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CHARACTERISATION OF ENDOTHELIN RECEPTORS IN GUINEA PIG GALLBLADDER MEMBRANES

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Endothelin isopeptides (ET-1, -2, -3) and sarafotoxin 6c (SX6c) are potent contractors of the guinea-pig isolated gallbladder. Based on the relative potencies of ET agonists and the effects of several ET receptor antagonists, we have reported that two receptors, an ETB and an additional uncharacterised receptor, mediate these contractions. Here, we have characterized the binding of ET to guinea-pig gallbladder membranes. Guinea-pigs (250-350g) were killed by cervical dislocation and the gallbladder rapidly excised, trimmed free of connective tissue and fat, minced to small pieces and homogenized for 10 min in 10 volumes of ice-cold assay buffer. The homogenate was centrifuged for 15 min at 1500g (4°C), the supernatant collected and centrifuged for 60 min at 100,000g (4°C). The pellet formed was resuspended in 3 ml of ice-cold assay buffer (as above) and its protein content determined. The membranes (20µg of protein) were incubated in binding buffer with ¹²⁵I-ET-1 (2000 Ci/mmol) in the presence of 10⁻¹⁵M to 10⁻⁶M ET-1, ET-3 or SX6c. After 240 min at room temperature, the binding was stopped by filtering the incubate through a Whatman glass filter followed by washing with 3 x 4 ml of ice-cold binding buffer. The amount of radioactivity present on the filter was measured in a gamma counter for 60 sec. In competition binding studies using membranes prepared from the guinea-pig gallbladder 10⁻¹¹ M and 10⁻⁶ M ET-1 inhibited by 76.9±3.1 and 95.7±1.1% respectively, the binding of [¹²⁵I]ET-1 (n=3). The displacement of ¹²⁵I-ET-1 by ET-1 was biphasic. A very high affinity site (IC₅₀: 35fM) and a high affinity site (IC₅₀: 0.18 nM) were observed. They represented 75% and 25% respectively, of the total population of ET receptors. Using ET-3 as the cold ligand, a biphasic displacement was also observed (IC₅₀: 0.10 nM and 70 nM, each representing about 50% of total binding). Using SX6c, a monophasic curve was observed with one site of low affinity (IC₅₀: >70 nM). This study shows that ET agonists in the guinea-pig gallbladder act through at least two high affinity sites and one lower affinity site. This supports our previous reports showing the existence of at least two receptors in this tissue. Further studies will be conducted to determine the exact nature of these receptors.

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IS THE FERTILITY OF FEMALES REDUCED IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE?

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Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC) are associated with a diverse group of extra gastrointestinal features. However, unlike patients

with irritable bowel syndrome (IBS), there have been few reports of gynaecological abnormalities in IBD. The aim of our study was to observe the effect of IBD and IBS on the age of menarche and menopause in females.

153 subjects (mean age 36 yr, range 19-95 yr), resident in Dublin, participated in the study. These included UC (n=40), CD (n=26), IBS (n=52) patients and 35 healthy female controls. The different subgroups were matched for age and marital status. Information from subjects was obtained by a written questionnaire. Menarche was at a significantly later date (student t test, p value < 0.05) in patients with CD (13.9 ± 1.14 yr) and UC (13.5 ± 1.4 yr) than in normal controls (12.8 ± 1.5 yr). No significant differences in age of menarche were observed between the IBS (13.2 ± 1.6 yrs) and control groups or between the CD and UC groups (p value > 0.05). The date of menopause was also significantly early (Mann-Whitney U-test) in patients with CD (41.8 ± 2.4 yr) and UC (43.4 ± 2.1 yr) than in normal controls (46.8 ± 3.3 yr). The differences between the IBS group (42.8 ± 5.6 yr) and the controls or the CD and UC patients were not statistically significant.

Conclusion: The fertile period of females with IBD is significantly reduced compared to healthy controls. This difference is not observed in IBS patients. The underlying mechanism is not known but may involve the abnormal immune responses observed in these patients.

TRANSCELLULAR CHLORIDE SECRETION IN HUMAN COLON

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Chloride (Cl⁻) secretion by the human colon epithelium provides the osmotic driving force for fluid secretion into the colon lumen. Under 'normal' conditions this fluid is readily reabsorbed. In secretory states the volume of fluid secreted secondary to Cl⁻ secretion may exceed the absorptive capacity of the epithelium and diarrhoea results (secretory diarrhoea). Increase in secretion by the colon epithelium is also thought to play a role in the diarrhoeas which occur in inflammatory disease of the colon. In this study we examined the pathways for Cl⁻ secretion in human colon and some aspects of their cellular control.

Normal human colon epithelium was dissected from colectomy specimens, mounted in Ussing chambers and voltage-clamped to 0 milli-volts. When necessary a single membrane of the epithelial cell was permeabilised with nystatin in order to study the 'opposite' membrane in isolation.

Entry of Cl⁻ across the basolateral membrane of the epithelial cells was inhibited by basolateral addition of a combination of bumetanide and DIDS. Entry of Cl⁻ at the basolateral membrane was found to be rate limiting for transcellular Cl⁻ secretion. This suggests that basolateral Cl⁻ entry to the cell occurs via Na⁺/K⁺/2Cl⁻ cotransport and/or Cl⁻/HCO₃⁻ exchange. Cl⁻ diffuses across the cell and is extruded at the apical membrane via Cl⁻ channels which were inhibited by a combination of anthracene-9-carboxylic acid (A-9-C) and 5-nitro-2-(3-phenylpropylamino)-benzoic acid NPPB. The NPPB inhibited channel was stimulated by experimental manoeuvres designed to increase intracellular

Ca²⁺. The A-9-C inhibited channel was stimulated by an increase in cellular cAMP (addition of forskolin). This demonstrates that Cl⁻ secretion across the apical membrane occurs via AMP and also by Ca²⁺ activated pathways.

Vectorial trans-cellular Cl⁻ transfer occurs in a basolateral to apical direction. Cl⁻ uptake across the basolateral membrane is the rate limiting step in this process and occurs via Na⁺/K⁺/2Cl⁻ cotransport and/or Cl⁻/HCO₃⁻ exchange. These mechanisms of Cl⁻ uptake are inhibited by bumetanide and DIDS respectively. Cl⁻ secretion across the apical membrane occurs by two separate types of Cl⁻ channel (which may be active simultaneously), a cAMP stimulated Cl⁻ channel which is inhibited by A-9-C, and a Ca²⁺ stimulated Cl⁻ channel which is inhibited by NPPB. The understanding of the transcellular mechanism of Cl⁻ secretion provided here provides a basic model for Cl⁻ (and fluid) secretion in human colon epithelium and allows for study of how these processes are affected by neuro-humoral and pharmacological agents.

DNA PLOIDY ANALYSIS IN METASTATIC GASTRIC ADENOCARCINOMA

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Gastric carcinoma is the most common malignancy in China, the fourth most common in Western Europe and a major cause of cancer morbidity and mortality worldwide. It is well known that metastases, especially distant organ metastases through the blood vascular system, have been the most important cause of patients' death. So far, markers valuable in predicting the metastatic potential of gastric carcinoma have not been identified and the mechanism of metastasis has been unclear. The aim of this study was to determine whether DNA ploidy analysis had any objective value in predicting the metastatic potential of gastric carcinoma to distant organs. DNA ploidy of 57 gastric carcinomas with metastases (12 liver, 1 adrenal, 4 ovary and 48 lymph node) was measured by flow cytometry. DNA aneuploidy was significantly related to liver metastases: 9 out of 12 gastric carcinomas with liver metastases were aneuploid (75%) compared with 13 out of 45 (28.8%) cases without liver metastases (p<0.01); the one gastric carcinoma with adrenal metastasis was also aneuploid. DNA ploidy was not related to ovarian or lymph node metastases. The results suggest that an aneuploid DNA pattern is a predictor of high risk potential for metastases to the liver and may be a useful tool in the "follow-up" of patients with gastric carcinoma in detecting those at high risk of developing metastases following surgical resection.

