated the potential role of antibody titre to Sc in the diagnosis of Crohn’s disease. An ELISA, with results (mean ± SEM) expressed as optical density at 410 nm, measured class IgA and IgG antibodies to Sc in sera of patients with Crohn’s disease (n=112), patients with other gastrointestinal disease (n=124) and healthy controls (n=44). Differences between the groups were analysed using the Wilcoxon test.

IgA to Saccharomyces cerevisiae was found to be significantly raised both in patients with Crohn’s disease (0.180 ± 0.012; P<0.001) and other patients attending the clinic (0.126 ± 0.005; P<0.001) when compared with healthy controls (0.041 ± 0.003). Similarly significant signs of IgG to Sc were shown in patients with Crohn’s disease (0.515 ± 0.227; P<0.001) and clinical patients (0.326 ± 0.019; P<0.001) compared with healthy controls (0.153 ± 0.026). However, immunoglobulin titres neither showed any relationship to site or activity of Crohn’s disease (Harvey Bradshaw Index) nor offered a reliable diagnostic marker for Crohn’s. IgA, considered the most discriminant, produced only a sensitivity of 54% for diagnosis when an optical density of 0.15 was used as diagnostic threshold. In addition further analysis revealed raised titres of IgA in patients with Irritable Bowel Syndrome (n=22) (0.117 ± 0.011; P=0.001) compared with healthy controls (0.041 ± 0.003).

Elevated antibody titres to Saccharomyces cerevisiae are not a specific diagnostic marker for Crohn’s disease. The cause and significance of an elevated immune response to Sc remains unknown but raises questions about the pathogenesis not only of Crohn’s disease but also Irritable Bowel Syndrome.
EVIDENCE THAT TROPOMYOSIN IS NOT THE MAJOR ANTIGENIC TARGET OF THE DAS ANTIBODY YE12H12 IN ULCERATIVE COLITIS

B. J. Rankin, E. D. Siviyutaya, C. O. Record, J. P. Pearson and A. Allen
Department of Physiological Sciences, University of Newcastle-upon-Tyne. Department of Gastroenterology, Royal Victoria Infirmary, Newcastle-upon-Tyne.

The adherent mucus gel barrier is thinner and discontinuous in patients with ulcerative colitis (UC) compared to the continuous layer in normal controls. This correlates with elevated activity of the mucolytic colonic proteases in UC. Here we describe the covalent polymerization of mucin from the adherent mucus gel in patients with UC.

Surface adherent mucus (brushings) and total cellular and adherent mucus gel (biopsies) were obtained during colonoscopy of UC patients and normal controls. Samples were collected into a cocktail of protease inhibitors and frozen at -20°C. Mucin was separated from the tissue by homogenization and CsCl density gradient centrifugation. Following exhaustive dialysis and freeze drying, polymetric and degraded mucin were separated using SDS PAGE on 4-15% gels, stained with PAS and scanned at 555nm.

There was a significant decrease in the fraction of polymerised mucin in the adherent mucus gel from patients with UC, mean = 50.6% SEM = 2.9 ± 5 compared to that from normal controls, mean = 64.5% SEM = 2.4 n=15 p=0.01. In contrast, there was no significant difference in polymeric mucin in colonic biopsies from patients with UC mean = 51.2% SEM = 2.9 ± 5 and normal controls mean = 59.0% SEM = 2.9 ± 2.

These studies show (i) a structurally weaker adherent colonic mucus gel barrier exists in UC (ii) this structural weakness is not reflected in the intracellular pre-secreted mucus (iii) this structural weakness may be explained by increased mucolytic faecal protease activity in UC.


The clear fluid passed per rectum after drinking an isotonic PEG-electrolyte solution is essentially a whole gut perfusate. Neutrophils can be detected in whole gut lavage fluid (WGLF) by cytofluorimetric analysis of granulocyte elastase (GE). We have previously found that neutrophils in WGLF characterise active colonic IBD, but not active small bowel CD. The aim of the present study was to investigate whether IL-8 is the chemotactic factor for neutrophil migration into the gut lumen in CD and/or UC. We studied 22 healthy volunteers, 60 CD and 28 UC patients, fully characterised clinically, and 58 with other GI diseases. IBD activity was objectively assessed by WGLF IgG content. GE was measured after sonication using the specific chromogenic substrate 1-pyrroglutamyl-L-prolyl-L-valyl-p-nitroanilide (QuadracTech). IL-8 was measured by a sandwich ELISA kit (Quantikine, R&D).

GE and IL-8 are normally undetectable in WGLF. WGLF GE and IL-8 concentrations were significantly higher in active CD and UC compared to inactive CD and UC. IL-8 correlated significantly with GE in UC (r=0.8; p<0.0001) but not in CD patients as a whole (r=0.19;p>NS), nor in colonic CD alone (p>NS).

The chemokine IL-8 is present in high concentrations in the gut lumen in active UC and CD, and IL-8 is likely to be the stimulus for luminal neutrophil migration in UC. However in CD, other candidate chemotaxants such as bacterial peptidoglycans and other more relevant, and their ablative gradient may explain the difference in luminal neutrophil migration between large and small bowel CD.
ASSessment by local micro for various py either active med)

Rectal blood flow in healthy controls was found to be 158 ± 8 PU. In patients with ulcerative colitis the values (mean 125 ±6) were significantly reduced (p <0.01) in all ulcerative colitis patients, both in active phase or in remission, regardless of the clinical and endoscopic severity of the disease, when active.

Conclusions: Impaired local blood flow may have a pathogenetic role in ulcerative colitis. Remission of the disease is not accompanied by normalization of local microcirculation, which may predispose to relapses.


Two hundred and twenty five patients with perianal Crohn's disease (PACD) were studied from 1979 to 1993 (sex: 126M - 76F, 96F - 43%; age: mean 49 yrs, median 36 yrs, range 12 - 86). Perianal lesions included minor cutaneous lesion (52.2 or 10%), ulceration (23 or 10%), fissure in ano (53 or 23%), anal endoscopy revealed bowel excorrision because of 25%, gluteal abscess (5 or 2%), fistula in ano (16 or 73%), rectovaginal fistula (30 or 13%), other(s) (11 or 5%). Medical therapy was first attempted in 148 pts being effective in 54 (36%), while surgery alone was employed in 77 pts (33%). Surgery was performed in 164 pts (73%). The employed procedures were: elective colorocutaneous resection in 53 pts (31%), resection of PACD or fistula in ano (43 or 26%), laparotomy (32 or 34%), proctectomy (8 or 5%), sphincterectomy and drainage (4 or 4%), drainage of abscess (78 or 46%), splenectomy (1 or 0.5%), other(s) (38 or 22%). The surgical therapy was followed by no change in 59 pts (35%), in 39 subjects 24% there was full recovery, while no change was observed in 27 (18%). Re-operation because of recurrence of PACD was necessary in 40 pts (26%).

The results of the follow-up (median: 6 yrs) are as follows: recovery (68 or 39%), no change (30 or 20%), improvement (33 or 20%) asymptomatic PACD (14 or 6%), recurrence (severe)(21 or 1%), mortality (9 or 4%), lost at follow-up (6 or 3%). Our study shows that PACD can be successfully treated by surgery.


Perianal involvement in Crohn's disease is a common and distressing condition, often refractory to medical or surgical treatments. Recent reports suggested the efficacy of hyperbaric oxygenation (HBO) in the healing of perianal lesions. We here report our experience of HBO in 10 consecutive patients with severe perianal CD.

Methods: Ten consecutive patients (8 women, 2 men, mean age 30 yrs) referred to our Hospital for severe perianal CD were studied. The average duration of CD was 6.5 yrs and the average duration of perianal lesions was 3 yrs (2 month - 10 yr). There were 4 superficial fissures, 4 admitting ulcers, 6 low or superficial fistulas, 2 high fistulas and one irreversible anal stenosis. All patients had received one or more medical treatments without healing the perianal lesions. Patients were treated twice a day, 5 days a week, in a multiphase chamber pressurised at 2.5 absolute atmospheres. Patients breathed 100% oxygen through a face mask. Oxygen duration was 2 hours starting with 15 minutes compression and ending with 15 minutes decompression. Results: Two patients discontinued HBO after a few sessions: the first one due to a bilateral ear-drum perforation, and the second one because of a bad psychological tolerance of the treatment. Eight patients completed at least 30 HBO sessions and were evaluable. At the end of the procedure, 60 of 8 patients treated were cured, 3 completely and 3 partially. The 3 patients who healed completely received HBO as an additional treatment to local surgical procedures. Two patients were not improved: the first one had an unhealed ano-rectal fistula and the second one a rectovaginal fistula.

Conclusion: HBO might be useful as a last resort treatment of chronic perianal Crohn's disease resistant to other treatments or as a complement to surgery.

Dexamethasone inhibits ulceration and promotes healing of experimental indomethacin-induced jejunal ulceration. A. Anthony, A. P. Dhillion, R. Sim, R. E. Pounder, A. J. Wakefield. Inflammatory Bowel Disease Study Group, Royal Free Hospital School of Medicine, London, N3, UK.

Background: Glucocorticosteroids are effective in the treatment of inflammatory bowel disease but their mechanism of action is not clear and is probably multifactorial.

Aim: We investigated the effect of dexamethasone on indomethacin-induced small intestinal ulceration in the rat.

Methods: A group of rats (n=6) received oral indomethacin (15mg/kg) and were killed 24 h later. The jejenum was examined and scored for mucosal ulceration. Other groups received oral dexamethasone (1, 3 and 6mg/kg) 0.5 h prior to indomethacin. Histological evaluation was performed, and ulcer scores were assessed both histologically and immunohistochemically.

Results: Indomethacin caused jejunal ulceration that was only reduced by the highest dose of dexamethasone 6mg/kg (ulcer score: dexam = 292 ± 80 mm² (P<0.01) vs control = 1860 ± 239 mm²). Dexamethasone caused a significant fall in the haemoglobin concentration that was prevented by dexamethasone at all doses. The ulcers induced by indomethacin were more severe, while the ulcers arising in rats pretreated with dexamethasone (all doses) were lined by a white fibrino-purulent exudate. Histologically, this ulcer exudate contained bacteria, fibrin, mucus and neutrophils. Dexamethasone alone had no significant histopathological effect on the small intestine.

Conclusions: Dexamethasone at high doses inhibits experimental indomethacin-induced jejunal ulceration. At low doses, it allows the stabilisation of the ulcer base with bacteria, fibrin, mucus and neutrophils with a reduction of ulcer haemorrhage. These observations may indicate a potential mechanism of action of glucocorticosteroids on human inflammatory bowel disease.
Inflammation T177–T187

DOWN-REGULATION OF INTERLEUKIN-8 PRODUCTION FROM PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH CROHN’S DISEASE VS. CONTRASTED PATIENTS WITH CD AND UC
AC de Bea, JA Ross, KCH Fearon, DC Carter
University Department of Surgery, Royal Infirmary, Edinburgh

Leukocyte activation and pro-inflammatory cytokine release is thought to contribute to the systemic sequelae of acute pancreatitis. Resistance to normal down regulation such as IL-10 may also contribute to the inflammatory response. To examine this hypothesis, peripheral blood mononuclear cells (PBMC’s) were isolated from healthy controls and during the first day of admission in 16 consecutive patients with acute pancreatitis and cultured in the absence or presence of lipopolysaccharide (LPS) (5 ug/ml) for 24 hours. Both spontaneous and LPS-stimulated IL-8 production, measured by enzyme-linked immunosorbent assay, in the pancreatitis group was elevated compared with the controls (238±26 vs. 103±17 ng/ml, meansEMAN, p=0.003; 424±35 vs 192±20 ng/ml, p=0.005 respectively). Subsequently, PBMC’s in 6 patients and 6 controls were incubated as above with human recombinant IL-4 or IL-10. The results are shown in the table and are expressed as a percentage of IL-8 production in the absence of IL-4 or IL-10.

