THE MECHANISM OF ACID-INDUCED DUODENAL MUCOSAL DAMAGE A Garner, *AC Hunter, JM Wilkes, MO Tanila, R Dib, SA Barztki, G Abu-Hillah Dept of Pharmacology, Faculty of Medicine, UAE University, Al Ain, UAE *Dept of Physiology, Medical School, University of Newcastle upon Tyne, U.K.

We compared mucosal barrier function after exposure of rat duodenum to 10 mM HCl in the form of HCl, a relatively lipophilic acid with a permanent anion, or HSO₄⁻, a transient hydrophilic acid occupying twice the molecular volume (47 cf. 23 Å³). Intrinsic anion permeabilities were determined in purified brush border vesicles by measuring changes in scattered light intensity following a hypertonic solute pulse. The mean (SD) rate constant for SO₄²⁻/HSO₄⁻ was 8.2 ± 0.2 × 10⁻³ sec⁻¹. CT permeability (>10⁻¹ sec⁻¹) was not rate-limiting for vesicular resealing. Exposure of duodenal mucosa in vivo to 10 mM HCl for 10 min caused a reversible increase in junctional permeability as evidenced by a rise in luminal appearance of the non-transportable probe ¹⁴C-urea (MW 76). Blood-to-lumen ura 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 junctional 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 cellular deaggregation. 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 CT ion pair via apical membrane carrier proteins combined with non-ionic diffusion of molecular HCl through the lipid bilayer. H⁺ permeability of 10⁻⁴ M HCl, pKa -6, contains 1×1M undissociated acid). The impermeability of SO₄²⁻ and the much lower lipophilicity of HSO₄⁻ would account for the relative lack of toxicity of this acid.

H. PYLORI: SYNTHESIS OF FATTY ACIDS FROM GLUCOSE IN-VITRO. S. Ahmed, HA Ahmed, MA Mendall, P Patel and TC Northfield. Department of Medicine, St. George's Hospital Medical School, London, UK.

Introduction: Metabolism of glucose through the penrose-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 phospholipids from glucose in liquid culture. As a control, we used a fatty acid deficient medium.

Method: H. pylori was incubated in Brucella broth with 10% horse serum and antibiotic supplement (vancomycin, trimethoprim, cefotulin 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. Polychrome extraction was used to isolate H. pylori lipids, 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 nmol of glucose/mg of H. pylori protein.

Incubation period: Incorporation of glucose into H. pylori:

<table>
<thead>
<tr>
<th>Period (hrs)</th>
<th>24hr</th>
<th>48hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell mass</td>
<td>3.6±0.3</td>
<td>3.3±0.3</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>0.19</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Conclusions: ¹⁴C-glucose is incorporated into H. pylori and can be utilized 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.
IDENTIFICATION AND ENDOSCOPIC SCREENING OF FIRST DEGREE RELATIVES (F.D.R.) OF COLORECTAL CANCER (C.R.C.) PATIENTS. 

J.M. Hunt, P.S. Rooney, K.A. Gifford, R.J. Mascari, J.D. Harpcastle and N.C. Armitage Department of Surgery, University Hospital, Nottingham. NG7 2UH

Endoscopic screening is recommended for F.D.R. of C.R.C. patients. To estimate mortality benefit of screening we must (1) identify those at risk, (2) know compliance and (3) neoplastic yield of screening. Identifying those at risk through the index patient has disadvantages. In a single general practice we identified F.D.R. of C.R.C. patients by questionnaire. We assessed compliance, workload, feasibility and yield of endoscopic screening.

Results: 1361 (all 40-75 years on general practice list) received questionnaire. 1118 (82%) replied. 102 declared a F.D.R. had C.R.C. and were invited to interview. 23 (23%) did not attend (D.N.A.). At interview 58 had F.D.R. with C.R.C. In 21 (20%) cases the F.D.R. with C.R.C. was refuted or unsure. Estimated total identified with F.D.R. with C.R.C. (including 80% of those who D.N.A. was 76 (78%). This is the same as index based studies.

Fifty three individuals were recommended endoscopic screening (45 at intermediate risk recommended 60 cm flexible sigmoidoscopy and 8 at high risk recommended colonoscopy). 34 accepted. Overall compliance was 47% Adenomata were detected in 4 (14%) of those screened. Endoscopic costs were low.

Conclusions: A questionnaire effectively identifies those with F.D.R. with C.R.C. Screening is feasible and relatively cheap.

IS SCREENING FOR BARRETT’S CANCER WORTH IT? 

TA Wright**, MR Gray**, AL Morris†, JT Gilmore†, JL Smart*, A Ellis*, AN Kingsworth**, Departments of Surgery** and Gastroenterology, Broadgreen† and Royal Liverpool Hospitals, Liverpool.

Screening for Barrett’s cancer is controversial due to a large variation in its reported incidence and lack of evidence that screening improves its prognosis.

We have reviewed the data from our surveillance program between 1983 and 1992 to assess the incidence of malignant change, its stage and its cost. Of 348 patients with Barrett’s oesophagus 47 presented with Barrett’s cancer (91% male). 166 patients (65% male, mean age 59.8; 35% female, mean age 72.4) had annual endoscopy and biopsy for a mean period of 2.8 years. Six patients (5 men) developed cancer - an incidence of 1 cancer per 59 male and 167 female patient-years of follow up. All had oesophageal resections. The stages were compared with 25 consecutively resected age/sex matched symptomatic patients with Barrett’s cancer.

RESULTS

<table>
<thead>
<tr>
<th>Stage</th>
<th>Unscreened</th>
<th>Screened</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>IIa</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>IIb</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

TOTAL 25 6

The stage in the screened group was significantly better (p<0.05). The cost of detecting one cancer was £11 016 for men and £27 900 for women.

CONCLUSIONS

1) Men with Barrett’s oesophagus were significantly younger (p<0.001) and at higher risk than women.

2) Screening for Barrett’s cancer is justified due to the high incidence and earlier stage of tumours detected.

3) The cost of detecting early Barrett’s cancer compares well with the high cost of palliative therapy for more advanced disease.

LENGTH OF BARRETT’S SEGMENT MAY BE DEPENDENT ON THE EXTENT OF ORAL TRANSPORT OF THE REFLUXATE

P. Singh, R.H. Taylor, D.G. Collin-Jones

Royal Naval Hospital, Haslar, Gosport, UK and Queen Alexandra Hospital, Portsmouth, UK

Twenty four hour oesophageal pH monitoring was performed in 11 patients with Barrett’s oesophagus. None of them had simultaneous two-level pH monitoring with the pH probes at 5 (D) and 10 cm (P) above the lower oesophageal sphincter (LOS). In one patient only distal pH monitoring was possible. In another, only the proximal pHmetry data were available because of electrical failure of the distal probe.

The median age of the patients was 54 years (range 20-72 years). There were 9 men. The median length of the Barrett’s segment was 6.0 cm (range 3-14 cm). There was significantly greater acid exposure at the distal level (D) than at the proximal level (P) with the median % of total, supine and upright times pH <4 being 48.2, 61.0, and 49.0 at D and 23.2, 23.2, and 21.9 for P (p<0.001). Linear regression analysis was done to test association between the length of Barrett’s segment and the degree of oesophageal acid exposure. At the distal level, only the % of supine time pH <4 had a weakly significant positive association (r=0.635; p=0.048). There was no significant association with the % of total or upright time pH <4 (r=0.63; 0.46 and p=0.08; 0.175 respectively). On the other hand, all measures of acid reflux at the proximal level showed a strong correlation with the length of Barrett’s segment (r=0.846; 0.81; 0.725; and p<0.001; 0.005; 0.018 for the % of times pH <4 for total, supine and upright periods respectively).

This new observation suggests that the length of the Barrett’s segment may be dependent on the extent of oral transport of the refluxate.

ALTERED OESOPHAGEAL SENSORY THRESHOLDS IN PATIENTS WITH SYMPTOMATIC BUT NOT EXCESS GASTRO-OESOPHAGEAL REFLUX - A DEMONSTRATION OF THE "IRRITABLE OESOPHAGUS".

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Department of Medicine, Royal Infirmary, Edinburgh EH3 9YW.

Ambulatory oesophageal pH monitoring in patients with symptoms suggestive of gastro-oesophageal reflux disease (GORD) identifies those with excessive oesophageal acid exposure, and allows correlation of symptoms with reflux events. A sizeable minority of patients studied however (12% in our service) demonstrates close correlation of symptoms to episodes of reflux but has a normal degree of oesophageal acid exposure (<6% of 24-hour periods). It was hypothesised that such patients might have a low threshold for perception of normal reflux events, akin to abnormalities of visceral sensation found in some patients with functional GI disorders. To test this we have measured the sensory threshold for oesophageal balloon distension in 12 patients with symptoms of GORD, without oesophagitis, in whom 24-hour pH monitoring had revealed normal acid exposure times, but with a >50% correlation of symptoms with reflux events (ie symptom index of 50% or more), and compared these with 13 healthy volunteer controls. The patient group demonstrated significantly lower thresholds both for initial perception of oesophageal distension, and for pain, compared to controls. 7.8±4.4 mmHg vs. 19.9±6.3 mmHg (p<0.01), and 10.5±4.1 vs. 16.3±6.0 (p=0.003) respectively. In addition, significantly smaller increments in balloon volume were required in the patient group to alter the sensation from a mild perception of distension to that of discomfort: 2.7±1.7 vs. 4.9±2.9 (p=0.016). Our study suggests that symptoms in these patients may result from a heightened perception of normal reflux events, and supports the concept of an “irritable oesophagus” in some patients with reflux symptoms. Rational therapy for such patients may involve pharmacological manipulation of visceral sensation, rather than that of acid secretion or motility.
SENSITISATION AND PHOTODYNAMIC THERAPY FOR OESOPHAGEAL, DUODENAL AND RECTAL TUMOURS WITH THE NEW PHOTSENSITISER - 5 AMINOLEVULINIC ACID.


Photodynamic therapy (PDT) is a non thermal technique for producing localised tissue necrosis with light after systemic administration of a photosensitising agent. In the gastrointestinal tract, it has the major advantage that full thickness effects heal well and do not lead to perforation. 5 aminolevulinic acid (ALA) induces endogenous production of the photosensitiser - protoporphyrin IX (PPIX) predominantly in normal and normal mucosa. It is eliminated from the body in 24-48 hours in contrast to haemoptoporphyrin derivative, which leaves patients sensitive to sunlight for weeks. Fourteen patients (10 males, 4 females) with a median age of 76 years (range; 38-89) had oesophageal (4 patients) or colorectal tumours (7 patients). There were 11 carcinomas and 4 with adenomas - one patients had familial polyposis coli with both duodenal and rectal adenomas. Patients were given 30-60 mg/kg ALA orally and biopsies of tumour and normal mucosa taken 6 hours later. They stayed in subdued lighting for 24 hours after ALA. The specimens were examined by fluorescence microscopy, with digital quantification to give tissue levels of photosensitiser. Three patients were given a second dose of ALA a few weeks later and their tumours were treated with red light (625 nm) from a gold vapour laser (50-100J). After 30mg/kg ALA the highest fluorescence levels were detected in the duodenum (tumour and normal mucosa median values in counts per pixel: 128 and 106). Slightly lower levels were observed in the oesophagus (104 and 88) but the lowest were in the large bowel (11 and 8). Doubling the dose of ALA in patients with colorectal tumours gave results similar to those in patients with upper gastrointestinal tumours and improved the tumour: normal mucosa ratio to 4:1 (116:34). The treated patients (oesophagus - 2, rectum -1) showed superficial necrosis in the areas (normal and tumour) exposed to laser light. 6 patients had transient rises in serum aspartate aminotransferases, 2 mild skin photosensitivity on the second day and 2 mild transient nausea and headache. We conclude that after oral ALA, photosensitiser levels are higher in duodenum and oesophagus than in the large bowel and that application of up to 100J of red light results in superficial mucosal necrosis. This has considerable potential for treating small tumours and areas of dysplasia as in Barretts oesophagus.

COST AND EFFICACY OF THROUGH 'SCOPE BALLOON DILATION OF OESOPHAGEAL STRICURES - AN AUDIT OF FIVE YEARS EXPERIENCE IN A DISTRICT GENERAL HOSPITAL. P.D. Mullans, G. K. Youngs, Department of Gastroenterology, Countess of Chester Hospital, Chester CH2 1 BQ.

The cost and efficacy of guide wire balloon dilation of oesophageal strictures has been questioned but since the introduction of through 'scope balloons in 1986 they have been the instruments of choice in our unit. We have audited mortality, morbidity, efficacy and cost of the procedure over a five year period in 59 patients: peptic stricture (n=41), malignant stricture (n=15) and post radiation stricture (n=3). We used Huibbs (12,16,18, and 20 mm diameter) and Rigiflex (25 mm diameter) balloons, each 8 cms long.

One patient with malignant stricture perforated and died following balloon dilation. Two subjects (one peptic, one malignant) recovered after conservative treatment. The peptic stricture subjects attended for dilatation: once (n=25), twice (n=8), thrice (n=6), and four times (n=2). Follow up (4 to 54 months) shows the majority have minor or no dysphagia providing they continue longterm omeprazole. Only five of the subjects with benign strictures needed additional bougieing. Seventeen balloons have been required of which five are still in use. Overall each balloon has survived a median of 12 patient-treatments although this improved to a median of 21 in the latter part of the study period. On average 5.5 balloons (of different sizes) were used per patient visit. The cost of balloons per visit over the five years is £32. Through 'scope balloon dilatation of oesophageal strictures is acceptable safe, simple to perform, and cost effective.

OESOPHAGEAL METALLIC STENTS: A PRELIMINARY REPORT ON 64 CASES.

Romagnese Diego, M.D., Di Toma Francesco, M.D. (*) (introduced by M.O. Farthing)

3rd Dept. of Surgery and (*) 3rd Dept. of Radiology - Regional General Hospital of Treviso - 31100 Treviso, Italy.

We report about the implantation a metallic stent in 64 patients with oesophageal carcinoma (34 cases) or oesophageal compression by a thoracic mass (21 cases). In 44 cases the stricture was in the middle or the lower oesophagus, while in 20 cases the stricture was located in the cardio region (gastric adenocarcinomas). We used two types of device: metallic selfexpandable stents (Wallstent®) in 34 cases, and metallic tubular stents inflatable by a balloon (Microstent®). We used the most part of the autoexpandable stents in the oesophageal compressions. We prefer use the dilatate stents in the long carcinomas. All these attempts were successful, and patients could eat a soft diet the day after the procedure. We observed 4 complications (6.5%): 2 stent dislocation (the day after the positioning procedure), 1 severe bleeding (a week after the insertion of a dilatable stent) and 1 tracheo-oesophageal fistula a month after the insertion of an autoexpandable stent. This last patient died for the perforation (overall mortality of 1.5%). In the follow-up period (a range from two to 11 months) we observed only two stent occlusions (3.1% on 64 cases) by carcinoma overgrowth through the metallic mesh. In these cases a laser treatment was completely successful, and the patients were able to eat a soft diet. In conclusion the metallic stents appear to be a good palliative treatment for the oesophageal malignant strictures, with a low complication rate. The neoplastic growth inside the metallic mesh does not appear a real problem.


Omeprazole 20mg once daily is effective in the treatment of gastro-oesophageal reflux disease (GORD): this study was designed to assess whether omeprazole treatment reduces the recurrence of oesophageal strictures complicating GORD. We randomised 366 patients with oesophageal stricture to either omeprazole 20mg o.m. or ramitidine 150mg b.d. for 1 year following dilatation to 12-15mm (36-34G). Subsequent endoscopy and dilatations were performed whenever clinically indicated and endoscopy on completion of treatment. Symptoms were assessed at clinic visits every 3 months.

Fewer patients on omeprazole required re-dilatation, compared to those on ramitidine (49/163 (34%) vs 78/143 (55%), p<0.001, the time to re-dilatation was longer (life-table analysis, p<0.001; see Figure), and patients in the omeprazole group required fewer re-dilatations (mean 0.04 per month vs 0.09 per month, p<0.01). On completion, symptom relief favoured omeprazole with ramitidine with 91/139 (65%) vs 52/122 (43%) asymptomatic (p<0.001).

This study shows that omeprazole is more effective than ramitidine in providing symptom relief and reducing the need for repeat dilatations in oesophageal stricture.
NITRIC OXIDE AND THE HAEMODYNAMIC CHANGES IN LIVER FAILURE.


Liver failure is characterised by arterial vasodilatation and reduced sensitivity to vasoconstrictors. Nitric oxide (NO), synthesised by nitric oxide synthase, has been implicated in pathological vasodilatation. Hepatic arterial rings were obtained from donors and recipients at liver transplantation. Dose-response curves to phenylephrine (PHE, 10⁻⁶ – 3x10⁻⁴M) were obtained and repeated in the presence or absence of L-NMMA (10⁻⁵M), an inhibitor of NO synthase. Nineteen rings were obtained from 7 donors (ring diameter 4.1 ± 0.4 mm (mean ± SEM), patient age 44.6 ± 4.5 years) and 25 rings from 9 recipients (diameter 2.7 ± 0.3 mm, age 38.9 ± 5.5 years). Recipients were vasodilated, systemic vascular resistance index 828.9 ± 180.5 dyn.sec/cm².m³. All rings contracted to PHE but the recipient response was shifted to the right of the donor response and achieved a lower peak developed tension. At 3x10⁻⁴M PHE the donor artery increased in tension by 5.8 ± 0.9g and the recipient artery by 2.3 ± 0.8g, L-NMMA had no effect on the PHE response in donor artery but increased the response of recipient artery by 131 ± 39% at 3x10⁻⁴M PHE. Rings from both groups did not relax to acetylcholine after contraction with PHE but relaxed to glyceryl trinitrate.

In conclusion, hepatic artery from patients with liver failure is resistant to vasoconstriction probably due to NO synthesis in its smooth muscle layer. This may contribute to the haemodynamic changes in liver failure.

MEASUREMENT OF OESOPHAGEAL VARICEAL PRESSURE USING A NOVEL ENDOSCOPIC PROBE D WILLIAMS-B ARNOLD, P ROBINSON, DC GLEESON, AG JOHNSON. Dept of Medicine and Surgery, Royal Hallamshire Hospital, Glossop Rd, Sheffield S10 2JF.

Variceal pressure has been measured noninvasively by the attachment of a pressure-sensitive fine gauge endoscope to the biochip channel of an endoscope to measure intravariceal pressure noninvasively by the technique of tonometry. In varices using a convex model varix confirmed intravariceal pressures of 10-50mmHg could be measured with good unthickness with a ± 5% error due to the force and angle of application. In vivo, the tonometer was validated against our standard sclerotherapy/noodle manometer to assess variceal pressure in 16 patients undergoing sclerotherapy. The tonometer was applied under direct vision 6 times to each varix and to the nonvarical wall. The operator decided pressure if pressures were above 30mmHg (the lowest range of nonvarical wall pressures). Then a sclerotherapy needle attached to a perfused manometry system was inserted into each varix. The needle was positioned to record the lowest pressure fluctuating with respiration denoting pressure if below 50mmHg. The tonometer was only able to measure 21 of 32 (65.6%) of varices to be identified due to difficulty with correct application. The tonometer applied to the oesophageal wall measured pressures of 60-90-100mmHg (median range). Patent varices were correctly identified in 18/20 (90.0-50.0mmHg) and thrombosis in 1/1 (50-0-50.0mmHg).

In conclusion a simple method to determine variceal pressure has been designed. The pressure recordings are steady and may allow for more detailed pressure measurements in the future.
T81

SOMATOSTATIN FOR ACUTE BLEEDING FROM PORTAL HYPERTENSIVE GASTROPATHY AND COLAPATHY.
E. Kouroumalis, I. Koutoubakis, O. N. Manousos
Department of Gastroenterology, University Hospital Heraklion, Crete, Greece

Acute bleeding from portal hypertensive gastropathy (PHG) and colapathy has recently been described as a common cause of bleeding in cirrhotic patients. Somatostatin has been reported in a few studies to be an effective treatment for this non-variceal bleeding. Use of Somatostatin in this condition has not been reported so far.

Cirrhotics with PHG have increased gastric blood flow and gastric mucosal perfusion. Somatostatin reduces both, splanchic blood flow and gastric blood flow.

In conclusion, Somatostatin is a safe and effective treatment of acute bleeding from either PHG or portal colapathy and reverses the endoscopic appearance of these conditions.

T82

TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC STENT SHUNT. RESULTS OF 2 YEARS OF FOLLOW-UP.
K J Simpson, N Chalmers, D N Redhead, NDC Finlayson, and FC Hates
DEPT OF MEDICINE AND RADIOLOGY, ROYAL INFIRMARY, EDINBURGH

Transjugular intrahepatic portosystemic shunt (TIPS) is a new therapeutic option for the prevention and treatment of varical haemorrhage, bleeding portal hypertensive gastropathy and resistant ascites. However, data regarding the long term efficacy of TIPS is lacking.

TIPS was attempted in 41 patients (23 male, 18 female) to control or prevent bleeding from oesophageal varices (20 patients, 4 performed as emergency TIPS), gastric varices (8 patients, 4 emergency TIPS), rectal varices (1) and portal hypertensive gastropathy (6); resistant ascites (3), hypersplenism (2), to facilitate embolisation of a large spontaneous portosystemic shunt producing encephalopathy (1). TIPS was performed successfully in 34 patients (83%) with one procedure related death. During 16 “patient years” of follow-up (longest 2 years), 9 patients have died; varical haemorrhage (2), liver failure (4), chest infections (2), pulmonary embolus (1). 5 patients have been transplanted, 1 patient is awaiting transplantation. Nonfatal upper GI haemorrhage occurred in 9 patients, 7 of whom had inadequate shunts (thrombosed 2 cases, intimal hyperplasia 2, hepatic vein stenosis 1, dislodged shunt 1, inadequate shunt 1). One patient bled from a gastric ulcer and another from an oesophageal varix. Clinical encephalopathy occurred in 3 patients. Ascites improved in all patients, but reaccumulation in 2 patients was complicated by recurrent haemorrhage. However, continued surveillance is necessary to detect shunt dysfunction which is commonly complicated by recurrent bleeding or reaccumulated ascites.

T83

LIVER TRANSPLANTATION (LT) IN PATIENTS OVER 60: RATIONAL USE OF A SCARCE RESOURCE?
Liver and Hepatobiliary Unit, Queen Elizabeth Hospital, BIRMINGHAM.