T CELL SURFACE MOLECULES AND ACTIVATION STATUS DURING ACUTE CELLULAR REJECTION FOLLOWING LIVER TRANSPLANTATION

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Liver transplantation (OLT) is complicated by acute cellular rejection (ACR) in up to 80% of patients with a peak incidence in the first two weeks following surgery. A diagnosis of ACR is suspected on the basis of an acute deterioration in biochemical

liver function, confirmed histologically with liver biopsy and associated with recipient T cell activation. To examine a component of the immunological events associated with ACR we have assessed the changes in T cell surface molecules and T cell activation status following OLT.

Using flow cytometry a total of twenty four peripheral blood T lymphocyte profiles were analysed in six patients over a four week period following liver transplantation, during which four episodes of histologically confirmed ACR developed in three patients. Blood was stained with monoclonal antibodies against CD3, CD4 and CD8 molecules and with dual labelling of the same markers in combination with HLA-DR to define the activation status of the T lymphocyte subsets.

Results: 1. Consistent changes were not observed in the HLA-DR negative CD4+ and CD8+ populations or the ratio of CD4+:CD8+ cells during ACR, a finding which has been equivocal in other studies. 2. All four episodes of ACR were associated with an increase in the activation status (HLA-DR antigen expression) of the CD3+, CD4+ and CD8+ populations for each patient as compared to baseline: Mean % CD3+HLA-DR+, CD4+HLA-DR+ and CD8+HLA-DR+ at baseline and during ACR was 2.8 vs 8.1%, 2.0 vs 12.5% and 1.6 vs 20.8% respectively. 3. Successful treatment of 3 episodes of rejection was accompanied by a decrease in the activation status of the three populations: CD3+, CD4+ and CD8+. Mean % CD3+HLA-DR+, CD4+HLA-DR+ and CD8+HLA-DR+ during ACR and following treatment was 8.1% vs 0.86%, 12.5% vs 0.86% and 20.8% vs 4.8% respectively.

In two patients no episodes of ACR developed following transplantation. In neither of the subjects were there consistent changes in the activation status of the T cell subsets.

In conclusion, we have identified consistent patterns of change in T lymphocyte markers following liver transplantation related to significant clinical events. Serial measurements of these markers may be of use in evaluating patients following transplantation and may predict those at risk of ACR.

HEPATITIS C IN HAEMOPHILIA – THE GOOD NEWS AND THE BAD

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Haemophiliacs are a high risk group for Hepatitis C virus (HCV). Infection is present in 50-90% of patients, depending on age, factor VIII requirements and the source of factor VIII. The life span for haemophiliacs rose from 42 yr in the 1940's to 71 yr in the 1980's.

We have studied 37 haemophiliacs. None presented with liver related disease and all were contacted by postal questionnaire. 30 had haemophilia A, 3 haemophilia B, 4 Von Willebrand disease (VWD) and one was a carrier. There were 34 males and 3 females, ages ranged from 2 to 68 yr. All had ELISA and recombinant immunoblot (RIBA) testing.

20 tested positive by RIBA and 6 of these have circulating virus, demonstrated by polymerase chain reaction (PCR). 17 tested negative for HCV. Mean ALT in the positive groups was 1.59ukat/L, range 0.22 to 6.36, in those negative mean was 0.48ukat/L, and range 0.23 to 0.89. The positive group were older, (mean age 33 yr, range 18 to 68 yr), than those negative (mean age 20 yr range 2 to 51 yr).

Conclusions: HCV has been demonstrated in 54% of this group, which is at the lower end of the reported range. Liver function tests reflected the presence of virus. HCV was commoner in older patients, possibly reflecting increased exposure to factor VIII. Although HCV is a slowly progressive disease, because of the improved life span in haemophiliacs, it is likely to be clinically significant in such patients.

HISTOLOGICAL CORRELATION OF DIFFERENT PATTERNS OF ENDOSCOPIC DUODENITIS

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Whether duodenitis is a distinct clinical entity on its own or a stage in the evolution or resolution of duodenal ulcer disease is still controversial. The aim of our study was to observe the histology of different endoscopic patterns of these lesions.

The case records of 41 consecutive patients (M:F ratio 2:1, mean age 38 yr, range 21 - 48 yrs) with endoscopic evidence of duodenitis were studied. Patients on anti ulcer medication before endoscopy were excluded from the study. All the endoscopies were performed in a single GI unit. Biopsies for histology were taken from the endoscopically inflamed areas of the bulb. The presence of *Helicobacter Pylori* (Hp) infection was checked with the deltawest test. 4 different endoscopic patterns of duodenitis were observed: patchy (n=8, 19%), diffuse (n=16, 39%), erosive (n=11, 27%), and nodular (n=6, 15%). 12 (30%) had normal histology (4/8 of patchy, 6/16 of diffuse, 1/11 of erosive and 1/6 of nodular duodenitis). In the remaining 29 patients, histology showed acute inflammation in 17 (59%), chronic inflammation in 8 (28%) and gastric metaplasia in 4 (13%). Acute inflammation was most frequently seen in erosive duodenitis (90%), and gastric metaplasia in nodular duodenitis (40%). Of the 29 patients with abnormal histology, deltawest test was positive in 20 cases (69%), most frequently in erosive duodenitis (90% of the cases).

Conclusion: The endoscopic diagnosis of 'patchy' duodenitis is unreliable and therefore if suspected, should be confirmed on histology. Erosive duodenitis is the type most accurately diagnosed endoscopically, most frequently associated with underlying acute inflammation and the presence of Hp infection. Nodular duodenitis is most commonly associated with underlying gastric metaplasia.

NON-INVASIVE DIAGNOSIS OF H.PYLORI INFECTION; A SIMPLIFIED APPROACH

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Non-invasive tests to detect *H.pylori* are necessary for epidemiological studies, investigation of dyspepsia and for follow-up after eradication treatment. The ¹³Carbon urea breath test (UBT) is highly sensitive and specific. However, the current recommended protocol for the UBT, including an overnight fast, the use of large reservoir bags, multiple positional changes and sample collections render the test unsuitable for widespread use. The aim of this study was to assess the sensitivity and specificity of a modified, simple UBT.

Subjects undergoing endoscopy for investigation of dyspepsia or after a course of eradication therapy were recruited. Non-fasting patients were administered a standard motility-inhibiting

liquid meal (50mls Calogen + 50mls Ensure). Immediately after, breath samples were collected in duplicate in 20ml vacutainers via straws. Subjects then received 100 mg of carbon urea dissolved in 100 ml of water. After sitting for 30 min, repeat breath samples were collected in vacutainers. An excess of ¹³CO₂ excretion of 5 per ml was taken as a positive result. The UBT results were compared with antral and corpus histology (x4), antral and corpus culture (x2) and antral CLO test. The "gold standard" was defined by the results of any two of the biopsy based methods.

169 subjects were recruited (98 male), mean age 42 yr (range 17-19). 89 subjects had a positive UBT; 85 true positives and 4 false positives. 80 subjects had a negative UBT; 78 true negatives and 2 false negatives. Therefore the sensitivity was 97.7% and the specificity was 95.1%.

Conclusion: This modified UBT is user-friendly, ideal for use in a primary care setting and is as reliable as other methods for the non-invasive detection of *H.pylori*.

OESTRADIOL PROMOTES SALT AND WATER RETENTION BY HUMAN COLON

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High oestrogen states (pregnancy, the use of the oral contraceptive pill, phases of the menstrual cycle) are associated with hypertension and oedema secondary to salt and water retention. The natriuretic steroid aldosterone promotes salt and water retention by activation of the ATP regulated potassium channel (K_{ATP}) via a membrane-bound, protein kinase C dependant mechanism and a slower genomic pathway. We investigated whether oestrogens have similar effects.