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous IL-8 pancreatitis controls</th>
<th>LPS-stimulated IL-8 pancreatitis controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4:0</td>
<td>100(0)</td>
<td>100(0)</td>
</tr>
<tr>
<td>IL-4:0.05</td>
<td>93.1(13.9)</td>
<td>86.7(8.8)</td>
</tr>
<tr>
<td>IL-4:0.5</td>
<td>63.7(15.3)</td>
<td>71.3(10.3)</td>
</tr>
<tr>
<td>IL-4:5</td>
<td>24.3(7.6)</td>
<td>31.9(4.0)</td>
</tr>
<tr>
<td>IL-4:50</td>
<td>14.3(5.3)</td>
<td>21.6(3.2)</td>
</tr>
</tbody>
</table>

The results indicate that IL-10 down-regulates IL-8 production in PBMC’s from patients with acute pancreatitis.

T179


Why does H. pylori cause different diseases in different individuals? Atrophic gastritis directs the patient towards gastric cancer. Atrophy probably results from specific immunological events. H. pylori induces recruitment of antigen presenting cells and consequent T cell immune responses to potential antigens. Inherited differences in the HLA region often lead to different disease outcomes. This study asked whether differences in the DO groups of the class II complex predict the presence of atrophy in H. pylori infection.

Method: Northern Europeans were studied prospectively. H. pylori was diagnosed by culture, histology and rapid urease tests. 3 antral and 3 corpus biopsies were assessed for atrophy and intestinal metaplasia (IM). Class II alloantigen typing was performed by polymerase chain reaction amplification of DNA extracted from peripheral blood leukocytes with sequence specific oligonucleotide priming. HLA specificity frequencies were compared with correction for the number of alleles (7) tested.

Results. Expressed as specificity frequency (%) DQw 2 3 4 5 6 Frequency
Atrophy+/− M. I. (17 patients)
(17 patients)
(17 patients)
(17 patients)
(17 patients)

No atrophy+/− M. I. (17 patients)
(17 patients)

#Differences in frequency of DQs p<0.05.

No significant differences were found with other specificities.

Conclusions: The results suggest that DQ 5 predisposes to the development of atrophy. Inherited differences in the HLA region affect the clinical outcome of H. pylori infection.

T180

EOSINOPHIL RESPONSE TO OKT, THERAPY IN HUMAN LIVER ALLOGRAFT REJECTION. Ben Ari Z, Lane TA, Chillon A, Rolles K, Davidson B, Burroughs AK. Departments of Haematology and Liver Transplantation Unit. Royal Free, LONDON NW3 2QG

Steroid unresponsive acute cellular rejection remains a clinical problem in liver transplantation. We have previously shown that eosinophils are a specific feature of acute cellular rejection and BMK-13 (anti major basic protein, which has cytotoxic properties) is the best pan-eosinophilic marker. To assess response to a 5 day course of OKT, therapy we quantified eosinophils in 17 liver biopsies (17 patients) who were unresponsive to 2 courses of steroid boluses (1g daily x 3 each). Acute cellular rejection was diagnosed and graded histologically with clinical and biochemical correlation before and after OKT. Eosinophil count was assessed by specific monoclonal antibodies BMK-13. The patients fell into 2 groups. The average number of eosinophils per portal tract before OKT, therapy was 20.5(±7) in 7 patients, and 0.4 per portal tract post OKT, therapy and 7.0(±6) in 4 patients and 0.1 per portal tract post OKT, therapy. Interestingly 6 patients did not have eosinophils in their portal tract prior to OKT, therapy. Patients with high highs within acute rejection (OHAR), they had a worse course as they needed either additional courses of methylprednisolone or FK506 therapy or they developed chronic rejection whilst patients with low or zero eosinophils counts had a better course and responded very quickly to OKT. It may be that the initial lack of eosinophils may reflect resolution of acute cellular rejection despite the presence of a heavy portal infiltrate. This study suggests that the number of eosinophils in the portal tract reflects the response to anti rejection therapy. It may also reflect the later clinical course and further suggests it’s possible role as an effecter cell in immunologically mediated liver allograft injury. These areas need further study, as they may allow tailoring of immunosuppressive therapy in difficult cases.
**T181**

**RECRUITMENT OF MUCOSAL MONONUCLEAR CELLS IN THE SUBMUCOSAL AND INTRAMUCOSAL COMPARTMENTS AFTER IN VITRO EXPOSURE TO GIGADIN PEPTIDES OF THE TREATED CELLCIC INTESTINE EVIDENCE FOR A LOCAL IMMUNE RESPONSE TO GIGADIN.**

*Mari L., **Picarelli A., **Solivert M., **Feis S., **Colletta S., **Greco K., **Di Giammattei I., **Giorpil V., **Del Pozo M., **Rebagliato M., **Grossi P., **Di Nicolantonio G., **Baili M., **Cattaneo T., **Di Pinto E., **Federico II*

The activation of mucosal cell-mediated immune response is a well known feature of the untreated cellic intestine. This in vivo immune activation can be reproduced in vitro in culture from small intestine biopsies of treated cellic patients, as revealed by the expression of HLA-DM by crypt enterocytes (Gut 1992: 33: 472) and interleukin 2 receptor (Gut 1992: 33: 581) in lamina propria (LP) mononuclear cells. In the present study we have investigated the LP cell intramucosal cell populations involved in vitro challenge with gigaedin peptides (gp). Expression of CD4, CD8, alpha and transferrin receptor (TFR) on epithelial cells, of markers of mononuclear cell populations (CD4, CD8, CD68), of activation markers (CD45RO, CD25, B7) and of adhesion molecules (ICAM1I, ICAM2, ICAM3, LFA1) have been investigated by immunohistochemical techniques on a set of frozen tissue sections of 10 treated cellic intestinal samples and 5 control sections, under conditions such as 0.1–1.0 mg/ml or macs (1 mg/ml) or saline (3 mg/ml) polyamide.

The presence of gp in the culture medium induces: a) in crypt enterocytes expression of HLA-DR, IFN, and TNF; b) in the subepithelial compartment of the LP recruitment of activated monocytes (CD45RO+CD14+, CD68+) which express ICAM1, ICAM2 and ICAM3, CD68, B7; in the intraepithelial compartment recruitment of T lymphocytes which express CD45RO and/or CD8, LFA1, ICAM1, ICAM2, B7; in the intracellular compartment recruitment of B lymphocytes which express CD20 and/or CD68, LFA1, ICAM1, ICAM2, B7. Similar results have been obtained in untreated cellic mucosa. The gp3 polyamide proteins are devoid of such in vitro effect. Gigaedin peptides in treated cellic mucosa trigger a specific non immunological response involving gp3 expression, without gp3 damage and/or proliferative reaction. Cells involved in the lamina propria immune response are both lymphocytes and monocytes, but only a particular subset of T cells seems to be able to migrate into the epithelium. ICAM1, ICAM2 and CD8 intracellular lymphocytes may play a role in determining tissue damage.

**T182**

**HYPOXIA INDUCES IL-8 PRODUCTION IN COLONIC AND HEPATIC EPITHELIAL CELL LINES. C.-C. Schurer-Waly, W. Baier-Kustermann, C. Bauer, F.E. Maly (introduced by E.S. Parsons). Inst. M.E., Colombian University, Zürich, Switzerland.

We investigated if hypoxia induces or modulates production of the potent neutrophil chemattractant IL-8 in gastrointestinal epithelial cells. Three cell lines derived from the gastrointestinal tract, SW620, T84 and HepG2 were grown to confluency in 6 well plates in the appropriate medium and stimulated with 20ng/ml TNF-a or 10ng/ml IL-1 B. 1 ml cultures were exposed for 24 hours to an atmosphere of either 20% or 1% O2. IL-8 release into culture supernatants was assayed by ELISA and expressed in ng IL-8 per ml (mean ± SD of triplicates).

Under normoxic conditions all cell lines constitutively secreted small amounts of IL-8 which were markedly raised under hypoxia. Cell viability was not affected by hypoxia or deduced pH which also had insinificant effects on IL-8 release.

SW620

<table>
<thead>
<tr>
<th>TNF-a</th>
<th>IL-1B</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS 0.2%</td>
<td>8.9±0.3</td>
</tr>
<tr>
<td>PBS 2%</td>
<td>2.9±0.0</td>
</tr>
</tbody>
</table>

We conclude that gastrointestinal epithelial cells have a clear although differing capacity to release IL-8 under hypoxic conditions. Hypoxia also increases the response to inflammatory cytokines. These mechanisms may contribute to neutrophil recruitment and damage to the gut wall in ischaemic disease.

**T183**

**PROTECTION OF COLONIC EPITHELIAL CELLS AGAINST OXIDANT DAMAGE BY POLY-ADP-RIbose POLYMERASE INHIBITORS. Watson A.J.M., Askew J.N. Dept of Medicine, Hope Hospital, University of Manchester, Eccles Old Road, Salford M6 8HD.

**BACKGROUND:** Depletion of cellular ATP plays a dominant role in determining cell viability following oxidant damage in inflammation. We hypothesised that preservation of cellular ATP would enhance cell viability. ADP-riboseylation of nuclear proteins by poly-ADP-ribose polymerase (PARP) can result in significant ATP depletion. We therefore tested whether the PARP inhibitors 3-aminobenzamide and nicotinamide would protect human colonic epithelial cells against oxidant damage.

**METHODS:** HT-29 cells were damaged in vitro by H2O2 with or without the PARP inhibitors 3-aminobenzamide and nicotinamide. The inactive structural analogues aminobenzoic acid and nicotinic acid were used as specificity controls. Cellular damage was measured in two ways: 1) immediate damage was assayed by the MIT test 2) long term viability was assessed by measurement of cell number by crystal violet staining 72 hours after H2O2 exposure. Cellular ATP content was measured fluorometrically.

**RESULTS:** 1 mM 3-aminobenzamide or 5 mM nicotinamide gave complete protection from exposure to 1 mM H2O2 for 30 min (cell survival 108.3 ± 10.6% vs 46.9 ± 1.3% (H2O2 alone) and 114.8 ± 4.5% vs 49.9 ± 5.2% (H2O2 alone) respectively, both P < 0.0001). Long term protection was also enhanced by both agents. Cell numbers expressed as a percentage of undamaged control cells were 32.0 ± 1.5% (1 mM H2O2 plus 1 mM 3-aminobenzamide) versus 20.3 ± 1.1% (1 mM H2O2 alone), P < 0.0001. Both 3-aminobenzamide (EC50 1 mM) and nicotinamide (EC50 8 mM) significantly reduced ATP loss during exposure to 1 mM H2O2 for 60 min (P < 0.001). The inactive analogues had no protective effect against H2O2-induced cell death or ATP loss, supporting the hypothesis that the active compounds are acting through PARP inhibition.

**CONCLUSION:** These data suggest that PARP inhibitors protect colonic epithelial cells from oxidant injury, opening new opportunities for therapy in inflammatory bowel disease. (Funded by a BRF project grant).

**T184**

**THE PERITONEAL CYTOKINE PROFILE IN ACUTE PERITONITIS. JN Badia, SA Whawell, DM Scott-Coombes, A Waghorn, JN Thompson. Department of Surgery, Royal Postgraduate Medical School, Hammersmith Hospital. Dene Road, London W12 OHS.

Cytokines are biological mediators of acute inflammation and are also potentially responsive for the systemic acute phase response to surgery.

**Aims.** To correlate the peritoneal and systemic cytokine response in acute peritonitis.

**Methods.** Six patients undergoing emergency abdominal surgery were studied (5 acute appendicitis, 1 diverticulitis). Peritoneal fluid and venous blood samples were taken at the start of operation. The samples were centrifuged at 2500 g for 10 minutes at 4°C and the supernatant stored at -80°C until assay. Interleukin-1 beta (IL-1), Interleukin-6 (IL-6) and Tumour Necrosis Factor (TNF) were measured in plasma and peritoneal fluid using immunosays.