With the increasing success of LT, indications have widened and the upper age limit extended. With the increasing scarcity of donors we have examined the outcome of patients grafted over the age of 60 years. Between 1982 and 1993, 1,537 patients were referred for LT, 209 (13%) were 60 years or more. 495 (35%) aged <60 years and 64 (31%) aged >60 years were grafted. The two groups were dissimilar with respect to indications and selection criteria. For example, 25% of the younger referred patients with fulminant hepatic failure (FHF) were grafted compared with 14% of the older group; comparable figures for PBC were 66% and 51%. The main indications for transplantation were FHF in 18% and 5% and PBC in 35% and 65% of the younger and older patients respectively. The 1 year actuarial survival for all patients was 71% for the <60 and 82% for the >60 groups. Comparable figures for those transplanted since 1990 were 80% and 85%.

There were no significant differences in the length of hospital stay or incidence of complications. Most of the older patients enjoy a good quality of life with return to their normal activities. Thus, LT in the management of older patients can have similar or better results than in the younger population.

The improved results observed in the older age group suggest that stricter selection criteria with special emphasis on pre-operative cardiorespiratory reserve result in optimal use of a scarce resource.

T84

ORTHOLOCOPIC LIVER TRANSPLANTATION (OLT) FOR HEPATOCELLULAR CARCINOMA (HCC): RADIOLOGY vs PATHOLOGY AS PROGNOSTIC INDICATORS.
PM Rizzi, P Kana*, SD Ryder, JK Ramage, JR McPeake, J Karan* B Portmann, JC Tan and Roger Williams, Institute of Liver Studies and Dept. of Diagnostic Radiology, King’s College Hospital, London.

Recurrence of HCC after OLT has been shown to depend on tumour size and number. There is little work on the accuracy of radiology in predicting the real tumour bulk in the explanted liver or recurrence after transplantation. We compared tumour size detected by radiology and pathology in 44 patients with HCC (35 male, 9 female, median age 51 years) transplanted from January 87 to May 93 (median follow-up 18 months). Pre-transplant assessment included ultrasound (44 patients), CT scan (20 patients) and angiography (19 patients).

Results. Radiology failed to detect tumour in 11 patients but demonstrated 8 with small solitary tumours (<4 cm), and 27 with large (>4 cm) or multicentric lesions. Pathological assessment showed a significantly higher number of patients who had large or multicentric tumours (38 vs 27; p<0.02). Indeed most of the incidental and radiologically small tumours were demonstrated to be multicentric. Tumour bulk was calculated from diameter of all lesions; mean tumour bulk (MTB) was 55.72 cm³ in patients with incidental HCC, 63.7 cm³ in patients with radiologically small lesions, and 646.9 cm³ in patients with large or multicentric HCC. Patients with incidental or small MTB; no recurrence was seen in these categories. Tumour recurred in 8/44 patients, all with large or multicentric lesions on radiology and large tumour bulk pathologically, with 7 deaths.

Conclusions. Despite missing some small tumours and most satellite lesions, radiology otherwise categorized correctly patients with small or large tumour bulk. Recurrence was not seen in patients with incidental or radiologically small lesions, even though they were pathologically multicentric. Therefore we suggest that radiology can be used to predict prognosis in terms of risk of recurrence.
INOCYANINE GREEN CLEARANCE: AN EARLY PREDICTOR OF GRAFT FUNCTION

R. Jalan, J. Plevris, N. D. Finlayson, P. C. Hayes
Scottish Liver Transplant Unit & Liver Research Laboratories, Department of Medicine, Royal Infirmary, Edinburgh EH1 9YW

Primary graft dysfunction occurs in up to 10% of liver transplant recipients and is a major reason for early mortality and retransplantation. The conventionally used markers of early graft function i.e., correction of acidosis, glucose requirement, consumption of potassium, serum alanine transaminase (ALT), prothrombin time (PT), bile flow, resolution of encephalopathy and haemodynamic instability can be very misleading as they are dependent on numerous other factors. The aim of this study was to assess the use of indocyanine green clearance (ICG) as a measure of graft function.

Methods: Peripheral ICG clearance was measured between eighteen and twenty-four hours after liver transplantation in sixteen consecutive patients. Doppler ultrasound confirmed normal hepatic arterial blood flow. ICG clearance was correlated with the ALT, prothrombin time, bile flow, time to correction of acidosis, the time to normalization of prothrombin time and outcome.

Results: The median ICG clearance was 348 ml/min, but the range was wide (151-607 ml/min). Significant correlations were found between ICG and times to normalization of PT (p = 0.001) and to the correction of acidosis (p < 0.01). No correlation was found with ALT, PT, bile flow, glucose requirement or consumption of potassium. A value of less than 200 was associated with poor outcome.

Conclusion: ICG clearance measured on the day after liver transplantation accurately reflects graft function and may be used to predict graft survival and final outcome.

INAPPROPRIATE EXPRESSION OF BLOOD GROUP ANTIGENS IN HEPATIC ALLOGRAFTS. S. Bloom, K. Fleming, B. Chapman, J. Neuberger and S. Hubshers Department of Gastroenterology and Pathology, John Radcliffe Hospital, *Liver Unit, Queen Elizabeth Hospital, Birmingham and **Department of Pathology, University of Birmingham.

We have examined the expression of blood group antigens of the ABO, Lewis, and Kell systems using monoclonal antibodies and immunohistochemistry on 42 liver allograft specimens from 33 patients undergoing liver transplant from 1986 to 1991, in order to examine whether altered blood group antigen expression might be a bearing on the immunopathogenesis of transplant rejection. Specimens were obtained at intervals of 0 days to three years post transplant and had the following histological diagnoses: time zero (n=4), acute rejection (n=4), chronic rejection (n=4) end-stage chronic rejection (n=15) and miscellaneous late post transplant biopsies (n=7). An aberrant expression of blood group antigens was observed in 5 out of 15 cases with chronic rejection. Two transplants into the same group O patient showed aberrant expression of AB antigens on hepatocytes, with a canular pattern, in group O transplanted livers. In all cases where a group O liver was transplanted into a group A recipient and where there were histological signs of chronic rejection, antibody staining showed acquisition of recipient blood phenotype by the donor bile ducts and/or endothelium. Aberrant expression of ABO antigens was only seen in chronic rejection.

In seven cases there was canular leucocytic staining of portal hepatocytes with the anti-A and anti-B antibodies, normally confined to ducts and ductule.

These changes in carbohydrate cell surface phenotype may play a role in regulating hepatic allograft susceptibility to immune mediated damage.

DOES IVC BYPASS DURING ORTHOTOPIC LIVER TRANSPLANTATION (OLT) HAVE A BENEFICIAL EFFECT ON RENAL FUNCTION? J. D. Harrison, A. D. Mayer, J. C. Buchalter and P. Mc Master
Liver & Hepatobiliary Unit, Queen Elizabeth Hospital, Birmingham

It has been suggested that the addition of inferior vena caval (IVC) bypass to portal-arterial veno-venous bypass improves in perfusion pressure in the kidney during the IVC clamping phase of OLT, limiting renal injury. To evaluate this hypothesis we have examined the effect of IVC bypass on creatinine in 240 patients undergoing OLT. 91 patients had portal to arterial (PA) bypass only, while 149 patients had IVC bypass in addition to PA bypass (PA+C). Creatinine levels pre- and 2 months post op. were compared in the two groups. Cyclosporin (CyA) levels were recorded in each group. In the PA group the initial creatinine was 123 (54-451) mmol/l and 110 (50-933) mmol/l in the PA+C group (NS). Two months post op. there were no significant differences between the two groups for post-operative creatinine or change in creatinine (-21[-1201-1142] mmol/l for [3447-840] mmol/l, NS). The duration on bypass was a mean of 84.8 ± 44.1 mins in the PA group compared to 105.8 ± 39.2 mins in the PA+C group (p=0.14, p=0.0004). Median blood transfusion was greater in the PA group (8 (1-42) v 6(0-25) units in PA+C, (2=10.5, p=0.001). Colloid transfusion was greater in the PA group than the PA+C group, [30-8] v [18.0-6.5] litres (2=11.2, p=0.0008). There were no differences for usage of FFP, cryoprecipitate or platelets. A multiple regression analysis with creatinine change as the dependent variable showed no independent numerical relationship between renal function, bypass type and CyA level.

We conclude that there is no benefit gained in terms of renal function for the use of IVC bypass in addition to portal to arterial bypass during OLT; but there may be an advantage in reducing blood and colloid transfusion requirements.

CHANGES IN HEPATIC EXPRESSION OF HEPATITIS B VIRUS (HBV) AND HEPATITIS DELTA VIRUS (HDV) GENOMES FOLLOWING LIVER TRANSPLANTATION (LT) AND RELATION TO HEPATOCELLULAR DAMAGE. NV. Naoumov, KA. Antonov, PYN. Wong, D. Doherty, A. Rayner, B. Formann and Roger Williams. Institute of Liver Studies, King’s College School of Medicine & Dentistry, London.

The interaction of HBV and HDV following LT in patients with cirrhosis or HCC was studied. These two viruses are of major importance in determining variable clinical course and their relative role in liver graft damage. For this purpose we studied HBV and HDV replication, viral antigen expression and the clinical outcome in 16 LT recipients for HBsAg, antidieta positive cirrhosis - 7 without HBV reinfection 8 to 48 months after LT (Group 1) and 9 with HBV reinfection, followed 4 to 59 months (Group 2). Hepatic expression of HDV and hepatitis B virus-Delta-RNA (with corresponding riboprobes) and HBV genome (with HB-DNA probe) was detected using in situ hybridisation in 16 native livers and in 51 serial liver graft biopsies. Results at sequential time points were: Native liver: HBV replication did not differ between Group A and Group B, while HBsDNA and/or large areas of HBsAg (+) hepatocytes were detected in 8/9 patients in Group B. Day 7 after LT: Genomic (genD), antigenomic HDV-RNA and HD-Ag were expressed in 8/9 biopsies (Group A, Group B-5), while HBsAg, HBcAg and HBV-DNA (by in situ hybridisation) were negative. HBsDNA was found by PCR in 4/5 liver specimens from both groups. One patient who was HBsDNA-negative by PCR had strong expression of genomic and antigenomic HDV-RNA and HD-Ag. Months 2-4 after LT: Active HBV replication in Group B, revealed by HBV-DNA (in situ hybridisation) and/or HBsAg and HBcAg in 5/9 patients. HDV replication was markedly reduced in 7/9 patients and liver histology showed lobular hepatitis (n = 7) and fulminating hepatic failure (n = 2). Years 2-5 after LT: In Group B HBV+HDV expression was observed in 4 patients and HDV+HBV in 1, all of them with CAH.

Conclusions: i) HDV does not prevent HBV reinfection in the liver graft; ii) HDV replicates in the new liver early after LT without evidence of active HBV replication; iii) HBV status in the native liver and in the graft determines the clinical outcome after LT for patients with HDV positive cirrhosis.
ALTERATIONS IN HEPATOCYTE EXPRESSION OF EPIDEMAL GROWTH FACTOR (EGF) AND TRANSFORMING GROWTH FACTOR ALPHA (TGF-α) IN CHRONIC LIVER DISEASE AND HEPATOCellular CARCINOMA (HCC)

SD Ryder, N Naoumov and Roger Williams. Institute of Liver Studies, King’s College Hospital, London SE5 9RS.

EGF and TGF-α are potent mitogens for hepatocytes and share a receptor. TGF-α is overexpressed in hepatoma cell lines but little is known about their role in hepatocyte proliferation and their relevance to oncogenesis. We examined expression of TGF-α and EGF in a spectrum of liver disease (due to alcohol (ALD) or hepatitis B virus (HBV) infection) from normal liver to cirrhosis and HCC by immunohistochemistry in paraffin-embedded tissue using a monoclonal anti-TGF-α and a polyclonal anti-EGF (microwave pre-treatment) antibody in 5 normal livers, 17 patients with ALD (12 cirrhotic), 21 patients with HBV related liver disease (11 CPH, 5 CAH and 5 cirrhotic) and 11 patients with HCC (HBV n=7, ALD n=4).

TGF-α was uniformly expressed in normal hepatocytes and HBV associated liver disease of all stages but expression markedly increased in ALD, especially where cirrhosis was present. HCC with both alcoholic and viral aetiologies showed considerably less TGF-α expression than surrounding liver. EGF was not detected in normal liver cells or in HBV associated CPH and CAH. In the presence of alcoholic cirrhosis, EGF was markedly increased, mainly in a distribution around the edge of cirrhotic nodules. HCC showed EGF positivity but less than surrounding liver tissue in HBV and ALD. These results indicate that TGF-α is important in regulation of normal hepatocyte proliferation, and EGF in the pathogenesis of pre-neoplastic lesions. Both growth factors are increased in alcoholic liver disease; in contrast, malignant transformation is characterized by loss of TGF-α expression. This suggests that alterations in growth factor expression may be important in HCC development.

COELIAC DISEASE ASSOCIATES WITH A POLYMORPHISM IN THE PROMOTER REGION OF THE TGF-α GENE WHICH FURTHER DEFINES THE COELIAC HAPLOTYPE

J C Mansfield, H Holden, A G Wilson, C D Holdsworth, G W Duff. Section of Molecular Medicine, Department of Medicine and Pharmacology, University of Sheffield, and Department of Gastroenterology, Royal Hallamshire Hospital, Sheffield, S10 2JF.

Endogenous tumour necrosis factor-α (TNFα) expression has been associated with enteropathy in humans, and exogenous TNFα can cause enteropathy in mice. Immunogenetic studies have mapped the genetic susceptibility to coeliac disease (CD) to an HLA DR α/β heterodimer which is usually associated with an extended HLA DR3 haplotype. This study investigates a polymorphism in the promoter region of the TNFα gene is associated with CD, and how this polymorphism may further define the disease susceptibility haplotype.

Genomic DNA from 46 patients with CD was extracted from diagnostic biopsies and compared to DNA from 253 healthy controls. TNFα allele typing was performed by polymerase chain reaction and restriction enzyme digestion. HLA DR3 typing was performed by PCR with sequence specific primers.

Allele carriage rates: Controls CD

- HLA DR1 +ve 38% 82%
- TNFα allele 2 +ve 40% 96%

A further 145 individuals of known HLA type were also TNFα typed to assess the association between HLA A1 B8 DR3 (“the autoimmune haplotype”) and TNFα allele 2. Among HLA A1 B8 DR3 +ve individuals 95% were also TNFα allele 2 +ve whereas among HLA A1 B8 DR3 -ve individuals only 23% carried TNFα allele 2 (odds ratio of 7.9). This confirmed the very close association between these two.

Conclusion: CD is associated with allele 2 of a polymorphism in the promoter region of the TNFα gene. This allele also associates very closely with the autoimmune haplotype HLA A1 B8 DR3. This has potential functional significance as well as genetic interest.

Coeliac disease

T90–T97

T90

IS THE SECOND "COELIAC" GENE LOCATED ON CHROMOSOME 19?

A STUDY OF SECRETOR STATUS AND COELIAC DISEASE

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Dept. of Medicine, Queen’s University of Belfast; Royal Victoria, Belfast City, and Mater Infirmorium Hospitals.

The link between coeliac disease and HLA-related genes on chromosome 6 is well established, but other, as yet unidentified, factors are also involved in determining susceptibility. The ability to secrete blood group antigens into body fluids is controlled by a single gene on chromosome 19. Non-secretors are at increased risk of HLA-associated autoimmune conditions including Graves’ disease and ankylosing spondyloarthritis. By determination of red cell Lewis (Le) antigen phenotype, which correlates with secretor status, we assessed non-secretor prevalence in patients with biopsy-proved coeliac disease, in a blood donor population, and in healthy hospital staff.

Two (3%) of 75 patients with coeliac disease, 8 (6%) of 145 blood donors and 5 (7%) of 67 staff controls had the phenotype Le (a-b-): as secretor status cannot be inferred from this phenotype, they were excluded from further study. The prevalence of non-secretors (Le (a-b-)) was 48% (35/73) among the remaining coeliac patients, 27% (37/137) in blood donors, and 26% (16/62) in control staff. Non-secretor prevalence was significantly higher in coeliac patients compared with both blood donors (i.e. 8.36, p<0.001, odds ratio 2.49, 95% confidence intervals (CI) 1.27-4.51) and with staff controls (i.e. 6.08, p<0.014, odds ratio 2.65, 95% CI 1.27-5.50). Clinical characteristics did not differ between secretors (Le (a-b+)) and non-secretors with coeliac disease.

Thus, the secretor gene on chromosome 19 is linked with susceptibility to coeliac disease. However, like the HLA markers associated with coeliac disease, the non-secretor phenotype is common in the general population, suggesting that further genetic factors are important in determining susceptibility.


Our aim was to investigate the amount and extent of proliferating small intestinal crypt cells within the inflammatory lesion seen in coeliac disease by immuno histochemical staining with the monoclonal antibody Mi-B1 and to correlate this with known indicators of toxicity such as raised IgA anti-gliadin in all three groups.

There was a strong positive correlation between the secretor status (Le (a-b+)) and in all three groups (p<0.05) the increase in total crypt labelling index in CD/N/D (41.2+/-7.6, p<0.05) compared with both the CD/GFD (23.3+/-7.1) and DC groups (23.1+/-5.7) between which there was no significant difference. In all three groups the highest third of the crypt had the highest labelling index and in all three compartments there was a significant increase in staining in the CD/GFD group (p<0.05 in each case) VHlTMT was significantly lower in CD/N/D group (0.34+/- 0.1 p<0.05) compared with the CD/GFD (0.64+/-0.1) and DC groups (0.44+/-0.1) as was ECH (17.1+/-2.7micm (p<0.03) vs. 28.8+/- 2.5micm, and 31.4+/-2.5micm, respectively). There was a strong negative correlation between labelling index and both VHlTMT and ECH measurements in all three groups (p<0.01).

We have demonstrated a significant increase in the number and extent of proliferating crypt cells in CD/N/D. The rise in proliferation index correlated strongly with known indices of small bowel toxicity such as ECH and VHlTMT measurements. An expansion of the proliferating crypt cellular compartment together with premature shedding of villous epithelial cells may be responsible for the pathological features seen in this disorder.

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DETECTION OF INTERFERON-GAMMA mRNA IN THE MUCOSA OF PATIENTS WITH COELIAC DISEASE BY IN SITU HYBRIDISATION.


Interferon-gamma (IFNg) is a cytokine produced during immune reactions, mainly by activated T-lymphocytes. The production of this cytokine in the small intestine has been shown to increase expression of HLA class II antigens on surface enterocytes and adhesion molecules within the lamina propria. It also causes crypt cell proliferation and local tissue damage.

To study the role of this cytokine in the immunopathogenesis of coeliac disease, radioautography in situ hybridisation was used to detect and localize IFNg mRNA in the small intestinal mucosa of controls and patients with this condition. Jaunial biopsycs from controls (n=5), and patients with treated (n=5) and untreated coeliac disease (n=5), were snap frozen and stored in liquid nitrogen. Cryostat sections were cut, and hybridized with specific 35S-labelled DNA oligonucleotide probes. Positively stained cells in the superficial lamina propria were counted with an eye-piece graticule: values were expressed per 0.0025 mm2. There was no staining of the intramuscular lymphocytes or surface enterocytes.

The untreated patients showed a significant increase of IFNg-producing cells (mean count: 18.58 ± 2.7) compared with the treated patients (5.0 ± 1.83) and controls (4.1 ± 2.6). The number of IFNg-producing cells in the lamina propria of treated patients was similar to that found in controls. A correlation between the number of IFNg-positive cells and the degree of villous atrophy in the untreated compared to the treated coeliac patients implies that IFNg may be involved in damage to the villi that is observed in this condition.

The results support the hypothesis that lamina propria CD4+ cells are involved in the disease pathogenesis and suggest that IFNg is involved in the disorganisation of the villous architecture that occurs in untreated coeliac disease.

In Vivo Imaging of Small Bowel Lymphocytic Infiltration in Coeliac Disease by 123I-Interleukin-2

A. Picarelli, R. Ferrari, R. Bonneau, M. Greco, G. Romp, F. Corazzoni, E. Pozzilli, A. Signori (Introduced by A. Toraci).

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In Coeliac Disease jejunal biopsy provides basic indication for diagnosis. However, the extent of histological lesion can not be precisely evaluated by biopsy. Thus, the aim of our study was to image in vivo the extent of bowel lymphocytic infiltration by using 123I-labelled interleukin-2 (IL-2). We have studied 5 healthy volunteers and 5 patients with Coeliac Disease at time of diagnosis made by jejunal biopsy and anti-gladiin antibody measurement. None of the patients were under immunosuppressive therapy at the time of the study. Two ml of 123I-IL-2 (20gic) were injected i.v. and tomographic gamma camera images (SPECT) were acquired 1 hour later. No significant bowel activity was found in normal subjects whereas patients had accumulation of 123I-IL-2 of various degree and extent. Localized abdominal accumulation was observed in 3 patients and diffuse small bowel accumulation in other 2. Quantitative analysis of 123I-IL-2 accumulation was also performed on a 6 cm thick abdominal axial section located below the kidney lower pole, thus avoiding liver and kidney activity. Regions of interest were drawn in the right, middle and left part of this section and the bowel/bone radioactivity ratio was calculated for each area after normalization per pixels. A statistically significant difference was found between patients and controls in all regions, as in the table:

<table>
<thead>
<tr>
<th>Bowel/bone ratio in patients (CD) and normals (NS) after 123I-IL-2 injection</th>
<th>Right NS</th>
<th>Right CD</th>
<th>Mean</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.95</td>
<td>1.86</td>
<td>1.90</td>
<td>0.03</td>
<td>0.02</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

We conclude that by 123I-IL imaging it is possible to visualize in vivo the lymphocytic infiltration in Coeliac Disease. This non-invasive technique may be relevant to evaluate the degree and the extent of infiltration and may be applicable for therapy follow-up.
T97

AUDIT OF PATIENTS’ UNDERSTANDING OF COELIAC DISEASE

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A survey of all female coeliac patients in a district
with a population of 500,000 was carried out, to
audit the patients’ view of their condition and style
of management.

Out of 105 female patients, whose name is in the
Registry of Coeliac Disease in the area 80 agreed to
participate. The patients were interviewed by a
Research Assistant, who administered a questionnaire.

The mean age of the patients is 43.8 years (range
21 – 78 years). The mean age at the time of
diagnosis was 32.1 years (range 1 – 74 years).

13 patients have a relative with coeliac disease.

2 pairs of those are identical twins. 5 patients felt that the diagnosis was not clearly explained and 15 patients felt that they did not receive adequate dietary information at the time of
diagnosis. Most patients saw the dietitian only once, and
half of those thought further visits would have been
desirable.