Normal human colonic epithelium was mounted in Ussing chambers and the transmembrane potential was clamped to 0mV by application of a short circuit current (S.C.C.) which is then a measure of electrogenic ion transport. The apical membrane was rendered electrically silent by treatment of the mucosal surface with the ionophore nystatin and a mucosa to serosal potassium gradient was established. Under these conditions the S.C.C. is a measure of potassium ion transport across the basolateral K_{ATP} channel. Subsequent addition of the oestrogen β-oestradiol produced a 7.2 ± 0.8% increase in S.C.C. (n=5) within 30 min. 17-α-oestradiol was more effective, it produced a 10.2 ± 1.2% increase within 30 min (n=4). Addition of chloride ions to the apical membrane of the nystatin treated colon causes cell swelling, this activates a calcium regulated potassium channel (K_{Ca}) and inhibits the K_{ATP} channel. Under these conditions addition of 17-α-oestradiol caused a 7.1 ± 1% decrease in S.C.C. after 30 min (n=4). These effects on the K_{ATP} and the K_{Ca} channels were abolished by treating the basolateral membrane with the diuretic amiloride (P<0.01) or with sodium free Krebs (P<0.01), this suggests that these effects occur due to alkalinization of the cell by stimulation of the Na⁺/H⁺ exchanger.

Upregulation of the K_{ATP} channel promotes salt retention by generating a favourable electrochemical gradient for sodium absorption by surface cells. Inhibition of the K_{Ca} channels prevents chloride secretion by colonic crypt cells. Chloride secretion causes loss of salt and water from the colon. Oestradiol simultaneously activates sodium absorption and inhibits chloride secretion thus causing net salt and water retention in human

colon. This provides a model for oestrogen effects on salt absorbing epithelia and has important implications for the management of fluid balance disturbances associated with high oestrogen states.

INCIDENCE OF ULCERATIVE COLITIS AND CROHN'S DISEASE IN AN IRISH POPULATION

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The aim of the study was to estimate the incidence of symptomatic ulcerative colitis (UC) and Crohn's disease (CD) prospectively from 1st October 1991 to 30th September 1992 in the Eastern Health Board region.

Cases were defined using the Lennard-Jones criteria and were identified primarily by systematic examination of hospital medical records. Risk factor data was obtained via telephone interviews with the incident cases. Data on each patient were collected using a standardised questionnaire by one researcher.

A total of 135 UC cases and 56 CD cases were identified during the 12 month study period. Using population figures from the 1991 census, crude incidence rates for the diseases were 11.03/100,000 in UC and 4.58/100,000 in CD. Mean age at diagnosis was 41.2 yr in UC (range 16-85 yr) and 35.6 yr in CD (range 10-76 yr). Males accounted for 58.2% of UC and 39.3% of CD cases. The presence of blood or mucous in stools was the most frequent presenting symptom in all cases: 86.6% in UC and 50.0% in CD. Risk factor data demonstrated that 26.1% of UC and 51.8% of CD cases were smokers (males and females) and that 48.3% of UC and 50% of CD cases had ever used the oral contraceptive pill (females only). A positive family history of inflammatory bowel disease (in first degree relatives) was found in 1.5% of UC and 16.1% of CD cases.

Conclusions: This is the first study to estimate the incidence of UC and CD in the Irish Republic, from a study population of over 1 million people. Demographic and risk factor data among Irish cases are consistent with previously published studies. These incidence rates may be used as a baseline to detect any change in the risk of inflammatory bowel disease in future studies.

THE NON-FAS TRACK TO APOPTOSIS IN LYMPHOCYTES

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Apoptosis (a form of programmed cell death) of lymphocytes is an important mode of immune regulation in the mucosal immune system. Apoptosis also has a critical role in pathologic states including tumour-derived immune suppression, inflammation and acquired immune deficiency syndrome. The only physiological, receptor-mediated mechanism for lymphocyte apoptosis that has been characterized is the Apo-1/FAS (FAS) receptor-ligand pathway. We have used a partially purified oesophageal tumour-derived factor to demonstrate the presence of a hitherto unrecognised, non-FAS mechanism of apoptosis in mucosal and systemic lymphocytes. The factor is selective, non-toxic, and is inhibitory to activated, proliferative but not resting lymphocytes.

The FAS antigen-deficient MRL/lpr mice and their normal congenic control strain were obtained and bred under

conventional conditions. The MRL/lpr mouse has a specific genetic mutation leading to defective apoptosis through the FAS pathway and develops lymphoproliferative and autoimmune disorders. A partially purified preparation of oesophageal tumour-derived immune suppressor factor that has been shown to be free of all known cytokines was tested in dose-ranging studies on cell proliferation and apoptosis using lymphocytes from the mutant and control mice. Proliferation was shown using thymidine incorporation and apoptosis demonstrated by flow cytometry (propidium iodide/Hoechst reagent).

The lpr mice were confirmed to have deficient FAS signalling as shown by defective labeling with FITC-tagged monoclonal anti-FAS and by defective inhibition of proliferation in functional assays with FAS antibody. Lymphocytes from the congenic control strain expressed the FAS receptor appropriately and had no functional deficit. When the responsiveness of lymphocytes from the two strains of mice to the tumour-derived immunosuppressor were compared, it was found that suppression leading to apoptosis occurred equally in a concentration-dependent manner in both the FAS-mutant (lpr) and the FAS-positive congenic control.

Conclusion: There exists a non-FAS-mediated pathway for triggering apoptosis in lymphocytes as shown by the ability of a human tumour-derived immune suppressor to mediate apoptosis equally in FAS-negative (mutant) and FAS-positive murine lymphocytes. This system can be now used to probe the non-FAS pathway and associated secondary signalling events.

UROKINASE TYPE PLASMINOGEN ACTIVATOR (uPA) PROFILE IN COLORECTAL NEOPLASIA AND RELATIONSHIP TO PATIENT SURVIVAL

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The malignancy of colorectal cancer (CRC) is related to its ability to disseminate as evidenced by the observation that the majority of CRC deaths are due to the development of metastases. Urokinase-type plasminogen activator (u-PA) is a serine protease implicated in tumour invasion. We previously employed semi-quantitative immunohistochemistry to demonstrate that u-PA antigen level is of prognostic importance in Duke's B colorectal cancer (CRC)¹.

The aim was to quantitate u-PA levels in colorectal tissue and to determine the relationship between u-PA level, clinical features, tumour stage, tumour grade and patient survival following CRC resection.

There were 105 CRC cases (63 males, 42 females mean age 68 yr, range 36-83). Tissue level of u-PA antigen was measured on detergent extracts of CRCs and corresponding colorectal mucosa remote from the cancer using an ELISA assay (American Diagnostica). There was a wide variation in the range of u-PA levels in normal mucosa and a significant difference between normal colonic and normal rectal levels ($p=0.001$). Results are expressed as ratio of cancer u-PA to normal mucosal u-PA to correct for this variation. u-PA ratio was independent of age ($p=0.6$), gender ($p=0.23$) tumour site ($p=0.77$), tumour size ($p=0.75$) or tumour stage ($p=0.16$). u-PA cancer/normal ratios were greater in poorly differentiated tumours ($p=0.03$). Patients with a high cancer/normal u-PA ratio had a significantly shorter survival than those with lower ratio (logrank test $p=0.0009$).

Cox regression analysis identified tumour stage (relative risk [RR] 2.6, $p < 0.0001$) and cancer/normal u-PA ratio (RR 2.3, $p = 0.05$) as independent prognostic features.

Conclusion: u-PA antigen measurement is an independent variable which is predictive of survival in colorectal cancer.

Reference

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COST-EFFECTIVE H. PYLORI ERADICATION

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In recent years, the efficacy of treatments aimed at the inexpensive, one week Bologna regime (omeprazole 20 mg daily + clarithromycin 250 mg b.i.d. + metronidazole 400 mg b.i.d.) and the two week Bordeaux regime (omeprazole 20 mg b.i.d. + amoxicillin 1 gram b.i.d + clarithromycin 500 mg b.i.d.) achieve eradication in 90-100% of cases. There is as yet, however, no standardised approach to eradication therapy. A first line treatment should be reliable, well-tolerated, inexpensive and efficacious.

The aim was 1) to assess the suitability of the Bologna regime as a first line eradication treatment. 2) To determine the factors that lead to treatment failure. 3) To evaluate the Bordeaux regime as a second line eradication treatment.

Subjects with H.pylori-associated duodenal ulceration (DU) or non-ulcer dyspepsia (NUD) were recruited at endoscopy. H.pylori status was assessed before and 4 weeks after treatment by histology (antral + corpus x 2), culture (antral + corpus) and CLO-test (antral); subjects were positive if 2 or more tests were +ve and negative if all tests were -ve. All subjects were treated with the Bologna regime.

