of an experimental evaluation of endoscopic techniques. The aim of the study is to estimate costs of gastrointestinal endoscopic techniques in a unit as-
sumed to perform 1) all the diagnostic and therapeutic procedures as well as
various biopsies, ultrasonography, 2) more than 3000 procedures
(2 level endoscopic unit). The model for costs estimation took into account
the following: 1) depreciation of instruments (video-endoscopes, automatic
washing machines, computers and so on) 2) cost of disposable and consum-
able, 3) cost of antiseptic use and disinfection and repairs 3) medical, nursing and secretarial staff salaries. Building costs and
the overhead charges (telephone, water, electric power) were not considered.
A "weight" was given to each procedure according to the time required
to perform it (i.e. colonoscopy-TC = 1, oesohago gastroduodenoscopy-EGD = 0.5,
sigmoidoscopy-RS = 0.6, biopsy-BIO = 0.1, polypectomy-POLIP = 0.4).
Number of procedures after which the instrument needs to be replaced
was established to use past experience of unit. Cost distribution was
based on 1993 activity. Costs in U.S. dollars are reported in table:

<table>
<thead>
<tr>
<th>Costs</th>
<th>EGD</th>
<th>CT</th>
<th>RS</th>
<th>BIO</th>
<th>POLIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staff</td>
<td>53(1%)</td>
<td>107(8%)</td>
<td>64(77%)</td>
<td>10(72%)</td>
<td>53(74%)</td>
</tr>
<tr>
<td>Depreciation</td>
<td>16(22%)</td>
<td>22(16%)</td>
<td>14(17%)</td>
<td>0.8(6%)</td>
<td>4(6%)</td>
</tr>
<tr>
<td>Disposables</td>
<td>5(7%)</td>
<td>5(4%)</td>
<td>5(6%)</td>
<td>3(22%)</td>
<td>15(20%)</td>
</tr>
<tr>
<td>Total (US dollars)</td>
<td>74</td>
<td>134</td>
<td>83</td>
<td>13.8</td>
<td>72</td>
</tr>
</tbody>
</table>

In spite of the widespread use of expensive instruments, staff salaries
account for the biggest percentage of costs. Costs were found to be compara-
tible with those reported in European studies and lower than those found
in the USA. Cost-benefit analysis of different diagnostic and therapeutic proce-
dures must therefore take into account different local situations.

**638** *Liver Function and interferon (IFN) Treatment in Chronic Hepatitis C (CHC)*

F. Livirios, F. Monica 1, A. Beccealla, L. Bortolato, C. Triches, C. Patani 2,
G. Crepaldi, L. Okolicancan 3, Institute of Internal Medicine, Italy; 2 Institute of
Clinical Chemistry, University of Padua, Italy; 3 Gastroenterology, University of
Padova, Italy. There is some evidence that IFN depresses antipryrine and theophylline clear-
ance thus impairing the drug metabolizing activity (DmA) (Eur J Clin Pharmacol,
39, 365, 1980; Hepatology, 17, 65, 1993). We therefore: i) studied the effect of IFN
and of IFN withdrawal on DmA as assessed by monoethyl-
glycine xilide (MEXG) formation and ii) investigated whether there was any
relationship between changes of MEXG values and response to IFN. Twenty-
five cases with liver biopsy-proven HCV-related chronic active hepatitis
(CAH) n = 16) or cirrhosis (n = 9) were included in the study and received rIFN
2a-2b (Intron-A), 6 MU tiw for 4 mo followed by 3 MU tiw for 8 mo). MEGX
formation (45 min sample and AUC 0-60 min) were measured in 14/25 pts
at mo 0, 4 and 12 and in 6 pts 6 mo after IFN withdrawal. Results: 11 pts
showed complete response (CR = ALT normalization), 4 partial response (PR =
ALT reduction > 50%) and 10 were non-responders (NR). MEGX values
(mg/ml/10 min) significantly during IFN treatment in both responders
(ECA = 5; cirrhosis = 3, 63 ± 8 SEM at time 0 68 ± 8 at mo 4 and 85 ± 8
at 12, and in NR (ECA = 3; cirrhosis = 3): 50 ± 12 at time 0 66 ± 7 at
mo 4 (p = ns). AUC values (mg/ml per min) paralleled these changes in both
groups. Similarly, IFN withdrawal was associated to a slight, but not signifi-
cant, increase of the 45-min MEGX concentration (from 56 ± 6 to 88 ± 7)
and of AUCs (2821 ± 334 vs 3644 ± 427) which was independent from treatment
outcome (CR 2; PR 2; NR). In conclusion: i) response to IFN was observed
in about half of treated pts; ii) DNA was not affected by IFN administration,
and in at least when the drug was given at a weekly dose of 9-18 MU for 6-12 mo,
and then was no relationship between response to IFN and pre-, during-, or
post-treatment MEGX formation.

**639** *Natural History of Cholelithiasis – A Prospective Study*

W. Johanns, N. Gniffe, C. Jakobert, L. Greiner. Medical Clinic A, Municipal
Hospital Wuppertal, University of Witten-Herdecke, Germany. Introduc-
 tion: Cholelithiasis is one of the most common abdominal find-
ings and diseases. Its natural history, which is still controversial, was investi-
gated in a prospective study.

Patients/Methods: 250 patients (f = 193, m = 57) who were examined in 1986/87
with a view to performance of extracorporeal shock wave lithotripsy
(ESWL) and a history of 50% of gallstones was performed in 1987/88
about the course of their gallstone disease.

Results: In 1986/87 84% of the women and 51% of the men had colics,
24% (59%) and 17% (15%) had specific symptoms and 29% (23%) were asymptomatic.
In the further course cholecystectomy was performed in 62% of the women
and 69% of the men. According to the 1986/87 preoperative data 75% of the
women undergoing surgery and 68% of the men had colics. 18% (21%) had
non-specific symptoms and 22% (23%) were asymptomatic.

**641** *Ultrasound Investigations on the Development of Gallstones in Patients on Chronic Haemodialysis*

W. Johanns, C. Tobis, L. Greiner. Medical Clinic A, Municipal Hospital
Wuppertal, University of Witten-Herdecke, Germany. Introduction: The current
theory of gallstone formation ("stolithogenic bile") and the clinical observation of gallbladder sludge in dialyzed patients
prompted us to examine whether periodic iatrogenic dehydration in the
context of chronic haemodialysis leads to increased sludge and subsequent for-
mation of gallstones.

Patients/Methods: 85 dialysis patients (n = 41, f = 44) with an average age of 68
(20-85) years were examined by abdominal ultrasound. As refer-
ce group, 85 patients from the Dermatology Department in Wuppertal
with approximately the same age and sex distribution were also examined
by ultrasound. The duration and frequency of dialysis, diarrhoea related fluid losses,
body weight and blood lipids were also determined.

Results: 24 of the 85 patients on dialysis had gallbladder calculi at the
time of the examination or had already undergone cholecystectomy because
of symptomatic cholelithiasis. Only 5 patients had developed gallstones
after beginning dialysis. In the reference group there were 16 patients
with gallstones or a history of cholecystectomy. There was no statistically
significant difference between the two groups with regard to the prevalence
of cholelithiasis. We found no evidence of increased formation of gallstones in
relation to either the degree of the periodic fluid loss or the overall duration
of dialysis. In both groups the incidence of gallstones increased with increasing age
and in women. Elevated triglyceride values in patients with gallstones
were also found in both groups.

Discussion: The hypothesis that periodic fluid deprivation in connection with dialysis is a contributory factor in the development of gallstones was
refuted. An association between cholelithiasis and chronological age and gender
was confirmed. Elevated triglyceride values can influence gallstone deve-
lopment.

**642** *Does Jejunoscopy Improve the Diagnostic Yield in Patients with Diarrhoea and/or Malabsorption of Unclear Origin?*

M. Pennazio, A. Arrigoni, F.P. Rossini. Gastroenterology, Gastrenterologi-
Endoscopy Service, S. Giovanni A.S. Hospital, Turin, Italy. Aim: patients
with diarrhoea and/or malabsorption of unclear origin (DMUO), having previously excluded a colonic disease, to evaluate in 27 patients
whether jejunoscopy improves the diagnostic yield in comparison to an explo-
ration restricted to the second portion of the duodenum (D) (routine upper
endoscopy). Methods: we prospectively evaluated 43 cases (24 men, 19
women, aged 18-71 years) with an established diagnosis of DMUO (o phase
length 1000-2500 mm long. At least four biopsies were taken in D as well as in the jejunum (J) explored within 10 cm past the ligament of Treitz (checked by fluoroscopy). Results:

*diarrhoea was due to other causes: pancreatic insufficiency (8), diabetic di-
arrhoea (3), small bowel bacterial overgrowth (3), hyperthyroidism (3), CMV
colitis (1), unknown (23). ** villous atrophy with pseudosarcoidotic granulo-
mas. *** Microsporidian infection in AIDS patients (2). Cyclospora infection
(1) — both confirmed by electron microscopy. lymphangectasis (1), villous
atrophy in AIDS patient (1) .:* 09: cobblestone (1): Crohn's disease (1); "glac-
ic icing like" mucous pattern (2); lymphangectasis (2); "mosaic" mucous pattern
(12); villous atrophy (10); inflammatory and non-inflammatory small inister disease stage A (1),
collagenous sprue (1); diffuse nodules (1): villous atrophy and lymphoid
hyperplasia (1). An abnormal duodenal and jejunal mucosal appearance was
found in 16 patients. In all these patients also histological findings was ab-
normal. A normal duodenal and jejunal mucosal appearance was found in
27 patients. 6 out of 27 had an abnormal histological finding only in the
jejunum, one patient both in the duodenum and in the jejunum. In the remaining 20
patients both duodenal and jejunal histological findings were normal. A du-
arrahoea was ultimately due to other causes. If we assume the jejunal
histology as diagnostic in patients with DMUO, the sensitivity of the duode-
nal histology was 74%. Conclusions: histological abnormalities of the jejunal
mucosa can be found in absence of abnormal duodenal histology and they
could represent a patchy nature of mucosal changes of the disease. Our results support the use of enteroscopy in the management of DMUO patients in whom inconclusive endoscopichistologic duodenal findings were found.

**645 Endoscopic Transanal Microsurgery for Local Excision of Rectal Tumors**

Ch. Kurth, F. Glaser, Ch. Herfarth. Dept. of Surgery, University of Heidelberg, Germany

For local excision of rectal tumors the Endoluminal Technique of transanal endoscopic Microsurgery (TEM) has been used. This study contains the advantages, possibilities and side effects of this technique.

**Methods and patients:** The operative equipment for TEM consists of a rectoscope with a working diameter of 40 mm in different lengths. A stereoscopic endoscope for direct vision is to be brought through the rectoscope. Several surgical instruments which cover all needs of dissection and suturing in the rectum can be placed in the rectum for ar-tight working channels in the cover of the rectoscope. A unit for insufflation, rinsing, defined suction and coagulation completes the equipment.

**Results:** From January 1987 to December 1994 82 patients with rectal tumors were operated by TEM: 60 sessile adenomas, 12 pt1 carcinomas, 6 pt2 and 11 pt3 carcinomas. 2 carcinoid tumors and 1 neurofibroma were locally excised. 79% of the tumors were localized in the middle third and 11% in the upper third of the rectum. Histological examination showed in 71 of 82 patients a rectal tumor free resection line. In 11 cases excessive coagulation prevented exact histological classification. The mortality rate was 0%. 2 patients had a dehiscence in the running suture. 1 postoperative bleeding occurred. In 4 cases a transitory fecal incontiency was observed and 1 patient developed a rectovaginal fistula. Of the 60 patients with sessile adenomas 1 developed a stenosis of the rectum. Of the 21 patients with a carcinoma only 3 patients had a recurrence after local excision (2 pt1, 1 pt2).

**Conclusion:** TEM is the treatment of choice for sessile adenomas and low risk T1-carcinomas of the rectum, especially the middle and upper third of the rectum where conventional surgical methods don’t reach the tumor. It has a low morbidity and meets the requirements of minimal invasive surgery.

**647 Primary Sclerosing Cholangitis in Ulcerative Colitis — Risk Factor for Colorectal Cancer**

Gundi Timmermanns, U.A. Heuschen, J. Stern, Ch. Herfarth. Department of Surgery, University of Heidelberg, Germany

**Introduction:** An estimated 6–10% of patients with ulcerative colitis (UC) suffer from primary sclerosing cholangitis (PSC). PSC is known to be a risk factor of malignancy of the common bile duct. The incidence of carcinomas of the common bile duct ranges between 1.5 and 3.0% in patients with PSC and is significantly increased in comparison to the risk in the general population. A significant number of studies discuss an increased risk for malignancy not only for the common bile duct but also for colorectal cancer.

**Results:** Between January 1982 and December 1994 a total of 443 patients with UC were treated surgically at our hospital (mean age 37.5 years (range 6–74). Restorative proctocolectomy with IAP was feasible in 301 patients (68%). A total of 34 patients (7.7%) suffered from UC-associated colorectal cancer (mean age 41.3 years (range 19–72). Carcinomas were located in 36% in the rectum, in 34% in the left-sided colon. Advanced tumor stage (T4) was found in 16%, lymphnode infiltration in 35.5%. Synchronous multifocal carcinomas (2 to 6 tumors) were found in 32.3% (11/34). High correlation was found between dysplasia and CRC. CRC was associated with epithelial dysplasia in 79%. In only 4% of the patients without CRC dysplasia was detected. Restorative IAP could be performed in 11 of 34 (32.3%) patients with colorectal cancer. In 8 of 34 patients (23.5%) malignant deterioration was unknown preoperatively. 5-year survival rate (Kaplan-Meier) was about 54% (78% in a group of well surveilled patients in contrast to 41% in the patients without consequent surveillance).

**Conclusions:** Preventive proctocolectomy in: In contrast to sporadic-CRC UC-associated CRC affects younger patients. Risk factors are: extent of disease, early age of onset, increased number of adenomas, familial disease and possible increased risk for malignancy not only for the common bile duct but also for colorectal cancer.

**650 Natural History of Asymptomatic Gallstones in the Elderly: A Multicenter Study**

G.I.S. Co. (Interdisciplinary Group for Study of Cholelithiasis). Italy

**Introduction:** An increased incidence of gallstone disease in the elderly as compared to young adults has been reported in literature. While in young adults the asymptomatic gallstone disease seems to be benign and surgical treatment is controversial, in the elderly some authors suggest a more aggressive approach since the mortality for complications and the operative risk seems to be higher as compared to the general population. However no prospective studies are reported to support such a hypothesis. The aim of the study was to evaluate the natural history of the asymptomatic gallstone disease in the elderly.

**Materials and methods:** We enrolled 196 subjects aged more than 65 years (M = 57, F = 139, mean age = 76 years, range = 65–88) with asymptomatic gallstones, diagnosed by ultrasonography. The following data were recorded: body weight, alcohol intake, coffee and cigarettes consumption, number of pregnancies, use of oestroprogestin drugs, family history of gallstones and diabetes, number and diameter of the stones, routine blood tests. All subjects were followed up every six months for a period of 3 years by means of clinical and biochemical evaluations.

**Results:** 152/196 patients were followed-up for 1 year (79/1912 (4.1%) died for diseases not related to gallstones; 6/192 (3.3%) presented biliary pain which required medical treatment in 3 patients (1.9%) and endoscopic...
treatment in other 3 cases (1.9%) without complications and/or relapses. 98 patients reached the 2 years control: 12 (12.2%) had died from diseases not related to gallstones, 2 patients (2.0%) presented biliary pain treated with medical therapy. At 3 years 59 subjects were checked: 3 (6.1%) died from diseases not related to gallstones and 2 (3.4%) presented biliary pain medically treated. The mean time period of follow-up was 1.68 years; the incidence of calculated biliary pain was 3.91% a person/year, while the incidence of surgical endoscopic treatment was 1.17% a person/year.

Conclusions. Asymptomatic gallstones in elderly patients became symptomatic in a low number of cases and present rarely complications. Therefore a conservative approach may be suggested also in elderly people.

651 Perioperative Tenasin C Serum Levels in Ulcerative Colitis and Total Colectomy
St. Riedl 1, A. Tandara 1, U. Hinz 1, P. Möller 2, H. Bodenmüller 4, H.J. Buh 1, A. Fiascone 1,2, G. Menegatti 1,3, M. Miglioli 1, A. Sciarrone 1, F. Depaoli 1, P. Holton 1, G. Borsotti 2,3, P. Zapparoli 1, M. Gatto 1, F. Lanzi 1, B. Massarri 2, P. Mulè 2, C. Ricci 2, M. Vergura 2, L. Barbata 1.
1 Department of Gastroenterology and Hepatology, Padua, Italy; 2 Department of Pathology, University of Padua, Italy; 3 Department of Biophysics, University of Padua, Italy; 4 Department of Radiology, Padua, Italy.

Tenasin (TN) is an extracellular matrix molecule mainly produced by fibroblasts. In normal colorectal mucosa TN is expressed in low concentration. Its distribution is restricted to parts of the basal lamina and lamina propria adjacent to the mucosal surface. In inflammatory and neoplastic discuses TN production is increased. We investigated serum concentrations of TN in patients with ulcerative colitis and determined perioperative TN serum levels in cases of total colectomy.

TN serum levels were analyzed in 54 patients using a double-sided sandwich ELISA. The results were compared to conventional parameters of inflammatory bowel disease and the clinical course. Statistical analyses were performed using Mann-Whitney U-test and Fisher’s exact test.

We found a significantly increased level of TN in the serum of patients with ulcerative colitis compared to normal controls (p < 0.001*). Two weeks after total colectomy TN serum levels decreased (p < 0.05**). Twelve weeks postoperatively the TN serum levels were lower (n.s.), but did not reach normal values (p = 0.005**).

652 Upper Gastrointestinal Bleeding in the Elderly: Role of Helicobacter pylori Infection and NSAIDs-Intake
A. Piottolo, M. Franceschi, G. Leandro 1, R. Fabrello, L. Bozzola 2, F. Di Mario 4, V. Meli 2, G. Valerio. 1 Dept. of Geriatrics, Vicenza, Italy; 2 Dept. Gastroenterology, Castellana Grotte (BA); 3 Serv. Clinical Pathology, Vicenza, Italy; 4 Dept. of Gastroenterology, University of Padua, Italy.

With the aim to evaluate whether NSAIDs use and HP infection may interact to increase risk of upper gastrointestinal (GI) bleeding in the elderly we studied 155 patients aged >70 years who have undergone upper GI endoscopy in our Geriatrics Dept. The patients were divided in 3 groups: 1) 67 patients (M = 24, F = 43, mean age = 79.5 years, range = 70-98) who had used NSAIDs at least 1 week before the examination; 2) 53 patients (18 NSAIDs-treated) who presented bleeding lesions at endoscopy (M = 27, F = 26, mean age = 79.0, range = 70-96); 3) 53 subjects age, sex and endoscopic diagnosis (but not bleeding) matched as controls group 2. HP positivity was confirmed in all cases by histology (2 antral and 2 body gastric biopsies, Giemsa and HE stains) and rapid urease test. Statistical analysis was performed by means of logistic regression.

Results. Group 1: 80.59% of patients resulted affected with active upper G.I. lesions: gastric ulcer (GU) = 38.6%, duodenal ulcer (DU) = 25.37%, GU + DU = 4.47%, erosive gastritis = 14.92% and 32.93% presented with bleeding lesions (GU = 50%, DU = 33.3%, DU + GU = 11.1%, erosions = 5.5%); 72.2% of bleeding and 83.3% of non-bleeding NSAIDs-users resulted HP+ve (n = 32); As regards Groups 2 and 3 the results are illustrated in Table 1.

<table>
<thead>
<tr>
<th>HP</th>
<th>NSAIDS</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (70)</td>
<td>B (228)</td>
<td>P</td>
</tr>
<tr>
<td>IgG A</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>IgG (µg/ml)</td>
<td>93.3</td>
<td>82.7</td>
</tr>
<tr>
<td>G (µg/ml)</td>
<td>27</td>
<td>30</td>
</tr>
</tbody>
</table>

Conclusion: 1. asymptomatic IgG seropositive blood donors showed a surprisingly high prevalence of gastro-duodenal ulcers. 2. PGI but not IgG or G levels were higher in ulcers compared with non-ulcers asymptomatic HP seropositive blood donors.

653 Quantification of Liver Microcirculation by Thermodiffusion: First Clinical Application After Transplantation
E. Klutz, T. Kraus, M. Bredt, B. Oeswald, N. Senninger, C. Herfarth, G. Otto.
1 Dept. of Surgery, University of Heidelberg, Germany.

Purpose: Experimentally, thermodiffusion has been shown to allow continuous long term monitoring of hepatic microcirculation after liver transplantation. It was the aim of this study to transfer the method to the clinical setting investigating a possible correlation of liver perfusion with graft dysfunction.

Method: Intraoperatively the thermodiffusion probe (ø 0.9 mm) was inserted into segment IV and exited through the abdominal wall in 7 patients undergoing orthotopic liver transplantation. Thereafter liver perfusion was quantified continuously (one measurement/sec) until postoperative day 6. The probes were then removed transcutaneously. Perfusion data are expressed as mean values of 20-min sampling periods starting 90 min after arterial reperfusion as well as in 12-hour intervals on the following days.

Results: Throughout the observation period the reliability of measurements was documented by stable conductivity of the probes. Intraoperative liver perfusion ranged from 56 to 118 ml/100 g/min. On day 1 a maximum increase of hepatic perfusion by 25% of the initial value was recorded, followed by stable measurements in four patients with uneventful course postoperatively. In patient 5 a second peak of plasma transaminases ascribed to reperfusion injury was paralleled by a decrease of liver perfusion from 103 ml/100 g/min on day 2 to 23 ml/100 g/min on day 3 recovering within 36 h. Patient 6 and 7 developed a R2-rejection on day 6 preceded by a reduction in liver perfusion 24 h earlier from 85 and 92 ml/100 g/min to 37 and 45 ml/100 g/min, respectively.

Conclusion: Thermodiffusion was proven to be a reliable method for the continuous monitoring of liver perfusion in patients after transplantation. Intraoperative perfusion, dererased on postoperative days, were recorded in patients with uncomplicated course. Prolonged reperfusion injury or rejection were characterized by secondary impairment of microperfusion.

654 Serum Pepsinogen I and Gastrin Levels in Helicobacter pylori IgG Seropositive Asymptomatic Blood Donors
1 Medical Clinic, University of Bologna, Italy; 2 Dept. of Paediatric, Turin; 1 Dept. of Microbiology, University College, London, UK.

Aim: To evaluate serum pepsinogen I (PGI) and gastrin (G) levels in 298 anti-Helicobacter pylori (HP) IgG seropositive asymptomatic blood donors (BD) according to endoscopic findings.

Methods: IgG were assayed by ELISA (Absorbance Index > 0.3 defined positivity) and PGI (µg/ml) and G (pg/ml) by RIA. Endoscopy with biopsies was carried out in all 298 asymptomatic blood donors (M/F: 173/125, age range 18-65, mean 45 yrs).

Results: Seventy out of 298 donors (24%) (group A) were found to have duodenal (N = 50) or gastric (N = 29) ulcers (M/F: 55/15, age range: 24-63, mean 47 yrs). In the remaining 228 asymptomatic donors (group B) (M/F: 119/109, age range: 21-67, mean 46 yrs) the endoscopic findings were as follow: gastric cancers (N = 2), erosive duodenitis (N = 41), gastritis and/or erosions (N = 139), macroscopically normal (N = 48). The mean serum IgG PGI and G levels in the 298 endoscoped blood donors were: 0.7 A, 85 µg/ml and 29 pg/ml respectively. The Table shows the mean IgG, PGI and G levels in the asymptomatic blood donors with gastroduodenal ulcer (group A) compared to the those without ulcer (group B).

<table>
<thead>
<tr>
<th>A (70)</th>
<th>B (228)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>IgG A</td>
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</tr>
</tbody>
</table>

Conclusion: 1. asymptomatic IgG seropositive blood donors showed a surprisingly high prevalence of gastro-duodenal ulcers. 2. PGI but not IgG or G levels were higher in ulcers compared with non-ulcers asymptomatic HP seropositive blood donors.
660 Incipient Neuroendocrine Complexes in the Gastric Mucosa of Patients with Pernicious Anaemia: Ultrastructural Aspects

H.G. Schmidt, A. Schmid, W. Domschke 1, Klinikum Nürnberg, Germany; 1Universität Münster, Germany

In patients (P) with pernicious anaemia (PA), the lamina propria of the gastric mucosa contains neuroendocrine complexes (NEC's), that is, neuroendocrine cells which are in direct contact with nerve fibres (Gut 1986, 27, 789). To date no studies dealing with the cytogenesis or histogenesis of NEC's have been published. Method: In a prospective study, 6 step biopsies were obtained via the endoscope from along the greater curvature of the stomach (4 times non-antral and 2 times antral mucosa) in 21 P with PA. Results: In several biopsies, most of which obtained from non-antral mucosa, 16 NEC were found to have NEC's. Five of the NEC-positive P additionally had, in the lamina propria, cell complexes that were interpreted as incipient NEC's. For the most part, these complexes comprised mature neuro-endocrine cells which were in direct contact with nerve fibres, and also other cells that were classified as proliferating neuroendocrine cells in that they contained no neuroendocrine granules, but a prominent rough endoplasmic reticulum, and a prominent Golgi apparatus. Proliferating neuroendocrine cells were usually in contact with one another as well as with mature neuroendocrine cells and nerve fibres only via strictly circumscribed points of contact, that is, the incipient NEC's represented cell complexes, the cells of which were characteristically only loosely connected with one another. In contrast to mature NEC's that are completely enveloped within a basement membrane, incipient NEC's showed either no or only fragmentary basement membrane at the periphery. Some incipient NEC's had no mature neuroendocrine cells, but were made up exclusively of proliferating neuroendocrine cells that were connected with nerve fibres. Summary: 1. This electron microscopic study revealed, for the first time, incipient neuroendocrine complexes in the lamina propria of the stomach, which are in contact with pernicious anaemia. 2. In five out of 16 NEC-positive patients, such incipient NEC's were found in the lamina propria of the non-antral gastric mucosa.

661 Interleukin 1 (IL-1) and Interleukin 1 Receptor Antagonist (IL-1ra) mRNA Levels in Pileal Ileal Pouches

M. Ferretti, P Gionchetti, M. Campieri, S. Galli 1, A. Beluzzi, F. Rizzello, C. Brignola, A. Venturi, M. Miglioli, L. Barbara. Istituto di Clinica Medica e Gastroenterologia, Università di Bologna, Italy; 1Laboratorio Centrale, Policlinico S. Orsola, Bologna, Italy

Pouchitis is major long term complication of ileal-anal anastomosis which occurs mostly in patients operated for ulcerative colitis (UC). We evaluated the mucosal levels of IL-1 and IL-1ra mRNA in 3 patients with not inflamed pouch and in 3 patients with pouchitis. As a control group we used 3 patients with UC in remission and 3 patients with active UC.

Mucosal biopsies were taken, during endoscopy, and immediately frozen in liquid nitrogen. The RNA was extracted and reverse transcribed into cDNA that was then amplified by PCR using specific probes for IL-1, IL-1ra and β-Actin (ACT). The amplification products were run on a common agarose gel and on capillary electrophoresis (CE), a sensitive technique allowing accurate quantification of small amount of DNA.

Results:

<table>
<thead>
<tr>
<th>Gel</th>
<th>Remission UC</th>
<th>Active UC</th>
<th>Pouch</th>
<th>Pouchitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACT</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>IL-1</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>CE (Average ± SD)</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

Conclusions: These preliminary results show IL-1 and IL-1RA gene expression in pouchitis as in the case of active UC, but with lower intensity. Furthermore we observed IL-1 gene expression also in pouches without pouchitis, different from UC in remission. These results, in conclusion, seem to support that pouchitis etiology is mediated by immune mechanisms as in the case of UC.

664 Comparative Bioavailability of 5-Aminosalicylic Acid (5-ASA) from Dipentum and Asacol in Patients with Ulcerative Colitis (UC) in Remission

P. Gionchetti, M. Campieri, F. Rizzello, A. Venturi, M. Ferretti, A. Belluzzi, C. Brignola, M. Miglioli, L. Barbara. Istituto di Clinica Medica e Gastroenterologia, Università di Bologna, Italy

Knowledge of the bioavailability of 5-ASA from different 5-ASA containing drugs is important for rationale therapy of UC. We compared the systemic uptake of 5-ASA and acetyl-5-ASA (AC-5-ASA) at steady-state during treatment with either an azo-bound preparation, olsalazine [Dipentum (D)] or a delayed release mesalazine [Asacosil, AS] at recommended doses for maintenance treatment. An open cross-over design with randomized sequence was applied and 15 patients (10 male, 5 female, age range 21-72) with UC in remission were given 7-day courses of D (1.0 g/day) and AS (1.6 g/day). Plasma and urine were collected on day 6 and 7 of each course and concentration of 5-ASA and AC-5-ASA were determined by HPLC.

Results:

Mean (SD) plasma and urine 5-ASA and AC-5-ASA

<table>
<thead>
<tr>
<th>Gel</th>
<th>Remission UC</th>
<th>Active UC</th>
<th>Pouch</th>
<th>Pouchitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>5-ASA (mmol/L)</td>
<td>0.55 (0.18)</td>
<td>7.10 (6.3)</td>
<td>14.37 (7.47)</td>
</tr>
<tr>
<td>AC-5-ASA (mmol/L)</td>
<td>2.61 (1.57)</td>
<td>20.83 (14.56)</td>
<td>24.27 (17.98)</td>
<td></td>
</tr>
<tr>
<td>Unine</td>
<td>5-ASA (mmol/L)</td>
<td>30.23 (15.79)</td>
<td>528 (277)</td>
<td></td>
</tr>
<tr>
<td>AC-5-ASA (mmol/L)</td>
<td>942 (577)</td>
<td>2002 (864)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 5-ASA (mmol/L)</td>
<td>972 (545)</td>
<td>2530 (1027)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery of 5-ASA + AC-5-ASA (% of given dose)</td>
<td>17.9 (9)</td>
<td>24.1 (10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.0001 compared to AS (after logarithmic transformation) * p < 0.01 compared to AS (after logarithmic transformation)

As results in significantly higher levels at steady state compared to D of both 5-ASA and AC-5-ASA in urine as well as in plasma. The low systemic load provided by D may increase efficacy and reduce the potential risk of nephrotoxicity.

665 Oral Budesonide Competes Favourably with Prednisolone in Active Crohn’s Disease

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An oral controlled release formulation of the corticosteroid budesonide (BUD) has been developed for the treatment of Crohn’s disease. The aim of this study was to investigate the efficacy and safety of two different dosage regimens of BUD, 9 mg once daily (om) and 4.5 mg twice daily (b.i.d.) in comparison with prednisolone (PRED) 40 mg o.m. 12 weeks in this double-blind, multicenter trial, 177 patients with active CD (CDAI > 200) were randomly assigned to one of the three regimens and treated for 12 weeks. BUD was tapered to 6 mg after 8 weeks and to 3 mg after 10 weeks. PRED was tapered to 30 mg over two weeks and then gradually to 5 mg during the last three weeks. Efficacy was measured as remission rate, remission being defined as CDAI ñ 150. The disease activity decreased rapidly in all groups. After two weeks, the highest remission rate (48%) was observed in the BUD o.m. group compared with 37% in the PRED group. At 8 weeks the same remission rates (60%) were found in the BUD o.m. and PRED groups compared with 42% in the BUD b.i.d. group (NS). At 8 weeks mean morning plasma cortisol levels were significantly (P = 0.005) less suppressed in both BUD groups (P = 184 nmol/L for BUD 9 mg o.m. and BUD 4.5 mg b.i.d.) than in the PRED group (P = 256 nmol/L). An impaired adrenal function, as assessed by a short ACTH stimulation test, was significantly (P = 0.0023) more common in the BUD group (84%) than in the BUD o.m. (58%) and the BUD b.i.d. group (50%). This study shows that, in patients with active CD affecting the distal ileum and/or ascending colon, both 9 mg o.m. and 4.5 mg b.i.d. of BUD are effective in inducing remission, and comparable to PRED 40 mg. BUD is generally well tolerated and cause less disturbance of the adrenal function than PRED.

666 IF10 Immunomodulation in Inflammatory Bowel Disease — A New Marker Specificity only for Continuous Endothelial Cells

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The aim of the present study was to investigate in detail the immunohistochemical properties of the two endothelial specific markers IF10 (continuous
endothelial and MS-1 (discontinuous endothelial) in bowel tissues of patients suffering from chronic inflammatory bowel disease (IBD). Immunohistochemical techniques were employed to study the morphology and phenotypic expression of inflammatory cells in routinely processed bowel tissues from 27 patients with Crohn's disease (CD), 18 patients with ulcerative colitis (UC), and from 20 normal controls. All patients with IBD and controls showed a low to moderate immunohistopathological staining restricted to the lamina propria and submucosa. In contrast to UC patients and healthy controls, 11F0 immunoreactivity was strongly de novo expressed in the muscularis propria of the small and large bowel in CD patients regardless of the histological severity of the inflammatory process. Neither in Crohn's disease nor in ulcerative colitis we observed immunoreactivity for MS-1 on endothelial surfaces. From this we conclude that endothelia in patients with IB do not undergo metastas. The high immunoreactivity of 11F0 antigen in the muscularis propria of CD indicates a state of functional immunological activation and may be important in the maintenance of chronic inflammation by facilitating leukocyte migration into sites of Crohn's disease involvement. Further studies of the factors controlling endothelial cell differentiation in the bowel of CD patients may help to explain the features observed in this study.

668 Immunohistochemical Distribution and Serum Levels of the Ca2⁺-Binding Proteins MRPA8 and MRPA14 and their Heterodimeric Form MRPA8/14 in Crohn's Disease

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In previous histochemical studies the distribution of the two Ca²⁺-binding proteins MRPA8 and MRPA14 has been demonstrated in different inflammatory diseases. Monoclonal antibodies against MRPA8 and MRPA14 and their heterodimer MRPA8/14 (27kDa epitope) were used to investigate immunohistochimically the distribution of these proteins in routinely processed bowel tissues from patients with Crohn's disease (CD). Furthermore we used a sandwich immuno assay to measure serum concentrations of MRPA8 in 52 patients with CD, 14 patients with diverticulitis, 10 patients with Salmonella gastroenteritis, and in 20 healthy controls. Disease activities of the patients were assessed by the Crohn's disease activity index (CDAI). In our immunohistochemical study MRPA8, MRPA14 and the heterocomplex MRPA8/14 were demonstrated in the majority of granulocytes and macrophages in active CD. Additionally, the heterogeneous complex MRPA8/14 immunoreactivity was present in epithelial cells of adjacent to ulcerative and fissuring lesions in the bowel. Serum MRPA8/14 concentrations were significantly (p < 0.0001) increased in patients with active CD (CDAI > 150), acute diverticulitis and Salmonella gastroenteritis with the highest levels being in patients with active CD. No significant correlations were found for levels of MRPA14 and MRPA8 alone, respectively. The follow-up of individual patients with initially active CD showed a further increase in MRPA8/14 levels during acute attacks of the inflammatory process. We suggest that our assay for MRPA8/14 immunoreactivity discriminates well between active and inactive CD and may have considerable potential in the analysis of clinical disease activity in CD patients. Our morphological results confirm the finding of increased MRPA8/14 serum levels in patients with active Crohn's disease.

669 Immunohistochemistry — Initial Experience with Intraluminal Immunofluorescence Diagnosis of Colorectal Carcinoma

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Introduction: Immunohistochemistry, a combination of endoscopy and immunofluorescence, has been developed in order to be able to diagnose and biopsy malignant epithelial early changes of the colonic mucosa more reliably. For evaluating the status of this new diagnostic procedure up to now 20 patients suffering from colorectal carcinomas have been examined pre-operatively by means of immunohistochemistry as part of a prospective study.

Method: For immunohistochemistry an available antibody against CEA was purified and coupled with a fluorescent staining. During pre-operative colonoscopy the fluorescent coupled antibody was applied by means of a catheter via the operating channel of the endoscope onto the tumor and the surrounding mucosa. Following an incubation period of 10 minutes the entire mucosal area was irrigated with a buffer. For visualization of the specific fluorescence a special narrow band filter with a wave length corresponding to the excitation maximum of the used fluorescent staining was additionally inserted in front of the standard source of light. Furthermore, a second narrow band filter which could only be penetrated by the emitted light was positioned in the light path in front of the investigator's eye.

Results: In most cases a fluorescence limited to the tumor could be excited in the excitation light after rinsing the unbound antibody loads. In the region of tumor necroses, however, no fluorescence could be identified. A significant difference between marginal tumor tissue and macroscopically unaffected mucosa were obvious.

Discussion: As investigations of fresh tumor material have already shown a differentiation between tumor areas and macroscopically normal mucosa is also possible with the in vivo immunohistochemical diagnosis of macroscopically clear-cut carcinomas, however, it is not intended to be the target of immunohistochemistry. By means of this method malignant early changes, especially in long-standing ulcerative colitis or in tubulo-villosus colonic adenomas shall rather be demonstrated. Further investigations are being carried out.

673 Retrograde Colorectal Lavage — Cytological Material for Continuing Diagnosis of Ulcerative Colitis

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Introduction: The risk of carcinomas in patients with long-standing extensive ulcerative colitis can only be assessed by evaluation of epithelial dysplasias in successive biopsies. Areas with epithelial dysplasias may be small and very limited. Apart from histological biopsy diagnostics referring to localisation cytological diagnosis as well may contribute to the detection of malignant and pre-malignant changes.

Method: As part of follow-up colonoscopy colorectal lavage was performed in addition to removal of successive biopsies in 10 patients suffering from long-standing ulcerative colitis. During this colonoscopy PBS buffer was added to the colon. Afterwards the entire fluid was drawn off and centrifuged. In a gradient centrifugation the enrichment of colonic epithelial cells was performed. After dispensation of cytological preparations the epithelial cells were assessed by means of routine cytology and also examined by means of DNA image cytometry. Correspondingly, DNA cytometry was performed in routine biopsies as well.

Results: In all cases good cytologic assessment was possible. Dysplasia could not be demonstrated. In direct comparison of cytology and histology an increased proliferation could be detected in individual cases exclusively in cytological cell material by means of DNA image cytometry.

Discussion: Combined colorectal lavage is a procedure which independent of localisation makes it feasible to detect potential pre-malignant changes qualitatively. An increased proliferation diagnosed in this cell material may indicate the begin of a malignant change even without epithelial dysplasias. Further prospective studies for validation of the procedure are presently being carried out.

674 Antiflammatory Cytokine Interleukin-10 in Patients with Inflammatory Bowel Disease

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Inflammatory bowel disease is characterized by an increased serum concentration of different proinflammatory cytokines. In the present study we investigated serum concentrations of the antiflammatory cytokine human Interleukin-10 (IL-10) as well as supernatants of peripheral blood mononuclear cells (PBMC) in patients with inflammatory bowel disease (IBD).

Human IL-10 was measured by our own established ELISA-system using PharMingen-antibodies. Serum antibodies were assessed in 44 patients with ulcerative colitis (UC), 40 patients with Crohn’s disease (CD) and in 30 healthy controls. Supernatants of LPS-stimulated PBMC of 12 patients with CD, 8 patients with UC and 11 healthy controls were determined for IL-10.

IL-10 serum levels were significantly increased in patients with active UC (mean ± SE, 144 ± 34.8 pg/ml; p < 0.001) and in CD (mean ± SE, 132 ± 32.7; p < 0.001) compared with healthy controls (mean ± SEM; 44 ± 9.5 pg/ml). Patients with inactive disease didn’t show any significant increase in IL-10 levels. Compared with clinical disease activity indices there was a significant correlation between IL-10 serum concentrations and CDAI in patients with CD (r = 0.45, p < 0.01) and UC (r = 0.39, p < 0.05).

Comparing IL-10 serum levels with serum concentrations of other proinflammatory cytokines there was a significant correlation to serum levels of interleukin-2 (IL-2) (r = 0.417, p < 0.05) and IL-6 (r = 0.387, p < 0.05) in patients with CD but not in UC. LPS stimulated PBMC of CD patients (mean ± SEM; 157 ± 172, n = 12, p < 0.05) and patients with UC (mean ± SEM; 193 ± 284, n = 8, p < 0.01) produce significantly more IL-10 than healthy controls (mean ± SEM; 1108 ± 121, n = 16, p < 0.01).

In summary, IL-10 is elevated in serum of patients with active CD and UC suggesting that IL-10 acts as a naturally occurring damper in the acute inflammatory process of IBD.

676 HCO₃⁻ and Cl⁻ Flux Through Guanylin-Stimulated Apical of Basolateral Anion-Importers in Rat Duodenum

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Background: Guanylin is a recently characterized peptide that is expressed in the gastrointestinal tract including the duodenum. It binds to a putative guanylyl cyclase receptor which is also occupied by the heat stable E. coli

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enterotoxin STa and stimulates the guanylate cyclase C and electrogenic Cl⁻-secretion. We recently found that guanylin is one of the most potent stimulators of duodenal bicarbonate secretion Aim: To further characterize the anion secretory process stimulated by guanylin, we have investigated whether basolateral anion importers are activated during guanylin stimulation and how their activity influences luminal JHCO3⁻ and JCl⁻. Methods: Rat parietal cells in primary culture were used. Guanylin-stimulated HCO₃⁻ secretion was measured by pH-stat. The contribution of luminal Cl⁻ and additive to gluconate- and carbobol-stimulated JHCO3⁻. The percentage of guanylin-stimulated JHCO3⁻ to JCl⁻ was approx. 70:30 at half-maximal guanylin concentration (5 × 10⁻⁸ M) and shifted towards a larger contribution of JHCO3⁻ at higher concentrations. JHCO3⁻ cotransport by bumetanide (10⁻⁵ M) decreased basal JCl⁻, but increased basal JHCO3⁻. The same response to bumetanide was observed in a more pronounced fashion after guanylin or STa stimulation in submaximal concentrations. At maximal stimulation, bumetanide decreased JCl⁻, whereas JHCO3⁻ remained unchanged, indicating that basolateral HCO₃⁻ uptake and CO₂ hydration was rate-limiting. Inhibition of Na⁺-HCO₃⁻ cotransport by SITS (10⁻⁵ M) did not affect basal JHCO3⁻, but inhibited guanylin (10⁻⁷ M) and STa (10⁻⁵ M) stimulation by up to 50%, respectively, indicating an equivalent reduction in JCl⁻, indicating that JCl⁻ increased in a compensatory fashion. A stepwise increase in apical anion secretion by guanylin or STa was paralleled by an increase of the subsequent inhibitory effect of bumetanide on JCl⁻, and that stimulation of the basolateral anion conductance also increased the ion flux rates through the basolateral anion importers. Conclusion: In rat duodenum, guanylin and STa stimulate an electrogenic secretion which in turn activates basolateral anion-importers such as the Na⁺-HCO₃⁻ and the Na⁺,K⁺,2CI⁻-cotransporter. The relative percentage of JHCO3⁻ and JCl⁻ through the apical conductance is largely determined by the transport capacity of these basolateral anion importers, and blockade of one leads to a partially compensatory increase in the flux rate of the other anion.

679 Characterization of Two Different Anion Exchangers in Rabbit Duodenum

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Introduction: In the duodenum, a percentage of basolateral HCO₃⁻ and the Na⁺,K⁺,2CI⁻-cotransporter. The relative percentage of JHCO3⁻, and JCl⁻ through the apical conductance is largely determined by the transport capacity of these basolateral anion importers, and blockade of one leads to a partially compensatory increase in the flux rate of the other anion.

682 Bone Mineral Density is Low in Both Longstanding Ulcerative Colitis and Crohn's Disease but only in Crohn's Disease at Diagnosis

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Purpose: We have recently shown that, at diagnosis, low bone mineral density (BMD) is a feature of Crohn's disease (CD) but not ulcerative colitis (UC). Corticosteroid therapy over a 1 year period did not result in any further bone loss. A number of previous studies, while demonstrating low BMD in IBD, did not find CD to have lower BMD than UC. An explanation for different results in these studies might be recruitment of frequently attending patients with a bias towards longstanding complicated disease. In such cohorts, confounding variables such as corticosteroid therapy or surgery may become more important than the systemic effect of disease alone.

Subjects and methods: We tested this hypothesis by measuring BMD in a cohort of longstanding IBD patients. The inclusion criteria for the study were: (a) UC or CD for at least 5 years; (b) at least 6 months of systemically corticosteroid therapy; (c) age between 20-65 years; (d) Inactive disease (CDAI < 150, Powell-Tuck index <4<15); (e) absence of cholestatic liver disease or other diseases affecting bone mineral density; (f) patient's age > 40 years; and (g) the study was performed on adult patients (median age 34, range 22-64 years) and 13 patients (7 M, 6 F) with UC (median age 33, range 21-64 years) were recruited into the study. BMD of lumbar 1-4 vertebrae was measured by Dual Energy X-ray Absorptiometry using a Hologic QDR-1000W scanner. The normal values were provided by the Holologic reference database which is similar to that of the local Scottish population.