At the time of the survey the majority were adhering
to the gluten free diet. Of 4 patients definitely not
keeping to the diet, 3 had the diagnosis on only
clinical grounds as infants. Only 71% of patients are members of the Coeliac
Society, but we would support establishing a local Branch.

The majority are willing to participate actively.

78% are not satisfied with the range of gluten free
products available on the market.

Half of the patients rely completely on prescription
to obtain the products. Following this survey, measures have been taken to
improve the information and dietary advice given to
the patients. Contacts are being made to establish a
local Branch for the Coeliac Society.

T99

VITAMIN E DEFICIENCY AND HIGH DIETARY POLYSATURATED FATS COMPROMISE SMALL INTESTINAL FUNCTION

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There is increasing evidence to implicate increased fluxes of free radicals in the pathophysiology of protracted diarrheal disease (PDD) in malnourished infants. Diets high in polysaturated fats (PUFA) are often used in the treatment of such infants and may provide an additional substrate for oxidative damage to the enterocyte. To study this, the water rates were fed 10% fat diets which were sufficient (E) or deficient (E-) in vitamin E (40 mg/kg feed).

The fat source was either corn oil (PUFA+) or lard (PUFA-). E-
PUFA+ animals developed an enteropathy with a significant (P<0.005) reduction in villus height, crypt depth villus / crypt ratio and mucosal thickness when compared with each of the other 3 groups (one way ANOVA - Scheffe multiple range test). These changes were associated with a significant reduction in mucosal hydrolase activity (alkaline phosphatase, sucrase and lactase, P<0.01) and elevated indices of oxidative damage (levels of thiobarbituric acid reactive substances and free malondialdehyde) in the jejunum. Jejunal transport was studied in vitro in an Ussing chamber and intestinal short circuit current (Isc) measured in E-PUFA+ and E-PUFA- groups. Isc data in E+PUFA- and E-PUFA- have been reported previously. E-PUFA+ animals exhibited a raised basal intestinal short circuit current [77±6 (95% CI), vs 70±2±5 cm2 n=30, P<0.05 (unpaired t test)] and increased secretory responses to acetylcholine (Isc max 193±27 vs 116±9 μA cm2, n=8, P<0.01), betahaneol (Isc max 119±26 vs 90±19 μA cm2, n=8, P<0.05), 300 μM dbcAMP
[median isc 54 (95% CI 38-78) vs 32 (24-53) μA cm2, n=8, P<0.05 (Mann Whitney U test) and E-PUFA- animals exhibit an increased secretory response to betahaneol (Isc max 85±13 vs 49±8 μA cm2, n=8, P<0.05 (Mann Whitney U)].

A diet high in polysaturated fat exacerbated both the oxidative damage and the hypersecretion which occurs in the jejunum in vitamin E deficiency and resulted in an enteropathy in this group of animals. This provides further evidence for the administration of formulae high in PUFA during the nutritional rehabilitation of infants with PDD and compromised antioxidant defences may predispose to the perpetuation of the diarrheal state.


T98

NUTRITION T98–T102

ANTIOXIDANT DEPLETION AND PROTRACTED DIARRHOEA OF INFANCY

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In severe protracted diarrhoea of infancy (PD), a vicious cycle of malnutrition and malabsorption has been proposed as a perpetuating mechanism. The factors linking these events have not been clearly defined. There are, however, some suggestions that depletion of nutrients important in oxidative defences could lead to increased intestinal permeability. We have therefore studied nutrient status in 23 malnourished children under the age of 2 years with PD, and the effect of an oxidative stimulus on intestinal transport in an animal model of impaired oxidative defences. In the children with PD, plasma glucose and Ca** were within the normal range. 10/23 were hypoalbuminaemic, 5/23 hypophosphataemic and 6/23 hypo-
magnesaemic. 20/23 were significantly depleted in one or more micronutrients required for oxidative defences. In: 16/23, 5.8±11.0 mol/L median 9.7; Ca: 5/23, 7.5-12.3 median 9.9; Se: 16/23 <0.05-0.2 median 0.09; vitamin E: 5/7, 5.0-10.2 median 6.0. The effects of an oxidative stimulus on small intestinal secretion was studied in an
Ussing Chamber in a rat animal model. 2,3-azobis
(2-aminopropane) dihydrochloride [ABAP] was added to the serosal surface and intestinal short circuit current [Isc] measured. 10mM ABAP increased Isc (median 8lac 19±4 cm2 95/9512-23) and 60mM ABAP produced a greater increment, in Isc (8 lac 63 (53-78)).

These data demonstrate that oxidative stress gives rise to increased intestinal secretion and that the defences to such stimuli may be depleted in children with PD. Particular attention needs to be paid to micronutrient replacement during the nutritional rehabilitation of these infants.

T100

SHORT CHAIN FATTY ACIDS REVERSE THE COLONIC SECRETION INDUCED BY ENTERAL FEEDING

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Short chain fatty acids (SCFA) stimulate salt and water absorption in the colon. Previous in vivo perfusion studies in normal subjects have demonstrated a secretion of salt and water in the ascending colon in response to enteral feeding, which may be relevant to the pathogenesis of enteral feeding related diarrhoea. The aim of this study was to investigate the influence of SCFA on this secretory effect.

6 healthy volunteers underwent a colonic perfusion study, which measured water and electrolyte movement in the ascending and distal colon, by sampling both at the hepatic flexure and from the rectal effluent. Once a steady state had been achieved after two hours perfusion of an isotonic electrolyte solution, the study consisted of 3 stages. Stage 1 established baseline fasting colonic water and electrolyte movement. In stage 2 samples were taken every 20 min for 3 hours during the nasogastric infusion of a standard polymeric enteral diet (1.4 ml/min; 1.4 kcal/min; 8.75 mg/N/min). In stage 3 similar samples were taken during diet infusion, but the electrolyte solution infused into the caecum, instead of containing mannitol to achieve isotonicity, as in stages 1 and 2, contained SCFA in physiological concentrations (acetate 50mM, propionate 20mM, butyrate 20mM).

The electrolytes concentrations and osmolality of these two solutions were identical.

Water movement

<table>
<thead>
<tr>
<th>Stage</th>
<th>Median/ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>2</td>
<td>1.9</td>
</tr>
<tr>
<td>3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

p = net absorption

Na, Cl and K movement were similar to that of water, but HCO3 was secreted during SCFA infusion.

Infusing SCFA directly into the caecum reversed the fluid secretion seen in the ascending colon during enteral feeding. This could have implications in the management of enteral feeding related diarrhoea.
Molecular and cell biology  T103–T107

CYTOKINE PRODUCTION AND ANTIGEN EXPRESSION BY SMALL AND LARGE INTESTINAL CELL LINES
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Gastroenterology Unit, Radcliffe Infirmary, Oxford

The role of intestinal epithelial cells in the gut's mucosal immune system is yet to be established. One of the major problems has been the isolation of a pure population of these cells. The aim of this study was to investigate the production of cytokines and cell surface antigens by three human epithelial cell lines.

Methods: Two well-differentiated human colon cell lines, CaCo2 and HT29/19A, and the small intestinal cell line, INT407, were seeded in flasks at 1x10^5/ml. Cytokine and antigen mRNA synthesis was assessed by PCR before and after stimulation with IFN-γ. Antigen expression was also assessed by FACS. Total cellular RNA was extracted using RNAzol B, an aliquot reverse-transcribed, and the cDNA amplified with specific primer pairs for β-actin, IL-6, II-1B, II-6, II-8, TNFα, IFN-γ, TGF-β, MCP-1, MHC Class II and CD4.

Results: As shown in the table below, transcripts were detected for IL-1B, IL-6, II-8, TNFα, TGF-β, MCP-1, MHC II and CD4. The TH1/TH2 cytokines were not detected. HLA-DR expression, as shown by FACS, was only found on HT29 cells in the presence of IFN-γ. No CD4 was detected.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>CaCo2</th>
<th>HT29</th>
<th>INT407</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1B</td>
<td>c</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>IL-6</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-8</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TNFα</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>MCP1</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>MHC II</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD4</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

Conclusions: Human epithelial cell lines can produce transcripts for a range of cytokines and for two surface antigens. Further analysis of protein products is essential before any further conclusions can be made.

ANTHROPOMETRIC AND QUALITY OF LIFE MEASUREMENTS IN A GASTROENTEROLOGIST CLINIC
T102

Departments of Human Nutrition and Dietetics, Royal Infirmary, Edinburgh, EH13B.

In 1988, we set up a specialist clinic targeted specifically at the treatment of chronic undernutrition. In March 1992, we carried out a case note review of the 62 patients who had attended the nutrition clinic to date. Patients were assessed clinically, anthropometrically (arm circumference, triceps and biceps skinfolds), and for weight gain, and were asked to complete a quality of life (QOL) questionnaire using the Nottingham Health Profile (Hunt et al 1990). Their QOL data were compared with those of 28 new patients who were asked to complete QOL profiles at referral. A total of 90 patients had attended the nutrition clinic by February 1993 and the most common disease categories were neoplastic (n=17), short bowel syndrome (n=15), severe gut motility disorders (n=15) and inflammatory bowel disease (n=12). 78 patients (89%) required prolonged follow up. Weight gain was determined in 63 patients of whom 54 had gained weight with an average weight gain of 5.5 kg (p<0.05). Serial anthropometric measurements were incurred by a mean of 15.5% (range 20.6% to 93%). OOL scores at presentation (n=28) were determined and compared with OOL scores obtained at follow up (n=47). A significant improvement in QOL was seen in the areas of energy (p<0.0027), emotional reaction (p<0.01), sleep (p<0.05), social isolation (p<0.05), social life (p<0.05) and sex life (p<0.05). Weight gain can be achieved in the chronicity 111 patients and such weight gain can be related to improvement in quality of life.

DIFFERENTIAL EXPRESSION OF E-CADHERIN IN NORMAL, METAPLASTIC, AND DYSPLASIA OCIEPHAGEAL MUCOSA: A PRACTICAL BIOMARKER
J A Jankowski, P M Newham, O Kandemir, M N Pignatelli
Imperial Cancer Research Fund Laboratories and Department of Histopathology, Royal Postgraduate Medical School, London.

Barrett's mucosa is a potentially premalignant columnar lined metaplastic epithelium in the oesophagus about which little is known regarding maintenance of cell adhesion. In this regard E-Cadherin (E-cd) is a 120kDa polypeptide present in all epithelial tissues and it is the prime mediator of cell-cell interactions.

An immunohistochemistry technique utilised the monoclonal antibody HEC-1 to identify the presence or absence of E-cd in microtome- treated paraffin- embedded sections from 114 patients with normal tissue (12), metaplastic (36) and dysplastic mucosa (14) and invasive squamous carcinoma (15) adenocarcinoma (27) and metastatic deposits (10).

Surface expression was high in non-metaplastic tissue and in non-dysplastic metaplasia but was reduced extensively in dysplasia. In addition, sections with adenocarcinoma characteristically expressed E-cd in the cytoplasm and this was associated with poor morphological differentiation. Metastatic deposits invariably displayed weaker staining than the primary tumour and membranous staining was seen only in different metastases.

SDS-page gel protein analysis revealed the characteristic 120kDa- sized protein in normal squamous oesophageal tissue. In Barrett's metaplastic tissue and carcinoma, however stronger bands at 108kDa and 50kDa were also present, suggesting either a truncated protein or decreased glycosylation in the metaplastic and neoplastic tissue.

In conclusion it appears that changes in E-cd immunoactivity and cellular localisation occur in premalignant metaplastic epithelium of the oesophagus. Further studies are in progress to evaluate whether the E-cd E-protein reflects both loss of function and also a biomarker in premalignant lesions.

CHOLECYSTOKININ IS A SATIETY HORMONE AT PHYSIOLOGICAL POST PRANDIAL CONCENTRATIONS.
AB Bellinger, L Michielu, S Methow, ML Clark. Dep. Gastroenterology & Chemical Endocrinology, St Bartholomew's Hospital, LONDON.

Exogenous administration of large amounts of cholecystokinin (CCK) reduces food intake in animals and humans. It is however not known whether doses which stimulate physiological post prandial levels would produce satiety and reduce food intake of a standard meal. The aim of this study was to investigate whether physiological concentrations of CCK produced by iv infusion would reduce caloric intake in a test meal. Methods: On separate occasions and after an overnight fast, 6 subjects received either iv saline or synthetic CCK-8 (40 µg/kg/hr, Squibb Diagnostics) to reproduce physiological post prandial concentrations determined in previous experiments. 25min after the start of the infusions subjects were presented with a standard test meal of known caloric content, far in excess of the amount subjects were likely to eat. Blood was taken before the infusion (baseline) and at 10min intervals thereafter for 40min. Plasma concentrations of CCK were measured by a sensitive and specific radioimmunoassay which, following Sep-pak extraction of plasma peptides, shows 0.1% cross-reactivity with gastrin.

Results. Calorie intake was significantly less (p<0.03) during CCK infusion than saline (figure) and this was associated with an increase in CCK levels from 5.1±0.07 pg/ml baseline to 8.3±2.28 pg/ml at 20 mins. There was no increase with saline infusion at 20 mins.

Conclusion. An infusion of CCK producing plasma levels within the physiological range reduces food intake. This shows that CCK is indeed a true satiety hormone.
T105

TRANSECTION OF E-CADHERIN INTO A HUMAN COLON CARCINOMA CELL LINE INDUCES DIFFERENTIATION AND INHIBITS GROWTH IN VITRO

D Liu1, AK Nigam2, EN Lalani3, GHW Stamp1, M Piemonti3 (introduced by Prof. NA Wright)  
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E-cadherin (E-cd) is a transmembrane glycoprotein which not only mediates homotypic cell-cell adhesion but also controls the differentiation of epithelial cells. Loss of E-cadherin is seen frequently in poorly differentiated colorectal tumours in which the architecture and glandular configuration is greatly impaired. It has been proposed that its normal function suppresses the invasive phenotype of carcinoma cells.  

A E-cd negative poorly differentiated colorectal carcinoma cell line (LS174T) was cotransfected with the plasmid BATEM2, expressing mouse E-cd cDNA and with the plasmid pSV2 conferring neomycin resistance. The expression of E-cd in transfected cells was detected by immunoperoxidase staining, ELISA and Western blotting. Inter cellular adhesion was increased (35%) in the transfecants compared to the parental line and this was completely blocked by an anti-E-cd monoclonal antibody (DECM1-1). Transfectants (LS-c2d-2) showed a polygonal epithelial phenotype in monolayer compared to a diffused fibroblast-like form in the parental cell line. In collagen gel well differentiated organoids in which nuclei were polarised towards the periphery were seen in the transfected line. The expression rates of LS174T and LS-c2d-2 were determined under anchorage-dependent (plastic) and independent (soft agar) conditions. There was a 5.8 fold reduction in the number of colonies of LS-c2d-2 cells growing in soft agar compared to the parental cells. The proliferation rate on plastic was also increased in the transfecants (p<0.0006).  

In conclusion, we have shown that transfection of E-cd cDNA into a poorly differentiated colon carcinoma cell line increased intercellular adhesion and led to alteration in the phenotype towards a more differentiated form and inhibited growth in vitro. However, the development of fully differentiated glandular structures may require other adhesion molecules or biological modifiers such as growth factors for the exhibition of the complete phenotype.

T106

USE OF DISEASE-SPECIFIC MONOClonAL ANTIBODies TO CLONE GENEs OF INB DURING THE INTRAMURAL approach TO THE COMPLEMENT REGUlATORY PROTEIN DAF. S Bloom, R Pignot1, D Simmons and DP Jewell. Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, and *British Biotechnology Ltd, Oxford, UK.  

Monoclonal antibodies to molecules upregulated in IBD (Gut 1993, 32(2s):2) have been used to screen cDNA libraries made from inflamed colon mucosa. The aim of the study was to identify genes encoding cell surface antigens which are upregulated in IBD. Methods: cDNA libraries made from inflamed and non-inflamed colon of patients with ulcerative colitis (UC) and Crohn's disease (CD) were ligated into the vector pCDM8 and transiently expressed in COS cells, derived from primate fibroblasts. Transfected cells were incubated with mouse monoclonal antibodies previously shown to recognise inflamed colonic mucosa. Antibody labelled cells were "patted" on dishes coated with goat anti-mouse Ig. Panned cells were lysed, epipocene DNA and used to transform MC1061/p3 E. coli. Plasmid DNA from individual colonies was purified and transfected into COS cells. Immunofluorescence confirmed that the transfected cDNA produced a molecule recognised by the antibody. cDNA clones giving positive fluorescence were sequenced and analysed using the Genbank database. Results: Seven antibodies yielded cDNAs giving positive immunofluorescence. One antibody stained COS cells transfected with a 1.4 kb cDNA fragment, revealed on sequencing to be the gene for the complement regulatory protein CD55 or decay accelerating factor (DAF). This antibody shows apical staining of colonic epithelium, a pattern very similar to that published reports of complement deposition, in 15/18 sections of UC mucosa, 5/7 sections of CD, and 0/9 sections of histologically normal mucosa. A second antibody staining both normal and inflamed colonic epithelium was transfected with a 1.3 kb cDNA which sequence matches that of immunoglobulin superfamily member CD66 (biliary glycoprotein 1), which has not previously been demonstrated in human colonic epithelium. Antigen staining was cell transfected with three cDNAs of 2.7, 2.3 and 2.9 kb. Sequencing of the 3' end of these cDNAs reveals identity with the human poly Ig receptor. Conclusion: We have identified cDNAs whose expression is upregulated in inflamed compared with normal colon, and 1 cDNA, originally isolated from liver, not previously known to be expressed in the colon. The cloning of DAF has relevance in the light of reports of complement deposition on the luminal epithelial surface in UC. This technique may shed light on the molecular pathogenesis of inflammatory bowel disease.

T107

APPROACHES TO GENE THERAPY IN THE GUT: RETROVIRUS MEDIATED GENE TRANSFER IN A MODEL OF GASTROINTESTINAL DEVELOPMENT R Del Buono and *NA Wright  
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For gene therapy to become a reality in the gut, methods must be developed to stably transfect gut stem cells in vivo. We have developed a novel and highly adaptable in vivo model of gastrointestinal development and differentiation. Here we describe the use of a retroviral vector to introduce the lacZ (β-galactosidase) gene to foetal intestinal endodermal cells in our model, in a first attempt to develop a system in which to assess the feasibility of gene transfer in gut stem cells, and to study genes of developmental interest in the gut.  

We have shown that the retroviral vector, MFG-Neom/LacZ, which contains a single BamHI site into which an insert can be inserted, will transfect the gut of the rat embryo at E15.5. The organs recovered from the gut at this time were transduced by the vector, and the β-galactosidase reporter gene was detected in the gut, liver, spleen, kidney and muscle. There was no detectable β-galactosidase activity in the control vector.  

Thus, β-galactosidase expression can be driven in vivo by the retroviral vector, MFG-Neom/LacZ. We are now developing a protocol for ex vivo gene transfer by this system, and for in vivo gene transfer into other organs using a modified vector.  

Inflammatory bowel disease T108-T115

NITRIC OXIDE IS CYTOTOXIC TO CULTURED CACO-2 INTESTINAL CELLS. FH Mourad, LJD O'Donnell, AM Cavaillon, ML Clark, MJG Farthing. Department of Gastroenterology, St Bartholomew's Hospital, London EC1A 7BE  

Nitric oxide (NO) is cytotoxic to vascular endothelial cells and the inhibition of nitric oxide synthase (NOS) has a protective effect in chemically-induced colitis in animals. Whether nitric oxide has a direct cytotoxic effect on enterocytes or colonocytes is not known. Our aim was to study the effect of the nitric oxide donor L-arginine and the nitric oxide synthase inhibitor nitro L-Arg methyl ester (L-NAME) on the survival of Caco-2 cells, an intestinal carcinoma cell line.  

Caco-2 cells were cultured in 6-well plates in Dulbecco's modification of Eagle's medium, and which contains glutamine, antibiotics and foetal calf serum. Twelve days after reaching confluency, when Caco-2 cells have differentiated and acquired the enterocyte phenotype, they were incubated with the same culture medium to which the following were added: (1) L-Arg 800μM; (2) D-Arg 800μM; (3) L-NAME 100μM or (4) L-Arg 800μM+L-NNAME 100μM. After 24h incubation the supernatant was removed and replaced with 0.5% Trypan blue in PBS and the cells incubated for a further 10min. Cell death was then determined microscopically (200x) by two blinded observers who counted those cells that failed to exclude the dye in four representative fields in three replicate wells. The number of dead cells in the control group (culture medium only) was 34.7±2.1 (mean±SEM). Addition of L-NAMe alone produced no significant change in cell death rate (39.7±3.5). Incubation with L-Arg significantly increased the number of dead cells (44.8±2.3; p<0.01); however, D-Arg was without effect (28.3±3). Addition of L-NAME with L-Arg significantly protected the cells from L-Arg-induced cytotoxicity (26.8±1.6; p<0.001 compared to L-Arg and p<0.01 compared to control)  

Thus, nitric oxide has a direct toxic effect on the cells of this carcinoma cell line, a process that can be prevented by nitric oxide synthase inhibition. NO may contribute to the cytotoxicity of intestinal cells in conditions such as ulcerative colitis in which NO production is known to be increased.
DUAL ACTION OF A NITRIC OXIDE SYNTHASE INHIBITOR ON ENDOTOXIN-INDUCED RAT INTESTINAL MICROVASOULAR INJURY


Nitric oxide (NO) formed by a constitutive enzyme plays an important role in modulating intestinal microvascular integrity. By contrast, excess production of NO by an inducible enzyme is implicated in the microvascular injury associated with endotoxin shock. We have now investigated the time-course of the effects of the NO synthase inhibitor, N\(^5\)-monomethyl-L-arginine (L-NMMA), administered at different times following endotoxin challenge, on intestinal microvascular injury.

Under halothane anaesthesia, 2\(\times\)10\(^5\) lipopolysaccharide (LPS,3mgkg\(^{-1}\)) was administered to rats. \([\text{125}^i]\text{Hunan serum albumin (2}\mu\text{g kg}\(^{-1}\)) was used for the determination of vascular albumin leakage, an index of endothelial injury. Concurrent administration of L-NMMA (50mgkg\(^{-1}\), s.c.) substantially provoked albumin leakage seen 1h after LPS in the ileum (from 142 to 532626Ul plasma g\(^{-1}\) tissue respectively, n=8; P<0.01). This increase in vascular leakage declined over the subsequent 1-2h, as was likewise found in the colon. It is known however, that activity of the inducible enzyme is only detected in intestinal tissues from 3h after LPS challenge, a time when changes in vascular permeability are initiated by low doses of LPS. L-NMMA (12.5:50mgkg\(^{-1}\), s.c.) administered 3h after LPS, caused a dose-dependent reduction in the albumin leakage in both ileum and colon, determined 2h later, being abolished with the higher dose (n=8, P<0.01 for both). L-NMMA (50mgkg\(^{-1}\)) did not significantly affect albumin leakage under control conditions 1h after administration in ileum or colon (n=8 for each).