162 subjects were enrolled (79 male), 141 NUD and 21 DU, mean age 49 yr (range 18-78). 150 patients completed the follow-up. H.pylori was eradicated in 121/150 (80.6%). Pre-treatment sensitivities were available in 20 of the 29 patients in whom treatment failed. 18/20 (90%) had primary metronidazole resistance, 1/20 had metronidazole and clarithromycin resistance and the remaining patient was sensitive to both antibiotics. 14 of the 29 subjects were subsequently treated with the Bordeaux regime. H. pylori was eradicated in 13/14 (92.9%).

Conclusion. The inexpensive, Bologna regime eradicated H. pylori in 80.6% of patients. Primary metronidazole resistance is an important factor in treatment failure. The more expensive Bordeaux regime is a highly effective second-line treatment.

A PROSPECTIVE COMPARISON OF LAPAROSCOPY AND COMBINED IMAGING IN THE STAGING OF DISTAL OESOPHAGEAL AND GASTRIC CANCER PRIOR TO SURGERY - FINAL REPORT

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A prospective comparison of laparoscopy and combined imaging (computed tomography and ultrasound) was made in the pre-operative staging of distal oesophageal and gastric cancer in patients who were selected for surgery.

Patients with clinically overt metastases or a contraindication to major surgery were excluded from the study. All patients underwent chest radiography, computed tomography of mediastinum and abdomen, ultrasonography of abdomen and laparoscopy.

Of 145 patients assessed consecutively, 39 (26.89%) had metastatic disease to the liver or peritoneal cavity, 35 of whom were detected pre-operatively. Metastases were detected by laparoscopy in 31 patients, and by combined imaging in 16 (ultrasound 11, contrast enhanced CT Scan 14). The distribution of metastases detected pre-operatively were peritoneal cavity in 23 (laparoscopy 23 versus combined imaging 7) and hepatic 12 (laparoscopy 9, combined imaging 7). Laparoscopy was significantly more sensitive than combined imaging in detecting metastases in patients with adenocarcinoma (laparoscopy 29, combined imaging 12) than in those with squamous cell carcinoma (laparoscopy 2, combined imaging 4).

Conclusion. Patients with cancer of the oesophago-gastric region who are selected for surgery, have metastases to the peritoneal cavity more frequently than to the liver. These metastases are more frequently detected by laparoscopy than by imaging methods. The addition of laparoscopy to the staging protocol prevented unbeneficial thoraco-abdominal exploration in 28 patients. Laparoscopy should be used in the assessment of these patients immediately prior to performing excisional surgery.

LAPAROSCOPICALLY-ASSISTED COLECTOMY: IS THERE AN INDICATION?

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Laparoscopic surgery has appeal for the performance of intra-abdominal procedures with minimal invasion, however the range of its applicability requires further definition. The utilisation of laparoscopy in colorectal surgery has been limited by concern regarding the adequacy of 'clearance' of malignant disease with this technique. A series of patients in whom laparoscopic mobilisation of the colon was performed to assist colectomy, facilitating a less invasive abdominal incision, is reported.

From July 1994, 16 patients have undergone a laparoscopically-assisted colectomy (LAC). (10 males, 6 females; mean age: 57.5 yr, range: 20-81 yr). The indication for surgery and the procedure performed are listed in the table. LAC was only undertaken for benign disease, or for low colorectal malignancies, with the 'cancer operation' being performed at open surgery. Operative technique was as follows:

INDICATION		PROCEDURE	
Ulcerative Colitis	3	Proctocolectomy + Pouch	1
		Proctocolectomy + Ileostomy	1
		Panproctocolectomy	1
Crohn's Disease	1	Subtotal Colectomy + Ileostomy	1
Polyposis Coli	1	Subtotal Colectomy + Ileorectal Anastomosis	1
RIF/Caecal mass (histology benign)	1	Right Hemicolectomy	1
Diverticular disease/ Fistula	1	Sigmoid Colectomy	1
Diverticular Disease/ Occult rectal carcinoma	1	Rectosigmoid Colectomy	1
Rectosigmoid carcinoma	4	<u>Anterior Resection</u>	8
Rectal carcinoma	4	+ End Colostomy	4
		+ Loop Colostomy	1
		+ Small bowel resection	1

With the patient in the Lloyd-Davis position, pneumoperitoneum was induced in the standard fashion. Laparoscopic access was via 4 ports (10mm x 3; 5mm x 1). Depending on the procedure, the splenic flexure alone, or with the transverse colon and hepatic flexure, was mobilised laparoscopically. Thereafter, access was via a transverse lower abdominal incision for completion of the procedure.

In all patients, colonic mobilisation was successfully achieved at laparoscopy. There were no complications related to this procedure. In 1 patient, a major pelvic bleed occurred during the open dissection. Mean operating time (total) was 222.5 min (range: 180-380). Colonic mobilisation was completed in less than 1 hr in all cases. 3 patients developed specific post-operative complications (anastomotic dehiscence: 1; Pouch sepsis / excision: 1, pelvic abscess: 1). Initial results indicate reduced post-operative respiratory complications, analgesia requirements and hospital stay in patients undergoing laparoscopically assisted colectomy.

POTASSIUM RECYCLING REGULATES FLUID SECRETION BY THE HUMAN AND OTHER MAMMALIAN COLONS

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Colonic fluid hypersecretion is an important and life threatening consequence of diseases such as inflammatory bowel disease and infectious diarrhoeas. Secretion of chloride ion occurs in the crypts while sodium and water follow passively. The chloride ions leave the cell through an ion channel, this involves a net loss of positive ion from the apical membrane which must be balanced by an exit of positive across the basolateral membrane. In this study we investigated the role of basolateral potassium channels in regulating charge balance thus maintaining secretion of chloride and ultimately salt and water.

Samples of colon were obtained from humans, rabbits and mice. Sheets of isolated epithelium were mounted in Ussing chambers. The short circuit current was measured under voltage clamped conditions. Bathing solutions were adapted to study chloride secretion and potassium recycling in isolation.

Addition of forskolin (a secretagogue which increases intracellular cAMP) produced an immediate and sustained increase in chloride ion secretion in human (Δ S.C.C. = 45 ± 8 uA/cm² n=10) rabbit (Δ S.C.C. = 30 ± 5 uA/cm² n=4) and mouse (Δ S.C.C. = 30 ± 5 uA/cm² n=4). Treatment of the basolateral membrane with 10 umol tetra-pentyl-ammonium (T.P.A. an inhibitor of the K_{Ca} channel) produced a complete inhibition of the response in all three species, whereas tolbutamide (an inhibitor of the K_{ATP} channel) produced less than 5% inhibition in all colons. Addition of carbachol (a secretagogue which increases intracellular calcium) produced an immediate but transient increase in chloride secretion which was completely inhibited by pre-treatment with T.P.A.

Chloride secretion in human and other mammalian colons is dependent upon potassium exit through the K_{Ca} channel on the basolateral membrane, this is essential to maintain charge balance within the cell. Fluid secretion into the lumen of the colon may be prevented by inhibition of these potassium channels. The basolateral K_{Ca} channel may be a useful target for the pharmacological control of fluid secretion in the colon.

CONSTIPATION NOT ANAL SPHINCTER HYPERTONIA PREDISPOSES TO POSTPARTUM ANAL FISSURE

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Anal canal pressures may be raised in patients with acute anal fissure. A prospective study of the effect of parturition on the pelvic floor has allowed study of the hypothesis that anal sphincter hypertonia predisposes to postpartum anal fissure.

313 primigravid women were studied. Anal manometry was performed six weeks before and after delivery in 209 women. Postpartum studies only were performed on a further 104 women. A bowel symptom questionnaire and detailed obstetrical record were kept. Anal fissure was diagnosed on history and examination.

29 (9.3%) women developed postpartum anal fissure. Antenatal anal canal pressures were identical in those who did and did not develop fissure. Postpartum resting and squeeze anal pressures were decreased equally (table). Postnatal constipation was more common in those with fissure (62%) than those without (29%, χ^2 5.2, $p < 0.02$). Caesarean section (11%), operative delivery (23%), perineal tear (22%) or epidural anaesthetic (54%) did not effect the incidence of fissure.

