POSTPRANDIAL GALLBLADDER FILLING (PPF) AND TURNOVER RATE IN HEALTH AND GALLSTONE DISEASE

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Impaired gallbladder emptying is implicated in gallstone disease, but methods (scintigraphy, BSG, ultrasonography:USG) have been conflicting results, and none can measure PPF. We reasoned that since SGG measures absolute emptying (AE) independently of PPF), whereas measures net emptying (NE, dependent on PPF), their combined use should therefore assess gallbladder filling in the postprandial period (PPF). We have therefore to assess PPF in health and in cholesterol gallstone disease. We validated the combined SGG and USG procedure in vitro using a phantom gallbladder, showing close correlation between observed and theoretical counts and volumes. We studied 14 gallstone patients and 11 healthy controls matched for age, sex and BMI. Following overnight fast and iv "TORIDA", basal measures of GB counts by SGG and volumes by USG were followed by a standard meal and repeat measurements at 10 min intervals for 90 min. We measured AE and NE (reduction in GB counts and volumes by SGG and USG at each time interval). We also calculated PPF (AE - NE) and gallbladder turnover rate (AE + PPF). In health, values for AE were higher than those for NE at all time intervals, confirming that PPF occurred immediately after the meal. Gallstone patients had a significant impairment in AE (p<0.005) and NE (p<0.05). PPF was also reduced (p<0.05) mainly in the latter part (50-90 min). Turnover rate was markedly reduced in gallstone patients by comparison with controls (p<0.05 at all time intervals between 10-90 min) and the cumulative turnover rate at 90 min was 10% in gallstone patients compared with 370% in controls (p<0.01). We conclude that subsequent use of SGG and USG can validly assess PPF: that PPF starts immediately after a meal and that it is impaired in gallstone patients. Gallbladder turnover rate, another novel parameter of gallbladder motor function, is markedly reduced in gallstone patients. Both these new parameters should be useful in studying pathogenesis of gallstone disease.

Biliary and pancreas disease W1–W8

W2

DYNAMIC CHOLECYSTOKININ (CCK) STIMULATED GALL BLADDER SCINTIGRAPHY IN ACALCULOUS BILIARY PAIN: CHRONIC CHOLECYSTITIS v CHOLELITHIASIS

John W. Perry, MD, Lincoln, 1993.

The management of patients with chronic acalculous biliary pain is difficult. We have evaluated 55 such patients whose symptom duration was <24 (12-120) months. The patients were symptomatically assessed by a modified visit score and followed up 3-6 monthly for >24 (12-60) months. The patients were divided into 3 groups according to the gallbladder emptying function (EF) determined using CCK stimulated scintigraphy: "Low" EF (<35%) n=29, "normal" EF (35-50%) n=10, "High" EF (>50%) n=16. Thirty five patients with visit scores of 3 or 4 underwent cholecystectomy; 20 did not have surgery because of symptomatic improvement (94%) or an alternative diagnosis (n=16).

Twenty-two cases with a low EF underwent cholecystectomy, of whom 21 (96%) improved symptomatically with visit scores of 1 or 2 (p<0.01) compared to only 4 out of 9 (44%) improved after cholecystectomy with a high EF (8%). All 4 patients with a normal EF that underwent cholecystectomy improved symptomatically and all 4 had EF values of 35-40%. Gall bladder histology which was available in 32 cases revealed chronic cholecystitis in 32 (100%) and cholelithiasis in 20 (63%) patients. Only 7 out of 12 (58%) patients with chronic cholecystitis alone improved symptomatically after cholecystectomy compared to 19 out of 20 (95%) patients with cholelithiasis (p<0.02).

This study demonstrates the use of CCK stimulated gallbladder scintigraphy in patients with acalculous biliary pain, being successful in alleviating pain in those with a EF < 40% and cholelithiasis.

SUBSTANCE P INCREASES SPHINCTER OF ODDBALL BASAL PRESSURE AND SPHINCTERIC FLOW IN THE AUSTRALIAN BRUSH-TAILED POSSUM

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Substance P containing nerves are present in the neural plexus of the sphincter of Oddi (SO) in the Australian Brush-tailed possum (Trichosurus vulpecula). The aim of this study was to determine the effect of Substance P on SO motility and transsphincteric flow in the possum.

Methods: Possums were studied under general anaesthesia (thiopentone infusion). Transsphincteric flow was measured gravimetrically and M3 motility was measured with manometry. In 7 possums Substance P (1.200 ng/kg) was administered by close intravenous (IA) injection. Recordings were taken at 30 sec intervals for the maximal changes in SO basal pressure (mmHg), contraction frequency (contractions/min), contraction amplitude (mmHg) and transsphincteric flow (ml/min). To determine the possible neural mediation of Substance P action, 5 possums received Substance P prior to and following neural blockade with tetrodotoxin (TTX, 9 ug/kg IA). Repeated measure ANOVA and paired t tests were used for statistical comparison.

Results: Substance P produced a significant [F(4,48)=7.35, p<0.01] dose dependent rise in basal pressure, with a mean control basal pressure of 3.6 +/- 0.4 mmHg rising to 22.3 +/- 6.5 mmHg following 2.000 ng/kg of Substance P. There was an associated dose dependent reduction in transsphincteric flow [F(4,48)=17.26, p<0.0001]. At doses higher than 2.000 ng/kg Substance P abolished transsphincteric flow in 6 of 7 possums. Substance P had no significant effect on contraction frequency [F(4,48)=3.46, p=0.06] or contraction amplitude [F(4,48)=2.66, p=0.12]. The effect of Substance P on SO motility and flow were not altered by TTX administration.

Conclusions: In this species Substance P increases the SO basal pressure and reduces the transsphincteric flow by a non-neural mechanism.

Obstructive jaundice impairs the neutrophil response to bacterial wall products.

SM Plusa, IN PRIMROSE, N WEBSTER.

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Patients with obstructive jaundice are prone to septic complications. Biliary obstruction has an inhibitory effect on reticuloendothelial function resulting in decreased clearance of endotoxin by the liver, but its effects on neutrophil function have not been fully assessed. We have therefore examined the expression of neutrophil adhesion molecules in jaundiced patients and studied the response to endotoxin and the bacterial wall peptide FMLP.

Eight patients with obstructive jaundice (mean age 64, bilirubin 198 g/L, range 84-360), were compared to 8 controls (age 55). The basal expression of adhesion molecules L-Selectin, CD11a, CD11b, CD11c and CD15 on neutrophils was studied in whole blood using fluorescein-conjugated monoclonal antibodies and flow cytometry.

Basal expression of L-Selectin, CD11a and CD15 was decreased in jaundiced patients (median channel fluorescence, L-Selectin 18.4 vs 42.0, CD11a 38.6 vs 62.7, CD15 106 vs 256, p<0.02). Expression of CD11b in response to FMLP at 10-6M and 10-9M was less in jaundiced patients (50.5 vs 102 with 10-6M, p<0.01, and 60.1 vs 131.2 with 10-9M, p<0.01). The response to endotoxin (100 ng/mL) in whole blood was also reduced (50.5 vs 124, p<0.01) and this lack of response occurred also in the absence of plasma (57.5 vs 212.9, p<0.01).

We have demonstrated that neutrophils from patients with obstructive jaundice are resistant to the effects of endotoxin and FMLP. FMLP has a direct effect on neutrophils without interaction with plasma and the persistent lack of response to endotoxin with washed cells indicates that the resistance to endotoxin is not due to plasma factors. The increased incidence of infection in jaundiced patients may result from the poor neutrophil response to bacteria.
W6

DEMONSTRATION OF TUMOUR NECROSIS FACTOR IN PANCREATIC ACINAR CELLS BY IN-SITU HYBRIDISATION IN A MODEL OF ACUTE PANCREATITIS.
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Clinical similarities between the systemic complications in acute pancreatitis (AP) and the sepsis syndrome (SS) may be the result of neutrophil activation. Tumour necrosis factor (TNF) is considered to be a primary trigger for neutrophil activation generated by the host in SS and may also play a part in the development of systemic complications of AP, particularly the acute respiratory distress syndrome. The early cellular events in acute pancreatitis remain to be established. Circulating blood levels of TNF may be an inappropriate reflection of its role in the pathogenesis of AP.

To investigate the possibility that TNF may be produced in the pancreas and be the trigger for neutrophil activation in AP, rat pancreatic tissue sections were examined by in situ hybridisation for expression of TNF synthesis.

Normal rat pancreas showed no signal for TNF. Strong expression of TNF was seen in the basal peri-nuclear region of the acinar cell in a model of acute pancreatitis induced by a method of microvascular ischaemia and was specific for this site, indicating de novo synthesis within the pancreatic acini. As far as we are aware this is the first time TNF synthesis has been demonstrated within pancreatic acinar cells.

This finding strongly suggests a role for TNF in the pathogenesis of acute pancreatitis. Variable detection or absence of this cytokine in the blood stream, which hitherto has been assumed to be derived from the mononuclear phagocyte series, is a misleading finding if local tissue expression represents the stimulus for neutrophil activation and the systemic effects resulting from their activation.

W7

MICRONUTRIENT ANTIOXIDANT STATUS IN CONTROLS AND CHRONIC PANCREATITIS PATIENTS AT JOHANNESBURG, RSA.
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The emergence and rising trend of chronic pancreatitis in South Africa led us to question whether poor antioxidant intake in the black population may be a factor facilitating damage from increasing exposure to xenobiotics such as alcohol, cigarette smoke and volatile hydrocarbons. Concentrations of antioxidants, or their metabolites, were therefore measured in fasting blood or urine samples from African controls and patients with chronic pancreatitis.

The striking finding was a very low level of ascorbic acid in control plasma (median and range 2.4, 0-10.4 mg/l) and values were even lower in patients, irrespective of disease activity or residual exocrine function (0.8, 0-2.1, 2p=0.001). Serum levels of selenium, vitamin E and B-carotene in controls were comparable to those reported in European controls and, likewise, exceeded the respective values in patients (2p=0.001 for each). Serum zinc and vitamin B12 levels were similar in controls and patients, but the latter group had lower levels of inorganic sulphate in urine (2p=0.01).

Thus, the recognised interaction between vitamin C and sulphur amino acids in protecting cells from xenobiotic-induced damage, and the reported therapeutic benefit from high-dose vitamin C in European patients with painful chronic pancreatitis (Aliment Pharmacol Ther 1990; 4: 357) suggest that antioxidant supplements may have prophylactic value in this community.

[Studies supported by Keatings/SAGES award].

W8

EXPRESSION OF LITHOSTATIN IN PANCREATITIS
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Northern blot analysis has recently identified mRNA from a novel gene (‘reg’) in regenerating rat islets, normal rat pancreas, kidney and gastric mucosa. This is now recognised to be identical to lithostatin ‘pancreatic stone protein’ which is postulated to prevent calcium precipitation in pancreatic secretions. It had been considered that depletion of this protein in chronic pancreatitis facilitated stone formation.

Using a monoclonal antibody to reg protein we have studied its expression in normal adult pancreatic tissue (7), chronic pancreatitis (8) and also in an animal (porcine) model for acute pancreatitis (10). The latter was effected by a combination of bile injection and ischaemia. Reg protein (lithostatin) expression in normal human pancreatic tissue was very faint. The human pancreatitis cases shared a varying pattern of increased expression in all but one case. In 3: it was generalised and the remainder showed a more focal increase. The pig pancreatitis cases showed intense expression especially in areas with more severe inflammation and glandular damage. Remaining areas of normal tissue showed only weak positivity.

The failure to detect significant amounts of protein in normal human tissue and its up regulation in both human and animal pancreatitis calls into doubt the hypothesis that reg protein (lithostatin) depletion is integral to the development of calculi in pancreatitis.
Gastrointestinal motility W9–W16

SOME REASONS WHY WOMEN HAVE SLOWER INTESTINAL TRANSIT THAN MEN
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It has been shown that women tend to have slower intestinal transit than men. The reasons for this are unknown. In this study, we performed multiple stepwise regression on the estimated transit time against possible dietary, anthropometric, physiological and pharmacological determinants in a community-based study of 883 women and 676 men. The determinants included age, BMI, waist–hip ratio, alcohol, oral contraceptive, dietary fibre and food group intakes. Transit time was estimated from prospective recording of three stools (Gut 1992; 33: 868). Diet and alcohol were assessed using a validated food frequency questionnaire.

Transit time in women taking the pill was significantly longer than in men of the same age not doing so (70.7 ± 6.3h, p < 0.05). Excluding pill-takers, mean transit time was relatively constant with age in pre- and postmenopausal women (62.6–63h and 58.9–59h respectively); this difference was significant (p < 0.05).

In both sexes multiple regression showed that alcohol increased transit; for example, in men drinking >40g/day alcohol mean transit time was 49h but 54h in those drinking 20g/day (p < 0.0001).