**Results.** Mean duration of preoperative symptoms was (mean ± standard error) 25 ± 3 hours. There was no postoperative complication. The peritoneal fluid cytokine levels were: TNF, 33 ± 14 pg/mL; IL-1, 452 ± 164 pg/mL; IL-6, 133 ± 52 ng/mL. Plasma IL-6 levels were substantially lower: 69 ± 24 pg/mL. Plasma TNF and IL-1 concentrations were very low or undetectable (<10 pg/mL).

**Conclusions.** High levels of peritoneal fluid IL-1 and IL-6 were detected in established acute peritonitis. They mediate the local inflammatory changes and may also contribute to the systemic response to sepsis. They are also potentially useful as a diagnostic test of peritonitis.
INACTIVATION OF RECTAL LTβ IN VIVO AND BY CACO2 CELLS IN VITRO. Cole AT Garlick NM *Entwistle N *Nassim M Jalvin A Robins A Hawkey CJ. Division of Gastroenterology and DInfection, University Hospital, Nottingham, Trent, and R D Lissans, Bakewell Rd, Loughborough Leics.

In patients with active ulcerative colitis (UC) there are raised colonic levels of the inflammatory mediator leukotriene B4 (LTβ) to its inactive form, 6-oxo-LTβ, an important anti-inflammatory mechanism. We therefore measured the levels of oxidized products of LTβ (which are relatively inactive) present in rectal dialsates and tested the hypothesis that colonic epithelial cells inactivate LTβ.

Methods Rectal dialysates from 15 patients with active UC (sigmoidoscopic grades 1 to 3) were analyzed by HPLC (C18 Bond Elut extraction, Waters HPLC-Novapak C18olumn; 280 nm UV & Diode array detectors). Inactivation of LTβ by Caco2 cells was examined by incubating washed cell monolayers with 10ml LTβ 1x10⁴M, for 4h, and removing sequential 1.2ml aliquots at 1h intervals, frozen to -70°C. For assay, Residual LTβ bioactivity was measured using a flow cytometric neutrophil shape change (NSC) assay (Garlick N Clin Sci 1994: abstract in press).

Results Dialysate LTβ levels were <2.4 to 47 x 10⁹M; 1 contained 20-OH LTβ, (1x10⁴M), 3 contained 20-COOH LTβ, (36 to 161x10⁴M) and 3 a product eluting between these tentatively identified as 20-CHO LTβ (19 to 14x10⁴M). In vitro, 10¹⁷M LTβ induced 84% NSC (background 10.2%; EDS0=4.7x10¹M; n=4). Incubation of LTβ with Caco2 monolayers (apical surface) but not cell free controls, caused a time dependent decrease in LTβ bioactivity from 84%M (mean, SD; ± 1) at t=5min to 83% (±8.0) at 1h, 60% (±20.4) at 2h, 33% (±24) at 3h and 8% (±2.5) at 4h (n=4).

Conclusions We have detected ω-oxidation products of LTβ in rectal dialysates of patients with active colitis and have shown inactivation of LTβ by monolayers of colonic epithelial cells in culture. Incubation (ω-oxidation) of rectal LTβ occurs in vivo and may be by the action of epithelial cells.

INTERLEUKIN-13 ENHANCES IL-1α BUT NOT TNF-α-INDUCED CHEMOKINE EXPRESSION IN HUMAN COLONIC EPITHELIAL CELLS.

G. Kolios, D.A.P. Robertson and J. Westwick. Pharmacology Dept., University of Bath and Gastroenterology Dept., Royal United Hospital, Bath.

Inflammatory bowel disease is characterised by neutrophil infiltration of the intestinal mucosa. The transmigration of selected populations of leukocytes are probably regulated by an interplay of adhesion molecules and chemokine production. The aim of this study was to examine the factors which regulate the expression and release of IL-8, a neutrophil and T-lymphocyte chemotactic cytokine, by the colonic epithelial cell line HT-29.

HT-29 epithelial cells were cultured at 37° in McCoy’s 5a medium with 10% fetal calf serum in 6-well plates until formation of confluent monolayers. Then, IL-1α, TNF-α, γ-IFN, IL-4, IL-10 and IL-13 were added in fresh medium in various concentrations either alone or in combination and the monolayers were incubated for 0.5-48 hours.

IL-1α (0.1-10ng/ml) and TNF-α (1-100ng/ml), but not γ-IFN, IL-4, IL-10, IL-13, added alone induced a dose and time dependent expression of IL-8 mRNA (Northern analysis) and release of IL-8 peptide (ELISA). In contrast to other cell types (i.e. monomac cells, synovial fibroblasts) we have examined, TNF-α induced a greater production of IL-8 (up to 149 ± 2.8ng/ml/24 hours) compared to that produced by IL-1α (up to 71.5 ± 1.8ng/ml/24 hours).

Examination of combinations of cytokines revealed that the TNF-α cell-derived cytokine IL-13 produced a dose-dependent (0.1-10ng/ml) significant (p<0.01) enhancement of IL-1α, but not TNF-α-induced IL-8 generation. The IL-1α cell-derived cytokines, IL-4 and IL-10 had no effect on either IL-1α or TNF-α-induced IL-8.

Thus, this synergistic action of IL-13 with IL-1α in normal colonic epithelial cells suggests a pro-inflammatory role for IL-13 in the pathogenesis of inflammatory bowel disease.

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"THE SENTIENT GUT": ENHANCED VISCERAL SENSATION IN THE OESOPHAGUS AND RECTUM IN FUNCTIONAL DYSESPHIA.

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Alteration in visceral sensation at the site of presumed symptom origin in the gut tract has been postulated as an important aetopathological mechanism in the so-called "functional" bowel disorders. Lowered sensory thresholds for the perception of balloon distention have been demonstrated in patients with intractable bowel syndrome, in the oesophagus in non-cardiac chest pain, and in the stomach in functional dyspepsia. Patients presenting with one functional GI syndrome however, frequently have additional symptoms referable to other parts of the gut. and this has led to the suggestion that enhanced visceral nociception may be a pan-intestinal phenomenon. We hypothesised that patients presenting with functional bowel disorders and with heightened perception of stimuli applied to parts of the gastrointestinal tract other than the stomach.

The sensory thresholds for initial perception (IP), desire to defecate (DD), and urgency (U) in response to rectal balloon distention, and the thresholds for initial perception and for discomfort in response to oesophageal balloon distension were measured in 10 patients with FD (7F, mean age 41 yrs.), and to those obtained in healthy controls. Somatic nerve sensory thresholds for perception and discomfort evoked by electrotactile stimulation at the index finger were also recorded in both groups. FD patients had significantly lower rectal sensory thresholds than controls (n = 32) (ml [SEM]): IP 45.0 [17.6] vs 59.3 [1.5] (p < 0.001), DD 98.0 [17.4] vs 298.7 [9.0] (p < 0.001), U 177.2 [25.4] vs 418.1 [12.6] (p < 0.001). A similar, significantly lower sensory thresholds for oesophageal balloon distension were demonstrated in the FD patients as compared to controls (n = 15) (ml [SEM]): perception 8.1 [10.9] vs 12.1 [1.5] (p > 0.01), discomfort 18.1 [11.1] vs 16.4 [1.1] (p < 0.01). Mean perception and discomfort thresholds for somatic nerve stimulation were slightly higher in FD patients than controls (n = 12), but the difference was not statistically significant (mA [SEM]): perception 21.1 [3.4] vs 16.4 [1.1] (p < 0.11), discomfort 44.4 [7.3] vs 42.3 [4.9] (p < 0.09).

These findings indicate that altered visceral sensation in patients with FD effects the gut tract other than the putative organ of symptom origin. This support the concept of generally heightened gut perception in patients with functional gastrointestinal disorders. Enhanced sensation additionally appears to be specific to visceral pathways.

OMEPRAZOLE MARKEDLY DELAYS GASTRIC EMPTYING

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The effect of acid on gastric emptying is controversial. Conflicting results have been obtained using H2-blockers, probably for the interfering effect of their anticholinesterase and motor actions. To clarify the effect of acid juice, gastric emptying of a solid meal (800 kcal; 15% from proteins, 45% from fats, 40% from carbohydrates) was measured by ultrasound scanning in 21 healthy subjects with and without a 4 day pretreatment with Omeprazole, 40 mg man. Antral sections were measured before and at 30, 60, 120, 180, 240 and 300 minutes after the meal. The last dose was taken 24 hour before the test, to avoid any non-secretory effects of the drug.

Results: basal and maximal postprandial antral cross-sectional areas (mean 1 SEM) were the same during the two tests (basal section: 343 (19.9) vs 345.6 (18.5) mm2 after the control (C) and the omeprazole (Om) test meal respectively; p:NS, paired data t test; maximal postprandial: C 1165 (41.5) vs Om 1132 (43.5) mm2, p:NS). A significant difference in antral sections between the two tests was found at time 120 [C 648.4 (45.6) mm2 vs Om 719 (43.0), p<0.05] and 240 [C 346.5 (24.6) mm2 vs Om 310 (21.1) mm2, p<0.03]. A larger proportion of the meal was retained in the antrum throughout the study after omeprazole, again the difference being significant at time 120 and 240. Omeprazole induced a highly significant delay in gastric emptying [C 198.6 (12.6) vs Om 230.9 (12.7) minutes; mean paired difference (95% CI): 42.3 (12.3-50.3), p<0.003]. The delay was not due to a prolonged lag phase [C 21.4 (6.1) vs Om 24.5 (7.7) minutes, NS], but rather to an effect on the slope of the emptying curve [C 4.38 (0.28) mm/min vs Om 3.6 (0.23), p<0.03]. In conclusion, omeprazole delays gastric emptying of a digestible solid meal. This fact should be taken into account when antisecretory drugs are used in duodenal ulcer patients, in whom a fast gastric emptying may increase the duodenal acid load, and, conversely, in dyspeptic patients, in whom gastric emptying is often delayed.

CONTINUOUS AMBULATORY pH MONITORING IN PIGS

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Acid secretion is one of the most studied physiological variables with much of our knowledge coming from work on rodent or canine models. However more no differences exist between the pig and man, including the development of gastric ulcers.

We studied 7 Large White pigs by 48 hr continuous ambulatory pH monitoring using an intragastric radiotelemetry capsule (RTC), attached endoscopically using a modified gastroscopy method. Recordings were made using a specially designed jacket. Standard meals were given to all animals.

Similar traces to those obtained in man were seen with specific parameters in the table below. Brief alkaline episodes were also seen in the early morning consistent with possible bile reflux.

<table>
<thead>
<tr>
<th>Pig</th>
<th>Baseline fasting pH</th>
<th>Inter-quartile range</th>
<th>Peak pH</th>
<th>Time to peak pH (min)</th>
<th>Time to baseline pH (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.9</td>
<td>1.7-2.0</td>
<td>6.4</td>
<td>54</td>
<td>8.0</td>
</tr>
<tr>
<td>2</td>
<td>1.8</td>
<td>1.7-2.0</td>
<td>4.4</td>
<td>20</td>
<td>5.3</td>
</tr>
<tr>
<td>3</td>
<td>1.7</td>
<td>1.6-1.8</td>
<td>4.6</td>
<td>32</td>
<td>10.5</td>
</tr>
<tr>
<td>4</td>
<td>1.4</td>
<td>1.3-1.5</td>
<td>5.6</td>
<td>78</td>
<td>6.5</td>
</tr>
<tr>
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<td>1.9-2.2</td>
<td>5.6</td>
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<td>7.5</td>
</tr>
<tr>
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<td>1.7-2.0</td>
<td>5.2</td>
<td>14</td>
<td>9.5</td>
</tr>
<tr>
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<td>0.8-0.9</td>
<td>3.6</td>
<td>32</td>
<td>12.0</td>
</tr>
</tbody>
</table>

This work provides further evidence for the similarity of gastric physiology in man and pig. Long term assessment of PCI is possible as the RTC has a lifespan of 60 days and is easily replaced.
**T194**

**THE RELATIONSHIP BETWEEN GASTRIC ACID OUTPUT & GASTRIC METAPLASIA (GM) IN THE DUODENUM**

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**Introduction:** Gastric metaplasia (GM) in the duodenum has been reported in 20% of patients with duodenal ulcer (DU) and up to 64% of normal subjects. Animal models indicate that GM develops in the presence of duodenal injury & acid. This study describes the relationship between acid secretion, presence & extent of GM in the duodenal bulb in Helicobacter pylori (Hp+) DU patients & Hp- healthy controls.