Conclusion. Anal canal pressures are normal prior to development of postpartum anal fissure, and decrease postpartum.

mean (CI) (mmHg)	MAX RESTING		MAX SQUEEZE INCREMENT	
	ante	post	ante	post
FISSURE (n=21)	58 (52, 72)	49 ^b (45, 56)	50 (29, 65)	30 ^c (18, 35)
NO FISSURE (n=188)	57 (55, 59)	54 ^a (51, 57)	60 (56, 64)	39 ^c (36, 42)

a $p = 0.05$, b $p < 0.01$, c $p < 0.001$ (v antepartum, paired t test)

PASSIVE IMMUNISATION OF THE GASTROINTESTINAL TRACT WITH HEN EGG IMMUNOGLOBULIN

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Gastrointestinal (GI) infection is a common cause of morbidity in young animals. Evidence is emerging that passive oral immunisation of the G.I. tract with specific immunoglobulin (Ig) provides protection against intestinal pathogens. Hens immunised against such organisms lay eggs containing large amounts of specific Ig.

The aim of this study was to test the ability of specific hen egg Ig to protect against *Salmonella* infection, in the young calf.

Twelve Isa Brown hens were immunised with heat inactivated *Salmonella*. An ELISA was developed to measure specific Ig levels, which reached a peak 20 days post immunisation. Specific Ig rich eggs were collected, the yolks harvested and pooled.

An animal model of *Salmonella* induced gastroenteritis was developed using young calves. Forty two animals were divided into three groups. Two of these groups acted as controls, one receiving no egg yolk supplement, while the other received normal egg yolk from non-immunised hens as a supplement. The third group received a supplement of specific-Ig rich egg yolk from immunised hens. After five days each animal was orally challenged with 5×10^8 live *Salmonella*. In the 2 groups of control animals, 14/16 animals given no egg supplement, and

9/13 animals fed normal egg, succumbed to infection within three days of challenge. However, in the group fed Ig rich eggs, only 4/13 animals succumbed to infection after challenge. These preliminary results suggest that specific hen egg yolk Ig has a potential use in passive oral immunisation against GI infections.

SURGICAL AND SMOKING HISTORY IN I.B.D.; A CASE CONTROL STUDY

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An inverse relationship between appendectomy and ulcerative colitis (U.C.) and smoking and U.C. has been proposed. Our study examines the frequency of common surgical interventions and of smoking in a group of IBD patients attending a twice weekly G.I. clinic.

440 patients were interviewed in a prospective, questionnaire based case control study. The subjects comprised 143 patients with U.C., 117 patients with Crohn's and 175 controls derived from an orthopaedic traumatology clinic matched for age, sex and socio-economic group. Subjects were questioned on all previous surgery and on smoking history.

The appendectomy rate amongst controls was 18.2% (32/175) which was significantly greater than that of U.C. patients 10.1% (15/148 $p < 0.05$) 11 of the U.C. patients had appendectomy prior to disease onset. The appendectomies were evenly distributed through the 3 categories of disease extent (proctitis, left sided and extensive colitis). There was no significant difference in appendectomy rate between Crohn's patients and controls. The 3 groups had comparable rates of tonsillectomy and cholecystectomy. 83.2% of the U.C. patients were non-smokers at the time of diagnosis in contrast to 50.4% amongst the Crohn's patients ($p < 0.01$).

Conclusions: U.C. is associated with significantly lower appendectomy rate than in controls. This is not true for other surgical procedures unrelated to the treatment of U.C. The role of the appendix in the evolution of U.C. requires definition and clarification with large multicentre studies.

GLIADIN STIMULATED CYTOKINE PRODUCTION

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The immune response to gliadin has been proposed to play a central role in the pathogenesis of coeliac disease. In this study, sensitivity to gliadin was investigated in 20 coeliac and 14 normal individuals. PBMCs were cultured with soluble gliadin and latex bead coated gliadin (latex-gliadin) at varying concentrations. PPD was included as a control antigen. Culture supernatants were removed after three days and cytokines (IL4, IL5, IL6, IFN γ) measured using capture ELISAs. Proliferation was assessed on day 6 by measuring thymidine incorporation.

Gliadin induced proliferation in cell cultures from 16 of twenty coeliac and 9 of fourteen normal individuals. IL6 production was seen in 11 of thirteen coeliacs and 8 of nine normal controls and these responses were independent of

proliferation. Gliadin failed to induce IL4 or IL5 production. Whereas PPD induced IFN γ production in association with proliferation in all individuals, gliadin induced IFN γ production was less frequently observed. Of 15 CD patients, four (all with active disease) demonstrated gliadin induced IFN γ production (with both soluble and latex-gliadin). In the normal controls an IFN γ response was only observed with latex-gliadin in two of 8 individuals tested. Latex-gliadin also seemed more potent at inducing proliferation with 4 subjects (3 normals, 1 coeliac) responding to this preparation alone. In preliminary experiments, cells cultured with gliadin for ten days when restimulated with gliadin demonstrated a suppressed response: in this cell proliferation, IL4 and IL6 production was inhibited but IFN γ was increased.

These results indicate that gliadin specific T cells are present in the peripheral blood of both coeliac and normal individuals. It is postulated that cells may be of the Th2 phenotype in normal subjects and coeliac patients with inactive disease (due to the observed increase in IL6 in the absence of IFN γ production) whereas in patients with active disease Th1 responses may predominate.

ADULT COELIAC DISEASE IN AN IRISH PROVINCIAL HOSPITAL

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Previous reports on adult coeliac disease in Ireland came from teaching hospitals. These often serve as tertiary referral centres with a possible resulting different patient type from hospitals serving a district general type function. We report on all patients diagnosed over a 10 year period in a county hospital serving a mixed urban/rural population of about 85,000.

A retrospective study of all patients newly diagnosed with coeliac disease between 1/1/85 and 31/12/94 who were over the age of 16 years was carried out. The sex, age, delay from onset of symptoms to diagnosis and mode of presentation were established and lab. parameters included haematology screen, serum calcium, serum albumin and specific antibodies were also recorded.

There were 46 patients diagnosed over the 10 years at a mean age of 51 (range 16 - 79 yr). The duration of symptoms prior to diagnosis ranged from three weeks to 30 yr (average 17 months). Those with mild non specific symptoms and in particular females, were most likely to have long delayed diagnosis. The principal modes of presentation were diarrhoea 26 (56%), weight loss 22 (47%), anaemia 18 (39%) of which 30% was iron deficiency, hypocalcaemia 5 (11%), steatorrhoea 3 (7%). In addition to haematological abnormalities, other abnormal lab. tests included low serum calcium in 9 of 36 tested, low serum albumin in 4 of 20 tested. Alpha gliaden antibodies were raised in 16 and normal in 10 patients with definite coeliac disease before starting on a gluten free diet. Endomysial antibodies in our population also seemed to be a poor marker for the condition, being raised in only 1 of 4 patients with definite coeliac disease before starting on a gluten free diet.

Conclusions: In adults the symptoms of coeliac disease may be non specific but certain symptoms such as diarrhoea with or without weight loss, inadequately explained anaemia (particularly iron deficiency) and hypocalcaemia should suggest early small bowel biopsy to rule out coeliac disease. Our study demonstrates a very long delay in diagnosis in some patients and particularly in women of child bearing years with anaemia.

Despite early optimism alpha gliaden antibodies have poor specificity and sensitivity in adult patients with coeliac disease in our area and it appears that endomysial antibody may not be any great improvement.

THE CYTOTOXIC POTENTIAL OF PERIPHERAL BLOOD MONOCYTES AND COLORECTAL TUMOR INFILTRATING MACROPHAGES IS MEDIATED THROUGH ENHANCED ARGINASE PRODUCTION

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Activated MØs produce O₂⁻ and TNF, known tumouricidal mediators. TIMs have been reported to demonstrate impaired production of these mediators suggesting that these effector cells are suppressed. We postulated that TIM cytotoxicity is not dependent on the release of these cytotoxic agents but is mediated via the release of the enzyme arginase. Currie *et al* have demonstrated that arginase is cytotoxic to tumour cells by depletion of the essential amino acid L-arginine, therefore the aim of this study was to determine the role of this enzyme in colorectal tumour-derived MØs. Human peripheral blood monocytes (PBM) were isolated from aged-matched controls (CON) and from blood pre-operatively obtained from patients undergoing surgery for colorectal cancer. Colorectal tumour specimens (5-10gm) were freshly procured and digested. The percentage of TIMs was then assessed flow cytometrically, and both PBMs and TIMs were seeded at a concentration of 2 x 10⁵/well. Cytotoxicity was assessed using a standard ⁵¹Cr release assay against the SW707 colorectal cell line. TNF, O₂⁻ generation and cytosolic arginase were also assessed.