Results: The median duration of disease for both UC and CD was 7 years. Median duration of corticosteroid use was 21 months for CD and 19 months for UC. Physical activity scores were similar in both groups. The mean T-score for spine BMD in CD was −1.1 (SD 1.0) and in UC was −1.0 (SD 0.6), p = N.S. Similarly, mean femur T-scores in both CD and UC were −1.0 (SD 1.3 and 0.9 respectively), p = N.S.

Conclusion: We conclude that, in longstanding IBD, there is no difference in BMD between CD and UC, unlike that at diagnosis. This explains why previous results, which studied patients with average duration of disease 6-12 years, could not find any difference. The absence of a similar systemic effect of CD on BMD may be best studied at diagnosis before institution of therapy.

683 Downregulation of Na/H Exchange Activity and NHE1 mRNA Expression in HT29/276 Cells During Culture in Acidic Conditions

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Introduction: Proximal tubule cells upregulate the Vmax of the brush border Na/H-exchanger (a NHE3 isoform) during acidosis, thereby stimulating HCO₃⁻ reabsorption. Surprisingly, in cultured renal epithelial cells an upregulation of basolateral Na/H exchanger and of the corresponding NHE1 isoform occurs during culture in acidic medium. Not only re- nal but also colonic epithelial cells are involved in total body pH homeostasis.
and the colonic epithelium is frequently exposed to acidic conditions. Aim: We therefore investigated the influence of culture in an acidic surrounding of dissociated Na+-exchange activity and NHE1 mRNA expression in HT29-B6 cells (a differentiated and polarized colonic cell line established by K. Kreusel). Methods: Intracellular pH (pHi) was measured fluorometrically in BCECF-loaded HT29-B6 cells after culture in the confluent stage at pH 7.4 or 6.9 for 48 h. Na+-exchange was recorded as the dimethylamidinium sensitive pHi-recovery after intracellular acidification to various pH-values using the ammonium pre-pulse technique. Maximal H+-flux rates at a given pH were calculated by multiplying the initial pHi-recovery rate with the intracellular buffering capacity at that pH. NHE1 mRNA expression was studied with the ribonuclease protection assay using expression of the human GAPDH as an internal control. Results: During culture in pH 7.4, pH was 7.26 ± 0.10 and dropped to 6.95 ± 0.01 after 30 min in 6.9 pH. After culture for 48 h at 6.9, pH was 6.98 ± 0.09, indicating that some adaptive response had taken place. A similar was observed between cells cultured in normal and acidic medium. Basolateral Na+-exchange rates in cells cultured at pH 7.4 showed the characteristics of the adaptive response (pHi-flux rates). It was surprising that Na+-exchange rates in cells cultured at pH 6.9 (but measured with pH 7.4) were low and almost not activated by a low pH. GAPDH mRNA expression was mildly reduced in cells cultured at pH 6.9, but NHE-1 mRNA expression was reduced by 50% compared to GAPDH expression. Cell viability was maintained and cells regained their original Na+-exchange characteristics in culture at pH 7.4. Conclusion: Prolonged exposure to an acidic surrounding results in a markedly higher pH than observed during acute exposure to acidic pHi in the cell line. These observations indicate that a cellular adaptive response to acidosis had occurred, basolateral Na+-exchange activity was downregulated and NHE1 mRNA expression reduced, showing that the major pH-regulator during selective intracellular acidosis is related to the adaptive response to an acidic surrounding in this cell line. In fact, downregulation of its activity may serve as a useful mechanism to reduce the proton influx into the cell at reduced extracellular pH.

868 F XIII Substitution in Therapy-Resistant Ulcerative Colitis
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Severe ulcerative colitis is often associated with intestinal blood loss and a deficiency of coagulating factor XIII which is important for clot formation and wound healing. Consequently, the substitution of F XIII may be beneficial.

In a prospective pilot study, 12 patients (7 f, 5 m; 34 ± 8.1 years) with therapy-resistant ulcerative colitis were treated with F XIII concentrate (Fibrogammin HS, Behring). Therapy resistance was defined as a lack of clinical remission after three weeks in spite of a consequent therapy with corticosteroids and 5-aminosalicylic acid. At this time the colitis activity index (CAI) and the endoscopic score (ES) according to Rachmilewitz were elevated to 10 ± 1.5 and 8.3 ± 2.3 points, resp. All patients suffered from hematochezia. F XIII concentrate (1250 u/day i.v.) was additionally administered for 10 days. F XIII activity and F XIII substrate a and b had been measured before substitution and thereafter.

The initial values of F XIII were markedly decreased (F XIIa ct.: 47 ± 17%; FXIIia : 61 ± 50%; FXIIIb: 72 ± 28%, each p < 0.01 vs. controls) and showed a significant increase after substitution (F XIIa ct.: 161 ± 46%; FXIIia : 210 ± 78%; FXIIIb: 197 ± 62%, each p < 0.01 vs. initial values). The stool frequency decreased from 3 to 2.1 after substitution (p < 0.002). The average increase in body weight amounted to 1.0 ± 0.8 kg and no further hematochezia was detected with the exception of two cases with positive haematoctit-results. Tests of nuclear factor activity were normal by Dual Energy X-ray absorbometry using a Hologic QDR-1000W scanner. The normal values were provided by the Hologic reference database which is similar to the local Scottish population.

Results: In adults, the mean Z-score for spine was −1.1 (SD 1.0) and this was not different from the values obtained at diagnosis in a different cohort previously reported by us. In adolescents, the mean spine Z-score was −2.7 (SD 1.7), significantly lower than adults (p = 0.02). Two of these adolescents had multiple vertebral collapses. The TDG-calcified vertebral parameters were normal in adults, two adolescents were vitamin D deficient.

Conclusions: Adolescents with Crohn’s disease form a special risk group for severe osteopenia. Growing bones are more likely to suffer from the effects of disease, nutritional deficiency and steroids. Monitoring for osteopenia should be standard practice in adolescents with Crohn’s disease.

688 Validation of the Leeds Dyspepsia Questionnaire in Detecting the Presence and Severity of Dyspepsia in General Practice and Hospital Populations
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Introduction: Many studies have used a questionnaire to assess dyspepsia but few have validated this instrument. The Leeds Dyspepsia Questionnaire (LDQ) was designed to detect the presence and severity of dyspepsia. There is no “gold standard” by which to measure dyspepsia so we have used the diagnosis reached by 1 of 3 informed GPs or 1 of 4 experienced gastroenterologists.

Validity: The LDQ was administered to 98 general practice patients chosen at random. The LDQ detected 35/41 patients with dyspepsia according to the GP (sensitivity = 85%). The LDQ detected 32/36 cases of dyspepsia, but few have validated this instrument. The Leeds Dyspepsia Questionnaire (LDQ) was designed to detect the presence and severity of dyspepsia. There is no “gold standard” which we measure dyspepsia so we have used the diagnosis of dyspepsia reached by 1 of 3 informed GPs or 1 of 4 experienced gastroenterologists.

Reliability: The LDQ was re-administered to 62 hospital patients after 4–7 days in 29/33 patients and all questions were concordant (gamma = 0.70–0.96; p < 0.001). The LDQ had good internal consistency with an alpha coefficient of 0.69. Responsiveness: The LDQ was repeated in 12 patients after one month of treatment. The median dyspepsia score fell from 22.5 (range 9–26) to 4.5 (range 0–27) (p < 0.001).

Conclusion: The LDQ is a valid instrument for detecting the presence and severity of dyspepsia.

689 Efficacy of Ten Days of Clarithromycin, Amoxycillin and Omeprazole in Eradicating Helicobacter pylori Infection
Introduction: Eradication of Helicobacter pylori (H. pylori) is important in the management of peptic ulcer disease. Recently clarithromycin, amoxycillin and high dose proton pump inhibitor for two weeks have been shown to be effective in eradicating this infection. We investigated the efficacy of a shorter duration regimen using lower doses of proton pump inhibitor.

Method: This was an open, single centre study. Patients with H. pylori infection at endoscopy as assessed by histology, rapid urease test, microbiology and 14C urea breath test (14C-UBT) were invited to participate. Patients were given omeprazole 20 mg od, clarithromycin 500 mg bd and amoxycillin 1 g bd for ten days. H. pylori eradication was assessed by 14C-UBT four weeks after completion of therapy. Adverse events and compliance were evaluated at patient interview.

Results: 34 patients were enrolled into the study (median age 49 years, age range 21–69, 20 males). One patient failed to attend follow-up and was not evaluable. The infection was eradicated in 28/33 patients (84.8%, CI 72–97%). 15 patients experienced side effects; the most common complaint was taste perversion (n = 8). All side effects were mild and no patient discontinued treatment due to adverse events. All patients harboured H. pylori strains that were sensitive to both antibiotics before treatment. In three patients in whom treatment failed, one demonstrated a strain resistant to clarithromycin.

Conclusion: Omeprazole 20 mg od, clarithromycin 500 mg bd and amoxycillin 1 g bd for ten days is an effective regimen in eradicating H. pylori.

691 Detection of PS3 and K-RAS Mutations in Pancreatic Juice and Diagnosis of Pancreatic Carcinoma
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Purpose of the study: There are still problems in differential diagnosis of chronic pancreatitis and pancreatic carcinoma.

Methods used: In a prospective study pancreatic juice from 32 patients with affection of the pancreas was obtained by endoscopic retrograde cholangio-pancreatography at time of diagnosis. The prompt preparation

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of DNA was necessary to avoid enzymatic destruction. After PCR amplification the samples were examined for mutations of the p53 suppressor gene at exon 5, 7, and 8. Mutant K-ras oncogenes at codon 12 and 13 in exon 1. The mutations for p53 were detected non-radioisotopically by SSCP–gelelectrophoresis and DNA sequencing. K-ras point mutations were analyzed by RFLP (restriction fragment length polymorphism). The results were compared to historical findings and clinical follow-up.

Summary of the results: No patient with histologically confirmed chronic pancreatitis had p53-ras mutations. Until now p53 mutations were found in five of twelve adenocarcinomas of the pancreatic head at codon 175, 271 and 273. In more than 80 percent of patients with carcinoma we detected a point mutation at codon 12 of the K-ras oncogene.

Conclusion: The additional detection of p53 and K-ras mutations from pancreatic juice might be a new promising method in differentiation between chronic pancreatitis and pancreatic carcinoma.

694 Reduced Gallbladder Emptying Is a Risk Factor for Gallstone Recurrence

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Following successful non-surgical therapy of gallbladder stones, some patients (pts) form recurrent stones whereas others do not. We therefore evaluated whether gallbladder emptying, a well-known laboratory marker for primary gallstone formation, is differently expressed in pts with gallstone recurrence and those without recurrence. Methods: Gallbladder emptying was studied in 36 pts 0.6–3.9 yrs (mean 2.0 yrs) after complete clearance of a single radioluculent gallstone (18 ± 1 mm, mean ± SE) by shock wave lithotripsy. Expression and cessation of adjuvant bile acid therapy. 18 consecutive pts at the time of diagnosis of recurrent stones (n ≤ 3 ± 1, largest stone: 8 ± 1 mm) were compared with 18 matched pts without recurrence. Matched pairs were obtained with regard to age, gender and age. Further, 18 healthy subjects served as controls. Gallbladder volumes were obtained sonographically (Dornier AI 3200, 5 MHz) at 5 min intervals for 90 min after a standard liquid test meal. Results: Residual volume (RV) (ml) and residual volume (RF) (of) the gallbladder were 50% larger in pts with recurrence than those without recurrence (p < 0.05). Compared to controls, RV and RF were even doubled in pts with recurrence (p < 0.01). Multivariate logistic regression analysis identified RF as an independent risk factor for gallstone recurrence (p < 0.02).

Recurrent No recurrence Controls

<table>
<thead>
<tr>
<th>Fasting volume (ml)</th>
<th>27 ± 3.7</th>
<th>23 ± 1.1</th>
<th>24 ± 2.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ml)</td>
<td>11 ± 1.3</td>
<td>9 ± 1.1</td>
<td>10 ± 0.9</td>
</tr>
<tr>
<td>(RF) (%)</td>
<td>45 ± 5%</td>
<td>33 ± 3%</td>
<td>25 ± 3%</td>
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</table>

< 0.05 vs controls, *p < 0.05 controls

Conclusion: Patients with gallstone recurrence after lithotripsy and adjuvant bile acid therapy are characterized by markedly impaired gallbladder emptying. Thus, gallbladder emptying may be considered as a selection factor for non-surgical therapy of gallstone stenosis.

696 Characterization of Thermal Stress Response of Endocrine and Exocrine Pancreas by 1-2 D-Analysis

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Although heat shock proteins are known to function as molecular chaperonins in many systems, next to nothing is known about their role in pancreatic physiology and in the response of pancreatic acinar cells to acute stress. To study the exocrine pancreatic stress response we have labeled acutely isolated rat pancreatic acini with [35S] - [35S] methionine labeled RNitin5 cells served as endocrine pancreas model. Separation of proteins by two-dimensional gel analysis followed by autoradiography allowed identification of 80–100 different proteins as well as assessment of the degree of expression of individual proteins under a variety of experimental conditions. Due to the strong induction of these proteins expressed at higher levels in response to heat, their identification could easily be accomplished. Mechanical stress through preparation of acutely isolated acini had no apparent effect on protein expression. Thus, prolonged preincubation of acini at 37 °C, ranging from 30–80 min, to allow cells to recover from preparation stress before labelling and/or exposure to heat, had no effect on [35S] methionine incorporation. Incubation of acini at 42 °C led to strongly increased expression of at least five distinct proteins of 92, 72, 56, 30 and 28 kDa molecular weight, while incubation at 37°C, or without heat, showed no major changes of protein expression. Western blotting and autoradiography analysis identified two of these proteins as HSP 65 and HSP 70. Similar effects could be observed in the endocrine minor cell line RIH56. Western analysis led to the identification of HSP 60 and HSP 70. Interestingly, RNITm5F HSP 60 was detected to translocate to the membrane fraction in response to thermal stress. This system will allow identification of additional stress proteins using western analysis or microsequencing techniques. The investigation of the alteration of protein expression in response to a variety of other forms of metabolic and chemical stress including models of acute pancreatitis should further prove interesting insights into the role of these proteins for pancreatic stress response.

697 Hyperthermia Leads to Expression of HSP 70 in Pancreas and Also Protects Against Acute Pancreatitis Induced by Cerulein Treatment

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To test the exposure of living tissue to hyperthermia can have protective effects, leading to increased tolerance of further stress episodes. It has also been shown that a number of proteins, later termed “heat shock proteins” are concomitantly expressed in response to hyperthermia. This protective effect has led to improved recovery of rat cardiac muscle, liver, lung as well as to better function of renal transplants in rats. We have found that acutely isolated rat pancreatic acini as well as RNITm5F cells also respond to thermal stress with expression of heat shock proteins. We have now transferred these in vitro data to an in vivo system. Rats were anaesthetized and body temperature was increased to 42 °C for 20 min, monitored with a rectal thermometer. Control rats underwent anaesthesia without hyperthermia. 24 h after hyperthermia, pancreatitis was induced by intraperitoneal injection of cerulein. Rats were sacrificed and pancreas were examined for evidence of pancreatitis and expression of heat shock proteins. A reduction of cerulein-induced edematous pancreatitis after hyperthermia was already macroscopically apparent. Thus, pancreata from rats not exposed to hyperthermia had a white-coloured and firm yellowish for primary pathology, whereas pancreata from rats exposed to hyperthermia appeared essentially normal. Light microscopically, interstitial edema as well as size and number of vacuoles were accordingly decreased in rats exposed to hyperthermia. Compared to non-treated controls, revealed expression of HSP 70 only after hyperthermia. These results strongly indicate that exposure to heat confers protection against pancreatitis and at the same time leads to increased expression of HSP’s. Using this approach we can now investigate the role of heat shock proteins in other models of pancreatitis. We will also be able to investigate whether down regulation of pancreatic heat shock protein response to stress plays a role in development of pancreatitis.

701 Epidermal Growth Factor Inhibits Vasoactive Intestinal Peptide- and Forskolin-induced CAM-production and Amylase Release by Pancreatic toxin-sensitive G-proteins in Rat Pancreatic Acini

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Evidence is accumulating that epidermal growth factor (EGF) regulates secretory function of pancreatic acini in addition to cell growth and differentiation. In the present study we investigated the effect of EGF on basal and secretagogue-induced activation of adenyl cyclase and amylase release in isolated rat pancreatic acini and the role of pertussis toxin-sensitive G-proteins in EGF-induced regulation of CAM pathway. Methods: Pancreatic acini were prepared by collagenase digestion. To examine the role of pertussis toxin-sensitive G-proteins in EGF-induced signal transduction, the acini were pretreated with pertussis toxin or vehicle for 2 h. Complete ADP-ribosylation of the α-subunits of the toxin-sensitive G-proteins was confirmed by a second pertussis toxin-catalyzed ADP-ribosylation of the per-

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on the scan and the blood results. In five the scan suggested activity but the blood results were normal, three of these had disease confined to the distal colon, one had early active colitis and one had small bowel Crohn's disease. One scan was negative although the blood results were abnormal (raised platelets), clinically this patient was in remission.

In 64 patients the diagnosis of IB was suspected but had not been confirmed previously and no radiologic scans were seen in 31 cases, of which 27/30 had normal blood results, 10/10 normal colonoscopy, 8/8 normal histology and 7/8 normal radiology. Positive scans suggesting IB were seen in 33 patients, of these 16 had IP. IP was also suggestive of active IB and 17/46 had raised blood tests. In this latter group further investigation confirmed a definite diagnosis of IB in five.

In summary Tc HMPAO scanning can reliably identify active IB which may be missed by normal blood results or radiology. In new patients the scan and sigmoidoscopy can be used to exclude IB. Abnormal scans enable further investigations to be efficiently targeted.

503 Epidermal Growth Factor Causes Tyrosine Phosphorylation of Several Substrates in Isolated Rat Pancreatic Acinar Cell Membranes

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Epidermal growth factor (EGF) regulates pancreatic acinar cell growth and differentiation as well as secretion. The aim of the present study was to correlate EGF-induced second messenger generation and receptor-mediated tyrosine phosphorylation in isolated rat pancreatic acini. Methods: Pancreatic acini were prepared using collagenase digestion. Amlyase released from the acini during 30 min incubation was determined using colorimetric assay. Cyclic AMP accumulation was detected by radioimmunoassay, and release of intracellular calcium was determined by measuring Fura-2 fluorescence. Membranes were isolated from the acini by differential centrifugation, and tyrosine phosphorylation of membrane proteins was detected by immunoprecipitation and Western-blotting using monoclonal anti-phosphotyrosine antibodies. Results: EGF caused a dose-dependent increase in amylase release from the acini with an EC50 of 15 nM. EGF (80 nM) increased the intracellular calcium concentration from 95 to 270 nM and caused a fourfold increase in cAMP level after five min of incubation. Probing Western-blots of isolated pancreatic acinar membrane proteins with an EGF receptor-specific antibody revealed the presence of the receptor with a calculated molecular mass of 170 kDa. Phosphatase treatment of the membranes with EGF (80 nM) caused a rapid and marked increase in tyrosine phosphorylation of the EGF receptor with a maximum 15 s after beginning of the incubation, followed by a substantial decline within one min. Tyrosine phosphorylation of two other proteins migrating at 48 and 90 kDa, respectively, increased with a slower kinetics than EGF receptor tyrosine phosphorylation and showed no decline within the period of observation of one min. Conclusions: EGF causes release of amylase from pancreatic acini and activates both adenyl cyclase as well as phospholipase C pathway. Phosphorylation of the EGF receptor occurs within 15 s, whereas tyrosine phosphorylation kinetics of two other yet unknown membrane proteins is slower. These data show that EGF causes short term regulation of pancreatic acinar cell function.

704 Inhibition of Growth of Human Colorectal Cancer by Gastrin Receptor Antagonist

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Gastrin is a potent growth factor for colorectal cancers. The aim of this study was to determine the effect of the gastrin receptor antagonist CR 2093 on basal and gastrin-stimulated growth of short term in vitro cultures of primary human colorectal adenocarcinomas and to relate this to gastrin receptor expression.

Tumour cells from surgical specimens were grown on matrices of type I collagen and irradiated fibroblasts. Cell proliferation was assessed by [3H]-thymidine uptake. Gastrin receptor expression was determined by an immunocytochemical technique using the mouse monoclonal antibody 2C1.

Increased growth in the presence of gastrin[17x 10^-10 and 5 x 10^-11 M] was shown in 18/34 (47%) tumours. The gastrin receptor antagonist significantly reversed this stimulated growth (p < 0.05) in 13/16 (81%) of the gastrin-sensitive tumours. In addition, CR 2093 inhibited the basal growth of 11/34 (32.4%) tumours and this inhibition could be reversed by gastrin-17 in that the sensitivity of tumours to gastrin-17 was not altered by the receptor antagonist, but there was no correlation between the intensity of expression and the degree of response. In addition, tumours which did not show an in vitro response to the hormone were also receptor positive. However, there was a significant correlation between intensity of receptor expression and inhibition of basal growth by CR 2093 (p = 0.05, r = 0.54).

Gastrin receptor expression in colorectal adenocarcinomas does not predict sensitivity to gastrin, and does not predict sensitivity to gastrin receptor antagonist on basal growth. Gastrin receptor expression may be related to endogenous gastrin production by colorectal tumour cells.

705 Prognostic Value of the Preoperative Serum Levels of the New Tumor Marker TUM2-PK in Pancreatic Cancer


The monoclonal antibody pyruvatekinase type tumor M2 (TUM2-PK) has been shown to have a high binding capacity to pancreatic cancer.

In the present study TUM2-PK serum levels were measured in pancreatic cancer and compared with the reference tumor markers CA 19-9, CA 50, CA 24-2 and CEA.

Overall 100 patients were included in this study, 64 patients had a historically confirmed pancreatic carcinoma, 36 patients gastrointestinal cancer (stomach, colon), 185 healthy volunteers served as controls.

For the healthy blood donors a cut-off value of 17 U/ml was determined, corresponding to 95% specificity. In patients with pancreatic cancer the sensitivity for TUM2-PK, CA19-9, CEA, CA 72-4 and CA 50 were 90%; 89.2%; 58.6%; 49% and 63.4% respectively. Linear regression analysis indicated that there was a positive correlation (r = 0.79).

According to the results of our study TUM2-PK was a comparable sensitivity but a higher specificity than the reference tumor marker CA 19-9.

706 Alleloype Analysis of Oesophagal Adenocarcinoma

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The development of oesophageal adenocarcinoma is thought to be associated with at least with a number of known tumour suppressor gene loci, 18q (11/34), 16q (4), 12q (1), 12p (1), and 5q (1). To determine additional chromosomal loci containing putative tumour suppressor genes involved in tumour development, loss of heterozygosity (LOH) studies were performed on 17 cases of oesophageal adenocarcinoma, arising on a background of Barrett's metaplasia. Using 138 microsatellite markers, all autosomal arms, excluding acrocentric arms, were examined.

The average fractional allele loss observed for the 17 tumours was 0.42. Frequent LOH of >40% of informative cases was observed on chromosomes 17p (4), 13q (1), 3q (85%), 4q (85%), 5q (59%), 6p (44%), 6q (59%), 9q (50%), 9q (47%), 11p (47%), 12p (47%), 12q (65%), 13q (47%), 16q (44%), 17p (71%), 17q (44%), 18q (75%), 20q (41%) and 21q (41%). Sequence analysis was carried out to determine the association between 17p allelic deletion and p53 gene mutation. Mutations were detected in 69% (11/16) of oesophageal adenocarcinomas and there was a close association between 17p allelic loss and p53 gene mutation (p = 0.00879, Fishers Exact Test). The tumours demonstrated a specific p53 mutation spectrum, with G-C to A-T base transitions at CpG dinucleotides accounting for 80% (8/10) of single base substitutions.

A low level of microsatellite instability was a feature of all tumours, with individual tumours demonstrating rearrangements ranging from 0.8%-45% of markers analysed. The precise nature and function of multiple genetic alterations underlying the development of oesophageal adenocarcinoma and several putative tumour suppressor genes may be implicated in tumorigenesis.

707 Pancreatic Therapy and Ileocolic Stenosis. An Ultrasonography Study in 200 CF-patients


For pancreatic steatorrhea high-strength pancreatin (HSP) preparations have been developed. Recently, strictures of the ascl. colon and caecum in cystic fibrosis (CF) patients have been attributed to HSP enzyme therapy (Lancet 1994; 344: 85-86).

Aim: To investigate gut wall morphology with respect to pancreatin supplementation in our cohort of 260 CF-patients.

Method: 214 CF-patients (1-38 years), 12 pediatric and 8 adults with chronic exocrine pancreatitis (CP) were studied by real time ultrasonography (US). 123 CF-patients had HSP-preparations (20,000 units lipase/capsule), 69 normal-strength pancreatin (NSP: 10,000 units/capsule), and 8 CP-patients did not require pancreatin. In 14 cases US was unsatisfactory. All 8 adults with CP received HSP US was performed by a single investigator (B.L.) who did not know the patients before and their therapy.

Wall thickness in the terminal ileum, caecum, asc. and desc. colon are measured of longitudinal and transverse wall thicknesses.

Results: Patients with CF receiving pancreatin had a unique ileocaecal US pattern with a prominent submucosal layer which is not observed in CP. Gut wall thickening was significant in the ileum with NSP and HSP in the caecum additionally, HSP being more effective than NSP only. The effect was correlered to HSP (or conditions requiring them), but not to the daily dose pancreatin.
Conclusion: US detects unique ileocecal wall lesions in the majority of CF-patients on pancreatitis. These lesions may lead to significantly increased ileocecal wall thickness, which is correlated but not restricted to HSP-preparations.

Table: Means ± SD [mm] (italics: signif.)

<table>
<thead>
<tr>
<th>Period</th>
<th>Ileum</th>
<th>Cecum</th>
<th>Asc.</th>
<th>Desc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 12)</td>
<td>1.23 ± 0.20</td>
<td>1.29 ± 0.14</td>
<td>1.21 ± 0.21</td>
<td>1.15 ± 0.17</td>
</tr>
<tr>
<td>Ø Supp. (n = 8)</td>
<td>3.1 ± 0.49</td>
<td>1.60 ± 0.02</td>
<td>1.07 ± 0.13</td>
<td>1.41 ± 0.10</td>
</tr>
<tr>
<td>HSP (n = 69)</td>
<td>1.60 ± 0.55</td>
<td>1.94 ± 0.75</td>
<td>1.54 ± 0.70</td>
<td>1.32 ± 0.41</td>
</tr>
<tr>
<td>HSP (n = 123)</td>
<td>1.76 ± 0.55</td>
<td>2.38 ± 0.97</td>
<td>1.72 ± 0.77</td>
<td>1.50 ± 0.63</td>
</tr>
<tr>
<td>CP (n = 68)</td>
<td>1.29 ± 0.22</td>
<td>1.41 ± 0.16</td>
<td>1.49 ± 0.22</td>
<td>1.79 ± 0.26</td>
</tr>
</tbody>
</table>

Diazepam Binding Inhibitor is a CCK Releasing Peptide in the Intestine

K.H. Herzig, S. Barodzec, G. Grant, R. Nustede, U.R. Fölsch, A. Pusztai.Dept. of Internal Medicine, Christian-Albrechts-University, Kiel, Germany; Dept. of Molecular Physiology, Gunna University, Maastricht, Japan; Dept. of Internal Medicine, University of Michigan, Ann Arbor, USA

Pancreatic exocrine secretion is tightly regulated by the cholinergic system and the presence of active proteases in the duodenum. Exclusion of proteases from the duodenum either by diversion of bile-pancreatic juice or addition of pancreatic protease inhibitors does not significantly affect pancreatic secretion. This mechanism became known as the negative feedback regulation of exocrine pancreatic secretion. Yet, how the duodenal protease concentration and cholecystokinin release are regulating each other, is unknown. We report here the isolation and characterization of a duodenal release-activated pig intestinal mucosa. 700 kg pig intestine was processed, the mucosa scraped off and acid extracted, peptides adsorbed to alginic acid, salt precipitated and dissolved in methanol. This protocol yielded 70 g of crude peptide material, which was further purified by HPLC. Each purification step was monitored for stimulation of pancreatic secretion by intraduodenal application of the extract in anesthetized and atropinized rats. After four HPLC steps we obtained a pure peptide which was identical to porcine diazepam binding inhibitor by peptide sequencing and mass spectrometry analysis. Porcine DBI was then synthesized using an automated peptide synthesizer on p-alkox benzyl alcohol resin with Fmoc protection strategy. Intraduodenal infusion of 200 ng of synthetic porcine diazepam binding inhibitor in anesthetized and atropinized rats significantly stimulated pancreatic amylase output. Infusion of the cholecystokinin antagonist MK-328 completely blocked diazepam binding inhibitor stimulated amylase output. Using a perfusion system containing isolated minumum mucosa from the duodenum, we stimulated and blocked diazepam binding inhibitor dependence of small cell pancreatic cancer cell lines, in anesthetized and atropinized rats. Yet, how amylase output is stimulated by diazepam binding inhibitor is unknown in duodenal washout in a rat of 7.5 x 10^{-11}M suggesting that diazepam binding inhibitor is released into the duodenum. In conclusion, we have isolated and characterized a cholecystokinin releasing peptide known as diazepam binding inhibitor from pig intestinal mucosa which stimulates cholecystokinin release intraduodenally in rats.

Identification of Differentially Expressed Genes Linked to the Mitogenic Action of PACAP on AR4-2J Cells

A. Trauzold, H. Schäfer, U.R. Fölsch, W.E. Schmidt. Laboratory of Molecular Gastroenterology, Dept. of Medicine, Christian-Albrechts-University of Kiel, Germany

Introduction: Beside its important role as secretagogue and neuromodulatory peptide in various tissues, i.e. intestine, pancreas and brain, the peptide hormone Pituitary adenyl Cyclase Activating Polyptide (PACAP) has also been shown to promote cell growth. In this study, novel gene products linked to the mitogenic activity of PACAP on the rat pancreatic acinar tumor cell AR4-2J were identified.

Methods: AR4-2J cells were stimulated with 10 nM PACAP[1–38] for various time periods. Total RNA was isolated and submitted to the mRNA differential display procedure employing low stringency RT-PCR with different combinations of anchored oligo-T primers and arbitrary decamer primers (BIOMETR). Analysis of the generated 32P-labelled amplification products was carried out using DNA sequencing gels and subsequent autoradiography. Bands of interest were excised, reamplified and submitted to DNA sequencing.

Results: The autoradiographic pattern of reverse transcribed and amplified mRNA isolated from AR4-2J cells stimulated with PACAP[1–38] revealed a considerable degree of identity indicating high reproducibility. However, individual primer combinations produced a few bands differentially regulated and were selected for further analysis. A six hour stimulation of AR4-2J cells with 10 nM PACAP[1–38] led to the appearance of three bands and to the disappearance of two bands. DNA-sequencing of these bands revealed in three cases unknown sequences of >200 bp in length encoding the 3' UT regions of the mRNA. Specifically designed primers targeted to the sequenced products were used for high stringency RT-PCR in order to verify the differential expression of the corresponding genes.

Conclusions: Employing our novel mRNA differential display technique, three gene products were identified that are differentially expressed in AR4-2J cells stimulated with 10 nM PACAP[1–38] when compared with unstimulated cells. Using the five' race technique these identified mRNAs were further amplified and sequenced in order to obtain structural data from 5' parts of these growth related unknown genes.

PACAP Induces Growth of AR4-2J Cells Involving Increased Expression of Cfos, Cjun and AP1-Activation

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Introduction: The peptide hormone Pituitary adenyl Cyclase Activating Polyptide (PACAP) plays an important role as secretagogue and neuromodulatory peptide in various tissues, i.e. intestine, pancreas and brain. PACAP has also been shown to promote cell growth. In this study, the mechanisms of the mitogenic activity of PACAP on the rat pancreatic acinar tumor cell AR4-2J were characterized.

Methods: Growth of AR4-2J cells was analyzed by means of 3H-thymidine incorporation. mRNA-levels of cfos and cjun in AR4-2J cells were determined by quantitative RT-PCR. Functional activity of the mature transcription factor AP1 was analyzed using gel retardation assays of nuclear extracts from AR4-2J cells treated with PACAP [1–38].

Results: At a dose of 1–10 nM, PACAP [1–27] and PACAP [1–38] maximally stimulated cell growth of AR4-2J cells. RT-PCR revealed that the mRNA levels of cjun were enhanced in AR4-2J cells treated with PACAP [1–10] nM. PACAP-1 receptor occupation was followed by a rapid (10 min), transient and dose dependent stimulation of cjun expression. Compared to PACAP [138], PACAP [1–27] was 20 fold less potent and VIP (1 nM) revealed no effect. Administration of dBcAMP (10 μM), forskolin (10 μM) or PMA (100 ng/ml) revealed also lower effects, even in a simultaneously fashion. Expression of cjun was abolished in the presence of the antagonist PACAP [6–38], the calmodulin inhibitor W7, the PKC inhibitor calphostin or the tyrosine kinase inhibitor genestein (all at micromolar doses). Gel retardation assays revealed an equipotent activation of AP1 by PACAP [1–27] and PACAP [1–38] but not by VIP or secretin. Activation of AP1 was less rapid and persisted for several hours in PACAP[1–38] treated cells with a delay of >5 minutes.

Conclusions: Via interaction with the PACAP-1 receptor, PACAP [1–38] and to a lesser extent PACAP [1–27] stimulate cell growth of AR4-2J cells. This effect includes the elevation of mRNA levels of cfos and cjun as well as the activation of the transcription factors AP1 and CREB. These findings indicate that internalized upstream signals involving the actions of PKC, calmodulin and tyrosine kinases.
**715 Distinction Between Steatosis and Early Liver Cirrhosis Using Computer Program for Tissue Characterization**

M. Krstic, S. Stamenkovic, A. Mosijovic, M. Popovic, N. Kovačević, R. Jelić, G. Janković. Institute of Digestive Diseases, Clinical Center of Serbia and Faculty of Electrical Engineering, Belgrade, YU

Echosonographic images of steatosis and early liver cirrhosis are very similar and distinction between these two different diseases can be usually made only on liver biopsy. In order to investigate texture properties of steatosis and cirrhosis we have developed the computer program for liver tissue characterization from sonographic images.

The program uses several statistical and spectral methods for texture characterization such as Spatial Gray Level Coocurrence, Fourier descriptors and texture energies obtained from Wavelet image decomposition, to compare tissue samples (taken from examined patients) with determined steatosis and cirrhosis texture prototypes. The program is men designed and has two operating modes. In the auto-diagnose mode, after calculating selected texture parameters for a minimum distance calculation, the program performs a comparison of input tissue samples into the steatosis or cirrhosis class. In the user diagnosis mode, after calculating selected texture measures, program provides numerical and graphical presentation of obtained results, and may be used also for further texture examinations and investigations.

The program runs under Microsoft Windows on IBM 386/486 compatibles. It accepts ultrasound images stored on disk by commercial frame grabbers. In validation study 42 patients with histologically proven steatosis and cirrhosis were included, with 55% present in our program gave statistically significant results as histology. These results are encouraging but further testing are necessary for its clinical application.

**716 Transcription Factor AP-1 is Activated by Physiological Concentrations of CCK-B and Gastrin Via Map-Kinases in the Rat Pancreatic Tumor Cell Line AR42J**

R. Gunther, F. Bauer, W.E. Schmidt. Laboratory of Molecular Gastroenterology, Gf Unit, I. Dept. of Medicine, Christian-Albrechts-University, Kiel, Germany

**Introduction:** The members of the fos and jun protein families constitute the subunits of the transcription factor complex AP-1. Binding of AP-1 to its consensus sequence is responsible for increased transcription of various genes in response to extracellular signals, such as TPA and serum factors. Enhanced AP-1 activity is mediated by two different mechanisms: transcriptional activation of jun and fos gene expression and posttranslational modifications. Physiological concentrations of the potent secretagogue CCK-B induce the transcription of c-fos, c-jun and c-myc in pancreatic acini. In order to characterize the functional significance of this CCK-B induced increase of c-fos and c-jun mRNA, we investigated the formation and activity of AP-1 in the rat pancreatic tumor cell line AR42J.

**Methods:** Transcription of c-fos and c-jun genes was measured by RT-PCR with appropriate primers. Formation and binding of AP-1 was analysed by Electrophoretic Mobility Shift Assays (EMSA) and Supershift-EMSA. CCK receptor mRNA and inosines involved in the activation of AP-1 were characterized by incubation with specific inhibitors (L-364, 718, L-365, 280, genistein, staurosporine, H-7) and a peptide based MAP-kinase assay. The plasmid 5X-TRE-TATA-CAT was used for transient transfection experiments.

**Results:** CCK-B and gastrin strongly induced transcription of c-fos mRNA, whereas c-jun transcription was not significantly affected. TPA and protein kinase A activators DBcAMP or forskolin showed only minor effects. CCK-B, gastrin and the phorbol ester TPA led to an increase of active AP-1 in CCL2 cells which were characterized by incubation with specific inhibitors (L-364, 718, L-365, 280, genistein, staurosporine, H-7) and a peptide based MAP-kinase assay. The plasmid 5X-TRE-TATA-CAT was used for transient transfection experiments. CCK-A and CCK-B receptor antagonists diminished the CCK-B and gastrin stimulated effects, respectively.

**Discussion:** The activity of the AP-1 complex is induced by the PLC coupled secretagoues CCK-B and gastrin and the PKC activator TPA via the MAP-kinase pathway. Current studies try to identify the set of genes regulated by AP-1 in AR42J cells in order to characterize its role in pancreatic adaption and growth.

**717 Short Term Transcriptional Regulation of the CCK-A/B Receptors by their own Ligands in AR42J Cells**

O. Carstens, R. Günther, E.G. Siegel, H. Schäfer, U.R. Fölsch, W.E. Schmidt. Laboratory of Molecular Gastroenterology, Gf Unit, I. Dept. of Medicine, Christian-Albrechts-University, Kiel, Germany

**Introduction:** Radioligand binding studies have demonstrated the regulation of CCK-A and CCK-B receptor expression on the plasma membrane of AR42J cells. However, the underlying molecular mechanisms for CCK receptor regulation remain unclear. In order to characterize these mechanisms, the CCK receptor mRNA in AR42J cells was analysed by RT-PCR.

**Methods:** AR42J cells were incubated with different concentrations (10-9 M to 10-12 M) of CCK-B and gastrin for various times (0-48 h) and with dexamethason (10-9 M). Preincubation was performed with actinomycin D (25 μg/ml or actinomycin D (10-9 M)). Whole RNA was extracted and reverse transcription was performed with oligo-dT primers and MMLV-RT. Specific PCR primers for the rat CCK-A (CCK-RA) and rat CCK-B (CCK-RB) receptors and rat GAPDH as a control were designed. PCR products were run on a PAGE, stained with ethidium bromide and analysed by the Bio imaging "whole band" software. Alternatively PCR products were evaluated by HPLC.

**Results:** Dexamethason increased CCK-A and -B mRNA up to 200% within 6 h. While CCK-RB mRNA decreased to starting level after 24 h, CCK-RB mRNA remained at the high levels of stimulation by 10-10 M CCK-B or gastrin the CCK-RB mRNA was down-regulated to 50% of its normal content and increased to starting level after 6 h. In contrast, CCK-RB mRNA was upregulated to 200% of normal content after 2 h and decreased to starting level after 6 h. Actinomycin D inhibited these effects. Ooctrecit had no effect on CCK-RB mRNA but inhibited CCK-B mRNA upregulation.

**Discussion:** Dexamethason elevates mRNA levels for the CCK-A and CCK-B receptors. Expression of CCK-A and CCK-B receptors is differently regulated by their own ligands. Experiments are in progress to study the signal transduction cascade involved in this regulation.

**719 Controlled Randomized Multicenter Trial of Urgent Endoscopic Retrograde Cholangiopancreatography (ERCP)/Papillotomy (EPT) for Acute Biliary Pancreatitis**

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In two monocenter trials the impact of urgent ERCP with EPT in case of bile duct stones on lethality and local and systemic complications in acute biliary pancreatitis (ABP) remained controversial (Lancet 1979, 1988; NEJM 328, 226, 1993). Therefore, we conducted a multicenter trial of ERCP/EPT vs. bile duct stones (CBD) with ABP without obstructive jaundice. In 22 German hospitals 238 pts have been randomized between 1989 and 1994 either to receive urgent ERCP within 72 hours since the beginning of abdominal pain (107 pts) or conventional management (99 pts). In 32 pts the CBD had to be excluded because of inclusion criteria had not been followed. In the conventional group ERCP was only performed in case of a) persistent biliary colics, b) septical fever, or c) increase of serum bilirubin of more than 3 mg % (51 mmol/l) in 5 days. If common bile duct stones (CBD) were identified at ERCP EPT was undertaken and the stones extracted. Results: According to all measured parameters both treatment groups were comparable. In the invasive treatment group urgent ERCP was successful in 102 (95%) of the 107 pts. In 51 pts (50%) CBD were detected and EPT performed. EPT was successful in 49 pts and in 44 pts stones were successfully removed. Local and systemic complications as well as lethality in both treatment groups are given in the table.

<table>
<thead>
<tr>
<th>Invasive group</th>
<th>N = 107</th>
<th>Convent. group</th>
<th>N = 99</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resp. fail.</td>
<td>12</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Renal fail.</td>
<td>7</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Chol. cyst.</td>
<td>10</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Choleran</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Newly devel. icterus</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sepsis</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lethality</td>
<td>10</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>

*p < 0.05

As indicated, patients with icterus in the conventional treatment group, gave reason for a delayed ERCP Conclusion: Overall, complications and lethality of acute biliary pancreatitis could be reduced significantly by urgent ERCP in the vast majority of cases of acute biliary pancreatitis (without biliary sepsis or obstructive jaundice) elective ERCP is sufficient when remaining bile duct stones are assumed.

**721 Hepatocyte Growth Factor is a Stimulator of DNA Synthesis and Amylase Secretion in AR42J Rat Pancreatic Carcinoma Cells**


Hepatocyte growth factor (HGF) has been implicated in liver regeneration and growth processes. The HGF-receptor is identical with the c-met oncogene. Recently, HGF and the HGF-receptor have been shown to be highly expressed in pancreatic carcinoma, which is in contrast to normal pancreatic cells where only the HGF-receptor is slightly detectable (Cancer Res. 54, 1775-1778, 1994). These results suggest the existence of an autocrine loop in pancreatic carcinoma, promoting tumor growth by HGF. To test this hypothesis, we investigated the actions of HGF towards AR42J rat pancreatic carcinoma cells. In a DNA synthesis assay, we found that these cells reacted to a HGF stimulus in a dose and time dependent manner with incorporation of [3H]thymidine into DNA. The maximum [3H]thymidine incorporation induced by 30 nM HGF was similar to the 100% induction by maximum cholecystokinin stimulation. The mitogenic action of HGF was mediated by the induction of the transcription factor c-fos. Addition of HGF to the cells resulted in a marked induction of c-fos mRNA, which was 2-3 fold stronger than the c-fos induction by cholecystokinin. The maximum stimulation of c-fos mRNA was also induced by 30 nM HGF and peaked after 45 min of incubation. By employing Tyrosphrin 25 and GF109203X, which are specific inhibitors of tyrosine kinase and protein kinase C activity, respectively, we could show that the induction of c-fos mRNA by HGF was mediated by these two kinase activities. In addition to...
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its mitogenic activity. HGF induced amylase release from AR4-2J cells but not from normal rat pancreatic acinar cells. In conclusion, our results provide further evidence that xenopsin could participate in an autocrine stimulatory loop leading to pancreatic tumor growth.

**723 Xemin Induces Relaxation of Rat Ileum In Vitro: Mode of Action**
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The 25-residue protein human xemin (Xe) is a xenopsin-like novel member of the neurotensin (NT) peptide family. This study characterizes the effect of Xe and related peptides on rat ileum.

Methods: Xemin peptides were synthesized by Fmoc chemistry and HPLC-purified. Full-length thicknessional rat ileal muscle strips were mounted on a Ussing gigaohm organ bath. Basic physiological recordings were obtained as previously described. The ileum strips were precontracted with 30 mM of TTX and 20 µM of Xe (1-23) (20 µM) and the concentration-effect curves were drawn. NT (1 µM) and the antagonist SR48692 (1 µM) were used as test compound.

Results: Xemin peptides showed a relaxant effect on the ileal preparations. The C-terminal fragment of the xemin molecule is important for binding and biological activity.