These findings confirm that initial suppression of the constitutive NO synthase by L-NMMA following challenge with LPS provokes early vascular injury in the ileum and colon. By contrast, administration of L-NMMA at a time of detectable expression of the inducible NO synthase, protects against the subsequent vascular damage, which may reflect inhibition of the excessive local generation of cytotoxic NO or its metabolites.

STUDIES OF EPITHELIAL METABOLISM AND MUCIN SYNTHESIS IN ULCEERATIVE COLITIS AND CONTROLS

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Introduction Butyrate is an important substrate for the colonic epithelium, and a defect in its metabolism has been reported in ulcerative colitis (UC). We postulated that pouchitis in UC might reflect a similar metabolic defect in the ileum. In addition, reports of therapy with butyrate enemas in UC have been favourable, and we have examined whether this response might reflect favourable metabolic conditions resulting in increased mucin synthesis.

Methods & Results Using a tissue culture technique \(^{14}\)C butyrate and \(^{14}\)C glucose metabolism were quantified in ileocolonic biopsies from quiescent UC and control patients. In UC rates of butyrate metabolism in the terminal ileum (79/139.3 \pm 56.2 nmol/hour/\mu g protein) (mean \pm SD, n=8), ascending colon (AC) (92.5 \pm 58.3, n=12) and descending colon (DC) (93.3 \pm 115.1, n=12) were not significantly different from those in controls (TI 118.1 \pm 57.2, n=10, AC 62.6 \pm 44.2, n=12, DC 51.5 \pm 32.2, n=12). In UC the rate of glucose metabolism in the DC (7.6 \pm 7.8 nmol/hour/\mu g protein) was greater than in controls (1.4 \pm 0.7 (P<0.01); rates of metabolism of glucose in the AC (6.2 \pm 7.7) and TI (23.3 \pm 13.8) in UC did not differ significantly from control values (AC 4.9 \pm 3.2, TI 15 \pm 13.6). In AC we have assessed the effect of butyrate on mucin synthesis by cultivating colonic biopsies (from UC and controls) in the presence of butyrate at concentrations 0-10mM; in controls a dose-dependent increase in \(^{3}H\) N-acetylgalactosamine incorporation into mucin was found in AC with maximum effect at concentrations of 0.1mM to 400 \pm 85% (mean \pm se, n=16) of control values (n=16). A similar response was obtained in UC biopsies. The effect of butyrate was blocked by 50mM bromo-octanate.

Conclusion Our studies confirm that there is regional variation in butyrate and glutamine metabolism in the colonic epithelium, but do not support the hypothesis that impaired butyrate metabolism is responsible for UC or pouchitis. Butyrate increases mucin synthesis in the colon and this could be an important physiological and therapeutic mode of action.

LACTOFERRIN IS PRESENT IN NORMAL MUCOSAL MAST CELLS, IS SELECTIVELY EXCRETED IN FAECES IN INFLAMMATORY BOWEL DISEASE (IBD) AND MAY BE RELEVANT TO LEUCOCYTE RECRUITMENT

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The raised faecal lactoferrin in I.B.D. has been assumed to reflect leucocyte shedding. Lactoferrin, however, enhances leucocyte adherence and can itself cause inflammation. We have therefore compared faecal excretion of lactoferrin with myeloperoxidase, the product of a different leucocyte granule, and assessed the mucosal localisation of lactoferrin.

Freshly voided faecal samples from normal controls (n=8), and inflammatory bowel disease (n=32). Crohn's disease (n=13) and ulcerative colitis (n=18) were homogenised, centriufuged, filtered and assayed by ELISA for lactoferrin and myeloperoxidase.

Although faecal myeloperoxidase was increased in both IBD (mean 47.0ng/mg protein, SD 36.0) and infective diarrhoea (mean 98.0, SD 113.0) compared with controls 1.0, SD 0.4, p<0.001) there was much greater excretion of lactoferrin in IBD so lactoferrin/myeloperoxidase ratio completely discriminated between IBD (mean 10.4, SD 6.2.) and infective diarrhoea (0.84, SD 0.76, p<0.01) with no significant difference between UC and CD.

This implies a selective release of leucocyte secondary granules in IBD.

Immunohistochemistry (n=20 UC, 20 CD, 20 controls) shows myeloperoxidase to be present as expected in all phagocytic cells but lactoferrin has a quite different distribution with expression mainly in macrophages the normal mucosa with intense staining of crypt abscesses in active IBD.

The selective release of lactoferrin from the mucosa in IBD may be a potent factor in the inflammatory process.

NEW APPROACHES IN CROHN’S DISEASE: RAPID LOCALISATION OF GRANULOMAS FOR PARALLEL LIGHT MICROSCOPIC (LM) AND ELECTRON MICROSCOPIC (EM) STUDIES

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The granuloma, a hallmark lesion of Crohn's disease, may localise to the antigenic stimulus for persistent cellular immunity in this condition. Thus these structures are important targets for detailed study. However, there are no techniques that permit both the identification of, for example, viral particles at the EM level and co-localisation of these structures with signals obtained by hybridisation or immunohistochemical techniques at the LM level. Limitations include fixing and staining techniques the focal distribution of granulomas. We have devised techniques to overcome these problems.

Methods: 1) Arterial perfusion-fixation at 100 mmHg of 10mins using 4% paraformaldehyde/0.1% glutaraldehyde in 100mM phosphate buffer at pH 7.2. 2) Tissue is sliced circumferentially into 5mm lengths, that are divided into 10 radial segments, to include the full thickness of the bowel wall. 3) Radial sections are embedded in agar and sliced on a McIlwain tissue chopper into 500-700um widths. 4The 500um sections are separated and stored in order, with alternate sections either being processed into paraffin, or stored in paraformaldehyde for subsequent EM processing. 5) Areas of interest, such as granulomas, are identified in H and E sections taken from the paraffin processed blocks. 6) The adjacent section is orientated, and the area of interest dissected out and cut into 1mm blocks for Lowicryl embedding. 7) Granulomas can be screened by LM immunohistochemistry and in situ hybridisation, for example for infectious agents, prior to immunogold/in situ gold at the EM level on the same tissue lesion. This technique has increased our hit rate of granulomas from 1:200 to 12 tissue sections suitable for EM study, representing a great saving in time and costs.
ABSENCE OF M. PARATUBERCULOSIS BUT PRESENCE OF M. AVIUM RFLP TYPE A/1 IN TISSUE EXTRACTS ISOLATED FROM PATIENTS WITH CROHN’S DISEASE EXAMINED BY THE POLYMERASE CHAIN REACTION


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Introduction: If Crohn’s disease in humans is similar to Johne’s of ruminants, then the infectious agent would be expected to be concentrated in foci of granulomatous inflammation. Johne’s disease may be caused by two agents: M. paratuberculosis and M. avium RFLP type A/I (the wood-pigeon bacillus). Both these bacteria have been isolated from Crohn’s disease. Methods: Granulomas were identified in single H and E sections from 10 resected Crohn’s tissues. A serial 30 μm section was cut, followed by a further section for H and E, to check that the same granuloma was still present. In the same tissue the base of an ulcer was also identified: all tissue on the 30 μm section was cut away leaving just the granuloma and ulcer base separately. These tissues were coded, de-waxed and DNA extracted using mini-bead beater. PCR was performed using primers from IS900 (specific for M. paratuberculosis) and from IS901 (specific for M. avium RFLP type A/I) and the results decoded.

Results: All sections were negative for M. paratuberculosis. One section taken from the base of an ulcer was positive for M. avium RFLP type A/I. Conclusions: The absence of M. paratuberculosis DNA in the granulomas indicates that either M. paratuberculosis is not involved in the disease in this group of patients, or it is concentrated in discrete foci that were not sampled in this experiment. The detection of M. avium A/I in a single specimen does, however, provide some evidence for the continued association between Johne’s disease bacilli and a minority of Crohn’s disease patients, although this may be due to secondary infection of ulcers.

NEOPLASIA T116–T123

THE PROGNOSTIC VALUE OF C-ERB B2 IN ADENOCARCINOMAS ARISING IN BARRETT’S OESOPHAGUS

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The oncogene c-erb B2 has sequence homology to the internal domain of the epidermal growth factor receptor. It is postulated that it causes autonomous phosphorylation of tyrosine kinase leading to altered growth regulation. It has been found in up to 25% of breast cancers, 15% of gastric cancers, 38% of non-small cell lung cancers and 13% of colon cancers. Over-expression of this oncogene has been associated with a poor prognosis in ductal breast carcinomas but appears to be related to the more well differentiated gastric carcinomas.

The aims of the present study were to determine the proportion of Barrett’s cancers over-expressing c-erb B2 and to relate this to stage, grade and survival to see if it has any prognostic value.

Archival sections of tumours from histologically confirmed Barrett’s oesophagus were stained immunohistochemically for the presence of c-erb B2. Sections were scored positive if greater than 10% of cells had membranous staining. The case notes and original pathological record were obtained to assess grade, stage and prognosis. A good prognosis was arbitrarily determined by survival more than one year from diagnosis.

The results confirmed that a poor prognosis was significantly associated with the poorly differentiated tumours (p<0.05) and stage IIB, III or IV (p<0.01). However, there was no relation between c-erb B2 over-expression and grade, stage or prognosis. C-erb B2 was positive in 19 (68%) of the 28 tumours.

We conclude that c-erb B2 does not appear to have any prognostic value in adenocarcinomas arising in Barrett’s oesophagus. It is, however, present in over 2/3 of tumours: a much higher proportion than in any other type of tumour studied so far. It may therefore have value as a pre-malignant marker in surveillance programs.
**OESTROGEN RECEPTOR AND OESTROGEN INDUCIBLE GENES ARE EXPRESSED IN GASTRIC MUCOSA AND CANCERS.**


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Gastric cancer is more common in men. It is also more common in males in some experimental models of gastric carcinogenesis. The anti-oestrogen tamoxifen appears to have some benefit in treatment of advanced gastric cancer. Furthermore, the beneficial effect of cimetidine in gastric cancer may also be endocrine in origin. The presence of oestrogen receptor in gastric mucosa and cancers is controversial. The aim of this study was to determine expression of oestrogen receptor and, as evidence of receptor function, expression of oestrogen inducible genes (pS2 and ERDS) in paired samples of normal gastric mucosa and cancer.

Oestrogen receptor expression was measured using a commercially available enzyme immuno-assay. Oestrogen receptor expression was significantly lower in cancers, 1.8 fmol/mg protein, compared to paired normal mucosa, 13.7 fmol/mg (n=9). Males and females had similar levels. In contrast, oestrogen receptor mRNA determined by Northern blot analysis was present in equal concentrations in normal mucosae and cancers. Northern blot analysis also demonstrated differential expression of pS2 and ERDS in cancers and normal mucosae. pS2 was undetectable in 13 out of 14 cancers relative to the paired normal mucosal tissue. ERDS was overexpressed in 12 out of the 14 cancers. In Situ hybridisation and immunohistochemistry showed pS2 and ERDS expression in epithelium.

Differences in expression of oestrogen receptor and the oestrogen inducible genes, pS2 and ERDS, between normal gastric mucosa and cancers may indicate a role for oestrogen in gastric cancer.

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**N-NITROSO COMPOUNDS LEVELS IN FRESH GASTRIC JUICE IN RELATION TO CLINICAL DIAGNOSIS**

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N-Nitroso compound (NOC) levels have been assayed in 510 samples of fresh gastric juice obtained at endoscopy using a newly published method (Xu et al, The Analyst 1993 in press) which utilised Thermo Energy Analysis.

When the levels were related to the gastric juice pH there were two clear peaks, one at acidic pH and the other higher peak at near neutral pH. The levels were shown to decrease markedly with storage of the samples.

The patients were subdivided according to previous gastric surgery and endoscopic diagnosis. The table shows the results in patients taking no relevant medication (n=172) [* p < 0.05 compared with normal (T test)].

<table>
<thead>
<tr>
<th>Patient group</th>
<th>n</th>
<th>pH nitrate nmol/l mean (SEM)</th>
<th>NOC nmol/l mean (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>54</td>
<td>2.5 4.07 (2.96)</td>
<td>1.63 (0.31)</td>
</tr>
<tr>
<td>DU</td>
<td>20</td>
<td>1.9 0.25 (0.13)</td>
<td>1.64 (1.15)</td>
</tr>
<tr>
<td>GU</td>
<td>8</td>
<td>2.4 2.91 (2.58)</td>
<td>2.46 (0.99)</td>
</tr>
<tr>
<td>V&amp;P</td>
<td>19</td>
<td>3.4 6.44 (3.87)</td>
<td>2.23 (0.72)</td>
</tr>
<tr>
<td>Partial g/ectomy</td>
<td>40</td>
<td>6.5 19.62* (3.51)</td>
<td>2.12 (0.44)</td>
</tr>
<tr>
<td>Gastric atrophy</td>
<td>11</td>
<td>5.5* 23.99* (11.83)</td>
<td>2.82* (0.96)</td>
</tr>
<tr>
<td>PA</td>
<td>11</td>
<td>7.5* 66.62* (11.74)</td>
<td>2.79* (0.83)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>9</td>
<td>9.4* 149.50 (78.02)</td>
<td>4.70* (2.05)</td>
</tr>
</tbody>
</table>

These results are in close qualitative agreement with the results reported in 1981 (Lancet, ii, 550-2) using a different method. All the NOC levels are higher than they include the unstable as well as the stable NOCs. Using this improved method we have been able to show higher NOC levels in fresh gastric juice from patients at increased risk of developing gastric cancer.

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**INHIBITORY EFFECTS OF THE GASTRIN RECEPTOR ANTAGONIST CR2093 ON GROWTH-FACTOR STIMULATED GROWTH OF A PANCREATIC CELL LINE**

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DEPARTMENT OF SURGERY, UNIVERSITY OF NOTTINGHAM.

AR42J, a rat pancreatic cell line, is known to express both gastrin and epidermal growth factor (EGF) receptors. In this study, we have examined the effect of a gastrin receptor antagonist, CR2093 on basal and gastrin-17 (G17), EGF and transforming growth factor (TGF) alpha-stimulated growth in vitro. At 5x10^-6 M, CR2093 reduced the basal growth of AR42J to 65% of control values (p<0.01). This growth inhibition was reversed to 91% by G17 at 10^-5 M, and also to 94% by EGF and to 75% by TGF alpha at 10 ng/ml. Alone, G17, EGF and TGF-alpha stimulated growth to 112%, 187% and 137% of control values respectively, and marked synergy was observed when G17 was used in combination with either growth factor. Furthermore, the combination of G17 and TGF-alpha reversed the inhibitory effect of CR2093 to 115% of control values. When the ability of CR2093 to bind to EGF receptors was measured, it was found that the antagonist could displace up to 23% of unlabelled EGF.

CR2093 has potent inhibitory effects on the basal growth of AR42J which can be reversed by G17 and EGF receptor ligands, and this action may in part be related to the antagonist's ability to inhibit binding to the EGF receptor.
CURRENT USE OF ASPIRIN AND NON-STEROIDAL ANTI-INFLAMMATORY DRUGS MAY PROTECT AGAINST SYMPTOMATIC COLORECTAL CANCER: A CASE CONTROL STUDY

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In vitro prostaglandin inhibition by non-steroidal anti-inflammatory drugs (NSAIDs) reduces colon carcinogenesis in rat models. Recent epidemiological reports suggest that salindac protects against polyp formation in familial polyposis and that aspirin use reduces colorectal cancer incidence and mortality. We report data from a case control study.

Method

273 consecutive cases of histological colorectal cancer were identified. A community dwelling control was found from each colorectal cancer propositus general practitioner’s register, matched for age (+/-2 years) and sex. Cases and controls were interviewed by one observer using a structured questionnaire including a section concerning medication use. Aspirin and NSAID use was classified as "never", "used to" (not in the last 12 months) and "current". Frequency of use was noted as "regular" (at least weekly) or "occasionally" (less than weekly). The odds ratio (OR) and 95% confidence intervals (CI) were calculated.

OR for drug ingestion "ever versus never" were: aspirin and/or NSAID 0.76 (0.58-0.99), aspirin only 0.77 (0.54-1.08), NSAID only 0.62 (0.25-1.36). OR for "current versus never" were: aspirin and/or NSAID 0.64 (0.43-0.93), aspirin only 0.67 (0.43-1.04), NSAID only 0.5 (0.16-1.20). OR for "used to versus never": aspirin and/or NSAID 0.92 (0.60-1.43), aspirin alone 0.86 (0.57-1.30), NSAID alone 1.9 (0.48-7.49).

We conclude from this study that there is some support for the hypothesis that colon carcinogenesis in man may be reduced by use of aspirin or NSAIDs. This requires further careful examination.

MUTATION ANALYSIS IN FAMILIAL ADENOMATOUS POLYPOSI5, 5 BASE PAIR DELETION AT CODON 1309 RESULTS IN A SEVERE PHENOTYPE. KP Nugent, BKS Phillips, SV Hodgson, S Cotrell, J Smith-Ravin, W Bodnar
St. Mark's Hospital , City Road, London ECIV 2PS

Patient phenotype in familial adenomatous polyposis (FAP) varies in the number of colorectal polyps and the presence or absence of a variety of extracolonic manifestations. Specific germline deletions within the coding sequence of the APC gene have been identified and may explain this heterogeneity.

The phenotype of 27 members from 7 different families with an identical 5 base-pair (bp) deletion at codon 1309 was compared with:

- a group of 61 age and sex matched patients with FAP where the mutation has not yet been typed; and
- b) 8 other different mutations in 25 individuals.

Patients with codon 1309 deletion have significantly more colorectal polyps at the time of colectomy than the 61 age and sex matched FAP patients with unknown mutations (p=0.0001, Mann Whitney U test).

The median number of polyps in their colectomy specimens was 4000 (interquartile (IQR) range 3000-4857), compared to 600 (IQR range 488-1400) in the 61 matched patients. 6 of 27 patients with the 5bp deletion at codon 1309 developed extracolonic cancers, compared with 1 of 61 controls (p=0.003, Fishers Exact). Other manifestations such as desmoid tumours were variably present in all groups.

These findings suggest that there is a correlation between a specific germline mutation and the number of large bowel polyps. The residual heterogeneity in phenotypic expression may be influenced by constitutional or environmental epiphenomena.


Minimum criteria for a tentative definition of HNPCC have been recently proposed by an International Collaborative Group on HNPCC (ICG-HNPCC) (Vasen et al., Dis Col Rectum 1991;34:424). A large group of families with a marked aggregation of tumors (including colorectal cancer) and other peculiar clinical features (early onset and/or multiple primaries) in which an inherited susceptibility is likely to occur, do not meet the above mentioned tentative definition and theoretically should be excluded from the diagnosis and screening procedures of HNPCC. In this study six clinical and pathological characteristics of vertical transmission of colorectal or early-onset cancers, familial aggregation (i.e. more than 50% of cancer cases in the nuclear pedigree), proximal localization of colon tumors, early age of onset, multiple cancers (including synchronous and metachronous colorectal cancers- and mucinous histology) were analyzed in 132 consecutive patients previously resected for colorectal cancer, in order to calculate the relative contribution of each of them to the diagnosis of HNPCC. The relative risk for HNPCC diagnosis for each parameter was calculated for all 176 colorectal cancers found in the kindreds by means of a stepwise logistic regression. Results: Six families (4.5%) met the minimum criteria of the ICG-HNPCC and could therefore be considered HNPCC. Twenty-three families (17.4%), having at least 3 of the above mentioned features, although not satisfying the ICG-HNPCC criteria, remained highly suspected for HNPCC. The multivariate analysis showed the following variables to be associated with HNPCC diagnosis: vertical transmission of colorectal cancer (relative risk R.R. =17.9, 95%CI=1.1-727), early age of colorectal cancer (<50 yr) (R.R=6.8, 95%CI=1.8-25.8), aggregation of tumors in the nuclear pedigree (R.R.=5.3, 95%CI=1-3.22), proximal localization of colon tumors (R.R.=3.8, 95%CI=0.9-15.77). No association with HNPCC diagnosis was found for the presence of multiple tumors or mucinous histology. Conclusions: The presence of two consecutive generations affected by colorectal cancer or early primaries seems to be the major risk factor associated with hereditary colorectal cancer syndromes, advising careful evaluation of the pedigree and strict family screening procedures.

THE ONTOGENIC ROLE OF EGF AND TGF ALPHA IN THE DEVELOPING HUMAN STOMACH.

EJ Kelly, SJ Newell, KG Brownlee & JN Primrose
Academic Unit of Paediatrics and Surgery, St. James’s University Hospital, LEEDS.

Epidermal Growth Factor (EGF) is produced by a variety of glands within the gastrointestinal tract and in experimental animals has been shown to facilitate increased growth of gastric mucosa. In human the concentration of EGF in amniotic fluid is seen to rise during gestation. EGF binds to a receptor which mediates tyrosine phosphorylation of the receptor. Transforming Growth Factor alpha (TGF alpha) also exerts its action via the EGF receptor. We postulate that EGF and TGF alpha may play a role in the development of the stomach, and have examined the expression of EGF, TGF alpha and the EGF receptor in fetal and infant stomachs.

Twenty fetal (13 to 28 weeks gestation) and 5 infant (2 to 21 weeks) stomachs were obtained and fixed in buffered formalin. Sections were examined for the presence of EGF, TGF alpha and the EGF receptor using monoclonal antibodies by the peroxidase anti-peroxidase method.

We have also examined amniotic fluid and fetal urine to determine whether EGF in the liquor is fetally or maternally produced. Amniotic fluid and urine samples were collected from 12 unstrressed, term infants born by elective cesarian section for maternal reasons. Samples were stored on ice, spun and frozen. Samples were analysed using a radio immunoassay to determine EGF concentration.

EGF immunoreactivity was not detected in any of the specimens examined. EGF receptors were detected from all specimens after 16 weeks gestation, but not before. Receptors were noted on the surface epithelium and not in the glands. Urine and amniotic fluid contain large amounts of EGF.

The site of the EGF receptor suggests that from 18 weeks gestation luminaly acting peptides produced during fetal life are important in the maturation of the human stomach.
A SCID MOUSE XENOGRAFT MODEL FOR HUMAN INTESTINAL DEVELOPMENT AND FUNCTION.

The study of human intestinal physiology is complicated by the relative inaccessibility of this organ for investigation, as well as a multi-facet of ethical considerations. In an attempt to study human intestinal development and function under more controlled experimental conditions we have established a mouse xenograft model that has provided an opportunity for long-term investigations.