Other variables less significantly associated with faster transit were BMI. In both sexes, soluble non-starch polysaccharide intake was shown to be associated with slower transit. In men and insoluble non-starch polysaccharide intake in men was associated with faster transit. In women, the food groups which were related to transit were potatoes and cooked fruit in men and pulses and bread products in women. Alcohol and sex hormones have a greater influence than dietary fibre on transit in people eating a typical English diet and go some way to explaining the sex differences.

W10

PATTERNS OF COLONIC MOTILITY IN PATIENTS WITH POSTCHILDBIRTH/HYSTERECTOMY CONSTIPATION
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Patients with postchildbirth/hysterectomy (PC/PH) constipation have been shown, using dynamic radiolabeled isotope scanning and segmental marker studies, to have isolated hindgut dysmotility and hindgut cholinergic denervation has been proposed as the underlying pathophysiology. We therefore used colonic manometry to examine the underlying motility pattern in response to cholinergic stimulation.

Patients (n = 10) and controls (n = 10) were studied using an 8-channel multilumen, water perfusion catheter (Andreworff), passed via a colonoscope. Motility (area under curve) in the transverse, descending and sigmoid colon was measured for 1 hour each before (rest) and after the administration of neostigmine 0.01 mg/kg ec.

Results

<table>
<thead>
<tr>
<th>Motility Type</th>
<th>Mean (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neostigmine</td>
<td>252(161)</td>
</tr>
<tr>
<td>Rest</td>
<td>156 (53)</td>
</tr>
<tr>
<td>Control</td>
<td>207(106)</td>
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</tbody>
</table>

These results demonstrate that in controls, an increase in hindgut motility is observed following cholinergic stimulation. In contrast, in patients with PC/PH constipation, cholinergic responsiveness is not seen due to the presence of an elevated motility index during the rest phase.

W12

LAXATIVE EFFECT OF BRAN ON SMALL BOWEL TRANSIT: DUE TO ITS PARTICULATE NATURE?
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It has long been known that the laxative effect of coarse bran is largely due to its physical properties, although this increases its water holding properties and its rate of fermentation. We analysed the laxative effect of bran by comparing its effect on particle size and viscosity with the potential gelling agent Ispaghula. On each of 4 days in a cross-over design study, comparing control, 15g bran, 15g irregularly shaped plastic particles <2mm size, and 7g Fybogel, 13 normal subjects ingested a low residue 1360kcal (325kcal) rice based meal radiolabelled with technetium to measure gastro-intestinal transit.

Control gastric emptying (mean t0 = 47.8 (7.6) mins) was not altered by ispaghula (mean delay 2.3 (7.8) mins), was slightly delayed by plastic (11.8 (6.8) mins p < 0.05) and also by bran (21.8 (9.2) mins p < 0.05). Colonic filling (tco) was significantly hastened from a control of 419 (sem 28.8) mins by plastic (mean hastening 50.6 (20.9) mins p < 0.05) and bran (73.5 (sem 24.5) mins p < 0.02) but not by ispaghula (6.5 (sem 17.2) mins p = ns). Small intestinal transit (colonic filling tco – gastric emptying tco) delayed gastric emptying and had a marked effect on small bowel transit. The gelling agent ispaghula had little effect on gastric emptying or small bowel function in this study.

Bran's neglecting effects on the upper gastrointestinal tract appear to be predominantly due to its particulate nature. Such effects on the small intestine may explain many of the laxative properties of bran.
**SYMPTOMS IN THE IRRITABLE BOWEL SYNDROME DO NOT CORRELATE WITH COLONIC TRANSIT ABNORMALITIES.**

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To investigate the hypothesis that the predominant symptom of irritable bowel syndrome (IBS) is associated with abnormalities of colonic transit, we measured colonic transit scintigraphically in 24 patients with constipation-predominant symptoms, 12 patients with diarrhea-predominant symptoms, and 12 patients as controls. Using a 3-day transit study, patients were categorized into one of five transit patterns.

Among the 24 patients with constipation-predominant IBS, the pattern of colonic transit was normal in 16 (67%), rapid in 5 (21%) and delayed in 3 (12%). The delay was generalised in one, right-sided in one and left-sided in another. Among the 12 patients with diarrhea-predominant IBS, the pattern of colonic transit was normal in 5 (42%), rapid in 6 (50%) and one patient had right-sided colonic delay. These patterns of transit abnormalities did not differ significantly between the groups and there was no correlation with the predominant symptoms.

We conclude that IBS may be associated with various abnormalities of colonic transit, but that predominant symptoms of IBS do not correlate with these objective abnormalities. Therefore, classification of IBS into constipation-predominant and diarrhea-predominant subtypes remains a clinical distinction with no basis in pathophysiology. Objective assessment is necessary to identify colonic transit abnormalities in IBS.

**EFFECT OF ZAMIFENACIN ON COLONIC M MOTILITY IN PATIENTS WITH IRRITABLE BOWEL SYNDROME (IBS)**

LA Houghton, J Rogers, PJ Whorwell, FC Campbell, A Kaimundo, J Goka, University Hospital of South Manchester, Royal London Hospital, Ninewells Hospital, Dundee, Pfizer Central Research, UK.

Colonial motility in patients with IBS appears to be exaggerated, particularly after meals. However, current anti-spasmodics lack tissue selectivity and often cause unwanted side-effects. Zamifenacin is a new potent gut specific M3 muscarinic antagonist developed for use in IBS. In this double-blind, parallel group, placebo controlled study, the effect of a single dose of zamifenacin 40mg on both fasting (30min) and fed (60min) colonic motility was assessed in 36 patients (aged 25-64, 19 male). Colonial motility was recorded using a 5-channel (channnels 5 cm apart) gold-radiopaque marker introduced to a depth of 35 cm by flexible sigmoidoscopy in the unprepared colon. Patients who showed a colonial motor response to the meal on the screening assessment were randomised to treatment with a single oral dose of zamifenacin or placebo.

**RESULTS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Motility Index(μm/s)</th>
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<tbody>
<tr>
<td>Pre-placebo</td>
<td>0.38±(6.62-8.85)</td>
</tr>
<tr>
<td>Zamifenacin</td>
<td>-0.73±(2.71-1.18)</td>
</tr>
<tr>
<td>Post-placebo</td>
<td>2.09±(5.18-15.63)</td>
</tr>
<tr>
<td>Zamifenacin</td>
<td>2.18±(9.37-0.63)*</td>
</tr>
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</table>

Results expressed as median change(range) of activity from screening to study day. *Denotes statistically significant difference in paired comparison with placebo, p<0.05. Three patients each on placebo and zamifenacin reported minor side-effects. In conclusion, zamifenacin reduces colonial motility, particularly postprandially in patients with IBS without side-effect and may be useful for treatment of this condition.

**EFFECTS OF ONDANSETRON ON SYMPTOMS AND MOTOR CORRELATES OF POST OPERATIVE EMESIS**

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Conventional treatment of post operative emesis is unsatisfactory. This double blind study has evaluated ondansetron, a 5HT3 receptor antagonist on symptoms and motor correlates of emesis. In 20 patients undergoing cholecystectomy (9 ondansetron, 11 saline). Gastric duodenal and jejunal manometry were assessed pre and post operatively for 24 hours. Symptoms and emetic episodes were assessed by linear analogue scale by an attendant observer.

**RESULTS**

Post operative nausea scores were lower (ondansetron 0.86±(0.22) vs placebo 1.51±(0.26)), general well being greater (ondansetron 7.04±(0.22) vs placebo 6.55±(0.22)) and emetic episodes per patient fewer (ondansetron 1.0±(0.44) vs placebo 3.8±(1.08)), in treatment than control arms. Post operative motility studies showed 410 isolated or stationary and 326 migratory phasic bursts (PB) of small bowel contractions. Ondansetron treatment was associated with fewer retrograde (RPB) and more antegrade (APB) phasic bursts (ondansetron 21 RPBs, 152 APBs vs placebo 38 RPBs, 115APBs, p<0.05) and reduced phasic burst contraction frequencies at both small bowel sites (p<0.001).

**Conclusion** Ondansetron alters postoperative small bowel motility by increasing antegrade propagation and reducing contractility of phasic bursts, and improves emesis symptoms.

+ chi² test
+ Mean +/- SEM
+ pair t test
+ Wilcoxon
+ unpaired t test
A DOSE RANGING STUDY TO DETERMINE THE ANTI-TIVRAL ACTIVITY AND SAFETY OF LAMIVUDINE (2'-DEOXY-3'-THIACYDINE) IN PATIENTS WITH CHRONIC HEPATITIS B INFECTION. de Man RA1, Schalm SW1, Main J, Thomas HC2, Everey J, Nevens F3 and Stakey H4. 1Dijkzigt Hospital, Rotterdam, The Netherlands; 2St Mary’s Hospital Medical School, London, United Kingdom; 3University Hospital, Gasthuisberg, Leuven, Belgium; 4Glaxo Group Research Limited, Middlesex, United Kingdom.

Lamivudine (GR190714X) is the single (-) enantiomer of the racemic mixture of 2'-deoxy-3'-thiacytidine. Lamivudine has been shown to be a potent inhibitor of human and duck hepatitis B virus replication in vitro.

A phase II randomised placebo controlled dose ranging study was conducted to evaluate the antiviral activity and safety of lamivudine in patients with chronic hepatitis B infection. Patients with histopathologically proven chronic hepatitis B, HBsAg present in serum for ≥6 months, HBsAg positive and stable hepatitis B virus DNA (HBV DNA) ≥10pg/ml were considered for entry. Forty-eight patients were randomised to placebo or lamivudine at one of five dose levels (5, 20, 100, 300 and 600mg/day). Lamivudine was administered orally once daily for one month and patients were subsequently followed for a further two months. Antiviral activity was assessed by measurement of serum HBV DNA (Abbott Genosys Assay) during treatment and follow-up.

Currently 42 patients have completed treatment and 33 have completed the study. Preliminary results confirm that lamivudine has an inhibitory effect on hepatitis B virus replication in patients with chronic hepatitis B infection. All doses of lamivudine produced a reduction in serum HBV DNA; however, there is a suggestion of a dose dependent effect. HBV DNA results available to date show that for 17/25 (68%) patients who received a dose of 20mg/day or greater, HBV DNA was reduced to <5pg/ml during treatment; the median (interquartile range) HBV DNA prior to treatment was 115pg/ml (64-450). Rebound of HBV DNA was observed after treatment completion. No serious adverse events have been observed, one patient (in the placebo group) was withdrawn from the study.

Lamivudine is an oral nucleoside analogue which shows substantial activity against hepatitis B and is well tolerated. Studies to investigate the effects of increasing the duration of treatment are now being undertaken.

PATHOGENESIS OF LIVER INJURY FOLLOWING HEPATITIS B VIRUS RECURRENT AFTER LIVER TRANSPLANTATION (OLT): A MAJOR ROLE FOR TUMOUR NECROSIS FACTOR-α.

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In HBV transgenic mice TNF-α and INF-γ have been shown to be important mediators in the lysis of HBsAg containing hepatocytes. To elucidate the immune mechanisms responsible for liver injury following HBV recurrence after OLT, characterised by a marked increase in the abundance of HBsAg, we studied the expression of TNF-α and INF-γ at the site of hepatic inflammation and serum levels of TNF-α and the two TNF receptors (R-55 and R-75).

TNF-α and INF-γ were detected by immunohistochemistry using corresponding monoclonal antibodies in 14 liver biopsies from 12 patients with HBV recurrence after OLT and were compared with the findings in 11 patients with chronic HBV infection (CLD). Serum levels of TNF-α and R-55 and R-75 were quantitated by ELISA. TNF-α was expressed in the liver in 10 (71.4%) of post-OLT and 9 (81.8%) of CLD biopsies while it was undetectable in the absence of HbsAg. To determine the role of TNF-α and the histological activity in the CLD group (R=0.89, p<0.01) but not in the post-OLT group (R=0.38, p=0.24). The post-OLT group serum levels of R-55 (median 3.0, range 1-10.2; normal <1.8ng/ml) and R-75 (range 0.6-35.8; normal <4.5ng/ml) were markedly elevated in contrast to that in the CLD group (R-55: median 1, range 0.5-2.2; R-75: 7.1, 2.2-13.8 respectively). Expression of INF-γ was less marked and was observed in only 5 (35.7%) of the post-OLT and 3 (27.3%) of the CLD cases.