**725 Effect of the Novel Regulatory Peptide Xemin and Related Peptides on Human Colonic Motility**
S. Katouzis, A. Clemens, C. Morys-Wortmann, R. Neumann, U.R. Fölsch, W.E. Schmidt. Laboratory of Molecular Gastroenterology, Dept. of Medicine, Christian-Albrechts-University, Kiel, Germany; 1 Max-Planck-Institute for Experimental Medicine, Dept. Immunoochemistry, Göttingen, Germany

Xemin was recently isolated from human stomach and is present in the whole gastrointestinal tract. Because of its structural homology to the amphipathic peptide xenopsin, it is thought to be the human form of xenopsin. The role of xenopsin regulation of gastrointestinal motility is unknown. Therefore, the aim of our study was to determine its action on motility of the human colon. Additionally, several fragments of xemin were used for structure-function analysis of their contractile action on isolated human colonic muscle strips.

Methods: Xemin and its fragments were synthesized by solid-phase Fmoc strategy. Peptides were purified by HPLC and characterized by mass spectrometry. Isolated full thickness circular muscle strips of the human colon without attached mucosa and submucosa were used. Results: Xemin, xenopsin, and neurotensin (NT) contracted human colonic muscle strips in a dose-dependent fashion, while kentensin was not active at concentrations up to 20 µM. The efficacies of the three peptides were not significantly different (p > 0.05; 71 ± 10; 74 ± 11 of the maximal effect of acetylcholine).

However, xenopsin was slightly more potent than xenin and NT (EC50 of 15 ± 1; 62 ± 4; 44 ± 12). The C-terminal fragments of xemin 14-25, 12-25, 19-25 and 20-25 were fully active and showed approximately the same potency and efficacy as xemin while the fragment 21-25 was also fully active but showed a marked decrease in potency. In contrast, N-terminal fragments exhibited no (1-23) or little (1-24) activity. The contraction induced by xemin and NT was partially inhibited by TXB (1 µM) (46%; 36%), atropine (1 µM) (32%; 28%), hexamethonium (100 µM (46%; 45%) and the recently developed NT-antagonist SR48692 (1 µM) (46%; 59%). Higher doses of the antagonist did not lead to significant increase of inhibition. Conclusion: (1) Xemin and NT produce a partially neuromodulatory contraction in the human colon by activation of cholinergic nerves (2) The C-terminal part of xemin molecule is essential for biological activity.

**729 Significance of Gastrointestinal Symptoms During Long-Distance Flights**

Introduction: Flights across several time-zones are combined with a lot of troubles in the sphere of cognition, psychomotoric, well-being and sleep. Some of the phenomena are conditioned by the flight itself (e.g. the decrease in air moisture), others are a result of the need to reset the biological clock. For all of these symptoms the term "jet lag" has been introduced. In the course of an analysis of the various troubles during and after transmeridian flights we investigated a group of gastrointestinal symptoms.

Materials and methods: A series of 40 consecutive day flights covering about 100 items of many ranges in connection with jet lag. In a scale from 1 to 4 (1 = rarely; 4 = frequently) the frequency of occurrence of each symptom was specified. From 1090 until 3801 this questionnaire was answered by 500 light attendants of the "Deutsche Lufthansa AG" (Frankfurt, Germany). The valuation was carried out anonymously. All statistical analysis were conducted using the Statistical Package for the Social Sciences (SPSS).

Results: Through approximately 20 symptoms, each having an loading <0.2, could be found out. The first 4 factors render the following items: mental efficiency, sleep-wake-rhythm, emotionality and physical symptoms. The 5th factor as a heterogenous one can be described as a jet-lag-effect, gastro-intestinal factor, that contains 10 symptoms (e.g. flatulence, hunger, thirst with a stand, item alpha of 0.714).

Discussion: The gastrointestinal factor 5 is the only one, that can be explained completely by physiological processes. E.g., most of the flight attendants complained about meteorism, which can be explained by the statement of "Boyle-Mariot" about the proportional expansion of gases in gut following decreases of air pressure. Thus, all of these factor-5-symptoms can be illustrated by physiological procedures.

Conclusions: Our trial confirms, that during and after transmeridian flights a variety of gastrointestinal symptoms occur. Future prevention strategies will have to focus on this area.
ded tissue were studied. MHC class I antigen was detected by an immuno-
histochemical method (APAAP, Biotin-Streptavidin) with the monoclonal anti-
body W6/32, which reacts with a polyclonal antibody against #2 microglobulin, the
nonpolymorphic light chain component of human MHC class I antigens.
Results: Complete loss of MHC class I expression was detected in 3
(11%) resectable and 2 (16%) advanced pancreatic carcinomas. Reduced MHC
class I expression was seen in 9 (33%) resectable (>80% negative tu-
tumor cells, n = 4; 20–79% negative tumor cells, n = 5) and 5 (42%) advanced
 (>80% negative tumor cells, n = 1; 20–79% negative tumor cells, n = 4) car-
cinomas. In the remaining cases no significant loss of MHC class I antigen
expression was detected. There was no correlation between loss of MHC
class I antigen expression and patient survival time in resectable pancreatic
carcinomas.
Discussion: Existence of tumor specific MHC class I restricted CTLs in
pancreatic cancer patients is evidence for an ongoing immune response to this
tumor. Anti MHC class I antibodies directed against a monomorphic de-
terminant does not seem to be suitable to detect possible selective losses of M
malignant cells in pancreatic cancer. Other studies with single chain
monoclonal antibodies against HLA class I allotypes are necessary to
determine the relevance of the abnormalities in MHC class I expression in
pancreatic cancer demonstrated in this study.

Are Pancreatitis-associated Proteins (PAP) Protective Factors in Acute Experimental Pancreatitis?
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Institut für Immunologie, Pathologie, Klinikum Mannheim, Germany; 2. Med. Klinik II, Universität Leipzig, Germany

Background: During the last few years the acute phase reaction of the ex-
ocrine pancreas was characterized (Digestion 1994;42(5)). After induction of experimental pancreatitis so-called pancreatitis-associated proteins (PAP) are expressed in the gland within 2 hours and 7 days after induction of acute pancreatitis no PAP can be detected whereas at 2 days PAP amounts 2%
of secretory (0.4% of total cellular) protein. The following experiments were
designed in order to elucidate a potential protective effect of PAP in the
pancreas.
Methods: In 6 groups of rats (n = 72 animals) edematous pancreatitis
was induced by intraperitoneal injection of cerulein (2 x 40 μg/kg b.w.). In 6
control groups (n = 72 animals) only vehicle (NaCl) was used. After 2 hours, 2
days or 7 days pancreatitis was induced by retrograde infusion of Na-taurocholate (4%, 1 μl/g b.w.) into the pancreatic duct in all animals.
After further 6 hours, 3 days or 7 days the surviving rats were sacrificed and
tissues were taken for histological and biochemical measurements.
Results: After induction of the second pancreatitis, in animals with pre-
existing PAP expression (2 days after cerulein) survival was much better than
in all other groups (92% vs. 33%; p < 0.001). Preliminary results of the histo-
logical examinations showed, that rats with pre-existing PAP survived despite
a more severe degree of inflammation (21.3 s vs. 15.4 histological score-points;
p < 0.01).
Conclusion: A protective factor seems to be expressed in the pancreas
during the course of cerulein-pancreatitis. There is evidence that this protec-
tive factor is identical to PAP but further studies have to be performed to
prove this hypothesis.
V.K. was supported by grant Ke 347/3-2 from the DFG

Expression of Heatshockprotein GP96, a Tumor Rejection Antigen, by Gastrointestinal Tumors
Universität Mainz, Germany; II. Medizinische Klinik, Krankenhaus Nordwest, Frankfurt am Main, Germany; Dept of Biological Sciences, Fordham
University, Bronx, New York, USA

Heatshock protein gp96 was identified as a tumor rejection antigen on chem-
ically induced mouse tumors and was investigated to express the human
homologue of the gene by gastrointestinal tumors and the stimulation of
tumor reactive T cells by autologous tumor gp96. Methods: Expression of
gp96 was analyzed on protein level (Westernblot, immunohistochemistry) and
by RT-PCR. Purification of gp96 from formalin固定 tumor tissue was performed by sequential concanavalin A affinity chromatogra-
phy and Mono Q cationic exchange chromatography. T cells from tumor pa-
patients were cultured in presence of gp96 derived from autologous tumor cell
cells, a melanoma and a sarcoma cell line, and tested for recognition of the
autologous tumor cells by TNF release assays. Results: Gp96 was detected in
fresh tumor tissues and cell lines derived from colon, gastric and pancre-
atic cancer by Western blot and Northern blot analysis. Gp96 purified from
electrophoresis extracts of fresh gastric cancer and colon cancer revealed the same physio-
chemical properties as mouse gp96 binding to concanavalin A and to Mono Q
cationic exchange resins at pH 7 and a typical elution profile. Normal colon
malignomas possessed a strong expression of gp96 in contrast to the autologous
colon carcinomas in an immunohistochemical analysis of tissues from five pa-
tenfants. Experiments on the regulation of gp96 expression by interferons will
be reported. Tumor reactive class I and II restricted T cells could be expanded in
vitro by stimulation with gp96 from autologous tumors. Conclusions: Heat-
shock protein gp96 is expressed by human gastrointestinal tumors of differ-
ent origins and can be purified from fresh tumor tissues. Reduced expression of
gp96 by colon carcinomas may indicate a tumor escape mechanism. The
in vitro stimulation of tumor reactive T cells by tumor gp96 points to a spe-
cific immunostimulatory role of this molecule against human tumors, as it
was observed in mouse models. (Supported by DFG, SFB 311/C9)

736 Postmortem Ductography in Equivocal and Mild Chronic Alcoholic Pancreatitis
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Digestive Diseases, School of Medicine, Belgrade, Yugoslavia

This study presents morphometric analyses of the pancreatic ductal sys-
rem in equivocal and mild chronic alcoholic pancreatitis (CAP) based
on the history of alcohol intake, histological findings and results of CAP
by anatomic pancreatography. The study was based on 29 pancreatic necropsy
specimens, with the diagnosis of equivocal and mild chronic alcoholic pan-
creatitis (CAP) based on the history of alcohol intake, histological findings and
results of POST mortem ductography. CAP was subdivided into two groups
according to the Cambridge classification: the controls consisted of 132 normal pancreatic specimens. The mean cal-
iber/length of the main and the accessory pancreatic duct measured in the
CAP group were 2.8/18.9 and 1.63/6.6, respectively. The accessory
ducts in patients with chronic alcoholic pancreatitis (CAP) have a larger average
than that of the control group. In addition to the controls, significant differ-
ences were detected in the mode of basal pancreatic ductal union (separated orifices more frequent in CAP), and in the patency of the minor papilla (more frequent in controls). These two features seem to be closely related to the development of CAP

737 Molecular Biological Related Immunostaining of the DCC-Geneproduct in Gastrointestinal Tissues
I. Department of Internal Medicine, Johannes-Gutenberg-University, Germany

Purpose: The role of cellular adhesion molecules seems to be important for
progression and metastasis in tumorigenesis. The DCC (Deleted in Colo-
rectal Cancer)-Tumorsuppressor-Gen encodes for a homologue of the neural
cell adhesion molecule. As shown by recent studies, loss of heterozygos-
ity (LOH) for DCC and deletion of both alleles are frequent in advanced
stages of colorectal and other malignancies. Precise details about the protein
expression pattern are lacking still. The aim of this study is the correlation
between the DCC expression in gastrointestinal tissues and its molecular-
biological background.
Methods: 22 tissue samples (stomach, colorectum) with matched pairs of
normal and malignant areas have been immunostained with a specific anti-
DCC-Mab. DCC-RNA-expression has been analysed by RT-PCR. Detection
of genomic polymorphism and LOH were performed by VNTR- and RFLP-
producing PCR-assays like a PCR-based Codon 201-mutation-assay from
tissue- and PBL-DNA.
Results: Correlating staining localization, intensity and tumor grading, the
colorectal tissue data are as follows:

<table>
<thead>
<tr>
<th>Normal tissue</th>
<th>Adenoma</th>
<th>G1-Tumor</th>
<th>G2-Tumor</th>
<th>G3-Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>mucosal cells</td>
<td>80-100%</td>
<td>70-100%</td>
<td>80-100%</td>
<td>100%</td>
</tr>
<tr>
<td>60-100%</td>
<td>70-100%</td>
<td>80-100%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>tumour-cells</td>
<td></td>
<td>+ stained</td>
<td>+ stained</td>
<td>+ stained</td>
</tr>
<tr>
<td>tumour-cells</td>
<td></td>
<td>+ stained</td>
<td>+ stained</td>
<td>+ stained</td>
</tr>
<tr>
<td>(crypts and surf.)</td>
<td>+ stained</td>
<td>+ stained</td>
<td>+ stained</td>
<td>+ stained</td>
</tr>
<tr>
<td>(crypts and surf.)</td>
<td>+ stained</td>
<td>+ stained</td>
<td>+ stained</td>
<td>+ stained</td>
</tr>
</tbody>
</table>

In one adenoma, a point mutation at codon 201 was detectable; in a G1-
case down regulated DCC-RNA-expression could be demonstrated by spe-
cific RT-PCR-hybridization. In gastric tissue samples neither in normal nor in
malignant areas DCC-expression was evident. DCC-LOH was found in one
case.
Conclusions: The DCC-expression has been analyzed in a large panel of
gastrointestinal tissue samples. Comparison of the staining localization and
the tumor grading and different genetic alterations have been found consis-
tently.

739 Fasting Gallbladder Volume and Emptying is Different in Patients with Symptomatic vs. Asymptomatic Stones
B. Brand, J. Groth, E.F. Stange. Division of Gastroenterology, Department of Internal Medicine, University of Lübeck, Germany

Purpose: Patients with cholecystolithiasis are known to have a large fast-
ingallbladder GB-volume and a reduced ejection fraction compared with
controls. This motility defect is established as one of the pathogenic factors
gallstone disease. To determine whether the presence of hypomotility is
expressed with the presence or absence of biliary colics, we studied fasting
GB-volume in symptomatic patients, patients with asymptomatic gallbladder
stones and controls. In addition, contractility was tested using a standard
liquid test meal (250 ml Nutrodip).

Methods: GB-volumes were obtained sonographically and calculated by
the rotation ellipsoid method. Biliary related pain was defined by the pres-
ene of 3/8 typical symptoms, pain description: 1: colic-like, 2: in the upper right abdomen, 3: radiation, 4: paincore >4/10 on an analog scale, 5: lasting >30 min, 6: duodenal; 7: at night, 8: precise definition of the time of onset and pain relief. Patients with asymptomatic stools were defined by the absence of all typical symptoms. Patients without biliary disease served as controls. Patients with unspecific complaint or <3 typical symptoms were defined as unspecific group. Exclusion criteria were episodes or present signs of cholecystitis, cholecystostomy or pancreatitis.

**Results:** Patients with symptomatic gallstones have a significantly shorter fasting period (p < 0.05) compared with asymptomatic patients and have a significantly higher biliary prandial volume (p < 0.006) compared with asymptomatic patients. GB contractility also differs between group 1 and 2 (p < 0.05). Patients are older in group 2 with a mean of 66 years compared to group 1 with 52 years. Distribution of sex is different in group 1 (77% female) vs. 2 (36% female) and 3 (38% female). Broca index, number and size of stones and wall thickness do not differ between the groups. (statistics: sex tester by Chi-square test, the other parameters tested by Kruskal-Wallis i-Way Anova)

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 43) x ± SD</td>
<td>(n = 11) x ± SD</td>
<td>(n = 21) x ± SD</td>
<td>(n = 14) x ± SD</td>
</tr>
<tr>
<td>Fasting (ml)</td>
<td>26.4 ± 13.1</td>
<td>59.6 ± 42.4</td>
<td>30.0 ± 19.2</td>
</tr>
<tr>
<td>45 min (ml)</td>
<td>7.8 ± 5.6</td>
<td>47.4 ± 54</td>
<td>10.8 ± 9.8</td>
</tr>
<tr>
<td>Contrib. (%)</td>
<td>67.1 ± 25.5</td>
<td>33.9 ± 40.8</td>
<td>64.4 ± 17.0</td>
</tr>
</tbody>
</table>

**Conclusion:** Asymptomatic but not symptomatic cholecystolithiasis is associated with impaired gallbladder function.

**774 The Contribution of Cholesterogenesis to Biliary Cholesterol Secretion in Healthy Humans**

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**Purpose of the study:** The hepatic HMG-CoA-reductase is upregulated in obese subjects and patients with cholesterol gallstones while their biliary cholesterol saturation is increased. Possibly, the cholesterogenesis is a determinant of the biliary cholesterol secretion in humans. In this context, a study was undertaken to measure the fractional synthesis rate (FSR) of cholesterol in bile compared to that in plasma of healthy humans with an intact enterohepatic circulation. In contrast to older techniques using radiolabeled precursors of cholesterol the mass isotopomer distribution analysis (MIDA) using [13C]acetate allows the measurement of the absolute cholesterol synthesis rate (ASR) of the whole body under known cystolic precursor enrichments in a short term kinetic.

- **Methods:** Healthy volunteers (5 males/3 females) were infused intravenously with [1-13C]acetate for 15 h to label newly formed cholesterol. During this period samples of duodenal bile were collected hourly while an enteral formula was infused. Blood was taken every hour during the acetate infusion and in regular time intervals up to 57 h after its stop. The cholesterol of duodenal bile or plasma was extracted by isopropanol and methanol/ether or acetone/ethanol, respectively. Thereafter, the free cholesterol was silylated and the distribution of the masses 368 (M + 0), 369 (M + 1), 370 (M + 2), 371 (M + 3) and 372 (M + 4) was analysed by gas chromatography-mass spectrometry.

**Results:** After 6 hours the [13C]label of the cystolic acid pool remained constant. At the end of the acetate infusion (15 h) the FSR reached 4.3% in the free (p < 0.05) and the total cholesterol fraction, which represents a compartment of the rapidly exchangeable cholesterol pool. The decay rate of this compartment amounted to 1.4%. From these values an ASR of 9.1 mg/kg/day was derived. Compared with the plasma, the FSR of cholesterol was higher in the bile during the whole infusion experiment and reached 5.3% after 15 h. Finally, the FSR of plasma cholesterol correlated with that of biliary cholesterol (r = 0.91; p < 0.01) in healthy humans.

**Conclusion:** In humans newly synthesized cholesterol is secreted with a higher preference in bile than in plasma. However, its contribution to biliary cholesterol is small.

**775 Gallbladder Stones in Children without Hemolytic Disease**

N. Radlović, R. Lukav, V. Perićić, G. Gribić, Z. Krestić, D. Šekepanović, J. Šadaković, Ž. Smoljančić, V. Lekl. *University Children's Hospital, Belgrade, Yugoslavia*

Over a 7-year period symptomatic cholelithiasis without hemolytic disease was diagnosed in 8 children (6 female and 2 male, aged 7.7 – 11.8 years (x = 12.18). In 7 cases cholelithiasis was manifested by recurrent abdominal pain located in the right upper quadrant (4) or epigastrium (3), and only in one case by acute abdominal pain under the right costal margin. Bilirubin of stools was associated with nausea in 7, vomiting in 6 and paor with sweating in 4 patients. In only 3 children their complaints were provoked by a fatty meal. In 5 patients cholelithiasis was characterized among close relatives, in 3 mothers, in one father and in one grandmother. Four of 8 patients were Rh negative. None of the children had either clinical or laboratory parameters indicating cholecystitis, cholecystostomy or pancreatitis. The diagnosis of cholelithiasis was made by ultrasonography, and in 5 patients it was confirmed by cholecystography. In all cases gallstones were radio-lucent; cholesterol or mixed; multiple in 6 and solitary in 2. All patients were treated surgically with cholecystectomy and all underwent intraoperative cholangiography. Biliary anomaly was found in 4.8 patients; in 2 curved gall bladder and also in 2 twisted cystic duct, while in 3 patients the cause of cholecystitis remained unclear. All patients have been under check-up for 1.37–6.92 years (x = 3.01) and all are still without complaints.

**776 Stent Implantation for Palliation of Malignant Gastric Stenosis**

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**Background:** Insertion of selfexpanding metal stents is well established in palliation of biliary and esophageal malignant stenoses. We evaluated the therapeutic effect of metal stent placement in 3 consecutive patients with advanced gastric cancer and intestinal obstruction following nonsurgical cure.

**Methods:** 2 women and 1 man aged 51, 68 and 84 years presented with symptoms of upper gastrointestinal obstruction. All 3 patients had preceding noncurative resection of gastric cancer. Site of malignant stricture was esophageojunostomy in 1 case and gastrojejunoostomy in 2 cases. Stenosis extended to 4, 5 and 12 cm of length. We used wallstents (Medtronic S.A., Lausanne, CH, 9 cm length, 14 mm diameter) for recanalisation of the 2 short strictures and a 15 cm selfexpanding metal stent (ultraflex, Boston Scientific, USA, 18 mm diameter) for the long stricture. Correct stent placement was achieved in all 3 cases without any complications and enabled passage of the endoscope into the jejunal limb.

**Results:** All patients immediately were able to resume oral nutrition. Nausea and vomiting ceased. The recanalisation or stent placement even after some trials of balloon dilatation. Implantation of a second stent (wallstent) was required in this case. Stent occlusion occurred in 2 patients due to tumor ingrowth and could successfully be treated by laser dilatation. Survival time was 11 weeks in 1 patient and 26 weeks in 2 patients, presumably due to a tumor infiltration in the jejunum in both cases. The 84 year old patient died 2 weeks after stent placement by means of untreatable mechanical ileus due to a second tumor fixation of colon.

**Conclusion:** Insertion of a selfexpanding metal stent provides high effective palliation in isolated cases with advanced gastric cancer following non-surgical surgery. Complications are related to malnutrition of different types of stents and to tumor ingrowth and obstruction requiring reinterventions in particular cases. Further exercises are warranted to find out which group of patients and which types of metal stents ensure most effective long-term palliation with this new method.
751 The Effect of Tile Eradication of Helicobacter pylori on Duodenal Ulcer Healing and Ulcer Relapse. A Randomized Controlled Study
T. Shirato, M. Ikeda, H. Murayama, K. Maeda, M. S. N. O. K. Oh, Y. Nakayama. Department of Internal Medicine 1 and the Department of Pathology 1, Fukushu Univ., Fukuoka, Japan

Object: The aim of this study was to investigate the effect of the eradication of Helicobacter pylori (HP) on the healing and relapse of duodenal ulcers (DU).

Design: Treatment with 400 mg cimetidine, twice daily alone (cimetidine group) versus 6 or 10 weeks of the same treatment and 2 weeks from the 8th day to the 21st day of treatment with 300 mg amoxicillin granule three times daily and 250 mg metronidazole three times daily (double therapy group).

Method: Fifty patients with an active DU and HP infection were randomly allocated into the two treatment groups and 42 patients completed the study. After confirming that the ulcer had completely healed, all patients were followed up for 1 year after treatment with tegafur, a mucosal protective agent not affecting HP. 50 mg three times daily.

Result: The healing rates at 6 weeks were 90% in the cimetidine group and 95% in the double therapy group, and the difference was not significant. HP eradication occurred in 0% of the patients treated with cimetidine alone and in 73% of the patients treated with the double therapy (P = 0.004).

The cumulative relapse rates at 6 months were 64% in the cimetidine group and 11% in the double therapy group (P = 0.0001). In the double therapy group, cumulative relapse rates at 6 months were 99% (24%) in patients in whom HP had persisted and 0% (0/14) in patients in whom HP had been eradicated (P = 0.04). Moreover, eradication significantly improved both the grade of gastritis and the quality of ulcer scarring. Conclusions: The eradication of HP markedly decreases the relapse rate in DU patients, however it was not found to affect the acute healing process in cases simultaneously treated with cimetidine.

753 Clinical Features of Primary Gastric Non-Hodgkin’s Lymphoma (pGHNHL) of the MALT, Results of the Prospective German-Austrian Multicenter Trial
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pGHNHL of the MALT is a rare tumor in western countries, where it accounts for about 1% to 5% of all gastric malignancies. In 1993 we initiated a prospective multicenter trial. We here present the clinical features of the first 91 patients from the ongoing study. There were 35 female and 56 male patients (mean age 59 years, range 29 to 74 years). Diagnostic work-up was done by case history and medical examination, endoscopy and endoscopic ultrasound of the upper GI tract, biopsy specimens assay, and by histopathological analysis of tumor resection material. Patients were staged according to the Musshoff modification of the Ann Arbor classification.

In 21/91 cases (23%) a low-grade NHL was diagnosed, whereas 70/91 cases (77%) revealed a high-grade NHL. Of these 15 showed low-grade components (secondary high grade NHL), and 55 were primary high-grade NHL. 17 of all lymphoma were staged E112 (19%), 26 E121 (29%), 32 E121 (35%) and 6 E121 (7%). Stage E121 and EV with multifocal manifestation of the lymphoma were found in 6/17 (35%) and 6 (7%) cases of pGHNHL. The diagnosis of pGHNHL in the upper GI tract was as follows: gastric cancer 57 (71%), antrum 59 (83%), fundus 9 (13%) and pyloric region 7 (7%). 5 lymphoma (5%) were localized in the duodenum, and 11 (11%) extended to the esophagus. The endoscopic appearance of the lymphoma showed evidence of a low-grade component in 29 (31%) and 30 (32%) cases, respectively. 34 (38%) showed an ulcerative infiltrating behavior, and 14 (15%) lymphoma revealed diffuse infiltration of the gastric mucosa.

Predominant clinical symptoms reported were abdominal pain (80%), anorexia (39%) and nausea (37%), followed by weight loss (35%), vomiting (19%) and gastrointestinal bleeding (19%). In 13% of all cases constipation occurred, 6% of the patients suffered from diarrhea.

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754 Prevalence of Helicobacter pylori Infection in Primary Gastric Lymphoma of the Malt: There is a Difference Between Low Grade and High Grade Lymphoma
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There is increasing evidence from epidemiological, morphological and molecular studies for a pathogenetic role of Helicobacter pylori (HP) in primary gastric lymphoma of the Mucosa-Associated-Lymphoid-Tissue (MALT). We studied the prevalence of HP-infection in 133 consecutive patients with histologically proven primary gastric lymphoma. The lymphomas were typed according to the MALT-classification (Issaescu PG, 1987) on the basis of endoscopic-biopsic and/or surgical resection material. There were 43 low-grade B-cell lymphoma (pG-1), 17 high-grade B-cell lymphoma (pG-2), 17 high-grade T-cell lymphoma (pG-3), and 13 primary high-grade lymphoma (without low-grade component, group III). HP-infection was assessed using urease test and histology.

Results:

<table>
<thead>
<tr>
<th>Group</th>
<th>Non-infection</th>
<th>Infection</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(n = 4)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td></td>
<td>+ Prazosin</td>
<td>+ Vagotony</td>
</tr>
</tbody>
</table>

Ulc. index (%): 9.4 ± 2.2 16.6 ± 2.1 1.5 ± 0.7 1.1 ± 0.6

(mean ± SEM). Ulcer index: ulcer area/ground area, *p < 0.05 (ANOVA)

[Conclusions] Acute cerebral ischemia impairs gastric mucosal integrity by reducing gastric mucosal blood flow through the activation of vagal adrenergic pathway in SHR.

755 Photochemically Induced Ischemic Colitis in Rats

Background and purpose: Recent compelling clinical studies suggest that ischemic colitis is caused by: at least in part, thrombotic occlusion in micro- rather than large-vessels of colon. However, no animal model of colonic thrombosis has been established. Here we report a new model of photochemically-induced ischemic colitis in rats, using krypton laser. Materials and Methods: Fourteen male Wistar rats (weighting 250-400 g) were anesthetized with amobarbital (100 mg/kg, i.p.). Right femoral vein was cannulated for rose bengal infusion, and laparotomy in the mid-lower abdomen was performed. In a krypton laser beam (10 x 2 mm) operating at 566 nm (20 mW, 4 mm) was used to irradiate surface of the colon at the anal side of ileocecum. The photosensitizing dye rose bengal (20 mg/kg b.w.) was administered intravenously over 90 sec starting simultaneously with the 4-min laser irradiation. The rats were sacrificed at one day (n = 7), 3 days (n = 3) or immediately (n = 2) after the laser irradiation. Two control rats were given laser-irradiation of the colon without rose bengal infusion. The irradiated sites were evaluated histopathologically with hematoxylin eosine stain and phosphotungstic acid hematoxylin (PTAH) stain. Results: Although ulcerative change was not evident immediately after laser irradiation, all rats, examined at one or 3 days later, showed localized ulcers. Microscopic examination demonstrated ulceration with mucosal necrosis, ghost-like appearance and edematous thickening of the submucosal layer, which was compatible with ischemic colitis. In addition, most thrombi in micro-vessels were not stained by PTAH (i.e., lacking fibrin). Conclusions: Reproducible ulcerative lesions were produced by phototrombosis at the level of microcirculation in the colon. This thrombotic model will be useful to further investigate the pathophysiology of ischemic colitis.

757 Acute Cerebral Ischemia Impairs Gastric Mucosal Integrity Through the Activation of the Vagal Adrenergic System in Rats
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Purpose: Cerebral ischemia is often accompanied by acute gastric lesions. To clarify the underlying mechanism, the influence of acute ischemic insult to the brain, induced by bilateral carotid artery occlusion (BCO), on gastric mucosal integrity was examined in spontaneously hypertensive rats (SHR).

In our previous study, cerebral blood flow decreased to less than 10% and gastric mucosal blood flow decreased to about 60% of the control after 1 hour of BCO in SHR.

Method: Twenty-two female SHR, aged 5-8 months, weighing 170-200 g, were used. Four hours before the experiment, were anesthetized with amobarbital. After bilateral common carotid arteries were exposed, the stomach was gently pulled out through an incision on the abdominal wall and the pylorus was ligated. Prazosin (an α1-adrenoceptor antagonist, 10−5 M) was given intragastrically to five rats in order to examine the involvement of the sympathetic nervous system. Seven rats were vagotomized. The rats were subjected to 1-hour cerebral ischemia induced by BCO. 0.6N HCl (1 ml) was injected into the stomach. The gastric mucosa was examined macroscopically after 1 hour of recirculation.

Results:

- ULCER INDEX [%]: 4.9 ± 2.2 16.6 ± 2.1 1.5 ± 0.7 1.1 ± 0.6
(mean ± SEM). ULCER INDEX: ULCER AREA/GROUND AREA, *p < 0.05 (ANOVA)

[Conclusions] Acute cerebral ischemia impairs gastric mucosal integrity by reducing gastric mucosal blood flow through the activation of vagal adrenergic pathway in SHR.
761 Mutations in a Hydrophobic Part of the Core Nucleotide Sequence of Hepatitis C Virus from Patients with Primary Biliary Cirrhosis in China

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Preliminary epidemiological studies indicated that the inshore area of the Yangtze River has one of the highest incidences of hepatocellular carcinoma (HCC) in China, and that the incidences of antibodies to hepatitis C virus (HCV) and HCV RNA are low in most cases of chronic liver disease and HCC in this area. Therefore, to obtain information on the association between HCC and the chronic carrier state of HCV, we carried out this study. The aim of our study was to investigate the core region of the HCV genome and to determine whether the mutational pattern of this HCV isolate is in fact the same as that of other Chinese and Japanese HCV isolates, and a tendency for more nucleotide (nt) substitutions in the core gene in HCC patients than in those with chronic hepatitis. In addition, we showed that nt substitutions were unevenly scattered along the genome with a cluster of missense mutations and transversions in the second hydrophobic domain from the 5’ end of the core region. The rates of the occurrence of missense mutations for each nt change and transversions were greater in the clustering variable region (CVR) than in other core region.

These findings suggested that the mutations occurring in this region of the HCV genome may be responsible for a selective pressure from cytotoxic T lymphocytes and aflatoxin B1 in patients with chronic HCV infection during hepatocellular carcinogenesis.

762 Omeprazole Plus Amoxycillin in Helicobacter pylori Infection: Role of Gastric pH-metry in Predicting Successful Eradication

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Combined therapy with omeprazole (OM) and amoxycillin (AMOX) has been shown to produce high rates of H. pylori (Hp) eradication. The aim of this study was to evaluate whether an optimal threshold of gastric pH exists for Hp eradication. Methods: 37 consecutive patients (29 M, 8 F; 19-79 yrs.) with Hp positive peptic ulcers were treated with different daily doses (20 (1 pt), 40 (9 pts), 80 (18 pts) and 120 mg (9 pts) of OM plus AMOX (750 mg id) for 14 days. On day 5 intragastric (ig) 24 h-pH-metry was performed. Subsequently omeprazole monotherapy (20-40 mg daily) was given until ulcer healing occurred and then discontinued. After further 6 weeks endoscopy was repeated and Hp status assessed by biopsy (2 antrum, 2 corpus; HE is modified Giemsa staining). Statistical analysis: Wilcoxon test, discriminant analysis, receiver-operating-characteristic (ROC) analysis. Data are given as means ± SEM. Results: Hp eradication was achieved in 29/37 pts. The igm pH < 4 was 3 lower in patients with successful Hp eradication compared to the failure group (5.6 ± 1.9 vs 17.1 ± 4.9; p = 0.07). Similar but insignificant differences between both groups were seen in 9times with pH < 4.5 and 6 in pH < 4.11: 1.2 ± 2.6/2.3 = 8 vs 0.26; pH < 5: 19.1 ± 3.2/39.1 = 12, p = 0.02; pH > 6: 37.7 ± 4/5.9 = 13.1, p = 0.17 and median pH (6.3 ± 5.6: vs. 0.22). Patients with successful Hp eradication were significantly older than the failure group (57.7 ± 2.7 vs 36.5 ± 4.5 yrs, p = 0.0006). Although OM dose was lower in the failure group this difference was statistically not significant (p = 0.17). ROC analysis demonstrated that the threshold pH × time = 3 (p = 0.002) and advanced age (p = 0.0009) predict eradication whereas median pH resp. 9times with pH < 4.5 and 6 do not influence the success. ROC analysis showed that a threshold of 9% time pH < 3 defines best (false - 25%, true - 75%, sensitivity 79%, specificity 75%) successful Hp eradication. Conclusions: (1) Gastric pH-metry seems to be a useful tool in Hp eradication. The threshold time pH 3 seems to be an important factor for successful eradication. (2) Eradication therapy is in particular successful in old patients.

766 Predictive Value of LES-Pressure Parameters in Gastro- esophageal Reflux — Asymmetry is not the Bad Guy


Pathogenesis of gastro-esophageal reflux (GER) is of multifactorial origin. The aim of our study was to evaluate the importance of basal LES pressure in GER with respect to sphincter asymmetry. Methods: 34 consecutive patients (15/M,19 F; 40-70 yrs) with gastro-esophageal reflux disease (GERD) were prospectively followed by ambulatory 24 h-pH-metry. LESP was assessed by a catheter with four side holes orientated at the same level at 90°. It was drawn through the LES at 1 cm steps. At each level, minimum, maximum and mean LESP were measured. At expiration was assessed. At the level exhibiting maximum LESP we integrated the circumferential pressures to form a 2-dimensional ‘vector area’. At this level the 4 pressure values were plotted in ascending order. The angle of the regression line through these points represented LESP asymmetry. Statistical significance (correlation coefficients and linear discriminant analysis) was performed. Results: Maximum pressure (LESPM) was best in showing an inverse correlation (p < 0.01) with total reflux time (Pearson’s corr. coeff. −0.48); vector area (r = −0.43) and asymmetry (r = −0.38) exhibited weaker correlations (p < 0.05). Analysis of subgroups i.e. supine and upright refluxes gave no additional information. Discriminant analysis in GER patients as classified according to DeMeester criteria revealed that patients with a maximum LESP > 17 mmHg were unlikely to have GER. Due to the overlap between the 2 groups this prediction overestimated GER whereas the prediction ‘no GER’ was rather accurate. Inclusion of the overall sphincter length (LESL) improved the accuracy of this classification, while further stepwise inclusion of other parameters such as vector area and sphincter asymmetry did not. Table 1 shows the classification matrix.

Table 1: Predictive value of LESPM + LESL in GER

<table>
<thead>
<tr>
<th>Predicted</th>
<th>Actual</th>
<th>All</th>
<th>No refl</th>
<th>reflux</th>
</tr>
</thead>
<tbody>
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<td></td>
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</tr>
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<td></td>
<td></td>
<td>30</td>
<td>13</td>
<td>17</td>
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<tr>
<td></td>
<td>0.0001</td>
<td>3</td>
<td>3</td>
<td></td>
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<td></td>
<td></td>
<td>14</td>
<td>12</td>
<td></td>
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</table>

Conclusions: Sphincter asymmetry does not weaken sphincter strength as previously proposed, it is common in high LESP. High maximum LESP (> 17 mmHg) predicts sphincter competence. Since the location of maximum LESP cannot be predicted, multilumen catheters are necessary for proper assessment.

769 Interferon-alfa Treatment for Chronic Active Hepatitis B in Cirrhosis

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Between 1990 and 1994 18 patients (pts) with chronic active hepatitis B in cirrhosis have been treated with interferon alfa (Ifn). All pts have been negative for Delta-virus, HCV and HIV. At the start of Ifn 12 pts were class A (Child-Pugh), 6 pts. had class B cirrhosis. Before treatment 7 pts. were positive for Hbs-Ag and Hbe-Ag in blood tests (group I), 11 pts (group II) were Hbs-Ag positive but negative for Hbe-Ag. The transaminases (TAs) in both groups did not differ significantly (ALT-medium 94 UI). The median duration of Ifn-therapy was 6 months, the dose range from 2 to 5 MU, three times a week. Results: loss of Hbs-Ag and normalization of TAs was achieved in 5 pts. (71%) in group I. In group II 3 pts. (27%) came out with normal TAs and negative testing for HBV-DNA-PCR. Serious effects of Ifn with deterioration to Child’s class C Were seen in only 2 pts. with interruption of Ifn in 1 pt. both survived. In HBV-cirrhosis is well tolerated in compensated pts. with an astonishing high response rate in wild type disease.

772 Epithelial Cell Death in Crypts of Ulcerative Colitis

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Aim: to determine whether abnormal epithelial cell death in crypts precedes the inflammatory change in ulcerative colitis (UC). We examined colonic biopsy specimens by immunohistochemical and in situ staining for apoptosis. Methods: Colon biopsy samples were obtained from untreated UC patients (n = 30), from inflamed areas ([I] and from the adjacent uninvolved areas ([II], and from unaffacted areas of patients with colon polyposis (control n = 20) ([III]). All specimens were examined histologically by in situ nick and TdT staining and anti-Fas antibodies immunohistochemical staining. We tabulated the frequency of TdT-positive and -negative cells in the bottom two-thirds of crypts in 100 longitudinal sections. Electron microscopy was used to evaluate the morphology of UC. Results: We found that some epithelial cells in crypts of uninvolved, as well as involved, areas in UC showed positive for all three histochemical markers, whereas the markers were virtually absent in controls. It was found that the percentage of TdT-positive nuclei per crypt section in descending order was ([I], ([II] and ([III], while the number of nuclei per crypt section in descending order was ([III], ([II] and ([I]. Morphological features typical of apoptosis were confirmed in the crypts of uninvolved, as well as involved UC by the electron microscopy. These findings suggested that some epithelial cells in crypts of UC were lost by an abnormally high occurrence of apoptosis, causing a shortening in the depth of crypts, and that the inflammatory change in the mucosa was caused by the activation of apoptotic epithelial cells may thus be considered to be a causative factor in the onset of UC.

773 Characterization of the Autoantigens in Coeliac Disease

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Although it’s obviously that gliadin is involved in the pathogenesis of coeliac disease (cd), the molecular mechanisms are still unknown. Furthermore, cd
appears to be associated with intestinal T-cell lymphoma. The serum of untreated CD patients contains antibodies belonging to the IgG and IgA classes that react with the K-mercuric matrix (ECM) of normal human tissue. For the diagnosis of CD, antibodies to endomysium, reticulin and gladin are detected by indirect immunofluorescence or ELISA. Especially the IgA anti-endomysium antibody is highly sensitive and related to the active phases of CD. His disease target antigen is distinguishable histologically because they are not detectable by methods such as Western blotting.

In order to identify the ECM antigens of CD, we metabolically labelled HT 1080 human fibrosarcoma cells with 35S-methionine. The culture medium, as well as the cell lysate, were immunoprecipitated with IgA antibodies of patients with active CD.

In SDS-PAGE/autoradiography two proteins with an apparent Mr of 90 kDa (low-molecular) and Mr 300 kDa (released at the medium) were identified. Colloagglutinin treatment suggested the absence of collagenase sequences in both molecules and partial digestion with V8-protease shows two distinct peptide patterns. Sufficient quantities of the proteins are currently available for detailed information.

Conclusion: Using the mesenchymal human cell line HT 1080 and immunoprecipitation we identified two native autoantigens of coeliac disease.

Expression of p53 and Its Association with Nuclear DNA Ploidy Pattern and Clinicopathologic Findings in Colorectal Adenomas and Carcinomas

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Purposes: The association of p53, a tumor suppressor gene, with DNA ploidy pattern and clinicopathologic features, was assessed in colorectal adenomas and carcinoma.

Patients and Methods: This study included 77 patients who underwent endoscopic polypectomy or surgery for colorectal adenomas (14 patients) and carcinomas (63 patients) at Tokyo Medical School Hachiioji Medical Center between December 1993 and December 1995. The adenomas showed mild atypia in 4 patients, moderate atypia in 5, and severe atypia in 5. With carcinomas, invasion of bowel wall was “m” in 2, “sm” in 5, “mp” in 13, “ss” in 23, “s” in 15, and “si” in 5, and the clinicopathologic class, rated on dukes stage, was A in 16, B in 17, C in 20, and D in 10. p53 were detected by the RT-PCR-SSCP method. The quantity of DNA ploidy pattern was determined by flow cytometry.

Results: p53 were not detected in any of the colorectal adenomas, but they were detected in 53% (34/63) of the colorectal carcinomas. The rates variation of the bowel wall for “m” and “sm” carcinomas were 50% (12/24) and 80% (4/5), respectively. It was estimated that p53 may play a role in some processes involved in malignant transformation from adenoma to carcinoma. The point mutations occurred in exon 5 in 12 cases, exon 6 in 2, exon 7 in 5, and exon 8 in 15, with no association with any other factor. With the association with DNA ploidy pattern, the point mutations occurred in 35.5% (6/17) of diploidy and 72.7% (6/8) of aneuploidy. With clinicopathologic features, p53 occurred in 56.2% (9/16) of Dukes A, 64.7% (11/17) of Dukes B, 80.0% (12/15) of Dukes C, and 20.0% (2/10) of Dukes D. p53 were detected less frequently in patients having distant metastases, but they were not associated either with stage of the disease or with lymph node involvement. They were associated with progression in incidence of “mp” or more advanced. The association of p53 with recurrence and prognosis remained unchanged because of the shortness of follow-up.

Conclusion: 1) It was estimated that p53 have a role in the process of malignant transformation from adenoma to carcinoma. 2) p53 were associated more frequently with aneuploidy, indicating that p53 are associated with prognostic factors. 3) p53 were not associated with clinicopathologic factors. 4) Their association with relapse and prognosis remains to be studied.

Different Priming of Neutrophil Granulocytes for an Enhanced Respiratory Burst by Crohn’s Disease and Ulcerative Colitis Seras

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During active inflammatory bowel disease (IBD) the respiratory burst of neutrophil granulocytes has been shown impaired using isolated circulating neutrophils. The aim of this study was to examine the priming effect of homologous sera of patients with active IBD on normal neutrophil granulocytes.

Materials and Methods: The supernatant (O2 release) of normal neutrophils in response to N-formyl-methionyl-leucyl-phenylalanine (FMLP) has been investigated after incubation with sera of patients with active and inactive Crohn’s disease and ulcerative colitis. O2 release was measured using the superoxide dismutase inhibitable reduction of ferricytochrome c.

Results: Normal neutrophils cultured with sera of patients with inactive Crohn’s disease (n = 10) showed an enhanced O2 release (607.1 ± 218.2 nmol/60 min) when compared with normal neutrophils incubated with sera of normal controls (n = 10, 318.8 ± 86.5 nmol/60 min) or with active Crohn’s disease sera (n = 5, 481.0 ± 113.0 nmol/60 min). In contrast preincubation with sera of patients with active ulcerative colitis (n = 5) results into an increased O2 release (750.0 ± 258.0 nmol/60 min) when compared with normal neutrophils incubated with sera of patients with active ulcerative colitis (488.1 ± 115.1 nmol/60 min).

Conclusions: This present investigation shows that Crohn’s disease sera with quiescent disease activity are able to prime neutrophils for an enhanced respiratory burst and like Crohn’s disease sera with active disease activity.

The results indicate furthermore a different priming of neutrophils in quiescent Crohn’s disease and ulcerative colitis.