**Methods:** We have studied 19 subjects (11 Hp+ DU patients & 8 Hp- healthy controls; 10 male, mean age 36, range 22-58). Hp status was determined by histology, bacterial culture & "C-urea breath test. All quadrants of duodenal bulbs were biopsied (>1cm away from the edge of any ulcer). The biopsies were routinely processed & stained with PAS. Extent of GM was measured by one observer (AWH) using a computer-enhanced image intensifier (Seescan Imaging, Cambridge) & calculated as % of epithelial surface of each biopsy specimen. The mean extent of GM was calculated for each subject. After an overnight fast, acid output (mmol/h) was measured as basal (BAO), peak acid output during gastrin releasing peptide (GEP) infusion (40 pmol/kg/h for 45 minutes) (PAO), & after a 30min washout period, following pentagastrin (6 μg/kg i.m.) (PAO).

**Results:** Median BAO, PAO & PAO, (mmol/h) in Hp-controls & Hp- DU were 2.4, 10.5, 22 & 12, 31, 37, respectively. The differences in BAO, PAO & PAO, between the two groups were significant (p<0.05). Mean GM extent was significantly higher in Hp+ DU (13-100%) than in Hp- controls (6%). There was a significant correlation between mean extent of GM & acid output (r= 0.76, r=0.8 & r=0.6 for BAO, PAO, & PAO, respectively & p<0.05).

**Conclusion:** This study is the first quantitative demonstration of the extent of GM in the duodenum is related to acid output.

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**T195**

**TOPICAL NSAIDS AND UPPER GASTROINTESTINAL BLEEDING AND PERFORATION**

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**Introduction:** The relationship between topically applied non-steroidal anti-inflammatory drugs (TNSAIDs) and gastroduodenal complications is uncertain. The aim of this study was to investigate the hypothesis that TNSAIDs are associated with upper gastrointestinal bleeding and perforation (UGIBP) using a record-linkage database containing all hospital event and dispensing data between 1989 - 1992 in a population of 400,000 people.

**Method:** A nested case-control study was conducted using all 1,458 cases hospitalised with UGIBP between Jan 1990 & Dec 1992, and six community controls matched for age and sex. Four exposure variables (i) exposure in the 45 days prior to hospitalisation (ii) ever exposure, (iii) number of prescriptions and (iv) prescription rate, for TNSAIDs, oral NSAIDs (excluding aspirin) and any ulcer-healing drug (UHD) were obtained using conditional logistic regression. Odds ratios (OR) were calculated with 95% confidence intervals.

**Results:** The OR for UGIBP with exposure to TNSAIDs 45 days prior to hospitalisation was 2.64 (1.66-4.21). For ever exposure to TNSAIDs the EOR was 1.58 (1.27-1.96). There was no relationship between UGIBP and the number or rate of TNSAID prescriptions. After adjusting for dispensed oral NSAIDs and UHDs which were independently associated with an increased risk of UGIBP, the OR for any exposure to TNSAIDs was 1.14 (0.90-1.43). Similar results were obtained for bleeding alone and perforation alone.

**Conclusions:** These data suggest that the association between TNSAID use and UGIBP is a result of confounding by concomitant oral NSAIDs and ulcer-healing drugs (presumably prescribed for pre-existing gastric-duodenal conditions). Topical NSAID use does not appear to be a risk factor for UGIBP.

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**T196**

**LOCALIZATION OF BASIC FIBROBLAST GROWTH FACTOR IN GASTRIC ULCERS. MA. Hull, K Morrell, CJ Hawkay and D Jenkins. Divisions of Gastroenterology and Pathology, University Hospital, Nottingham NG7 2UH.**

Basic Fibroblast Growth Factor (bFGF) has potent angiogenic properties and is also mitogenic for fibroblasts and intestinal epithelial cell lines. Exogenous bFGF promotes angiogenesis and healing of experimental gastric ulcers (GUs) in rats. In normal gastric mucosa, bFGF is present in blood vessels, lamina propria and muscularis mucosa. We have previously shown an increase in immunoreactive bFGF in homogenates from the GU rim compared with intact mucosa (Gut1993;34:S51). Therefore we investigated bFGF localization in chronic gastric ulcers by immunohistochemistry.

**Method:** Formalin-fixed sections from 9 surgically resected GUs (3 antrum, 2 incisura, 4 body; 3 NSAID, 6 non-NSAID) and normal gastric mucosa were stained using a murine monoclonal anti-recombinant human bFGF antibody (Ma552, Wako) and an ABC peroxidase technique.

**Results:** In intact mucosa there was patchy staining for bFGF in parietal cells of gastric glands and bFGF was detected in surface epithelial cells immediately adjacent to the ulcer edge. Basic FGF was detected throughout the muscularis mucosae in increased staining adjacent to granulation tissue. There was intense staining of endothelial cells in granulation tissue microvessels and small arteries but only sparse staining of macrophages and myofibroblasts in granulation tissue. Immunoreactive bFGF was detected at similar intensity in NSAID and non-NSAID GUs.

**Conclusion:** There is increased local expression of bFGF in chronic GUs. Basic FGF is present in epithelial cells at the ulcer edge as well as endothelial and smooth muscle cells. bFGF may promote epithelial mitogenesis as well as angiogenesis in GUs.
Endoscopy T199–T208

Changes in blood pressure and duodenal atony during ERCP: Studies of the influence of buscopan and glucagon. Ramesh B, Jabbar M, Morden A, Huddy SPJ, Wyke RJ – The Ipswich Hospital NHS Trust, Heath Road, Ipswich, Suffolk, IP4 5PD.

Hycosamine N-butylbromide (Buscopan) is more commonly used in the UK than glucagon for duodenal paralysis during ERCP. This study is the first to compare the effect of these drugs on blood pressure (BP) during ERCP. Methods: The BP of 29 patients (aged 37-84 years), who had ERCP, was continuously measured with finger photoplethysmographic monitor. Data was recorded directly onto computer at 30 second intervals for the duration of the procedure. The duration of duodenal atony was measured separately by three observers from videotapes of the procedure. Patients were randomised to receive Buscopan 20 mg (8 ±15) or Glucagon 1 mg (8 ±14) intravenously. All received supplemental oxygen and were sedated with pethidine and midazolam.

Results: Both groups were similar for age, physical fitness, doses of sedation and duration of procedure. None of these factors has significant correlation with BP changes. Duodenal atony was significantly [p < 0.05] longer after Glucagon than Buscopan injection [mean = 17.6 ±6, 8.2 min, vs 13.1 ±4.4]. Baseline (BP, HR) data was similar for both groups. HR increased significantly [p < 0.05] after Buscopan injection from [mean = 94.4 ±6.1, to 126.0 ±19.5]. The mean systolic, diastolic and mean arterial blood pressure fell [p < 0.05] in 8/15 (53.3%) by 20-50 mm Hg of the Buscopan group. Four of these patients (aged 76-80 years) had hypotensive episodes (SBP<100, DBP, MAP < 60 mm Hg) immediately after Buscopan injection which lasted 1-7 min, whereas no significant changes in HR or BP were recorded after Glucagon.

Conclusion: Glucagon produced longer duodenal atony and had no effect on blood pressure. It may be a better choice in the elderly with low baseline blood pressure.


IM is recognized as a precancerous change but the mechanisms underlying its development and evolution are still unclear. In this study, we evaluated risk factors and associated mucosal biochemical changes in a series of 134 patients (69 m, 65 f, mean age 55, range 25-82, after informed consent), affected by chronic non-atrophic or atrophic gastritis, 37 of which (28%) presenting IM of gastric mucosa. Smoking habit, alcohol and vitamin C (VITC) intake were ascertained by using a dietary questionnaire. Helicobacter pylori (Hp) infection was looked for using H&E and Glems in antral biopsies. Gastric juice vitamin C (VITC), mucosal reduced and oxidized glutathione (GSH,GSSG) and malondialdehyde (MDA) (a measure of lipid peroxidation and of free radicals production), were determined by HPLC (the first 3) or fluorometry (MDA), using 4 biopsies. Statistics was performed by univariate and stepwise discriminant analysis. By univariate analysis, age (p < 0.009), VITC intake (p < 0.02), gastric juice GSH (p < 0.05), Hp infection (p < 0.04), atrophy (p < 0.01) and, inversely, alcohol consumption (p < 0.02) were significant. By stepwise discriminant analysis, the risk factors examined, only age (p < 0.008), gastric mucosa MDA (p < 0.05), Hp infection (p < 0.008) were related with IM. In summary, age, atrophy and free radicals production, as measured by MDA, are the major determinants for IM. Free radicals, which are potentially genotoxic, beyond playing a role in causing IM, might also be involved in determining IM evolution into cancer.

A Prospective Randomised Trial of 10 and 11.5 FG Endoprostheses in malignant bile duct obstruction. IA Finnes, PA O'Tooole, JM Rhodes, MG Lombard, R Sutton, IT Gilmore. Gastroenterology Units, Walton and Royal Liverpool University Hospitals, Liverpool, UK.

Introduction. Previous studies have highlighted the advantages of 10FG over 7FG stents in the relief of malignant obstructive jaundice, but whether there is any further advantage to be gained by increasing the stent diameter even further is not clear. We have compared 10FG and 11.5FG stents.

Patients, methods, and results. 94 patients with jaundice due to likely or proven malignancy were prospectively randomised at ERC (n=88) or at combined procedure (n=6) to treatment with a single 10 (n=45) or 11.5 FG (n=49) prosthesis. The trial was stratified into hilar & non-hilar groups to take account of the poorer prognosis reported previously in the former. The patient groups were matched for age, serum bilirubin at entry, diastases, length of stent inserted, and similar proportions of patients from each group subsequently underwent attempted surgical resection (3/45 and 8/49). Jaundice resolved in 85% and 88% of cases respectively. Median patient survival was 125 days (range 3-7000) for 10FG and 143 days (11-700) for 11.5FG. Median stent survival (excluding patients who did not undergo subsequent resection) was almost identical (83 days and 80 days). Stent replacement for blockage was similar in the two groups (10/45 vs 10/49), and 3 patients in each group required stent replacement for migration. The proportion of patients jaundiced at time of death were similar (56% vs 64%). Although the median stent survival in the hilar group was lower (85 days, range 12-320) than in the non-hilar (128 days, range 3-700) this did not achieve statistical significance (p = 0.11, Mann-Whitney). Stents could not be placed at the initial ERC in two cases (one 10FG, one 11.5FG), and a single complication occurred in the 10FG group (retroperitoneal perforation which resolved conservatively).

Conclusions. In this study there was no difficulty inserting the 11.5FG stents, but stent survival and patient survival were almost equal in the two groups.

Low Basic Fibroblast Growth Factor Levels in Intact Mucosa of Gastric Ulcer Patients: Evidence for a Constitutional Deficiency. MA Hull and CJ Hawkey. Division of Gastroenterology, University Hospital, Nottingham NG7 2UH.
The an-id-labile peptide Basic Fibroblast Growth Factor (bFGF) may play an important role in gastric epithelial restitution and repair as well as in ulcer healing. Gastric ulcer (GU) patients appear to have a profound reduction in intact mucosal bFGF levels compared with non-ulcer patients. We tested whether GU healing was associated with changes in mucosal bFGF levels.