Intact segments (2-3 cm lengths) of human fetal intestine (10-15 weeks gestational age) were xenotransplanted into sub-cutaneous tunnels on the back of 6-8 week old C.B-17 scid mice, for durations of up to 6 months. Xenograft visualisation and the presence of all 4 major intestinal epithelial cell lineages were evident within 2 and 4-6 weeks following transplantation respectively. Enteroocyte differentiation was well advanced by 10 weeks, as indicated by enzyme cytochemistry showing brush border alkaline phosphatase, aminopeptidase-N, lactase, a-glucosidase and dipeptidylpeptidase IV activities comparable to those measured in children. Double-label in situ hybridisation employing biotinylated and digoxigenin-labelled whole mouse and human DNA probes, performed at both light and electron microscopical levels, demonstrated a human origin for a majority of cellular components comprising the xenograft, including intra-epithelial leukocytes. Host mouse connective tissue and some leukocytes were shown to invade a predominantly human lamina propria. In addition, the endothelium supplying the xenograft was of a murine origin.

In summary, we have developed an intestinal xenograft model that closely resembles human neonatal gut. We are currently assessing the suitability for this model to mimic human enteropathy in vivo, with the aim of providing a novel means for drug testing at a pre-clinical level.

GASTRIC EMPTYING IN THE PRETERM INFANT: BREAST IS BEST
Ever A.K., Darby GM, Morgan MEI, Booth DW.
Birmingham Maternity Hospital and Institute of Child Health, University of Birmingham, Birmingham, UK.

Failure of adequate gastric emptying (GE) frequently prevents successful breast feeding in the preterm infant. However, its determinants are poorly understood. We have therefore used a novel ultrasonic technique to compare GE in a cross-over study of infants receiving expressed maternal breast milk (EMBM) and a whey-based formula milk.

14 infants (9 males, 5 females) were studied on 46 occasions. Median (range) gestation of the group was 33w(30-35), birthweight 1650g(1130-2130). Each infant received a bolus nasogastric feed (median/range) volume 21(13-29)ml/kg/feed. Of either EMBM or formula, and the alternative at the next feed. With the infant in the right lateral position, ultrasonic images of the gastric antrum were obtained using the aorta and vertebrae as constant landmarks. Measurements of antral cross sectional area (ACSA) were made before the feed and then sequentially following its completion until ACSA returned to its pre-feed value. Half-emptying time (t1/2) was calculated as the time taken for the ACSA to fall to half the maximal increment. Half-emptying time for EMBM was half that for formula feeds: Mean(SEM) t1/2 EMBM 35.9 min(3.57);formula 72.4 min (9.90); p<0.0001. Moreover, the emptying curve for formula fed infants was usually exponential. There was no significant difference between emptying rates of feeds of the same type for an individual baby. These data demonstrate that feed type has a major effect upon GE which is difficult to explain on the basis of differences in energy density or fat content. These observations have important implications for the management of infants who are intolerant of feeds.


IMPACT OF CYTOMEGALOVIRUS AND EBSTEIN BARR VIRUS INFECTION IN CHILDREN FOLLOWING LIVER TRANSPLANTATION
S.W. Davidson, N.S. Murphy, G.O. Adeoye, D.A. Kelly.
The Liver Unit, The Children's Hospital, Ladywood Midway Birmingham, UK.

The high seroprevalence of CMV and EBV in adults is well known. In contrast, most children are seronegative for these viruses at transplantation, and are therefore at a greater risk of subsequent disease. The incidence of CMV and EBV infection and associated morbidity were studied prospectively in 92 paediatric OLT recipients (median age 2.1 years, range 0.3 to 14.8 years). At OLT 69 (75%) were seronegative for CMV and 44 (48%) children received CMV positive grafts. Of the 30 negative children a received a positive graft. Of 70 survivors, 24/55 CMV seronegative recipients seroconverted to CMV, 18/24 had received seropositive grafts and despite Acyclovir prophylaxis 14/18 developed significant disease. Seroconversion was asymptomatic in 6 patients who had received seronegative grafts. Median time to CMV seroconversion was 51 days (range 8-872). All children with CMV disease responded to treatment with Ganciclovir and hyperimmune globulin.

EBV serology was available in 69 patients at transplantation 54 (63%) being seronegative. 43/68 survivors were seronegative at OLT and 56/63 seroconverted to EBV at a median of 203.5 (range 12-746) days. Reactivation occurred in 9/25 seropositive recipients at a median of 508 (range 51-810) days. There was significant morbidity in 7 patients, one of whom developed lymphoproliferative disease and in addition 1 patient with EBV and Hepatitis C co-infection developed acute hepatic failure requiring retransplantation.

CONCLUSION: As most children undergoing OLT are seronegative for CMV and EBV, subsequent infection is frequent and has a significant morbidity. Effective antiviral prophylaxis or vaccination in this high risk population is essential.

ONE YEAR LONGITUDINAL COMPARISON OF STOOL POSITIVITY AND SERUM IMMUNOREACTIVITY TO GIARDIA LAMBLIA IN CHILDREN FROM RURAL SOUTH VIETNAM.
Char S, Kachanich PH, Robertson G, Tippett G, Hao DO, Farthing MJG. Dept Gastroenterology, St Bartholomew's Hospital, London, U.K., Dept Microbiology, Concord Hospital, Sydney, Australia & Center for Pediatrics, Ho Chi Minh City, Vietnam.

Seropositivity of children to Giardia in endemic areas is commonly reported to be 20-50% by ELISA while 10-30% are stool positive at a given time. To evaluate whether this underestimates the exposure of the population to the organism and to analyse the antibody profile of children from an endemic area, we compared stool positivity (wet film and concentrate, n=125) and immune responsiveness to Giardia in children 2-11yrs from a rural village in Vietnam on two occasions, 1 yr apart.

Stool positivity was 25.6% on the first occasion. Prevalence was greatest in children 4-6yrs (38%) and lowest in those >8yrs (7.5%). Stool prevalence was 15.4% 1 yr later. Antibody responses to 'native' antigens were determined by immunoprecipitation of biotinylated Giardia antigens and responses to 'denatured' Giardia antigens assessed by SDS-PAGE and immunoblotting. Sera from all children, regardless of stool positivity, recognised Giardia antigens using both techniques on both occasions. A variety of antigens ranging from 31-170kDa were recognised by IgG, IgA and IgM antibodies and corresponding IgA and IgM recognition (but not IgG) decreased with increasing age of the subject. Bands corresponding to 170, 88, 71, 45 and 33kDa were seen by immunoprecipitation in all children: the 88kDa major surface antigen was immunodominant, although not prominent by immunoblotting, indicating the importance of conformational epitopes on OMPs. No major changes in antibody profiles occurred over the 1yr period irrespective of changes in stool positivity.

Exposure to Giardia in this population is ubiquitous and suggests that previous measures of seroprevalence in endemic regions are underestimates. The serum immunoanalysis indicates that despite detectable specific anti-Giardia antibody responses, persistent and/or recurrent infections with Giardia are common in children in the developing world.
T129

RETORSINE INGESTION PREVENTS NEONATE LIVER COPPER EXCRETION. Morris P.A. and Turner, M.S., Department of Paediatrics, Children’s Hospital, Sheffield, S10 2TH.

Copper (Cu) and the pyrolyzidine alkaloid retorsine (RET) cause synergistic liver damage and hepatic Cu accumulation in rats (Out 1992; 33: S3). This study investigated the transfer of RET from mother to pup via lactation and the effect on the neonatal liver, which is already high in Cu at birth. 6 rats with newborn litters were fed either control (CONT) diet (3 dams, 28 pups) or diet containing RET at 50mg/kg food (3 dams, 33 pups) for 3 weeks. At weaning, 35 pups were sacrificed and livers taken for Cu analysis. Pups whose mothers had received RET had lower body weights and higher liver Cu levels than pups from CONT dams (mean=SEM. p<0.01, Mann-Whitney test). Liver Cu levels at weaning were normal (19±1ug/dry wt) with no histological damage.

<table>
<thead>
<tr>
<th>NO OF PUPS</th>
<th>BODY WEIGHT (g)</th>
<th>LIVER Cu (ug/dry wt)</th>
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<tbody>
<tr>
<td></td>
<td>CONT</td>
<td>RET</td>
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<tr>
<td></td>
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</tbody>
</table>

The remaining pups (13 male, 13 female) were weaned and fed either CONT diet or Cu loaded (2g CuSO4/kg diet) for 15 weeks. Weight gain for each sex was normal as were LFTs which were performed every 4 weeks. At sacrifice, liver Cu was as shown (mean=SEM).

The liver of adult rats with raised liver Cu showed mild focal necrosis but were otherwise normal. Conclusions: (a) RET was transferred to pups in the milk of lactating rats (b) RET in milk prevented the normal excretion of neonatal liver Cu (c) this effect persisted, later causing an inability to handle dietary Cu which may be a possible explanation for the dramatic liver Cu levels and damage seen in Indian Childhood Cirrhosis.

T130

Biliary T130-T140

Peroperative Cholangiography Through the Laparoscopic Bladder during Laparoscopic Cholecystectomy

AD Fou, AJ Baigrie, R Cobb, BL Douling

Departments of Surgery, Northampton General Hospital and John Radcliffe Hospital, Oxford.

Laparoscopic cholecystectomy has rekindled the controversy surrounding the need for peroperative cholangiography. The increasing use of endoscopic retrograde cholangiopancreatography when stones are clinically suspected has meant that cholangiography during laparoscopic cholecystectomy is primarily used to provide a so-called "roadmap" of biliary anatomy.

Fifty patients underwent gall bladder cholangiography (GBC). There were no deaths and no morbidity relating to bile duct injury. It was successful in 48(88%) patients. This compares with a success rate of 56% to 88% for cystic duct cholangiography (CDC). Although normal anatomy was demonstrated in all patients, identification of a short or particularly wide cystic duct simplified dissection, providing reassurance that the common bile duct (CBD) was not being inadvertently ligated.

This study demonstrates that GBC has a high success rate while being a safe and simple procedure. It is undoubtedly quicker and there is virtually no learning curve. Unlike CDC, difficult anatomy is demonstrated prior to dissection and possible injury of the cystic duct. Thus GBC is a satisfactory alternative to CDC and its speed and simplicity will encourage the use of peroperative cholangiography which has been shown to reduce the risk of CBD injury in laparoscopic cholecystectomy.

T131

RESULTS OF AGGRESSIVE SURGICAL MANAGEMENT OF KLTASKIN TUMOURS

JP Harrison, AD Meyer, JAC Buchels and P MCMaster.

Liver & Hepatobiliary Unit, Queen Elizabeth Hospital, Birmingham.

The management of high bile duct carcinoma remains a difficult clinical problem. We have reviewed the results of the treatment over the last ten years of 39 patients with Klatskin tumours. 22 patients (56%) were females and the median age of the patients was 57 (26-78) yrs. Presentation was most commonly with painless jaundice in 30 patients (77%). 8 patients had pain in addition and one patient presented with a mass. The patients undergoing liver transplantation had a history of primary sclerosing cholangitis prior to developing deeper jaundice as a result of their hilar cholangiocarcinoma. 28/39 patients (69%) had lost weight prior to presentation. The biliary strictures were imaged at ERCP alone in 22 (56%), PTC in 14 (36%) and both of these investigations in 3. Three patients had extended resections of liver and extra-hepatic bile ducts, whilst 7 patients had orthotopic liver transplantation. The median survival of this group was 45 (2-292) weeks compared to 12.5 (2-192) weeks for the patients with irresectable tumours (p=0.001). Laparoscopy was employed as a staging procedure in 7 patients (16%). 22 patients (56%) had surgical bypass, either with a stent in the first 5 years or a segment IV duct bypass recently. 4 patients had a stent placed at ERCP and 4 had a radiologically placed stent. Chemotherapy was given to 3 patients (8%). Median bilirubin at presentation was 256 (8-754)umol/l with an alk. phos. of 1040 (362-4570)iu/l. Our results suggest that an aggressive approach to the management of Klatskin tumours can result in satisfactory palliation for the majority of patients and relatively prolonged survival in patients with resectable tumours.

T132

THE ROLE OF MAST CELLS IN THE PATHOPHYSIOLOGY OF OBSTRUCTIVE JAUNDICE

R Clements, M Emiris, L Halliday, O Campbell and D Forlanda

Dept of Surgery & Clinical Chemistry, The Queen’s University of Belfast.

 Mast cells are activated by a wide range of immunological and non-immunological factors. In obstructive jaundice, systemic concentrations of endotoxin and bile acids are increased, both of which potentiate the release of histamine and other mediators from mast cells in vivo. In this study we investigated the relationship between bile acid profiles, systemic endotoxemia, blood and organ histamine levels in vivo in experimental biliary obstruction.

Method: Forty-five male Wistar rats(250-300g) were allocated equally to one of three groups - no operation, Sham operation and Bile Duct ligation (BDL). After 3 weeks, splanchnic blood and bile were assayed for histamine, serotonin, histamine concentrations with and without histamine (H1)-receptor blockade. Peritoneal mast cells (PMC) were isolated after lavage with Tyrode’s buffer and assayed for Histamine content per cell.

Results: BDL rats had significantly elevated histamine, TCA, AGCA, and histamine concentrations compared with control animals (Mann-Whitney U). Blood histamine concentrations correlated with increased plasma TCA (P<0.011) and AGCA (P=0.001)[Spearmen Rank]. Organ histamine content (mg protein) was significantly increased in the BDL rats compared with control groups (Liver (P<0.0001), Lung (P<0.05) and Kidney (P<0.011). Mann-Whitney]. Peritoneal mast cells from BDL rats had decreased histamine content per cell (pg) and PMC histamine content correlated inversely with both blood and organ histamine concentrations. (Data expressed as mean±SEM. *P<0.001. ±P<0.05).
T133

EARLY COMPLICATIONS AND MEAN 8 YEAR FOLLOW-UP AFTER ENDOSCOPIC SPHINCTEROTOMY IN YOUNG FIT PATIENTS. T.C.K. Thom, R. Kennedy, F. A. O’Connor. Dept. of Medicine, Altnagelvin Area Hospital, Londonderry, N. Ireland, UK.

Endoscopic bile duct sphincterotomy (ES) is the preferred treatment for many elderly and high-risk patients with common bile duct stones. However, the high popularity of laparoscopic cholecystectomy has the potential to increase the number of young, fit patients referred for ES. However the role of ES in these patients has yet to be established, largely because of the early complication rate and long-term effects of sphincter ablation are determined, the former being apparently unrelated to age and medical problems. We evaluated the early complications and incidence of long-term biliary symptoms in patients aged less than 55 yrs.

All patients aged 55 or less who had ES from 1980-92 were identified from a database and details were obtained from their notes. ES and stone extraction where appropriate were performed in the standard manner using standard equipment. Follow-up was performed by review of notes and patients and their general practitioners (GP) were asked to complete a standardized questionnaire.

During 1980-92, 45 out of 394 patients (11%) undergoing successful ES were aged 55 yrs or less (mean 44 yrs, range 22-55). The indications for ES were for stones in 26 (62%) and papillary stenosis in 16 (38%). None had serious medical conditions. 2 had diabetes and 1 had hypertension. 19 of 26 had stones successfully extracted. Complications occurred in 2 patients who had haemorrhage, 1 requiring surgery, 2 patients had baskets impacted requiring surgery. None died. Thus the early complication rate was 9.5%, surgery was required in 7.1% and there was no mortality.

Data from 30 of these patients were obtained with a mean follow-up period of 8 years (range 10 mths-12 yrs), 29 had cholecystectomies; 28 electively and 1 during laparotomy for an impacted basket in 2 patients (10%) had further biliary problems: 1 with a stricture due to chronic pancreatitis also had a pancreatic sphincterotomy 9 yrs ago had no problems until last year, 1 had an unplanned repeat ES 6 mths later for restenosis and 1 had possible cholangitis. 4 (13%) had recurrent abdominal pain of uncertain cause. The rest had no problems related to the biliary tract. This suggests that in young fit patients, ES may be a relatively safe procedure and long-term results may be comparable with those of surgical procedures.

T134

THE IMPACT OF ENDOSCOPIC EPHINPHITOTOMY AND LAPAROSCOPIC CHOLECYSTECTOMY ON THE MANAGEMENT OF GALLSTONES IN A DISTRICT GENERAL HOSPITAL. AF BARMGREN J A EYRE-REES. TAUNTON AND SOMERSET HOSPITALS. TAUNTON TAI 5DA.

The impact of endoscopic sphincterotomy (ES) and laparoscopic cholecystectomy (LC) upon the management of gallbladder disease in a single health district of 300,000 has been assessed by prospective audit of all NHS and private patients treated in the 5 year period 1989 to 1993. The district in 1989 and LC in 1991. The cholecystectomy rate increased from 51 per 100,000 population in 1989 to 61 per 100,000 in 1992.

The number of patients treated for duct stones (SD) increased from 11 per 100,000 in 1988 to 29 per 100,000 in 1992, due to increasing numbers of patients > 65 years having ES of whom 62% had their gall bladder left in situ. Policies of early ERCP in gallstone pancreatitis and jaundice and selective ERCP before LC increased ERCP workload for gallstone disease beyond that predicted by the British Society of Gastroenterology to 44 procedures per 100,000. Thirty day mortality was 0.2% for 615 open cholecystectomies (OC), 0% for 213 LC, 2.7% for 147 surgical explorations of common bile duct (EBD), and 0% for 137 ES. Major duct injury occurred in 0% after OC, 0.5% after LC, 0.7% after EBD and 0% after ES. Mean Hospital stay was > 2 days for OC and 4 days for OC.

Thus more patients have been treated for gallstone disease since ES and LC became available. More patients have undergone choledochoscopy, more elderly patients had ES with the gall bladder left in situ and more patients had a diagnostic ERCP. Duct injury rates after minimal access surgery (LC and/or OC) were similar to those after laparotomy (OC and/or EBD).

T135

IATROGENIC BILE DUCT INJURY COMPLICATING LAPAROSCOPIC CHOLECYSTECTOMY: TJ Boyle, AG Bean, ME Roddie, HJ Scott, RCN Williamson, NA Habib. The Department of Surgery, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 ONN.

Laparoscopic cholecystectomy has revolutionised the management of symptomatic and complicated cholelithiasis, but early reports indicate an increased incidence of bile duct injury (up to 1%) versus the conventional operation (0.1-0.4%). Eight patients referred to a specialist hepatobiliary unit with this complication for following laparoscopic cholecystectomy are reported and a protocol for the management of these patients is described.

All patients underwent elective laparoscopic cholecystectomy for biliary colic (6) or following acute cholecystitis (2). Intra-operative difficulties were reported in 6 patients, which included poor view or "anomalous" anatomy, and inflammation or impacted gallstones. In 7 patients a bile leak was reported in the early post-operative period. Four patients were referred early (5-22D) and 4 late (3-6 mo). Three patients had unsuccessful attempts at biliary reconstruction prior to referral.

Following admission the biliary tree was imaged by PTC alone, or in combination with ERCP. Visceral angiography was performed to identify concomitant vascular injuries. Patient details are shown:

<table>
<thead>
<tr>
<th>Pat. Age</th>
<th>Sex</th>
<th>Delay to Injury</th>
<th>Procedure</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35F</td>
<td>2D</td>
<td>Biliary peritonitis</td>
<td>Rt. hepatico-jejunostomy</td>
</tr>
<tr>
<td>2</td>
<td>62M</td>
<td>5D</td>
<td>Biliary peritonitis</td>
<td>Drainage</td>
</tr>
<tr>
<td>3</td>
<td>5F</td>
<td>9D</td>
<td>Controlled bile leak</td>
<td>Insertion of T-tube</td>
</tr>
<tr>
<td>4</td>
<td>62M</td>
<td>10D</td>
<td>Biliary peritonitis</td>
<td>Hepatico-disrupted bile tree</td>
</tr>
<tr>
<td>5</td>
<td>62F</td>
<td>3 mO</td>
<td>Jaundice</td>
<td>Hepatico-jejunostomy</td>
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<tr>
<td>6</td>
<td>3F</td>
<td>4 mO</td>
<td>As above</td>
<td>As above</td>
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<tr>
<td>8</td>
<td>59F</td>
<td>4 mO</td>
<td>Jaundice</td>
<td>Hepatico-disrupted bile tree</td>
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</tbody>
</table>

Biliary reconstruction was via Roux-en-y hepaticojejunostomy. The anastomosis was stented and a jejunal access loop fashioned. Following a normal HIDA scan at 10 days the stents were clamped and the patient discharged. Sints were removed at 6 weeks following normal stentography. Patients are seen at 6-monthly intervals thereafter. To date all remain well.

T136

HISTOLOGICAL AND RADIOLOGICAL ABNORMALITIES OF THE BILIARY TREE IN HIV RELATED CHOLANGIOPATHY. Hunt JB, Taylor GP, Thomas HW, Wilkins J, Goldin R, Summerfield JA. Departments of Medicine, Communicable Diseases and Pathology, St Mary's Hospital Medical School, Imperial College of Science Technology and Medicine, London W2 1PG.

Biliary abnormalities are well recognised in HIV infection. Both Cytomegalovirus (CMV), Cryptosporidiosis (CS) and Microsporidiosis (MS) have been found in biliary aspirates or ampullary biopsies and implicated in the disease process. We have used biliary tree imaging, histology and biopsy to determine the extent of biliary involvement in HIV. Biopsy samples were taken during ERCP in HIV positive patients undergoing endoscopic retrograde cholangiography (ERC).

8 male patients (ages 28-64 yrs), 7 with AIDS and 1 stage IV E, were studied. Liver function tests were bilirubin median 11 (range 7-36) umol/l, alkaline phosphatase 445 (163-1290) u/l, alanine transferase 48 (29-108) u/l. Biopsy was performed after routine ERC. Sphincterotomy was performed in 4 patients. In the remaining 4 cases biopsies were obtained through an intact sphincter.

4 patients had intrahepatic and extrahepatic duct irregularity. 1 each had intrahepatic disease or common bile duct dilatation to the ampulla alone. 2 studies were normal.

Adequate tissue for histological examination was obtained in 7 cases. The tissue was examined at multiple levels by light microscopy using haematoxalin and eosin stained sections and in addition stained with Giemsa, periodic acid Schiff and Ziehl-Neelsen stains.

4 patients had a chronic inflammatory infiltrate and in 3 CS were identified. In 1 of these 3 CMV inclusion bodies were also seen. Mycobacterium avium intracellulare was not identified. In 2 cases, 1 with a normal ERC, no opportunistic agents were identified.