The Japanese Herbal Medicine “Sho-saiko-to” (TJ-9) Regulates Abnormal IL-4 and IFN-γ Production In Peripheral Blood Mononuclear Cells from Patients with Chronic Viral Liver Diseases

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“Sho-saiko-to” (TJ-9) is the most widely prescribed herbal medicine for treatment of chronic liver disease (CLD) patients in Japan. Previously, we reported (i) monoamine production capacity of CLD patients is significantly reduced and (ii) TJ-9 strongly induces production of cytokines such as IL-1β, TNF-α and IFN-γ in peripheral blood mononuclear cells (PBMC) in vitro. The current study investigated (i) IL-4 and IFN-γ production capacity in PBMC and effects of TJ-9 on their production, (ii) whereby 7 herb components of TJ-9 are responsible for the effects, and (iii) whether TJ-9 works directly on T lymphocytes or the effects appear indirectly through its effects on monocytes.

At first, either a stimulant, T-9, or each herb component (supplied from Tsumura, Tokyo) was added to the PBMC obtained from 20 CLD patients (type B8-C12) and 17 healthy volunteers, then incubated for 48 hours, and IL-4 and IFN-γ levels in the supernatant were measured using the ELISA method. In the next examination, a stimulant with T-9 or each herb component, or a stimulant alone, was used for the same measurements. As a control study, these measurements were repeated using only the T lymphocyte fraction of healthy volunteers.

As a result, lectin-induced IL-4 production and anti-CD3-induced IFN-γ production in PBMC of CLD patients were significantly higher than those of healthy persons (p < 0.01). On the other hand, T-9 and each herb component did not induce IL-4 or IFN-γ. By adding T-9, Con-A-induced IL-4 production and anti-CD3-induced IFN-γ production were suppressed by 35% and 55% on the average, respectively. This suppression was most remarkable when using scutellaria root on IL-4 and when using glycyrrhiza root and scutellaria root on IFN-γ. These were also confirmed in the tests using only the T lymphocyte fraction of healthy persons.

In CLD patients, IL-4 and IFN-γ productions were significantly increased compared to those of healthy persons. T-9 did not induce IL-4 and IFN-γ productions, and markedly suppressed overproduction of these cytokines in PBMC of CLD patients. This work shows that TJ-9, i.e., scutellaria root and glycyrrhiza root, has the potential to regulate functional abnormalities of T lymphocytes in CLD patients.

Mutations of the Hepatitis B Virus Genome in Patients with Fulminant Hepatitis B Infection

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The pathogenesis of fulminant Hepatitis B virus (HBV) infection is well not understood so far. Aim of this project is to investigate if there is an association between specific viral variants and a fulminant course of HBV infection.

Material and methods: The entire nucleotide sequence of the HBV genome was analysed from the serum of 9 patients with fulminant HBV infection. After isolation and amplification of HBV DNA by PCR the plus- and minus strand were directly sequenced.

Results: Analysis of the complete genome showed new, previously not described mutations in all regions of the virus. Most mutations were found within the enhancer II-core promoter region and within the core region. There were no identical mutations in the 9 patients examined. Moreover no mutation was identical with the previously described mutations in 2 patients with fulminant HBV infection (Ogata 1993, Hasegawa 1994). A precoex stop codon mutation at nucleotide position 1896 was found in 4 of 9 patients.

Conclusions: Due to these observations fulminant HBV infection is not caused by a single mutation, especially not by the precoex stop codon mutation at nucleotide position 1896 which previously has been associated with a fulminant disease course. Whether the newly described mutations after antigenicity, replication efficiency or infectivity of the virus and thus may cause a fulminant disease course will be further investigated.
We investigated effects of estradiol in vivo on hepatic fibrosis induced by dimethylthiourea (DMN) in rats. Methods: Animals received a single i.p. injection of DMN at a dose of 20-50 mg/kg wt (group D), and were treated with estradiol valerate (group E), timiclonor acetate (group T), or anti-estriol antibody (group A) for 2 weeks. After anesthesis, each liver was promptly removed and cut into small pieces, some pieces were used for histohemical study, other were for direct determination of collagen contents, and semiquantification of mRNA expression of type I and III procollagen, collagensetase (MMP-1) and collagenase inhibitor (TIMP-1) by RT-PCR, compared with beta actin gene expression. Results: In group D, hepatic fibrosis was induced mainly around the central veins and liver collagen contents rose in a DMN-dose dependent manner. However, in group E and T, hepatic fibrosis improved, and collagen contents were decreased, although group T showed higher serum ALT and lower levels of the body and liver weight than group E. The group A collagen contents were the highest in all groups. Group E expressed mRNA of MMP-1 and TIMP-1 at a lower level than group D. Conclusions: Estradiol suppressed the formation of collagen and gene expression of collagenase. These results and improved histological findings in fibrotic rats, suggesting that estradiol may accelerate collagen degradation.

Collagen Accumulation Before Histological Changes in Hepatic Fibrosis

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The mechanism of hepatic fibrosis remains obscure. In this study we established a simple and sensitive assay of collagen contents in fibrotic liver induced by dimethylthiourea (DMN) in rats, and examined immunohisto logically and genetically collagen expression.

Methods: Animals received a single i.p. injection of DMN at a dose of 20-50 mg/kg wt, and were anesthetized 3, 9, 14, and 42 days after the DMN injection. Each liver was promptly removed and cut into small pieces; some pieces were used for immunochemistical study, other were for direct determination of soluble collagen contents achieved by homogenizing, freezing-thawing and sonicating, and semiquantification of mRNA expression of type I and III procollagen, collagensetase (MMP-1) and collagenase inhibitor (TIMP-1) by RT-PCR, compared with beta actin gene expression.

Results: Serum ALT levels and poly I hydroxylase activities rapidly increased for 3 days and normalized after 9 days, whereas liver collagen contents gradually rose in a DMN-dose dependent manner and remained until 42 days. Type I and III collagen fibers were formed mainly in necrotic areas and portal regions, and increased after 9 days and persisted thereafter. However, collagen contents were significantly accumulated even in the liver before appearance of hepatic fibrosis. Gene expression of procollagen type I increased dose-dependently, but those of procollagen type III, MMP-1 and TIMP-1 were inhibited at a high liver weight.

Conclusions: Collagen accumulation appeared before histological changes in hepatic fibrosis, suggesting that collagen degradation system may play a role during liver fibrogenesis.

Cases of Esophageal Varices Complicated by Hepatocellular Carcinoma After Endoscopic Injection Botox

S. Okamura, N. Muguruma, S. Hibino, S. Hayashi, M. Yasuda, T. Yokoi, Y. Kakehashi, T. Okahisa, H. Shibata, S. Ito, M. Yano, I. Shimizu, S. Ito. Second Department of Internal Medicine, School of Medicine, The University of Tokushima, Tokushima, Japan

Following the recent spread of the use of endoscopic injection sclerotherapy (EIS), etc., the prognosis of esophageal varices has been improving. However, post-EIS hepatocellular carcinoma (HCC) is one of the important determinants of the prognosis of esophageal varices. We recently analyzed cases of esophageal varices which were complicated by HCC after EIS.

From the patients who underwent EIS at our department after 1987, 84 patients who satisfied the following requirements were selected for this study: (1) HCC was absent before the first EIS; and (2) patients who could be seen for 1 year after the last EIS. There were 56 males and 28 females, with ages ranging from 18 to 77 years (mean: 57.6 years). The underlying disease was liver cirrhosis in 75 cases, primary biliary cirrhosis in 6 and idiopathic portal hypertension in 3. The average follow up period after EIS was 3 years and 6 months.

During the follow-up period, HCC developed in 17 (20.2%) of the 84 cases. The incidence of recurrence of varices was significantly higher in the HCC complications of the non-HCC-complicated group. The survival rate was also significantly lower in the HCC-complicated group. Thus, the prognosis of esophageal varices after EIS was poor in cases complicated by HCC.
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mucosa. 0.5 ml of MMSC (100 mg/kg) or saline (as a control) was administered via the tail vein. 1 ml of 75% ethanol was administered intragastrically 15 min after MMSC or saline injection. Changes in mucosal blood volume (IHb) and mucosal blood oxygenation (ISO2) were measured using organ reflectance spectrophotometry (TS-200, Sumitomo electric Co., Japan) every 5 min from 15 min before MMSC injection to 30 min after ethanol administration.

Results: MMSC did not induce significant changes in IHb and ISO2 before ethanol administration. In control rats (n = 6), 75% ethanol decreased IHb by 7.1 ± 1.8% (Mean ± SEM) after 30 min and markedly decreased ISO2 by 30.2 ± 2.6%, indicating mucosal hypoxia. In MMSC pretreated rats (n = 8), the decrease in IHb was 9.1 ± 1.4%, significantly smaller compared with controls (p < 0.05). MMSC prevented the development of gastric mucosal lesions induced by ethanol.

Conclusions: 1) MMSC inhibited the gastric mucosal hypoxia induced by ethanol. 2) This action might be involved in the protective effect of MMSC on the gastric mucosal lesions.

805 Effect of Methotrexine Sulphonium Chloride on the Alcohol-Induced Gastric Mucosal Hypoxia in Rats
T. Ichiara, H. Mikami, K. Endo, M. Hirose, N. Sat0. Department of Gastroenterology, Juntendo University, Medical School, Tokyo, Japan

Methotrexine sulphonium chloride (MMSCC), a component of cabbage extract, has long been in clinical use as a mucosal protective drug. Its mechanism of action is unclear. Gastric mucosal blood flow plays a role in the pathogenesis and the healing of mucosal lesions. It is important to evaluate the effect of anti-ulcer drugs on gastric mucosal hemodynamics. Intragastic administration of ethanol in high concentrations is known to induce the disturbance of mucosal microcirculation. We thus studied the effect of MMSCC on the ethanol-induced changes in gastric mucosal hemodynamics in rats.

Methods: After anesthesia (pentobarbital, 3.5 mg/100 g) and laparotomy of fasted Sprague-Dawley male rats weighing 250 g, a small incision was made in the forearmost to position the optical probe on the gastric mucosa. 0.5 ml of MMSC (100 mg/kg) or saline (as a control) was administered via the tail vein. 1 ml of 75% ethanol or saline injection. Changes in mucosal blood volume (IHb) and mucosal blood oxygenation (ISO2) were measured using organ reflectance spectrophotometry (TS-200, Sumitomo electric Co., Japan) every 5 min from 15 min before MMSC injection to 30 min after ethanol administration. Results: MMSC did not induce significant changes in IHb and ISO2 before ethanol administration. In control rats (n = 6), 75% ethanol decreased IHb by 7.1 ± 1.8% (Mean ± SEM) after 30 min and markedly decreased ISO2 by 30.2 ± 2.6%, indicating mucosal hypoxia. In MMSC pretreated rats (n = 8), the decrease in IHb induced by ethanol was 8.1 ± 1.4%, similar to the controls, while the decrease in ISO2 induced by ethanol was 21.0 ± 2.7%, significantly smaller compared with controls (p < 0.05). MMSC prevented the development of gastric mucosal lesions induced by ethanol.

Conclusions: 1) MMSC inhibited the gastric mucosal hypoxia induced by ethanol. 2) This action might be involved in the protective effect of MMSC on the gastric mucosal lesions.

806 Gastrin-Releasing Peptide Receptor (GRP-R) Gene: Its Complete Structural Characterization and Basis of Its Altered Expression in Gastric Cancers

GRP alters many GI functions including stimulation of numerous GI hormones release, motility and normal tissue and tumor growth. Its actions are mediated by the GRP-R which is a member of the G-protein coupled 7 transmembrane superfamily. Similar to most GI hormones/neurotransmitters little is known about its gene structure or its regulation. We have recently provided preliminary information on its gene structure and evidence that it is regulated by dexamethasone (DEX) and CAMP. In the present study we have fully characterized its gene structure and investigated further its regulation by DEX and CAMP. The entire 3-exon gene spans 24 kilobases (Kb) and was isolated from a P1 bacteriophage genomic clone. All exon-intron boundaries and 3 Kb of 5 untranslated region (UTR) were sequenced. The intron lengths were 20 and 2 Kb, respectively. Two TATA boxes exist at 496 and 585 upstream of the ATG start codon. With ribonuclease protection assays and inverse PCR of DNA clones, mRNA initiation sites were localized to 58, 64, 68 and 76 bp downstream of the TATA box at +496. RNase protection analysis with Swiss 3T3 cells demonstrated that the 7 Kb and 3 Kb mRNA species had identical initiation but different polyadenylation sites. DEX (0.1 μM) treatment of Swiss 3T3 cells resulted in a decrease in mRNA levels at 24, 48 and 72 hrs, respectively, to 45, 60 and 35%, respectively. In contrast, treatment with 8 Br-CAMP (1 mM) increased mGR-R mRNA levels by 50, 50, and 85% at 24, 48, and 72 hrs, respectively. A similar time course was seen for changes in receptor number for both agents with binding studies. The half-time of GRP-R mRNA in control cells was 5.5 hrs and was unchanged by DEX or CAMP treatment. This study reports the full characterization of the molecular structure of mGR-R gene for the first time. Furthermore, it demonstrates that the expression of this gene can be transcriptionally regulated by CAMP and by dexamethasone.

807 Establishment of a Human Rectal Adenocarcinoid in Xenograft in Nude Mice

Adenocarcinoid which has both carcinoid and adenocarcinoma features is rare in the large bowel. This composite tumor has not well characterized, but prognosis of adenocarcinoid is generally worse than typical carcinoid. In order to improve the survival rate of patients of adenocarcinoids, the further characterisation is needed. At our hospital there were four cases of adenocarcinoids in the colorectal lesion. From a 58-year-old female patient of the rectal adenocarcinoid, we have succeeded xenografts in nude mice and tumorigenesis has been established for the first time in the world. Using immunohistochemical methods, we examined both primary tumor of the rectum and aggregations in nude mice. The results of immunohistochemical finding show below.
The expression of various hormonal peptides in the xenograft are almost same as primary tumor. But gastrin is expressed only in xenograft and inversely somatostatin only in primary tumor. These results suggest that adenocarcinoid has multi-neuroendocrine potential and the expression of each endocrine products may vary in the circumferential environment. The establishment of adenocarcinoid in xenograft provides an excellent model for study further the biological behavior of adenocarcinoid and the in vivo of chemotherapeutic agents on tumor growth.

**808**

The Natural History of Patients with Zollinger-Ellison Syndrome (ZES): Evidence for Two Different Clinical Forms, an Indolent and Aggressive Form

H.C. Weber, R.T. Jensen. NIH, Bethesda, MD, USA

Pancreatic gastrinomas are thought malignant in 60–90% of cases in older studies. As part of a long-term natural history study, we analyzed data from 185 patients with ZES followed prospectively at the NIH. Detailed anatomic data on 139 patients was available. Mean follow-up from onset to death was 12.4 yrs and from diagnosis to last follow-up 7.1 yrs. An equal number of patients had either a duodenal or pancreatic tumor only (29%). 19% of patients had liver metastases at initial evaluation and 5% developed liver metastases. The clinical course was correlated with other clinical parameters. Metastases to the liver occurred significantly more often with pancreatic than duodenal tumors (52% v. 5%, p < 0.00001) whereas the metastatic rate to lymph nodes was not different. Metastases to the liver correlated with the size of the tumor (r = 0.0001). 10-year survival in patients with liver metastases was 30% and without liver metastases was 96%. Survival of patients with liver metastases (p < 0.00001) but not with lymph node metastases, was shortened. Analysis of the survival data demonstrated in a quarter of patients the tumor had an aggressive clinical course whereas in 75% the gastrinoma pursues an indolent clinical course. The aggressive course is associated with the presence of liver metastases on presentation, a poor survival, absence of MEN-1, a large primary (>3 cm) tumor which is predominantly located in the pancreas (92%), female gender (67%), markedly elevated gastrin levels (mean=5100 pg/ml), and a shorter disease duration. Correlation with results of flow cytometry demonstrate the aggressive clinical course is associated with a high phase percentage, a low percentage of metastasized neoplastic aneurysm and a high multiple stem line aneurysm pattern compared to those whose disease pursued an indolent course. With earlier diagnosis of this disease, this study provides for the first time parameters that may be used to predict the course. However, to provide optimal therapy it will be important to identify additional clinical characteristics and tests that can predict the clinical behavior reliably in an individual patient.

**809**

The Safety of Dual Therapy with Clarithromycin and Omeprazole in the Treatment of Patients with Duodenal Ulcer Disease Associated with *H. pylori* Infection

C. Olson, M. DeBartolo, R. Hippensteel, J.C. Craft. Abbott Laboratories, Abbott Park, IL, USA

Clarithromycin (CL) was administered in combination with omeprazole (OM) in four large, well-controlled studies (two European and two U.S.) to assess the safety and efficacy of this combination in the eradication of *H. pylori* from the gastric mucosa and prevention of duodenal ulcer recurrence. A total of 346 patients received CL 500 mg TI D and OM 40 mg QD for the first 14 days, followed by OM 40 mg QD (in one study) or 20 mg QD (in three studies) for an additional 14 days. Most frequently reported adverse events (excluding taste perversion)

<table>
<thead>
<tr>
<th>Event</th>
<th>All adverse events</th>
<th>Excluding concurrent conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea</td>
<td>18 (5%)</td>
<td>11 (3%)</td>
</tr>
<tr>
<td>Headache</td>
<td>16 (5%)</td>
<td>6 (2%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>15 (4%)</td>
<td>12 (3%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>12 (3%)</td>
<td>5 (1%)</td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td>11 (3%)</td>
<td>8 (2%)</td>
</tr>
<tr>
<td>Infection</td>
<td>9 (3%)</td>
<td>1 (&lt;1%)</td>
</tr>
<tr>
<td>Total</td>
<td>142 (41%)</td>
<td>74 (21%)</td>
</tr>
</tbody>
</table>

*Number of patients reporting each adverse event.*

<table>
<thead>
<tr>
<th>System</th>
<th>All adverse events</th>
<th>Excluding concurrent conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute gastritis</td>
<td>5 (1%)</td>
<td>4 (1%)</td>
</tr>
<tr>
<td>Taste perversion</td>
<td>1 (&lt;1%)</td>
<td>1 (&lt;1%)</td>
</tr>
</tbody>
</table>

Taste perversion, an adverse event commonly observed throughout the development of CL, was reported by 54 of the 346 patients (CL 16%). The percentage of CL + OM treated patients reporting adverse events in the U.S. studies (49%, 81/164) and European studies (41%, 75/182) were statistically similar (p = 0.131).

Twelve (13%) of the 346 patients were prematurely terminated from study treatment due to adverse events. For each laboratory parameter, less than one percent (<1%) of the patients had laboratory values considered possibly insufficient per criteria developed by Abbott Laboratories. Overall, clarithromycin 500 mg TI D in combination with omeprazole 40 mg QD is safe and well tolerated.

**812**

A Comparison of Culture, Histology, and 13C-Urea Breath Tests for Detection of *H. pylori*

N. Siepman, S. Cox, C. Olson. Abbott Laboratories, Abbott Park, IL, USA

Three diagnostic techniques for detecting *H. pylori* were employed in four large, well-controlled, treatment studies. Cultures (Cx), histology (Hx), and 13C-Urea breath tests (UBT) were performed in 500 mg CL and OUD ulcers and the pretreatment and 4–6 week follow-up results are displayed below:

- Total: 53 / 775 / 828 / 245 / 380 / 625

The sensitivities for UBT and Cx using Hx as the gold standard were 91% and 83% at pretreatment and 89% and 82% at 4–6 weeks respectively.

The US and European results were similar when comparing Cx versus Hx; however, the UBT was considerably more sensitive in the European studies than in the US (98% compared to 86% in the US pretreatment).

The results from these studies suggest that Hx was the most sensitive test for detecting *H. pylori* Cx is useful for verification positive results but is limited because of the number of false-negatives due to the asymmetric distribution of *H. pylori* in the mucosa and the difficulty in transporting and culturing the organism. In UBT was less successful in patients with negative ulcers. No false-negatives in the US studies indicates the need for standardization.

**814**

Primary Susceptibility of *H. pylori* to Clarithromycin Compared to Metronidazole in Patients with Duodenal Ulcers Associated with *H. pylori* Infection

C. Olson, A. Edmonds. Abbott Laboratories, Abbott Park, IL, USA

Triple therapy regimens containing clarithromycin (CL) and/or metronidazole (MET) have been used for the treatment of *H. pylori* (Hp) in patients with duodenal ulcer disease. Therefore, it is of interest to examine Hp susceptibility to CL and MET. Data from four large, well-controlled studies (one conducted in the UK, one in Europe, and two in the US) were analyzed. Pretreatment culture data were used, and susceptibility was assessed using MIC results from agar dilution. CL MICs were interpreted as: susceptible (S) ≤3 mcg/mL; intermediate (I) 4 mcg/mL; resistant (R) >8 mcg/mL. For MIC, MET were interpreted as: S ≤8 mcg/mL; I 16 mcg/mL; R ≥32 mcg/mL. Pretreatment Hp susceptibility results are presented below:

<table>
<thead>
<tr>
<th>Total strains</th>
<th>Clarithromycin</th>
<th>Metronidazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>S I R</td>
<td>S I R</td>
<td>S I R</td>
</tr>
<tr>
<td>Overall</td>
<td>626 604 3</td>
<td>19 489 55 82</td>
</tr>
<tr>
<td>UK Study</td>
<td>83</td>
<td>0 81 6 5 17</td>
</tr>
<tr>
<td>European Study</td>
<td>152</td>
<td>150 0 2 2 23</td>
</tr>
<tr>
<td>US Studies</td>
<td>391</td>
<td>371 3 17 301 48 42</td>
</tr>
</tbody>
</table>

Primary resistance to CL was low. Overall, only 3% of the patients had Hp resistant to CL prior to enrollment. In contrast, resistance to MET was observed in 13% of patients. More specifically, resistance to MET in the UK and Europe (20% and 15%, respectively) was markedly higher than CL resistance in the UK and Europe (0% and 1%, respectively). Though less striking in the US studies, a notable difference between the MET (11%) and CL (4%) resistance was evident.

In addition, due to the large number of intermediately susceptible MET isolates, overall susceptibility to CL was much higher than for MET (95% vs. 77%, respectively). Thus, while primary resistance of *H. pylori* to clarithromycin is minimal, metronidazole resistance is higher and could limit its usefulness as an anti-Hp agent.

**816**

Clarithromycin Resistance in U.S. and European Patients Treated with Clarithromycin (CL) and Omeprazole (OM) for Duodenal Ulcer Disease Associated with *H. pylori* (HP) Infection

J.C. Craft, C. Olson, N. Siepman, A. Edmonds. Abbott Laboratories, Abbott Park, IL, USA

HP resistance to CL was examined using data from four large, well-controlled studies (two European and two U.S.). A total of 346 patients received CL 500 mg TI D and OM 40 mg QD for the first 14 days, followed by OM 40 mg QD (in one study) or 20 mg QD (in three studies) for an additional 14 days. Culture (Cx) was performed pre-RX and post-RX, and resistance to CL was assessed using primary and MIC results. Disk diffusion data were also collected but were only used in the analysis if MIC results were unavailable. CL MICs were interpreted as: susceptible (S) ≤2 mcg/mL; intermediate (I) 4 mcg/mL;
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resistant (R) ≥ 8 mcg/mL. Zone size for CL was interpreted as: S ≥ 15 mm; 11.12-14 mm; R ≥ 11 mm. Pre- and post-Rx susceptibility data for patients in whom HP was isolated pre-Rx are presented below:

<table>
<thead>
<tr>
<th>Pre-Rx</th>
<th>S</th>
<th>I</th>
<th>R</th>
<th>No isolate</th>
<th>Cx not done</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>U.S.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td>4</td>
<td>5</td>
<td>17</td>
<td>81</td>
<td>19</td>
<td>126</td>
</tr>
<tr>
<td>Resistant</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td><strong>Europe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>77</td>
<td>18</td>
<td>109</td>
</tr>
<tr>
<td>Resistant</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*If more than one post-Rx result was obtained for a patient, the most conservative one (i.e., resistant) was used in the analysis.

Pre-Rx resistance to CL was low. In U.S. studies, only 4% of patients (5/126) with pre-Rx susceptibility data had resistant isolates, whereas none of the 109 patients in the European studies was resistant pre-Rx. Among patients with susceptible pre-Rx isolates, development of resistance 4-6 weeks post-Rx occurred in 17 of 126 patients (13%) in the U.S. studies and 7 of 109 patients (6%) in the European trials. Thus, resistance patterns differed slightly between the U.S. and European studies.

**819**

A Comparison of Biopsy Specimens from the Antrum and Corpus for Culture of *H. pylori*

N. Siepman, R. Green, G. Ayinlian. Abbott Laboratories, Abbott Park, IL

Four well-controlled treatment studies in patients with duodenal ulcers and *H. pylori* infection were conducted, and the *H. pylori* culture results for the antrum and corpus sites were compared. Antrum and one corpus biopsies were taken at each endoscopy. The pretreatment and 4-6 week follow-up results are displayed below:

<table>
<thead>
<tr>
<th>Pretreatment Corpus</th>
<th>4-6 Week follow-up corpus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg neg</td>
<td>Avg pos</td>
</tr>
<tr>
<td>Antrum sample # 1</td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>200</td>
</tr>
<tr>
<td>positive</td>
<td>113</td>
</tr>
<tr>
<td>total</td>
<td>313</td>
</tr>
<tr>
<td>Antrum sample # 2</td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>190</td>
</tr>
<tr>
<td>positive</td>
<td>112</td>
</tr>
<tr>
<td>total</td>
<td>302</td>
</tr>
</tbody>
</table>

Table includes all patients with both DU and HP pretreatment who had the appropriate post-Rx visit.

**821**

Dual Therapy with Clarithromycin and Omeprazole Delays Ulcer Recurrence Despite Persistence of *H. pylori* Following Treatment

N. Siepman, C. Olson, A. Edmonds, G. Ayinlian. Abbott Laboratories, Abbott Park, IL, USA

**Background:** Numerous studies have documented the relationship between *H. pylori* (Hp) and peptic ulcer disease, demonstrating that treatment with an antibiotic in addition to anti- ulcer medications can be used in DU patients for healing ulcers, eradicating Hp, and preventing ulcer recurrence, thereby curing the patient of ulcer disease. But what happens if an antibiotic is used and Hp is not eradicated? It is of interest to determine whether the addition of an antibiotic can have a positive impact on the clinical outcome in patients in whom Hp was not eradicated.

**Methods:** Data from two well-controlled European trials were examined to determine if patients who remained Hp positive after dual therapy with clarithromycin (CL) and omeprazole (OM) had better clinical efficacy results than patients who received OM alone. A total of 182 patients received CL 500 mg TID and OM 40 mg OD for 14 days, followed by OM 40 mg OD (in one study) or 20 mg OD (in the other study) for an additional 14 days. An additional 187 patients received OM monotherapy. Endoscopy (including culture and histology) for antrum and corpus, and 13C-UBT were performed prior to treatment (Rx), post-Rx, and at follow-up (4-6 weeks and 6 months after Rx). 78% of CL + OM patients and 3% of OM patients showed eradication of Hp 4-6 weeks following Rx. The 36 CL + OM and 162 OM patients who remained Hp positive are used in this analysis.

**Results:** Among patients who remained Hp positive following Rx, CL + OM patients demonstrated significantly lower ulcer recurrence than OM patients (6/29 (19%) and 77/148 (52%), respectively, p < 0.02). In addition, CL + OM patients had a significantly higher resolution/improvement of epigastic pain and burning 6-months after Rx than OM patients (22/24 (92%) and 83/121 (69%), respectively). Chronic gastritis variables showed similar patterns, with CL + OM patients demonstrating higher resolutions of inflammation and atrophy, and a lower incidence of ulcers in patients who received OM alone. Thus, despite failing to eradicate Hp in some patients, Rx with CL + OM was still more effective than OM alone in preventing DU recurrence.

**822**

Effect of Smoking on *H. pylori* Eradication and Duodenal Ulcer (DU) Recurrence in Patients Receiving Dual Therapy with Clarithromycin (CL) in Combination with Omeprazole (OM)

M. De Bartolo, R. Reitmayer, C. Olson, A. Edmonds. Abbott Laboratories, Abbott Park, IL, USA

**Background:** Several studies have suggested that smoking affects the eradication of HP for patients treated with OM and amoxicillin (AM). Dual therapy with OM and AM has shown, in one study, a lower eradication rate in smokers (21%) than in non-smokers (52%). Another dual therapy, CL and OM, has also shown to be effective for eradication of HP. Therefore, it is of interest to examine whether HP eradication using CL + OM is affected by smoking. Methods: Patients from randomized, double-blind, multi-center studies who received CL 500 mg TID in combination with OM 40 mg QD for 2 weeks (days 1-14), followed by OM 40 mg QD (in one study) or OM 20 mg QD (in three other studies) for an additional 2 weeks (days 15-28), were analyzed by their smoking status for the duration of the study and post- eradication of DU recurrence. Patients with endoscopically verified DU and evidence of HP pretreatment were enrolled. DU was assessed by endoscopy and HP was assessed by culture, histology, and 13C-UBT. Results: 318 patients with DU and confirmed HP pretreatment were evaluated (mean age 48 yrs, mean DU size 9.5 mm).

**Table**: Smoking status, Ulcer healing post-Rx, HP eradication at Rx, and Ulcer recurrence at Rx.

<table>
<thead>
<tr>
<th>Smokers</th>
<th>Ulcer healing at Rx</th>
<th>HP eradication at Rx</th>
<th>Ulcer recurrence at Rx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td>96% (147/154)</td>
<td>71% (104/146)</td>
<td>22% (26/118)</td>
</tr>
<tr>
<td>Non-Smokers</td>
<td>94% (128/136)</td>
<td>77% (106/138)</td>
<td>16% (19/117)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.59</td>
<td>0.48</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Table includes all patients with both DU and HP pretreatment who had the appropriate post-Rx visit.

**824**

Treatment with Hydrocortisone Activates Gastric Mucosal Genes Encoding for EGF, bFGF and FGF Receptor — The Molecular Basis for Its Ulcer Healing Action

A. Tanawski, K.J. Wahlstrom, T.H. Nguyen, I.J. Sarfah. DVA Medical Center, Long Beach, and the University of California, Irvine, CA

Previous studies (Gut 1994; 35: 896-904) demonstrated that the antacid-hydrocortisone (HTAL) Tildac accelerates gastric ulcer healing and provides better quality of mucosal restoration than omeprazole (OME). However, the mechanisms of HTAL’s induced ulcer healing are not clear. Since growth factors promote cell proliferation, re-epithelialization, angiogenesis and ulcer healing, we studied whether HTAL and/or OME affect expression of genes encoding for EGF, bFGF, its receptors and/or their respective proteins in both normal and ulcerated gastric mucosa.

**Methods:** Rats with or without acetic acid-induced gastric ulcers (n = 64) received intragastrically 2 x daily 1 ml of either: A) Placebo (PLA), B) HTAL or C) OME 50 mg/kg for 7 or 14 days. Studies of gastric specimens: (1) Quantitative histology (2) Expression of EGF, bFGF, its receptor-1 and -2 (FGFR-1 and -2), and β-actin mRNAs was determined by reverse transcription polymerase chain reaction (RT/PCR) and quantified with a videomage system. (3) Gastric sections were immunostained with antibodies against EGF, bFGF, FGFR-1 and -2, and fluorescein isothiocyanate (FITC) and rhodamine isothiocyanate (TRITC) was assessed and quantified. 4) Western blotting. Results: In non-ulcerated gastric mucosa of PLA treated group, EGF expression was minimal, while bFGF and its FGFR-1 and -2 showed moderate levels. Gastric ulcer mucosa HTAL treatment enhanced expression of EGF, bFGF and FGFR-1 by 50% ± 4, 70% ± 5% and 80% ± 6% (p < 0.01). OME treatment reduced expression of EGF in ulcerated mucosa by 55 ± 3% (p < 0.01) and increased bFGF expression by 550 ± 40% (p < 0.001). Conclusions: 1) Treatment with HTAL activates genes for EGF, bFGF and FGFR-1 in normal and ulcerated gastric mucosa resulting in increased mucosal concentration of these growth factors. 2) Since EGF promotes growth of epithelial cells and bFGF growth of connective tissue and angiogenesis, the above actions of HTAL provide the mechanism for its ulcer healing action and improved (vs OME) quality of mucosal restoration.
High Frequency of Colonic Neoplasms in Patients with Duodenal Adenomas

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Purpose: To precise the prevalence of colorectal neoplasia in patients presenting with adenomatous polyps of the duodenum and of the ampulla of Vater.

Methods: Presence or absence of colonic neoplasia was retrospectively and prospectively assessed in 29 patients with adenomatous polyps of the duodenum. There were 1.7 amputary adenomas (AA) (8 with moderate dysplasia, 9 with severe dysplasia) and 12 duodenal adenomas at a site distinct from the papilla (DA) (7 with moderate and 5 with severe dysplasia). For each case, reports on endoscopy, surgery and pathology were obtained, or colonoscopy was performed after diagnosis of duodenal adenoma.

Results: Colonic neoplasms were present in 19 out of 29 patients (65%), in 7 out of 12 DA (58%) and in 12 out of 17 AA (70%). There were 7 patients with severe dysplasia or cancer of the colon or rectum (24%, 2 patients with DA and five patients with AA). Nine out of 29 patients (31%) had 3 or more adenomatous polyps. There was no difference in median age between patients with duodenal and colonic neoplasms, and patients with only duodenal neoplasia (59 and 56 years). However, patients with PA and colonic adenomas were slightly older than patients with PA and without colonic neoplasia, but without statistical significance (median age 66 versus 56 years).

Conclusion: Colonic neoplasms are present with a high frequency in patients with duodenal adenomas. Severe colonic dysplasia or cancer is also frequently present in such patients. Endoscopic examination of the colon should be performed in patients with duodenal adenomas, especially before aggressive treatment of these adenomas.

Clinical Significance of Hepatic Scintigraphy with 99mTc-DTPA-Galactosyl-Human Serum Albumin

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Objective: Hepatic scintigraphy using 99mTc-DTPA-galactosyl-human serum albumin (GSA) allows us to examine the amount and distribution of hepatic receptors (i.e., the total amount of functioning liver parenchyma)and is expected to be useful for evaluating the total capacity of residual liver cells (the hepatic reserve). The present study is important for diagnosing the extent of hepatic regeneration, but its adequate information is not always yielded by conventional methods. We compared the GSA-based evaluation methods with the indocyanine green (ICG) test in terms of their correlation to the histological activity index (HAI) score studied by Knodell et al.

Subjects and Methods: The subjects of this study comprised 31 patients with chronic liver disease. The ICG test was employed to determine its 15 minutes retention rate (ICGR15). Using GSA according to the compartment model, we could perform compartment analysis which allows separation of the maximal binding to ASGP receptors and the hepatic blood flow. From thus obtained GSA Rmax (maximal removal rate for the ligand), H15GSA (retention ratio in blood) and L15GSA (hepatic GSA uptake ratio), we calculated the LHH/LAH ratio as a modified indicator of the amount of receptors. These parameters were analyzed in relation to the total HAI score and the HAI score for each of the following 4 categories; (I) periportal +2; bridging necrosis, (II) intralobular degeneration and focal necrosis, (III) portal infiltration, and (IV) fibrosis.

Results: (1) The correlation between total HAI score and each GSA-based parameter was significantly higher than that between total HAI score and ICGR15. (2) The correlation between GSA Rmax and each of four HAI scores was higher than that between each HAI score and L15H, H15H, LHH15 or HHH15. (3) The GSA Rmax exhibited a higher correlation with each HAI score than did ICGR15. Its correlation was particularly high with HAI scores for necrosis and fibrosis. (4) The GSA Rmax was found to be more useful than the ICGR15 in distinguishing chronic hepatitis from hepatic cirrhosis.

Discussion: The clinical usefulness of the analysis of the fate of GSA in blood and its accumulation in the liver was assessed, in comparison to ICG. GSA had a particular high correlation with HAI scores of necrosis and fibrosis, suggesting that the fate of GSA is closely related to a decrease in the number of liver cells due to liver injury.

Conclusion: GSA was found to be useful as a means of assessing hepatic reserve, sensitively reflecting the severity of liver injury.

Intensive Chemo-radiation Treatment in Advanced Esophageal Carcinoma: A Feasibility Study

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Purpose: To evaluate the efficacy and acute toxicity of an intensive concurrent chemoradiotherapy protocol in patients with advanced esophageal carcinoma. This regimen was proposed as a conservative procedure for inoperable patients or as neo-adjuvant in other cases.

Methods: Ten male patients, mean age 62 years old (range 50–72) were treated between October 1993 and May 1994. There were 9 squamous cell carcinomas and one adenocarcinoma. The US staging was as follows: 1 T3NO, 6T3N +, 3T3N + M +, 1T4N +. The treatment protocol included one initial chemotherapeutic course (SFU 1013 mg/m2 D1-4-DDP 100 mg/m2 D2) followed by concurrent chemo-radiation association: same chemotherapy regimen associated with a continuous accelerated hyperfractionated radiation scheme (1.5 Gy, BID, 5 days/week, 3 weeks, for a total dose of 45 Gy. using focalized fields). This regimen was followed by surgery in two patients and by one to four chemotherapy courses in 8 patients. Acute toxicity and response (endoscopy and biopsies) were evaluated after completion of radiotherapy and 6 month after treatment initiation.

Results: Acute toxicity, following chemo-radiation, consisted of dysphagia grade 3 (Atkinson scale) in two patients, for 9 days, weight loss 6 kg, and grade 4 in 1 patient for 14 days, other patients experienced grade O to 2 dysphagia for a mean duration of 7.6 days, mean weight loss 5.8 kg. Tumor response after radiation was complete (macroscopic and histological) in 6 patients (60%) and partial in 4 (40%). One of the 2 operated patients died of post-operative complications. Nine months after the last radiotherapeutic regimen, 4 patients are dead (1 of post-operative complications, 3 from the progression of the disease, I severe recurrences and 1 brain metastasis without local recurrence). Two patients have no evidence of disease. The other 4 patients are still alive with local progression, and with bone metastasis in 1 case.

Conclusions: This chemo-radiation regimen resulted in a high local response rate with an acceptable toxicity. However, early tumour recurrence was frequently observed, which was disappointing in regard to this aggressive combined treatment. Further investigations and longer follow up are required to evaluate this treatment regimen.

Phenotypic Severity of Familial Adenomatous Polyposis: Relationship to Polypl Pattern and to Mutation Site

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Background: Familial adenomatous polyposis (FAP) is caused by various mutations within the APC gene of chromosome 5. Recent studies suggest that a different severity in the clinical course of the disease results from different mutations of this gene. We here attempted to correlate clinical courses of FAP patients with the macroscopic pattern of colon and duodenum, and with the site of APC mutation when determined.

Methods: Six criteria of severity were proposed, consisting of disorders related to FAP and excluding colorectal carcinoma. 17 FAP families were classified accordingly to the presence or absence of one of these criteria in almost most one of their members (1 to 5 patients per family). Endoscopic and post-operative aspect of colon in 39 patients, and endoscopic aspect of duodenum and ampulla of Vater in 21 were reported. Clinical history and macroscopic aspect of colon and duodenum were compared with the site of APC mutation in these families.

Results: 7 out of 17 families were classified as “severe phenotype” families. Confluent polyposis with a majority of non diminutive polyps was characteristic of these families (confluent polyposis in 15 out of 15 patients versus 4 out of 20 patients in other families, p<0.0001). Polypoid type papilla was also characteristic of these families (6 out of 8 patients versus 3 out of 14 patients in other families, p = 0.026). Confluent colonic polyposis was characteristic of exon 15 mutation in 7 families versus 1 family in patients with mutations in exons 1, 2, and 3 (p = 0.017) versus 2 out of 15, p < 0.01) as was a polypoid type papilla (p < 0.01).

Conclusions: A subset of FAP families (40% in our series) gather all extra-colonial disorders related to FAP. Members of these families are characterized by a confluent colonic polyposis even at young ages and a polypoid type papilla after 20 years old. The results suggest that these macroscopic patterns of colon and duodenum, and the localization of the responsible mutation in exon 15 of the APC gene, may be of help to select patients who require early prophylactic colectomy, early and regular follow up of duodenum, and closer follow up in order to detect other extra-colonial disorders related to FAP.

Contribution of “Antral Sweep” on Liquid Emptying — Measurement with Color Doppler Ultrasonography

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Although gastric emptying of liquid has long been thought to be driven by the changes of fundic tone, recent studies suggest the importance of antral contraction on liquid emptying. The Aim of this study was to determine the contribution of “antral sweep” on liquid emptying by using color Doppler ultrasonography.

Subjects and Methods: Total of 10 healthy volunteers were examined by ultrasonography after drinking 400 ml of cowsmose soup (1 kcal). The ultrasonic probe was attached to the level of antrum, pylorus, and the duodenal bulb in a plane. For 5 minutes, antral contractions and the transpyloric color signals into the duodenum were ob-
served. The "antral sweep" was defined as the antral contraction associated with the simultaneous transpyloric color signal into the duodenum. During the 5 minutes of observation, the sweep accounted for 4.5% of the antral contractions in the patient group and 2.8% in the control group. Sonographic Doppler imaging was employed was SSA-260A, Toshiba, Japan. Results: Total of 89 transpyloric flow into duodenal bulb was detected by color Doppler. Eighty-nine percent of transpyloric flow were associated with the contraction of proximal antrum (3 cm or more from pylorus). None of the contractions of distal antrum (less than 3 cm from pylorus) was associated with transpyloric flow. In another word, in total 164 contractions of proximal antrum, 80 (48.8%) worked as "antral sweep". Other antral contractions as well as "antral sweep" were associated only with retrograde plulsion.

Conclusion: Color Doppler ultrasonography was successful in the detection of antral contractions and the transpyloric flow of liquid meal. Approximately half of antral contractions work as "antral sweep" in liquid emptying.

Effect of Eradication of Helicobacter pylori with Lansoprazole and Clarithromycin


The Aim of the study was to evaluate the effects of lansoprazole and clarithromycin in patients with Helicobacter pylori (Hp) associated peptic ulcer. Methods: Forty-five patients (29 M, 16 F, mean age 46.2 yr) with peptic ulcer (DU n = 34, GU n = 8, GDU n = 3) were included in this study. The patients received lansoprazole 30 mg daily for 6 weeks and clarithromycin 400 mg/day during last 2 weeks. Both of the Hp infection as well as eradication of the infection were confirmed by rapid urease test and histological examination of biopsy specimens. Antral gastritis was assessed histologically by the amount of inflammatory cells infiltration (between grades 0 and 3). Serum gastrin (G) and pepsinogen (PG) were determined by radioimmunoassay. In addition, we studied the effect on antral G-Cell and D-Cell number using immunohistochemical technique. Results are presented as a mean ± SEM. Results: Hp clearance was obtained in 32/45 (71.1%) and Hp eradication was obtained in 22/40 (55.0%). After the therapy antral gastritis improved significantly (from 2.1 ± 0.1 to 1.3 ± 0.1, p < 0.01). Serum G, PG, and PGII decreased significantly and the ratio increased significantly (G; from 145.9 ± 8.4 to 100.2 ± 18.5 μg/ml, p < 0.05, PG; from 73.5 ± 7.7 to 60.7 ± 9.1 ng/ml, p < 0.05, PGII; from 21.9 ± 3.0 to 14.1 ± 2.7 ng/ml, p < 0.01, PGII; from 7.3 ± 0.2 to 4.9 ± 0.2 ng/ml, p < 0.01). Antral D-cell counts increased significantly (from 2.11 ± 0.31 to 2.86 ± 0.40 (p < 0.05). Antral G-cell counts also increased from 9.62 ± 1.51 to 13.13 ± 1.49, but no significant difference was found.

Conclusion: These results suggest that lansoprazole (30 mg/day) and clarithromycin (400 mg/day) may be one effective methods for patients with Hp associated peptic ulcer.

High Incidence of Duodenogastric Reflux in Patients with Non-ulcer Dyspepsias (NUD)


Motility disorders of the upper gastrointestinal tract have been implicated in the pathogenesis of NUD. However, the role of duodenogastric reflux (DGR) in NUD remains controversial. The Aim of this study was to investigate DGR in patients with NUD, along with gastric motility. Methods: The study population consisted of 121 asymptomatic healthy volunteers (86 men; mean age, 37.9 yr) and 122 NUD patients (59 men; mean age, 44.9 yr). After drinking liquid meal, DGR, antral contraction and gastric emptying were evaluated by ultrasonography with color Doppler. For the evaluation of DGR the probe was positioned at the level of the transpyloric plane. Frequency of DGR was expressed as the number of episodes during 5 minutes. Reflux index was expressed as the multiplication of the mean distance (cm) of detected color signal from the pyloric antral edge per 5 minutes. The frequency of antral contraction was measured as the number of contractions during 3 minutes intervals. The amplitude of antral contraction was calculated from the reduction of the antral area (difference between relaxed and contracted area). Motility index was calculated as amplitude X frequency of antral contraction.