Method: Four biopsies from intact antral mucosa were obtained from 8 patients with a GU (7 NSAID, 1 non-NSAID) before and after healing (at 2 months) and 52 non-ulcer controls (33 NSAID, 19 non-NSAID). Following initial biopsies patients received standard ulcer healing therapy (n=5) or were enrolled into a blinded trial of omeprazole 20/40 mg daily versus misoprostol 200 mcg qds for GU healing (n=5). Drug therapy was continuing when the repeat biopsies were taken. Immunoreactive bFGF levels were measured using an in-house ELISA.

Results: Intact mucosal bFGF levels increased significantly following GU healing (GU 8.9±3.0 pg/mg vs healed 41.5±12.3 pg/mg, p<0.02; means±SE, Student’s paired t-test) but were still significantly lower than bFGF levels in non-ulcer patients (211.5±95 pg/mg, p<0.004 vs healed GU values). There was no significant difference in bFGF levels in non-ulcer patients using (210±03 pg/mg) and not using NSAIDs (200±88 pg/mg, SAID, 75).

Conclusion: Low mucosal bFGF levels persisted even after GU healing. This suggests a constitutional bFGF deficiency in these patients which may predispose to ulceration. The increase in mucosal bFGF levels after healing may be due to acid inhibition which would reduce bFGF breakdown. Mucosal bFGF levels seem to be independent of NSAID usage in non-ulcer patients but the possibility that NSAIDs alter intact mucosal bFGF levels in GU patients will require further study.
ENDOscopic SPHINCTerOtoMY in Young Patients: A FOLLOWup STUDY

Endoscopic sphincterotomy (ES) is part of the accepted management of choledocholithiasis. The aim of this study was to establish whether ES is associated with adequate long-term relief and whether it is appropriate in young patients.

41 patients aged 50 yr underwent ES between 1985 and June 1993. All were sent a questionnaire concerning biliary symptoms: abdominal pain and jaundice (major symptoms) and dark urine and intolerance of fatty foods (minor). All were asked to provide blood for liver function tests. 29 replied to the questionnaire (response rate 71%). Median age of the respondents was 44 yr (range 21-69yr), 66% were female and the median follow was 5 yr (0 mo - 8.6 yr).

The majority reported no major biliary symptoms since sphincterotomy. 70% were pain free and 83% had not been jaundiced. However, 31% had experienced darkening of the urine and 45% had intolerance of fatty foods. 17 patients provided blood samples (response 41%) and, of these, 15 (88%) had normal liver function. In the remaining 2 patients who had slight (< 2 x ULN) elevation in the serum alkaline phosphatase (SAP), follow up ERCP was clear in 1 and revealed a bile duct stone in the other (7 yr after the initial ERCP).

These preliminary data show that endoscopic sphincterotomy is well tolerated in the majority of patients in the long term with few reporting major symptoms of biliary disease. However, repeat ERCP should be considered if there is elevation of SAP, even if slight. Furthermore, biochemical assessment is particularly valuable when given the common and frequent reporting of minor symptoms of biliary disease.

T203

THE USE OF HIGH FREQUENCY ULTRASOUND MINIPROBES IN ROUTINE ERCP

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The various methods (ERCp, Endoscopic Ultrasonography (EUS), conventional ultrason, Spiral CT) use imagological bile tree and pancreatic duct have their limitations. Therefore in the early 90’s ultrasound-miniprobes were developed that could be passed through the working channels of endoscopes. They have the opportunity to perform intraductal ultrasound of the bile tree and pancreatic duct with some new, improved probes.

Patients and methods: During routine ERCp in 54 patients the catheter-based ultrasonic devices (outside diameter 1.6-2.4 mm, frequency range 12-20 MHz, Olympus/KeyMed) were passed through the working channel of a standard duodenoscope. Ultrasound scanning from the intraductal bile duct (patients n=45) and main pancreatic duct (n=9) was performed.

Results: Ultrasound scanning revealed high resolution pictures of the bile tree, pancreatic duct, surrounding liver and pancreatic parenchyma. The depth of ultrasound wave penetration was limited to 15-25 mm. In 22 patients with biliary strictures, the miniprobe ultrasound was able to differentiate between different causes (n=7) corresponding to the results of Spiral-CT, EUS, cytology and/or histology. In 3 patients with a filling defect suspicious of an impacted stone, miniprobe scanning demonstrated hilar papillary adenomas (n=2) and a polyposid cholangiocarcinoma (n=1), preventing, therefore, the intraductal use of a basket for stone removal with the possibility of major bleeding. Pre-sphincterotomy scanning with miniprobe of patients with suspected choledocholithiasis revealed stones that were missed on ERCp in 5/16 patients. In patients with chronic pancreatitis (n=3), images revealed a heterogeneous, lobulair echopattern different to that of patients with (n=3) or without chronic pancreatitis (n=3). Examinations were extremely easy to perform and only took between 3-5 minutes. No complications were observed.

Conclusions: Intraductal ultrasound has been found to complement ERCp and conventional US and CT scanning. The examination can be performed quickly, the catheters are simple to use and do not require expensive purpose built endoscopes like the EUS. However, depth of penetration is limited and therefore accurate staging of larger tumours is impossible. We conclude that the miniprobe techniques are extremely useful during ERCp and have great potential in other areas of endoscopy.

T204

LASER lithOTriPY of LARGE stones in the BILE DUCT with a STONE RECOGNITION SYSTEM. CP Swain, M Glynn, C Ainley, N Van Someren, E Bewick. The Royal London Hospital, Whitechapel, London E1 1BB

We studied the efficacy of a new laser system featuring a stone recognition system to fragment large gallstones both in bench experiments and in the human biliary tract compared to a mechanical method. Sequential high powered Nd:YAG laser lithotripsy had failed at endoscopic retrograde cholangiography. The value of laser and electrohydraulic lithotripsy has been previously limited by seedling direct visual evidence of probe contact with the stone using mother/baby endoscope systems since misdirected pulses can perforate the bile duct wall. The new laser stone recognition system (Telemix, Telesop, Advanced Medical Systems) allows fragmentation of the stone without delivering pulses to bile duct wall. The Rhodamin 6G dye laser pulse, wavelength = 595nm striking stone or bile duct wall induces a plasma with a characteristic fluorescence signal which is passed back up the single return laser fiber (1mm in diameter) to the laser system. If the signal is not recognised as stone the pulse is aborted before a 10th of the total energy is delivered. The pulse accelerates the surface of the stone reaching transient peak temperatures >10,000 degrees C with photoacoustic damage knocking small fragments off the stone. In bench experiments the stone recognition system was highly effectively able to distinguish post mortem biliary duct from all types of biliary stone tested (n=50) in over 1,000 experiments. The system sometimes failed to recognize white pancreatic stones as stones i.e. it failed to fire at them but always distinguished pancreatic duct from stone. It fragmented all stones tested working better in liquid phase than air. It distinguished stent (Blue, black, red and white) from stone (we left pigtail stents in situ during treatment). 10 patients with common duct stones of varied size (2.5-6cm) the laser was used without cholangioscopy to fragment stones when mechanical lithotripsy had failed and was used to retrieve an impacted stone with a 1.5 cm stent in the bile duct. Bile ducts were cleared in 9/10 patients requiring a mean of 2.1 laser procedures. 1,700-30,000 pulses of 80-120 mJ were needed per patient - 78% of pulses were in contact with the stone. Patients were aware of laser pulses but none remembered the treatment and three offered surgery instead of repeated endoscopic laser treatment all chose the endoscopic procedure. Laser lithotripsy with this stone recognition system is safe to use without cholangioscopy, easy to perform, effective but is expensive. It represents a significant advance in the management of large retained stones in the bile duct.
A COMPARISON OF THE VALUE AND SAFETY OF PERCUTANEOUS ENDOSCOPIC GASTROSTOMY IN MALIGNANT AND BENIGN DISEASE.


There is little data on the value and safety of percutaneous endoscopic gastrostomy (PEG) in the management of patients with dysphagia due to malignant disease. We compared results of gastrostomy/jejunoscopy placed in 28 patients with malignant disease (M) 20 Head/neck, 8 oesophageal (upper G3 cancer) with results in 92 patients with benign disease (B) (34 stroke, 20 head injury, 16 MDS/MS, 16 mouth disorder, other 6) from 1990-1994. PEG or PEJ were successfully placed in 26/30 B (86%) and 28/28 M patients (85%). Although median survival and stornal feeding time was significantly (p<0.05) shorter for patients with malignant disease (5 months) than benign disease (16 months), some patients with malignancy had prolonged periods of home feeding (8pts>6/12, 1>12/12, 1>36/12) and one patient whose swallowing was restored after radiotherapy had his gastrostomy removed. Total feeding times were 154 months (M) vs 842 (B). PEG placement in malignant disease also allowed effective access for drug treatment/pain control, avoidance of aspiration, management of fistulae, perforation and distal obstruction and helped overcome difficulties of mastication, poor saliva production, andrelated difficulty with staggering/dyskinesia. PEG placement was technically more demanding because of bizarre anatomy sometimes requiring small scopes/mouthguards, a variety of kits, dilators, radiography. 4 direct percutaneous endoscopic jejunostomies (PEJ) using a new technique were placed for gastro duodenal obstruction using an enteroscope puncturing the small bowel directly. Peristomal infection rates were significantly more frequent in M (7/28) than B (2/92) (p<0.05) were reflected by antibiotic prophylaxis, oral antisepsics and post procedure care. Better results were achieved by earlier referral of patients with head and neck cancer prior to radiotherapy. All patients remained able to swallow their saliva (4 had laser treatment, 2 had prostheses, 18 had radiotherapy, 8 had surgery).

These studies give new data concerning the efficacy and safety of PEG in malignant disease, describe new techniques and show that PEG can be highly effective in supporting nutrition and palliating symptoms of selected patients with malignant dysphagia especially of head and neck origin.

SHORT AND LONG TERM OUTCOME AFTER PERCUTANEOUS ENDOSCOPIC GASTROSTOMY PLACEMENT.

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Percutaneous endoscopic gastrostomy (PEG) is an accepted method of enteral feeding. However, there are few analyses of parameters of outcome. We therefore retrospectively determined the outcome of consecutive evaluable subjects in whom PEG was undertaken. Patient files and endoscopic records were reviewed and the diagnosis, previous feeding history, indication, duration of PEG use, complications and outcome were recorded. Follow up was for 6-52 months.

PEG was attempted using the 'pull' technique in 80 patients (49 male), median age 75yrs (range 19-90yrs). Indications were neurological dysphagia (51), mechanical dysphagia (14), and inanition (15). Nasoenteric feeding had been tried in 70% for a median of 16 (4-79) days. Prior evidence of aspiration was evident in 72% of patients. PEG insertion was successful in 93%. The 6 failures were due to patient factors (oesophageal stricture, obesity, subcostal stomach, ascites). No immediate procedure-related complications occurred. One major complication (leakage with peritonitis) and 11 minor complications (superficial stornal infections) occurred within 30 days. One major complication (tube migration requiring PEG removal) and 9 minor complications (6 tube blockages, 3 infections) occurred after 30 days. In 6 patients, aspiration of feeds remained a problem. The PEG was replaced with a gastrostomy button (GB) in 23 patients. Thirty nine patients had died (none tube related) with a PEG or GB in situ, after a median of 2 months (2 days-60 months), 19 patients are alive with a PEG in situ and a median of 8 (6-52) months, while 15 subjects recovered and had the PEG or GB removed after a median 3 (0.5-13) months.

PEG is a safe and effective mode of access for prolonged enteral feeding. Patient selection is important to avoid failure and inappropriate short term feeding. The frequency of aspiration appears reduced but PEG-jejunoscopy may be useful in those with prior aspiration.

DIAGNOSTIC VALUE OF LAPAROSCOPY IN PERITONEAL DISEASES PRESENTING AS ASCITIS OF UNKNOWN ORIGIN.


Introduction: Both peritoneal involvement in systemic conditions and primary diseases of the peritoneum usually present as ascites without an obvious cause. Ascitic fluid analysis can but suggest etiology in these cases. Small and flat peritoneal nodules are not often able to be detected by abdominal ultrasonography and/or CT scan.