	Cytotoxicity %	Superoxide nmols/µg BSA	TNF pg/µg BSA	Arginase µmols/µg BSA/hr	Arginase Inhibition % Cytotoxicity
CON	16.3±6.8	0.028±0.018	37.30±10.26	57.57±18.49	6.2±5.6\$
PBM	43.4±19.4*	0.020±0.003	15.80±2.02*	91.20±17.04*	18.4±9.2\$
TIM	65.8±7.9*	0.016±0.003	12.34±1.53*	154.0±17.28*@	20.6±15.7\$

Data = Mean±SD, Stats.= ANOVA, *p<0.05 Vs CON, @p<0.05 Vs PBM, \$p<0.05 Vs cytotoxicity.

These results indicate that both PBM and TIMs have impaired production of TNF and O₂⁻, however, cytotoxicity is enhanced above CON and is associated with a paralleled increase in arginase activity. The arginase-dependent nature of this cytotoxicity was confirmed by addition of the inhibitor L-lysine which significantly abrogated cytotoxicity in all groups. These results suggest caution in the use of the arginase substrate L-arginine in the management of colorectal cancers.

MØ=macrophage, O₂⁻=superoxide anion, TNF=tumour necrosis factor, TIMs=tumour infiltrating macrophages.

Reference

Curry, G. A. Activated macrophages kill tumour cells by releasing arginase. *Nature* 1978; vol. 273, p. 758.

L-ARGININE INCREASES TUMOUR BURDEN IN A RAT MODEL OF COLORECTAL CANCER

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The antitumour effects of L-arginine has been demonstrated in a number of experimental models including mammary and

transplantable solid tumours. This study was to investigate the effect of dietary arginine in procarcinogen 1,2-dimethylhydrazine (DMH) induced colorectal cancer model.

Colorectal tumours were produced in male Wistar rats by 20 weekly subcutaneous injections of DMH at a dosage of 20 mg/kg body weight. L-arginine was given in a 1% solution of drinking water. Animals were sacrificed 2 weeks after the last DMH injection. Animals were divided into 4 groups. Group I was given DMH injections, Group II was given DMH and 1% arginine for 22 weeks, Group III DMH and arginine for the first 10 weeks only, Group IV DMH and arginine for the last 12 weeks.

Results (Mean±SEM)

	total tumours	incidence (%)	tumour/animal	tumour/tumour bearing animal	tumour area	tumour volume
Group I (24)	74	96	3.08±0.28	3.22±0.26	12.11±1.63	40.67±7.48
Group II (24)	41	79	1.71±0.34**	2.16±0.37**	8.05±1.67	23.11±6.80
Group III (24)	24	58*	1.0±0.25**	1.71±0.3**	4.82±1.52*	12.02±5.69*
Group IV (21)	98	90	4.67±0.66*	5.16±0.62*	7.02±0.94	18.56±4.70

*p<0.05, **p<0.005 compared with DMH control (Mann-Whitney U test).

Fourteen out of 24 animals developed tumours in Group III compared to 23 out of 24 in Group I (p=0.006, Yates-corrected Chi² test), 19 out of 24 in Group II and 19 out of 21 in Group IV. Decreased incidence concomitant with reduced tumour area and volume were found only when dietary arginine was given in the first 10 weeks of colorectal carcinogenesis i.e. during the initiation stages. Arginine supplementation during the promotion phases appeared to significantly enhance the tumour burden.

Conclusion. These results suggest that dietary arginine may be used for tumour prevention only in the early stages of colorectal carcinogenesis before tumour formation but that its effects may be harmful in the later stages of tumour development.

INTERFERON ALPHA THERAPY IN HAEMOPHILIACS WITH CHRONIC HEPATITIS C

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Early experience with interferon therapy in haemophiliacs chronically infected with hepatitis C suggested that they respond as well as non-haemophiliacs to interferon alpha. The aims of this study were to examine the effects of interferon therapy in hepatitis C in an Irish haemophiliac population.

We prospectively studied 29 haemophiliac patients who received 32 courses of interferon alpha 2b (IFN), using HCV-RNA PCR to guide dosage and duration of therapy as well as to determine virological response.

All patients were asymptomatic, HCV RIBA and PCR positive, and had abnormal liver enzymes. Seven had concomitant asymptomatic HIV infection; none had hepatitis B. Genotyping in 20 patients revealed Type 1 in 14, Type 2 in 2 and Type 3 in 4. Treatment was initiated with IFN 3-5 MU s.c. alt die and increased to a max of 6 MU if LFT's remained raised or PCR +ve after 3 months. Blood was drawn monthly for liver enzymes and PCR. Responders were encouraged to continue treatment for 12 months.

Three patients had sustained biochemical normalisation and

negative PCR on treatment of which 1 remains in remission 1 year off treatment. Fourteen became virologically negative by PCR on treatment but relapsed within 3 months of stopping interferon. Three were retreated with interferon and again responded while on treatment. Twelve had no evidence of biochemical or virological response.

We conclude that response to interferon is common in an Irish haemophilic population. However, sustained virological and biochemical response rates appear <10% using interferon monotherapy in this cohort. These results suggest that either combination therapy or maintenance therapy might be worthwhile treatment options.

ARGININE INDUCES PANCREATITIS BY A NITRIC OXIDE INDEPENDENT MECHANISM

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Arginine is used therapeutically in humans to correct hypertensive states, but has been shown in high doses to induce pancreatitis in rats. As arginine is a producer of nitric oxide (NO) this has been thought to be the cause of the pancreatitis. This study was designed to test this hypothesis. 500mg per 100g body weight of arginine was injected intraperitoneally either alone or with nitric oxide synthesis blockers (l-NAME and l-NNA) or a breakdown inhibitor (superoxide dismutase-SOD). 90 Sprague-Dawley rats were studied for 24 or 72 hr, before estimation of serum amylase, wet:dry lung weight ratio and histology of lung and pancreas.

Results are as shown; the relevant inter-group comparisons will be discussed.

group	n	pancreatitis		24hr amylase	ARDS	w/d ratio
		mild	severe			
control	5	0	0	4072(588)	0	4.4(0.7)
arginine	15	8	6	5574(1357)	12	5.5(1.3)
l-NAME	15	0	0	5400(980)	0	4.7(0.6)
l-NNA	5	0	0	4065(1085)	0	4.3(1.1)
SOD	10	3	0	3334(711)	0	4.3(0.9)
arg + l-NAME	15	3	12	12200(6449)	5	4.2(0.6)
arg + l-NNA	16	1	15	8932(3897)	1	5.3(0.5)
arg + SOD	9	1	8	6888(1980)	8	6.4(1.7)
p value		< 0.0001		<0.001	<0.0001	<0.0001
statistics		χ^2		ANOVA	χ^2	ANOVA

Arginine induces pancreatitis in rats, irrespective of drugs acting on NO metabolism. Histological changes are more severe after 72 hr and with l-NAME and l-NNA. Pancreatitis-induced lung changes however appear to be NO mediated.