Results: (Mean ± SE, significance at the 0.05 level)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy volunteers (n = 121)</th>
<th>NUD (n = 122)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenogastric reflux</td>
<td>19.6 ± 2.0</td>
<td>54.4 ± 4.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Frequency (no/3 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reflux index</td>
<td>6.5 ± 5.8</td>
<td>18.5 ± 16.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Antral contractions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency (no/3 min)</td>
<td>9.6 ± 0.7</td>
<td>9.2 ± 1.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Amplitude (%)</td>
<td>83 ± 14.0</td>
<td>71.5 ± 24.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Motility index</td>
<td>8.03 ± 1.54</td>
<td>6.67 ± 2.58</td>
<td>0.01</td>
</tr>
<tr>
<td>Gastric emptying</td>
<td>62.3 ± 16.6</td>
<td>43.6 ± 19.1</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Conclusions: DGR was increased and gastric emptying of liquids was decreased in NUD. Amplitude of antral contractions and motility index were also decreased. These findings demonstrated that DGR, along with gastric dysmotility, may be related to the pathogenesis of NUD.

Glucose Metabolism in Rat CCl4-induced Cirrhosis: Interference of CCI4 Toxicity with the Observed Effects

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Glucose intolerance and insulin resistance are the main features of cirrhosis-induced glucose metabolism impairment. The rat CCl4-induced cirrhosis model is widely used, but conflicting results have been reported with regard to the mechanisms of alteration in glucose metabolism. The purpose of this study was to evaluate the influence of the delay between performing metabolic studies and the last CCl4 administration. Methods: Cirrhosis was induced in rats by chronic phenobarbital and intra-gastric CCl4 treatment, and estimated by 13C-aminoxyacetic breath test and liver pathologic studies. Glucose tolerance was measured by iv glucose tolerance tests, insulin sensitivity and insulin responsiveness by euglycemic hyperinsulinemic clamp techniques in 2 groups of CCl4-induced cirrhotic rats: the first group (acute cirrhosis) was tested 3 days after the last CCl4 administration, and the second group (delayed cirrhosis) was tested 2 weeks after the last CCl4 administration. The results were compared to body weight-matched control rats. Results: Hepatic nodular architecture was present in cirrhotic rats, and a 22% decrease of 13C-aminoxyacetic breath test was observed compared to controls (p < 0.05). Basal glycemia and insulinemia, as well as glucose-stimulated insulin response were identical in both cirrhotic groups and controls. Insulin sensitivity, as measured by the slope of the regression line between glucose infusion rate (GIR) for physiologic insulin perfusions (2 and 6 mU/kg.min) was unchanged in acute and delayed cirrhosis compared to controls. On the opposite, insulin responsiveness, as measured by the GIR for high insulin infusion (30 and 60 mU/kg.min), was decreased for the acute cirrhosis only, but not for the delayed group. Compared to controls (19.7 ± 1.2 mg/kg.min vs 24.27 ± 1.13 and 23.35 ± 1.18 respectively, p < 0.05). Conclusions: CCl4-induced cirrhotic rats were not found glucose intolerant. However, the delay between CCl4 administration and performance of the clamp studies appears to play an important role, as insulin responsiveness, but not insulin sensitivity, was decreased in the acute cirrhosis group. Thus, CCl4 toxicity may per se modify glucose metabolism, warranting caution when using the experimental CCl4-induced cirrhosis as a model of human cirrhosis.
844 Preneoplastic Lesions and Hepatocellular Carcinoma
Foci (HCC) on Liver Explant Specimens of Patients
for Liver Transplantation

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The frequency of HCC on cirrhosis discovered on liver explant specimens is
estimated between 8% and 16%. It is assumed that these HCC develop on pre-
neoplastic lesions: macroregenerative nodules with no dysplasia (MNR type I),
dysplasia (MNR type II or borderline nodules), small cell type severe
dysplasia.

The objective of this study was: 1) to evaluate the frequency of unde-
tected HCC found on cirrhotic livers removed at liver transplantation (LT); 2) to
specify the size and type of these HCC; 3) to determine the frequency of
preneoplastic lesions, either isolated or associated to HCC.

Materials and methods: From October 1980 to March 1994, 80 patients
(21 women and 59 men, mean age 43.6 years), were transplanted for cirrho-
sis, among whom 25 were alcoholic cirrhosis, 35 posthepatic (17 HCV, 18
HBV +/- HDV), 7 postcholestatic and 13 other causes; with regard to the
severity of the cirrhosis, they were 5 Child A, 39 Child B and 38 Child C. A
systematic 3 mm section pathologic study was conducted on explanted
livers, focusing on: MNR, isolated severe dysplasia, borderline nodules and
HCCs.

Results: MNR were found in 34/80 cases (42.5%), severe dysplasia in
15/80 cases (18.7%), borderline nodules in 25/80 cases (31.3%), HCC in 14/80
cases (17.5%) (one focus only: 6 cases, 5:5:13 cases, size < 20 mm: 13 cases).
The presence of HCC was significantly associated with MNR (p = 0.006), severe
dysplasia (p = 0.006), and borderline nodules (p = 0.02). The patients with
HCC foci were in average 9.5 years older than patients without HCC (p = 0.02).

Conclusion: 1) The frequency of HCC undetected before LT was 17.5% in
our series; 2) the frequency of preneoplastic lesions was 42.5% for MNR,
18.7% for severe dysplasia, 31.3% for borderline nodules. 3) The frequency
of undetected HCC and preneoplastic lesions in cirrhotic liver should be taken
into account in the screening of cirrhosis and for liver transplantation’s
indication.

845 Nutritional Therapy in Crohn’s Disease; Factors
Influencing Induction of Remission

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Background: Although enteral nutrition with elemental diet (ED) and total par-
enteral nutrition (TPN) has been shown to control disease activity of Crohn’s
disease, remission could not be induced in up to one third of patients with active
disease. The aim of this study was to clarify factors influencing the
induction of remission.

Subjects and methods: From 1985 to 1992, in 194 patients treated with nutri-
tional therapy at our hospital, 160 active patients (69 ileitis, 66 ileocolitis, 23 colitis,
16 ileocelecolitis and 3 with sotomy small bowel abscess) treated with
enteral nutrition with ED or TPN were analyzed. These 160 patients were
divided to two groups (patients with remission and non-remission) based on clinical
parameters excluding the presence or absence of edematous ileitis. The
induction of remission was defined as an ED score reduction of more than
50% 4 weeks after starting therapy.

Conclusion: 1) The frequency of remission achieved a significant
reduction of radiographic findings of cobblestone appearance (small intestine: 3.6-1.92,
colon: 4.0-3.3) and longitudinal ulcer (small intestine: 3.8-2.5; colon: 2.6-2.1). However,
stenosis score was unchanged. Comparing the remis-
sion group and the non-remission group, the stenosis score signifi-
cantly higher in the latter group but the scores of small intestine not significant.
Cobblestone score improved after ED and all the scored findings were significantly
higher in the non-remission group (cobblestone appearance, remission group: 2.6-2.0,
non-remission group: 8.5-7.8). In addition there was a positive correlation between scored findings of the colon and CDAI.

Conclusions: From the results of this study it is suggested that the colonic
involvement of the intractable group is much severer and the response to ED is
much less than the remission group. Therefore, colonic involvement seems
keystone to enteral nutrition.

847 The Usefulness of Quantitative Analysis of
Radiographic Findings of Crohn’s Disease

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Introduction: Since we have reported that the double contrast study of the
small bowel and the colon were excellent methods of detecting mucosal le-
sions of the intestine in inflammatory bowel disease, we found that significant
radiographic improvement of bowel lesions was obtained in patients with
Crohn’s disease of the small bowel who received nutritional therapy (either enteral diet or prednisolone). This study was undertaken in order to find out usefulness of quantitative analysis of radiographic findings of Crohn’s disease before and after enteral nutrition (EN). In addition, we intended to find the differences in radiographic findings between the remission and intractable patients by EN.

Materials and Methods: Eighty-seven patients of active Crohn’s disease who
had been admitted to Chikusho hospital between 1985-1992 and un-
dertreatment four weeks of treatment were selected as Crohn’s disease.
Inclusion of the 83 pa-
tients were divided to two groups (65 patients with remission and 18 non-
remission) based on clinical response to the treatment. Remission was de-
fined as Crohn’s disease’s activity index (CDAI) < 150 and ESR < 20 mm/hr
after 4 weeks of treatment. CDAI at the pre-treatment was 223 ± 75 (M ±
SD). Double contrast X-ray examination of the small intestine and colon
were performed before and at 4 weeks after treatment. Radiographic findings
were quantified in each 4 segments of the small intestine and each 6 segments of
the colon; important three findings as follows were scored: cobblestone ap-
pearance (0-4), longitudinal ulcer (0-4) and stenosis (0-3). The sum of
total score was defined as radiographic activity score at the time.

Results: Patient achieved a significant reduction of radiographic
findings of cobblestone appearance (small intestine: 3.6-1.92, colon: 4.0-3.3) and
longitudinal ulcer (small intestine: 3.8-2.5; colon: 2.6-2.1). However, stenosis score
was unchanged. Comparing the remis-
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cantly higher in the latter group but the scores of small intestine not significant.
Cobblestone score improved after EN and all the scored findings were significantly
higher in the non-remission group (cobblestone appearance, remission group: 2.6-2.0,
non-remission group: 8.5-7.8). In addition there was a positive correlation between scored findings of the colon and CDAI.

Conclusions: From the results of this study it is suggested that the colonic
involvement of the intractable group is much severer and the response to EN is
much less than the remission group. Therefore, colonic involvement seems
keystone to enteral nutrition.

849 Chronic Cholestatic In Patients Supported by
Prolonged Parenteral Nutrition (P-PN)

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The aim of this study was to determine the prevalence of chronic cholestatic
in patients with intestinal failure treated by P-PN, and to analyze predictive
factors of its occurrence.

Methods: Between January 1985 and June 1992, 86 pts with intestinal failure,
without cancer or HIV infection, were treated by P-PN in our institution.
Among them, 61 fulfilled the following inclusion criteria: (1) no liver disease or
liver function tests at least A1 (ALT≤40, AST≤60, GGT≤70, alkaline phosphatase
≤200), no causative factor of liver abnormalities during the follow-up period (ie: virus B and C infection, drug-
toxicity, biliary disease). Chronic cholestasis was defined as 2 out of the 3
following liver abnormalities, for at least 6 mo: bilirubin, alkaline phosphatase
≤350, GGT≤150, ALP≤200. Probability of chronic cholestasis was calculated using the
Kaplan-Meier method. Multivariate analysis was made using the Cox model
to identify factors associated with a higher risk of chronic cholestasis.

Results: Patients were followed for a median of 36 mo after initiation of
PN (range: 6-133 mo). Probability of chronic cholestasis in pts treated by P-PN
was 38 and 58% at 1 and 3 yr respectively. Jaundice developed in 12 and 30% of
the pts at 1 and 3 yr. No pt developed liver failure, but liver biopsy performed
in 16 of the 32 pts with chronic cholestasis found extensive portal fibrosis in 6
pts and cirrhosis in 2 pts. Multivariate analysis indicated that the following
tests were associated with a higher risk of chronic cholestasis: ileal resection, whatever the length of resection, (odds ratio: 3.9, 95% CI 1.3-11.4, and exclu-
sion of the liver, 95% CI 0.2-9.6). Other factors such as age, sex, primary diagnosis, chronic intestinal occlusion, residual lesions on
small bowel, were not predictive of chronic cholestasis.

Conclusion: Prevalence of chronic cholestasis increases with duration of
PN, and reaches 58% at 3 yr. Patients with ileal resection and exclusion of the
colon are at higher risk to develop this complication.

850 Detection of Asymptomatic, Small Pancreatic
Carcinoma by Mass Survey

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To evaluate the efficacy of mass survey for early detection of pancreatic car-
cinoma, 201,550 subjects which were examined by ultrasonographic mass
survey at our co-operative institutions during the period from 1986 through 1993 were analysed. Forty-six cases of symptomatic pancreatic carcinomas diagnosed at our out-patient clinic during the same period were also analyzed. CA19-9 was assayed in the patients with pancreatic carcinoma and 2,864 subjects randomly selected from the mass survey group. The CA19-9 positive cases of the two groups were examined and followed-up for 5 years on average. Result: We found 11 cases of pancreatic carcinomas by ultrasonographic mass survey. All of them were resectable, except the case which was of sclerosing type. Five were less than 2 cm in diameter, in which 4 were of stage I, one and a half year survival rate was significantly higher than the mass survey group than in out-patient group (45.4% vs 9.5%, p = 0.001). CA19-9 was positive in 3.0% of the mass survey group. Positive rate increased along with age of the subjects. No pancreatic carcinomas were identified in 80 of 86 cases below 40 years old who survived for 5 years on average. Conclusions: The mass survey by ultrasonography is useful for the detection of asymptomatic, small pancreatic carcinoma which has a good prognosis. CA19-9 is not useful for the early detection of pancreatic carcinoma, because of its low positive rate in the asymptomatic patients and its increase of false positive rate along with age.

852 Cytoskeletal Architecture of Ganglionic Cells in Myenteric Plexus of Rat Duodenum Revealed by Quick-freezing and Deep-etching Method
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The ganglionic cells in the myenteric plexus may have special cytoskeletal structures suitable for the role in regulation of gastrointestinal motility. We applied a whole mount preparation technique to the quick-freezing and deep-etching (QF-DE) method. Platinum replicas for the plexus were prepared after fixing, embedding, and observed cytoskeletons of ganglionic cells in the myenteric plexus three-dimensionally.

Nerve profiles in the plexus, which contained neurofilaments much less than other types of neurons, had varicose contours, and were bundled by Schwann cells. The cytoskeletons were hardly observed in varicose regions, while slender nerve regions contained some neurofilaments in parallel with the fiber profiles. Schwann cells had poor short cytoskeletons running across the axolemma, and their cytoskeleton became thinner around varicose regions of the nerve profiles. They wrapped the neurons incompletely, and neuronal membrane had direct cross-links in naked regions. Moreover, varicose regions often showed close association with neighbouring nerve fibers. Excessive matrix was not rich between the bundled nerve fibers, though it existed more around peripherically located fibers. Fewer cytoskeletons of the neurons would be unsuitable for rapid signaling. Additionally, neuronal association especially at the varicosed regions, or synaptic vesicle-accumulating sites, suggested close intercommunication of each neuronal cell in the plexus. Our QF-DE method would be useful to examine their three-dimensional structures of the plexus.

854 Dual Therapy with Lansoprazole and Tranexamic Acid for Upper Gastrointestinal Bleeding: A New Approach to Endoscopy
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Introduction: Meta analysis suggests that acid suppression and fibroinolysis inhibition may improve outcome in upper gastrointestinal bleeding, but an impact on infrequent end points such as death is difficult to show. We, therefore, evaluated lansoprazole (LAN) or tranexamic acid (TRAN), by a new approach to drug screening using blood in the stomach at endoscopy as main end point.

Methods: All patients with suspected upper GI bleeding were randomized to oral lansoprazole (60 mg then 30 mg qds), tranexamic acid (1.5 g then 1 qds), both drugs or placebo for up to 4 days. Two hundred and twenty-eight patients with a definite upper GI bleed met eligibility requirements. Logistic regression analysis was used to identify determinants of blood in the stomach at endoscopy.

Results: Patients were endoscoped 19 (median, IQR 12–23) hours after admission. The odds ratio for blood in the stomach was 3.04 (95% CI 1.16–8.01, p = 0.024) for high risk patients (old, shocked or liver disease) compared to others. The odds ratio increased by 0.03 (0.0–0.06) for each year of age (p = 0.021) and by 0.02 (95% CI 0–0.04) for each beat per minute of the initial pulse (p = 0.070). Treatment reduced the odds of blood in the stomach:

<table>
<thead>
<tr>
<th>Placebo</th>
<th>LAN</th>
<th>Both</th>
</tr>
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<tbody>
<tr>
<td>% with blood</td>
<td>p vs placebo</td>
<td>% with blood</td>
</tr>
<tr>
<td>35.2% (19/54)</td>
<td>0.0244</td>
<td>13.9% (11/75)</td>
</tr>
<tr>
<td>1.04 (0.0–0.03)</td>
<td>0.26</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Only 5 of the 228 patients died.

Conclusion: Blood in the stomach at endoscopy is a valid and sensitive end point (compared to conventional ones such as death) which shows lan-

855 Cytoskeletal Structure of Smooth Muscle Cells Along Myenteric Border of Rat Duodenum Revealed by Quick-freezing Method
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Smooth muscle cells along boundary regions of rat duodenum have close association with the myenteric plexus and may have special cytoskeletal architecure. We used the quick-freezing and deep-etching (QF-DE) method to examine the cytoskeletal structure of smooth muscle cells along whole mount strip specimens. Using platinum replicas for longitudinal muscle along boundary regions, we were able to investigate their cytoskeletons.

The cytoskeletons in the smooth muscle cells consisted mainly of thin or thicker filaments. They were cross-linked each by shorter filaments, and changed their arrangements in cortical regions or extruded areas. Membranous organelles such as mitochondria were cross-linked with unidentified cytoskeletons. A few filaments existed around caveolae, which were interconnected with cytoskeletal filaments or plasma membrane by thinner filaments. Intricated filaments existed around gap junctions between smooth muscle cells. It seems that the filaments of actomyosin contractile systems, basement membranes around smooth muscle cells consisted of collagen fibers and networks of other thin filaments. However the basement membranes were relatively homogeneous, and dense layers, which can be observed commonly in conventional ultrar thin sections, were obscure. The cytoskeletal architectures of smooth muscle cells have been revealed to consist of contractile systems and other ultrastructures which would have roles for signal transduction and be independent of the contraction. They are proposed to be appropriate for regulated gastrointestinal motility.

856 Validation of Two New Rapid Blood Tests for H. pylori
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Introduction: A rapid, reliable blood test for H. pylori would reduce unnecessary investigations for dyspepsia since duodenal ulcer disease and gastric cancer are both rare in the absence of H. pylori. We therefore assessed two new rapid tests, Flexsure and the Helisal against routine tests for H. pylori. Centrifugation of blood is recommended for Flexsure. Since this is not routinely available in primary care, we evaluated its performance on serum formed from blood specimens allowed to stand for a short period.

Methods: Blood was taken from 100 patients presenting for endoscopy. To reflect conditions in primary care the Flexsure was tested on the serum formed from blood allowed to stand for a half, one and three hours. Helisal was performed on 53 of these specimens. Both tests were performed blind to the H. pylori status; H. pylori was considered present if histology (entra
cus and corpus; H&E, toluidine blue) and CLO culture, or C14 urea breath test were positive. Results that were negative on Flexsure or Helisal were retested using routine ELISA serology (Helic-G).

Results: On standard testing 57 patients were positive for H. pylori, 41 negative and 2 were indeterminant.

<table>
<thead>
<tr>
<th>Helisal</th>
<th>Flexsure 1/2</th>
<th>1 hour</th>
<th>3 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invalid</td>
<td>0%</td>
<td>10%</td>
<td>0%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>63%</td>
<td>75%</td>
<td>66%</td>
</tr>
<tr>
<td>Specificity</td>
<td>88%</td>
<td>92%</td>
<td>89%</td>
</tr>
</tbody>
</table>

Invalid and false negative Flexsure results were more likely to occur on early testing, suggesting insufficient serum had formed. Only 1 patient with H. pylori was negative on all serological tests.

Conclusion: Serum formed from unspun blood is an alternative if centrifuging facilities are not available. It has greater sensitivity at 3 hours and similar specificity to Helisal.

858 Is Endoscopic Diagnosis Necessary Before H. pylori (HP) Eradication Therapy?
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Introduction: Current advice is that endoscopic diagnosis of peptic ulceration and Hp status should be confirmed prior to attempting eradication of infection. We have investigated if symptoms and serological testing for Hp, alone and combined, would be successful at directing eradication treatment, without endoscopic diagnosis. Methods: 131 patients (66 males, 56 females, age 15–87 median 57 years) with simple dyspepsia (divided into ulcer-like, reflux-like or atypical dyspepsia) and not on NSAID treatment, underwent diagnost-
cal upper GI endoscopy. Hp status was separately assessed by Helisal Rapid Whole Blood Test. Results: 76 patients were Hp +ve and 51 Hp –ve, 23 patients had peptic ulcers (all Hp +ve). No gastric cancers were seen. Three
Pathology of Human Idiopathic Intestinal Pseudo-obstruction and W/W Mice in Gut Motility: A Gut Pacemaker Disease?

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We report about resemblance between pathology of human chronic idiopathic intestinal pseudo-obstruction syndrome (IIP) and the abnormalities in the gut motility of W/W mice, both probably resulting from the defects of gut pacemaker activity. Small intestine of W/W and human IIP was morphologically and electrophysiologically examined. As for W/W mice, macroscopic examination detected anemia and the lingering of their intestinal contents. Light microscopy with toluidine blue staining and electron microscopy revealed defect of the network of interstitial cells of Cajal (ICC) in the region of myenteric border. ICC development was insufficient in the region adjacent to the submucosal plexus, and outer longitudinal muscle layer showed 3-4 fold hyper trophy. Slow phasic contractions, which were exhibited in control smooth muscle strips, could not be detected in the specimens; and also the slow waves. Bulk smooth muscle contractility, however, did not change. In human IIP gut of a case, 27 years-old female with family history of her brother with fatal intestinal obstruction by unknown etiology, the cells surely regarded as ICC had not been examined in IIP gut by electron microscopy. Moreover, their outer muscle layer also showed hyper trophy. Structures of autonomic nerves showed no pathological changes both in W/W and W/W gut.

ICC network in gut muscularis externa are suggested to have an essential role in regulation of intestinal motility. Our examination strongly suggests that slow wave activity originates in ICC network, and that ICC defect may induce intestinal pseudo-obstruction if in severe states.

Change of Rats Mast Cell Ultraplacement During Exocytosis Revealed by Quick-Freezing Techniques

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Mast cells are important in the pathological response which forms the inflammatory reaction, and the release of chemical mediators from the secretory granules is required to maintain the extracellular milieu and the cellular cascade of reactions. Inflammatory bowel diseases. Here we investigated changes of rat mast cell ultrastructures during exocytosis using various quick-freezing (QF) techniques.

Mast cells were stimulated with compound 48/80 for 0180 sec. Conventional electron microscopy could detect mast cells started exocytosis, although it was hard to examine other events during exocytosis. In contrast, the QF revealed some cytoplasmic events; actin filaments in stimulated mast cell cytoplasm decreased around the secretory granules before degranulation. Perigranular membranes in stimulated mast cells formed membrane docking between adjacent granules. E-face of perigranular membranes had diffuse granular appearance, while P-face of the perigranular membranes had tiny holes and showed no complete sheet-like structures. Compact morphological appearance and skeletal structures of discharged granules, indicating existence of considerable granule contents, has been revealed.

As for freeze-fracture immunochemistry, resting mast cells incubated with anti-Mast cell degranulation. The images were the same in their initial, circular matrices on the fractured surface, while stimulated mast cells exhibited seroton in addition to their intergranular cytoplasm; cytoplasmic immuno reaction was located especially in perigranular regions. These findings revealed by QF would be reasonable to rapid degranulation and keeping inflammatory response.

Result of Gastric Mass Survey with Reference to Analysis of Cases with Obvious: Photofluorographic Abnormalities

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Gastric carcinoma is a frequent type of cancer in Japan. Mass survey has been widely performed to detect this cancer. As a part of general medical check-up, we examined 97,700 subjects in total over the past 5 years, using specially modified buses installed with photofluorographic apparatus. We pointed out some abnormal findings in 17.21 patients (17.6%), and among them 191 cases of gastric cancer (0.2%). In addition, 238 of the above 17.21 subjects had obvious abnormality by photofluorography; we urgently suggested those cases to undergo further diagnostic procedures.

The 238 subjects of this group included 65 cases of gastric cancer (27.3%), 67 cases of gastroduodenal ulcer (28.2%), and 10 cases of submucosal tumor (4.2%). That is, 34.0% of mass-survey-detected gastric cancer were found. The examination leads to a certain degree of over-treatment. Moreover, twenty-seven of 65 cancer cases (41.5%) were at an early stage. The average age of cancer cases was 64.5 ± 10.1 years in age. As for blood chemistry, cancer cases showed decreased hemoglobin (Hb) and serum triglyceride (TG) in comparison with their data one year prior (p < 0.026). Non-cancer cases showed decreased Hb and Ht (p < 0.0007), but no changes about serum TG levels (p = 0.72).

The cases with obvious photofluorographic abnormalities had a high cancer detection rate. Even in this group, early cancer ratio was considered high and made the mass survey cost-effective.

Result of Colorectal Cancer Screening by Immunological Fecal Occult Blood Test in 122,292 Subjects

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Colorectal carcinoma has become increasingly frequent in Japan. Mass screening has been performed by using immunological fecal occult blood test. As a part of medical check-up, we examined 122,292 subjects.

Cases with Obvious: A86

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Background and Aim: Long term parental nutrition (PN) in early infancy is inevitably involved in liver dysfunction. Limit in the duration of PN was investigated utilizing serial liver biopsy specimens. Methods: Nine infants (neonatal and early infancy) having undergone surgery for the alimentary tract or abdominal wall and having been maintained with parental nutrition (PN) were studied. All infants developed cholestasis jaundice shortly after the commencement of PN. Liver specimens were obtained from 6 infants at the time of the initial and further surgeries (i.e. Hepatocytes, gastrostomy, atresia of the upper jejunum and postoperative intestinal obstruction) and from 3 points at the time of autopsy. The specimens for light-microscopy were formalin-fixed paraffin-embedded and stained with hematoxylin and eosin (H&E). PAS, PAS/Giastase, Masson-Tichrome, Colloid-Iron, Aiolin-Blue, Resulin, Sudan IV test. The duration of PN was from 96 to 270 days (mean 119 ± 5 months). Six infants gave liver specimens at 30, 52, 65, 70, 97, 147 days from the beginning of PN. The specimen at 30 days PN showed ballooned hepatocytes and Kupfer cell hyperplasia. PAS positive hepatocytes and bile pigments were seen in the hepatocytes and sinusoids. A few inflammation cells infiltrated into the glisson sheath. The specimens at 65 or 70 days showed ballooned hepatocytes and narrow sinusoids. Bile duct proliferation and inflammatory cell infiltration of variable degree predominated. The histological features were the same in all specimens. In the autopsy specimens, cirrhosis featured by prominent duct cholestasis, hepato-cell necrosis, portal bridging fibrosis was seen. Conclusion: There was a relationship between the duration of PN and the degeneration of course of liver architecture. The ultimate result of long term PN was cirrhosis. Long term PN should be undertaken with a meticulous care.
4th UEGW Berlin 1995

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The Role of Serotonin in Ucerogenesis
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It has been shown (Hashizume, 1978) that serotonin causes steady ischemia in rats and possesses strong ulcerogenic activity. We investigated the role of serotonin in intestinal ulcerogenesis, caused by different factors. Experiments were made on rats. Ucerogenesis was induced by serotonin (10 mg/kg intraperitoneal), ethanol (96% intragastric), immobilization. Lesion severity was observed by gastroscopy with differentiation of ulcers, erosions and gross haemorrhages. The lipooxidation activity grew by the thiobarbituric acid method, was evaluated by the thiobarbituric acid method. Serotonin led to erosions and haemorrhages in gastric mucosa in three hours after having been injected. In 24 hours serotonin produced ulcers; erosions increased 4.7 fold, haemorrhage index did not change. The lipooxidation activity grew by the method of the thiobarbituric acid method, decreased erosions by 32%, haemorrhage 2.6 fold. Periton by immobilization ulcerogenesis eliminated erosion formation, reduced haemorrhages 6 fold. Periton also twice lessened the mucosal damage in ethanol treated rats. Blockade of serotonin receptors (9 mg/kg) by serotonin ulcerogenesis, decreased erosions by 32%, haemorrhage 2.6 fold. Periton by immobilization ulcerogenesis eliminated erosion formation, reduced haemorrhages 6 fold. Periton also twice lessened the mucosal damage in ethanol treated rats. Blockade of serotonin receptors (9 mg/kg)

Absence of Genomic Sequence of Mycobacterium Paratuberculosis from Resected Intestinal Tissues of Crohn's Disease by Nested Polymerase Chain Reaction
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Recent studies have suggested the etiological role of Mycobacterium paratuberculosis (M. PTB) in Crohn’s disease. To determine whether M. PTB has the possibility to cause Crohn’s disease, we investigated for M. PTB by highly sensitive nested polymerase chain reaction (PCR).

Method: DNA was extracted from resected intestinal specimens from patients with 13 Crohn’s disease or 14 ulcerative colitis and from 3 non-inflammatory controls using phenol/chloroform method. The only resected specimens at surgery were selected for this study to avoid the contamination in endoscopic procedures. Nested PCR was performed using primer pairs complementary to sequences in insertion element IS 900 gene of M. PTB. Nested PCR techniques provided high sensitivity to detect 1 fg of control DNA templates from M. PTB. After nested amplification, PCR products were evaluated by agarose gel electrophoresis with ethidium bromide staining. Identity of PCR products was confirmed by subsequent cleavage with restriction endonuclease enzymes at predetermined sites to yield fragments of predicted sizes.

Results: No M. PTB specific PCR product was detected in any of these specimens for M. PTB, although control positive DNA template from M. PTB consistently gave products of the appropriate size, which were identified by restriction enzyme analysis.

Conclusions: Despite enhanced sensitivity of nested PCR, the failure to detect M. PTB genome in intestinal tissues in Crohn’s disease argues against the pathogenic role of M. PTB in Crohn’s disease.

Colorectal Adenomas Containing Invasive Carcinoma (ACIC): Review of Histological Features and Follow-Up Results
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Conservative management by endoscopic snare polypectomy (ESP) is a generally accepted policy for ACIC, providing that unfavorable histological features (carcinoma at the margin of resection, poorly differentiated carcinoma, lymphatic or blood vessel invasion by cancer cells) are absent.

The purpose of this study is to verify reliability of these histological criteria as indicators of risk of residual cancer after ESP.

We reviewed the records of 22 patients, who had had 22 ACIC and polypoid carcinoma removed by ESP in our Endoscopy Unit from 1985 to 1994. 8 patients received additional surgical treatment and colectomy specimen was examined. In 14 patients who underwent no further surgical treatment, more than 3 years follow-up endoscopic and ultrasound investigations were performed. In 14 ACIC (13 patients) favorable histological criteria were fulfilled. 10 of these patients had further treatment after ESP, without recurrence or metasis at follow-up (range 36–114 months, average 62). 2 were operated on, and colectomy specimens showed neither residual cancer in colon wall nor lymph node metastasis. 8 ACIC and 1 polypoid carcinoma (9 patients) had one or rare unfavorable histological features (S had carcinoma at the margin of resection, 2 had vascular invasion by cancer cells, 1 had both, and 1 had poorly differentiated carcinoma at the margin of resection). 4 of these were treated by ESP alone: successive follow-up (range 37–78 months, average 48) showed no recurrences or metastasis. 5 (4 ACIC and 1 polypoid carci- nomma) underwent additional surgical treatment: in this group too colectomy specimens were negative.

There is general agreement on conservative management by ESP of ACIC with favorable histological features. Our data suggest that this may be a treatment choice, also when unfavorable histological features are present, in high-risk surgical elderly patients, comparing risks of death from operation with risk of residual cancer.

Role of Granulomatous Enterocolitis and its Extraintestinal Manifestations
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Animal model of inflammatory bowel disease (IBD) could be useful to promote a pathogenetic understanding of IBD and provide a clue of the possible causes. We have developed rabbit granulomatous enterocolitis and its extraintestinal manifestations by a long-term administration of muramyl dipeptide (MDP) emulsified with Freund’s incomplete adjuvant, which is a minimal structure of bacterial cell wall. MDP emulsion was injected submucosally at 6 portions of the rectum and colon, 10 cm proximal from the anus, using a flexible endoscope. Seven rabbits were injected 8 times or 9 times a month, and were sacrificed one month after the last injection. Histological changes of the colon in the 7 rabbits were mononuclear cell infiltration, epithelioid granulomas, granulomatous lesion and prominent fibrosis, although they were different in degree. In 3 of 7 rabbits, the colon epithelia showed degeneration, necrosis, extensive denudation and regeneration. Histological examination of the liver in 5 of 7 rabbits showed pericholangitis and periductal fibrosis like sclerosing cholangitis seen in IBD patients. In 4 of 7 rabbits, fibrosis bridging mainly between portal and portal veins, and sometimes between portal and central veins, was seen. Two of 7 rabbits developed polyarthritides. The histological changes in our model has led to the suggestion that a continuous stimulation with bacterial wall fragments may be involved in chronic intestinal inflammation and its extraintestinal manifestations like IBD.

Diagnosis of Pancreatic Cancer Including Mucin Producing Pancreatic Cancer
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Using position emission tomography (PET), tumor images and pancreas parenchyma images are available for cancer cells which physiologically require glucose and for pancreas parenchyma tissue highly accumulated by amino acids, by administrating 2-deoxy-2[18F]fluoro-D-glucose (F) (half time 110 min.), and 11C-l-methionine (M) (half time 20 min.), respectively. We report in this work the results obtained by the PET observations in 28 pancreatic cancer cases as a part of abdominal cancer diagnosis system using PET. In addition, the preoperative diagnosis of 2 cases with mucin producing pancreatic cancer by PET will also be reported, although it has been extremely difficult to diagnose.

Subjects: 28 patients with pancreatic cancer were investigated by PET with M and/or F. PET instruments: ECAT II (EGG/Ortec, USA) or PET391/04(C1: ECAT, USA). Radiopharmaceuticals: M and F were supplied by Tohoku University Radio Isotope Center. Procedures: After transmission scanning M (148–592 MBq) and F (111–259 MBq) were injected for 30 sec. By ECAT II, they were scanned after 20–30 min. injection of M and after 35–45 min. injection of F. By PET391/04, they were scanned after about 20 min. injection of M and/or F.

(1) Image analysis was successful in 22 cases out of 28 pancreatic cancer patients given PET examination. 4 cases showed negative images of tumor only by M-PET, 2 cases displayed positive images of tumor only by F-PET and by both M & F-PET, the tumor was visualized as M-negative and F-positive, that is, mirror images in 15 cases. The cases confirmed by PET diagnosis were 21/22 (sensitivity 95%). (2) We succeeded in diagnosing 2 mucin producing pancreatic cancer cases before operation by PET.

Along with the various current diagnoses, PET using 11C-l-methionine and/or 2-deoxy-2[18F]fluoro-D-glucose was useful for pancreatic cancer di-agnosis. 2 cases with mucin producing pancreatic cancer could be preoperatively diagnosed by PET.

Usefulness of 99mTc-GSA in Patients with Chronic Hepatitis C
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Liver scintigraphy with 99mTc galactosyl human serum albumin (99mTc-GSA) which binds to the asialoglycoprotein receptors on the hepatocytes is a newly developed method for evaluating hepatic function. Its clinical usefulness in patients with chronic hepatitis C was investigated.
Forty-one patients were studied. After injection of 99mTc-GSA, uptake ratio (U/L) and histological activity index (HAI) scores proposed by Knodell et al comparing other liver function tests.

LHL/HH had negative correlations with hyaluronic acid (HAI) (Rs = -0.632), ZT (Rs = 0.534), type IV collagen (IV-C) (Rs = 0.532), y-globulin (Rs = -0.530) and IgC-R15 (Rs = -0.450). LHL/HH correlated with prothrombin time (PT) (Rs = 0.504), albumin (Rs = 0.428) and so on. No significant correlation was observed between "biliary tract enzymes" and LHL/HH. Moreover, LHL/HH (Rs = -0.640) represented HAI scores accurately as ZT (Rs = 0.730), IV-C (Rs = 0.701) and y-globulin (Rs = 0.650) did. HA (Rs = 0.629), PT (Rs = -0.533) and IgC-R15 (Rs = -0.473) fell behind in this regard.

These results indicated that LHL/HH reflects histological changes of the liver in patients with chronic hepatitis C as well as ZT and IV-C and that 99mTc-GSA is clinically useful to evaluate severity of chronic hepatic diseases.

**HCV Infection, Mixed Cryoglobulinemia and Cryoglobulinemic Syndrome**

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**Aim of the work:** to verify the incidence of the presence of cryoglobulins in the serum and histological changes of the cryoglobulinemic syndrome.

**Patients and Methods:** 130 subjects resulted anti-HCV+ using RIBA second generation, affected by chronic active hepatitis histologically detected, were also investigated for the presence of cryocrit.

The research of the cryoglobulins has been performed on samples withdrawn and separated at 37°C and successively stored at 4°C for 48 hours, looking the presence of a precipitate quantitated as cryocrit (% of total serum volume) by centrifugation in hematoct tubes. The cryoglobulins were identified by immunoelectrophoresis. The HCV RNA by RT-PCR was not performed. A group of 235 anti-HCV negative subjects was tested as a control. The chi-square method was employed for the statistics evaluation.

**Results:** the presence of cryocrit was found in 29/130 subjects (22.3%). The cryoglobulins were identified as type II in 18/29 sera (65.5%) and type III in 11/29 cases (37.9%). Only 4/29 patients (13.7%) had symptoms (arthritus, vasculitis, kidney failure, hypertension) high levels of circulating immunocomplexes and rheumatoid factor.

**Conclusions:** the presence of cryocrit was found in 22.3% of cases and shows a significant prevalence (P > 0.001) in patients anti-HCV+. The cryoglobulins were not found in the sera of subjects anti-HCV negative of the control group. The presence of the symptoms appear more rare and few, significant. On the contrary anti-HCV positivity has been reported in 90% of cases affected by mixed cryoglobulinemia.

**Evaluation of Severity and Management in Acute Pancreatitis**

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The medical treatment of acute pancreatitis should be started without delay and be arranged in accordance with the severity of pancreatitis. In this study, we evaluated the prognostic signs to predict the severity and prognosis of acute pancreatitis, based on clinical findings and laboratory data in relation to mortality. Using the clinical criteria by Intractable Pancreatic Disease Research Group (IPDRG) in Japan (1990), 364 patients with acute pancreatitis admitted to our department: and affiliated hospitals in 1984-1994 were evaluated. In our series, 74 patients were judged as severe pancreatitis. All 29 patients (39% of severe pancreatitis) who died were assessed as severe pancreatitis according to the IPDRG criteria. However, 5 died patients were not regarded as severely ill by Ranson’s and Miehe’s criteria, which do not include clinical findings. A mean age of death group (66 ± 18) was higher than that of recovery group (52 ± 17). Among laboratory data, levels of LDH and base excess were significantly different between both groups (LDH: recovery group 626 ± 514 IU/L vs death group 933 ± 587 IU/L, BE: -2.4 ± 5.9 mEq/L vs -9.6 ± 7.8 mEq/L). The mortality rate in severe pancreatitis was lower in patients who had biliary tract procedure within 48 h after the onset of disease (33%) than in those who did not (63%). The mortality rate was higher in patients who had surgical treatment within 7 days (63%) than in those after 8 days (31%). The dosage of protease inhibitors given within 48 h after the admission was 1.09 mg/kg in recovery group. The mortality rate in 9 patients managed with peritoneal lavage and/or hemofiltration therapy was 44%. In conclusion, we should assess the severity of acute pancreatitis based on the clinical findings and laboratory data. In severe cases of acute pancreatitis, intensive care is absolutely necessary at the early stage of pan- creatitis. Surgical treatments should be considered for later complications such as infected necrotic foci.

**Histopathology of Liver Disease in HCV Positive Patients According to the Different Viral Genotypes**

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Hepatitis C virus (HCV) is a common cause of a chronic and end-stage liver disease. There is evidence that severity and progression of the disease and the response to interferon therapy may be related to different virus genotypes.

The aim of our study was to evaluate in our area the rate of different HCV genotypes and the related liver diseases. We detected HCV-RNA in serum by using RT-PCR in the 5’ external region and assessed the severity of the disease by liver biopsy. Genotype was assessed according to Okamoto et al. in a series of 193 consecutive patients with HCV viremia, 108 of whom (56%) underwent to liver biopsy.

**Results:** Genotypes 5-7 were genotype 1A 3.4% type II 20.3% type III 0.5% type IV 7.3% type V 8.8% mix and 23% indeterminate.

Of the remaining biopsies not listed in the table 2 had normal tissue, 2 had acute hepatitis, 4 had associated acute disease and 8 had chronic hepatitis not otherwise classified because of the poor quality of biopsy.

Correlation of liver disease severity with HCV genotype indicated that most severe disease is associated with HCV viremia. The progression towards cirrhosis and HCC seems to be more frequently associated with genotype II, but the evolution of mild lesions associated with other genotypes need to be evaluated in a prospective study. Liver biopsy remains an essential tool for the management, diagnosis and grading of HCV chronic hepatitis.

**The Changes of Markers of Hepatic Fibrosis on Interferon Treatment of Hepatitis C**

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**Objectives:** We investigated the changes of markers of hepatic fibrosis (pro-collagen III peptide (PIIIIP) and type IV collagen 7S domain (IVTS)) on interferon treatment for Hepatitis C, in relation to effects of treatment. Methods: Sixty-one patients (32 male, 29 female, mean age 51) with HCV-RNA positive and anti-HCV positive were studied. We used recombinant interferon a 2b 600 or 1000 million unit per day. First 14 days injection was done everyday. After end of that period treatment was continued for 26 weeks three times a week. We checked the HCV-RNA and manay day hepatic fibrosis markers (Coll IVTS, PIIIIP six months after treatment, and HCV genotype and volume of HCV before treatment. Results: HCV-RNA negative patients were 73% at end of treatment, 38% six months after treatment. Most interferon effective geno- types were type II, HCV-RNA negative patients were 75% six months after treatment. PIIP mean concentration was 0.76 U/ml before, 0.61 U/ml at end of, 0.64 U/ml six months after treatment. IVTS mean concentration was 5.782 ng/ml before, 5.078 ng/ml at end of, 4.668 ng/ml six months after treatment. PIIP six months after treatment was significantly decreased compare with before treatment (mean 0.52 vs 0.76 U/ml P < 0.01). In IVTS before treatment, that of HCV-RNA positive at six months after treatment group were significantly higher than that of negative group (mean 6.242 vs 5.231 ng/ml P < 0.05). Neither PIIP nor IVTS had significant difference before treatment in HCV-RNA genotype and in volume of HCV. Conclusion: PIIP is useful for estimating effects of treatment. IVTS is useful for predicting effects of treatment.

**Ocstatatin (RC-160) — Continuous Infusion Therapy in Patients with Carcinoid Tumors — European Multicentre Trial**

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Somatostatin analogues have previously demonstrated beneficial value in the treatment of patients with carcinoid tumors. In several cases of carcinoid tumors. In the present study a new somatostatin analogue was administered as continuous subcutaneous infu- sions to look for antimutator effect.

Totally 25 patients, median age 67 years were included in the study. Pri-
mary tumor located in the midgut region in the majority of the patients and 26 of the patients presented liver metastases. U-5-HIAA median 616 μmol/24 h, chromogranin A median 25 000 μg/l. Octatstatin (RC-160) was administered s.c. via disposable minipumps giving a dose of 1.5 mg/day for 3 months, increasing up to 3 mg/day for another 3 months, total treatment period 6 months. Results: Among 19% of the patients showed complete disappearance of tumor related clinical symptoms whereas 43% showed significant amelioration.

Biochemical reactions: One patient showed a complete normalization of urinary 5-HIAA, whereas 20% showed significant reduction (<50%) and 66% stabilization.

Tumor size: One patient showed significant tumor reduction, whereas 19 patients (76%) showed change and 20% had significant tumor progression. Chromogranin A + B levels decreased significantly for the whole group in the 3 month period (p = 0.042).

The treatment was well tolerated, minimal adverse events were pain at injection site and 3 patients developed gall stones.

In summary the new somatostatin analogue Octatstatin demonstrates significant clinical and biochemical effects in patients with carcinoid tumors. Despite giving the drug as continuous infusions no significant increase in the number of tumor responses was encountered.

881 Premalignant Mucosal Changes in the Pelvic Pouch in Patients with Ulcerative Collitis

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Total colectomy followed by formation of a pelvic pouch (PP) is an established treatment for patients with ulcerative colitis (UC). Some UC-patients with a PP develop dysplasia in the PP-mucosa.

Purpose: To determine if moderate and severe atrophy in the PP-mucosa is associated with subsequent development of dysplasia and/or DNA aneuploidy.

Methods: Eleven UC-patients (7 men) developed progressive, moderate to severe atrophy in the PP. The median age was 37 years (range 29-52), and the median UC-duration before colectomy was 10 years (range 0.1-25). The median duration of the PP in function was 9 years (4-12). Proctocolectomy was performed due to colorectal dysplasia in 5 patients, chronic continuous symptoms in 4, and due to severe, intractable activity in 2 patients. Five patients had relapsing acute attacks of pouchitis and 3 patients had chronic continuous pouchitis symptoms requiring long-term metronidazole therapy.