No patient experienced haemorrhage, 1 developed pancreatitis which discharged by 5 days.

Endobiliary biopsy is safe and can be performed without sphincterotomy. A range of infecions may be found. These results suggest the biliary changes in HIV are not due to a single pathogen.
GALLBLADDER INERTIA TO BOLUS INTRAVENOUS AMINO ACIDS IN PATIENTS RECEIVING INTRAVENOUS NUTRITION. BP Sanders, S Halligan, G Zoli, SM Camacho, LID O'Donnell, MRI Farthing. Departments of Gastroenterology and Radiology, St Mark's and St Bartholomew's Hospital, London E1.

Previously it has been shown that bolus administration of intravenous amino acids promotes gallbladder contraction in healthy volunteers by inducing cholecystokinin release. We have now examined how bolus administration of intravenous amino acids affects gallbladder motility in patients receiving intravenous nutrition (IVN).

Patients on IVN received rapid intravenous infusions of an amino acid mixture (Synthamid 14 without electrolytes, 85g/l) at doses of either 125ml in 5 min or 250ml in 20 min. Gallbladder volumes were determined by ultrasonography before and at 5 min intervals for up to 60 min after commencing the infusion.

Five patients received infusions of 125ml in 5 min (a regimen which previously has been shown to produce an ejection fraction (EF) of 64% in healthy volunteers); with this regimen the mean EF was only 2% (range 35 to 84%) - none of these patients had EF's > 50%. Five further patients received infusions of 250ml in 20min (which previously produced an EF of 61-77% in healthy volunteers); with this regimen the mean EF was 16% (range 66 to 61%) - 2 of the patients in this group had EF's > 50%

Thus inducing gallbladder contraction by means of bolus intravenous amino acids is more difficult in patients receiving IVN than in healthy volunteers and factors which reduce gallbladder motility such as opiate consumption, hyperglycaemia and visceral myopathy need to be identified prior to commencing prokinetic therapy.

SIMVASTATIN 40 MG PLUS URSODEOXYCHOLIC ACID 750 MG DAILY FOR GALLBLADDER STONE DISSOLUTION. MALCOLM C. BATESON

GENERAL HOSPITAL, BISHOP AUCKLAND, COUNTY DURHAM.

Ursodeoxycholic acid (UDCA) has a moderate efficacy, and HMG-CoA reductase inhibitors such as simvastatin have a low efficacy, for dissolution of radiolucent cholesterol-rich stones. The drugs work synergistically to reduce biliary cholesterol content and the combination might be expected to produce more rapid and effective stone dissolution.

Ten patients aged 28-83 years (5 female) with radiolucent gallbladder stones 3 to 10mm in diameter in a gallbladder that was not functioning on oral cholecystography, and with no evidence of calcification on CT scanning, were treated with UDCA 750 mg plus simvastatin 40 mg, for up to 24 months. None had severe recurrent symptoms or complications and all had refused surgery. Review ultrasonography was performed at 2-3 months, 6 months and then 6-monthly. Complete dissolution was confirmed by repeat ultrasonography. All patients were reviewed frequently by the same gastroenterologist throughout therapy. There were no side-effects and symptoms improved or disappeared. No patient developed cholestasis. Serum cholesterol fell in every patient (mean 6.14 to 4.40 mmol/l, p < 0.01).

One female aged 28 with 3 mm stones had complete dissolution at 2 months. One male aged 59 with 5 mm stones had complete dissolution at 6 months. One female aged 49 with 4 mm stones had partial dissolution at 3 months and stones were completely dissolved at 2 years. One female aged 28 with 5 mm stones has partial dissolution at 6 months. One male aged 44 with 7 mm stones had partial dissolution at 6 months but no further change at 2 years. The other 5 patients showed no change in stones 3, 9, 10, 10 and 10 mm at 6 - 24 months.

CONCLUSION

These patients were selected to be ideally suited to dissolution therapy, but the results were not better than those obtained with UDCA only. Small stone size is the main criterion for good results.

STENTING AS A DEFINITIVE PROCEDURE IN BENIGN BILIARY DISEASE

BT Johnston and PA O'Connor

Altsgeavie Area Hospital, Londonderry, N. Ireland

Aim: To assess the outcome of patients who had a biliary stent inserted as a definitive procedure for benign disease.

Methods: Follow-up of 31 consecutive patients, 13 male, of mean age 75 years (range 35-90) for a mean period of 26 months (range 1-123). Stents were inserted for intractable stones in 20 patients, benign strictures in three and both in eight.

Results: Ten patients died a mean of twelve months after the procedure. One stricture patient died from causes related to the biliary tract. The remaining 21 patients were followed for a mean of 29 months and all are asymptomatic. Because of stent blockage or cholangitis, two patients underwent open surgery and four required a median of one (range 1-4) further ERCPs. A stricture was present at initial stenting in five of these six patients. Requirement for further procedures were statistically more frequent (45% v. 9%, p < 0.05) in patients with strictureing.

Conclusions: Stenting for intractable biliary stones in patients unfit for surgery is a safe procedure with 0% mortality and only 9% morbidity. Stenting in the presence of biliary strictures carries a significantly greater risk of future complications.

THE IMMUNOCYTOCHEMICAL CHARACTERISTICS OF HUMAN GALLBLADDER INNervation

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Various immunocytochemical characteristics of the human gallbladder innervation and epithelial secretory status were studied in an attempt to identify whether consistent changes might be found in gallstone formation and cholecystitis.

Methods: Four gallbladders were studied, 32 had been removed for symptomatic disease and 12 gallstone free gallbladders were obtained from donors of hepatic transplants. The gallbladders were assessed by haematoxylin and eosin staining and by immunocytochemical staining for neuronal and axonal structures, nerve sheaths, neuroendocrine cells and markers of epithelial secretion and differentiation.

Results: A significant reduction in the number of stainable nerve fibres and ganglion cells in the lamina propria was observed in inflamed gallbladders as compared with normal. No differences between normal and inflamed gallbladders were found in neuroendocrine markers (VIP, substance P, somatostatin, Synaptophysin) and markers of secretory and differentiation status (Carbonic anhydrase, Prostaglandins E2, F2α, GST's α, γ, δ class).

Conclusion: In the abnormal gallbladders there are less nerve fibres and ganglion cells as compared with normal. Whether this has relevance for abnormal gallbladder function as a causal factor, or if a result of tissue damage remains to be determined.
Surgery T141–T149

T141

ACUTE APPENDICITIS - WHY DOES IT HAPPEN AND HOW IS IT MANAGED?
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GENERAL HOSPITAL, BISHOP AUCKLAND, U.K.

Acute appendicitis is one of the commonest causes of abdominal surgery in Britain. The cause of this condition is not properly understood and there may be room for improvement in managing suspected cases.

METHODS
All appendix histology was identified from the histopathology department at Bishop Auckland General Hospital for 12 months from 1st April 1991. Patients’ case records were identified and notes requested and scrutinised by a consultant gastroenterologist who had personal involvement with the cases. True diagnosis, date of operation, place of residence and time of operation were identified. Results were subjected to subgroup analysis.

RESULTS
There were 156 pathology reports, and 148 case records were seen (95% retrieval rate). 93 cases had true acute appendicitis, 46 cases had false acute appendicitis, and 9 cases had appendectomy as an elective procedure. No definite diagnosis was established in 33 out of 46 cases who had urgent appendectomy but no acute appendicitis; there were a variety of other diagnoses in the other 13. Time of day of operation was not related to correctness of diagnosis.

Appendectomy was commoner in the summer quarter July - September (10.3 cases per month) than in the first six months of the calendar year (6.2 cases per month). There was no geographical clustering.

Luminal contents were identified in 5/93 true acute appendicitis and 8/46 false acute appendicitis.

CONCLUSIONS
- False-positive rate for diagnosis of acute appendicitis is 33%.
- There is no evidence to support the obstructive hypothesis of aetiology.
- There is no evidence of clustering.

T142

MOTOR ACTIVITY AND INTRAGASTRIC PH CHANGES ASSOCIATED WITH POST OPERATIVE BILE VOMITING
PK Small, MA Loudon, FC Campbell. Department of Surgery, Ninewells Hospital and Medical School, Dundee, Scotland.

Vomiting is a common post operative complication despite preoperative fasting, but has obscure pathophysiology.

Hi: 24 hour solid state monitoring, this study has sought (i) gastric, duodenal and jejunal motatur activity and (ii) intragastric pH changes indicative of enteral bile shifts, in 13 patients both pre operatively and for 24 hours after cholecystectomy. Motility alone was assessed in 7 further patients.

RESULTS: Preoperative studies demonstrated stable pH and normal fasting motility in all subjects. Post operatively, abnormal motor patterns comprising frequent incomplete contracture bursts (termed phasic bursts [RBs]) were observed in all patients. Phasic bursts which migrated retrogradely [RPBS], were associated with bile vomiting (35 RBPS occurred in 9 vomiting vs 7 RBPs in non vomiting, p<0.05).

Post operative gastric pH (pH = 4.7 +/- 0.52) was higher than preoperative pH (PH = 2.9 +/- 0.43, p<0.01), but a fall in pH was recorded after bile vomiting episodes (5.2 +/- 0.47 [before] vs 4.8 +/- 0.43 [after]. p<0.05).

Conclusion: Retrograde phasic bursts are associated with post operative bilious vomiting and probably induce entero gastric bile reflux. The consequent rise of intragastric pH falls after bilious vomiting.

Chi-square test
Mean +/- SEM
Paired t test

T143

INTRAVENOUS CHOLANGIOGRAPHY IN THE PREOPERATIVE ASSESSMENT OF PATIENTS FOR LAPAROSCOPIC CHOLECYSTECTOMY.
S.J. Wigmore, K. Wood and D.A.D. Macleod

Departments of General Surgery and Radiology, St. John's Hospital at Howden, Livingston, West Lothian.

Considerable controversy surrounds both the need to investigate the common bile duct (CBD) for gallstones preoperatively in patients undergoing laparoscopic cholecystectomy and the method of investigation. This paper examines the role of intravenous cholangiography (IVC) in the detection of CBD stones in the first 100 patients considered for laparoscopic cholecystectomy at this institution. The clinical diagnosis of cholelithiasis was confirmed in all cases either by ultrasound scanning (92) or by oral cholecystography (8). Liver function tests (LFT) and (IVC) were performed preoperatively on all patients. IVC detected CBD stones in 10 patients. 5 of these patients had a dilated CBD and 5 did not. Only 2 cases of CBD stones were detected by ultrasound scanning. All CBD stones were confirmed by either endoscopic retrograde cholangiopancreatography or by exploration of the CBD. 25 patients who did not have CBD stones on IVC had elevated LFT's. 4 patients who had normal LFT's and a normal ultrasound on USS did have CBD stones detected by IVC. No patients had a reaction to the contrast medium used.

IVC is a safe investigation with high diagnostic accuracy in the assessment of CBD stones and a very low false positive rate (0 in this study). Ultrasound scanning and LFT's are unreliable at predicting or detecting duct stones.

T144

RESTORATION OF INTESTINAL CONTINUITY FOLLOWING HARTMANN'S PROCEDURE: TIMING, METHODS AND PATIENT SELECTION
S.J. Wigmore, G.S. Duthie, I.E. Young, E.M. Spalding and J.B. Rainey

Department of General Surgery, St. John's Hospital at Howden, Livingston, West Lothian.

Restoration of intestinal continuity following Hartmann's procedure (HP) is important for patient-related high morbidity; (anastomotic leak rates 4-17%) and mortality (1-4%). 148 patients, under the care of 7 different surgical units underwent reversal of HP over a 5 year period, which represents the largest series yet studied.

The patients who underwent reversal represented 53% of those undergoing HP during the time period. The mortality of the study group was 0.6%, anastomotic leak rate was 4% and incidence of anastomotic stricture was 8%. The mean time interval between HP and reversal was 142 days and no relation was found between timing and complications. Anastomotic stricture occurred significantly more commonly in stapled than sutured anastomoses (p<0.05), however leaks were equally common in both types. The group who developed major complications were of the same age as the rest of the study group and there was no difference in preoperative state.

Of the patients undergoing HP who were not reversed a significant proportion were excluded from further surgery due to advanced malignancy, short life expectancy or personal preference for permanent colostomy. We believe that the low complication rates reported in this study are attributable to the high level of experience of operator performing this technically difficult procedure consultant 66% and senior registrar 33%.
CONVERSIONS AND COMPLICATIONS IN LAPAROSCOPIC CHOLECYSTECTOMY: AN INDEPENDENT AUDIT.
R.J.C. STEELE, K. MARSHALL, M. LANG, J. DORAN
DEPARTMENT OF SURGERY, UNIVERSITY OF NOTTINGHAM.

Commencing with the introduction of laparoscopic cholecystectomy (LC), a detailed, independent, prospective audit of all cholecystectomies carried out in a teaching hospital with eleven general consultant surgeons has been in operation for two years. Of 410 operations, 283 were intended LCs; in the first year, 58% were intended LCs, and this rose to 79% in the second year. Conversion rates were 20.5% for both years. Mean operating time for LC was 123 mins for year 1, and 123 mins for year 2, compared with 92 mins and 161 mins respectively for open cholecystectomy (OC). The overall complication rate was 10% for LC and 20% for OC. This included 6 bile duct injuries in the LC group (18), and one in the OC group (6.8%).

Six consultants had performed over 20 LCs personally, and among these, conversion rates varied from 7-29%, and the complication rate varied from 2-23%. A statistically significant inverse correlation was found between the complication rate and the conversion rate (r = -0.91, p<0.01).

This unselective audit of a group of general surgeons introducing LC into their practices has revealed complication rates and conversion rates which are higher than those reported in most of the published literature, but it may be more representative of practice throughout the UK. It also suggests that complication rates may be related to willingness to convert to open operation.

THE PERITONEAL CYTOKINE RESPONSE TO SURGERY
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Cytokines are biochemical mediators which control the acute phase inflammatory response. Although the human systemic cytokine response to sepsis and trauma has been extensively studied, the local response to injury in man has not been fully investigated. This study was to investigate the peritoneal cytokine response to operation.

Peritoneal fluid was sampled from patients 1h, 3h, 5h and 18h following elective abdominal operation for non-inflammatory disease (n=6, median age 69y; range 55-80). Bacteriological studies of peritoneal fluid were negative in all patients. High concentrations of TNF were detected at 1h (median 137pg/ml; range <10-532) and 3h (272; <10-300) after which levels rapidly declined (5h: 28; <10-304, 18h: <10; <10-16). Low concentrations of IL-1 were detected 1h after surgery (18pg/ml; <10-104), but this was followed by a rapid increase at 3h (154; 77-261) which was sustained at 5h (153; 66-291) and 18h (152; 41-253) (P<0.05, ANOVA). A more rapid and sustained rise in IL-6 was seen, 1h: 40pg/ml; 3-88, 3h: 137; 115-285, 5h: 241; 14-346 and 18h: 238; 118-416 (P<0.05).

This study gives an insight into the local dynamic cytokine response seen in human peritoneal inflammation. Peak concentrations of TNF and IL-1 are shown to precede maximal concentrations of IL-6, which is present in much higher concentrations. These results are consistent with the sequence of release of cytokines initiated by inflammatory cells localised to the peritoneum following operative injury.

(Submitted for publication).

INFLUENCE OF OBESITY ON OPERATIVE DIFFICULTY AND POSTOPERATIVE COMPLICATIONS IN OPEN AND LAPAROSCOPIC CHOLECYSTECTOMY
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Obesity is generally considered to increase the risks and technical difficulty of operations. Its influence in laparoscopic surgery is not clear, and this prospective study addresses the relationship between obesity, technical difficulty and outcome in open (OC) and laparoscopic (LC) cholecystectomy.

Body mass index (BMI=kg/m²) was used to classify patients undergoing both OC (n=116) and LC (n=216) as: normal weight (BMI <25), overweight (OW) BMI 25 - 29.9 and obese (OB) BMI > 30. The technical difficulty of each operation was graded by the operator and complications were recorded.

Access (x²=16.6 p<0.001) was improved, dissection was easier (x²=9.7 p<0.01) and the operation was a whole less difficult (x²=11.5 p<0.001) in LC vs OC. Access in LC was better in NW vs OB patients (x²=11.1 p<0.01) but other measures of technical difficulty were not affected by BMI. For OC bleeding was more problematic and operations were more difficult in OW vs NW patients (p<0.05). BMI had no effect on the time required to complete LC (median 120 min) but in OC the operative time was longer for OB (median 100 min) vs NW (median 75 min) patients. The need for conversion from LC to OC (44/216=20%) and the bile duct injury rate (3/216=1.4%) were not influenced by BMI. The overall complication rates in the NW group was 11/147 (7.5%), v OW=15/122 (12.3%) and OB=5/63 (7.9%) (NS).

Mean follow-up was 14.8 months.

Obesity does not increase the technical difficulty or complication rates of LC and should not be regarded as a contraindication for this procedure.

LAPAROSCOPIC NISSEN FUNDOPLICATION
C Royaton, M Lansdown, Hull Royal Infirmary, Hull.
W Brough, Stepping Hill Hospital, Stockport.

Anti-reflux surgery is ideally suited for the minimally invasive approach and several laparoscopic anti-reflux procedures have therefore been described. We have used the laparoscopic approach to perform a floppy Nissen, the wrap being made around a 58 French gauze bougie.

Between December 1991 and May 1993 we attempted 61 procedures - 29 male, 12 female, age 27-43 years.

Concomitant procedures have been performed in 3 patients. 2 a highly selective vagotomy, 1 a cholecystectomy. 4 patients had previous surgery in the region, 3 a prior vagotomy and pyloroplasty, 1 a trans thoracic repair of hiatus hernia. Of the 61 patients, 1 were converted to open, 2 because of a very short thickened oesophagus, 1 because of uncertain anatomy.

Complications. 1 death has occurred from acute pancreatitis and appeared unrelated to the direct surgical procedure. 1 patient had a perforation of the stomach which required a laparotomy. 2 have required further surgery for dysphagia due to too tight a crural repair.

Of the 57 patients who had a laparoscopic Nissen satisfactorily performed, 35 were discharged home on the 2nd post operative day, 16 on the 3rd, 8 remained in hospital for longer than 3 days, the majority for chest infections.

Follow up. 34 patients are available for follow up at one year. 1 has mild gas bloating, the remaining 13 are asymptomatic. Of a further 14 patients at 6 months 1 has moderate dysphagia, 2 mild gas bloating, 11 are asymptomatic. Mild to moderate dysphagia has been observed in half of the patients in the early post operative phase, usually settling by 3 months.

Pre-operatively 24hour ambulatory pH studies have been performed. So far 10 patients have had repeated studies at 6 months post operatively. 9 patients have no reflux at all, 1 has minimal reflux but is asymptomatic.
Traditional elderly patients (70 years) who present as surgical emergencies with colorectal cancer have poor outcome. The purpose of this study was to examine a large consecutive population of patients with colorectal cancer. (n = 93, males 36%, mean age 71.3, age range 43-97) who underwent scheduled or emergency surgery in order to identify from preoperative data risk factors that may be associated with 30 day mortality.

Patients were subdivided by age (70 years vs 70 years) mode of admission (elective vs emergency), and nature of surgery (scheduled list vs emergency). Variables measured for all patients included: operative HB, nutritional status (serum albumin), smoking status, other pre-existing disease, family history, ASA score, grade of operating surgeon and anaesthetist, perioperative blood transfusion and Duke's stage of primary. The 30 day mortality rate is shown below:

<table>
<thead>
<tr>
<th>Age</th>
<th>Elective adm.</th>
<th>Emergency adm.</th>
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<tbody>
<tr>
<td>70</td>
<td>3/67 5%</td>
<td>3/24 13%</td>
</tr>
<tr>
<td>70</td>
<td>10/100 10%</td>
<td>5/33 15%</td>
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</tbody>
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*Fisher's Exact Test*

This shows that the higher mortality in the older age group is not statistically significant and of all the factors measured, the only correlation with 30 day mortality was preoperative nutritional status (serum albumin >30g/dl) status in patients undergoing scheduled surgery.

We conclude that emergency surgery following emergency admission for colorectal cancer carries significant risk, however, since we are unable to determine factors associated with poor outcome from routine preoperative data, all patients who present with this should be offered surgery regardless of age.

### Endoscopy T150–T158

A NOVEL ENDOSCOPIC METHOD FOR DILATING DIFFICULT BILIARY STRUCTURES. RAM van Someren, MJ Bengt, CC Ainley, MJ Glyn, CP Swan. Department of Gastroenterology, The Royal London Hospital, London E1 1BB.

During ERCP, an impasse may be reached when a tight biliary stricture is encountered which allows passage of a guidewire, but not of 7 Fr polyurethane or taper-tipped biliary dilators, nor of other type balloon dilators. In such cases, there is no alternative to immediate percutaneous transhepatic drainage, or non-sterile contrast medium has usually been introduced proximal to the stricture, and the risk of subsequent cholangitis is considerable.

The reason for failure to dilate such strictures is the inability to exert enough force at the stricture because of looping of the dilating instrument in the duodenum between the ampulla andoduodenoscope. We have therefore developed a method which relies on traction rather than a pushing force, thus avoiding duodenal looping.

A floppy-tip 400cm "tracer" wire (Wilson-Cook) is usually used to cross the stricture. A 11.5 Fr Soehendra stent retriever (Wilson-Cook) is then introduced via the duodenoscope, and abutted against the distal edge of the stricture. The tip of the stent retriever has a "self-clamping" external thread, designed to thread the interior of plastic stents prior to stent removal. In the case of strictures, clockwise rotation of the torque transmission Soehendra instrument pulls the tip into and across the stricture. Once across, the device is removed by pulling it down through the stricture, leaving the guidewire in situ and thereby maintaining access. Such a procedure allows an Olbert balloon dilator to be subsequently placed across the stricture, maximising the dilatation prior to conventional stent insertion. An additional advantage is that samples of tissue are often recovered in the screw thread, and can be sent for histopathological analysis.

In the four months we have used the new method, we have performed a total of 238 cases of which 6 followed the category of tight malignant strictures allowing passage of a guidewire only (4 hilar and 2 low common bile duct). 5 were successfully stented at the initial ERCP, and 1 went on to stenting by combined procedure due to loss of position of the guidewire across the stricture.

This technique appears to be a useful additional method to enable drainage of obstructed system, or progression to a malignant biliary stricture. The role of this technique in the management of benign biliary strictures has yet to be ascertained.

### T152

THE OUTCOME FOLLOWING SELECTIVE ENDOCUTIC FOLLOW-UP OF HEALED GASTRIC ULCERATION. M Winstel, Y Mohsen, M Hallissey, WH Allum, JW Fielding. Department of Surgery, Queen Elizabeth Hospital, Edgbaston, Birmingham.