THE EFFECT OF THE HEAT SHOCK AND ACUTE PHASE RESPONSES ON E. COLI INDUCED PMN APOPTOSIS

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The neutrophil (PMN) has been implicated in sepsis-induced tissue injury. This may result from PMNs dying by cell lysis rather than apoptosis (apop) with extrusion of deleterious cellular contents. The principle role of the PMN is to ingest and kill

bacteria and we have previously shown that the ingestion of *E.coli* results in the induction of PMN apop. During systemic inflammatory response syndrome (SIRS), there are elevations in both the acute phase and heat shock proteins, however the effects of these on *E.coli* induced PMN cell death is unknown. So the aims of this study were to determine the effects of heat shock and acute phase responses on *E.coli* induced PMN apop. PMNs were isolated from 10 healthy volunteers. 1×10^6 PMN/ml were divided into three groups, control, LPS (10ng/ml for one hour) or heat (42°C for 45 min). PMN activation was assessed after LPS co-culture by ingestion of *E.coli* and CD11b expression. Heat shock protein 72 was assessed in the heated group by western blotting. LPS resulted in a significant increase in both ingestion (control PMN 416 ± 53 LnMCF and LPS primed PMN 518 ± 23 LnMCF) and CD 11b expression (control PMN 983 ± 94 LnMCF and LPS primed PMN 2103 ± 515 LnMCF). Heating resulted in the expression of HSP 72. The PMN were then further divided into two groups, one incubated with medium alone and the other *E.Coli* (1:25 PMN:*E.coli*) for 12 hr. Apop was then assessed by Prodidium iodide DNA staining and FcγRIII receptor.

	% Apoptosis			FcRIII (LnMCF)		
	Control	LPS	Heat	Control	LPS	Heat
PMN + Medium	28±7	14±6	48±18*	1028±200	1059±87	253±33*
PMN + E.coli	63±9	65±5	82±15§	242±43	139±74	47±19§

Data = Mean ± SD. Stats = ANOVA with Scheffe correction.

*p<0.05 vs Control PMN + Medium. §p<0.05 vs Control PMN + *E.coli*.

These results demonstrate that *E.coli* induces PMN apop, as indicated by an increase in apop and decrease in FcγRIII receptor expression. On pre-exposure to LPS there was a significant reduction in the rate of spontaneous apop. However on heat shocking the PMN there was a significant increase in spontaneous and inducible apop. This indicates that a heat shock response as can occur with pyrexia increases the rate of PMN apop. Therefore, the presence of a pyrexia during the septic state may play an important role in the removal of PMN by apop, thus avoiding tissue injury.

DOES MALTECTOMY (APPENDICECTOMY/ TONSILLECTOMY) INFLUENCE THE OCCURRENCE OR MODE OF PRESENTATION OF ADULT COELIAC DISEASE?

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Recent reports suggested that appendicectomy may protect against the development of ulcerative colitis. Depletion of mucosal helper T cells is the suggested mechanism. Since mucosal T cell activity has an even more critical role in the pathogenesis of coeliac disease, we investigated whether the development or mode of presentation of this disorder is influenced by appendicectomy and/or tonsillectomy.

One hundred and fifty consecutive adult coeliac patients diagnosed at three hospitals by the accepted international criteria were studied [105 females and 45 males; mean age at diagnosis 45 (median 43, range 16-86) yr]. The control group consisted of 138 consecutive patients examined at orthopaedic clinics in the two geographic areas [mean age 45 yr (median 39, range 17-86)].

Contrary to the findings in ulcerative colitis, where appendicectomy was rare before the onset of the condition, 28 of our 150 coeliac patients had undergone appendicectomy before the diagnosis of their disease, in comparison with 15 of the 138 controls ($p=ns$). Similarly, there was no significant difference in pre-diagnosis tonsillectomy rate (26 of 150 patients vs 26 of 138 controls). In the coeliac patients, the age at presentation did not differ between those who had previous appendicectomy [mean 45 (median 42) yr] and those who did not have previous appendicectomy [mean 45 (median 43) yr]. Presenting features, predefined in the study protocol, were compared in the appendicectomy and non-appendicectomy coeliac patients and did not differ significantly [diarrhoea/steatorrhoea (64% vs 48%), weight loss (46% vs 36%), anaemia (35% vs 41%), dermatitis herpetiformis (4% vs 5%), other (4% vs 6%)].

Conclusion: Resection of mucosa-associated lymphoid tissue (MALT) does not influence the occurrence or the mode of presentation of adult coeliac disease. Therefore, in this classical example of a T cell-mediated disorder, the hypothesis that surgical depletion of mucosal T cells has a protective effect or influences the clinical manifestations cannot be supported.

GUT PERMEABILITY CHANGES FOLLOWING DISTANT ORGAN INJURY

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Abnormal gut permeability to enteric flora or their toxins is considered to be an aggravating factor in a variety of illnesses. This study investigates the effect of isolated lower limb ischaemia-reperfusion injury on gut permeability.

Male Wistar rats ($n=30$) were randomised into three groups: control, 3 hr of bilateral hind limb ischaemia alone and 3 hours of bilateral hind limb ischaemia followed by 2 hr of reperfusion. At the end of the experiment blood samples were taken for measurement of systemic endotoxin concentration. Four sacs from the terminal ileum (10 cm each) were removed, everted over a glass rod, tied at both ends and filled with a stock solution of polyethylene glycol (M wt. 4000) in bicarbonate buffer. The sacs were then placed in a beaker filled with ^{14}C -labelled polyethylene glycol in buffer solution and incubated for one hr in a shaking water bath at 37°C and continuous oxygenation. At the end of the incubation period 200 μ samples were taken from the each sac and its radioactivity counted. The count was then calculated as a percentage of the radioactivity in the incubation flask and taken as a measurement of sac permeability to polyethylene glycol.

Results (Mean \pm SEM)

	Endotoxin (pg/ml)	% Permeability
control	2.5 \pm 0.91	3.1 \pm 0.26
3h ischaemia	1.2 \pm 0.68	2.8 \pm 0.18
2h reperfusion	44.8 \pm 19*	4.5 \pm 0.62*

* $P < 0.05$ (vs control and ischaemia) *t* test.

Conclusion: Gut permeability is altered by distant organ injury. This supports the hypothesis that the gut plays a central role in the development of multiple organ dysfunction in the critically ill or injured patient.

THE EMERGENCE OF HAEMOPOIETIC CHIMERISM FOLLOWING LIVER TRANSPLANTATION - A NOVEL MECHANISM FOR TOLERANCE INDUCTION?

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The development of tolerance following orthotopic liver transplantation [OLT] has always been of interest and mechanisms postulated to explain its development include clonal deletion and peripheral T lymphocyte anergy. Although donor chimerism is traditionally associated with successful outcome following allogeneic bone marrow transplant, a partial or mixed chimeric state may exist following solid organ transplantation, and patients who exhibit stable mixed haemopoietic chimerism may not experience acute cellular rejection following OLT. Haemopoietic chimerism has been suggested as a principal component in the development of tolerance after OLT, i.e. two way cellular trafficking from the donor organ to recipient and vice versa. The aim of this project was to examine the incidence of chimerism after OLT using a polymerase chain reaction [PCR] based method for microsatellite analysis of highly polymorphic simple tandem repeat [STR] sequences. The number of repeats varies between individuals and can be used to identify haemopoietic cells from different individuals. Patients were studied prospectively, and retrospectively 8 to 11 months after OLT.

DNA was extracted from the donor organ and explanted organ at the time of transplantation. DNA was extracted from peripheral blood at serial points post OLT. Donor and recipient cells were examined for chimerism by PCR-based amplification of STRs using a panel of microsatellites which included Cyp-19, Int-2 and vWF. A radioactive label, $\alpha^{32}P$ was incorporated into the PCR reaction. Following amplification, gel electrophoresis was performed and subsequent autoradiography identified the amplified STRs.

One patient, who required retransplantation following hepatic artery thrombosis, has demonstrated complete donor chimerism at 2 and 3 months post OLT, which may reflect the presence of haemopoietic progenitor cells in the donor liver. This patient did not experience cellular rejection after OLT. A second patient exhibited transient chimerism 2 weeks post OLT. Three patients studied retrospectively at 8, 10, and 11 months post OLT did not demonstrate peripheral blood chimerism.

Conclusions: Preliminary results indicate that chimerism occurs following OLT, and that PCR based microsatellite analysis is a sensitive method of detecting chimerism. Donor derived lymphocytes may exert beneficial immunomodulatory effects in OLT recipients.