Results: The patients were examined with a flexible video endoscope and biopsies were taken from 5 locations (the effenter small intestine, upper portion of the pouch, mid portion (2 biopsies), and lower portion of the pouch. From each location 1-2 biopsies were taken for histological assessment of dysplasia and 1-2 biopsies were taken for flow-cytometric DNA-analyses.

Conclusions: 1. Although this study confirms the association of steatosis with alcohol, obesity, diabetes mellitus and hyperlipidaemia and the association of nonalcoholic steatosis with female gender, obesity and diabetes, in 8 (26.6%) patients histological changes weren’t explained. 2. The biochemical characteristics were similar with only GGT levels being higher in the alcoholics. 3. Histology confirms that nonalcoholicics can develop liver disease identical to that of alcoholic liver disease but cirrhosis was not present in the former.

889 Acute Drug-Induced Liver Injury

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Introduction: Toxic responses of the liver are not uncommon, and hepatic drug reactions may mimic almost any kind of liver disease. So, early recognition of hepatic drug reactions is very important.

Aim: To analyse all cases of acute drug-induced liver injury observed during the last 5 years.

Patients and methods: Between January 1990 and December 1994, 12 (63%) out of 19 cases of acute drug-induced liver injury, histologically proven, were analysed concerning age, sex, aetiological associations, biochemical parameters, histology and course of liver disease. Seven (37%) cases with insufficient clinical data were excluded. Acute hepatotoxicity (elevation of liver tests for less than 3 months and above twice the upper limit of normal) was defined as hepatocellular necrosis when R > 5, cholestatic when R < 2 and as mixed when R > 5 and R = ALT:alkaline phosphatase). All other causes of liver injury were excluded. After considering a drug-induced etiology, all drugs potential offending were discontinued.

Results:

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Drug</th>
<th>Time&lt;</th>
<th>ALT&lt;</th>
<th>Total</th>
<th>Liver</th>
<th>Histology&lt;</th>
<th>Time of</th>
<th>normalization of liver tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>59</td>
<td>Nisatol</td>
<td>30</td>
<td>8</td>
<td>7</td>
<td>90</td>
<td>C</td>
<td>S + I</td>
<td>45</td>
</tr>
<tr>
<td>F</td>
<td>40</td>
<td>Dilofenac</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td>18</td>
<td>C</td>
<td>S + I</td>
<td>35</td>
</tr>
<tr>
<td>F</td>
<td>21</td>
<td>Ketokonozol</td>
<td>15</td>
<td>1</td>
<td>8</td>
<td>55</td>
<td>C</td>
<td>C</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>61</td>
<td>Reppelcine1</td>
<td>14</td>
<td>1</td>
<td>4</td>
<td>65</td>
<td>C</td>
<td>I</td>
<td>15</td>
</tr>
<tr>
<td>F</td>
<td>56</td>
<td>Flutaxolcin</td>
<td>7</td>
<td>1</td>
<td>7</td>
<td>90</td>
<td>C</td>
<td>C</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>46</td>
<td>Prinol</td>
<td>15</td>
<td>10</td>
<td>30</td>
<td>85</td>
<td>M</td>
<td>C</td>
<td>5</td>
</tr>
<tr>
<td>M</td>
<td>52</td>
<td>Rafteran +</td>
<td>20</td>
<td>10</td>
<td>25</td>
<td>25</td>
<td>M</td>
<td>C</td>
<td>5</td>
</tr>
<tr>
<td>M</td>
<td>52</td>
<td>Rafteran</td>
<td>20</td>
<td>10</td>
<td>25</td>
<td>25</td>
<td>M</td>
<td>C</td>
<td>5</td>
</tr>
</tbody>
</table>

1Immunoallergic hepatitis. 2From beginning of liver administration (days). 3Increase the upper limit of normal. 4Hepatocellular; C: Cholestatic; M: Mixed. 5S = Steato-; I = Inflammation; C = Cholestasis; I = Cirrhosis; I = Days
Conclusions: (1) Usually, acute hepatic necrosis clinically resembles other severe febrile illnesses caused by bacterial or viral infections, and early recognition of hepatic drug reactions remains a clinical challenge.

890 Spermine Inhibits Gastrointestinal Motility Activity and Induces Emesis

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Background and Aim: Spermine, one of polyamines ubiquitously occurring in living tissues, has been reported to be fatal; (3) Cholestasis can persist for long periods of time. (4) Early may be seen in patients with overt jaundice; (2) Drug-induced hepatitis associated with overt jaundice lifetimes. (3) The aim of this study was to compare results of colonyoscopy and further courses of chemotherapy in patients with HNPCC features (mean age 59) were recorded. Results: In the first group initial colonoscopy found 16.2% and adenomas in 32.1%, in the second group cancer in 61% (8% multilobar) and adenomas in 30% (villosus structures twice as frequent). Of the 73% of the cancers and 73% of the adenomas were in the right colon in the first group; 58% and 52% in the second group. Surgery was palliative in 22% of the second and in no case of the first group. The cancers were Dukes A or B in 8296 and C in 16% in the first A or B in 67% C in 18% and D in 15%. In the second group. All of the screened and one symptomatic group had 5 year survival. Durino colonoscopic surveillance 6 more cancers in initially negative cases (Dukes A or B) were found in the first group. Conclusions: Until modern techniques are available a profound family history is the key to detect HNPCC. Colonoscopic surveillance of the subjects prior to symptoms is fully justified to achieve an early diagnosis.

894 Polyamine Transport Into Rabbit Enterocyte Basolateral Membrane Vesicles

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Isolated basolateral membrane vesicles (BLM) of the rabbit enterocyte were used to investigate polyamine transport across the basolateral side of intestinal epithelial cells. Vesicles were characterized in terms both purity and orientation. Marker-enzyme assays showed a 12-fold enrichment of ouabain-sensitive Na+K+-ATPase, a basolateral-membrane marker. The use of immunoblotting techniques further confirms the absence of brush-border-membrane contamination. The orientation BLM vesicles was predominantly inside out on the determination by two independent criteria. The uptake of spermine and spermidine into basolateral membrane vesicles was rapid within the first minute, reaching approximately 30% of equilibrium values; within the first five minutes about 60% of the equilibrium uptake was achieved. However, osmopressor showed binding to the membrane of about 80% and 35% for spermidine and spermine. There was no evidence for sodium cotransport. At a pH of 7.5 the degree of polyamine uptake was significantly higher than at a pH of 6.5, indicating that polyamines are transported across the basolateral membrane of the enterocyte under pH conditions at which they are fully charged. Analysis of polyamine uptake over increasing concentrations of unlabeled polyamines showed saturable kinetics, with Km values of 32.2 and 20.83 mM for spermidine and spermine, respectively. Polyamine uptake was inhibited by di-, tri and tetracations. Transport of putrescine was not inhibited by spermidine and spermine, although spermidine and spermine inhibited the uptake of each other in a competitive manner. These results imply a saturable polyamine transport system does exist on the basolateral side of the enterocyte.

895 Endoglin is Expressed Universally in Gastric Carcinomas in Comparison to the Selective Expression of TGF-β Type I and II Receptors

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Endoglin is a membrane protein with the capability to bind transforming growth factor-βs (TGF-βs) which is considered as a substitute for TGF-β type III receptor, especially in endothelial cells. However, its role in carcinomas has not been clearly elucidated. In order to clarify this point, we performed immunohistochemically the expression of three distinct TGF-β receptors, endoglin and TGF-β type I (TGF-βRI) and type II receptor (TGF-βRII) with specific antibodies that had been kindly donated by Dr. K. Miyazono (Ludwig Institute for Cancer Research). Sixteen cases of gastric carcinoma (8 intestinal type and 8 diffuse type), and two gastric cancer cell lines, KATO III and MKN-28, established from signet-ring cell carcinoma and well differentiated adenocarcinoma, respectively, were examined. In clinical cases, the frequency of expression of endoglin, TGF-βRI, and TGF-βRII was 81%, 63%, and 63%, respectively. Both cell lines expressed endoglin, however TGF-βRII and TGF-βRII were expressed only in KATO III. In summary, endoglin was universally expressed, while TGF-β RI and TGF-βRII were frequently expressed in intestinal type of gastric carcinomas. These results suggest that endoglin may play an important role in signal transduction of TGF-βs when TGF-βs exert its activity in tumor cells which do not express TGF-βRI and TGF-βRII.

896 Transethosomal Transport of Short Chain Fatty Acids and their Metabolism in Pig Hindgut

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In vitro experiments in Ussing chambers were performed in order to study the transport as well as the intraepithelial metabolism of short chain fatty acids in the caecum, proximal and distal colon. Stripped epithelial tissues were incubated in isocatic buffer solutions. SCFAs were only present in the mucosal solutions at a concentration of 60 mM, consisting of 60% acetate, 25% propi-
onate and 15% butyrate at the beginning of each flux rate measurement. For osmotic reasons SCFAs were completely replaced by gluconate in serosal buffer solutions. The tissues were incubated under short-circuit current conditions for 1 h. In addition, with epithelia from the proximal colon flux rate measurements were also performed after setting the transepithelial potential difference (PD) to 25 mV with the serosa being positive. At the end of each 1-h period of flux measurements, the tissues were taken from mucosal and serosal buffer solutions for HPLC analysis of SCFA-metabolism. SCFA transport and rate of intracellular metabolism were calculated from serosal uptake and serosal release during the experimental periods. In the presence of a 2 cm² serosal tissue area and mucosal buffer solutions, the ratio of molar SCFA proportions irrespective of hindgut segment. Voltage clamp conditions did not influence mucosal SCFA uptake indicating the presence of electrogenic transport. The SCFA release was calculated from the difference between two flux measurements and thus exceeding mucosal loss. In serosal solutions molar butyrate proportions were significantly lower in comparison with mucosal solutions. This was accompanied by slight increases of molar acetate proportions indicating incorporation into lipids. The next day,gut might have lost an additional SCFA release from the epithelial tissues within the experimental period which had still been present from the in vivo situation.

**989 Mast Cells Are Involved in the Disruption of Intestinal Barrier Function in Experimental Colitis**

**J. Stein, J. Ries, K.E. Barrett.** Dept. of Medicine, University of California, San Diego

**Background/Aim:** Various immune cells and mediators have been suggested to play crucial roles in alterations of intestinal barrier function and water absorption in inflammatory bowel diseases. The objective of our study was to determine whether mast cells (MC) may contribute to mucosal barrier dysfunction in experimental colitis. **Methods:** Colitis was induced in male Sprague Dawley rats with intracolonic trinitrobenzene sulphonic acid (TNBS, 30 mg, 5% in 0.5 ml saline). Controls were saline-treated animals. In vivo loop permeability studies were used to assess colonic water flux (μl/mcm²) and lumen to blood ratio (51Cr-EDTA clearance (% administered dose)) 4 h, 12 h, 2, and 4 days after TNBS administration. Myeloperoxidase (MPO) was used as an index of granulocyte influx. A role for MC was investigated with the mast cell stabilizer daxoxamine (Dox; 5 mg i.p.) as a single dose 2 h before TNBS. Some rats were treated with dexamethasone (Dex; 1 mg i.p.) 24 h prior to the induction of colitis. Dex was a positive antiinflammatory control; it also depletes mucosal MC. Data are given as means ± SEM for 4 rats in each group. Results: TNBS or its vehicle resulted in a marked alteration in water absorption (0.82 ± 0.12 μl/mcm²) and permeability (4.68 ± 1.12%) at 4 h after administration compared to controls (154 ± 32 μl/mcm², 0.064 ± 0.012%). Neither Dexamethasone nor Dox was able to attenuate these early changes, likely related by the ethanol vehicle. At later times, TNBS, but not its vehicle, also increased 51Cr-EDTA permeability and decreased water absorption; both effects were significantly attenuated by Dox and Dex (table). Dexamethasone also significantly (p < 0.05) attenuated TNBS-induced MPO accumulation. Levels at 4 days were 12.9 ± 1.5 (TNBS); 5.3 ± 1.4 (Dex) and 6.3 ± 1.2 (Dex) μg/m tissue. In conclusion, these data suggest that MC play an important role in altering mucosal barrier function in colitis.

**51Cr-EDTA clearance (%)**

<table>
<thead>
<tr>
<th>TNBS alone</th>
<th>TNBS + Dex</th>
<th>TNBS + Dox</th>
<th>TNBS + Dex + Dox</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 h</td>
<td>1.34 ± 0.24</td>
<td>0.88 ± 0.19</td>
<td>0.92 ± 0.36</td>
</tr>
<tr>
<td>24 h</td>
<td>2.24 ± 0.69</td>
<td>2.80 ± 0.08</td>
<td>0.96 ± 0.36</td>
</tr>
<tr>
<td>48 h</td>
<td>0.96 ± 0.32</td>
<td>0.04 ± 0.09</td>
<td>0.04 ± 0.09</td>
</tr>
<tr>
<td>72 h</td>
<td>0.68 ± 0.13</td>
<td>0.32 ± 0.07</td>
<td>0.32 ± 0.07</td>
</tr>
</tbody>
</table>

**P < 0.05 or better compared to TNBS alone.

**990 Mastoparan Regulates Permeability in Intestinal Cell Lines Via Apical Chloride Conductances**

**R. Gerhard, A.J. Ries, S. Zeuzem, W.F. Caspary, J. Stein.** II. Medical Department, University Hospital, Goethe University, Frankfurt/Main, Germany

The ability of Mastoparan, a teadecapeptide purified from wasp venom, to activate both heterotrimeric G-proteins and monomeric small molecular weight (smw) G-proteins of the rho subfamily (rho, rac), was exploited, to investigate the possible role(s) of G-proteins in regulation of epithelial paracellular permeability. The human colon epithelial cell line Caco-2 was used for all studies. Methods: Cells were grown with DMEM/H12 media supplemented with 5% neonatal calf serum, 100 μg/ml streptomycin and 100 U/ml penicillin. Confluent monolayers were subcultured with 0.025% trypsin only in DMEM/H12 supplemented with 5% heat-inactivated fetal calf serum. CM- and LGF-grown, cell cultures were used in Ussing chambers. After stabilization of electrical parameters monolayers were stimulated with 10, 20 and 50 μM Mastoparan. The interaction of mastoparan with smw-G-proteins (rhoA) was investigated by ATP-ribosylation of rho by Clostridium botulinum exoenzyme C3. Results: Apical, but not basolateral, addition of mastoparan (10, 20 and 50 μM) increased IC₅₀ and decreased transepithelial resistance 7, 10 and 12 fold. Both IC₅₀ and transepithelial resistance were reversed by Ca²⁺-free medium or and pertussis toxin (12 h; 14 ng/ml). In contrast mastoparan had no effect on IC₅₀.

**991 Sensitization Due to Aspergillus-Derived Enzymes and Papain In Patients with Crohn's Disease**

**R. Wiewrodt, J. Stein, A. Kühn, W.F. Caspary, R. Buhl. 2nd Medical Department, J.W. Goethe University, Frankfurt/Main, Germany**

**Background/Aim:** It has been claimed that food sensitivities occur often in Crohn's disease and that exclusion of foods with an elevated diet-prolins remission. The objective of this study was to compare the occurrence of sensitizations due to enzymes used in the food industry in patients with Crohn's disease in remission (CD; n = 9, CDAI < 150) with controls (A; n = 9) and healthy nonanotops (NA; n = 9). Methods: Skin prick tests (SPT) were performed with 14 allergen groups and a total of 71 allergens (pollen, plants; flour; animal dander; foods; dust mites; papain; α-amylase etc.) Skin tests to aspergillus derived enzymes (cellulase, amyloglucosidase, hemielulose, pectinase, protease) were performed using a 10 mg/ml solution in 0.9% NaCl. Wheal with diameters > 2 mm were considered positive, and were quantitated. A sensitization index (SI) was calculated for each allergen based on the mean wheal size relative to a histamine control test performed in the same subject, and expressed as histamine equivalents. Results: All subject groups had comparable responses to histamine (NA = 3.5 mm, A = 3.44 mm, CD = 2.52 mm). The results for SI with selected allergens are shown below. Statistical significance was assessed between responses in A and CD subjects.

<table>
<thead>
<tr>
<th>SI</th>
<th>NA</th>
<th>A</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollen</td>
<td>0.07</td>
<td>1.18</td>
<td>0.67</td>
</tr>
<tr>
<td>Asp. enzyme</td>
<td>0.00</td>
<td>0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>Papain</td>
<td>0.00</td>
<td>0.11</td>
<td>0.34</td>
</tr>
<tr>
<td>All allergens</td>
<td>0.28</td>
<td>0.24</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**994 AIF, Sensitive G-Proteins Regulate Transport and Barrier Functions of IECs Via Different Pathways**

**J. Stein, C. Traynor-Kaplan, K.E. Barrett.** Dept. of Medicine, University of California, School of Medicine, San Diego

G-proteins are involved in many signal transduction pathways. The ability of aluminum fluoride (AIF) to activate G-proteins is of interest, since other members of the G-protein superfamily (SM) of GTP was exploited, to investigate the possible role(s) of G-proteins in epithelial function. The human colon epithelial cell line, Caco-2, was used for all studies. Methods: Cells were grown with DMEM/H12 media supplemented with 5% neonatal calf serum, 100 μg/ml streptomycin and 100 U/ml penicillin. Confluent monolayers were subcultured with 0.025% trypsin onto collagen-coated permeable supports. For experiments, cell monolayers were mounted in Ussing chambers. After stabilization of electrical parameters, 5 mM NaF + 10 μM AlF₃ were added to generate AIF. Cells were preincubated with pertussis toxin (14 ng/ml) for 12 h, or the intracellular Ca²⁺ chelator Bapta (0.25 mM) for 45 min. 200 μM deferoxamine, a heavy metal chelator, was added 10 min before NaF. Short circuit current (Isc) and potential difference were measured. Transepithelial resistance was calculated using Ohm's law. Chloride secretion was assessed as changes in ISc. Intracellular CAMP was determined in cell extracts via a commercial ELISA. All values are means ± SEM for n experiments, and were measured 20 min after addition of NaF. Resistance values are given as fractions of the starting resistance. Results: Basolateral, but not apical, addition of AIF increased ISc (2.6 ± 0.5 vs. 13.5 ± 2.4, p < 0.05) and decreased resistance (1.03 ± 0.03 vs. 7.0 ± 0.03, p < 0.05). Both effects were reversed by pertussis toxin and deferoxamine. The effect on ISc, but not resistance, was reversed by Bapta and chloride-free medium. Neither addition of AIF was altered by staurosporine (0.1 μM, n = 3), trifluoperazine (0.1 μM, n = 5). Glucose (10 mM) or 6-phenylisopropyl captopharma C-07312 (10 μM, n = 4). AIF also had no effect on CAMP levels relative, compared with VIP added as a positive control (14.5 ± 1.1; AIF 16.1 ± 1.3; VIP 7.9 ± 1.1 pmol/g protein; n = 4). Discussion: We conclude that AIF-sensitive G-proteins can regulate the transport and barrier function of intestinal epithelial cells. The regulation of the two function

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occurs via different pathways and depends on the side of AIFα addition. The effect on chloride secretion, but not that on resistance, involves calcium but does not appear to require phospholipase C or an unknown mechanism. The effect on resistance is independent of calcium. However, both effects require aluminum and are not mediated by fluoride alone.

905 Superoxide Radicals and Hydrogen Peroxide Increase the Permeability of Caco-2 Cells

J. Ries, B. Gehbandt, Anja Schmitt, W.F. Caspary, J. Stein. Dept. of Medicine, J.W. Goethe University, Frankfurt/Main, Germany

Increased free radical production in the intestine is associated with many pathological conditions such as shock, ischemia or chronic inflammatory bowel disease. These conditions are also associated with increased permeability and mucosal infection. Thus, the aim of this study was to investigate whether various oxygen-derived free radicals were able to alter the intestinal barrier. Methods: Caco-2 cells were grown in DMEM supplemented with 10% fetal calf serum, 100 µg/ml streptomycin and 100 U/ml penicillin. For permeability studies, cells were subcultured with 0.025% trypsin and seeded at a concentration of 400 000 cells/cm² on collagen-coated permeable supports. After 14 days, cells were mounted in Ussing chambers. As permeability marker, fluoroescein isothiocyanate (FITC)-labelled dextran, with a molecular weight of 4400 D, was added apically at a concentration of 0.2 mg/ml. The concentration of the marker in the apical or basolateral bath was measured using a fluorescence spectrophotometer. Xanthine and xanthine oxidase at various concentrations were used to generate superoxide radicals. Data were expressed as FITC-dextran basolateral concentration as percent of the apical side concentration. Results are given as means for 4 experiments ±SEM. Results: FITC-dextran progressively accumulated in the basolateral reservoir even at an extremely low concentration. The results show that the permeability of the intestine is increased by the addition of an oxygen-derived free radical. After xanthine/xanthine oxidase application, the increase is higher than xanthine/xanthine oxidase alone.

906 Nasoenteral Nutrition for HIV Associated Wasting — Comparison and Outcomes

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The role of nasoenteral nutrition (NEN) in the treatment of the HIV associated Wasting Syndrome is unclear, up to now only 4 case reports (each involving one patient) have been published as abstracts. The purpose of this study was to evaluate acceptance, outcome and management strategies for enteral nutrition in malnourished Aids patients. Methods: 89 Aids patients (CDC stage C) with weight loss > 5% of their usual body weight underwent a medical work up for treatable disease and an intensified nutritional counseling. 23 Patients had no acute treatable opportunistic infection, tumor or contraindication for enteral feeding and were offered NEN support. 12 patients declined NEN, 2 other patients did not finish the study due to cosmetic reasons. 23 Patients completed the 4 week study. 2000 kcal of a polymeric diet were given via a pump through a CH 8, polyurethan naso-gastric or duodenal feeding tube. Recently initiated AZT/Ddi therapy was an exclusion criterion. Results: Mean weight at the beginning of the study was 52.4 ± 7.0 kg (SD) and mean age was 43 ± 10 years (range 25 - 69). Patients were in the role of NEN intervention. The mean weight decrease was 1.8 kg, and 5 patients had to be reeducated and the CMV infection was reactivated. After starting CMV therapy he gained 5 kg after NEN was restarted. Albumin, transferrin, cholesterol and lipids tended to be increased, but this was not significant. CD count and p24Ag were unaltered. NEN induced weight gain was seen in 9 patients. Mean Body Mass Cell (MBC) increased (BMI, measured by BIA) increased weight significantly 0.9 ± 0.9 kg (SD) (P = 0.003) from 20.5 ± 2.4 kg to 21.4 ± 2.8 kg. One of the 9 patients who lost 1.8 kg, was reeducated and had CMV elimination. After starting CMV therapy he gained 5 kg after NEN was restarted. Albumin, transferrin, cholesterol tended to be higher after NEN, but this was not significant; CD count and p24Ag were unchanged. No NEN induced complications were seen. Conclusions: For patients not gaining weight with nutritional counseling (step 1), enteral nutrition (step 2) is indicated before institution of more costly and risky parenteral nutrition. Step 3 is a less invasive and alternative procedure to NEN. The increase in weight and BCM in all patients not having an active opportunistic infection. NEN was well tolerated, still all 49 patients who asked for continued nutritional support offered an alternative to NEN after completion of the study. We therefore recommend NEN in the initial phase of enteral nutrition therapy, to evaluate if enteral therapy is effective, or if contraindications to PEG exists. For long term use of enteral nutrition a PEG is preferred by patients despite being an invasive procedure.

907 Detection of Interferon-Gamma (IF-Gamma) in Mucosal Biopsies of Crohn’s Disease by RT-PCR

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Purpose of the study: There are still conflicting results in the role of T-cell mediated immune response in Crohn’s disease. Based on the cytokine expression it is possible to estimate the degree of T-cell-activation in inflamed intestinal mucosa. The aim of the present study is to detect locally expressed cytokine mRNA levels of Interleukin 2 (IL-2) and IF-gamma in the intestinal mucosa of patients with Crohn’s disease. Methods: RNA was isolated from the mucosal biopsies obtained by colonoscopy of 20 patients with Crohn’s disease. The mRNA levels of IF-gamma were determined by a semi-quantitative [RT-PCR] assay. The results were visualized by agarose gel electrophoresis and confirmed by Southern blot hybridization. The clinical activity was assessed using the Crohn’s disease activity index (CDAI). The degree of inflammation was scored. Results: In 60 CD patients with Crohn’s disease IL-2 and IF-gamma were detectable (2/8 with a CDAI 150–180 and 4/6 with a CDAI of 180–230). In the control group no sample was positive for IF-gamma but 2/6 of the control samples were positive for IL-2. Conclusions: To our knowledge this is the first in vivo detection of interferon gamma by RT-PCR in Crohn’s disease. The results showed that the T-cell mediated immune response in the mucosa of Crohn’s disease. According to the TH1/TH2 model the increase of IF-gamma could be connected with the absence of TH2 cytokines like IL-4 and IL-10. There was no significant correlation of a positive cytokine expression with clinical activity.

910 Neuropeptide Y Microinfused into the Paraventricular Nucleus of the Hypothalamus (PVN) Stimulates Colonic Transit by Central CRF-Pathways

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We have shown that Corticotropin-Releasing Factor (CRF) in the PVN is involved in mediating stress induced alterations of colonic transit. The PVN contains numerous NPY-IL positive nerve fibers, terminals, and NPY receptors. NPY projections to the PVN directly synapse on CRF-IL neurons. Central NPY exposure upregulates the hypothalamic CRF concentration and release. Also, NPY in the PVN is well established, to be involved in CNS regulation of feeding behavior and, CRF-antagonist administration into the CSF augments NPY-induced feeding. Thus, our Aim was first to determine, if the PVN is a sensitive site for different doses of exogenous NPY to influence colonic motor activity in fed and fasted animals, and second to investigate if central CRF is involved in this effect. Methods — Study 1: SD rats were chronically implanted with a silicone catheter into the proximal colon, and a microinjection cannula into the PVN. In fasted, freely moving rats a radioactive marker was injected into the colon, and NPY, CRF or vehicle was microinjected into the PVN (75 nl). Colonic transit was evaluated by the geometric center method 60 min after marker injection. Study 2: Rats were additionally implanted with an injection cannula into the lateral ventricle (ICV). The CRF-antagonist, aghical CCRF₉₋₄₁ was injected into the ICV (50 µg) 15 min before microinjection. Studies 2/4: In non-fasted, freely moving rats, central injections were performed as described, and 0.2 ml of an iso-osmolar dye was injected into the colon. Colonic transit time was evaluated as the time between marker infusion and discharge of the first colored pellet.

Results:

Studies 1 & 2

<table>
<thead>
<tr>
<th>Peaks into PNV</th>
<th>BSA</th>
<th>NPY</th>
<th>NPY (150 ng)</th>
<th>NPY</th>
<th>CRF</th>
<th>CRF (1.5 µg)</th>
<th>CRF (3.0 µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon transit (min)</td>
<td>33.4 ± 0.4</td>
<td>4.3 ± 0.7</td>
<td>5.5 ± 0.5</td>
<td>4.7 ± 0.2</td>
<td>4.8 ± 0.3</td>
<td>5.1 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Transit time (min)</td>
<td>36.9 ± 2.1</td>
<td>410 ± 11</td>
<td>247 ± 9</td>
<td>209 ± 2.0</td>
<td>322 ± 10</td>
<td>253 ± 31</td>
<td></td>
</tr>
</tbody>
</table>

Studies 3 & 4

<table>
<thead>
<tr>
<th>Peaks into Colonic fluid (CSF)</th>
<th>NPY</th>
<th>CRF</th>
<th>aghical CCRF₉₋₄₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA into Cerebrospinal fluid (CSF)</td>
<td>NPY</td>
<td>CRF</td>
<td>aghical CCRF₉₋₄₁</td>
</tr>
<tr>
<td>Colon transit (min)</td>
<td>34.0 ± 0.3</td>
<td>4.8 ± 0.2</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>Transit time (min)</td>
<td>348 ± 14.2</td>
<td>218 ± 9.2</td>
<td>209 ± 20</td>
</tr>
</tbody>
</table>

mean ± SEM; *p < 0.05 vs BSA; **p < 0.05 vs BSA-ICV; ANOVA & Student-Newmans-Keuls

Conclusions: These studies demonstrate, that the PVN is a sensitive site for NPY to induced a dose-related stimulation of colonic transit in fed and
fasted conditions. The data show, that the NPY-effect in the PVN is mediated by central CRF-pathways. The results suggest, that NPY in the PVN might play a role in central CRF-mediated stress-induced alterations of colonic motor activity.

911 Colonic Distention-Induced C-Fos Expression in the Nucleus Tractus Solitarii (NTS) is Diminished by the 5-HT3-Receptor Antagonist Granisetron
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The c-fos immediate early gene is acutely induced in many brain regions by relevant physiological stimuli. Thus, c-Fos immunoreactivity (c-Fos-IR) is a useful marker to identify activated neuronal systems. Intraluminal distension of intestinal organs is a well established technique to investigate visceral sensation. This study investigate the pathophysiology of diseases where alterations of intestinal perception are suspected, e.g. the irritable bowel syndrome (IBS). 5-HT3-receptor antagonists have been proposed as a therapeutic alternative in the treatment of IBS. The aims of our studies were, first to establish a rat model to investigate neurotransmission of colonic afferent information to the brain, and second, to determine if 5-HT3-receptor blockade affects central c-fos expression. Methods: Study 1: In awake, fasted, male SD-rats a balloon sealed to tygon tubing was inserted into the colon, and inflated with 10 mmHg non-nosius (10-40 mmHg) or nosius (70 mmHg) intraluminal pressures by use of a computerized barostat device. After two hours, animals were deeply anesthetized, transcardially perfused with buffer followed by Zamboni's fixative. After cryoprotection, sections (30 μm) were either stained for c-Fos-IR or after Nissl's method for anatomic control. Study 2: Animals were injected i.p. with granisetron (SmithKline Beecham; 500 μg/kg saline) at 18 hrs (650 μg/kg), 30 rain and 15 min (375 μg/kg each) before colonic distension (CD). Controls were injected with vehicle, Results: We observed an increase in c-fos-expression dependent on the intensity of CD in certain neuronal nuclei. In study 2 (N = 3) pretreatment with granisetron reduced CD-induced (70 mmHg) c-Fos-IR in the NTS by 38% (average number of c-Fos-IR positive cells section: vehicle: 57 ± 8, granisetron: 35 ± 6). Conclusions: Stimulus intensity of CD is reflected best by c-fos expression in the NTS and the peri-aqueductal gray. Whereas, in the amygdaloid and hypothalamic c-fos expression seems to be affected by other influences, e.g. handling. The data suggest that 5-HT3-receptors play a major role in conveyingafferent information about physical distension of the colon to the NTS.

912 CCK Induces C-Fos Expression in the Colon of Coeleus (LC), the Nucleus of the Solitary Tract (NTS) and the Paraventricular Nucleus of the Hypothalamus (PVN) Via Capsaicin-Sensitive Vagal Pathways and CCK-A-Receptors
H. Mönikes, G. Lauer, R. Arnold. Dept. of Medicine, Division of Gastroenterology and Endocrinology, Philips-University, Marburg, Germany
We recently showed that i.p.-injection of cholecytokinin-8S (CCK) dose-dependently activates neurons in the LC as measured by c-fos expression. Also, it has been demonstrated that i.p.-injection of CCK-8S induces c-Fos-IR in the PVN and the NTS; this effect on the NTS depends on vagal nerve activity. Further, we have demonstrated that corticotropic-releasing factor (CRF) in the LC mimics stress-induced alterations of gastric acid secretion and colonic function, suggesting that the LC might play a role in stress-related alterations of gastrointestinal (GI) function. In addition, it was hypothesized before that peripheral CRF acts on noradrenergic CNS-neurons. The Aims of this studies were first, to determine whether capsaicin-sensitive vagal pathways and CCK-A or CCK-B receptors are involved in the CCK-induced c-fos expression in the rat LC, and second, to determine if these neurons are catecholaminergic. Methods: In awake, non-fasted, male rats antagonists for CCK-A-receptors (MK-329, 1 mg/kg), CCK-B-receptors (L-365,260, 1 mg/kg), or vehicle were given i.p. at 45 min before i.p.-injection of CCK-8S (10 μg/kg) or vehicle. Two hours after peptide injection animals were transcardially perfused with Zamboni's fixative. After cryoprotection, brain sections (30 μm) were stained for C-Fos-IR. In a second study, rats were pretreated with peripheral application of the sensory neuron-specific enkephalinase sub 2 weeks before experiments. In the third study, we used a two-colour staining technique to visualize in the same neuron C-Fos-IR and the cytoplasmatic immunoreactive tyrosine hydroxylase (TH), as a marker for catecholaminergic neurons. Results: (mean ± SEM; *p < 0.05 vs NaCl 0.9% vehicle; #p < 0.05 vs CCK-8S/vehicle; ANOVA & Student-Newmans-Keuls). The CCK-A-antagonist markedly reduced C-Fos-IR in the NTS, LC and PVN, but the CCK-B-antagonist had no effect.

913 Regulation of the GLP-1 Receptor Expression by Glucagon
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Previously, we reported that pretreatment of insulina cells (RINm5F) with dexaemethasone resulted in a decreased binding of glucagon-like peptide-1 (GLP-1). Since the mechanism of this dexaemethasone effect was so far unclear, we now investigated glucagon receptors (GLP-1 receptor mRNA) expression in vitro in RINm5F cells. The cells were cultured in standard cell culture medium, containing 1% bacitracin and 10% fetal calf serum (FCS). After preincubation with dexamethasone (100 nmol/l) and progesterone (100 nmol/l) alone, or in addition with RU 486 (100 nmol/l), RNA was isolated and GLP-1 receptor mRNA quantitated by Northern blot and slot blot hybridization. Treatment with dexamethasone, RU486 or a combination of both for 6 hours led to a significant increased GLP-1 receptor mRNA levels (% of control: 151 ± 9, 177 ± 12, and 153 ± 18%, respectively; mean ± S.E.M). These increased mRNA levels returned to control levels after incubation for 12 hours (dexamethasone) and 24 hours (RU 486, dexamethasone + RU 486) and decreased continuously in incubations up to 72 hours (72 ± 4%, 72 ± 7%, and 72 ± 5%, respectively; mean ± S.E.M). Treatment with progesterone (100 nmol/l) had no effects on mRNA levels. Furthermore, binding experiments were performed. Scatchard analysis of the binding data revealed, that receptor numbers were increased 2.5 fold after 6 hours preincubation with dexamethasone (100 nmol/l), returned to control levels by 24 hours and became reduced to 18% by 48 hours.

Our data suggest that a decreased GLP-1 receptor expression may play a role in the development of an impaired glucose tolerance in steroid-induced diabetes mellitus.

914 Differential Effects of Erythromycin on Postprandial Antroduodenal Motility and Gastrointestinal Secretion in Humans
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Erythromycin (E) is a motilin agonist stimulating gastric motor and pancreatic but not gastric acid secretion in the interdigestive state. The Aim of this study was to characterize the effect of E on gastrointestinal secretion and antroduodenal motility in the postprandial state and to study if these effects are mediated via the cholinergic system. Methods: 8 healthy volunteers underwent 3 studies in random order. After a 30-min interdigestive basal period, an amino acid-solution was perfused into the duodenum at 1.2 kcal/min for 210 min in order to stimulate both, pancreatic enzyme and gastric acid secretion and to induce a fed motor pattern. On two days, the first 30-min postprandial period was followed by two subsequent 90-min study periods with E which was IV infused at 0.7 mg/kg and 3.5 mg/kg for 10 min, respectively. Background infusions were 0.154 M NaCl on one day and atropine (A; 5 μg/kg/h) after an initial bolus of 5 μg/kg on the other day. On the third day, the control study was repeated with background infusion of 0.154 M NaCl for 210 postprandial min. Antroduodenal motility and gastrointestinal secretion were measured throughout each experiment with transnasal cervical differential measurement monitoring correct transploric position of the duodenal tube. Parameters of motility and secretion were expressed as area under the response curve above basal. Results: In the fed state, 0.7 mg/kg E induced a gastric phase II-like activity within 10 min which propagated to both, antrum and duodenum in the following postprandial phase I. This motor phenomenon was accompanied by a pancreatic but not a gastric secretory peak. Therefore, low-dose E neither stimulated antroduodenal motility nor pancreatic secretion (Mean ± SEM; *p < 0.05 vs NaCl 0.9%, #p < 0.05 vs E + A):
The image contains a page from a scientific publication, likely from a journal, discussing various aspects of gastric emptying and its mechanisms. The text is filled with scientific terms and data, indicating a detailed study on the subject. However, without the specific content or context, it's challenging to provide a coherent summary or answer specific questions based on this image alone. The page includes tables, graphs, and detailed statistical analyses, typical of biomedical research articles.
each experiment, blood was regularly sampled to determine glucose, IR-C-peptide, IR-Insulin, IR-GIP and IR-GLP-1 (antibody GA 1178) over 180 min. Results: Timed Duodenal: The oral administration of glucose caused a common, load-dependent GIP release with the plateau being achieved after 30 (1 kcal/min) and 15 (2 kcal/min) min, respectively. The lower duodenal glucose load failed to increase GLP-1 plasma levels whereas the higher load brought about a steady-state of duodenal GLP-1 release after 15 min. Oral administration of the same glucose loads induced a dose-dependent GIP release peaking at 30 min. Afterwards, IR-GIP gradually declined returning to basal level at 150 min with 45 g and remaining elevated throughout the study period with 90 g. GLP-1 release to oral glucose was load-dependent, peaked at 15 min with 45 g and at 30 min with 90 g. Basal levels resuscitated at 75 min with 45 g and at 180 min with 90 g glucose.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC over basal</th>
<th>180 min</th>
<th>D: duodenal, O: oral load</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/45 g</td>
<td>0/45 g</td>
<td>0/45 g</td>
<td>0/45 g</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>1.47 ± 3.9</td>
<td>25.4 ± 3.0</td>
<td>15.3 ± 2.4</td>
</tr>
<tr>
<td>IR-GIP (ng/l)</td>
<td>1.3 ± 0.2</td>
<td>3.1 ± 0.5 *</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>IR-GLP-1 (mmol/l)</td>
<td>0.1 ± 0.2</td>
<td>15.9 ± 3.3</td>
<td>15.0 ± 2.8 ±</td>
</tr>
<tr>
<td>IR-insulin (mmol/l)</td>
<td>54.2 ± 25.6</td>
<td>59.2 ± 28</td>
<td>261.3 ± 39.7 #</td>
</tr>
<tr>
<td>IR-C-peptide (mg/l)</td>
<td>12.5 ± 12</td>
<td>23.5 ± 1.7 *</td>
<td>14.9 ± 1.0</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001 vs. 45 g glucose, same route of administration.

### Conclusions

A comparable GIP release was induced by oral administration and duodenal perfusion of 45 g glucose. With 90 g, the oral route released more GIP probably reflecting the initial rapid gastric emptying exceeding the duodenal delivery. The oral route of administration of identical glucose loads yielded a markedly higher GLP-1 release than the duodenal route. This finding would reflect higher duodenal delivery of glucose associated with the early phase of gastric emptying. By contrast with GIP, a threshold delivery of 1 kcal/state of the duodenum must be exceeded to release GLP-1. To test luminal presence or absorption of glucose is insufficient to induce secretion of GLP-1. We suggest that a threshold rate of duodenal nutrient flow and/or nutrient absorption would initiate a neural and/or hormonal signal to the distal gut glucose concentration. The incretin effect in response to low oral glucose loads would be mainly mediated by GIP. The markedly higher insulin release by oral as compared to duodenal administration of glucose would be explained by distinctly higher GLP-1 (lower glucose load) and GLP-1 (GIP higher load).

### Pattern of Intestinal Motility: Effects of Cisapride

M. Knuth, J. Schier, T. Becker, U. Wanka, R. Arnold, Dept. of Gastroenterology, University of Marburg, Germany

The spatial and temporal patterns of individual contractions in the human jejunum following a solid meal or pharmacological stimuli remain to be elucidated. Investigation of this issue necessitates a manometric technique with multiple, closely spaced recording sites. This study was designed (a) to compare the motor patterns in the interdigestive phase I and II and in the postprandial state after a solid meal and (b) to assess the effect of cisapride, a stimulator of cholinergic neural input, on these patterns.

Methods: Healthy male volunteers were randomized to placebo, 10 mg or 20 mg cisapride three times a day for 3 days according to a randomized, double-blind crossover design. On the 4th day (last dose 30 min before gastointestinal intubation), jejunal motility was recorded by perfusion manometry (8 ports spaced at 2 cm-intervals) for a complete interdigestive cycle and for 180 min following a solid 453 kcal meal (45% carbohydrate, 37.7% lipid, 17.3% protein). Analysis of motor events was computerized. The time windows defining propagation of single contractions were derived from the mean ± 2SD of propagation velocity in phase III. Individual migrating contractions (IMC) were visually identified as contractions of high amplitude (> 40 mmHg) and long duration rapidly moving across the entire recording segment.

### Results

Data represent the average of all 8 channels or the sum of all contractions from all channels. Mean ± SEM; *p < 0.01 or less vs. placebo; #p < 0.01 or less for placebo/postprandial vs. placebo/phase II

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>10 mg Cis</th>
<th>20 mg Cis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peaks/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude (mmHg)</td>
<td>2.14 ± 0.6</td>
<td>2.38 ± 0.3</td>
<td>23.6 ± 0.7</td>
</tr>
<tr>
<td>Mobility index</td>
<td>4.35 ± 0.24</td>
<td>4.11 ± 0.15</td>
<td>4.24 ± 0.09</td>
</tr>
<tr>
<td>% Propagated peaks</td>
<td>18.7 ± 2.8</td>
<td>27.7 ± 1.7</td>
<td>25.8 ± 2.1</td>
</tr>
</tbody>
</table>

### Postprandial

| Peaks/min |          |          |          |
| Amplitude (mmHg) | 2.0 ± 0.2 | 3.0 ± 0.25 | 2.7 ± 0.19 |
| Mobility index | 4.16 ± 1.0 | 4.60 ± 0.4 | 4.91 ± 0.09 |
| % Propagated peaks | 28.7 ± 2.0 | 33.5 ± 7.2 | 33.9 ± 2.3 |
| Sum of peaks | 2450 ± 229 | 3397 ± 255 | 3118 ± 213 |
| Sum of propagated peaks | 727 ± 109 | 1189 ± 123 | 1016 ± 116 |

### 10-7 M cisapride

| IMC: postprandial |          |          |          |
| Amplitude (mmHg) | 40.6 ± 1.7 | 7.3 ± 2.4 | 4.9 ± 2.3 |
| Amplitude (mmHg) | 40.6 ± 1.3 | 5.2 ± 2.4 | 52.9 ± 2.0 |
| IMC duration (s) | 2.6 ± 0.1 | 6.0 ± 0.1 | 6.2 ± 0.2 |

### Conclusions

In the human jejunum, the motor pattern following a solid meal differs from the interdigestive phase II in that contraction frequency and proportion of propagated contractions markedly rose. These phenomena are supposed to be important motor correlates of the accelerated postprandial transit. Stimulation of cholinergic neural input by cisapride primarily enhances motility parameters which govern intestinal transit (Am J Physiol 1991;259:G420-429). In interdigestive phase I and II in the postprandial state, cisapride clearly increases the total numbers of single contractions and individual migrating contractions. 10 and 20 mg cisapride are equipotent.

### P35 Expression in Colorectal Cancer

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Colorectal tumors appear to rise as a result of the mutational activation of oncogenes coupled with the inactivation of several tumor suppressor genes. The p53 gene, which is frequently mutated not only in colon cancer but in several other tumor types, has been shown to be the target of allelic loss events on chromosome 17. In this study, the expression of p53 antigen (p53 ag) in colorectal cancer was correlated with the occurrence of anti-p53 serum antibodies (anti-p53 ab).

92 patients who underwent surgical resection of colorectal cancer during 1990-1994 were studied. Tissue tumors were snap frozen and cut into 5 μm sections. P35 ag expression in tumor tissue was detected by western blotting (antibody H223, n = 92) and by immunohistochemistry (IHC) (antibody DO-1, n = 67). 40 sera were available for anti-p53 ab testing by western blot.

P53 was detected in 43 of 92 patients (46.7%) by immunoblotting. Out of 67 tumors (44.8%) showed positive staining by IHC. The correlation between the two methods was highly significant (p < 0.10, Fisher’s exact test, 2-tail). Anti-p53 ab were detected in 9 of 40 patients (22.5%). All of the anti-p53 ab positive patients revealed elevated p53 ag levels in their tumor tissue. 21 of the 31 patients, who were anti-p53 ab negative showed no elevated p53 ag levels in the tumor tissue. 10 patients, negative for anti-p53 serum ab, showed increased steady state levels of p53 in their tumor tissue. Overall the correlation between p53 ag expression in tumor tissue and the occurrence of anti-p53 serum ab was significant with p < 0.0003 (Fisher’s exact test, 2-tail).