These data indicate that TNF-α is present in a similar proportion of patients following HBV recurrence post-OLT, despite immunosuppression, and in CLD. The markedly elevated levels of TNF receptor post-OLT may lead to increased susceptibility to this inflammatory cytokine and contribute to liver damage in these patients.
HEPATOCELLULAR PROLIFERATION, TNFα AND INFLAMMATION IN PRIMARY BILIARY CIRRHOSIS (PBC)

For each patient, a biopsy was performed with a stepwise forward approach, used to select significant variables and to compute a Classification Index (CI, a function of the values of these variables) in order to define patients as those with or without high probability of cirrhosis. The resulting model was validated with the split sample technique. Results. Three clinical variables, age (yrs), presence (value 2) or absence (value 1) of HBsAg and of abdominal pain at presentation, and two laboratory variables, serum bilirubin (natural logarithm of μM/l) and albumin (g/l) were significant in the diagnosis of cirrhosis. Each variable was multiplied by its weighting coefficient (1.07, 1.78, 1.31, -1.24 and 0.17 respectively); the sum of these five products is the CI for the single patient. A CI less or equal to 0.65 means a high probability (87.7%) of cirrhosis, while a CI greater than this value indicates a high probability (73.7%) of no cirrhosis. Overall correct prediction of diagnosis or cirrhosis or no cirrhosis was achieved in 85.4% of our pts. Conclusion. This index can be useful in the management of patients in whom liver biopsy is not available.

CIRRHOSIS IN HEPATOCELLULAR CARCINOMA (HCC).

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When a patient first presents with HCC it is important to know whether or not cirrhosis is present. Methods. 226 patients with HCC and biopsy proven presence (162) or absence (64) of cirrhosis were reviewed. Sex, age, aetiology (alcohol, HBsAg, anti HCV), smoking habits, clinical picture at presentation (ascites, encephalopathy, abdominal pain or palpable mass), previous gastrointestinal bleeding, and liver function tests (bilirubin, albumin, INR, alkaline phosphatase, AST and total protein) were considered as explicative, continuous or categorical variables as appropriate. A logistic regression model, with a stepwise forward approach, was used to select significant variables and to compute a Classification Index (CI, a function of the values of these variables) in order to define patients as those with or without high probability of cirrhosis. The resulting model was validated with the split sample technique. Results. Three clinical variables, age (yrs), presence (value 2) or absence (value 1) of HBsAg and of abdominal pain at presentation, and two laboratory variables, serum bilirubin (natural logarithm of μM/l) and albumin (g/l) were significant in the diagnosis of cirrhosis. Each variable was multiplied by its weighting coefficient (1.07, 1.78, 1.31, -1.24 and 0.17 respectively); the sum of these five products is the CI for the single patient. A CI less or equal to 0.65 means a high probability (87.7%) of cirrhosis, while a CI greater than this value indicates a high probability (73.7%) of no cirrhosis. Overall correct prediction of diagnosis or cirrhosis or no cirrhosis was achieved in 85.4% of our pts. Conclusion. This index can be useful in the management of patients in whom liver biopsy is not available.

DO LIVER TUMOUR CELLS TAKE UP IODISED OIL? AN IN-VITRO STUDY

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Lipiodol (a contrast medium containing iodinated fatty acid esters) is selectively retained in hepatocellular carcinomas, allowing targeted intra-arterial delivery of radioisotopes and cytotoxic drugs conjugated with Lipiodol. The mechanism of retention and the effect of Lipiodol alone on tumour cells is unknown.

HepG2, a human hepatocellular carcinoma cell line, was exposed to 1%, 2% and 4% Lipiodol in culture medium for 4, 8, 24 and 32 hours, in logarithmic and plateau phases of growth. For each experiment (n=5) a growth curve was constructed, cell viability assessed by Trypan Blue exclusion, and media Lactate Dehydrogenase (LDH) estimated. Protein metabolism was assessed by [3H]-labeled Leucine incorporation. Cell monolayers were stained with Oil Red O and silver nitrate for lipid and Lipiodol respectively.

Lipiodol-exposure had no significant influence on cell number, growth curve configuration, cell viability, LDH levels or [3H]-Leucine incorporation compared to controls. Oil Red O revealed a significant increase in cytoplasmic lipid. Intracellular silver nitrate staining increased significantly (p<0.01, Rank Sum Test).

This suggests that Lipiodol is incorporated by hepatocellular carcinoma cells in culture, without altering cell viability and replication. Similar retention mechanisms may apply in-vivo. The study establishes a simple system for in-vitro cytotoxicity assays for drug-lipiodol conjugates.

TOWARDS A QUANTITATIVE DETERMINATION OF ANDROGEN RECEPTOR EXPRESSION IN ADULT HUMAN LIVER TISSUE

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Androgens have often been implicated in the aetiology of hepatocellular carcinoma but their role remains undefined. In particular the presence of androgen receptors (AR) in normal and diseased liver tissue is controversial. We have developed sequence specific reverse transcription PCR to quantify, for the first time, the expression of AR in six adult liver biopsies (two from patients with chronic liver disease). The resultant sequence data for both strands of the double stranded PCR AR fragment had 100% similarity with the published AR mRNA sequence (complete codons). The amount of AR was expressed as ratios of the level of AR mRNA to the level of two independent control genes, β-actin and glyceraldehyde phosphate dehydrogenase (GAPDH). The median AR:β-actin ratio was 0.53 (range 0.37-0.86) and the AR:GAPDH ratio was 0.85 (0.60-1.00).

AR:GAPDH ratios were lower in the patients with chronic liver disease. A recent study demonstrated a method to quantify AR expression in human liver biopsies which is both specific and sensitive. Application of this method has shown unequivocally AR expression in human liver tissue and these preliminary results suggest that AR mRNA varies significantly with the severity of disease.
ALtered Cortisol Metabolism in Alcoholic and Non-alcoholic Cirrhosis and Its Relationship to Clinical Severity of Liver Disease.


Chronic liver disease may reduce the clearance of adrenal corticosteroid metabolites because of diminished activities of hepatic oxidative and reductive enzymes, whilst circulating free cortisol levels may increase due to reduced synthesis of albumin and cortisol-binding globulin. In alcoholic liver disease cortisol production may be altered by direct effects of ethanol on the hypothalamic-pituitary-adrenal axis (pseudo-Cushing's syndrome). However, few studies have been performed on stable patients without recent alcohol withdrawal or severe intercurrent illness.

We investigated 24-hour urinary excretion of cortisol metabolites by gas chromatography in 22 male cirrhotic patients, 14 with alcoholic cirrhosis (AC) and 8 with Hepatitis C cirrhosis (HCV), in comparison with 20 male control subjects. The two patient groups were matched for age and Child-Pugh score. Total excretion of cortisol metabolites was reduced in the AC group in comparison with controls (4900 ± 2850 vs. 7620 ± 2760 μg/24 h, p < 0.05) but not in the HCV group (7670 ± 5160). There was a significant reduction in the ratio of 11-hydroxy to 11-oxosteroids (11OH/11OX) in the AC but not the HCV group (controls: 0.81; AC: 0.68, p < 0.05; HCV: 0.57, p > 0.05), indicating reduced production of 11-hydroxysteroid dehydrogenase. In contrast, a marked increase was seen in the ratio of 20-hydroxy to 20-oxosteroids (20 OH/20 OX) in both cirrhotic groups (controls: 0.35; AC: 0.73, p < 0.001; HCV: 0.61, p < 0.05) when compared to controls. Furthermore, total output of cortisol metabolites decreased in proportion to the severity of liver disease in the cirrhotic groups (Child-Pugh score: r = -0.43, p < 0.05; serum albumin: r = 0.65, p < 0.001; bilirubin: r = -0.56, p < 0.05). The 20 OH/20 OX ratio increased with severity (Child-Pugh score: r = 0.80, p < 0.001; albumin: r = -0.70, p < 0.001; bilirubin: r = 0.75, p < 0.001).

These results show that in chronic liver disease cortisol production is reduced in proportion to the degree of clinical decompensation. Increased reduction of the 20-oxy group appears to be a direct effect of liver damage, possibly reflecting reduced glucuronyl transferase activity. We found no evidence of pseudo-Cushing's syndrome in our alcoholic cirrhotic patients.

DO HYDROPHOBIC BILE ACIDS DAMAGESPECIFIC HUMAN HEPATOCYTE ORGANELLES?

AG Lim, HA Ahmed and TC Norfield. Dept. of Medicine, St George's Hospital Medical School, London.

In cholestasis, morphological changes of hepatocyte mitochondria, such as swelling and fragmentation of cristae have been observed. It has been hypothesized that this damage is caused by retained endogenous bile acids. We have previously shown that hydrophobic bile acids (BA) can damage human hepatocyte mitochondria. Our aim was to determine whether this damage is specific, by comparing it to the effect of bile acids on microsomes. Wedge liver biopsies were obtained from patients without liver disease. The liver samples were homogenized and organelles separated by differential and sucrose density gradient ultracentrifugation. The purity of organelle separation was assessed by specific enzyme markers and by electron microscopy. Mitochondria and microsomes were incubated with deoxycholic (DCA), chenodeoxycholic (CDCA), ursodeoxycholic (UDCA) and cholic acids (CA) at concentrations 0–10 mM. The activities of succinate cytochrome C reductase and NADPH cytochrome C reductase were measured as indices of mitochondrial and microsomal function respectively and expressed as % of control (mean±SEM). Effectiveness of BA in causing order of hydrophobicity is shown below.

<table>
<thead>
<tr>
<th>BA</th>
<th>mM</th>
<th>0.3</th>
<th>1.0</th>
<th>2.5</th>
<th>5.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCA</td>
<td></td>
<td>91.46 (9)</td>
<td>52.32 (7)</td>
<td>7.71 (1)</td>
<td>6.3 (1.7)</td>
<td>3.2 (0.8)</td>
</tr>
<tr>
<td>CDCA</td>
<td></td>
<td>106.18 (11)</td>
<td>81.25 (4)</td>
<td>14.6 (0.5)</td>
<td>9 (2.8)</td>
<td>6.9 (0.2)</td>
</tr>
<tr>
<td>CA</td>
<td></td>
<td>97.63 (5)</td>
<td>50 (5.7)</td>
<td>12.5 (2)</td>
<td>7.2 (0.5)</td>
<td>3.0 (1.2)</td>
</tr>
<tr>
<td>UDCA</td>
<td></td>
<td>113.60 (6)</td>
<td>109.51 (12)</td>
<td>92.9 (4.8)</td>
<td>88.5 (2.2)</td>
<td>78.3 (2.2)</td>
</tr>
<tr>
<td>Succ. cytochrome C reductase activity (Mitochondria)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCA</td>
<td></td>
<td>99.8 (3)</td>
<td>103.73 (7)</td>
<td>100 (0)</td>
<td>100 (2.7)</td>
<td>100 (2.7)</td>
</tr>
<tr>
<td>CDCA</td>
<td></td>
<td>108 (10)</td>
<td>116.8 (10)</td>
<td>98.4 (2.9)</td>
<td>120.6 (7)</td>
<td>117 (3.3)</td>
</tr>
<tr>
<td>CA</td>
<td></td>
<td>109 (6)</td>
<td>112.8 (1)</td>
<td>109 (1.4)</td>
<td>100 (2)</td>
<td>103.9 (2)</td>
</tr>
<tr>
<td>UDCA</td>
<td></td>
<td>101 (6)</td>
<td>102.2 (2)</td>
<td>104 (1.3)</td>
<td>111 (4.4)</td>
<td>108 (9.4)</td>
</tr>
</tbody>
</table>

We have shown that mitochondrial function is markedly affected by bile acids, and that the degree of inhibition is dependent on hydrophobicity. In microsomal function, mitochondrial inhibition is not affected to the most hydrophobic bile acid at the highest concentrations used. We conclude that mitochondrial function is specifically sensitive to bile acid damage; and we suggest that bile acid inhibition of mitochondrial energy production may be an important mechanism of hepatic injury and death in cholestatic liver diseases.
**W29**

**BILARY DECOMPRESSION RESTORES KUPFFER CELL FUNCTION IN OBSTRUCTIVE JAUNDICE**

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Depressed Kupffer cell clearance capacity (KCCM) predisposes jaundiced patients to systemic endotoxinosis and infectious complications. Biliary decompression remmances the main therapeutic strategy in managing biliary obstruction. In this study we investigated the efficacy of internal (ID) and external biliary drainage (ED) in achieving recovery of KCCM. Internal and external biliary drainage were established by way of a choledochocleodenoanostomy and choledochocutaneous respectively.

Methods Male Wistar Rats (250-300g) were assigned to one of six groups: Sham operated, Bile duct ligation (BDL) for 3 weeks and Sham operated or BDL for 3 weeks followed by biliary drainage for 21 days after ID or ED. KCCM was measured using an isolated in situ hepatic perfusion technique employing FITC-labelled late particles (0.75µm) as the test probe. Plasma was assayed for bilirubin (Bil), endotoxin and anti-cytoplasmic antibody (AACA) concentrations.

Results Increased rate had reduced KCCM (P<0.001) and increased concentrations of ACGA (P=0.001) and endotoxin (P=0.001) compared with control rats. Biliary drainage for 3 weeks produced a recovery in KCCM and normalisation of endotoxin and ACGA concentrations however external drainage was less effective than ID (P=0.05) in restoring Kupffer cell function.