Aim: To assess the efficiency of laparoscopy in the differential diagnosis of peritoneal disease.

Patients and methods: 2131 laparoscopies performed at our Endoscopy Unit between Nov 1969 and May 1993 were retrospectively reviewed. In 80 patients (3.8%) laparoscopy was indicated as part of a diagnostic protocol for ascites of unknown origin after other less invasive tests had failed to reach a diagnosis. 48 of these were performed prior to the introduction of CT and abdominal US in routine clinical practice. 12 out of these 48 patients were excluded for analysis together with the 32 patients in whom laparoscopy was indicated after a negative US and/or CT scan. These 80 patients were M/F 32/36, mean age: 62.3 (42-79). Laparoscopy was performed according to Kalk's technique. Biopsy specimens of the peritoneal nodules under direct vision was obtained in 34 (50%) patients. We evaluated the usefulness of laparoscopy in making a diagnosis (either providing a diagnosis or significantly contributing to the establishment of a diagnosis).

Results: Laparoscopy provided a diagnosis in 60 patients (88.2%) and only in 8 cases (12.8%) did not add a significant information. Biopsy specimens were taken from 34 of these patients (50%), and they helped to confirm the diagnosis in 28 of them (82.4%). Peritoneal carcinomatosis was the predominant aetiology (49 cases, 71%), and then peritoneal tuberculosis in 14 (20.5%), peritoneal pseudocyst in 4, and mesothelioma in 1. The procedure was safe and well tolerated.

Conclusions: 1. Laparoscopy is an effective and safe procedure in the diagnosis of peritoneal diseases. 2. Biopsy under direct vision is mandatory. provided contraindications and technical difficulties are absent. 3. Peritoneal carcinomatosis is by far the most frequent finding, although peritoneal tuberculosis still represents an important proportion of cases.

AN ENDORBOT FOR GASTROINTESTINAL ENDOSCOPY. F Gong, CP Swain, TN Mills. Department of Medical Physics and Bioengineering, University College London, UK.

We studied the feasibility of using existing technologies to design and develop a new type of self-moving endoscope - an endorbot which is small enough to be swallowed by the patient and can be navigated through the human gastrointestinal tract. The endorbot would propel itself and transmit images from inside the body without wires, fibroptic bundles or cables. The device would take the form of a swallowable capsule consisting of a CCD camera, xenon flashlamp, a propulsion mechanism and a power source. PROPULSION could be provided by movement of a "giant magnetostrictive alloy" in an externally applied rotating magnetic field. Alternative methods of robot walking, utilizing articulated legs or wigglng by pressure alteration in a curved double minisauage require electric, clockwork power or even electrically stimulated peristalsis or might be possible. VIDEOCAMERA could be an existing miniature low power CCD camera dissipating only 150 mW. LIGHT SOURCE could be provided by a flash light identical to that found in compact-cameras. VIDEO SIGNAL TRANSMISSION: a miniature microwave transmitter could be used to transmit the video signal. This microwave system is small in size and has an extremely low power requirement (60mW, output. frequency 10.310GHz -10.350GHz). POWER REQUIREMENTS: power to operate the miniature CCD camera -150 mW, power to operate the video transmitter -100mW and energy for 500 exposures of the xenon flash lamp could be provided by a 10 mm x 10 mm 1 Amp hour lithium cell for bright light or to run a CCD camera at low light levels by lower power flashes at 30Hz. CONCLUSION: our calculations of power to size ratios using best available battery power data suggest that the combination of existing technologies for micromachine electronic-video endoscopy could make it just possible to build a swallowable simple endoscope featuring a battery-powered CCD camera, 100mW lights, light source, and a transmitter weighing about 500 coloured images of the gut. Until there have been major improvements in existing technology, the power requirements of a more complex endoscope with robotic propelling the use of the thin, flexible cable to the outside world. These machines are likely to be first tested in gastrointestinal organs, the largest endoscopabale part of the human body.
T209

EXPRESSION SITES OF ISOFORMS (α and β) OF TYPE II TOPOISOMERASE IN HUMAN FOETAL TISSUES

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Passage of one duplex of DNA through another is essential for DNA replication, transcription, repair, and condensation. Human DNA topoisomerase II (topoII) mediates this topological rearrangement and is expressed from two genes as isoforms, topoIIα (p170) and topoIIβ (p180). These isoforms, are biochemically distinct and are targets for antineoplastic agents including doxorubicin, etoposide and suramin. Differential expression of the isoforms in mouse tissues has been reported, raising the possibility that the effectiveness of topoII inhibitors may vary as a consequence of the isoforms expressed in normal and neoplastic tissues. However, patterns of expression in mouse tissues were examined by northern blots, which do not allow the determination of specific sites of expression within these tissues.

We studied the expression sites of topoIIα and topoIIβ mRNAs in a range of formalin-fixed paraffin-embedded human foetal tissues by in situ hybridisation using 35S-labelled antisense riboprobes specific to C-terminal regions of human topoIIα or topoIIβ mRNA. We used a probe for ducts as a positive control.

TopoIIα and topoIIβ mRNAs were found to be expressed similarly in most foetal tissues studied; brain (very regional), kidney (nephrogenic zone, glomeruli, tubules), adrenal (cortex, medulla), scalp (bud of hair follicles), and spleen (uniform). Some differences were observed: in the stomach (topoIIα was expressed focally in the epithelium of the gastric glands and putative smooth muscle cells whereas topoIIβ was expressed more strongly in the stromal cores); in the intestine (topoIIα was expressed at the crypt/villus junction and focally in the stroma whereas topoIIβ was expressed more generally); and in the heart (topoIIα was expressed in the myocytes and smooth muscle whereas topoIIβ expression was not detected.

Studying expression sites of the two topoII isoforms has refined our understanding of their selective use during development. Selective expression of the isoforms in adult tissues and tumours may affect responsiveness to chemotherapeutic agents.

T210

DISTRIBUTION OF HUMAN PancreATIC SECRETORY TRYPSIN INHIBITOR (PSTI) USING IN SITU HYBRIDISATION AND IMMUNOLOGICAL STAINING TECHNIQUES

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Background: PSTI is a potent growth factor and protease inhibitor, which has previously been identified in the adult pancreas and gastrointestinal epithelium by immunological staining. We have examined the distribution of PSTI synthesis in multiple organ systems of human adult and foetus using in situ hybridisation.

Methods: cDNA produced by reverse transcription PCR from a human pancreas (PANC-1) cell line was used to make 35S labelled antisense riboprobes for in situ hybridisation. 35S riboprobes were used on a series of formalin-fixed paraffin-embedded human foetal and adult tissues followed by autoradiography. Immunostaining of the tissues was done using a polyclonal rabbit anti-human antibody.

Results: As expected PSTI is synthesised and secreted at high levels in the adult and foetal pancreas. Immunological evidence for PSTI expression in epithelial cells in the gastrointestinal tract was supported by in situ hybridisation data. Significantly, significant levels of PSTI mRNA were also found in the transitional epithelium of the kidney, a sub-set of cortical neurones of both the foetal and adult human brain, and the epithelioid cells of the testis.

Conclusion: PSTI is widely expressed in the human adult and foetus. The early expression of this growth factor suggests a role in the development of multiple organ systems.

T211

INDUCTION OF ANCHORAGE DEPENDENCE BY THE TREATMENT WITH DOXORUBICIN AND ETOPOSIDE UPON KATO-3 GASTRIC CARCINOMA CELL LINE.

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The trefoil family consists of a group of cysteine-rich peptides with trefoil-like domains that are disulphide-bonded. The trefoils are known to be mucin-associated and a role in mucosal healing is proposed. The biological function of the trefoil peptides is not clear.

The trefoil peptide, porcine pancreatic a-amylase/trypsin inhibitor (PSTI) was shown to stimulate cell proliferation in a human breast carcinoma cell line MCF7, and the activity was dependent on the presence of glutathione in the culture medium. MCF7 also secretes the trefoil peptide p52 upon oestrogen stimulation.

To elucidate the function of the trefoils, PSTI was used in an assay designed to promote cell attachment and spreading in an anchorage independent cell line Kato-3 a signet ring cell carcinoma. Immunohistochemical analysis showed p52 expression and the cell line was positive for both Alcian blue and periodic acid Schiff's reaction indicating mucin production.

Culture plates were simultaneously coated with poly-l-lysine and PSTI. Cell suspensions of Kato-3 were seeded in a 4hr adhesion assay and the effect assessed by measuring the total DNA. PSTI stimulated adhesion in a dose-dependent manner (12.5% increase at 100μM; 25% at 1μM; 50% at 10μM). At higher concentrations of 100μM and 1μM the adhesion was maintained to 25% above the control. Cell spreading and morphological changes were not observed due to the short time of the assay.

The results suggests that PSTI confers anchorage dependence upon Kato-3 which may be due upon the presence of mucin and could serve as a useful in-vitro model for the study of cell attachment, the initial event leading up to epithelial restitution.

T212

SODIUM NITROPRUSSIDE, A NITRIC OXIDE DONATING COMPOUND, STIMULATES HUMAN COLONIC ION TRANSPORT IN VITRO

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INTRODUCTION Nitric oxide has been implicated as a possible inflammatory mediator in the pathogenesis of Ulcerative Colitis. In experimental animal intestine, NO also been demonstrated to stimulate electrogenic chloride secretion, although it's role in regulating human intestinal ion transport has not been investigated. We have therefore used sodium nitroprusside (SNP), a source of exogenous NO, to study the effect of NO in regulating human colonic ion transport in vitro.

METHODS Normal distal human colonic was obtained at surgical resection for colonic carcinoma and stripped of underlying smooth muscle. Tissues were mounted in Ussing chambers and voltage clamped to zero potential difference by the continuous application of a short circuit current (SCC). A pharmacological approach was used to identify the charge carrying ions and to characterise mediators involved in the SCC response. All drugs were added to the serosal side and statistical analysis was carried out with paired Student's t-tests.

RESULTS SNP (10^-6-10^-4 M) evoked a concentration dependent increase in SCC (max SCC 51.2±11.6 uA/cm² at 10^-4 M; n=8). Bumetanide 100μM, an inhibitor of the Na/K/Cl co-transporter necessary for electrogenic chloride secretion, reduced the SCC response to SNP 100μM by 61.5±12.5%, n=6; p<0.05. The SCC response to the cholinomimetic agent carbachol 100μM was virtually abolished in the presence of bumetanide 100μM, but was unaltered by SNP 100μM. The cyclo-oxygenase inhibitor piroxicam 10μM, and the norepinephrine blocking agent tetraodotoxin 1μM reduced the SCC response to SNP 100μM by 40.8±13.2% and 45.7±7.7% respectively (both p<0.05).

CONCLUSION These results suggest that SNP stimulated increase in SCC in human colon mucosa in vitro is at least in part due to electrogenic chloride secretion, and mediated by local eicosanoid production and stimulation of enteric nerves. This study provides further evidence that NO may be an important physiological mediator of electrogenic transport in the human colon. As inducible NO synthase is increased in inflammatory bowel disease, NO stimulation of colonic electrolyte transport may contribute to diarrhoea in inflamed intestine.
CHOLERA TOXIN STIMULATES 5-HYDROXYTRYPTAMINE RELEASE INTO HUMAN JEJUNUM. CP Bearcroft, D Perrett, MIG Farthing, Digestive Disease Research Centre, Medical College of St Bartholomew’s Hospital, London EC1M 6BQ.

Cholera toxin produces intestinal secretion by activation of the adenylate cyclase complex. However, animal studies have shown that 5-hydroxytryptamine (5-HT) may be released from enterochromaffin cells of the small intestine following stimulation by cholera toxin. We have studied the release of 5-HT from the human jejunum in 7 male subjects following administration of a subclinical dose of cholera toxin (paired, controlled, randomised, double blind study).