In our study we could show p53 overexpression in about 45% of colorectal tumors. An anti-p53 antibody response was detected in 22.5% of the patients examined, all belonging to the group with elevated p53. These data support the hypothesis that p53 accumulation is a pre-condition for the induction of the anti-p53 antibody response. Further, an anti-p53 antibody response can be regarded indicative for an underlying p53 overexpression in the corresponding tumor tissue in colorectal cancer.

### Pre-treatment with Bioluminescence Sensitivity to Block APO-1/Fas Mediated Apoptosis in Hepatoma Cells

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Apoptosis is the most common form of eukaryotic cell death. The APO-1/Fas,
a type I transmembrane receptor, is able to mediate apoptosis. Triggering of APO-1/Fas by agonistic antibodies such as anti-APO-1 or anti-Fas or by the natural ligand (APO-1/Fas-L) induces apoptosis in a dependent manner. We have previously shown that treatment of primary human hepatocytes with anti-APO-1 resulted in rapid cell death. The aim of this study was to evaluate the effect of anti-APO-1 and APO-1-L on hepatoma cells.

Hep G2 cells were treated with anti-APO-1 or APO-1-L, with or without pretreatment with bleomycin. Fab-fragments against APO-1/Fas, which prevent binding of the ligand, were applied to assess the specificity of the apoptotic response. The induction of apoptosis was assessed morphologically and by DNA staining.

Untreated Hep G2 cells and Hep G2 cells treated with anti-APO-1 or APO-1/L did not show signs of apoptosis. Cells treated with bleomycin displayed signs of apoptosis in up to 5% of the cells. In contrast, sequential treatment with bleomycin followed by addition of anti-APO-1 or APO-1-L-L revealed condensation and fragmentation of the nuclei, typical for apoptosis, in up to 80% of the cells. This effect was inhibited in a dose dependent manner by anti-APO-1 Fab fragments.

Pretreatment of HepG2 cells with bleomycin induces responsiveness of hepatoma cells toward APO-1/Fas mediated apoptosis. This phenomenon can be specifically blocked. This argues for a role of the APO-1/Fas system in bleomycin induced apoptosis. Pretreatment of HepG2 cells can be used as in vitro model for the study of APO-1/Fas mediated apoptosis in the liver.

Pre-treatment with Bleomycin Induces Sensitivity Toward APO-1/Fas Mediated Apoptosis in Hepatoma Cells

M. Muller1, E. M. Aliño-Awam1, J. Hagelstein1, P. Krammer2, W. Stremmel2. 1Department of Internal Medicine IV, University of Heidelberg; 2German Cancer Research Center, Heidelberg

Apoptosis is the most common form of eukaryotic cell death. The APO-1/Fas, a type I transmembrane receptor, is able to mediate apoptosis. Triggering of APO-1/Fas by agonistic antibodies such as anti-APO-1 or anti-Fas or by the natural ligand (APO-1/Fas-L) induces apoptosis in a dependent manner. We have previously shown that treatment of primary human hepatocytes with anti-APO-1 resulted in rapid cell death. The aim of this study was to evaluate the effect of anti-APO-1 and APO-1-L on hepatoma cells.

Hep G2 cells were treated with anti-APO-1 or APO-1-L, with or without pretreatment with bleomycin. Fab-fragments against APO-1/Fas, which prevent binding of the ligand, were applied to assess the specificity of the apoptotic response. The induction of apoptosis was assessed morphologically and by DNA staining.

Untreated Hep G2 cells and Hep G2 cells treated with anti-APO-1 or APO-1/L did not show signs of apoptosis. Cells treated with bleomycin displayed signs of apoptosis in up to 5% of the cells. In contrast, sequential treatment with bleomycin followed by addition of anti-APO-1 or APO-1-L-L revealed condensation and fragmentation of the nuclei, typical for apoptosis, in up to 80% of the cells. This effect was inhibited in a dose dependent manner by anti-APO-1 Fab fragments.

Pretreatment of HepG2 cells with bleomycin induces responsiveness of hepatoma cells toward APO-1/Fas mediated apoptosis. This phenomenon can be specifically blocked. This argues for a role of the APO-1/Fas system in bleomycin induced apoptosis. Pretreatment of HepG2 cells can be used as in vitro model for the study of APO-1/Fas mediated apoptosis in the liver.

Pre-treatment with Bleomycin Induces Sensitivity Toward APO-1/Fas Mediated Apoptosis in Hepatoma Cells

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**Clinical Significance of Serum Thrombomodulin Levels in Inflammatory Bowel Disease**


The pathogenesis of Crohn’s disease (CD) and ulcerative colitis (UC) is still unclear. Nevertheless, the occurrence of anti-endothelial-cell antibodies, ANCA’s, and soluble V. Willebrand factor indicates a vascular component in active disease. This is especially true of UC. To further evaluate the importance of vasculitis in CD and UC we determined the levels of serum thrombomodulin (sTM). Thrombomodulin is the endothelial receptor for thrombin. sTM is only released upon endothelial cell damage.

60 serum samples from 31 patients with CD (18 females, 13 males, mean age 32 ± 11) and 40 serum samples from 22 patients with UC (7 females, 15 males, mean age 33 ± 11) with histologically and endoscopically proven disease were investigated. Disease activity was calculated using the Crohn’s disease activity index (CDAI) in CD and using the Truelove-index in UC. The sTM values were determined with a specific ELISA (Diagnostica Stago). Sera from 15 healthy laboratory staff members were used as controls.

sTM levels were only significantly elevated in patients with UC and high disease activity but not in CD or less active UC (mean ± SE):

<table>
<thead>
<tr>
<th>Activity</th>
<th>Low</th>
<th>Mild</th>
<th>High</th>
<th>Control</th>
</tr>
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<tbody>
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<td>high</td>
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<tr>
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<td>&gt;250</td>
<td>&lt;10</td>
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<tr>
<td>Number</td>
<td>6</td>
<td>32</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>sTM (ng/ml)</td>
<td>24±5</td>
<td>24±7</td>
<td>28±6</td>
<td>29±7</td>
</tr>
<tr>
<td>sTM range</td>
<td>18-32</td>
<td>12-42</td>
<td>20-33</td>
<td>26-45</td>
</tr>
</tbody>
</table>

*p < 0.001 (2-tailed Student’s t-test for unpaired samples, calculated between control and CD or UC samples)

In this study we showed that sTM is significantly elevated in patients with highly active UC only. These increased sTM levels indicate endothelial cell damage and the development of vasculitis in severe UC. sTM might be considered as sensitive serological marker in severe UC.

**Clinical and Pathological Evaluation of Small Carcinoma of the Pancreas**

T. Manago, H. Tekeyama, Y. Okuda. First Department of Surgery, Nagoya University, Nagoya, Japan

The clinical and pathological characteristics of 26 small carcinomas (less than 2 cm in diameter) of the pancreas were evaluated to identify the unfavorable factors that could affect the prognosis.

Twenty-five of 26 tumors were located in the head and one in the body of the pancreas. Microscopic finding revealed tubular adenocarcinoma in 20 patients (77%), papillary adenocarcinoma in 2 patients (8%), adenomasous carcinoma in 1 (4%), acinar cell carcinoma in I, cystadenocarcinoma in 1 and islet cell carcinoma in I. Capsular invasion (S) was found in 19% (n = 9) of the patients, retroperitoneal invasion (R) in 23% (n = 6), portal venous involvement (P) in 27% (n = 7) and liver metastasis in 4% (n = 1). Lymph node metastases (N) were found in 35% (n = 9) of the patients; posterior and anterior pancreaticoduodenal, superior mesenteric, common hepatic and portal/pancreatoduodenal ligament lymph nodes were each involved in 6-12% lymph node metastasis. In 25 patients with pancreatic head carcinoma underwent pancreaticoduodenectomy or total pancreatectomy and one with pancreatic body carcinoma underwent univisceral pancreatectomy.

Ten patients (38%) had a Stage I lesion (N0, S0, R0, P0, V0). Three patients (12%) were in stage II (N1, S1, R0, P0, V1) and 7 patients (28%) in Stage III (N2, S2, R2, P2 or V2) and 6 patients (23%) in Stage IV. The cumulative 5 and 10 year survival rate was 80% and 48% respectively. In 16 patients with stage II, III or IV survival rate was 42% after 2 years and 0% after 3 years.

The recurrence after surgery was found in the liver (21%), local resection (23%), peritoneal dissemination (18%) and lung (4%). In this study it was shown that more than half of small carcinomas of the pancreas have a fairly extensive spread through the extrapancreatic lymphatic, venous, and neural networks, and have a poor prognosis. However, a small localized lesion without any extrapancreal extension can be resected with a chance of cure.

**Recombinant Expression of Different UDP-Glucuronyl Transferases Identifies Autoepitope Differences of LKM-3 Antibodies in Autoimmune Hepatitis and Hepatitis D**

C.P. Strassburg 1, P. Obermayer-Straub 1, B. Alex 1, R. Tukey 2, M.P. Manns 1, 1 Dep. of Gastroenterology and Hepatology, Medizinische Hochschule Hannover, Germany; 2 UCSD Cancer Center, La Jolla, CA, U.S.A.

Liver-kidney microsomal antibodies termed LKM-3 are detected in approximately 10% of patients with chronic hepatitis D (HDV). The molecular target of LKM-3 was recently identified as family 1 UDP-glucuronyl transferase (UGT-1). LKM-3 autoantibodies appear to be an example of virus-associated autoimmunity. In hepatitis C a similar association with LKM-1 autoantibodies, that are typical for autoimmune hepatitis (AIH) type 2, has been characterized. In AIH type 2 LKM-1 is predominantly directed against the cytochrome P4502D6. There is evidence that LKM-3 autoantibodies also appear in AIH type 2 and are therefore novel markers of AIH. The aim of this investigation was to characterize LKM-3 in AIH and HDV. Family 1 UGT are a polymeric group of enzymes that consist of a variable amino terminal region (which is regulated between exon 1 and 10) and a constant cytochrome terminus encoded by exons 2-5. Human UGT 1.6 and 1.1 and rabbit UGT 1.6 were used as molecular targets to investigate LKM-3 antibodies. Methods: Recombinant baculoviruses expressing human UGT 1.1, 1.6 and rabbit UGT 1.6 were constructed, antigen purified, activity tested and used for immuno blotting (RIBA) and in the solid phase of an ELISA. Three LKM-3 positive patient sera identified among 50 German patients with HDV 50 normal controls, 26 hepatitis B patients, and 45 AIH patients (4/4 n=25; 3/4 n=10; 2/4 n=5; 1/4 n=10; 0/4 n=5). Markers of viral hepatitis were studied. Results: AIH sera recognized both UGT 1.1 and HDV-associated LKM-3 sera recognized human UGT 1.6, but not rabbit UGT 1.6. ELISA titers were higher in AIH than in HDV. Conclusions: Expression of several family 1 UGT identified autoepitopes differences between AIH and HDV-associated LKM-3 and demonstrates a fast strategy of determining autoepitope variability. The molecular epitope of HDV-associated LKM-3 appears to lie in the amino acid sequence dissimilarity between UGT 1.1 and 1.6. In AIH, in contrast, LKM-3 autoantibodies recognized all studied UGT and have higher titers. Additionally, LKM-3 appear to be novel markers of AIH that are found in 8% of our sera with AIH type 2, and in 6% of sera with HDV.

**Functional Relevance of Mutations in the Hepatitis B Virus Pre-S Genome Before and After Liver Transplantation**


Reinfection with the hepatitis B virus after orthotopic liver transplantation (OLT) is a serious complication associated with increased morbidity and mortality. The translation product of the preS region in the HBV genome has been shown to be immunodominant in host defense mechanisms and has been implicated in the attachment and uptake into the hepatocyte. The region p937-4 in the preS1-domain and p1016-12 in the preS2-domain are shown to be bound to hepatocyte membranes in vitro. The regions p21-47, p120-141 or p133-150 are all known to confer protective immunity in chimpanzees. The region p133-145 binds to the transferrin receptor of T cells and is able to inhibit T cell autorepresentation.

We investigated 20 patients who required OLT for HBV related disease. Only patients were selected suffering from reinfection of the graft. 7 of these 20 patients were found to have HCC in the explanted liver. All but 2 patients received anti-HBs prophylaxis post-OLT. Reinfection occurred in 6 of the investigated patients during anti-HBs prophylaxis with anti-HBs-levels between 91 and 169 U in 4 patients and anti-HBs-levels between 4 U and 17 U in the remaining 2 patients at the time of reinfection. HBV-DNA was extracted and the preS-region was amplified via PCR from HBSAg positive sera collected before and after transplantation. In order to analyse also minor virus populations PCR-products were cloned. After EcoRI digestion insert size was analysed by agarose gel electrophoresis. Sequencing was performed of at least 4 clones if no difference in insert size was detected. Clones with different insert size were sequenced additionally.

70% of the patients were infected with the preS-subtype adw2, the remaining 30% with the preS-subtype ayw as determined by sequence homology to published data. The region p21-47 was damaged in one patient in all sequenced clones and in one patient in 25% of the sequenced clones by in vitro mutation in material recovered pre-OLT. Post-OLT both patients show only wildtype (wt) virus after reinfection. Damage in the region p120-145 by either insertion or deletion was found in material recovered from 6/20 patients. In 5 of them at least one known human B cell epitope was affected. 4 of these patients showed major deletions in 100%, 75% and 25% of the sequenced clones within p120-145 post-OLT with reemergence of the wt virus post-OLT. One of the two remaining patients showed identical deletions in this region in 75% of the sequenced clones also post-OLT. The other patient showed total destruction of this region by a big insertion mutation in 25% of the clones post-OLT.
Most of the cases with deletions, insertions or mutations in B-cell epitopes return to virulence in the major virus population post-OLT due to immune suppression. The possibility of de novo infection instead of reinfection must be considered. These data indicate that either p21-47 or p120-145 alone are sufficient for virus attachment to host cells in vivo. Alternatively, additional mechanisms may exist for the uptake of the virus.

932 Anti-HCV IgM in Hepatitis C Reinfiction After Liver-Transplantation

Anti-HCV IgM levels were correlated with the activity of hepatitis C virus (HCV) induced liver disease and the response to interferon. The aim of the study was to investigate the influence of HCV-PCR-SSCP and direct nucleotide sequence analysis and therefore heterogeneous sub-populations of p53 mutated cells particularly at an early tumor stage might sometimes not be found.

20 patients suffering from HCC were included into the study. Tumor tissue was microdissected and DNA was prepared from tumor cells. Nucleotide sequence analysis of subcloned PCR fragments of exons 5-8 was performed in order to detect heterogeneous p53 mutations. In a screening procedure 4 clones and in a confirming procedure 12 clones were analyzed. p53 gene alterations were further investigated at the protein level by immunohistochemistry.

Sequence analysis confirmed a mutation in only two cases (10%). One at codon 220 was a homogenous transition in nearly all clones from TAT to TGT. The other mutation was a transversion from CGG to CAG at codon 248. It was found only in 25% of the clones. The immunohistochemistry showed a p53 overexpression only in the latter tumor with a positive staining of 30% of the nuclei.

We therefore conclude that the incidence of p53 mutations in European hepatocarcinomas is very low. A subpopulation of p53 mutated cells within a heterogeneous HCC is a rare event. The contribution of this genetic alteration to hepatocarcinogenesis in Europe seems of little importance.

934 A Hydrophobic Region in the Acidic Transactivation of LAP/IFN-L6 Is Important for the Activation of a Hepatitis C Virus (HCV) Promoter
C. Trautwein, D. Walker, M. Manns, Dept. Gastroenterology and Hepatology, Medizinische Hochschule Hannover, Germany

LAP/IFN-L6 is a liver specific activator of transcription. Besides mediating liver-specific gene expression, LAP is involved in regulating the acute phase response. As phosphorylation of LAP Ser160 in the N-terminal part of the protein enhances the activation of liver specific gene expression, we investigated the contribution of this region to the activity of LAP.

To investigate the interaction of LAP/IFN-L6 with the RNA-polymerase machinery, we constructed LAP mutant proteins containing a series of eight deletions. Increasing parts of LAP from the N-terminus of the protein. In the largest deletion LAP Δ1-170 the N-terminus was eliminated up to close proximity of the DNA-binding domain of LAP. All the deletion mutants were cloned into a CMV-expression vector. To test the effect on transactivation the different deletions were cotransfected with a LAP-responsive reporter plasmid (CRP-CAT) into HepG2 cells. A 10-fold reduction in transactivation compared to the wt was found with the CMV-LAP Δ1-40. All further deletions failed to activate the CRP-CAT higher than background level, even though they were strongly expressed in the nucleus as monitored by gel shift and western blot experiments. To further characterize the C-terminal part of the transactivation domain, multiple internal deletions were introduced into the LAP-ORF and tested for transactivation function in cotransfection experiments. The ability to stimulate the CRP-CAT reporter was closely related to the presence of aa 21-103 in the deletion mutant of LAP suggesting that this region mediates activation of LAP dependent genes.

To further confirm these results domain swapping of all the deletion mutants was performed with the LAP-DNA-binding domain and the GAL4 DNA-binding domain. As with the wt protein, the activation of the GAL4 reporter plasmid was linked to the presence of aa 21-104 of LAP in LAP/GAL4 fusion protein. Interestingly a 100% higher activity of the GAL4 construct was observed, when the LAP 21-104/GAL4 fusion construct was transfected instead of the wt LAP 21-152/GAL4 fusion protein, even though a similar affinity to the GAL4 binding site was shown by gel shift experiments.

Further analysis revealed that this region is rich in acidic amino acids and computer assisted analysis indicated two interesting regions, a helical (aa38-63) and a hydrophobic (aa85-95) region. Single and double mutations of acidic amino acids were introduced in both regions. Transfection experiments showed that mutations in the helical regions do not change the activation of the CRP-CAT, while mutations in hydrophobic region lead to a several fold decrease.

Our results demonstrate that aa 21-103 are the transactivation domain of LAP. This region is rich in acidic amino acids and a mechanism-similar to that described for VP16 or p53 could be important in mediating the activation of RNA-polymerase II. As the transactivation domain from aa 21-103 alone results in a stronger activation than in the consensus with the whole protein, additional inhibitory domains might exist in LAP.

936 Elevated Splanchnic and Systemic Transforming Growth Factor-β2 Levels Are Normalized after Liver Transplantation

Transforming growth factor β (TGF-β) is involved in the development of cirrhosis as a central mediator of fibrogenesis. To determine the relevance of TGF-β2 subtype we investigated circulating and splanchnic levels as well as splanchnic production.

Methods: 20 patients with liver cirrhosis (Child A = 4, Child B = 9, Child C = 7; alcohol = 5, hepatitis = 6, biliary = 6, cryptogenic = 3) and 6 patients in the longterm course after orthotopic liver transplantation were studied. All received hepatic venous catheterization, hepatic venous pressure gradients, and hepatic blood flow were calculated for transgenic mice C57Bl/6, were determined. Hepatic venous and arterial blood samples were assayed for TGF-β2 by ELISA, splanchnic production rate calculations.

Results: While in normal controls no circulating levels of TGF-β2 were detectable with the assay used, all cirrhotics had elevated levels of TGF-β2. Hepatic venous were in the same range as arterial levels (mean 63.3 pg/ml, range 18.7-133.5 pg/ml) and mean 62.3 pg/ml, range 17.2-113.1 pg/ml, respectively). Child A patients had lower levels than those in Child B or C state (p = 0.06 and p = 0.05, respectively). No significant TGF-β2 release in the splanchnic area could be shown, although some patients had marked arterio-venous heparin levels (maximal 20.4 pg/ml). After liver transplantation of patients showed slightly elevated levels compared to normals.

Correlations between TGF-β2 measures and inflammatory activity, hepatic venous pressure gradient, liver blood flow or ICG half-life time were detected.

Conclusions: Patients with liver cirrhosis show significantly elevated circulating and hepatic venous TGF-β2 levels. There is an increase with clinical worsening of liver function. No splanchic release of TGF-β2 as a possible site of production could be demonstrated. After liver transplantation the majority of patients has normalized, i.e. undetectable TGF-β2 levels.
937  
Hepatic Amino Acid Metabolism in Cirrhosis  
In cirrhosis there are characteric changes of peripheral amino acid levels with a decrease in BCAA and an increase in the branched chain amino acids (BCAA) decreased. Hypothetically, AAA and MET elevation has been attributed to lower hepatic clearance. Currently there are no data available where this assumption has been measured directly.  
Methods: We measured hepatic blood flow of BCAA: AAA and MET in 52 patients with cirrhosis of differing etiology (Child A = 7, Child B = 23, Child C = 22). Hepatic vein catheterization and hepatic blood flow measurement (ICG-steady-state-infusion method) were performed. Arterial and hepatic venous blood was collected in EDTA-anticoagulated citrate, glycine, sodium, chloride. Results: The hepatic blood flow was calculated. ICG half-time (ICG-t1/2) and systemic ICG clearance served as measures of liver function.  
Results: A highly positive correlation to lowing of patient's clinical state. There was a correlated decrease of ICG-t1/2 rate with a strongly reduced ICG-t1/2. This was observed with a large group of patients. No significant correlation between arterial and liver function (ICG-t1/2) was observed, too.

938  
Role of Antioxidative Glutathione and Cysteine in Peripheral Blood in Patients with Inflammatory Bowel Disease  
B. Sido, V. Hack, H. Eisenloh, M. Schychowski, G. Schürmann, Ch. Herforth, W. Drage. Dept. of Surgery, University of Heidelberg, and German Cancer Research Center, Heidelberg, Germany  
Oxidative stress due to chronic inflammation is known to play an important pathophysiologic role in inflammatory bowel disease (IBD). Cysteine and glutathione are important natural antioxidants with strong influences on immune activity. Aim of this study was to show, whether chronic IBD leads to a short-age of these antioxidative systems in peripheral blood.

30 surgical patients with IBD (18 with Crohn disease, 12 with ulcerative colitis) that were admitted for restection of the small or large bowel were included in the study. 30 healthy individuals served as a control group. Total intracellular glutathione (GSH) and glutathione disulfide (GSSG) were determined enzymatically in peripheral blood mononuclear cells (PBMC). Plasma samples were treated with sulfosalicylic acid and assayed for cysteine and glycine. Glutathione and cysteine concentration were determined spectrophotometrically as acid-soluble thiols. Values are presented as mean ± SD. T-test is used for statistical analysis.  
Cysteine levels were found to be decreased (11.4 ± 3.5 vs. 13.6 ± 3.2 µM) (P < 0.05) in PBMC from patients with IBD 8/30, compared with patients' PBMC (13.6 ± 3.2 µM). Cysteine was also shown to be decreased in PBMC from patients with IBD (11.4 ± 3.5 vs. 13.6 ± 3.2 µM) (P < 0.05).

939  
Effect of C-Kit Ligand, Stem Cell Factor on Mediator Release from Human Intestinal M cells  
S.C. Bischoff, S. Schwengberg, K. Wordelmann, A. Weimann 1, R. Raab 1, M.P. Manns. Dept. of Gastroenterology and Hepatology Medical School of Hannover, Germany; 1 Dept. of Abdominal Surgery, Medical School of Hannover, Germany  
Mast cells (MC) are capable of releasing multiple inflammatory and regulatory mediators. However, the regulation of mediator release in intestinal MC is largely unknown. Apart from IgE receptor-crosslinking (IgE-RC) no sec- retagogues have been described so far for this cell type. In a previous study, we found that the fibroblast-derived cytokine c-kit ligand or stem cell factor (SCF) strongly modulates mediator release in human lung mast cells. Here, we examined the effect of SCF, IL-3, CSF, FMLP and IgE-RC on human intesti

941  
"Intestinal Prick Test": A New Diagnostic Method for Intestinal Food Allergy?  
S.C. Bischoff, J. Mayer, A. Herrmann, G. Zeck-Kapp 1, P. Meier, M.P. Manns. Dept. of Gastroenterology and Hepatology, Medical School of Hannover, Germany; 1 Dept. of Dermatology, Medical School of Hannover, Germany  
The relevance of intestinal food allergy (IFA) has been questioned, because of the lack of diagnostic values, even when skin tests and IGE levels are not significant in these patients. We selected 29 patients (age 34 ± 12 years, 18 Crohn's disease, 11 ulcerative colitis, 12 unclassified disease) with signs of an intestinal manifestation of food allergy (abdominal pain, diarrhea etc. in reaction to particular foods, elevated serum IgE, eosinophilia, improvement after therapy with sodium cromoglicate or specific elimination diet). These patients underwent colonoscopy and endoscopic intestinal provocation by ingesting allergens selected according to patients history into the cecal mucosa. After 20 min the reaction (wheel and flare) was registered semiquanti-titatively, and biopsies were taken from the provocation areas. 21/29 patients (71%) showed at least one positive reaction. In total, 84 allergens were applied, 42/84 positive reactions (50%) occurred. The allergens most fre- quently inducing positive reactions were apple > hazel nut > milk protein > wheat > paprika > meat > egg. Preclinical studies suggest that eosinophil degranulation correlates with the visible wheel and flare reaction. The results did not correlate with skin Prick test (which was negative in 26/29 patients) nor with measurements of specific IgE (which were positive for at least one food allergen in 24/29 patients). 10/21 patients with positive IFA provocation test could be effectively treated by elimination diet, sodium cromoglicate, without further need of steroids. These data show that IFA provocation test may be useful to ensure the diagnosis of food hypersensitivity and to opti-mize treatment in selected cases of patients with inflammatory bowel disorders and inflammatory symptoms of unclear origin.

942  
Bone Density and Bone Metabolism in IBD. A Clinical Study in 90 Patients  
S.C. Bischoff, A. Herrmann, J. Evers, M. Göke, G. Brabant 1, A. von zur Mühlen 1, M.P. Manns. Dept. of Gastroenterology and Hepatology Medical School of Hannover, Germany; 1 Dept. of Endocrinology, Medical School of Hannover, Germany  
Several recent reports suggested a direct disease-mediated effect of IBD on bone metabolism. We investigated in 80 patients (pts.) with Crohn's disease (CD, n = 61), ulcerative colitis (UC, n = 29), or other IBD (n = 7) biochemical markers of bone metabolism and bone mineral density (peripheral quantita-tive computed tomography at the forearm). Only 32 pts. had a normal density (+SD) compared to an age- and sex-matched control group. In 17 pts. den-sity of the trabecular bone was more than 2 SD below the mean of controls (<60%). Calcium in serum was normal in all pts., but intact PTH serum levels were increased above normal in 15 pts. (mean ± SD: 8.7 ± 2.9 mmol/l). Bone Gly A protein, a serum marker of bone formation, was increased in 8 of the 11 pts. (11.1 ± 3.6 µg/l), whereas the pyridoline cross-linked carboxy-terminal telopeptide of type I collagen (ICTP), a recently described serum parameter of bone breakdown, was stimulated in 33 pts. (10.4 ± 13.1 µg/l). 12 pts. with increased ICTP showed a decreased bone mineral density below 60% and 3 of them never received steroids. An active status of the underlying disease in most of these 33 pts. with increased ICTP (CD: 167 ± 124) suggests a direct effect of the underlying IBD. In the whole series of pts. with inflammatory ac-tivity (CDIA > 15, UCIA = 32), 50% had an increase in ICTP (ICTP > 5 µg/l, mean: 12.9 ± 18.8 µg/l). Several mechanisms may be envi-sioned: 1. a high inflammatory activity directly affects bone metabolism via a yet unknown way; 2. inflammation of the small bowel may decrease intestinal calcium absorption; 3. the treatment with steroids may exert a further bone catabolism. A careful analysis of bone metabolism and supplementation with calcium seems therefore mandatory for the follow-up of pts. with IBD even when no steroids are used for treatment.
**943 Recognition of Three Different Epitopes on UDP-Glucuronosyltransferases by LKM-3 Antibodies in Patients with Autoimmune Hepatitis and Hepatitis D**

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About 10% of the patients from Italy with chronic hepatitis D infection and from the USA with autoimmune hepatitis produce liver-kidney microsomal antibodies type 3 (LKM-3). Recently, we identified family 1 UDP-Glucuronosyltransferases (UGTs) as the molecular target. This finding for the first time characterizes an enzyme of the phase 2 drug metabolism as the target of an autoimmune process. Epitope mapping experiments were performed using the human bilirubin-transferase UGT 1.1 expressed in E. coli. Six sera from patients with autoimmune hepatitis (AIH) and six sera of patients with hepatitis D (HIVD) were included in our study. In general, the titers of the autoantibodies proved to be higher in the sera of patients with autoimmune hepatitis than in HIVD patients. Based on the minimal peptide of recognition, three classes of autoantibodies were found: class 1 was only detected in one patient with AIH and recognizes a peptide consisting of aa 289 to 374, class 2 antibodies exhibit a larger minimal epitope comprising aa 265–374. These autoantibodies are typical for patients with AIH, where five out of six sera tested were consistent with this pattern, while only two out of six sera from HIVD patients exhibited the class 2 pattern of reactivity. Finally class 3 antibodies reacting with a minimal epitope of aa 265–374, are negative, if tested against aa 289–374, but surprisingly exhibit low reactivity against the full length C-terminus consisting of aa 289–332. Class 3 epitopes were found in four out of six HIVD sera and were not present in patients with AIH. In conclusion, we can distinguish between three autoepitopes on UGTs of family 1, that are all conformation dependent and are located in the region of aa 265–374. Experiments are under way to define the epitopes more closely, using hybrid-UGTs between a nonreactive family 2 UGT and UGT 1.1 to overcome the problem of conformation dependence of the epitopes.

**946 Course of Hepatitis C After Renal Transplantation**


Patients with chronic hepatitis C run a risk of developing liver function deterioration after kidney transplantation (KTx). To determine the impact of hepatitis C on long-term morbidity and mortality we analysed 152 anti-HCV positive (second generation test; >85% HCV-RNA positive) of 1236 renal transplant recipients (RTR; prevalence 12.3%), who were seen in our outpatient clinic between January 1992 and December 1994. In patients with hepatitis C age at KTx was 42.8 (6–69) years, mean time on dialysis 78 months and mean follow-up after KTx 88 months. Immunosuppressive regimens and frequency of rejection episodes in anti-HCV positive patients were comparable to the total population. Only 5 (4%) of the 110 anti-HCV positive patients living with functioning transplanted liver. 17 (11%) of the anti-HCV positive patients died; 9 of these (i.e. 2% of the total anti-HCV positive RTR) suffered from liver failure due to sepsis, but none died of terminal posthepatic liver cirrhosis.

Addition of a retrospective analysis of causes of death in all 43 anti-HCV negative (RTR 1991 patients over the period 1968–1992), including all RTR with hepatitis C, liver disease is only responsible for 2% (n = 7) of deaths (7 out of 324) after KTx. In contrast, 34% (26 out of 76) HBSAg positive RTR died; 15 of these (58%) died of terminal posthepatic liver cirrhosis and 12 (42%) suffered from liver failure due to extrahepatic sepsis following KTx.

KTx in patients with hepatitis C and normal liver function appears to be justified because of low morbidity and mortality due to chronic liver disease.

**947 Dopamine maintains the intestinal villus blood flow during Experimental Endotoxemia**

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The gut is considered the "motor of sepsis". Intestinal hypoperfusion with subsequent ischemia and breakdown of the mucosal barrier leads to translocation of endotoxin (LPS). We examined the effects of dopamine (DPX), a non-synaptic transmitter, on intestinal microvascular blood flow in normotensive sepsis in rats using intravital microscopy.

21 male Wistar rats (250–300 g) were randomized into 3 groups: a control group receiving saline 0.9%, a group receiving LPS 1.5 mg/kg over the first hour after our experiment and a third group receiving LPS 1.5 mg/kg over the first hour and DPX 2.5 mg/kg/min during the entire experimental period. After cannulation of the right jugular vein for drug administration and the left carotid artery for blood measurement of blood pressure, heart rate and oxygen saturation, a laparotomy was performed and a loop of the small intestine was exteriorized, opened along its antimesenteric border, and fixed on a plexiglas stage with the mucosa being upside. All animals received fluorescein isothiocyanate-labeled erythrocytes 30 min before the experimental procedure. In each rat, 5 arterial and 5 venous lines were microscopically at 0 min, 10 min, 60 min and 120 min. Blood flow was calculated by counting the number of labeled erythrocytes/min and correlating the counted erythrocytes with the systemic hematocrit of the labeled erythrocytes.

In all groups, the mean arterial blood pressure did not change during the observation period. In the LPS group, microvillus blood flow was reduced 120 min after the start of the LPS exposure (p < 0.05 vs baseline). In the LPS+DPX group, blood flow remained at baseline values despite the LPS administration (p > 0.05 vs LPS).

We conclude that DPX maintains intestinal mucosal perfusion during normotensive sepsis and therefore might be protective to the mucosal barrier.

**948 DBcAMP induces Hyperpolarisation of the Membrane Potential in Hepatocytes by Using Ca++/Calmodulin as Signal Transducer**

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DBcAMP (Dibutyryl-CAMP) led to an increase of the Na-dependent uptake of bile acids. This increase in uptake depends on the concentration of intracellular Ca++. Calmodulin and the cAMP-induced Ca++ efflux (Ca++/Calmodulin). To study the effect of DBcAMP on the CMPP in hepatocytes, we suppose that the DBcAMP induced hyperpolarization of the CMPP in hepatocytes or are the DBcAMP-induced changes just coincident events. Membrane hepatocytes were freshly isolated with the collagenase perfusion method. The MP was calculated with the Nernst-equation using the distribution of 36Cl- across the hepatocyte plasma membrane. We used Quin-2 for the determination of Ca++. Results: Administration of TMBB (a blocker of Ca++/Calmodulin rebound from intracellular stores alone) to hepatocytes did reduce the CMPP (Ca+++, but didn't change the resting membrane potential, TMBB administered to hepatocytes pretreated with DBcAMP significantly reduced the CMPP and lowered the expected DBcAMP induced hyperpolarization of the CMPP of Administration of Thapsigargin or Lonomycin alone increased the CMPP (Ca+++, but didn't change the MP). Each of them in combination with DBcAMP resulted in a sign higher CMPP (Ca+++) but in a lower hyperpolarization. Giving Calmodulin-antagonists (Calmidazolium and WV) alone didn't change the CMPP nor the MP in combination with DBcAMP the DBcAMP induced increase in CMPP wasn't influenced but the DBcAMP induced hyperpolarisation was totally eliminated. Conclusion: High (Ca+++) as well as low (Ca+++ led to a reduction in DBcAMP induced hyperpolarisation. The DBcAMP induced hyperpolarisation does depend on Calmodulin and is regulated by the hight of (Ca++/+). These results would be explained by a Ca+++/Calmodulin-dependent Na-K-ATPase which does change the MP in hepatocytes.

**949 Validation and Differentiation of Endothelin 1/2 and Big-endothelin Inmunoreactivity Detected in Human Bile by HPLC**

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Introduction and Methods: Hepatic secretion of Endothelin (ET) by sinusoidal and biliary epithelial cells has been documented. High levels of biliary ET, correlating with primary graft function were previously published after liver transplantation (OLT). ET-peptides possess a strong cholestatic effect in vivo. We evaluated ET-1 and big-ET-1 immunoreactivity by RIA (FA Biomedica Vienna) in two clinical collections (Samples from the gall bladder of 31 patients during cholescyctectomy (CC) and TUBE-samples from 21 patients after uncomplicated OLT). ET detected by RIA in bile extracts was compared with ELISA data (FA Biomedica Vienna) and validated with HPLC-technology, using purified ET-1, ET-2, ET-3 and big-ET peptides as references in chromatography.

Results: Mean ET-bigET in concentration during CC (ET: 153.7 ± 103.7 pg/ml, bigET: 322.9 ± 123.7 pg/ml) was significantly higher compared to values after OLT (ET: 37.3 ± 24.5 pg/ml; bigET: 136.1 ± 54.6 pg/ml), p < 0.0003. Plasma levels only ranged from 0.4–5.6 pg/ml (ET) respectively 6.0–35.4 pg/ml (big-ET) and were significantly lower compared to simultaneous biliary ET concentrations in both collections (p < 0.0001). Synchronous ET and big-ET levels in bile samples were correlated by linear regression (R = 0.8, p < 0.001). No correlation was found between bile and serum ET levels nor with the degree of local gall bladder inflammation during CC. Elution profiles of synthetic ET-peptides and biliary extracts were identical in HPLC. ELISA and RIA data in HPLC fraction showed a good correlation. According
to the HPLC elution profile. ET-1 is the dominant ET-peptide in human bile. Interestingly, no big-ET-fragment could be detected. The elution profile of bile extracts further demonstrated additional signals (in fraction 29-34), pointing to other ET-propeptides or hepatic ET-metabolites so far not specified.

Conclusions: HPLC bile elution profiles also state the existence of comparably high amounts of immunoreactive ET and big-ET in human bile. The data suggest a potential role of ET-peptides in the regulation of bile duct motility. High biliary ET levels could contribute to functional cholestatic syndromes, often encountered after OLT. Treatment with ET-antagonists could improve bile flow under such circumstances.

955 Effects of Neutralization of TNFα in Chronic Experimental Colitis in Mice

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The balance of pro-inflammatory and anti-inflammatory cytokines seems to be disturbed in inflammatory bowel disease (IBD). Neutralization of pro-inflammatory cytokines such as interleukin-1 and tumor necrosis factor (TNFα) may exert an anti-inflammatory effect by interrupting the cytokine cascade.

Purpose: In order to study the role of TNFα in IBD, we have established a mouse model of ulcerative colitis and used a monoclonal antibody to neutralize TNFα in vivo.

Methods: Chronic colitis was induced in female BALB/c mice by feeding 5% dextran-sulfate (DSS) in drinking water in 4 cycles consisting of DSS for 7 days and a 10 day free intermission. Colitis was detected by histology at 4, 6, 8 and 12 weeks after completion of the induction phase. Mice were used at 4-6 weeks after completion of the induction phase. For treatment (n = 5 per group) mice received either 100 μl saline, 3 mg/kg dexamethasone (DXM), or 100 μg monoclonal rat anti-mouse TNFα antibody by daily intraperitoneal injection for 5 days. Mice did not differ in weight and appearance after treatment. Mice were sacrificed on day 6. Colitis was assessed by histology. Parameters (epithelial dysplasia, ulceration, LP-infiltrate, lymph follicles) were scored individually (0-IV).

Results: Mice treated with DXM showed a significant reduction of scores of LP-infiltrate, lymph follicles and ulceration. Mice treated with anti-TNFα antibody showed a slight reduction of LP-infiltrate and epithelial dysplasia and a significant reduction of ulceration and lymph follicles.

Conclusion: The DSS induced chronic colitis in mice is a suitable model to study the effects of anti-cytokine strategies of therapy. Neutralization of TNFα reduced the histologic inflammatory score of colitis. Further studies on the effects of anti-TNFα therapies appear promising.

956 Specific Advantages of Dextran Compared to Other Colloids in the Treatment of Acute Necrotizing Pancreatitis of the Rat

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Necrotizing pancreatitis is characterized by a substantial decrease of pancreatic microcirculation. Previous studies have demonstrated a protective effect of dextran on microcirculation, acinar necrosis and mortality. This study investigates the effects of dextran compared to the standard therapy and alternative colloids.

Methods: Necrotizing pancreatitis was induced in 60 dextran-tolerant Wistar rats (dxxd Phr) by intraductal glycodeoxycholic acid (10 mM) and intravenous caerulein (5 μg/kg) for 6 hours. Control animals received intraductal and intravenous saline. 6 hours after the intra-ductal induction of pancreatitis animals were assigned to five groups (n = 10/group): Ringer (32 ml/kg); Gelatin, HAES or Dextran (8 ml/kg). Additionally isovolemic hemodilution (HID) with dextran was done by infusion of 4 ml/kg dextran before performing the HID with dextran (8 ml/kg). After death or sacrifice at 24 Hours acinar necrosis was assessed.

Results:

<table>
<thead>
<tr>
<th>Mortality (%)</th>
<th>Acinar Necrosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>IHD</td>
<td>2.0* ± 0.8</td>
</tr>
<tr>
<td>Dextran</td>
<td>6.2 ± 0.7*</td>
</tr>
<tr>
<td>HAES</td>
<td>13.5 ± 2.0</td>
</tr>
<tr>
<td>Gelatin</td>
<td>12.5 ± 1.1</td>
</tr>
<tr>
<td>Ringer</td>
<td>11.9 ± 1.2</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. Ringer, HAES, Gelatine

Conclusions: Intravenous infusion of IHD with dextran reduces acinar necrosis and improves survival rate in acute necrotizing pancreatitis. Treatment with Ringer or alternative colloids is significantly less effective.

958 New Aspects in Monitoring and Therapy in Severe Acute Pancreatitis: Anticytokines and Plasmapheresis

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Introduction: Due to the development of severe complications including multiple organ failure severe acute pancreatitis (SAP) is associated with a high mortality. Enhanced levels of cytokines and acute phase proteins reflect the systemic inflammatory process leading to extracorporeal complications. Our prospective controlled study is intended to investigate the effect of plasmapheresis on the outcome of SAP and the prognostic value of endogenous anticytokines.

Methods: SAP was defined by the presence of necroses together with failure of at least one organ system (cardiovascular, kidney, respiratory failure). Patients were given a standardized intense care treatment and randomized for plasmapheresis or conservative treatment. Serum levels of cytokines (IL-1, IL-6, IL-8, TNFα) as well as TNF receptors (TNFR-p75, TNFR-p75) as endogenous receptor antagonists of the systemic inflammatory response and IL1-receptor antagonist (IL-1RA) were measured during the course of SAP and as control group of mild acute pancreatitis (MAP).

Results: Initially enhanced cytokine levels were higher in SAP. The levels of endogenous antagonists rose to high values in patients with SAP whereas there was little increase of TNF levels in patients with MAP.

During the follow up cytokine and TNF levels decreased in both, MAP and SAP under conservative treatment. In patients with SAP without plasmapheresis TNF receptors decreased in the course of the disease and none survived. In contrast patients with SAP treated with plasmapheresis showed an increase of TNFR's and a better survival rate (75%).

Conclusion: Our data show a trend to better survival when patients with SAP are treated with plasmapheresis. Initially enhanced cytokine levels reflect the severity of the disease and correlate to the clinical course. TNF receptor levels appear to be of prognostic significance initially and in the course of SAP. Plasmapheresis seems to further increase the enhanced TNF receptor levels in SAP whereas cytokine levels decrease.

959 Microscopic Peritoneal Seeding of Gastrointestinal Malignancies — First Results of a Prospective Study

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Demonstration of single micrometastatic tumor cells, e.g., to the bone marrow or to the lymph nodes, by use of immunocytochemistry or PCR-based techniques has been shown to be of independent prognostic value in colorectal carcinomas (CRC). We therefore studied the prognostic implications of microscopic peritoneal seeding in gastrointestinal malignancies as determined by intraoperative intraperitoneal washings.

Methods: 207 patients entered the study. 197 with malignant, 10 with benign disease. Patients with macroscopic peritoneal seeding (n = 18) served as positive controls, patients with benign diseases as negative controls (n = 10). Intraoperatively 100 ml warm NaCl 0.9% were instilled in the abdominal cavity. 20 ml were reaspirated. For cytology H-E-staining and staining with HEA-125 antibody was performed. The results of cytology were correlated with TNM-stage and the postoperative follow-up over a 21 months period.

Results: 35.5% of patients had positive cytology: 3 of 18 patients with macroscopically visible peritoneal carcinoma were missed by routine cytology but identified by use of immunocytochemistry (sensitivity 83% vs. 100%). There was a close relationship between tumor stage and the occurrence of a positive cytology in those tumors without grossly visible peritoneal carcinoma (p < 0.05 for T and p < 0.02 for N-stage). No correlation was demonstrated with tumor grading (p = 0.32). Whereas 67.8% of patients with negative cytology were still alive after 21 months follow-up this was only the case in 45.2% of patients who had a positive cytology during surgery.

Conclusion: Peritoneal micrometastatic seeding of tumor cells is not only related to T- and N-stage of gastrointestinal malignomas. Most interestingly, in an univariate analysis positive cytology also influences postoperative survival time in gastrointestinal malignomas.

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Prevalence of the Course of Symptoms in Patients with Gastroesophageal Reflux

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Symptoms of gastroesophageal reflux are highly prevalent in the population, but no data are available on the predictive value of endoscopic findings and pattern of gastroesophageal reflux as assessed by 24-hr pH-metry on the long term prognosis of patients with grade 0–III esophagitis. Thus, we prospectively studied the variation between long term outcome and the initial findings of upper GI endoscopy and 24-hr pH-metry, respectively.

Methods: 59 patients (age 21–75 yrs, 29 f, 30 m) with symptoms of gastroesophageal reflux were included. At the time of enrollment, upper GI endoscopy and 24-hr pH-metry were performed. At this time, 31% of the study population had endoscopically confirmed esophagitis (Savary and Miller grade II–III). 53% had an increased proportion of pH-values below 4.0 (8%), individual patients were included upon the discretion of the referring physicians who were notified about the findings of endoscopy and the pH-metry. After a mean follow-up of 26 months the previously validated German version of the Bowel Disease Questionnaire was sent to all patients and additional symptoms and current treatment assessed. Data of 45 patients (76%) became available for final evaluation (6 had reloacted, 4 deceased, 4 non-responders). Logistic regression analysis was utilized to assess endo scopical findings and reflux pattern as predictors for symptomatic outcome.