**Data as Mean±SEM**

<table>
<thead>
<tr>
<th>Model</th>
<th>Bil/umol/l</th>
<th>ACGA µg/ml</th>
<th>Endotoxin µg/ml</th>
<th>KCCM % Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDL</td>
<td>168±12.3</td>
<td>340±35.7</td>
<td>105±21.9</td>
<td>16.2±2.8</td>
</tr>
<tr>
<td>Sham</td>
<td>1.2±0.3</td>
<td>120±24.5</td>
<td>7.1±3.2</td>
<td>38±5.1</td>
</tr>
<tr>
<td>BDL+SHAM</td>
<td>3±1.1</td>
<td>8±1.6</td>
<td>2.9±1.7</td>
<td>34±4.2</td>
</tr>
<tr>
<td>B/ED</td>
<td>2.7±0.8</td>
<td>140±16.5</td>
<td>3.7±2.5</td>
<td>40±3.4</td>
</tr>
<tr>
<td>B/ED</td>
<td>3.3±1.5</td>
<td>110±62.7</td>
<td>7.3±2.6</td>
<td>33±2.1</td>
</tr>
<tr>
<td>B/ED</td>
<td>3.3±1.5</td>
<td>110±62.7</td>
<td>7.3±2.6</td>
<td>33±2.1</td>
</tr>
</tbody>
</table>

Conclusions These data support the therapeutic role of biliary decompression, in particular by internal methods, in restoring Kupffer cell function and attenuating systemic endotoxinosis in obstructive jaundice.

**W31**

**SOUTHERN HYBRIDISATION ANALYSIS OF HBV DNA IN PERIPHERAL BLOOD LEUCOCYTES AND OF DIFFERENT CELL TYPES DURING DIFFERENT STAGES OF HEPATITIS B VIRUS INFECTION**

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*Dept of Gastroenterology Charing Cross Hospital London, **Dept of Academic Medicine Royal Free Hospital London.

Hepatitis B virus (HBV) has been found frequently in peripheral blood mononuclear cells (PBMC), but there seems to be no correlation with clinical status or serological profile. In this study we evaluated PBMC HBV DNA from 49 individuals utilising Southern hybridisation analysis following Hind III digestion. Southern hybridisation analysis revealed HBV DNA sequences in PBMC from 16/29 (55%) of chronic HBV carriers with serum HBsAg and HBV DNA, compared with 1/8 (13%) of carriers with anti-HBc and HBV DNA negative (p<0.05). Two of 7 patients with previous HBV infection and chronic liver disease had detectable PBMC HBV DNA. One patient with acute HBV infection and 6 controls were negative. Of the 19 patients with PBMC HBV DNA, 18 had high concentrations of HBV DNA. In addition 5 of these had free monomeric HBV DNA and 6 patients had low molecular weight bands. Following EcoRI digestion, 6/12 patients with high molecular weight bands developed bands at 3.2kb indicating free monomeric HBV DNA. The other 6 had a mixed picture of high molecular weight bands and a band at 3.2kb. Nine of the above patients originally had PBMC HBV DNA subsequently had total peripheral blood leucocytes separated into PBMC and polymononuclear cells. Four only had HBV DNA in PBMC, 2 only in polymononuclear cells and 3 in both types of cell.

Peripheral blood leucocytes often contain multimers of free monomeric HBV DNA and this is more common in patients with serum viral replication and may occur even in the absence of serum HBsAg. These findings have implications for recurrence of virus disease after hepatic transplantation.

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**W30**

**REGULATION OF KUPFFER CELL-DERIVED 95kD TYPE IV COLLAGENASE/GELATINASE BY GLUCOCORTICOIDS AND PROSTAGLANDINS.**

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University Medicine, Southampton General Hospital, United Kingdom

Kupffer cell-derived 95kDa type IV collagenase/gelatinase (95kDa C/G) degrades the normal basement membrane-like matrix in the subendothelial space of Disse with matrix-dependent changes in hepatocytes; fat-storing cell and endothelial cell functions. Thus, it may play an important role in the pathogenesis of liver injury and fibrosis. Regulation of this enzyme may occur at several levels including synthesis, extracellular pro-enzyme activation and inhibition. The effect of glucocorticoids, indomethacin and cytokines on regulation were investigated in this study.

Kupffer cells (KC) were isolated from normal rat liver by pronase/collagenase digestion and purified by density gradient centrifugation and centrifugal elutriation. After 3 days in culture KC were exposed to the following reagents in serum-free conditions: dexamethasone (1µM), indomethacin (10µM), interferon-γ (500u/ml), TNF-α (1500u/ml), myristate acetate (PMA) (50µg/ml), PDGF (10ng/ml). KC media was analysed by zymography and 14C gelatin degradation (expressed per µg cellular DNA).

Dexamethasone caused a significant decrease in detectable 95kDa gelatinase activity in KC media both with and without organomercurial activation (63% and 54% respectively, p<0.05, Wilcoxon). It also inhibited a 10-fold increase in release of 95kDa C/G in response to PMA which is known to occur at the level of protein synthesis. Indomethacin caused a decrease in the detectable gelatinase activity in crude KCCM (without proenzyme activation) but had no effect on total gelatinase release (assayed after proenzyme activation). Interferon-γ, TNF-α and PDGF had no significant effect on gelatinase release.

These data indicate that dexamethasone inhibits 95kDa C/G synthesis and release from KC which may be one mechanism for the protective effects of glucocorticoids in liver injury. They also suggest that there are prostaglandin-dependent mechanisms involved in the activation of this enzyme.

**W32**

**DEMONSTRATION OF IL-2 RECEPTOR EXPRESSION ON CIRCULATING LYMPHOCYTES IN ALCOHOLIC LIVER DISEASE USING FLOW CYTOMETRY (FACS).**

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The pathogenesis of alcoholic liver disease (ALD) is unknown, however there is some evidence to implicate perturbations of cell mediated as well as humoral immunity. We have utilised the new, highly sensitive technique of FACS to examine IL-2 receptor (IL-2r) expression (a marker of mononuclear cell activation) on peripheral blood mononuclear cells (PBMC) in ALD.

**METHODS**

In preliminary studies, 9 patients with biopsy proven ALD with no evidence of coincidental infective or autoimmune liver disease, and 19 normal controls, were studied. A whole blood lysate technique was used to obtain PBMC which were stained with antibody to IL-2 receptor. T cells and monocytes were separately identified using CD3 and CD14 respectively.

**RESULTS**

Significantly increased IL-2r expression (both percent of cells positive (p=0.014) and fluorescence intensity (p=0.036) was found on lymphocytes in ALD compared to controls. However no IL-2r expression on monocytes was found in either patients or controls.

**CONCLUSIONS**

The results demonstrate the presence of immune activation markers on circulating lymphocytes in patients with ALD. These findings lend further support for the suggestion that immunological mechanisms may be involved in the pathogenesis of ALD. The absence of circulating monocyte IL-2r does not exclude the possibility of immune activation of monocytes in the liver.
Colorectal

W33

INFLUENCE OF THE INVOLVEMENT OF THE CIRCUMFERENTIAL MARGIN OF EXCISION BY TUMOUR ON SURVIVAL FOLLOWING RESECTION FOR RECTAL CANCER

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Local recurrence remains a common problem following resection for rectal cancer, and may adversely influence survival. We have previously shown that involvement of the circumferential resection margin (CRM) of excision by tumour is an important factor in predicting the development of local recurrence, but it remains uncertain whether this translates to improved patient survival. In a prospective study, pathological specimens from 150 patients who had undergone resection for rectal cancer were examined for involvement of the CRM by tumour along with routine pathological variables.

At a median follow-up of 5 years, local recurrence had occurred in 43 patients (28.6%), with a cancer-specific 5 year survival of 47%. The influence of tumour involvement of the CRM is shown below.

Local recurrence 5 year survival

<table>
<thead>
<tr>
<th>CRM involved</th>
<th>(n=63)</th>
<th>CRM clear</th>
<th>(n=98)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>34 (54.0%)</td>
<td>9 (9.2%)</td>
<td>65.0%</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>Chi-square</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

Further analysis was aimed at determining if CRM involvement independently influenced survival. Cox's stepwise regression analysis showed the presence of metastases, nodal involvement and CRM involvement to be the only indices independently influencing survival (all p<0.0001). These results underline the importance of adequate circumferential excision during surgery for rectal cancer.

CRM = circumferential resection margin

W34

ARE BILARY BILE ACIDS DIFFERENT IN PATIENTS WITH FAMILIAL ADENOMATOUS POLYPOSIS?

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Familial adenomatous polyposis (FAP) is associated with APC gene mutations; the development of polyps and cancer is variable and may be promoted by bile acids (BA). One previous study also indicated an intrinsic BA metabolic defect with an increased cholate (CA):cholenoxycholate (CDCA) ratio in the bile of FAP patients, but comparisons were between FAP patients post-colectomy and untreated controls. Therefore we analysed biliary BAs using gas chromatography in both pre- and post-colectomy FAP patients and compared them with the appropriate control groups. The results showed no difference between the groups matched for intact colons (+) or colectomy (-) for total primary BAs and CA:CDCA; significant differences occurred with pre-colectomy vs post-colectomy irrespective of the groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Colon N</th>
<th>Primary BAs</th>
<th>CA:CDCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAP+</td>
<td>12</td>
<td>73 (61.87)</td>
<td>0.7 (0.5.1.1)</td>
</tr>
<tr>
<td>Control</td>
<td>86</td>
<td>49 (70.10)</td>
<td>0.6 (0.4.1.2)</td>
</tr>
<tr>
<td>FAP-</td>
<td>12</td>
<td>94 (87.99)</td>
<td>0.9 (0.5.1.1)</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>93 (75.100)</td>
<td>0.9 (0.6.1.1)</td>
</tr>
</tbody>
</table>

mole%, median (range); a vs b, c vs d, e vs f, g vs h = all NS; a vs c, b vs d, e vs g, f vs h = all Z=0.05 at least (McWhitney U test).

Differences of the same order were found with 10 individual BAs from each glycine and tauroine fraction of BAs.

CONCLUSION: There is no intrinsic BA metabolic defect in FAP; colectomy results in a dramatic reduction of secondary BAs in both FAP and non-FAP patients.

W35

FAMILIAL INFLAMMATORY BOWEL DISEASE IN RELATIVES OF PATIENTS WITH CROHN'S DISEASE

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Introduction: Although it is now generally accepted that relatives of patients with Crohn's disease (CD) are at increased risk of developing inflammatory bowel disease (IBD), the pathogenic significance of this finding is uncertain: genetic and environmental factors have both been implicated. Furthermore the size of increased risk is disputed, previous estimations from specialist centres may reflect a bias in patient selection (disease severity; age of onset; ethnic origin).

We evaluated the prevalence of familial IBD in an unselected population of adult CD patients in Oxfordshire. Less than 5% were tertiary referrals.

Methods: Questionnaire, supplemented by review of case notes and interview.

Results: Information was obtained from 433 patients, including 9 adopted at birth. Two hundred and fifty seven were female. Ages ranged from 17 to 85 years. In 78 families (18%), at least one first or second degree relative had IBD. Three relatives were affected in 14 families (3.2%), and four in three families. Both CD and ulcerative colitis (UC) occurred in these multiply-affected pedigrees.

In 50 families (11.5%), a first degree relative had IBD. Siblings were most affected (33 siblings in 29 families [67.0%]). CD (20 cases) was more common than UC (13). Nineteen parents and four offspring were affected.

In only one family was a spouse affected. IBD was not present in families of adopted CD patients.

Conclusion: These data confirm the high prevalence of familial IBD, and further implicate genetic susceptibility in disease pathogenesis.

W36

MUCOSAL AND INTERNAL SPHINCTER DAMAGE PREDICT INCONTINENCE BETTER THAN EXTERNAL SPHINCTER DIVISION AFTER FISTULA SURGERY

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Physiology and function have been assessed prospectively in 51 patients treated for suspected idiopathic anal fistula.

Patients: Interphincteric fistulae (15), transphincteric (22) controls (sepsis outside the sphincters with no sphincter division - 13). All were evaluated pre- and postoperatively by questionnaire and anoanal physiology.

Results: Controls: physiology and function unchanged by surgery. Interphincteric fistulae: Lay open reduced resting pressure significantly (92 v 48 cmH2O, p<0.005); no change in squeeze pressure. The 8 patients with worsened continence had lower postoperative resting pressures than the 7 with normal postoperative continence (60 v 38 cmH2O, p=0.01).

Transphincteric fistulae: Lay open reduced resting pressure (7) decreased resting pressure (100 v 60 cmH2O, p<0.0001). Squeeze pressure was decreased in the distal 2 cm anal canal when laid open, but only in the distal 1 cm for a loose seton. There were no significant differences in squeeze pressures between the 11 patients whose continence deteriorated (6 lay open; 3 loose seton; 2 tight seton) post-operatively with the 12 whose function was unaffected; but total pressures (releasing plus squeeze) in the distal 2 cm were significantly lower in the incontinence group (210 v 140 cmH2O, p<0.005).