SECRETOR STATUS AND CLASS 1 HUMAN LEUCOCYTE ANTIGENS IN COELIAC DISEASE

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The highest reported prevalence of coeliac disease is found in the West of Ireland. In this population, an extended major histocompatibility complex relating to coeliac disease has been determined, which includes human leucocyte antigens HLA-A1

and B8, determined by a single gene on chromosome 6. Secretion of blood group antigens including ABO and Lewis blood groups is determined by a single gene on chromosome 19. A possible increased prevalence of non-secretors amongst the coeliac population has recently been described. Using Lewis (Le) antigen phenotype, secretor status can be inferred. Of 112 coeliacs who had either Le(a) or Le(b) antigens, 36 (32%) were non-secretors Le(a+,b-), compared with 27% of 103 disease free controls ($p=0.313$, odds ratio = 0.703, 95% CI 0.388-1.267). 9% of coeliacs had the recessive Lewis phenotype Le(a-,b-) versus 2% of controls. HLA-A1, B8 and non-secretion of Lewis antigens were unrelated. An increased prevalence of complications and associated autoimmune disease was found in the non-secreting and recessive coeliac groups. The relationship between the non-secretor state and coeliac disease is not confirmed in this study, nor has an increased prevalence of class 1 HLA markers been demonstrated in non-secretors. The finding of an increased prevalence of complications and auto-immune conditions in the recessive and non-secretor groups may prove useful as a predictor of disease severity in coeliac disease.

UNCENSORED OPEN ACCESS GASTROSCOPY – LIMITED RESOURCES, UNLIMITED DEMAND

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Several recent reports from other countries suggest that open access upper gastrointestinal endoscopy (O.G.D.) reduces the burden on hospital out patient departments, offers the opportunity of earlier, accurate diagnosis and treatment and allows the family doctor to retain control of patient management. Despite the apparent advantages, a 1992 paper reported that only 10% of U.K. Units offered completely uncensored open access gastroscopy. Suggested factors discouraging the provision of this service included fear of excessive or inappropriate referrals, inadequate referral data and blurring of G.P./Consultant responsibilities. We established an uncensored open access service in 1994 and examined the data for the first year of operation with particular reference to the advantages and disadvantages outlined above.

Data was recorded on all patients attending for open access O.G.D. from 1/2/94 to 31/1/95. A comparator group of patients who attended for gastroscopy after consultant out patient assessment during the year immediately prior to the establishment of the open service were also studied.

In the first year of the open access service, the total number of O.G.D.s performed increased by 16% (729 - 846). Of the total 244 (29%) were open access. During the year open access as a percentage of total O.G.D.s increased from 22% in the first three months to 33% in the second six months. The mean delay time from referral to open access O.G.D. was 36 days versus 45 days in the comparator group. In the open access group, 64% (157) were referred directly back to the G.P.s versus 28% in the comparator group, 9% (22) of the open access group received O.P.D. appointments versus 57% of the comparators. Follow up O.G.D. appointments were given to 23% in the open access and 27% in the comparator group.

Endoscopy findings which will be submitted separately for reporting in detail at this meeting showed no significant difference between the open access and the comparator group.

Conclusion: Uncensored open access O.G.D. considerably

increased the endoscopy burden, it provided a disappointing reduction in delay time from referral to O.G.D., it significantly reduced out patient visits and for the majority allowed the G.P. to control management. Because we underestimated the human and technical resources required to provide this service, the increased workload without adequate resources, damaged staff morale and cooperation and resulted in a general reduction in the level of service offered by the Unit.

UPPER GASTROINTESTINAL ENDOSCOPY (UGE): SHOULD GENERAL PRACTITIONERS HAVE AN OPEN ACCESS TO THE ENDOSCOPY UNIT?

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Patients referred for UGE are usually assessed first by the medical team in the outpatients. Apart from extra cost, this sometimes involves unnecessary delays in diagnosis and treatment. An open access to the endoscopy unit can only solve this problem if this facility is used appropriately by the GP's. We compared direct referrals by GP's with outpatients referral by the medical team.

The case records of 184 consecutive patients (M:F ratio 110:76, mean age 48 yr) were analysed. All were referred for endoscopy to a single unit, either directly by the G.P's - group 1 (n=92, M:F ratio 50:42, mean age 41 yr) or by the medical team from the outpatients - group 2 (n=92, M:F ratio 60:34 mean age 55 yr). Patients referred for repeat endoscopy, inpatient referrals and casualty referrals were excluded from the study. The findings on endoscopy and the duration of symptoms and drug treatment prior to endoscopy were compared between the two groups.

In group 1, 17 patients had peptic ulcer disease (duodenal ulcer n=11, gastric ulcer n=6), 56 had either hiatus hernia or inflammatory lesions (oesophagitis/gastritis/duodenitis, alone or in combination), and 19 had normal findings. The mean duration of symptoms before endoscopy was 3 months. 65/92 (70%) patients received some sort of acid blockade treatment prior to endoscopy. In group 2, 14 patients had peptic ulcer disease (duodenal ulcer n=10, gastric ulcer n=4), 52 had either a hiatus hernia or inflammatory lesions and 26 had normal endoscopy. The mean duration of symptoms before endoscopy was 8 weeks. 45/92 patients (49%) received acid blockade prior to endoscopy. No significant differences between the two groups were observed.

Conclusion: An open access endoscopy unit can help cut down extra cost and unnecessary delays in diagnosis and management.

HELICOBACTER PYLORI AND SUBTYPES OF INTESTINAL METAPLASIA IN GASTRIC ANTRAL MUCOSA

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Helicobacter pylori (*H. pylori*) leads to gastric cancer through

a sequence of histological events, from chronic acute gastritis, through intestinal metaplasia (IM), dysplasia and finally cancer. IM is subdivided into three subtypes, type I, type II and type III. Type I may progress to type III, the latter being closely associated with adenocarcinoma. The aim of this study was to determine the relationship between the subtypes of IM and *H. pylori* in the gastric antral mucosa.

Patients undergoing endoscopy at the Meath/Adelaide Hospitals were enrolled. The aldehyde fuchsin - alcian blue technique was used to identify the subtypes of IM.

88 patients were included in the study. Type I was found in 81 patients (92.1%); type II in 61 patients (69.3%) and type III in 23 patients (26.1%). No significant difference in the prevalence of type I was found between *H. pylori* positive patients (n=49) and *H. pylori* negative patients (n=39). However, the prevalence of type II and type III was significantly lower in the *H. pylori* positive patients than in the *H. pylori* negative patients (see table).

IM Subtype	<i>H. pylori</i> +ve	<i>H. pylori</i> -ve	Significance
Type I	91.8% (45/49)	92.3% (36/39)	N.S.
Type II	61.2% (30/49)	79.5% (31/39)	p<0.05
Type III	14.3% (7/49)	41.0% (16/39)	p<0.01

Conclusions: With the progression of IM, the prevalence of *H. pylori* decreases. This may be due to the spontaneous clearance of the bacteria in the premalignant stomach. This study suggests that IM, once present, will progress independent of *H. pylori* infection.

COMMON BILE DUCT STONES: SIZE AND SHAPE: A RADIOLOGICAL REVIEW

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Whether endoscopic balloon sphincteroplasty is a better method than standard electrosurgical sphincterotomy for the removal of common bile duct stones is uncertain. However, since small (<15mm) faceted common bile duct stones (CBDS) are amenable to endoscopic sphincteroplasty, a potentially safer means of duct clearance than sphincterotomy, we decided to perform a radiological review of our common bile duct stones. Hence the size (maximum diameter largest stone), number and shape of common bile duct stones at E.R.C.P. in 234 consecutive patients (123 females: 111 males; mean age 69.3 yr; range 30-95 yr.) were reviewed independently by two radiologists. Correcting for an X-ray magnification factor of 1.5x, the mean stone size was 8.5mm. (Range 4-26mm). 20% (47pts.) were <5mm, 45% (105 pts.) were 5-10mm, 26% (61 pts.) were 10-15mm, 7% (16pts.) were 15-20mm while only 2% (5 pts.) were >20mm including one pt. >25mm. 76 stones were single and 158 were multiple (Mean 5; range 2-20). 59% were faceted, 36% were oval/round while 5% were piston shaped.

Conclusion: The majority (91%) of common bile duct stones are amenable to sphincteroplasty but a prospective comparative trial is required to establish if sphincteroplasty is superior to sphincterotomy.