Following intestinal intubation, a closed 10cm segment of upper jejunum was isolated between 2 occluding balloons and exposed to 15µg of purified cholera toxin for 2h prior to closed segment perfusion with a plasma electrolyte solution containing a non-absorbable volume marker (10³ Polyethylene glycol). 5-HT in jejunal effluent and 5-hydroxyindolacetic acid (5-HIAA) in urine (up to 6h after cholera toxin) was measured by high performance liquid chromatography with fluorometric detection.

In contrast to controls, all subjects secreted fluids in response to cholera toxin, mean -3.48 ml/cm²h (SEM +/- 1.36). During 6h following cholera toxin, 5-HT was secreted into the lumen at a mean concentration of 496 nM (+/-106). 5-HT was not released into the effluent in the control experiments and the difference was significant (p=0.0034, paired t-test). Before exposure to cholera toxin, urinary 5-HT was 14.8±4.3µg/mmol creatinine excreted (+/-1.61) and after exposure, 4.5±(+/-1.73). These were similar to control values, 6.6±(+/-1.07) before and 3.8±(+/-1.16) after perfusion.

Thus, cholera toxin promoted the release of 5-HT into the intestinal lumen, but quantitative changes in urinary 5-HIAA were not detectable. 5-HT is a known intestinal secretagogue and our findings that 5-HT may play a role in mediating cholera toxin-induced secretion in humans.

T211

T212

5-HT is a potent intestinal secretagogue. It is released from small intestinal enterochromaffin cells by cholera toxin (CT) and 5-HT receptor antagonists reverses the CT-induced secretion. Release of 5-HT may also be precipitated by intraluminal distension, manipulation and hypertonic glucose, and may be involved in the modulation of basal transport of water and electrolytes. The aim of this study was to measure tissue 5-HT levels in absorptive and secretory states.

Under anaesthesia, the entire small intestine of adult male Wistar rats (190-220g) was isolated between two cannulas. 75µg CT or saline was instilled into the segment. Thereafter, in situ small intestinal perfusion was performed with plasma electrolyte solution (PES; Na 140, K 4, Cl 104, HCO₃ 40mM) containing a non-absorbable volume marker, to assess net water and electrolyte movement. At the end of the experiment, full thickness segments of small intestine were obtained by freeze clamping. 5-HT levels were analysed by reversed phase high performance liquid chromatography with fluorometric detection. Results were compared with those of non-perfused segments which had been minimally handled (control).

CT induced net water secretion (median: -50±0.1µl/min/g intestinal dry weight [interquartile range: -59.5 to -29.8], n=13) and saline, net absorption (50 [±41 to 61], n=8; p<0.01). Tissue 5-HT levels in the CT group (37±0.3µM/mg dry weight [30 to 44.5, n=6]) were significantly lower than in the saline group (49.5 [±1.75 to 51.75], n=10; p<0.05).

Tissue 5-HT levels in the control group were significantly higher than in both the perfused groups (79 [71.75 to 91.25], n=4; p<0.01).

Our data show that, although manipulation of the intestine reduces tissue 5-HT content, the release of 5-HT induced by CT results in a further measurable lowering of tissue levels.

T213

T214

T215

T216

PENICILLIN TRANSPORT IN SMALL INTESTINAL BUSH-BORDER MEMBRANE VESICLES (BBMV).

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The mechanisms of antibiotic transport in human small intestine are poorly understood and thus antibiotics for oral use are selected on the basis of animal studies which do not always predict oral absorption in man. We have therefore studied antibiotic transport in rabbit and human small intestinal BBMV and here report our results with penicillin-G (Pen-G).

Pen-G uptake is a pH-dependent process with a pH optimum of 4.5. Under a pH gradient (pHoutside=5.0, pHinside=7.1) almost 90% of Pen-G is transported into the lumen of the vesicles.

Under the same pH gradient, Pen-G uptake shows a maximal overshoot (excess accumulation) after 2 min with more than 70% of Pen-G uptake being accounted for by an H⁺ coupled transport system. Equilibrium uptake occurs after one hour.

Pen-G uptake is mediated by a saturable carrier process; Vmax 59 nmol Penicillin-G/mg protein/30s, Kₗ 22.7 µM for rabbit BBMV. Similar values were found in human BBMV.

In rabbit BBMV inhibition studies showed that the following β-lactam antibiotics (10 mM) inhibit Pen-G (0.1 mM) transport: amoxicillin (14%), ampicillin (58%), penicillin-G (36%), penicillin-V (57%), cefadroxil (42%), cephalaxin (67%), cephalotin (29%) and cephradine (18%).

Most peptides had an inhibitory effect; L-carnosine (35%), gly-gly (18%), gly-gly (48%) and gloy-gloy (56%). Unexpectedly, gly-sar had a stimulatory effect of 92%, whereas both gly and sar on their own or combined had an inhibitory effect.

Studies of antibiotic uptake in vitro may define important differences between animals and humans. Characterisation of the uptake systems may be useful in the rational design of antibiotics for oral administration.

SIMULTANEOUS MEASUREMENT OF FLUID TRANSPORT AND TRANSIENTINAL PD IN VIVO USING AN ON-LINE TECHNIQUE.

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Intestinal transport is commonly investigated using methods which measure transmural electrical activity. These do not, however, detect changes in electroneutral ion transport processes which contribute to the driving force for net fluid movement. A gravimetric technique of measuring fluid transport on intact segments of ileum in vivo has been used to monitor transientinal PD in order to determine whether changes in electrical activity reflect alterations in net fluid movement.

Rats were anaesthetised with Sagatal (70mg/kg ip), and a segment of the proximal jejunum (approximately 8cm long) was cannulated and mounted on a chamber with intact blood and nervous supply. After the chamber was washed of its contents and filled with 154mM NaCl (approximately 2ml), the remaining intestines were removed. The chamber was suspended on a weight transducer 3mm above the body cavity and fluid transport was measured by the change in weight of the segment. Results are expressed as *±* indicating secretion, or +± indicating absorption in µl/min/g dry weight tissue. Transientinal PD was measured between a salt bridge electrode in contact with the luminal fluid and a wire reference electrode in the peritoneal cavity. Blood pressure, heart rate, transientinal PD and fluid transport were recorded on computer.

During saline infusion (femoral vein, 2ml/hr for 30min) transientinal PD remained steady at 5.5±6.6mV and fluid transport averaged 33.8±4.7µl/min/g. PGE₂ (200µM at 2ml/hr for 30min) induced a rapid rise in transientinal PD (to ±11.4±5mV, p<0.01 paired t-test n=4), and simultaneously reversed fluid absorption into a net secretion (+12.9±8.0µl/min/g; p<0.01 n=4). Although the rise in PD was sustained throughout the period of saline infusion which does not always predict secretion, fluid absorption gradually decreased. A comparison of fluid movement assessed gravimetrically or by phenol red dye dilution, with both saline and PGE₂-infusion, indicated that the changes in intestinal weight reflected alterations in the volume of luminal fluid.

These results suggest that with PGE₂-induced secretion, the initial rise in electronic ion movement may be closely linked with initial fluid secretion, however during sustained secretion a different mechanism may be involved.

GL motility T218–T228

T218


Sumatriptan (S), a 5HT receptor agonist, used to treat migraine is associated with chest symptoms in 3-5% of patients. In most cases the cause of these chest symptoms is unknown, but there have been isolated reports of cardiac involvement. This study examines the possibility of an effect of S on oesophageal function. Lower oesophageal sphincter (LOS), oesophageal body (OB) (4 sites), gastric and pylorogeal (to monitor swallowing) pressures were measured using a perfused manometric catheter with a sensor in 24 healthy volunteers (aged 19-44y, 19 male) after either subcutaneous (S 16mg) or saline control. Sequences of 6 consecutive 5ml water swallows with a minimum interval of 20 sec between each swallow were carried out 5, 15, 30, 45 and 60 min after injection. Treatment order was randomised and double blind; and throughout the study ECGs and symptoms were monitored.

Results: S significantly increased the amplitude [71.3(0.1,82.3); mean(95% CI) vs 87.0(72.3,100.7) mmHg: p<0.001] and duration [4.7(4.4,4.9) vs 5.7(5.3,6.2) sec: p<0.001] of OB contractions, the percentage of subjects exhibiting repetitive (2 or more) distal OB contractions (46% vs 7%: p<0.01) and basal LOS pressure [36.4(30.1,42.7) vs 50.8(38.7,62.9) mmHg: p=0.001]. However, S had no effect on the velocity of propagation of OB contractions (3.1(2.7,3.4) vs 3.3(3.0,3.6) cm/sec or on relaxed LOS pressure [3.3(3.4,4.4) vs 2.6(1.5,3.7) mmHg]. In OB impaired subjects, S induced gross increases in both amplitude (>200mmHg) and duration (7-20 sec) of OB contractions. Chest symptoms were experienced by 5 subjects (21%), and although all showed changes in motility, there appeared to be no correlation with the degree of change. No subject exhibited any ECG abnormalities.

Conclusions: This study shows that at supra-therapeutic doses, S significantly alters both LOS and OB motor function, without affecting ECG. These data suggest that oesophageal mechanisms may play a role in S-induced chest symptoms, although further work at therapeutic doses is required.

T219

GASTRIC EMPTYING OF A SOLID MEAL IS ACCELERATED BY THE REMOVAL OF FIBRE NORMALLY PRESENT IN FOOD. L Benini, G Castellani, F Brighenti, MT Brentegani, KW Heaton, M C Casiraghi, C Sembenini, A Fiorella, G Testolin, I Vintani. Dept of Gastroenterology, Rehabilitation Hospital of Valeggio sM, University of Verona, Italy; DISTAM, University of Milan, Italy; Dept of Medical Science, Royal Infirmary, England.

Exogenous fibre added to liquid meals delays gastric emptying. Its effect on solid meals is more debated, and nothing is known of the effect on gastric emptying of fibre naturally present in food. We therefore studied gastric emptying of two different solid meals in eight healthy subjects. The meals were exactly equivalent except for the total dietary fibre content (high-fibre 20 g, low-fibre 4 g per 1000 Kcal). They supplied 870 kcal (700 kcal females), 47% of which from carbohydrates, 35% from fats and 17% from proteins.

The meals consisted of inositol, antiserum, sheep's milk porridge and cooking oil in a 3:4:5:6 ratio. Subjects fasted 10h before study and ate the meals at 20°C, at an angle of 30°. Gastric emptying was measured by scintigraphy, with tracer activity estimated by a standardised isotope solution (0.5% active). Scintigrams were performed continuously for 24 h.

RESULTS: The gastric emptying of the high-fibre meal was significantly delayed as compared with the low-fibre meal: mean T1/2 was 105±128 min for the high-fibre meal vs 54±65 min for the low-fibre meal (p<0.001). In the high-fibre meal, mean gastric emptying time was 304±141 min vs 203±86 min for the low-fibre meal (p<0.05). No significant differences were seen in the amount of undigested residue remaining at 24 h. A significantly greater amount of fibre residue was found in the high-fibre meal. Stool weight was greater in the high-fibre meal (76±21 g vs 43±13 g).

In conclusion, fibre naturally present in food delays gastric emptying of a solid meal, reduces the glycemic response and delays the return of hunger feeling even after a single assumption.

T220


The predominant route for drug administration is orally, with the belief that the dose will be handled as food. Tablet dosage forms are normally taken either before a meal i.e. in the fasting state, or with food. Non-disintegrating tablets of 7mm in diameter or less are expected to empty with liquids and solid meals whereas larger tablets may be retained until propulsive contractions of interdigestive motility move the tablets across the pylorus. The aim of our study was to investigate the relationship of gastric emptying of small and large non-disintegrating dosage forms given in the fasted and fed state.

Method: Gastric emptying studies were performed in ten healthy male volunteers using gamma scintigraphy to measure the transit of two radio-labelled dosage forms (3mm [111In] and 10mm [99mTc] diameter) following one of three radio-labelled, physiological test meals (dextrolyte 5%, beef extract drink and shepherds pie [111In]). Scintigraphic images of 60s duration were collected for each radionuclide in turn without interruption until all radioactivity had left the stomach.