Incontinence was associated with locally decreased anal mucosal electrosensitivity (0.2 v 8.4mA, incontinent v controls, p=0.004).

Conclusions: Incontinence after fistula surgery is related to internal sphincter damage and mucosal insensitivity, but cannot be predicted on the basis of external sphincter division.
Ploidy, K-ras and p53 expression in Heredity Non-Polyposis Colorectal Cancer (HNPPC).


No morphologic or molecular biomarkers have been so far identified in HNPPC, which is usually defined on the basis of clinical criteria. On the other hand, identification of HNPPC is of fundamental importance for the management of these patients, which is different from that of sporadic colorectal tumours. Our purpose was to evaluate if ploidy, oncogenes and anti-oncogenes expression might be of help in the recognition of this syndrome. Paraffin embedded tumour specimens of 29 patients with HNPPC were compared with 123 (ploidy study) or 30 (ras expression study) cases of sporadic carcinoma. DNA ploidy was evaluated on nuclei suspensions after enzymatic digestion and staining with propidium iodide in a Becton-Dickson flow-cytometer; pan-ras expression was assessed by standard immunohistochemistry with the Ab-1 monoclonal antibody (Oncogene Sciences), p53 expression with monoclonal (PAB 1801 and DO7) and polyclonal (CM1) antibodies. In HNPPC, 52% of tumours were diploid versus 27% of sporadic cancers (X2 =4.381, p=0.036). Immunostaining for pan-ras was positive in 15 out of 29 patients with HNPPC (51%) as opposed to 20 out of 30 (66.6%) sporadic carcinomas (n.s.). Positive immunostaining for the p53 tumour-suppressor gene was shown in 45% of patients with HNPPC versus 55% of sporadic carcinomas. In conclusion, the present studies confirm previous observations indicating a prevalence of diploid tumours in HNPPC; in contrast, these preliminary observations did not show marked differences in K-ras and p53 expression between hereditary and sporadic colorectal cancers.

Determinants of prognosis in colorectal cancer: a population-based study.


Istituto di Patologia Medica, Istituto di Anatomia Patologica and Servizio di Igiene Pubblica, Universita' di Modena, Modena, Italy.

Dukes' stage at diagnosis is the most important variable related to prognosis in colorectal cancer. Other factors have been studied, but their influence on survival has not clearly been established. The main purpose of the present study was to examine some clinical and pathologic variables of the tumour in relation to 5-year survival after colorectal cancer diagnosis. Three hundred ninety-seven patients out of 406 diagnosed from 1984 through 1986 within a population-based colorectal cancer registry of Northern Italy were followed-up, and their status assessed at December 31, 1991 (survival after at least 5 years from diagnosis). The overall survival, after exclusion of colorectal cancer unrelated deaths, was 41.1%. Among conventional variables, univariate analyses showed that younger age was a determinant of more favourable prognosis (50.9, 45.0, and 26.9% survival in <56 yrs., 56-75, and >75 age groups, respectively, p<0.001) as was Dukes' stage at diagnosis. Several pathologic variables were evaluated in 274 neoplasms from the same series. Among them, pattern of tumour growth, degree of differentiation, lymphocytic infiltration of the tumour, and fibrosis were significantly predictive of survival. In Dukes' stage B1 tumours the number of involved lymphnodes was also related to survival. A multivariate Cox's hazard model was used in order to establish the independent value of each variable found to be significantly related to the clinical outcome in univariate analyses. Dukes' stage, pattern of tumour growth and age at diagnosis entered the model, suggesting independent influence on colorectal cancer prognosis. In conclusion, besides Dukes' stage, other clinical (patient's age at diagnosis) and pathologic variables (pattern of tumour growth) seem to be important prognostic determinants after colorectal cancer diagnosis.

Significant increase in negatively charged serine dependent proteinases in Ulcerative colitis


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Patients with ulcerative colitis (UC) have raised total faecal proteinase activity compared to healthy controls. A thinner and discontinuous mucus barrier in UC is associated with this rise in proteinases, which are mucolytic. This study describes the fractionation of faecal proteinases from controls and colitics. Human faecal extracts fractionated by ion exchange HPLC were eluted with a linear gradient, 0-1M NaCl in 67mM phosphate buffer, pH 7.5. Proteinase activity, assayed by measuring the formation of new NH2-terminal groups using succinyl alambrin substrate, was expressed in units of mmoles NH2- terminal min-1 g-1 dry weight of faeces (U). HPLC fractionation of faecal extracts produced two peaks of proteinase activity; pools 1 and 2. Pool 1 eluted in the unbound fraction (before the salt gradient) which was coincident with 98% of the pancreatic proteinase activity. Pool 2 eluted during the salt gradient (between 60-310 mmoles NaCl). Mean proteinase activity for pool 2 was significantly six fold higher for UC patients (6.8 ± 2.2U, n=9) than that for controls (1.2 ± 0.3U, n=10), p<0.05. Mean proteinase activity for pool 1 for UC patients (4.6 ± 2.1U, n=9) was also six fold greater than that for controls (0.7 ± 0.2U, n=10), although not significant. Specific inhibition showed that serine dependent proteinases were the major component (>76%) in pools 1 and 2 from both controls and UC patients. These studies show (i) that a spectrum of proteinases are raised in UC (ii) there is a significant increase in negatively charged faecal proteinases in UC compared to controls and (iii) this activity is predominantly from serine dependent proteinases presumably from both the endogenous microflora and host enzyme secretions (pancreas and white cells).

THE SOURCES OF INCREASED NITRIC OXIDE PRODUCTION IN ULCERATIVE COLITIS


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Nitric oxide (NO) synthesis is increased in rectal biopsies from patients with active ulcerative colitis (AUC) but its origin is unknown. The enzyme NO-synthase (NOS) exists in two forms, the calcium dependent constitutive (CNOS) and the calcium independent inducible (INOS). To determine the activities of these in macroa and faeces of 11 patients with AUC and 10 healthy volunteers, supernatants of homogenised rectal biopsies or sonicated faeces were incubated with L-arginine (300mM) and spiked with [14C]-arginine (319 MCi/mmol) in the presence of MgCl2 (1mM) CaCl2 (1mM), calmodulin 50 units, tetrahydrobiopterin (100mM) and L- or D- N6-monomethyl-arginine (NMMA) before application to silica 60 TLC plates. The scintillation of bands corresponding to arginine, citrulline and urea was counted. Total NOS activity was calculated from the difference between 14C-citrulline production with L-NMMA and that with D-NMMA in the presence of calcium, and INOS from that difference in the absence of calcium. The difference between total NOS and INOS gave the activity of CNOS.

NOS was not detectable in normal mucosa. Mean total NOS in AUC was 0.55±0.06, CNOS 0.325±0.075 and INOS 0.22±0.03 nmol/mg/min. Rectal biopsies in AUC showed increases in both CNOS and INOS but faeces increased CNOS alone. There was no CNOS activity in faeces from healthy volunteers. Increased INOS activity may come from leucocytes infiltrating the rectal mucosa but leucocytes do not contain CNOS. Faecal bacteria which possess CNOS, may be an important source of increased NO production in AUC.
Motility W43-W56

UPPER GUT TRANSIT IN THE IRRITABLE BOWEL SYMPTOM: ANALYSIS ACCORDING TO THE TYPE OF DYSPEPTIC SYMPTOMS PRESENT. DA Gwee, AF Collk and MW Read (Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Sheffield S5 7AU.)

Dyspeptic symptoms are common in patients with the Irritable Bowel Syndrome (IBS). So far no consistent abnormalities in upper gut transit have been noted when IBS patients are studied as a whole regardless of symptoms. We measured gastric emptying of 5克（GE）of a radiolabelled solid meal using a gamma camera, in a consecutive series of 17 IBS patients. In addition, we also measured the oro-caecal transit time (OCTT) with a hydrogen breath test. Patients were divided based on the presence (+) or absence (-) of the following symptoms: meal related pain (Meal+/-Meal-), nausea (Nausea+/Nausea-) and early satiety or postprandial fullness (Satiety+/Satiety-).

Symptom n GE(SE) (min) OCTT(SE) (min)
Meal+ 9 14.3 (3.8) 261.7 (34.2) **
Meal- 8 26 (4.2) 293.8 (30.9) **
Nausea+ 6 22.3 (6.1) ** 224.2 (25.5) **
Nausea- 11 18.4 (2.3) 305.5 (29.6) **
Satiety+ 9 15.8 (3.7) ** 270.5 (25.5) **
Satiety- 8 27.4 (3.4) 41.11 *
* p<0.05, ** p<0.01

Further, when the distributions were analysed, it was noted that the GE of IBS patients were either faster, or slower than Nausea- patients; in the Nausea- group, GE fell within the normal range.

These results suggest that when studying upper gut transit in IBS patients, it may be useful to (1) analyse them according to the type of dyspeptic symptoms present and (2) look at the distribution pattern.

W41

BOTH SENSATION AND PRESSURE IN THE ANAL SPHINX Are IMPAIRED AFTER EVERSION OF THE ANO-RECTAL STUMP DURING RESTORATIVE PROCTOCELECTOMY; M.W. Williamson, MG Lewis, PM Sagar, DJ Holdsworth, DJ Johnston; Academic Unit of Surgery and Centre for Digestive Diseases, The General Infirmary at Leeds.

In restorative proctocolectomy it is vital that the sensory and motor functions of the anal sphincter remain intact. We have used the ano-rectal eversion technique, which permits accurate placement of the transverse staple line at a chosen level above the dentate line.

Our hypothesis was that eversion would minimise the potential for retained colonic mucosa, without impairing the function of the anal sphincter.

36 patients (median age 33 years) underwent paired physiological tests, before, and 12 months after operation: 18 were also tested, 3 and 6 months after operation. Electro-sensitivity was measured in the upper, middle and lower anal canal using a constant current stimulator; anal sphincter pressure by micro-balloon and station pull-through technique.

RESULTS PRE-OP 3 6 12 MONTHS
Upper canal 10.0 9.1 9.8 9.1
Mid-canal 7.0 8.1 8.4 8.0
Lower canal 4.8 6.4 7.4 8.2
RAS 91 91 73 91
*p < 0.05 # p < 0.01

Resting anal pressure (RAS) decreased significantly after ano-rectal eversion. Sensation in the upper anal canal was preserved, precisely in the area affected most by mucosal stripping to the dentate line. However, sensation in the mid anal canal was reduced transiently, while in the lower anal canal it deteriorated with time, perhaps as a result of stretching of the pudendal nerve in the course of anal eversion.

W42

A DOUBLE BLIND RANDOMISED CONTROLLED TRIAL OF DIETARY CALCIUM SUPPLEMENTATION IN INDIVIDUALS WITH ADENOMAS (1 YEAR RESULTS). Rooney P.S., Gifford K.A., Clarke P.A., Hardcastle J.D. and Armitage N.C. Department of Surgery, University Hospital, Nottingham. NG7 2UH

Dietary supplementation with calcium has been shown to reduce rectal mucosal proliferation in individuals at high risk of colorectal cancer. However there are few long term, randomised controlled trials in individuals with sporadic adenomas.

One hundred and thirty eight individuals (age range 34-70) were colonoscoped, found to have adenomas and offered dietary supplementation. Seventy nine accepted and were entered into a double blind randomised controlled trial of 1500mg of calcium per day or placebo.

Rectal mucosal proliferation was measured by the in vivo metaphase arrest technique, Crypt Cell Production Rate (CCPR).

RESULTS Four individuals withdrew from the study in the first 12 months.

TIME/MONTHS calcium n=40 mean CCPR 12.2(5) * 9.0 (2.8) 9.3 (3.2) (sd) cc/m2/hr
(s) cc/m2/hr
placebo n=39 mean CCPR 10.6(5) 9.4(2.9) 8.9(3.3)
(s) cc/m2/hr

*** p=0.002 t=3.15 df=76 *** p=0.009 t=2.7 df=74

There were no significant changes in proliferation in the control group. Supplementation with 1500mg of calcium produced a sustained reduction in rectal mucosal proliferation even after 12 months.

It is too early to comment upon the effect of calcium on new adenoma formation; however there appears to be a role for dietary supplementation of calcium in longer and longer cancer prevention trials.

W43


National Spinal Injuries Centre, Gastroenterology and Radiology Dept, Stoke Mandeville Hospital, Aylesbury and Gt Science Research Unit, London Hospital Medical College, London.

Many patients with spinal cord lesions suffer from intractable constipation. Sacral parasympathetic denervation is the presumed cause of delayed transit. Clinical assessment gives no information regarding regional colonic transit and is subject to patient and physician bias. We have measured segmental and total colonic transit in patients with spinal cord lesions with constipation to assess the severity and site of faecal transit in the colon and rectum.