In a screened population of 1748 patients over 40 years of age with dyspepsia, a gastric ulcer was diagnosed in 82 (4.7%). (single = 68, multipie = 14, cardiac = 8, fundus = 3, body = 15, antrum = 56). Histological assessment of 6 adjacent mucosal biopsies revealed chronic atrophic gastritis (CAG) = 13 (15.9%), regenerative changes (RC) = 26 (31.7%), atypia (A) = 15 (18.3%), dysplasia (D) = 7 (8.5%) (mild = 5, severe = 2) and carcinoma (C) = 1 (1.2%). Following treatment, median = 12 weeks, 76 (92.7%) ulcers had healed. Four patients (4.9%) with a persistent ulcer underwent resection and two patients were lost at follow-up. 53 patients (64.6%) remained symptomatic.

48 patients (56.1%) with a healed ulcer but persistent histological abnormalities underwent repeat 6 monthly surveillance for a median 31 (7-69) months. 16 patients (34.8%) developed a recurrent ulcer (single = 11, multiple = 5) at a median of 16 (7-39) months which had healed at a median of 3 (2-11) months in 15 patients with one requiring resection at 24 months. Three patients (6.5%) developed carcinoma (1 stage I = 1, stage II = 1) at a median of 13 (4-36) months. 10 (37.0%) of 27 patients with no evidence of recurrence or neoplasia developed new potentially premalignant histological lesions, CAG = 1 (3.7%), RC = 5 (18.5%), A = 8 (29.6%), D = 6 (18.6%). D = 4 (14.8%). A selective follow-up programme for healed gastric ulcers based on initial adjacent histological features is associated with a high detection rate for recurrent ulceration, potential premalignant lesions and occult neoplasia.
MISDIAGNOSIS OF COELIAC DISEASE
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Small bowel histology remains the gold standard of diagnosis in coeliac disease. Following the introduction of upper gastro-intestinal endoscopy in the 1970s, endoscopic forceps biopsy of the first and second parts of the duodenum replaced peroral suction biopsy as the mainstay of diagnosis.

We report six female patients with a mean age of 26.3 years referred to us between 1989 and the present for a tertiary opinion with persistent symptoms. They had been diagnosed as suffering from coeliac disease on the basis of duodenal biopsies taken at endoscopy. These were interpreted as showing subtotal villous atrophy. All the patients had been on a gluten-free diet for a mean duration of 23 months (range 7-36 months) prior to referral.

The initial biopsies were reviewed by us in conjunction with a gastrointestinal histopathologist and found to be within normal limits. Misdiagnosis was associated with a small biopsy sample size and poor orientation of the fixed specimens. We found the original slides had been misinterpreted due to the sections being cut tangentially. The villus height : crypt depth ratios (4.1 +/- 1.4, normal range 3-5), surface enterocyte cell heights (34.4 mm +/- 6.3, normal range 29-34), and intra-epithelial lymphocyte counts (18.5/100 epithelial cells +/- 3.4, normal range 10-30) were normal in all the biopsies.

The eventual diagnoses made in these cases were the irritable bowel syndrome (n=3), post-inflammatory inflammatory bowel disease (n=1), migraine (n=1) and Crohn’s disease (n=1). The mean duration between presentation and the final diagnosis was 3.8 years (range 2-8 years).

To investigate for coeliac disease, we conclude that a minimum of three biopsies distal to the first part of the duodenum should be taken; the biopsies should be correctly orientated, of adequate size and reviewed by a histopathologist experienced in gastroenterological diagnosis. Where interpretation is not conclusive, previously biopsied specimens should be reviewed.

In our experience, the use of a Watson or Crosby peroral jejunal biopsy capsule may be useful. Should any doubt remain concerning the diagnosis, a further peroral suction biopsy of the jejunum following a gluten challenge is recommended.
ENTONOX AS MEDICATION FOR COLONOSCOPY.

St Mark’s Hospital, City Road, London, UK.

AIM The aim of the study was to assess the efficacy of Entonox, a 50:50 nitrous oxide/oxygen combination, as analgesia/sedation for colonoscopy compared with standard IV medication (paraldehyde 50mg + midazolam 2.5mg) and placebo.

METHOD 89 patients attending for day case colonoscopy were randomly allocated to receive either Entonox, standard medication or placebo. Two experienced colonoscopists performed the procedures. The study was double-blinded using normal saline injection in the Entonox and placebo groups and inhaled air in the standard medication and placebo groups. Recordings of pulse, BP and oxygen saturation were made before and during intubation. Episodes of pain, extra medication required and duration of stay within the Unit were recorded. The patients’ experience of the colonoscopy was assessed by a questionnaire.

RESULTS There was balance between the three study groups with regard to age, sex, cardio-respiratory history, findings at colonoscopy, patient anxiety before colonoscopy or the technical difficulty of the procedure. There were 20 episodes of 

T158 ARE CONSCIOUS SEDATION AND PHARMACIAL ANAESTHESIA USEFUL FOR GASTROSCOPY? A RANDONMEDISED DOUBLE-BLIND PLACEBO-CONTROLLED PROSPECTIVE STUDY


Conscious sedation and pharyngeal anaesthesia are widely and usually simultaneously applied for endoscopic procedures. However, randomized, double-blind placebo-controlled prospective study has yet been performed which compares different drugs for the combination of conscious sedation, benzodiazepine (midazolam) and pharyngeal anaesthesia. Two hundred consecutive outpatients undergoing diagnostic gastroscopy (G) were randomly assigned to four groups: Almidazolam (MD; 25 mg/kg iv) + xylocaine spray 10% (XYL;100mg) B(MID) + placebo XYL C(placebo MID + XYL) D (placebo MID + placebo XYL). Before G, patients were scored (visual analogue scale, 0-100) for anxiety and specific items of apprehension (e.g. pain, nausea). At least 2 after G, patients’ tolerance was reevaluated by the same psychologic items (VAS, 0-100; extremely uncomfortable). In addition, endoscopists assessed tolerance independently. All G and interviews were performed by 2 experienced endoscopists in a standardized environment, applying the same videodensocopy equipment (diameter 9mm).

Blood data from 198 patients (198a, 97f, median age 45y, range 18-87y) could be analysed. Patients in the 4 groups were similar regarding demographic data (sex, age, weight, habits [smoking, alcohol intake]), previous experience with 0 and pre-endoscopic anxiety scores. Amenorrhea occurred in the 2 MID groups in 33% (group A) and 25% (group B resp. C). Tolerance scores for 100 (mean SEM) patients endoscopists 4 MID + XYL n=49 17 2 10 1 placebo MID + XYL n=49 21 2 10 1 placebo MID + XYL n=51 26 2 13 2 placebo MD + placebo XYL n=49 22 2 13 2

SUMMARY AND CONCLUSIONS: This double-blind placebo-controlled prospective study shows that IV midazolam (35 mg/kg) and topical xylocaine have a distinct beneficial effect on patient’s tolerance in routine gastroscopy. In addition, beneficial effects of midazolam and xylocaine as additive. Base of the procedure judged by the endoscopists was mainly influenced by XYL.

BLOOD ANTIOXIDANT PROFILES IN RELATION TO PANCREATIC CARCINOMA WITH NON-GALLSTONE (RECURRENCE) RECURRENT PANCREATITIS

Sharer NM, Lee S, Taylor PM, Schofield D, McIntosh J, Mei G, Braganza IM

Deficiencies in several blood antioxidants including glutathione have been recorded during active episodes of pancreatitis (Digestion 1989; 40: 22-45; J Intern Med Soc Transact 1993: in press) and could represent antioxidant consumption secondary to inflammation. We now report finding blood antioxidant profiles in 20 consecutive patients (age 19-63 years; males 15, females 5) referred after a recurrence of pancreatitis 1-6 months earlier, or with constant pain. They had not taken antioxidant supplements; 12 drank alcohol in excess, 12 smoked cigarettes, and 3 did neither.

Plasma vitamin C of the group as a whole was lower than in controls (median 3.9, range 0.7-22 vs 14, 6.3-19 mg/l; 2p<0.001), as was serum selenium (79, 22-125 vs 119, 81-161 mg/l; 2p<0.001); whereas whole blood and plasma glutathione values were normal. Patients with pancreatic calculi invariably had negligible vitamin C (1.3, 0.7-3.9 mg/l), while non-calcific chronic pancreatitis was associated with intermediate levels (8.9, 1.4-23 mg/l; 2p<0.005 vs calcific disease). Inflammatory and/or cystic expansion of the gland on ultrasound/CT scan was found in several of these and others with constant pancreatic pain had lower vitamin C and selenium levels than those who were pain-free between attacks (vitamin C 2.4, 0.7-14 vs 11, 6.4-13 mg/l; 2p<0.05; selenium 64, 22-91 vs 102, 101-106 mg/l; 2p<0.005). Five patients with normal pancreatic morphology and cystic expansion, in 'recurrent acute pancreatitis', had normal vitamin C and selenium levels. These results suggest that the degree and persistence of vitamin C and selenium deficiency influence eventual pancreatic pathology after pancreatitis - whether calcific or non-calcific chronic pancreatitis, or recurrent acute pancreatitis.
T161

THE ROLE OF PLATELET ACTIVATING FACTOR IN A MODEL OF ACUTE PANCREATITIS
L.J. Formella, M. Whittaker*, A.N. Kingsnorth
Department of Surgery, University of Liverpool and *British Biotechnology Group plc, Oxford, England, U.K.

The role of platelet activating factor (PAF) a bioactive phospholipid which has effects on capillary permeability, was investigated for its role in the pathogenesis of acute pancreatitis by administration of BB-882, a potent selective PAF antagonist. Pancreatitis was induced by microvascular ischaemia in 24 Wistar rats, resulting in increases in serum pancreatic enzymes, pancreatic weight and histologic evidence of acute pancreatitis. Histological changes were assessed using an established point scoring system for each of the features of oedema, inflammatory infiltrate, fat necrosis, parenchymal necrosis and haemorrhage. Treatment with BB-882 (5mg/kg l.p. 30 min after induction of pancreatitis significantly inhibited elevation of serum amylase and histological changes of pancreatitis. Values are mean (range) p values Wilcoxon rank sum test.

<table>
<thead>
<tr>
<th>Serum amylase (IU/L)</th>
<th>Pancreatic weight (g)</th>
<th>Histology score (0-27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatitis</td>
<td>+ saline</td>
<td>(n = 12)</td>
</tr>
<tr>
<td>3928</td>
<td>2800-5900</td>
<td>(1.75-3.08)</td>
</tr>
<tr>
<td>Pancreatitis + BB-882</td>
<td>2477</td>
<td>(1.92-2.96)</td>
</tr>
<tr>
<td>p&lt;0.001</td>
<td>N/S</td>
<td></td>
</tr>
</tbody>
</table>
| Values are mean (range) p values Wilcoxon rank sum test.

Platelet activating factor may be released endogenously during the evolution of acute pancreatitis. Administration of BB-882, a potent PAF antagonist, after induction of acute pancreatitis alters the progression of acute pancreatitis in this model.

T162

TEMPORAL MUCOSAL MORPHOGENESIS AFTER SMALL INTESTINAL STEM CELL (SISC) TRANSPLANTATION
IS Tait, H Flint, GS Evans, D Hopwood, FC Campbell. Department of Surgery, Ninewells Hospital and Medical School, Dundee, Scotland.

Transplantation of SISCs generates neomucosa with small intestinal (SI) phenotype, but the event sequence of mucosal morphogenesis is unknown. This study examines temporal organotypic mucosal morphology and cytodifferentiation after grafting.

Methods: Epithelial aggregates were isolated from 6 day rat SI by enzymatic digestion and grafted subcutaneously to inbred AO rats. Eight grafts were retransplanted at each of 3, 5, 7, 10, 14 and 21 days. Graft morphology and cytodifferentiation were assessed by specific histo- and immunohistochemistry. Cell proliferation was assessed by Mitotic index and PCNA staining.

Results: At 3 Days, grafts were sparse tubular structures comprising stratified undifferentiated epithelium with a high mitotic index (10-20%), surrounded by intense neovascularisation. By 5 Days, cyst like structures developed, lined by an epithelial monolayer, containing a "cap" of proliferative hyperchromatic cells (Mitotic index = 10%) at one pole. Cells extending laterally differentiated and included scant goblet cells close to the "cap" region, but cell senescence occurred at greater distances. At 7 Days, crypt budding occurred adjacent to "caps". Senescence of peripheral cells induced cyst coalescence. By 10 Days, "caps" had enlarged and generated crypts and villi. Many cysts coalesced. All cell lineages were identified only at 14 Days or later.

Conclusions: A sequence of mucosal morphogenesis after postnatal SISC transplantation has been defined. Proliferation / differentiation patterns show a temporary change of asymetric stem cell division.

T163

INTESTINAL PROTEIN METABOLISM IN RESPONSE TO POST-OPERATIVE STRESS
I S Marway and V R Peevy. Department of Clinical Biochemistry, King's College School of Medicine and Dentistry, Besserner Road, London, U.K.

Periturbations in intestinal function are induced by surgical stress but the mechanisms are not clear. Changes in intestinal protein synthesis may be a contributing mechanism, but previous studies have indicated that protein synthesis in the whole small intestine is relatively insensitive to the effects of immediate (i.e., 1 and 2 days) post-operative stress. However, these previous studies only examined the whole intestine of starved rats and none of the principal protein fractions were examined.

The aim of this study was therefore to address the question of whether different regions and protein fractions of the small intestine were affected by surgical procedures. Male Wistar rats were subjected to surgical stress involving anaesthesia, bilateral lumbar incisions and suturing. After 1 week fractional rates of protein synthesis (defined as the percentage of the protein pool renewed each day by synthesis, i.e., k, %/day) were measured with labelled phenylalanine. In control rats, k, values for whole (i.e., combined mucosa and seromuscular layers) segments of the jejunum were not significantly altered (P>0.05, NS). In the seromuscular layer k, values for mixed proteins were markedly reduced by 28% (P<0.001), demonstrating regional sensitivity. The synthesis of contractile proteins in the seromuscular layer were also significantly reduced (P<0.001) reduced by 33% in surgically stressed rats, i.e., from 63 ± 6 to 42 ± 3%/day (all data mean ± S.E.M.; n=5-8) though synthesis of contractile protein synthesis in the whole-jejunum was unaltered, i.e., 101 ± 4 and 102 ± 3%/day in control and surgically stressed rats, respectively. The decline in the rate of seromuscular protein synthesis in response to post-operative stress involved transcriptional control mechanisms, k, values (defined as the translational index, or RNA activity, mg protein/day/mg RNA) were unaltered, i.e., 29 ± 3 and 29 ± 4 mg protein/day/mg RNA in control and surgically stressed rats, respectively.


A search for additional peptides of the trefoil family i.e. secretory peptides containing one or more conserved cysteine structural motifs (trefoil or P-domain), has resulted in the cloning and characterization of a peptide representing the human homologue of rat intestinal trefoil factor. This peptide has a single P-domain and may be called hP1.B.

Reverse transcription PCR using RNA from small intestine lung and uterus and PCR using genomic DNA with synthetic oligonucleotides based upon a well-conserved region of the trefoil family gave clones encoding a single trefoil pre-pro peptide, deduced to be of 80 amino acids, that has 67% amino acid homology with rat intestinal trefoil factor; 76% considering the mature 59 amino acid peptide sequence.

Southern blot analysis with a full-length cDNA probe indicated that only one copy of the gene exists per haploid genome.

RNA blot analyses revealed that positive hybridisation signals (approx. 500-550 bases long) were obtained using poly A+ RNA isolated from small intestine, uterus and stomach (weak).

Hybridisation in situ using a 35S-labelled antisense riboprobe and sections from blocks of formalin-fixed wax-embedded tissues revealed that in the intestine hP1.B mRNA is expressed in goblet cells, with expression at varying levels also in regions of ulceration associated cell lineage in the small intestine. In the human stomach, hP1.B mRNA was relatively scarce, but it was more easily detected near to gastric ulceration and in regions of intestinal metaplasia. Hyperplastic polyps of the large bowel also had significant levels of hP1.B mRNA.

Three distinct trefoil peptides, human intestinal trefoil factor (hP1.B), pS2 and human spasmolytic polypeptide are now known to be expressed in mucin-rich cells of the ulcer-associated cell lineage, hyperplastic polyps and ulcerated gastric mucosa. These results highlight the importance of the search for biological functions of this growing family of peptides and of investigations into their co-expression with mucins.

T164

**INTESTINAL FUNCTION AT ALTITUDES ABOVE 5000M**

**SPL Travis, C A Court, JS Menzies, M Stroud, JSA Edwards.**

John Radcliffe Hospital, Oxford, St Thomas' Hospital, London, Bournemouth University, APRE, Farnborough. ( Introduced by DP Jewell)

Weight loss at altitudes above 5000m cannot entirely be explained by reduced dietary intake. To investigate intestinal function, carbohydrate absorption and permeation were measured in 13 healthy volunteers (median age 32, 28-47yr) at sea level, 10 days after arrival in Nepal (3500m), then at 5400m before and after ascent up to 7900m on Mt. Everest. Dietary intake and energy expenditure (double labelled water) were measured in 4 subjects. The test solution contained xylose (5g Xyl), 3-O-methyl-D-glucose (3mg 2.5g), lactulose (Lac 3g) and L-threonine (Rh 1g) in 100ml water (280mOs). Serum (30 and 60min) and urine (5hr) were stored until analysis by thin layer chromatography. Weight loss between sea level and 5400m did not reach significance, but decreased (mean±sd) from 72.5±6.2 to 66.0±6.9kg (p<0.001, paired t) above 5400m. Body fat mass estimated from skinfold thickness decreased by 14.7±3.3% (p<0.012), largely below 5400m. Carbohydrate absorption assessed by the serum xyl/Rh ratio at 60min decreased from 1.9±0.6 to 0.9±0.2 (p<0.012, unpaired t) at 5400m, but was not significantly less than baseline values after the ascent. Intestinal permeability (Lac/Rh excretion ratio) increased from 0.03±0.02 to 0.03±0.01 at 5400m, possibly due to infection, but reverted to normal (0.03±0.01) at 7900m. Mean energy intake on 38 days during the ascent above 5400m was 8±6.0.7 MJ which did not always match energy expenditure. Results suggest that carbohydrate permeability above 5400m is normal. Whilst impaired absorption may contribute to weight loss, preliminary results indicate that net negative energy balance is also a factor.

**HUMAN SMALL INTESTINAL PHYTASE ACTIVITY**

**T H Josel, J.D. Lewis, B T Cooker.**

Dudley Road Hospital, Birmingham B18 7QH

Phytic acid is the major storage form of phosphorus in seeds and so is a common dietary constituent. Excessive ingestion of unfermented phytates has been shown to cause mineral deficiencies in man. Recently, however, phytic acid has been found to be antineoplastic in animal models of both colon and breast carcinomas. There have been no previous studies quantifying phytase activity in the human small intestine although animals are known to have this enzyme.

We measured phytase and alkaline phosphatase activity in vitro in mucosal homogenates from two human small intestinal specimens obtained from transplant donors. Rat intestine was also studied for comparison.

The phytase activity was measured at three levels of the small intestine by measuring the production of inorganic phosphate (using malachite green in acidified molybdate as chromogen) during hydrolysis of phytic acid. Alkaline phosphatase activity was measured by the breakdown of paranitrophenylphosphate.

We found phytase activity in human small intestine at low levels (30 times less than that in rat tissue and 1000-fold lower than alkaline phosphatase in the same tissue). The activity was greatest in the duodenum and lowest in the ileum.

We conclude that the normal human small intestine has very limited ability to digest undegraded phytates. Although this may have adverse nutritional consequences with respect to metabolic cation imbalances, the presence of undigested phytate in the colon may protect against the development of colonic carcinomas.

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**DOES SMALL BOWEL AUTOTRANSPLANTATION AFFECT FATTY ACID ABSORPTION?**

**Anne M Walters, M G Sav. (Introduced by J N Priorose.)**

Gastroenterology Research Unit, Mayo Clinic, Rochester Minnesota

Lymphatic play an important role in the absorption of long chain fatty acids. Small bowel transplantation results in a transaction of all lymphatic drainage from the small bowel and impaired absorption of long chain fatty acids would appear likely until reconnection commences at 14 - 28 days. This was studied using a model of small bowel autotransplantation which avoids graft injury from ischemia, reperfusion and rejection, while retaining portal venous drainage.

Four groups of 6 dogs had an 80 cm isolated loop created:

- Group 1: neurotically intact jejunum,
- Group 2: "autotransplanted" jejunum,
- Group 3: neurotically intact ileum,
- Group 4: "autotransplanted" ileum.

Dogs were studied at 2 and 9 weeks after "autotransplantation". The loops were perfused with 5 mM oleic acid in an isomolar solution containing bile salts. This was delivered at 3 ml/min at 39°C for 3 hours on 4 separate occasions at both time points.

Oleic acid absorption (table) was similar in both jejunum and ileum. Absorption in neurotically intact and "autotransplanted" dogs was similar and remained unaltered between 2 and 9 weeks.

<table>
<thead>
<tr>
<th>Percentage of Oleic Acid absorbed per 15 minute period</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 2</td>
<td>46.0±10.6</td>
<td>52.0±6.2</td>
<td>41.0±10.0</td>
<td>35.4±4.1</td>
</tr>
<tr>
<td>Week 9</td>
<td>41.8±1.7</td>
<td>47.0±2.9</td>
<td>41.1±5.5</td>
<td>35.7±5.5</td>
</tr>
</tbody>
</table>

Results expressed as Mean±SEM

Small bowel autotransplantation does not impair long chain fatty acid absorption in dogs. This essential nutrient may potentially be introduced in the early post operative period in small bowel transplant patients.

**INCREASED EPITHELIAL AND VASCULAR COMPONENTS OF INFLAMMATION AND REDUCED INFLAMMATORY CELL INFILTRATE IN HELICOBACTER PYLORI ASSOCIATED GASTRITIS AFTER ONE WEEK OF ANTIBIOTICS.**

**JC Aberton, D Jenkins, GE Kirk, MA Hull, DJE Collen, A Cockayne, RC Spiller, CJ Hawkey.**

Depts Gastroenterology, Histopathology & Microbiology, University Hospital, Nottingham, NG7 2UH

H. pylori associated gastritis appears to have less epithelial and vascular change than the "reactive" gastritis of chemical injury. By examining biopsies before and after antibiotic therapy we aimed to see whether this was due to a partial suppression of these changes.