Results: detailed in Table 1.

<table>
<thead>
<tr>
<th>Meal Type</th>
<th>Fasted</th>
<th>Liquid Nutrient</th>
<th>Solid Nutrient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tab size</td>
<td>3mm</td>
<td>3mm</td>
<td>10mm</td>
</tr>
<tr>
<td>Median</td>
<td>102.0</td>
<td>91.5</td>
<td>119.5</td>
</tr>
<tr>
<td>Range</td>
<td>56-187</td>
<td>(33-125)</td>
<td>(55-326)</td>
</tr>
<tr>
<td>Duration</td>
<td>156-360</td>
<td>(128-347)</td>
<td>(128-347)</td>
</tr>
</tbody>
</table>

There was a significant effect of meal type on gastric emptying. However, the effect on the transit of the solid dose was minimal and non-significant. In conclusion, our results suggest that single unit dosages of tablets is possible during a normal daily feeding regimen. The data suggests that 3 or 4 doses of medication taken with meals during the day could be retained for longer periods, causing potential problems such as gastric mucosal irritation or drug toxicity, when accumulated tablets empty from the stomach together.
T222

COLONIC POUCHES—MOTILITY MAY EXPLAIN EVACUATORY DIFFICULTY
Hughes SF, Scott SM, Pilot MA, Williams NS.
The Royal London Hospital, Whitechapel, London.

The incidence of frequency and incontinence following low anterior resection is reduced by the construction of a colonic pouch. However, inability to evacuate the pouch is common. This may be due to the lack of development of a coordinated, propulsive action in the pouch. We investigated the effect of pouch construction on the propagation of colonic migrating motor complexes in a canine model.

A colonic J pouch was constructed from an isolated loop of mid colon in 5 dogs. A strain gauge was sutured to the serosa of each limb of the pouch and two similar positioned strain gauges were sutured to the colonic serosa in 5 control dogs. The animals were allowed 2 weeks to recover prior to the commencement of recordings. Fasted motility was recorded via the strain gauges, relating to an amplifier, digital data logger and chart recorder. The direction of propagation of migrating complexes was analysed visually.

240 hours of pouch recording and 280 hours of control recording were obtained between 14 and 90 days postoperatively. The results are expressed as mean±SEM percentage of complexes recorded from the proximal strain gauge.

Pouch Control
Antegrade
18.8±3.1* 81.5±2.8
Retрогrade
52.2±5.8* 7.6±1.8
None
19.8±2.6 10.3±2.0
Simultaneous
2.1±0.2 0

*p<0.05 (compared with control) Student t test.

There was no change in the propagation of the complexes compared with 90 days. The results for the intact colon are in concordance with others. However, over 50% of complexes arising in the pouch were propagated aborally. This is considerably higher than previous reports in isolated colonic loops (Gastroenterology 1981;90:1057) and may be related to the side to side anastomosis required for pouch construction. The pouch demonstrated very little synchronisation and may be required for efficient propulsive action. These findings may explain the evacuation difficulties experienced by patients with colonic pouches.

T223

TWO YEARS EXPERIENCE OF SPHINCTER OF ODII MANOMETRY AS AN A CLINICAL SERVICE IN THE EVALUATION OF POST COLORECTAL PAIN
AJK Williams, A Prude, RC Heading, KR Palmer Centre for Liver and Digestive Disorders, Royal Infirmary, Edinburgh EH3 9YW

Sphincter of Oddi dysfunction is regarded as a cause of recurrent biliary pain following cholecystectomy. Biliary manometry is the best test for evaluating sphincter of Oddi dysfunction (SOD) Since January 1992, we have performed biliary manometry as a regional clinical service for patients with post cholecystectomy pain and suspected SOD.

Forty two patients (35 women, median age 53) with post cholecystectomy pain were referred for evaluation. All complained of recurrent biliary type pain. Five patients were classified as Biliary Type II dysfunction (biliary pain and 1 or 2 of dilated CBD >12mm, prolonged biliary drainage at ERCP, abnormal LFTs with pain) and 37 patients as Type III (biliary pain only).

Biliary manometry was performed as a day case using light intravenous sedation with benzodiazepines by one experienced endoscopist (performed > 3000 ERCPs). A triple lumen low compliance fluid perfusion polyethylene catheter (1.7mm diameter) was inserted across the ampulla. The procedure was successful in 29 patients (23 female, median age 51). Four patients had sphincter of Oddi stenosis (basal pressure > 40mm Hg) and 6 patients sphincter dyskinesia (high frequency contractions). The procedure was not technically successful in 13 patients (31 per cent).

No complications occurred as a result of manometry except one case of post procedure pain not due to pancreatitis, requiring overnight stay.

Biliary manometry is technically difficult. The procedure may be performed with a low complication rate. Only a minority of patients (14 per cent) with post cholecystectomy pain will have sphincter of Oddi stenosis.

T224

REGIONAL COLONIC RESPONSES TO STANDARD STIMULI.
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It is unclear whether differences in regional colonic motor responses to stimulation reflect differences in regional contractility. An aim: To compare colonic elastance and motor responses to cold pressor (CP), hyperventilation (HVT) and meal stimulus in the transverse and sigmoid colon in 22 healthy subjects. Methods: Tonic and phasic colonic motility and elastance were assessed using a combined barostat and multi-port manometry assembly tube with one balloon in the transverse colon and another in the sigmoid colon. Motility was studied before, during, and after 5-minute periods of cold pressor test and hyperventilation, and for 2 hours after a 1000-kcal meal (35% carbohydrate, 53% fat, 12% protein). Colonic tone, assessed as the mean barostat volume, and phasic contractility, as the area under the contraction curve (auc), during and post-stimulus were compared with baseline. Colonic elastance (dV/dP) was corrected for elastance of the barostat. Results:

Table 1.

<table>
<thead>
<tr>
<th>CP</th>
<th>HVT</th>
<th>Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Change in Tone (k):</td>
<td>Trans Colon</td>
<td>13.1±2.1* 10.2±2.1* 24.1±3.5*</td>
</tr>
<tr>
<td>Mean Change in Phasic Contractility (auc, mmHg.cm)</td>
<td>Trans Colon -28±7 -50±9 412±53</td>
<td>Sig Colon 51±8 229±27 1244±204</td>
</tr>
<tr>
<td>Tonic responses were greater in the transverse, and phasic responses greater in the sigmoid colon following a meal. Elastance was greater than the sigmoid colon (7.6±0.4 vs. 4.1±0.2 a/mHg, P&lt;0.001).</td>
<td>Conclusions: Phasic contractility of the colon is unaffected during CP and HVT. The increase in colonic tone in response to stimuli that inhibit (HVT) or stimulate (CP) central sympathetic outflow suggests that different neural mechanisms are involved. Qualitatively different, but qualitatively similar tonic responses appear to reflect differences in the physical properties of two colonic regions.</td>
<td></td>
</tr>
</tbody>
</table>
LUMINAL CONTENTS AND HUMAN COLONIC MOTILITY
S J Warren, M G Lord, J Rogers and N S Williams
Surgical Units, The Royal London & Newham General Hospital, UK

Most motility recordings are performed in the cleansed bowel following colonoscopy or are limited to distal regions in unprepared bowel, yet the fundamental question of the effect of bowel cleansing upon colonic motility has never been systematically studied.

Following standardised bowel preparation, a probe featuring multiple pressure sensors and balloons was placed into the transverse colon at colonoscopy in 7 normal subjects. Spontaneous and distension provoked intraluminal pressure activity was recorded over the subsequent 3 days. Computer derived motility indices and mean amplitudes over these 3 days were compared. Ethical committee approval was granted.

<table>
<thead>
<tr>
<th>DAY</th>
<th>wave amplitude(cm H2O)</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous</td>
<td>23 (19-27)</td>
<td>31 (27-32)</td>
<td>33.5 (27-39)</td>
</tr>
<tr>
<td>Distension</td>
<td>32 (19-41)</td>
<td>38 (31-41)</td>
<td>39 (22-47)</td>
</tr>
</tbody>
</table>

median (interquartile range)
Wilcoxon Ranked Sum * p<0.05

Within the first 24 hours, the amplitude of spontaneously occurring contractile waves is significantly less than on the subsequent two days. Distension provoked waves and overall motility indices however do not change significantly over the three day period. It should be appreciated that early colonic motility recordings may not accurately reflect later physiological recordings.

DOES ROUTINE ABDOMINAL ULTRASOUND ENHANCE DIAGNOSTIC ACCURACY IN IRRITABLE BOWEL SYNDROME?
C Y Francis, D F Martin, P J Whorlow
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It is currently postulated that irritable bowel syndrome (IBS) should be diagnosed positively using a minimum of investigation although this policy risks some disorders being overlooked. Abdominal and pelvic ultrasound (US) is of sufficient quality to allow assessment of all abdominal viscera and associated pathology and it was the purpose of this study to evaluate its role in substantiating the diagnosis of IBS.

125 consecutive patients (100 female, 25 male) in whom a confident diagnosis of IBS was made at first attendance were studied. All had normal haematological and biochemical indices and sigmoidoscopy. Those over 45 years had normal barium enema or colonoscopy. All had complete US assessment of abdominal contents including pelvic organs in women by one radiologist using a 3.5MHz transducer. 21 patients had an abnormality on US (6: gallstones, 1: dilated bile duct, 2: fatty liver, 1: benign ovarian cyst, 1: benign uterine cyst, 4: fibroids, 6: miscellaneous). No other disease or bowel abnormality was found. Review of those patients with abnormalities did not lead to a revised diagnosis although some with gallstones were referred for surgery.

It is concluded that in patients with IBS (1) A positive approach to diagnosis is a safe policy and routine diagnosis by exclusion is unnecessary. (2) Serious gynaecological pathology is unlikely to be overlooked. (3) Routine abdominal US is not necessary in the majority of patients.

NATURAL HISTORY OF BOWEL SYMPTOM REPORTS IN CONSULTANTS AND NON-CONSULTORS WITH IRRITABLE BOWEL SYNDROME. Gombarone IE, Dewnap PA, Libby GW, Farthing MIG. Dept Gastroenterology, St Bartholomew’s Hospital, London

Using a validated bowel disease questionnaire, we studied age and sex-matched groups of IBS consultants (n=27) and non-consultants (n=32) drawn from the same community, then carried out a one year follow-up. At entry all subjects had abdominal pain plus three or more Manning criteria. All of the Manning criteria were more frequent among the consultants but only abdominal bloating was significantly more prevalent (81% vs 44%, p<0.01). In addition, the consultants had a more accurate appraisal of their abnormal bowel habit than the non-consultors, who tended to normalise or trivialise symptoms. At follow-up there had been considerable changes in symptom reports in both groups. In only 1 (4%) of the consultants and 2 (6%) of the non-consultors did both the number and pattern of symptoms remain the same. Three (11%) consultants had an increase and 15 (55%) had a reduction in the number of symptoms, while among the non-consultors 11 (34%) had an increase and 13 (41%) had a reduction. The numbers fulfilling three or more Manning criteria at follow-up had decreased in both groups from 100% to 67% of the consultants and 72% of the non-consultors (NS). Four (12.5%) of the original non-consultants had become consultants, of which 2 reported an increase and 2 a decrease in their bowel symptoms. Seven (26%) of the original consultants had ceased consulting. Of these, 5 reported a reduction in their bowel symptoms, with 1 becoming symptom-free. A comparison of the new groups of consultants (n=24) and non-consultants (n=35) again found bloating to be more prevalent among the consultants (88% vs 46%; p<0.02). Eighteen (75%) of the new consultants and 25 (71%) of the new non-consultors had an abnormal bowel habit. Again more consultants than non-consultors (67% vs 34%) accurately reported this. Thus, we have found evidence of a considerable turnover of symptoms in IBS with a tendency towards symptom reduction, which is greater on balance among those who consult doctors.