Nineteen patients with spinal injury with varying degrees of constipation, and 18 age and sex-matched normal controls participated. Colonic transit was measured using a simplified method without the need for multiple X-rays or radioisotopes (Metcalfe et al, Gastroenterology, 1987; 92: 40-47). Three gelatin capsules, each containing 20 radio-opaque shapes were ingested on 3 consecutive days (day 1, circles, day 2, spicules, day 3 squares). On day 4, a single, plain abdominal X-ray was taken to identify the presence and location of the shapes within the colon. Total and segmental colonic transit for the right, left and rectosigmoid colon was calculated (Arhan et al. Dis, Colon Rectum, 1981; 24: 625-629).

Total colonic transit was significantly longer in patients than controls (median range); Patients: median 62.4 hrs (15.6 to 64.8); Controls: 22.8 hrs (8 - 55.2 ), p<0.001. Four patients with severe constipation had total colonic transit times of > 60 hours, which was in keeping with their symptoms and 3 patients had a total colonic transit shorter than 30 hours. Segmental colonic transit at all levels was also significantly longer than the normal controls but again with a wide variation. Right colon, patients median 9.6 hrs (3.6-44.4), control 5.3 hrs (1.2-20.4 ), p<0.007. Left colon, patients median 14.4 hrs (2.4-42), control 6hrs (2.4-18) (p<0.001). Rectosigmoid, patients 10.8hrs (1.2-56.4), controls 3hrs (1.2-38.4) (p=0.01).

This study has shown that total colonic transit was significantly slower than controls in the majority of spinal patients with symptoms of constipation seeking medical advice, although no significant differences were found in segmental transit. In addition, colonic delay was not limited to distal holdup but also seen frequently in the proximal colon. This information is useful in the optimum management of this patient group.

W44
DEFaecography in Patients with the Irreitable Bowel Syndrome.

R. Hutchinson, A. Mattan*, I. K. Harding*, & D. R. Amar. Department of Surgery, Queen Elizabeth Hospital, Birmingham, & Department of Physics & Nuclear Medicine, Dudley Road Hospital, Birmingham.

There are few studies of anorectal function in the irritable bowel syndrome (IBS). To investigate the hypothesis that colonic predominant symptoms of IBS may be attributable to differences in anorectal function, we studied rectal evacuation in 16 patients with IBS using scintigraphic defaecography.

9 patients (M: F = 6:1; median age 36, range 24-59 years) had constipation-predominant symptoms (CP-IBS) and 7 patients (M: F = 6:1; median age 50, range 39-65 years) had diarrhoea-predominant symptoms (DP-IBS). All fulfilled accepted criteria for the diagnosis of IBS.

The mean percentage rectal evacuation of 66% (SD = 16) in CP-IBS was not significantly different from that of 75% (SD = 24) in DP-IBS. Similarly, there were no differences in evacuation rates (mean 2.3%/sec; SD = 1.3 in CP-IBS; mean 2.6%/sec; SD = 3 in DP-IBS) or in mean evacuation angles (mean 128°, SD = 11 in CP-IBS; mean 137°, SD = 9 in DP-IBS). Pelvic floor descent was present in 7 (78%) patients with CP-IBS (mean 4.7 cm) and in 4 (57%) patients with DP-IBS (mean 5.1 cm). Rectoceles were demonstrated in 5 (62%) patients with CP-IBS (mean size 2.7 cm) and in 3 (43%) patients with DP-IBS (mean size 3.4 cm). There were no significant differences in the prevalence or size of pelvic floor descent and rectocoele between the groups.

We conclude that the different symptoms of patients with constipation-predominant IBS cannot be attributed to objective differences in anorectal function.

EFFECT OF RATE AND EXTENT OF STARCH DIGESTION ON GASTRIC EMPTING AND GLYCEMIC RESPONSE TO THE NEXT MEAL.

L. Benini, F. Brighenti, G. Castellani, M. T. Brentegani, C. Sambenini, N. Pellegrini, M. C. Carcaghi, I. Vantini. Dept of Gastroenterology, University of Verona at Valeggio sm DIStM, University of Milan, ITALY.

The ingestion of slowly-digestible fibre-rich foods flattens the glycaemic response to the next meal. High doses of starch in the distal ileum reduce the rate of gastric emptying of a meal. This study investigated a - if starches with different gastric properties eaten at breakfast influence gastric emptying and glycemic response to the following lunch; b - if this effect is related to carbohydrate fermentation in the colon or c - to the absorption of carbohydrates (CHO) in the distal small intestine. Ten healthy asymptomatic volunteers (26-58 years old) ate at breakfast, in random order, 4 Angel-bread cakes containing sucrose (15 g), cellulose (5%) and 60 g amylopectin (quickly digestible starch) (A); 60 g amylose (slowly digestible starch) (B); the same as (A), but with 100 mg of α-glucosidase inhibitor Acrabose as the same as A but with cellulose replaced by lactulose (C). Five hours later, they ate a 800 kcal solid meal (47% CHO, 17% protein, 36% fat). Ultrasonography was used to quantify gastric emptying of this lunch. Plasma glucose was measured half-hourly, breath hydrogen hourly over the day. RESULTS: At breakfast, glucose incremental areas under the curve (IAUC) were significantly lower for B and C (p < 0.05 for all contrasts against A) than A. Hydrogen IAUC were higher for C and D (p < 0.05 for all differences apart C vs D), confirming the arrival of similar quantities of fermentable CHO in the colon after Acrabose and lactulose ingestion. After lunch, D caused a significant delay in gastric emptying (p < 0.01; A: 74 ± 2.8 min; B: 91 ± 7; C: 809.7; D: 10917.3; p < 0.01 for D vs A and C). However, glycemic response was reduced, though not significantly, only by D (p < 0.01), which caused only a minimal delay in gastric emptying. Acrabose, which mimicked the post-breakfast glycaemia of slowly digestible amylose and the colonic fermentation of lactulose, had no effect on gastric emptying or on post-prandial glycaemia. We conclude that the second meal effect on glycaemia is due to slow-starch digestion coupled with distal CHO absorption rather than to the presence of CHO in the ileum or colon or to a delayed gastric emptying.
AUDIT OF TRANSIT ABNORMALITY IN CHRONIC IDIOPATHIC CONSTIPATION.

R Hutchinson, A Noughton, A B Moxley, L K Harding. 
Department of Surgery, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH & Department of Physics & Nuclear Medicine, Dudley Road Hospital, B18 7OH.

Optimum management of chronic idiopathic constipation depends on assessment of colonic transit and rectal evacuation patterns. Patients with slow colonic transit, impaired rectal evacuation, or a combination of both disorders. 36 patients (F:M 32:4, mean age 42, range 17-70 years) with chronic idiopathic constipation were studied by scintigraphic defaecography and colonic scintigraphy.

Using parametric images to classify colonic transit patterns, transit was normal in 12 (33%), delayed in 23 (64%) and one patient had rapid transit. The delay was generalised in 4 (11%) and right-sided in 19 (53%). Based on studies from normal subjects, impaired rectal evacuation was defined as less than 30% rectal evacuation or an inversion ratio greater than 1.5 s/100 s. Scintigraphic defaecography demonstrated impaired rectal evacuation in 25 (69%) patients. Using both investigations to identify the transit abnormality, 6 (17%) patients had slow colonic transit, 8 (22%) had physiological colonic transit, 17 (47%) had a combination of both dysfunctions. The physiological assessment was normal in only 5 (14%) patients.

These data show that 17% of patients with chronic idiopathic constipation have slow colonic transit which may be suitable for treatment by colectomy. A similar proportion are normal on these physiological tests, but almost 70% of constipated patients have impaired rectal evacuation. These patients are unlikely to benefit from colectomy. We believe that evaluation of chronic constipation should include tests to assess rectal evacuation as well as colonic transit.

INVESTIGATING THE EFFECT OF HYDROGEN PEROXIDE AND INTERLEUKIN-1 ON GUINEA-PIG DISTAL COLONIC SMOOTH MUSCLE FUNCTION.

I T Pipez, D G Thompson, S L A H Westman. 
Department of Physiology, University of Manchester, Salford M6. 

Levels of oxygen-free metabolites and interleukin-1 are elevated in colitis. The aim of this study was to determine the effects of both hydrogen peroxide and interleukin-1 on guinea-pig distal colonic smooth muscle function.

Strips of guinea-pig distal colon from either the longitudinal or circular muscle axis were incubated with 500 µM H2O2 for 20 min in a significant reduction of the response to K+ (40 mM) (% spasm: Longitudinal: Initial 100 ± 0; after H2O2 88.1 ± 8.0; after vehicle 125.3 ± 9.9; Circular: Initial 100 ± 0; after H2O2 66.4 ± 3.6, after vehicle 135.5 ± 12.8 p<0.05 n=6). However, incubation with H2O2 (500 µM) did not alter the responsiveness of either muscle layer to carbachol (CCh). H2O2 (500 µM) itself caused a transient contraction of both muscle layers which was partially sensitive to noradrenaline (TXA, 1 µM). The K+ (40 mM)-induced spasm evoked in either muscle layer was not sensitive to TXA (1 µM).

In further experiments muscle responsiveness to CCh was tested both before and after 4h incubation with H2O2 (1mg/ml). In longitudinally- and circularly-oriented strips incubation with H2O2 (1mg/ml) did not modify the concentration-effect curve to CCh (p>0.05, n=6). H2O2 (1mg/ml) itself was without effect on the basal tension of either longitudinally- or circularly-oriented strips.

In conclusion H2O2 contracts guinea-pig distal colon, in part, by a TXA-sensitive mechanism suggesting that it causes release of noradrenaline. The inability of H2O2 to reduce the response to K+ but not CCh suggests that it does not alter muscle function directly. It would also appear that it is not due to H2O2-induced disruption of neuronal function since the K+ spasm was TXA-insensitive.

The inhibition of interleukin-1 to change muscle function suggests that its role may be to cause inhibition of neutrophils which in turn release reactive oxygen metabolites. The mechanism by which H2O2 reduces response of smooth muscle to K+ but not to CCh requires further investigation.

Supported by the National Association of Colitis and Crohn's Disease.

S 1 Warren, D Burlesh, and N S Williams

Short Chain Fatty Acids Reduce Human Longitudinal Colonic Motility in Vitro

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The mechanism of action of dietary fibre upon colonic motility remains obscure. Colonic bacterial fermentation of fibre produces short chain fatty acids (SCFAs), mainly acetic, propionate and butyrate. As major anions of the large bowel and main determinants of pH, they are a putative luminal chemical stimulus to human colonic motility.

Resected normal human right (n=7) and left (n=7) colon were perfused intraluminally with a physiological SCFA mixture and varying concentrations (1, 10, 100 mM) of individual SCFAs. The longitudinal contractile activity was analysed by an activity index and compared to Krebs perfused control periods of spontaneous activity (control vs control)

<table>
<thead>
<tr>
<th>Right Colon</th>
<th>Left Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con 1M 10M 100M</td>
<td>Con 1M 10M 100M</td>
</tr>
<tr>
<td><strong>Butyrate</strong></td>
<td>5.7 5.3 6.6 6.3 7.0 5.4 5.6 4.5</td>
</tr>
<tr>
<td><strong>Propionate</strong></td>
<td>5.2 5.2 4.5 4.2* 7.8 7.5 4.7* 4.8*</td>
</tr>
<tr>
<td><strong>Acetate</strong></td>
<td>6.5 5.4* 5.2* 5.4* 7.9 5.7 4.6 2.7*</td>
</tr>
</tbody>
</table>

SCFA mixture Right: 5.3* (con 6.6) Left: 3.4* (con 5.4). Activity Index - median *p < 0.05 **p < 0.01 Wilcoxon Signed Rank

Luminal physiological concentrations of SCFAs reduce human longitudinal colonic contractile activity in vitro. Acetate and propionate reduce motility whilst butyrate has no effect at any concentration (suggesting their effect is independent of pH) and thus the colon demonstrates a regional variation in responsiveness to individual SCFAs.
EFFECT OF SWALLOWING AND VAGAL STIMULATION ON THE HUMAN OESOPHAGEAL EMG RESPONSES EVOKED BY MAGNETIC STIMULATION OF THE CEREBRAL CORTEX.


Depart. of Medicine, University of Manchester and *Institute of Neurology, London, U.K.