Methods: Twenty-two H.pylori infected duodenal ulcer patients, 18 male, median age 42 (range 22-68), not taking aspirin or non-steroidal anti-inflammatory drugs, had endoscopic biopsies obtained from gastric antrum and body before and immediately after 1 week of amoxicillin 500mg ids and cimetidine 800mg nocte. H.pylori eradication was assessed by 14C-urea breath test (UBT) 34-39 days later. Haematoxylin and eosin stained biopsies were graded 0-3 for acute and chronic inflammatory cell infiltrate, foveal hyperplasia, oedema and vasodilatation. Significance was assessed using Student's t test.

Results (mean±SEM):

- Epithelial/vascular Acute infl. (0-3) Chronic infl. (0-3) component (0-9)
- Antrum Body Antrum Body Antrum Body
- Before 1.1±0.1 0.4±0.1 1.6±0.1 0.6±0.1 3.6±0.3 3.8±0.3
- After 0.9±0.1 0.1±0.1 1.2±0.1 0.4±0.0 4.1±0.3 3.2±0.3
- p <0.001 <0.05 <0.05 <0.05 <0.05 <0.05

8 biopsies met criteria for reactive gastritis, 7 after and 1 before treatment. H.pylori was undetectable in all but 2 patients immediately after treatment (by modified Giemsa, CLO test or culture) but UBTs 1 month later were positive in all showing eradication did not occur.

Conclusion: The epithelial and vascular components of inflammation increase as the H.pylori associated inflammatory cell infiltrate subsides with antibiotic therapy. This suggests that these components may be suppressed by the organism or by the associated cellular infiltrate.
PORTAL HYPERTENSIVE GASTROPATHY AND CHRONIC GASTRITIS ARE RELATED TO DIFFERENT PATHOGENIC FACTORS

M Guandalini, M Sorghi, A Pittobello. Dept. of Gastroenterology, S.Raffaele Hospital, University of Milan, Italy.

Mild forms of portal hypertensive gastropathy (PHG) are endoscopically similar to chronic non-erosive gastritis (CNG) but the pathogenetic factors involved are thought to be different. In our study we investigated the role of local microcirculation and of H.pylori infection.

Three groups of patients were studied. 15 pts with liver cirrhosis (grade A or B by Child-Pugh classification), esophageal varices F1 or F2 and no previous sclerotherapy, with endoscopic signs of mild to moderate PHG; 15 pts without liver disease, and with endoscopic signs of CNG, histologically confirmed; 15 subjects with normal endoscopic appearance.

Mucosal blood flow was measured by means of laser Doppler velocimetry (PF3, Perimed, Sweden). Values were expressed in perfusion units (1 PU=10 mW). Biopsies from the gastric antrum were taken and H.pylori detected by a rapid urease test (CLO test, Brocadex).

Mucosal blood flow was found significantly reduced in patients with CNG (148±12) whereas in subjects with PHG was significantly higher (254±12) than in controls (192±12).

A positive CP test was found in 26.6% of pts with PHG, 90% of pts with CNG and in 30% of normal subjects.

Due to their different pathogenetic features treatment of CNG should be carried out with drugs strengthening mucosal defenses and active against H.pylori, while in PHG drugs reducing blood flow (i.e. beta-blockers) seem to be indicated.

BLEEDING PEPTIC ULCER IN ENGLAND AND WALES: EPIDEMIOLOGICAL TRENDS 1956-1985. Marius Z Pilor and Michael J S Langman. Department of Medicine, University of Birmingham Medical School, Queen Elizabeth Hospital, Birmingham, UK

We used national statistical data from the Hospital In-Patient Enquiry and the Office of Population Censuses and Surveys to calculate age-specific rates of hospital admission and hospital mortality for peptic ulcer with haemorrhage, in England and Wales between 1979-1985. We compared the results to data from 1956-7 (Johnson HD Gut 1962;3:106-117). Notable changes occurred in men and women over 65 years of age. There was a decrease in admission rates for bleeding gastric ulcer (GU) in 65-75 year old men (39 to 26/100 000 population) and those over 75 (87 to 66/100 000). Admissions for bleeding duodenal ulcer (DU) decreased only in men over 75 years (102 to 93/100 000) and did not change or increased slightly in younger men. In women, GU admissions trebled in those over 75 from 17 to 46/100 000 and admissions for DU doubled in those over 65 (8 to 17 in those 65-75 and 17 to 40/100 000 in those over-75 years). The proportion of patients over 65 years admitted for peptic ulcer bleeding doubled between 1956-1985: from 32 to 60% in men for GU, and 48 to 74% in women, with a decrease in mortality from 28 to 16% in men and 22 to 12% in women in this age group. For DU, the proportion of admissions over 65 years rose from 28 to 46% in men and from 37 to 72% in women. Although there was a decrease in mortality in men of this age group from 19 to 13%, in women there was no change at 22%. Our findings indicate that hospital mortality (with the exception of DU in elderly women) from bleeding peptic ulcer has decreased nationally and that this decrease is not confined to younger age-groups.

LONG-TERM STUDY WITH DIFFERENT DOSAGES OF OMEPRAZOLE FOR PREVENTING RELAPSES OF DUODENAL ULCER DISEASES

G.I.S.U. (Interdisciplinary Group for Ulcer Disease)

Omeprazole is present the most powerful drug in inhibiting gastric acid secretion, being 20 mg daily able to maintain intragastric pH > 3 for a period of at least 18 hours.

The same drug shows a long-acting capacity in the control of the gastric acidity, up to 48-72 hours.

Aim of the present study has been to verify if different schedules of administration of Omeprazole will be able to maintain the scarring phase of duodenal ulcer in a long-term study (6 months) after endoscopically proved healing of the acute lesions with a treatment of 40 mg daily of Omeprazole for 4 weeks.

360 consecutive DU patients were enrolled in the study, according to a randomized double-blind following treatments:

A) Omeprazole 20 mg daily (114 patients);
B) Omeprazole 20 mg each other day (121 patients);
C) Omeprazole 40 mg on Saturday and Sunday (125 patients).

Endoscopic and scintigraphic were performed at admission and at the 6th month of maintenance treatment and at every symptomatic relapses (pain for more than 4 days).

Statistics: data were analyzed by means of Pearson Chi Square with standardised deviates.

RESULTS:

<table>
<thead>
<tr>
<th></th>
<th>Scurred</th>
<th>Relapses</th>
<th>Drop outs</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10 (10%)</td>
<td>8 (8.5%)</td>
<td>7</td>
<td>0.0138</td>
</tr>
<tr>
<td>B</td>
<td>94 (91.7%)</td>
<td>21 (18.3%)</td>
<td>6</td>
<td>0.0138</td>
</tr>
<tr>
<td>C</td>
<td>100 (84%)</td>
<td>19 (16%)</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

X² = 8.656; D.F. = 2; p = 0.0138. The standardised deviates show a significant lower relapse rate in schedule A (-2.2).

Side effects of mild entity: constipation (4 cases), itch (1 case), weakness (1 case).

CONCLUSION: Omeprazole 20 mg daily represents the best choice in maintenance therapy of duodenal ulcer.

DO POST OPERATIVE SYMPTOMS AFTER HSV HERALD ULCER RECURRENT?

JM Wilkinson, KB Hosie, AG Johnson

University Department of Surgery, Hallamshire Hospital, Sheffield, S10 2JF.

Between 1979 and 1984, 141 patients (110 men, 31 women) underwent highly selective vagotomy(HSV) by a standard technique for duodenal ulcer. All patients had received pre-operative treatment with full dose H2-receptor antagonists (H2RA). 107 underwent HSV for persistent relapse on withdrawal of H2RA (relapsing responders), and 30 because of non response to H2RA (non responders). At four years follow up non responders were found to be more likely to be symptomatic post operatively (p=0.001). 125 patients are still alive at a median of 11 years 3 months post operation (range 8-14 years), and of these 114 (92%) were reviewed. 10 (9%) were found to have had an endoscopically proven recurrence and 8 (7%) still had symptoms without evidence of recurrence. The endoscopic recurrence rate and symptomatic rate at 11 years were no longer significantly different between relapsing responders and non responders. Only one of the non responders symptomatic at first follow up developed recurrent ulceration between 4 and 11 years. All other recurrences between 4 and 11 years (n=9) were in patients who were asymptomatic at first follow up.

The long term endoscopic recurrence rate after HSV is low. The pre-operative response to H2RA does not help in predicting the likelihood of ulcer recurrence or long term postoperative symptoms. Post operative symptoms do not necessarily predict long term ulcer recurrence.
THE EFFECT OF LONG TERM MISOPROSTOL CO-ADMINISTRATION WITH NSAIDs: A HISTOLOGICAL STUDY.

K Shah, AB Price, IC Talbot, KD Barthan, W Griffin, CG Fenn, J Bjarnason. Northwick Park Hospital, St Marks Hospital, Searle Pharmaceuticals and King's College School of Medicine, London, UK.

Misoprostol has been shown to be effective for the prevention and healing of macroscopic gastroduodenal lesions due to NSAIDs. The long term effect of misoprostol co-administration with NSAIDs on a histological level is however unknown. Methods: One hundred and eighty patients on long term NSAID treatment (80 Misoprostol [4-800 mcg/day] for 1-2 years) underwent endoscopy with two biopsies from duodenum, antrum and corpus. Biopsy appearances were graded and classified according to the Sydney System. Results: There were no significant demographic differences between the two groups of patients. The table summarises the main histologic findings in the stomach.

<table>
<thead>
<tr>
<th>Histology findings</th>
<th>NSAIDs</th>
<th>NSAIDs and misop.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic pan-gastritis</td>
<td>23 (20)</td>
<td>23 (21)</td>
</tr>
<tr>
<td>Chronic gastritis of corpus</td>
<td>5 (2)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Chronic gastritis of antrum</td>
<td>7 (6)</td>
<td>11 (9)</td>
</tr>
<tr>
<td>Minimal inflammatory change</td>
<td>7 (0)</td>
<td>4 (0)</td>
</tr>
<tr>
<td>Reactive gastritis (reflux, type C)</td>
<td>27 (0)</td>
<td>8 (0)*</td>
</tr>
<tr>
<td>Mixed group</td>
<td>5 (0)</td>
<td>3 (0)</td>
</tr>
<tr>
<td>Normal</td>
<td>16(0)</td>
<td>16(0)*</td>
</tr>
</tbody>
</table>

Within parenthesis: number HLO positive

* P<0.01

The results suggest two different patterns of damage which relate to HLO status. Patients on NSAIDs and misoprostol had a significantly less prevalence of reactive gastritis and were significantly more likely to have normal gastric biopsies. Misoprostol does not affect chronic gastric changes or the patients HLO status. Conclusion. Long term co-administration of misoprostol with NSAIDs is not associated with any adverse histological changes and specifically reduces the prevalence of reactive gastritis.

GASTROPARESIS, ACID SECRETION AND HELICOBACTER PYLORI COLONISATION IN FUNCTIONAL DYSPEPSIA

B Waldron, D Smith, FC Campbell. Department of Surgery, Ninewells Hospital and Medical School, Dundee, Scotland DD1 9SY

In functional dyspepsia (FD) gastroparesis is common, but its cause is obscure. In volunteers, gastroparesis may be induced by acid suppression. This study has investigated relationships between solid meal gastric emptying time (T50) and pentagastrin stimulated peak acid output (PAO), in 83 patients with FD, diagnosed after exclusion of organic disease. Fifty FD patients had gastroscopy and biopsy for assessment of gastritis severity and Helicobacter pylori (H pylori) colonisation. Control studies of gastric emptying were carried out in 15 volunteers.

Results: Gastric emptying was delayed in FD (T50 = 100 [90-111] min vs 67 [57-78] min; p<0.01). Gastritis was present in 34 patients and H pylori in 31. Gastritis severity was directly related to H pylori colonisation (r=0.73; p<0.01) and inversely related to PAO (r=-0.29; p=0.05). Gastric emptying time (T50) was inversely related to PAO viz.

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>PAO (mmol/hr)</th>
<th>T50 (min)</th>
<th>p&lt;0.01***</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0 - 8</td>
<td>115 (92-179)</td>
<td>3</td>
</tr>
<tr>
<td>II</td>
<td>9 - 25</td>
<td>29 (71-106)</td>
<td>1 vs II</td>
</tr>
<tr>
<td>III</td>
<td>26 - 40</td>
<td>27 (82-115)</td>
<td>1 vs III</td>
</tr>
<tr>
<td>IV</td>
<td>&gt; 40</td>
<td>65 (60-82)</td>
<td>1 vs IV</td>
</tr>
</tbody>
</table>

Conclusions: Gastroparesis is associated with hypochlorhydria in FD. The association between H pylori colonisation and gastritis on one hand and severe gastritis with hypochlorhydria on the other, suggests that hypochlorhydria may be an end stage phenomenon of H pylori colonisation.

* Mean (95% CI) ** Mann Whitney U test *** Median (IQR) | Pearson Product Moment test of correlation

SYMPOMS IN ORGANIC AND FUNCTIONAL DYSPEPSIA: IS A CLINICAL DISTINCTION POSSIBLE? M A Loudon, N Ahmed, PK Small, DM Smith, B Waldron, FC Campbell Department of Surgery, Ninewells Hospital, Dundee, Scotland DD1 9SY

Dyspeptic symptoms are conventionally used in the diagnosis of upper gastrointestinal disease, however the specificity and sensitivity of such symptoms is uncertain. This study aimed to establish if the nature and severity of symptoms correlated with the presence of upper gastrointestinal pathology at endoscopy.

Patients referred for endoscopy were assessed prospectively by questionnaire. Frequency and severity of 6 dyspeptic symptoms (epigastric pain, early satiety, heartburn, regurgitation, nausea and flatulence) were scored. Patients with positive endoscopic findings (N = 34) were classified as OD. Patients with negative endoscopy, abdominal ultrasound and other investigations, as clinically indicated, (N=35) were categorised as FD. A dyspepsia score (Max = 84) was generated using frequency and severity of symptoms. Patients were compared for all measures of dyspepsia severity (symptom number, symptom frequency, severity).

Results: OD patients were significantly older than FD = [52.5 (42-64) years (OD) vs. 37.4 (27-45) years (FD, p < 0.001**)] Dyspepsia occurred on at least 3 days weekly in 27 of 34 OD patients vs 30 of 35 FD patients (p = 0.06). Symptoms were similarly distributed in the two groups with heartburn the commonest symptom in both groups = [25.34 (OD) vs. 23.34 (FD)]. Mean symptom number = [4 (3 - 5)* OD vs. 4 (2 - 5) FD, p = 0.001**]. Dyspepsia scores = [21.9 (13.1 - 39.2)* OD vs 24.5 (10.4 - 43.8) FD, p = 0.001**].

Conclusion: The nature and severity of dyspepsia is a poor indicator of the presence of organic upper gastrointestinal disease

THE HISTORY OF ULCER DISEASE OVER THE PAST 19-20 YEARS

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Many changes have occurred in the diagnosis and management of peptic ulcer disease since the original study by Fry (1964). To assess what has happened to date, a consecutive series of ulcer patients first diagnosed endoscopically some 10-20 years ago (1972-1983) have been followed up.

A total of 345 cases were found. Medical records were obtained when available and ALL were sent questionnaire at their last recorded address which were analysed in terms of symptoms, Analogue score and Nottingham Health Profile score. 38 were known to have died and 77 did not reply. Results obtained in 230 patients (Male 148, Female 82 : DU 180, GU 50) are reported.

Since first diagnosis 59 patients (26%) have been operated upon for their ulcer. (Uncontrolled 39 : Stomach 8 : Perf. 5 : Bleed 4 : Ca. 3). The remaining patients have been analysed for their initial current treatment, present symptoms, Nottingham Health Profile score, latest endoscopic findings and complications.

Initial treatment was Cimetidine (C) in 81% : Ranitidine (R) 12% : Other 7%. Currently (18%) are still receiving treatment (C 52% : R 15% : Other 33%) while (48%) are off all therapy. 6 symptoms are still present in 51% of which the commonest is abdominal pain (43%). 21 patients have had complications since diagnosis (perforation 5 : bleeding 4 : peptic stenosis 10 : gastric carcinoma 3). The analogue score given by the patients on their current ulcer state was >70 (100 = Good) in 56%.
INTRODUCTION: De-Nol tab (tripotassium dicitrato bismuthate) is used for eradicating H. pylori, but bismuth (Bi), which is potentially neurotoxic, therapeutic Bi should not be absorbed, be well tolerated and still eradicate H. pylori. We have studied whether Roter (Bi)(sub)rate(carboxylate complex, Boots Healthcare) fulfills these criteria.

METHODS: a) Five healthy volunteers were given either two De-Nol tab (214mg Bi) or one Roter tablet (205mg Bi) and after a week, the other preparation. Blood and urine were analysed for Bi by - IC^3 mass was assessed with 13C breath tests. b) Mean Inhibitory Concentrations (MICs) for De-Nol and Roter were obtained in in vitro cultures using ten strains of H. pylori. c) Thirty H. pylori positive patients diagnosed by two antral rapid urease tests (CLO test) were given either two De-Nol tab or two Roter tablets tds for one month both with amoxycillin 250mg tds and metronidazole 400mg tds for one week. CLO tests were repeated four weeks after treatment.

RESULTS: a) Peak blood Bi levels occurred thirty minutes after taking two De-Nol tab (range 4.2 - 80ug/ml, median 64.6ug/ml). There was also a wide range in 48 hour Bi urinary excretion 87.2 - 1905ug (median 165.5ug). Following Roter there was no evidence of Bi absorption. There was no correlation between Bi absorption and H. pylori status. When De-Nol was given first baseline Bi in blood and urine was still elevated after one week.

b) MIC: De-Nol < 12.5ug/ml; Roter > 400ug/ml.

c) H. pylori eradication rates: De-Nol 7/15 (46.6%); Roter 9/14 (64.3%). One patient on Roter did not attend follow-up gastroscopy.

CONCLUSIONS: There is an unexplained wide range of Bi absorption from De-Nol (0.04 - 0.9% of that ingested excreted in urine). Bi is not absorbed from Roter. Both eradication regimes were well tolerated.

Although the MIC for Roter is high, in vivo eradication was equal or greater than for De-Nol. Eradication rates may be lower compared to other studies, perhaps due to high levels of metronidazole resistance in our local population. Further trials using Roter are needed.

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TRIPLE THERAPY IS BETTER THAN DOUBLE IN ERADICATION OF HELICOBACTER PYLORI

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In the following open clinical studies, 90 patients with Helicobacter pylori (HP)-associated ulcer (N = 82) or severe functional dyspepsia (N = 17) requiring therapy, in order to compare the efficacy of triple therapy vs double therapy and the effect of eradication on gastritis, were treated with:

1. 4 x 500 mg Amoxicillin for 15 days then 4 x 250 mg Metronidazole for 10 days (16 pts).
2. A plus 4 x 120mg Collodial Bismuth for 25 days (15 pts).
3. A plus 20 mg Omeprazole for 25 days (10 pts).
4. 40 mg Omeprazole for 2 wk plus 4 x 500 mg Amoxicillin the 2nd wk (27 pts).
5. 2 x 40 mg Omeprazole plus 4 x 500 mg Amoxicillin for 1 wk (25 pts).

Two biopsy samples from gastric antrum (pa) and body (pb) were taken for histology and Hp determination.

STATISTICS: results were analyzed by Chi Square test with Yates correction for continuity.

The Hp eradication rates - determined by histology after modified GiEMSA staining in the 9th wk after discontinuation of study medication - were 62.5% in Group A, 69.9% in group B, 58.2% in group C, 34.9% in group D, 24% in group E.

HISTOLOGY

<table>
<thead>
<tr>
<th>Hp+ve</th>
<th>Hp-ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>32</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>500</td>
<td>50</td>
</tr>
</tbody>
</table>

Hp eradication may significantly improve gastritis from deep to superficial and diminishes gastritis activity both in gastric antrum and body.

We conclude that triple therapy with Amoxicillin, Metronidazole and Collodial Bismuth represents - in our experience - the best choice, on the contrary, in spite of previous reports, double therapy with Amoxicillin and Omeprazole (both 40 mg and 40 mg/day) is not a useful and effective approach to the eradication of Hp from gastric mucosa.

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Colorectal cancer F180–F185

FAECAL OCCULT BLOOD SCREENING FOR COLORECTAL CANCER (CRC): THE TWO-TIER GUAIAC-IMMUNOCHEMICAL STRATEGY. GP Young, DJB St John. Introduced by M Farthing. Department of Gastroenterology and University of Melbourne Dept. of Medicine, The Royal Melbourne Hospital, Melbourne, VIC 3050, Australia.

Dietary exclusion of peroxido-rich foods is important when using guaiac-based faecal occult blood tests (FOBT), especially the more sensitive ones (e.g. HemoccultSENSA, HOS), to reduce false-positives and thus costs. Another approach to reduce false-positives is to confirm significance of positive results using a haemoglobin-specific immunochromat FOBT (e.g. HemeSelect, HSE) - this would remove the need for diet. Aim: Methods: To test sensitivity and specificity of a two-tier approach (TTA), 83 patients with mainly asymptomatic colorectal adenomas (55 single, 46 = 9mm) and 159 with mainly symptomatic CRC (29 proximal to splenic flexure) were tested simultaneously by HOS and HSE. Specificity was evaluated in 1,535 screeners; colonoscopy was performed in positive screeners. All were on an exclusion diet to optimise HOS performance before and during the testing of 3 serial stools, samples being prepared immediately after defecation.

Results: The Table shows the number of positive results for each test situation, and significance comparing HOS and HSE positive rates.

<table>
<thead>
<tr>
<th></th>
<th>HOS+HSE</th>
<th>HOS only</th>
<th>HSE only</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRC</td>
<td>99 (91%)</td>
<td>2 (1.8%)</td>
<td>6 (5.5%)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Adenoma</td>
<td>24 (28.9%)</td>
<td>12 (14.5%)</td>
<td>23 (27.7%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Screeners*</td>
<td>17 (1.3%)</td>
<td>51 (3.8%)</td>
<td>24 (1.8%)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Amongst the screeners, one had CRC and 19 had adenomas; these patients were removed before calculating corrected specificity shown in the Table. The CRC patient was positive by both tests but 13 of the adenomas were positive by only one of them and would have been missed by a TTA. Discussion: The TTA had a high sensitivity for symptomatic CRC although this would be expected to be lower for screen-detected CRC. In screeners, however, the TTA would miss half the adenomas detected by HSE and one-third by HOS. Corrected specificity of 1.3% was excellent with the TTA but two-thirds of adenomas would have been missed. Thus the TTA is limited by the sensitivity of the guaiac test; for the approach to be successful an even more sensitive guaiac test is needed.