BACKGROUND: animal studies suggest that the CNS control of oesophageal motility is modulated via sensory feedback; in man however, evidence for this is lacking. AIMS: to study the effects of vagal afferent stimulation induced either by swallowing or stimulation of the extracranial vagus nerve on the cortically evoked human oesophageal EMG responses. METHODS: oesophageal EMG was recorded in 7 volunteers, 2cm below the upper oesophageal sphincter after transanal insertion of a catheter containing bipolar ring electrodes. Magneto-electric pulses were discharged through a circular coil placed at the vertex of the skull for cortical stimulation. A further "figure of 8" coil was placed at the angle of the jaw for stimulation of the extracranial vagal afferent fibres. Study 1: cortical stimulation was performed without swallowing and at varying intervals (200ms to 5 sec) after the initiation of a swallow using the myelobody muscle EMG to trigger the cortical stimulator. Study 2: cortical and vagal stimulation were performed both separately and together with varying vago-cortical stimulation intervals (20ms to 200ms). RESULTS: Study 1: cortical stimulation without swallowing produced a triphasic oesophageal EMG response at a latency of 0.8±0.4ms (mean±SEM) and an amplitude of 66±16 μV. The amplitude increased (318±99.3 μV, p=0.02) with the arrival of the peristaltic wave, indicating modulation by an afferent pathway induced by the swallow. Study 2: Vagal Stimulation: produced a reflex latency response due to stimulation of the CNS via vagal afferents at a latency of 56.8±14.5ms. Combined Vagal and Cortical Stimulation increased the amplitude of the cortically evoked oesophageal EMG response with increasing vago-cortical stimulation delay and was maximal at 100ms (329.6±27.5 μV, p<0.01). CONCLUSION: Vagal afferents acting via the central nervous system modulate oesophageal motility evoked by cortical stimulation and play a role in the control of swallowing in man.

QUALITY OF LIFE IN FUNCTIONAL AND ORGANIC INTESTINAL DISEASES. A. Martin, L. Leone, R. Maccararetto. Divisioni di Gastroenterologia, Università di Padova, ITALY.

Evaluation of health-related Quality of Life (QL) is an important component of patients' assessment, especially in chronic gastrointestinal disorders. Aim of the study was to assess whether QL is affected in some common gastrointestinal diseases. Methods: a specific questionnaire analyzing features which are frequently altered in intestinal diseases, was developed and tested for reliability and validity in control subjects and patients with Ulcerative Colitis (UC). Four areas are explored by the instrument: Intestinal Symptoms (IS), Systemic Symptoms (SS), Emotional Function (EF) and Social Function (SF). We studied with this instrument 20 patients with Irritable Bowel Syndrome (IBS), 15 with treated Coeliac Disease (Coeliac) and 17 with mildly active UC. Results were as follows:

<table>
<thead>
<tr>
<th></th>
<th>IBS</th>
<th>Coeliacs</th>
<th>UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS</td>
<td>1.1±0.1</td>
<td>3.5±0.4</td>
<td>5.4±0.8*</td>
</tr>
<tr>
<td>SS</td>
<td>1.8±0.2</td>
<td>7.1±0.5*</td>
<td>4.7±0.1*</td>
</tr>
<tr>
<td>EF</td>
<td>2.1±0.2</td>
<td>6.6±0.8*</td>
<td>4.7±0.3*</td>
</tr>
<tr>
<td>SF</td>
<td>0.0</td>
<td>0.5±0.2</td>
<td>3.1±0.9*</td>
</tr>
<tr>
<td>TOT:5.5±0.6</td>
<td>18.2±1.38</td>
<td>13.3±3.08</td>
<td>21.5±2.4*</td>
</tr>
</tbody>
</table>

(*) P<0.0001; (†) P<0.01 vs controls Student's unpaired t-test.

Conclusions: 1. QL is altered in patients with functional and organic intestinal disorders; 2. even treated, Coeliac Disease is associated with significant impairment of QL; 3. patients with active UC are greatly affected in all areas.

PSYCHOLOGICAL DISTRESS AND COPING CAPACITY IN FUNCTIONAL AND ORGANIC GASTROINTESTINAL DISEASE

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Patients with inflammatory bowel disease (IBD) probably have no excess psychopathology. Psychological aspects to irritable bowel syndrome (IBS) are more obvious. In other conditions, coping capacity modifies the relationship between multiple minor stressful events and illness, and may influence objective markers such as natural killer cell activity. It has not been previously assessed in IBD or IBS.

Reported life stress (Hassles scale), coping style (COPE scale), psychological distress and social functioning (General Health Questionnaire) were studied in 84 consecutive out-patients with at least 2 years' well-documented IBS, uncomplicated ulcerative colitis or Crohn's disease, compared to 25 controls (diabetic and cardiac clinics). Patients on psychotropic drugs were excluded. Nine patients (5 IBS, 4 IBD) declined to participate fully leaving 25 reasonably well-matched patients in each group.

Although psychological distress and social dysfunction were present in all 4 patient groups, the Hassles score (0-4.0) was uniformly lower than normal (1.47±0.39). Psychological distress was greater in IBS (mean score 33.5) than in other patients (22.7±0.05). A tendency of IBS patients to have lower coping scores was significant only for a greater need to turn to religion (8.6 v 5.9, p<0.05). The psychological parameters studied appeared independent of each other.

A number of different psychological questions are raised by the study and the style of coping with stress exist between patients with IBS and IBD, but are minor. The disability from chronic IBS, which appears at least as great as that from IBD, is not therefore adequately explained by psychological inadequacy.

DISCRIMINATION AGAINST PATIENTS WITH INFLAMMATORY BOWEL DISEASE BY INSURANCE COMPANIES

G A Moody & J F Mayberry, Leicester General Hospital.

The aim of this study was to investigate the attitudes of insurance companies to patients with IBD. A standard letter requesting information regarding the likelihood of loading on life assurance in connection with a mortgage was sent to 50 major insurance companies from a fictitious patient. A similar letter from a consultant gastroenterologist requested simple guidelines for patients with IBD. Either form was sent to 50 companies for a total of 250 types of insurance. A questionnaire investigating the experience of patients with IBD was also sent to 100 patients with IBD selected at random from an epidemiological data base of patients with Crohn's disease and ulcerative colitis (n=10). 39 insurance companies responded to the request for information from the "patient", response rate 79%. 24 were split between those who thought the patient would be accepted at normal rates (n=7) and those who expected the patient to have an increased premium (n=17). Only 27 companies replied to the letter from a consultant requesting general guidelines, response rate 54%. There were only 17 overlapping replies from the 2 letters. Of these 17 replies 5 companies (30%) informed the patient to expect increased premiums whilst advising a consultant they could expect normal rates. 6 companies (39%) told both the fictitious patient and consultant to expect normal rates. Either one or both of the remaining companies (35%) felt unable to advise without either completion of an application form and/or a consultation, or a particular patient in mind. 59% of patients responded to the questionnaire. Over half the patients who replied (54%) had applied for an insurance policy. More than a third of these had required a medical (36%) or a report from their GP (41%) before being accepted. 39% of patients had received a letter on their policies because of IBD including 2 patients who had been turned down altogether. In conclusion patients with IBD are clearly discriminated against by insurance companies. Life tables should be appropriately amended and patients made aware of such difficulties.
SECRETION OF IMMUNOLOGICALLY AND BIOLOGICALLY ACTIVE INTERLEUKIN-8 BY A GASTRIC EPITHELIAL CELL LINE.

SPLUSA E, FARMERY, P FECHL, LID LINDLEY*, IN PRIMROSE, JE CRAWFORD*
Academic Units of Surgery and Medicine, St James's University Hospital, Leeds, UK. & Sandus Research Institute, Vienna, Austria.

Helicobacter pylori infection increases the gastric production of interleukin-8 (IL-8), a neutrophil activator. We have demonstrated IL-8 mRNA expression in the gastric epithelial cell line ST42 using RT-PCR, and investigated the effects of cytokines and cycloheximide (CHX) on IL-8 secretion. The biological activity of cell culture supernatants on neutrophil adhesion molecule expression has been studied.

Constitutive expression of IL-8 mRNA was demonstrated but minimal secretion occurred over 24 hours in the absence of stimulation. TNFa, IL-1α and IL-1β (0.1–20 ng/ml) induced a dose-dependent increase in IL-8 production. Optimal 24 hour IL-8 secretion was in the presence of IL-8 secretion (3.0 ng/ml) and increased cytokine-induced secretion (IL-1β alone 7.3 ng/ml, IL-1β & CHX 10.7 ng/ml, n=5, P<0.02).

Supernatants of CHX-stimulated cells significantly increased expression of neutrophil CD11b as detected by labelling with fluorescent antibody and flow cytometry (median channel fluorescence 23.7) compared to medium controls (P<0.02). This increased expression was blocked by pre-incubating the supernatant with anti-IL-8 monoclonal antibody (15.8 with antibody, P<0.01).

These results demonstrate the secretion of IL-8 by a gastric epithelial cell line in a cytokine-dependent manner and that the IL-8 is biologically active in terms of neutrophil activation. Mucosal IL-8 production may be significant in the production of neutrophil-mediated tissue damage in H. pylori infection.

ROLE OF SUPEROXIDE IN RAT GASTRIC MUCOSAL INJURY PROVOKED BY NITRIC OXIDE DONORS.

D LAMARQUE and B J W HILLS, Department of Pharmacology, Wellcome Foundation Ltd, Beckenham, Kent, U.K.

Local intra-arterial infusion of high doses of nitrovasodilators such as nitroprusside or S-nitroso-N-acetyl-penicillamine (SNAP) that spontaneously liberate nitric oxide (NO) cause extensive gastric mucosal injury. The involvement of systemic hypotension and of the superoxide radical in such NO-induced mucosal damage has now been investigated in vivo using different forms of superoxide dismutase (SOD).

In pentobarbitone-anaesthetised rats, with 2ml 0.1M HCl instilled into the gastric lumen, local infusion of nitroprusside (10–40μg kg−1 min−1 for 15 min) through a cannula in the left gastric artery caused dose-dependent haemorrhagic injury, involving 42±4% of the total mucosal area (n=19) with the higher dose, when evaluated 20 min later. By contrast, intragastric instillation of nitroprusside (50mg kg−1) which caused a similar fall in systemic blood pressure as that observed with the higher intra-arterial infusion (5AS±5 and 61±4 mmHg respectively, n=6 each) failed to induce macroscopic mucosal damage, indicating that the injury was not a consequence of systemic hypotension. The mucosal damage by local nitroprusside was dose-dependently reduced by i.v. administration of a systematically acting conjugate of SOD-polypehylene glycol (500-2000 IU, kg−1), to 6±4% of the mucosal area (n=6, P<0.001). Likewise, local concurrent infusion of bovine SOD (25-250 IU, kg−1 min−1 for 15 min), but not denatured SOD, dose-dependently reduced the nitroprusside-induced damage to 6±4% of total mucosal area (n=5, P<0.001). Local SOD (250 IU, kg−1 min−1 ia.), also abolished mucosal injury induced by local SNAP (40μg kg−1 min−1 ia.; n=6; P<0.01). These were not non-specific protective actions since the mucosal injury induced by endothelin-1 (5pmol kg−1 min−1 local ia. for 15 min) was not significantly inhibited by these doses of SOD.

These findings with SOD indicate an involvement of superoxide in the injurious actions of exogenously generated NO. This may reflect a role of the peroxynitrite radical derived from a combination of NO and superoxide in such mucosal tissue injury, or to a synergistic cytotoxic interaction between these radicals, in the microvasculature.

p53 EXPRESSION IN GASTRIC CANCER AND ITS CORRELATION WITH SURVIVAL.

B V Joy paul, E L Newman, D Hopwood* and A Cuschieri, Departments of Surgery and Pathology*, Ninewells Hospital, Dundee. DD1 9SY.

Mutations in the tumour suppressor gene p53, are the most common genetic alterations encountered in human cancer. Unlike wild type p53 protein, the mutant p53 gene products have a prolonged half-life due to their altered conformation. As a result, abnormal p53 accumulates in malignant cells and can be demonstrated immunohistochemically in tissue sections.

In this investigation, we studied the expression of the p53 oncoprotein in 206 cases of gastric adenocarcinomas. A standard immunohistochemical technique employing the CM-1 anti-p53 polyclonal antibody was applied to the formalin-fixed and paraffin embedded material from these tumours. Overexpression of p53 was defined as positive nuclear staining.

Our findings indicate that 46% (94/206) of gastric carcinomas express high levels of p53. There was no significant correlation between p53 positivity and the following histopathological parameters: tumour grade, Ming type (expansile or infiltrative) and the Lauren type. Survival analysis revealed a significant association (p=0.0062) between p53 expression and the survival time. The 5-year survival of the patients with p53-positive tumours was 6 months compared to 15 months for those with p53-negative tumours (median survival time being 5.6 and 11 months respectively).

These data suggest that over expression of the p53 oncoprotein is an independent marker of shortened survival in gastric carcinogenesis.

SUCRALFATE REDUCES THE INCIDENCE OF GASTRIC CANCER IN RATS FOLLOWING TRUNCAL VAGOTOMY AND GASTROENTEROSTOMY.

M LANSDOWN, R DIMANT, P QUIRE, M DIXON, D JOHNSTON, University Department of Surgery, Leeds General Infirmary, Leeds.

The aim of this study was to determine whether a mucosal protectant (Sucralfate) would reduce the risk that carcinomas would develop after truncal vagotomy and gastroenterostomy. Rats underwent either gastrostomy or truncal vagotomy and gastroenterostomy. Half the rats in each group received Sucralfate incorporated into their diet. All rats received MNU (75 μg/ml) in their drinking water for 16 weeks.

<table>
<thead>
<tr>
<th>Operative procedure</th>
<th>Incidence of carcinoma %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrostomy</td>
<td>2/49</td>
</tr>
<tr>
<td>Gastrostomy +</td>
<td>0/61</td>
</tr>
<tr>
<td>Sucralfate</td>
<td>0/61</td>
</tr>
<tr>
<td>TV+GE</td>
<td>11/46</td>
</tr>
<tr>
<td>TV+GE + Sucralfate</td>
<td>1/39</td>
</tr>
</tbody>
</table>

* P=0.01 compared to TV+GE or Sucralfate or gastrostomy.

The incidence of carcinoma after TV+GE is significantly increased compared to gastrostomy, whereas the incidence of carcinoma after TV+GE+SUCralfate is not significantly increased. Sucralfate may act by protecting the mucosa from the carcinogenic effects of MNU and enterogastric reflux.
INTRODUCTION: Little is known of ODU in the H2-RA era. 

INCIDENCE AND DEMOGRAPHY: Between 1976-81, of 537 patients (pts) seen with GU alone (no associated lesions) 129 had ODU (24%). ODU pts (compared to those with small HRA) were significantly (p < 0.05) older and had a more aggressive illness (as shown by a higher incidence of bleeding, anorexia, weight loss and emergency admission). More GUel joined malignant and most were located in the body of the stomach.

IMMEDIATE OUTCOME AND HEALING: 3/129 died immediately (bled n=2, unrelated cause [UC] n=1). 14 had urgent surgery (bled n=12, suspected cancer n=2). 6/12 were treated medically mainly with cimetidine (CIM) 1 g daily. Healing occurred in 9/112 (87%) of whom 13 had refractory disease. Healing took time > 3 mo + needed CIM 2-3 g. 8 others died (UC). 3 had surgery for failed medical treatment, 2 defaulted and 2 with refractory GU were on treatment. Refractoriness was more common in pts with associated major medical illness (42% vs 12%, p < 0.01). 14/96 (15%) of pts died within 3 mo. (2 bleed, 3 post-op, 9 UC). and 31 died later (2 gastric cancer, 28 UC).

CONCLUSIONS: The long-term outcome of the OGU itself treated with HRA alone is good. However, the condition remains serious with frequent complications and a high mortality due to the patients' poor general health.

SERUM CA 72-4 LEVELS IN GASTRIC CANCER PATIENTS: COMPARISON WITH CA 19-9 AND CORRELATION WITH RECURRENCE

Departments of Surgery and Biochemical Medicine*, Ninewells Hospital and Medical School, Dundee, DD1 9BY

Serum levels of the tumour markers CA 72-4 and CA 19-9 were measured in patients with benign gastric diseases (n=52) and gastric adenocarcinomas (n=52). To ascertain the correlation between recurrence and the serum levels of the tumour markers, 30 cancer patients were then followed up for a median post-operative period of 38 months (range 10-105).

Our findings indicate that in the pre-operative serodiagnostic evaluation of gastric carcinomas, CA 72-4 had a similar sensitivity to CA 19-9 (42% versus 44%) and there was a definite diagnostic gain from the combined determinations (63%). However, CA 72-4 had a much better specificity (in cases of benign gastric disease 100% were negative compared with 72% for CA 19-9).

17 of 30 patients in the longitudinal cohort are without recurrence; 16 (94%) and 10 (59%) of these patients have normal levels of CA 72-4 and CA 19-9 respectively.

13 patients have developed recurrent disease. Sera from 9 of 13 (70%) showed a characteristic pattern of CA 72-4 levels rising from near-normal after surgery to diagnostic values, with an approximate lead-time before clinical diagnosis of recurrence of six months. Only three patients (23%) showed this pattern with CA 19-9.

The results indicate that CA 72-4 is a reliable tumour marker in gastric cancer and is superior to CA 19-9. In addition, serial sampling of CA 72-4 in post-surgical gastric cancer patients may be a useful means of identifying those with recurrent disease.

A COMPARISON OF THE STAGE OF GASTRIC CANCER DETECTED IN UNITS WITH DIFFERENT REFERRAL MECHANISMS

N Hayes, D Scott, S Raineys and SM Griffin. 
Departments of Surgery and Pathology, 1 Newcastle General Hospital and Cumberland Infirmary, Carlisle.

Over thirty months the staging of gastric cancers detected in two units within the same region was examined. Each had a similar clinical workload and identical surgical management policies. In Unit A (Newcastle General Hospital) an open access endoscopy referral system had been established, whereas in Unit B (Cumberland Infirmary) general practitioners had no direct access to the endoscopy service.

One hundred and eleven consecutive patients (Unit A, N=56; Unit B, N=55) with adenocarcinoma of the stomach underwent surgical evaluation either with intention to cure, or to palliate. Ninety-six resections were performed (A=51; B=45) and the remainder (A=5; B=10) had either palliation or evaluation only. Cases were staged according to the TNM system adopted by the UICC.

There were 40 cases of zeroa-negative (T1 or T2) cancer (A=26; B=14) and 71 more advanced cases (T3 or T4) (A=30; B=41). The lower stage of tumours in Unit A was significant (p<0.01, Fisher's exact test). Combining T with N status to provide overall staging in the 96 resections, the ratio of Stage III to IIIIV in Unit A was 25 to 26; Unit B 15 to 30 (p<0.05). Fifteen (13.5%) early gastric cancer (EGC) cases were detected (A=10, B=5), including 5 with positive lymph nodes (A=4, B=1), the difference between the units was not significant (p=0.091).

i) EGC is being detected in increasing proportions, and with a higher incidence of lymph node metastases than is usually reported.

ii) This study suggests that open access endoscopy may influence the stage of detection of gastric cancer.

NOVEL SURFACTANT-LIKE LIPID (SLL) OF HUMAN STOMACH, DUODENUM AND RECTUM

IT Anderson, FE Murray, C Gallacher, FE Ross, G Miles & D Hopwood

A surfactant-like lipid (SLL) was recently found in rat small bowel and similar material described in human stomach: the function and nature of this material remains ill-defined. The aim of this study was to determine if SLL is found in human small bowel, stomach and large bowel biopsies and to compare this lipid composition measured by TLC and GLC.

Biopsies from oesophagus (n=8), stomach (n=10), duodenum (n=9) and large bowel (n=6) were examined electron microscopically using validated methods which preserve lipid. SLL was found between intestinal epithelial cells but not oesophageal squamous epithelium and had a typical detergent pattern of surfactant phospholipid. Membranous lipid was also present between cells. SLL was observed in enterocyte endosomes, compatible with absorption of SLL, and in Golgi apparatus of colonic Goblet cells, suggesting synthesis may be occurring here.

Large bowel and duodenal biopsies contained less phospholipid, non-esterified fatty acids and cholesteryl ester than oesophageal biopsies (mol/mol mg weight±SEEM) but the proportion of phospholipid to neutral lipid was similar (Table).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cholesteryl ester</th>
<th>Triglyceride</th>
<th>Free Fatty Acid</th>
<th>Phospholipid Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oesophagus</td>
<td>1.22±0.13</td>
<td>0.50±0.07</td>
<td>1.86±0.32</td>
<td>3.39±0.38</td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.59±0.19</td>
<td>0.60±0.19</td>
<td>1.12±0.23</td>
<td>2.07±0.63</td>
</tr>
<tr>
<td>Rectum</td>
<td>0.39±0.05</td>
<td>0.34±0.08</td>
<td>1.54±0.18</td>
<td>1.45±0.14</td>
</tr>
</tbody>
</table>

*p<0.05

The phospholipid saturated an unsaturated fatty acid ratio was lower in rectum (0.61±0.02) than duodenum (1.51±0.33, p=0.025) or oesophagus (2.32±0.31 p<0.001), indicating different fatty acid composition in biopsies from these sites.

In summary we have demonstrated a novel SLL in stomach, small bowel and large bowel but not in oesophagus. Total lipid content of biopsies was inversely related to the presence of surfactant which may have an important role in mucosal defence in columnar epithelium.
THE MECHANISM OF ACID-INDUCED DUODENAL MUCOSAL DAMAGE A Garner, AC Hunter, HM Wilkes, TO Tantra, RD Bib, SA Burski, G Abu-Mileh. Dept of Pharmacology, Faculty of Medicine, UAB University of Alabama, and Dept of Physiology, Medical School, University of Newcastle upon Tyne, UK.

We compared mucosal barrier function after exposure of rat duodenum to 10 mM HCl in the form of HCl, a relatively lipopholic acid with a permanent anion, or HSO₄⁻, a divalent hydrophilic acid occupying twice the molecular volume (47 cf. 23 Å²). Intrinsic anion permeabilities were determined in purified brush border vesicles by measuring changes in scattered light intensity following a hypertonic solution pulse. The mean (SD) rate constant for SO₄²⁻/H₂SO₄ was 8.9 ± 0.2 x 10⁻⁵ sec⁻¹. Cr permeability (10⁻⁴-10⁻⁵ sec⁻¹) was not rate limiting for vesicular reswelling. Exposure of duodenal mucosa in vivo to 10 mM HCl for 10 min caused a reversible increase in juncional permeability as evidenced by a rise in luminal appearance of the nontransportable probe ¹⁴C-urea (MW 76). Blood-to-lumen urea flux increased 98 ± 5 % over control 10-20 min after acid exposure then declined to basal levels commensurate with restitution of epithelial barrier function. The early phase of HCl-induced superficial injury was characterised by mucosal uptake of acid leading to a reversible loss of tight juncional integrity as determined by ultrastructural examination. Significantly, no evidence of mucosal injury could be discerned by light microscopic examination of the duodenum after brief exposure to 10 mM HCl although longer exposure (30 min) or higher concentrations (25mM) induced eventually epithelial cell desquamation. In contrast, exposure to 10 mM H⁺ in the form of HSO₄⁻ for 10 min did not influence barrier function and there was no significant change in the blood to lumen flux of ¹⁴C-urea. Cellular acidification after HCl could occur by proton uptake as a Cr⁺ ion pair via apical membrane anion carrier proteins combined with non-ionic diffusion of molecular HCl through the lipid bilayer (10mM HCl, pKa ~ 6, contains ~1mM undissociated acid). The impermeability of SO₄²⁻ and the much lower lipophlicity of HSO₄⁻ would account for the relative lack of toxicity of this acid.

H. PYLORI SYNTHESIS OF FATTY ACIDS FROM GLUCOSE IN-VITRO S. IRVOS, HA Ahmed, MA Mendsall, PP Patel and TC Northfield. Department of Medicine, St.Georges Hospital Medical School, London, UK.

Introduction: Metabolism of glucose through the penose-phosphate pathway has recently been demonstrated in H. pylori. Apart from pentose production, this pathway also produces reduced co-enzyme II (NADPH), a fundamental requirement for fatty acid synthesis. However, the ability of H. pylori to synthesise fatty acids from glucose has not been demonstrated. We aimed to determine whether H. pylori can synthesise fatty acids and phospholipid from glucose in liquid culture.

Method: H. pylori was incubated in Brucella broth with 10% horse serum and antibiotic supplement (vancomycin, trimethoprim, ceftiofur and amphotericin) at 37°C under microaerophilic conditions. ¹⁴C-glucose was added to cultures and incubated for 48hrs. H. pylori were separated from the culture medium by centrifugation and washing at 24 and 48hrs. Incorporation of radio labelled glucose was determined by scintillation counting of the separated H. pylori. Folch extraction was used to isolate H. pylori lipid, which was then separated on thin layer chromatography. Fatty acid and phospholipid bands on chromatograms were visualised by iodine staining and quantified by scintillation counting.

Results: Table shows the incorporation of glucose into H. pylori and its use in the biosynthesis of fatty acids and phospholipids at 24 and 48hrs. All units are in umol of glucose/mg of H. pylori protein.

<table>
<thead>
<tr>
<th>Incubation period</th>
<th>Incorporation of glucose into H. pylori:</th>
<th>cell mass</th>
<th>fatty acids</th>
<th>phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>24hr</td>
<td>3.6±0.3</td>
<td>0.13</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>48hr</td>
<td>3.3±0.3</td>
<td>0.07</td>
<td>0.14</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: ¹⁴C-glucose is incorporated into H. pylori and can be utilised for the biosynthesis of fatty acids and phospholipids. Further characterization of glucose metabolism may be important in the development of therapeutic agents against H. pylori.