Results: At follow-up, 33 out of 45 patients complained of persistent reflux symptoms; these patients had significantly lower mean pH-values (5.1 versus 5.6, p < 0.04) at the time of enrollment and an increased proportion of pH-values below pH 4.0 (20% versus 8%, p < 0.05). In contrast, prevalence of symptoms at the time of follow-up was not different for patients with and without esophagitis (82% versus 84%). Adjusting for medication during follow-up, reflux pattern at the time of initial work-up was the only predictor for symptomatic outcome. This holds true if patients without esophagitis were analyzed separately.

Conclusions: More than two years after initial diagnostic work-up the majority of patients with gastroesophageal reflux has persisting symptoms. Reflux pattern (mean pH-values, proportion of pH-values < pH 4.0) rather than endoscopic findings predict long term symptomatic outcome. The data emphasize the need for an intensified long term treatment strategy in reflux patients, even if esophagitis is absent.

Microsatellite Instability in Colorectal Cancer — Clinical and Pathomorphological Implications

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Recently, ubiquitous mutations in simple repeat sequences (microsatellite instability, MIN) have been described as a new mechanism of genomic instability in colorectal cancer (CRC) due to mutations in human mismatch repair genes (MSH2, MLH1, PMS1, PMS2). We have prospectively screened 81 colorectal carcinomas for MIN and have looked for the pheno- and genotypical characteristics of MIN positive tumors.

Methods: 81 tumors were investigated in DNA of 81 colorectal cancer speci mens and corresponding normal tissue. Five microsatellite loci were amplified by PCR: APC, p53, D10S89, D18S34, D9S171. Paraffin sections of the tumors were characterized pathologically, and examined with the antibody DO-1, an anti-laminin antibody. The proliferative activity was assessed by AgNOR staining, DNA ploidy and S-phase fraction were determined by flow cytometry, the family histories were registered in standardized family trees.

Results: MIN was found in 21% (17/81) of CRC. In comparison to MIN negative carcinomas, tumors expressing MIN were mostly localized proximal to the splenic flexure (76% vs. 20%, p < 0.01), and had a preponderance of high grade carcinomas (65% vs. 11%, p < 0.01). In addition, MIN positive tumors were mostly diploid (76% vs. 33%, p < 0.01) and had a lower value of p53 positivity (18% vs. 56%, p < 0.04). According to AgNOR and S-phase fraction there was a trend to lower proliferative activity (p < 0.05). Besides these phenotypical characteristics, MIN positivity was much higher in patients meeting the criteria of the National Commissio n of Colon and Rectal Cancer (NNCC) criteria (33%) and most interestingly, even patients with non-HNPPC fa miliar accumulation of gastrointestinal carcinomas had a higher incidence of MIN (31%).

Conclusion: Our data give strong evidence that MIN positive tumors form a distinct entity of CRC regarding localization, tumor type, ploidy, p53 immunoreactivity and proliferation rate. Moreover, screening of CRC for MIN may be a useful marker for familial cancer predisposition.

Measurement of Gastric Sensory Function and Compliance: Effects of the Distension Mode

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Gastric distension has been used to evaluate sensory function in humans but studies in vivo comparing different distension protocols are lacking. Two popuations of gastric mechanoreceptors have been postulated based on rapid and slow elastic balloon distension studies, but past results may simply reflect varying responses to distension. Thus, we aimed to compare the influence of the mode of gastric distension on sensation and gastric compliance using a barostat device. Methods: In 7 healthy volunteers we positioned a barostat bag in the proximal stomach and tested in random order (in triplicate) four different distension protocols: (a) standard ramp distension with 4 mmHg pressure increase increments of 20 sec duration; (b) slow ramp distension with 2 mmHg pressure increments of 40 sec duration; (c) slow random distension using a pressure ramp consisting of 2 mmHg increments of 40 sec duration with randomly interspersed pressure steps 50% below the preceding pressure step and (d) rapid random distension with 4 mmHg pressure increments of 10 sec duration. Results: The distension procedures yielded air flow rates between 2.4 mL/s for slow ramp and 18.4 mL/s for rapid random distension. First perception and maximal tolerable pressure were 10.9 ± 1.1 mmHg and 19.6 ± 1.5 mmHg, respectively. First perception and maximal tolerable pressures were significantly correlated (r = 0.93, p < 0.005). The gastric pressure at occurrence of perception and the maximal tolerated pressure were not significantly different for the different distension protocols. However, gastric compliance was significantly reduced during rapid random distension (p < 0.01 vs. slow ramp and p < 0.05 vs. slow random distension) but not during standard ramp distension. Conclusion: Gastric sensory pressure thresholds as assessed by isobaric distension are not influenced by the mode of distension, suggesting a single population of gastric mechanoreceptors. A decreased gastric compliance in response to rapid distension with high flow rates may reflect delayed adaptive gastric relaxation.

Induction of Hydroxynonenal-modified Protein Epitopes by Iron/Acetylsalicylic Acid-Induced Ito-Cells

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Introduction: Fibrinosis in chronic liver disease is characterized by the increased formation and accumulation of components of the extracellular matrix. Lipid peroxidation products like 4-hydroxynonenal (HNE) have been proposed to stimulate collagen gene expression in transformed cells. Here we react with a wide variety of proteins to form aldehyde-protein adducts. We used a specific polyclonal antibody to demonstrate the presence of pro-fibrogenic aldehyde epitopes in Ito cells after induction of lipid peroxidation by exposure of the cells to iron/acetylsalicylic acid.

Methods: Low density lipoproteins purified from WWHHL rabbit serum were modified in vitro with HNE. Rabbits were immunized with the autologous but modified proteins and polyclonal antibodies were gained. To cells were purified from rat liver and cultured for 10 days. Lipid peroxidation was induced by incubating these myofibroblast-like cells with 100 µM FeSO4 and 200 µM ascorbate.

Results: The polyclonal antibody specifically reacted with NaCNBH3-stabilized HNE epitopes as shown by Western-blotting using unmodified and HNE-modified BSA or ovalbumin as controls. Non-stabilized HNE-modified proteins could not be detected by the antibody after SDS-PAGE and Western-blots neither proteins modified in vitro nor in protein extracts from transformed Ito cells exposed to iron/acetylsalicylate. Presumably this was due to chemical disintegration of the epitope during SDS-PAGE. In transformed Ito-cells non-stabilized HNE-epitopes were detected by immunohistochemistry when the mitotic index was reduced or when HNE-modified protein immunoreactivity revealed HNE-adducts in the cytoplasm as well as in the nucleus of myofibroblast-like cells.

Conclusions: Exposure of transformed Ito-cells to iron/acetylsalicylate led to HNE-protein-adduct formation indicating that under these conditions lipid peroxidation can be induced in isolated Ito cells. The non-stabilized HNE-protein adducts detected by immunohistochemistry lost their antigenic properties during SDS-PAGE. Accordingly only stabilized HNE-modified proteins could be detected in Western blots. HNE-adduct formation detected in Ito cells can be the link between lipid peroxidation and fibrogenesis.

Modulation of Visceral Afferents: Effects of Insulin-Hyperglycemia on Duodenal Sensory Thresholds

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Altered sensory functions play a role in the development of symptoms in functional bowel disorders and it has been postulated that sensory function is modulated by central mechanisms. Aim: To analyze effects of insulin hyperglycemia on (1) thresholds for first perception and pain during small intestinal balloon distension and (2) small intestinal motility. Subjects: We studied 12 healthy subjects (7 f, 5 m, age 24–38 years). Duodenal perception thresholds were determined in triplicate in 15 minute intervals by a standardized segmental stepwise distension of the duodenum performed with a barostat device during the interdigestive phase and during intraduodenal nutrient infusion. Small intestinal motility was measured with

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a 5 channel low compliance perfusion system. In random order 6 subjects re-
ceived human insulin at a dose of 2 IU/kg body weight i.v. or the correspond-
ing volume saline. Plasma glucose levels were measured at 5 min intervals 
and an intraduodenal nutrient infusion started when blood glucose was < 30 
glucose intravenously to increase and maintain the plasma glucose levels 
between 80 and 120 mg/dl. 

Results: A bolus of PYY stimulated the release of insulin in the volume, but not the pressure threshold for pain significantly (*p < 0.05) increased as compared to control experiments without hypoglycemia (table). Small intestinal motor response to nutrients was not significantly affected by insulin hypoglycemia Mi 5.2 ± 0.2 vs 5.2 ± 0.1, p > 0.6).

Conclusion: Insulin hypoglycemia, a central actin cholinergic and adren-
ergic stimulus, increases the duodenal volume threshold for pain, while pres-
sure thresholds are unaffected. This effect is due to altered compliance of 
the small intestine that is independent of small intestinal phasic motor activity.

964 Bim 4004-1 Is a Novel Peptide YY (PYY) Analog Selective for Y2 Receptors 
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Essen, Germany; University of Basel, Switzerland 

Proabsorptive actions of PYY in animal models and preliminary human stud-
ies suggest that PYY analogs may be useful therapeutic agents in diarrhea 
and malabsorption. Recently proabsorptive effects in canine small intestine 
have been reported for a novel synthetic PYY analog, BIM 4004-1. Two re-
ceptor subtypes, Y1 and Y2, mediate the effects of PYY. It is neither known 
which of these receptor subtypes mediates the proabsorptive effects of PYY, 
nor to which receptor the PYY analog BIM 4004-1 binds. We studied the 
receptor affinity of BIM 4004-1 in model systems of Y1-like and Y2-like re-
ceptors. Methods: BIM 4004-1 was kindly supplied by P. Eden, Biomeasure. 
Binding of human Y1- and BIM 4004-1 to Y1 receptors was tested in a human 
enhryolecumina cell line (HEL) which exclusively expresses Y1 recep-
tors. Proxilc saline membrane preparations were used for studying binding 
to Y2 receptors. Competitive receptor binding studies were performed as pre-
viously reported (Grandt et al., EJP 1994). Data were fitted to a sigmoid func-
tion using a Hill slope of -1. Calculations were performed using an iterative 
non-linear regression analysis. All data are means of 3 experiments. 

Results: BIM 4004-1 has a similar affinity as PYY 1–36 to Y2-like receptors in porcine 
stripped membranes (7.1 ± 0.1 vs 7.1 ± 0.1 pmol/l, n = 3). BIM 4004-1 is less than 1000 fold more potent on Y1-like receptors of HEL 
cells (pKb 6.6 and 8.43 vs 6.09, right panel).

Conclusions: The PYY analog BIM 4004-1 is a highly Y2 selective ago-
ist. BIM 4004-1 is less than 1000 fold potent on Y1 receptors as PYY 1–36. The 
recently reported proabsorptive effects of BIM 4004-1 in a dog model suggest an 
importance of Y2 receptors for proabsorptive actions of PYY. Y2 selective agonists warrant further studies in model systems of diarrhea and 
malabsorption.

966 Peptide YY Inhibits Low-Dose but not High-Dose 
CCK-Stimulated Pancreatic Enzyme Secretion in 
Humans 
D. Grandt, J.M. Gschossmann, M. Schmiczek, C. Beglinger, H. Goebell, 
P. Layer. University of Essen, Germany and University of Basel, Switzerland 

The physiological role of peptide YY (PYY) for inhibition of exocrine 
pancreatic secretion in humans has been questioned since PYY does not inhibit 
exocrine pancreatic secretion stimulated by high-dose CCK iv. While the 
low-dose CCK iv stimulates pancreatic enzyme secretion by modulating va-
gustone high-dose CCK iv can directly act on acinar cells to stimulate enzyme 
secretion. We determined whether the effect of postprandial levels of PYY on 
CCK-stimulated pancreatic enzyme secretion depends on the dose of CCK 
used. Methods: The effect of PYY on basal and CCK-stimulated pancreatic 
enzyme secretion was studied using a marker perfusion/assistance technique in 
volunteers intubated with a multilumen tube. PYY 1–36 (45 pmol/kgh, n = 8) 
or saline (n = 8) was continuously infused during the experiment. CCK-Bs 
was infused in graded doses of 0, 3.3, 10 and 30 pmol/kgh for 45 min each. 
Pancreatic trypsin and lipase outputs were measured.

Results: CCK dose-dependently increased lipase and trypsin outputs. 
(1) PYY significantly inhibited the pancreatic tryptic output. 
(2) PYY significantly inhibited secretion due to 3.3 and 10 pmol/kgh CCK. 
(3) PYY did not inhibit enzyme secretion stimulated by 30 pmol/kgh CCK.

Conclusions: In humans PYY infused in doses mimicking postprandial 
plasma levels inhibits basal pancreatic enzyme secretion and secretion stim-
ulated by low-dose CCK iv. PYY does not significantly inhibit the stimulatory 
effect of high-dose CCK iv. We hypothesize that PYY potently inhibits vagally 
mediated stimulatory effects of CCK, but does not inhibit direct stimulation 
of acinar cells by CCK. This is in agreement with the previously reported in-
ability of PYY to inhibit enzyme secretion in isolated pancreatic acinar and 
the assumption that PYY acts by modulating vagal tone through Y receptors 
of the area postrema to which binding of PYY has been demonstrated.
with the onset of nocturnal sleep has been reported. The aims of this study were to investigate the effects on postprandial motor activity of the size of a meal consumed, the size of the preceding meal, and to compare the effect of a mid-day meal and a late evening meal.

8 healthy male volunteers, aged 19-38, underwent 5 separate 24-hr ambulatory manometry studies. After an overnight fast, subjects were intubated with a fine naso-jejunal catheter, incorporating 3 microtransducer and 15 cm apart. Its tip was positioned fluoroscopically, so that the middle transducer was at the Ligament of Treitz. 80 minutes after the start of the recording, subjects ate 1-5 portions (220-1100 kcal) of tagliatelle with vegetables. 10 hours later they consumed 2 or 4 portions of the same food and went to bed 30 min later. Pressure, sampled at a frequency of 2 Hz, was recorded on a portable digital recorder and downloaded for computer analysis. Flow activity (FA) was defined as the interval between the start of a meal and the phase III of the MMC. The integrated fed response (Area under the curve, AUC) was derived by computer analysis of the pressure records. (Mean ± SE).

After the midday meal, FA lasted 16±8, 30±6, 22±6, 23±8, 36±6, 38±8 min for the 1-5 portions respectively. The increment for each extra portion was significant (p < 0.05), except between 2 and 3 portions. AUC was 1086±235, 210±60, 158±70, 85±38, 32±17 mmHg x s x min⁻¹. After the late meal FA lasted 255±56, 286±59 or 266±63 min after the 2 portions, and 392±28 or 408±60 min after 4 portions, this difference was statistically significant, but not influenced by the size of the preceding meal. The respective AUC values (164±32, 183±23, 211±30 and 188±44, 173±36 mmHg x s x min⁻¹) did not differ, nor did AUC or FA differ between the mid-day and the late evening meals.

We conclude that meal size is the major determinant of FA. The size of the previous meal is irrelevant. We found that the onset of sleep rather than the time of eating determines the response to a late meal.

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TNB (30 mg in 50% ETQ). Rats were treated with UDCA (10 mg/kg) either for three days starting with the administration of TNB (group I, n = 8) or for eight days starting on day 4 after induction of colitis (group II, days 2–9, n = 6) and sacrificed at day 3 or day 9, respectively. Macroscopically visible injury was scored on a 0 to 10 scale. A blind histological assessment of mucosal integrity was also performed (scale 1 to 8). The colon was divided in 3 segments (proximal, middle, distal). All animals from the analyzed groups were expressed as mean ± SD. Results: UDCA prevented TNB induced macroscopic mucosal damage in the group I, especially in the area around the ulcer (S1: controls 3.22 ± 1.56 - UDCA 1.00 ± 0.89, p < 0.05; S3: controls 3.23 ± 1.22 - UDCA 0.81 ± 0.57, p = 0.02). Histological analysis confirmed a trend towards a decrease of necrosis and inflammation in all treated groups which was however not significant. Conclusion: Ursodeoxycholic acid attenuates the severity of TNB induced colitis when administered before, simultaneously or after the onset of the inflammatory area and protected the area with the most severe damage was reduced. In addition to our previous results in indomethacin induced ileitis, these data may provide a rationale for studies in patients with mild inflammatory bowel disease.

975 Effects of Keratinocyte Growth Factor on Mucosal Injury in Rat Colitis: A Dose-Response Study


Introduction: Keratinocyte growth factor (KGF) is a mitogen specific for epithelial cells known to enhance tissue repair in the skin. Its role in gastrointestinal repair is unknown. We studied the effect of exogenous rhKGF on mucosal integrity, mucosal proliferation and mucus production in the rat colon. Methods: Colitis was induced by enemas containing trinitrobenzenesulfonic acid/ethanol. In 3 groups of animals, KGF treatment was started 24 hours after induction of colitis at doses of 5 mg/kg (n = 20), 1 mg/kg (n = 10) and 0.1 mg/kg (n = 10) and continued daily for a week. Control animals received vehicle only. Colonic tissue samples were evaluated macroscopically and microscopically and assayed for myeloperoxidase (MPO) activity. To study the effect of KGF on mucosal proliferation another group of rats (n = 6) without colitis was treated with 5 mg/kg KGF for 7 days and received 50 mg/kg bromodeoxyuridine (BrdU) prior to sacrifice. Histological staining of colonic tissue sections with high iron diamine (HID) to determine mucin content, and immunohistochemistry using an anti-BrdU antibody to determine cellular proliferation was performed. Results: KGF at a dose of 5 mg/kg significantly reduced macroscopic necrosis and microscopic ulcerations by 46 and 53%. Similar significant results were obtained at a dose of 1 mg/kg with reductions in necrosis and ulcerations of 43 and 36%, respectively. A lower dose (0.1 mg/kg) was ineffective. MPO activity was not significantly different from controls in all groups. KGF significantly increased BrdU positive cells locally and in the lower 80% of the colon. KGF also increased the amount of HID-stained colonic mucus goblet cells. Conclusions: KGF ameliorates tissue damage in rat colitis suggesting that it plays an important role in mucosal integrity and repair likely by an increase in mucosal proliferation and mucus production.

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976 Sequence of Inflammation and Dysplasia in Dextran Sulfate Sodium Induced Colitis in the Rat

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Background: There is a need for a reliable animal model for premalignant lesions in the development of colonic cancer in chronic ulcerative colitis. A small number of experiments mentioned the carcinogenic effect of dextran sulfate sodium (DSS) on the large bowel of mice, rats, rabbits and Syrian hamsters. In this study we present the chronological sequence of DSS induced colitis and dysplasia in the rat. Methods: Male Wistar rats, weighing 90–120 g were used. All rats received water containing 5% (W/v) synthetic DSS (molecular weight 40000). Eight groups were formed: Al = four days DSS, Al = seven days DSS (B1); DSS feeding days DSS and ten days washout which is analogous to one cycle, Cl = two cycles...CV = six cycles). Colonic injury was assessed macroscopically and microscopically. Dysplasia was graded in accordance to Riddell et al. The grading system used was indefinite negative, negative, positive for dysplasia, D2 = positive for dysplasia, D3 = low-grade dysplasia, D4 = high-grade dysplasia). Results: In group Al we found active ulcerative colitis (UC) in 15 of 16 rats predominantly on the left side of the large intestine (13/15). D1 lesions were observed in 8 rats. In group B1 all rats and in group C1 only 2 of 6 had a severe UC. Only regenerative epithelial lesions (D1) were seen (n = 2). All rats of group B (n = 7) had a UC with the following dysplasia incidence rate: D6/7. D5/7. D2/7. D3/7. Dysplastic lesions were scattered over the total large bowel. In groups CI to CIII no dysplasia was observed. After five or six cycles of DSS (CI and CV) all rats (n = 6) showed dysplastic lesions at multiple sites ranging from D1 to D3. High-grade dysplasia was not detected. Conclusion: We showed that after administration of 5% DSS for seven days followed by the drinking water 70% of the rats showed significantly positive for dysplasia and 28% low-grade dysplasia. Multiple dysplastic lesions occurred after five cycles DSS. The dextran sulfate colitis model thus seems appropriate to study the development of dysplastic lesions in inflammatory bowel disease.

977 Colonic Smooth Muscle Cells in Rats as a Model Target of Insulin-like Growth Factor-I in Vivo and In Vitro

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Introduction: Insulin-like growth factor-I (IGF) is an important mediator gastrointestinal growth and tissue integrity. We have recently shown an increase in IGF binding sites after colonic injury exclusively in the muscularis propria. In this study we investigated the effects of exogenous IGF-I on colonic s.m. (SMC) in primary culture. Methods: Sprague-Dawley rats were treated with rhIGF-I via subcutaneous osmotic minipumps at a dose of 2.4 mg/day/kg for 14 days. The body weight of all animals was monitored before and after treatment. Colons were removed, rinsed and the wet and total length was measured. Cross-sections were stained for H&E and the thickness of the mucosa, the muscularis mucosa and the muscularis propria was measured. Cell proliferation in the muscularis propria was determined immunohistochemically using an antibody against PCNA. Growth rate of SMC in primary cultures isolated from rat colon was determined in a defined media containing transferrin and selenium (basal media) in the presence or absence of IGF-I (100 nM), EGF (10 ng/ml) and FGF (200 ng/ml). Results: Body weight of IGF-treated animals was significantly higher compared to controls (365 g ± 6 vs. 341 g ± 4). Both colon wet weight and length increased significantly in treated animals (2.5 g ± 0.1 vs 1.9 g ± 0.1 and 19.7 cm ± 0.5 vs. 18.5 cm ± 0.5). The thickness of the circular and longitudinal SM was markedly increased in treated animals (26 μm ± 2 vs. 13 μm ± 1 and 94 μm ± 6 vs. 64 μm ± 6, respectively). PCNA proliferation staining in the muscularis propria, showed a significantly higher number of positive cells in IGF-treated animals compared to controls (23 ± 3 vs. 9 ± 1.5). The thickness of the muscularis mucosa did not change. Growth rate of SMC when treated with IGF alone (0.15 ± 0.02) was not statistically different from control of cells in basal media (0.06 ± 0.01). However, IGF in combination with EGF and FGF produced a significant increase in cell proliferation (0.51 ± 0.04) in the growth rate compared to EGF alone and IGF alone. Conclusion: IGF has profound growth stimulatory effects on colonic smooth muscle cells. In vitro experiments in primary culture support our in vivo findings but suggest that IGF acts along with other growth factors such as EGF and FGF.

Ursodeoxycholic Acid Attenuates Leukocyte-Endothelial Cell Adhesion in Indomethacin-Induced Intestinal Inflammation in the Rat

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Ursodeoxycholic acid (UDC) has recently been shown to ameliorate the macroscopic and microscopic extent of experimental ileitis induced by indomethacin (INDO) in the rat. The objective of this study was to assess the effect of UDC on leukocyte-endothelial cell adhesion which is increased in this model of intestinal inflammation. Methods: INDO (7.5 mg/kg, s.c.) was injected 48 and 24 hours prior to the experiment. The mesenteric microcirculation was observed by intravital microscopy in animals treated with UDC (10 mg/kg via oral feeding tube) or its vehicle for 3 days starting with the administration of INDO. Leukocyte rolling velocity, the number of adherent and emigrated leukocytes, erythrocyte velocity, and vessel diameter were monitored in 30 μm diameter postcapillary mesenteric venules. Macroscopic visible injury was scored 0 to 10 on a blind histological assessment of mucosal integrity on a 1 to 6 scale. Results: Adherence (100 μm venule) Emigration (microcap. field) Macroscopy (1-6) Histology (0-10)

Control 3 ± 0.5 2.1 ± 0.6 0 1 1
INDO + UDC 14 ± 0.7* 6.5 ± 1.0* 8.7 ± 0.5* 7 ± 0.2**

* p < 0.05 vs. control, *p < 0.05 vs. INDO – UDC, **p < 0.005 vs. INDO – UDC

The INDO-induced increase in leucocyte adherence, macroscopic and histologic damage was significantly blunted by UDCA treatment (adherence –35%, macroscopy –35%, histology –23%), while the difference in leukocyte emigration between the INDO and UDC group was just not significant (p = 21%). UDC without INDO had no effect on the registered parameters.

Conclusions: UDC may attenuate macroscopic and microscopic severity of INDO-induced intestinal inflammation by reducing the leukocyte-endothelial cell interaction or by changing the exposure to endogenous bile acids necessary for the INDO-effect.
979 Epidermal Growth Factor Increases Colonic Mucosal Blood Flow by a Prostaglandin Dependent Mechanism in the Rat Model

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Background: Members of the EGF family of growth factors appear to mediate protection against acute gastric and colonic injury. The mechanisms responsible for the protective effects are currently under investigation. In gastrc mucosa EGF has been shown to induce an increase in mucosal blood flow in vivo. In vitro EGF induces prostaglandin production (PGE2). The aim of the current study was to examine the changes in colonic basal mucosal blood flow and injury induced hyperemia in response to EGF and whether these changes were sensitive to indomethacin pretreatment. Methods: 8 animals were either pretreated with indomethacin (5 mg/kg ip) or vehicle for one hour, and then treated with EGF (600 ng/kg ip) or vehicle for another hour before induction of colitis with topical administration of 30 mg trinitrobenzenesulfonic acid in 50% ethanol onto the colonic mucosa. Basal mucosal blood flow and injury induced hyperemia were determined using tissue spectrophotometry as described previously. Results: EGF treatment alone increased basal mucosal blood flow by 20 ± 6%, but did not change injury induced hyperemia compared to control. Pretreatment with indomethacin alone did not significantly change basal mucosal blood flow or injury induced hyperemia. Pretreatment with indomethacin completely abolished the EGF induced increase of basal mucosal blood flow and reduced injury induced hyperemia in EGF treated animals. Conclusion: We hypothesize that the EGF induced protection of the colonic mucosa is in part mediated by an EGF induced increase of mucosal blood flow. The increase in mucosal blood flow is mediated by increased prostaglandin production induced by EGF. Inhibition of prostaglandin production with indomethacin unmasks an additional vasoconstrictive effect in animals treated with EGF.

981 TGFα and EGF-Resistant but not EGF are Locally Increased Expression After Acute Colonic Injury in Rats

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Background: Members of the EGF family of growth factors appear to mediate protection against acute gastric and colonic injury. EGF and TGFα, a peptide within the kindred of the EGF superfamily mediate their biological activity by binding to a common receptor. Having shown that EGF protects colonic mucosa against acute injury the aim of the present study was to evaluate the endogenous expression of EGF, TGFα and the EGF receptor in response to mucosal injury in the same model of colitis in rats. Methods: Colitis was induced in Sprague Dawley rats by administration of 30 mg trinitrobenzenesulfonic acid in 50% ethanol. Animals (n = 6 each timepoint) were sacrificed before and at 2, 4, 6, 12 and 24 hours after induction of colitis. Colon tissues were examined for expression of TGFα, EGF and EGF receptor by Northern Blot and Ribonucleose Protection Assay. Protein translation was analyzed by Western Blot analysis and immunohistochemistry using specific antibodies for TGFα, EGF receptor and EGF. Results: A 3 times increase for TGFα expression and a 4 times increase in EGF receptor expression were observed within the first 24 hours after induction of colitis. TGFα protein was expressed as a 29 KDa precursor but not at the 50 amino acid TGFα metabolite. Only a weak expression of EGF mRNA expression was obtained by ribonucleose protection assay which did not increase during the course of colitis. TGFα and EGF receptor immunoreactivity was localized in the crypt and surface epithelium of the colonic mucosa. Conclusion: The early increased expression and translation of TGFα and EGF receptor during the course of colitis supports our hypothesis, that members of the EGF superfamily of growth factors play an important role in the early phase of colitis. We hypothesize that TGFα precursors but not EGF or the 50 aminoacid TGFα metabolite are the main locally expressed proteins that induce the EGF receptor in colonic inflammation mediating its protective effects.

982 Bile Duct Ligation and Fasting Reduce Microcirculatory Disturbances in Indomethacin-Induced Intestinal Inflammation in the Rat

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Indomethacin (INDO) induced intestinal inflammation is preceded by an increase in mucosal permeability and microcirculatory dysfunction. Direct exposure to luminal bile acids and INDO is necessary for mucosal damage. The objective of this study was to assess the effects of bile duct ligation and fasting on INDO induced intestinal inflammation in rat mesenteric venules. Methods: In 4 experimental groups a single dose of INDO (7.5 mg/kg, s.c.) was administered 24 hours prior to the experiment. Animals were treated with EGF for 18 hours (3 groups) or vehicle for the whole 24 hours (1 group). In 2 of these groups, animals underwent bile duct ligation or sham operation immediately before INDO injection. In 2 control groups, animals received one injection of indomethacin vehicle without or with sham operation prior to application. Ten venules (30 μm diameter) per animal were observed using intravital microscopy and the following parameters were monitored: number of adherent and emigrated leukocytes, leukocyte rolling velocity, erythrocyte velocity, venular blood flow, and shear rate. Results: INDO alone induced a significant increase in leukocyte adherence and emigration (4.8-fold and 4.2-fold, respectively) compared with untreated animals 24 hours after administration. Sham operation before INDO administration resulted in an even higher rate of adherence and emigration (2.1-fold and 1.6-fold vs. INDO without sham operation, respectively, and 0.4-fold and 6.2-fold vs. sham operation without INDO, respectively). The increase after sham operation + INDO was significantly blunted by bile duct ligation (adherence by 40%, emigration by 55%) to an extent similar to fasting rats after INDO injection. Sham + INDO resulted in a 1.6-fold increase in leukocyte adhesion without a significant rise in emigration. Conclusions: Bile duct ligation and fasting may contribute to the reduction of indomethacin induced microcirculatory dysfunction by prevening biliary cycling of INDO or by reducing exposure to luminal bile acids.

983 Adenine Nucleotides Stimulate Migration in Wounded Cultures of Small Intestinal Epithelial Cells

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Background: After various forms of superficial injury, the integrity of the gastrointestinal mucosa is reestablished by rapid migration of epithelial cells across the wound margins in a process termed restitution. Previous studies demonstrated that several cytokines physiologically present in the gastrointestinal mucosa promote intestinal restitution. The effects of adenosine nucleotides have been observed to enhance wound healing in wounded cultures of kidney epithelial cells in vitro. However, the mechanism of action remains unclear. Method: The effects of adenine nucleotides were studied in an in vitro model of intestinal epithelial restitution using the non-transformed rat intestinal epithelial cell line IEC-6. Standard "wounds" were established in confluent monolayers of IEC-6 cells and migration was quantitated in the presence or absence of AMP, ATP, cAMP and adenosine. Results: AMP and ATP significantly promoted epithelial cell restitution in vitro in a dose-dependent manner. Enhancement of epithelial restitution was observed as early as six hours after initiation of the wound. The minimum dose of AMP or ATP required to observe significance enhancement of restitution was 25 micro- mo/l, the maximum response was observed at a concentration of 250 micro-mol/l. In contrast, adenosine, cAMP and AMP had no effect on cell migration. Enhancement of IEC-6 cell restitution was independent of cell mass as assessed by pre-treatment of wounded monolayers with replication-inhibiting doses of mitomycin C. The promotion of IEC-6 restitution in the presence of ADP or ATP could be completely blocked by addition of immunoneutralizing anti-TGFβ1 to the culture media at the time of establishing the "wound". Conclusions: The findings suggest that certain adenine nucleotides which are released from injured or dying intestinal mucosal cells, or administered exogenously, may promote epithelial restitution after mucosal injury through a TGF-β dependent pathway.

984 Various Diets Affect Leukocyte Adherence and Emigration to a Different Extent in the Indomethacin Model of Chronic Intestinal Inflammation

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The rationale of the therapeutic effect of enteral nutritional treatment in Crohn's disease is unknown. The objective of this study was to assess the influence of various diets on the intestinal microcirculation in a model of long lasting ileitis in Sprague-Dawley rats. Methods: Animals were fed for 2 weeks with standard or special diet (poor of fibre, rich in protein, unsaturated or saturated fatty acids, or carbohydrates, poor or rich in cholesterol or fat) until intestinal inflammation was induced by two injections (7.5 mg/kg s.c. of) of INDO 24 h apart. After induction, rats were fed for other diets for 10d until the experiment. Ten postcapillary mesenteric venules (30 μm diameter) per animal were observed using intravital microscopy and the following microcirculatory parameters were monitored: number of adherent and emigrated leukocytes, leukocyte rolling velocity, erythrocyte velocity, venular blood flow, and shear rate. Macroscopically visible injury was scored 0 to 5. Results: Adherence Emigration Macroscore (per 100 μm venules) (per microsc. field) score
No INDO standard 3.1 ± 0.6 2.2 ± 0.6 0 1
INDO +
standard 4.7 ± 0.4 4.6 ± 0.4* 3.4 ± 0.5* 0.4
 fibre 4.9 ± 0.5 4.3 ± 0.5* 2.4 ± 0.6* 0.8* protein 53.9 ± 1 24.1 ± 0.3* sat. fatty acids 5 25.2 ± 0.2* 19.3 ± 0.3* 0.4 ± 0.2* carbohydrates/salts 45.6 ± 0.5 31.2 ± 0.6* 25.0 ± 0.5* cAMP 6.0 ± 0.5* 4.2 ± 0.5* 2.5 ± 0.9* fibre/standard 6.2 ± 0.7* 10.5 ± 1.3* 3.6 ± 1.0* *p < 0.05 vs. no INDO standard, **p < 0.05 vs. INDO + standard.

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The application of various diets without INDO had no effect on microcirculatory parameters.

Conclusions: The INDO-induced increase in leukocyte and emigration is differentially affected by certain dietary combinations (r = 0.37 of adherence vs. emigration). The correlation of emigration with macroscopic damage (r = 1.7y + 1.2, r = 0.65) indicates that at least emigration need to be affected in order to have a therapeutic effect.

986 Anorectal Function in Progressive Systemic Sclerosis: Another Parameter for Gastrointestinal Involvement?

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The aim of this study was to investigate anorectal function in patients with progressive systemic sclerosis (PSS) and to define the role of anorectal function parameters in the diagnosis of gastrointestinal involvement of PSS. Since esophageal motor disorder is the most common gastrointestinal manifestation in PSS, results of anorectal manometry were compared between patients with normal and disturbed esophageal function. Methods: 25 patients with PSS (22 f, 3 m; 17 x PSS, 6 x CREST-syndrome, 2 x overlap syndrome with predominance of PSS) were prospectively enrolled in the study from 1992 to 1994. All patients were referred for manometric assessment of esophageal function; none of the patients had originally been sent for evaluation of fecal incontinence. Esophageal function was judged abnormal when there was separation of the lower two thirds of the esophageal body. In anorectal manometry, anal resting pressure and maximal squeeze pressure were recorded with an 8 lumen water perfused catheter. Rectal perception threshold as well as both threshold and excitability of the rectal inhibitory reflex (RAIR) were determined by insufflation of a balloon attached to the tip of the probe. Results: (median and range)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients with normal esophageal motility (n = 8)</th>
<th>Patients with disturbed esophageal motility (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal incontinence</td>
<td>2 x (0-60)</td>
<td>3 x (0-120)</td>
</tr>
<tr>
<td>Anal resting pressure (mm Hg)</td>
<td>61 (35-121)</td>
<td>65 (27-96)</td>
</tr>
<tr>
<td>Max. squeeze pressure (mm Hg)</td>
<td>159 (64-254)</td>
<td>182 (63-315)</td>
</tr>
<tr>
<td>Perception threshold (ml)</td>
<td>20 (12-25)</td>
<td>20 (10-50)</td>
</tr>
<tr>
<td>Excitability of RAIR</td>
<td>6/8 (75%)</td>
<td>1/7 (14%)</td>
</tr>
<tr>
<td>Threshold of RAIR (ml)</td>
<td>20 (10-60)</td>
<td>20 (10-50)</td>
</tr>
<tr>
<td>Amplitude of RAIR (% of resting pressure)</td>
<td>50 (38-60)</td>
<td>60 (33-88)</td>
</tr>
</tbody>
</table>

Conclusions: Patients with PSS may suffer from fecal incontinence, but it was a rare symptom in an unselected group of patients. There was no significant difference in anorectal function in PSS patients with normal or disturbed esophageal motility; thus, anorectal manometry could not differentiate between patients with and without gastrointestinal involvement of PSS. In contrast to other observations, RAIR was excizable in nearly 90% of our patients.

987 Esophageal Manometry in Progressive Systemic Sclerosis: Screening Procedure or Confined to Symptomatic Patients?

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Esophageal manometry and transit scintigraphy are considered the most sensitive techniques for diagnosing esophageal involvement in progressive systemic sclerosis (PSS). Although there are no controlled data on the impact of manometric findings on therapeutic strategies and clinical outcome, diagnosis of PSS-associated esophageal involvement usually is an indication for anti-reflux treatment in order to prevent further complications like reflux esophagitis with stricture formation and dysphagia. The aim of this study was to prospectively evaluate the positive and negative predictive value of esophagus-related symptoms in patients with PSS by comparing the results of a standardized questionnaire with esophageal manometry.

Methods: 34 patients with proven PSS (30 females, 4 males; 21 x PSS, 9 x CREST-syndrome, 4 x overlap syndrome with predominance of scleroderma) were referred for esophageal manometry. Patients were asked for heartburn, dysphagia for solid and liquid food and chest pain in an standardized questionnaire. Manometry was performed with a water perfused 8-lumen catheter (Synectics medical). Manometric tracings were interpreted using standard criteria; PSS induced esophageal dysfunction was diagnosed when there was aperistalsis of the lower two thirds of the esophageal body.

Results:

<table>
<thead>
<tr>
<th>Asymptomatic</th>
<th>Symptomatic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manometry normal</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Manometry abnormal</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>12 (35%)</td>
<td>22 (65%)</td>
</tr>
</tbody>
</table>

Conclusions: Only 59% of patients with symptoms possibly indicative of esophageal involvement had a pathological manometric tracing; vice versa, patients without symptoms revealed esophageal involvement in 58%. Thus, screening examinations for esophageal dysfunction are mandatory in all patients with PSS.

988 Validity of a New Rapid Whole Blood Test for Helicobacter pylori Infection

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Different serological tests for Hp-infection are linked with a wide range of validity. A rapid, albeit valid serological test performed within a few minutes in the consulting office is essential for the treatment of the dyspeptic patient that is no candidate for endoscopy. The study aimed to validate the new Helisal rapid whole blood test (RWBT).

Methods: 77 patients undergoing upper gastro-intestinal endoscopy for different reasons with unknown Hp-status (without Hp suppressive treatment during the last 4 weeks) were meanwhile enrolled in the study. The assessment of Hp infection was performed by urease test, culture and histology from 4 antrum and 4 body biopsies. An Hp infection was excluded only by negative results in all of these three biopsic tests. The RWBT was performed by an independent person unaware of the endoscopic and bacterial findings.

Results: 47 patients proved to be Hp positive, 30 patients Hp negative as judged by endoscopic biopsic tests. Compared to these reference methods the RWBT was true positive in 42, false positive in 8, true negative in 22, false negative in 5 patients (specificity 89%, sensitivity 73%).

Conclusions: In our setting, the RWBT proved to be a sufficiently valid non-invasive method for diagnosis of Hp infection. It might be an useful means for screening young dyspeptic patients prior to endoscopy.

989 Small Bowel Bacterial Overgrowth After Gastric Surgery


Operations of the stomach may predispose to small bowel bacterial overgrowth (SBBO) by altered gastric motility, achlorhydria and diverted small bowel loops. The aim of this study was 1. to investigate frequency and symptoms of SBBO in patients with operated or resected stomachs and 2. to evaluate the role of the glucose H2-breath test in the diagnosis of SBBO by comparing the results of the breath test with a quantitative microbiological culture of small bowel contents. Methods: In 34 patients with gastric surgery (28 x subtotal stomach resection with gastrojejunostomy [25] or gastroduodenostomy [9]), 2 x Whipple’s operation, 1 x gastrectomy, 1 x esophageal resection (with stomach dislocation, 1 x vagotomy) who were referred for gastroscopy, small bowel content (preferably from the afferent loop or as far distal as possible) was endoscopically aspirated in a sterile technique. The aspirate was immediately cultured on different media, and cultures were analyzed for species and quantitative growth.

Conclusions: Patients undergoing upper gastro-intestinal endoscopy for different reasons with unknown Hp-status were referred for Hp suppressive treatment during the last 4 weeks. The assessment of Hp infection was performed by urease test, culture and histology from 4 antrum and 4 body biopsies. An Hp infection was excluded only by negative results in all of these three biopsic tests. The RWBT was performed by an independent person unaware of the endoscopic and bacterial findings. Results: 47 patients proved to be Hp positive, 30 patients Hp negative as judged by endoscopic biopsic tests. Compared to these reference methods the RWBT was true positive in 42, false positive in 8, true negative in 22, false negative in 5 patients (specificity 89%, sensitivity 73%).
990 Association of Increased Serum Concentrations of Lipoprotein (a) with Focal White Matter Lesions in the Brains of Ulcerative Colitis Patients

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Background: Recently, we have found a significantly increased frequency of focal hyperintense white matter lesions in patient with ulcerative colitis (UC) or Crohn’s disease (CD) by magnetic resonance imaging (MRI). Since MRI does not allow the differentiation between vasculitic and atherosclerotic lesions, we evaluated cardiovascular risk factors and autoantibodies (Ab) in all patients with or without white matter lesions.

Patients and methods: Patients with CD (age 29 ± 7 years, mean ± SD), UC (29 ± 6 years) were studied by MRI. All persons were examined using a 1.5 T magnet (SP36, Siemens, FRG) with T1 (TR 570 ms/TE 15 ms) and T2 (TR 2000 ms/TE 12-90 ms; TR 3700 ms/TE 80 ms) weighted axial and coronal images including gadolinium enhanced studies.

Results: Focal white matter lesions were found in 23/58 CD patients (40%), in 12/21 UC patients (59%), and in 65/62 age matched (30 ± 15 years) healthy volunteers (15%). The relative risk for white matter lesions was 2.6 (95% confidence interval: 1.3-5.3) in CD patients and 2.5 (1.2-5.5) in UC patients compared to healthy persons. 7/12 patients with UC and white matter lesion but only 2/20 patients without white matter lesions had lipoprotein (a) serum concentrations of >30 mg/ml (relative risk: 5.8, 1.4-23.6; p = 0.03). No significant difference was found in patients with CD with (7/23) and without (7/23) white matter lesions. No differences were found in UC or CD patients for cholesterol, LDL cholesterol, HDL cholesterol, blood glucose, HbA1c, fibrinogen, Broca indices, blood pressure, smoking habits, ANA, rheumatoid factor, carotid ab., pancreatic ab., goblet cell ab., or pANCA.

Conclusions: Focal white matter lesions in patients with UC, but not with CD are associated with elevated concentrations of lipoprotein (a). Lipoprotein (a) is a known risk factor for thromboembolic events. It has a strong structural homology to plasminogen and inhibits fibrinolysis.

991 Amino Acid-absorption from Protein-based (PB), Peptide (PFP), or Amino Acid Diet (AID) in the Isolated Perfused Rat Intestine


In pancreatocentralized humans 61% of nitrogen (N) are absorbed from intact lactalbumin after intragastric application despite the absence of pancreatic proteolytic enzymes. It was thus speculated, that the small intestine represents a hub of the N-absorption from intact protein. To exclude the effects of digestion due to gastric and pancreatic secretions we determined mesenteric venous amino acid appearance rates in the isolated vascularly and luminaally perfused intestine as a measure of nitrogen absorption.

After a control phase (C) small intestines of 5 rats each were luminaally perfused with either PB (Biosorb® Sonde), or PFP (Pepspirit®, or AAD (Nur2000®). Vascular exchange rates were calculated from concurrent mesenteric perfusate samples (measured by HPLC) and perfusate flow. Exchange rates of Gin, Ala, and Leu are given in table 1. In table 2 exchange rates of Leu, Ile, and Val are normalized on the basis of a luminal nitrogen content of 1 g N/100 ml diet.

Table 1. Vascular appearance rates of amino acids

<table>
<thead>
<tr>
<th>C</th>
<th>PB</th>
<th>PFP</th>
<th>AAD</th>
</tr>
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<tbody>
<tr>
<td>Gin</td>
<td>-85 ± 8</td>
<td>-50 ± 2</td>
<td>-83 ± 11</td>
</tr>
<tr>
<td>Ala</td>
<td>54 ± 4</td>
<td>70 ± 12</td>
<td>123 ± 15</td>
</tr>
<tr>
<td>Leu</td>
<td>5 ± 11</td>
<td>9 ± 1*</td>
<td>10 ± 2</td>
</tr>
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</table>

(mi mo l · min⁻¹ · g⁻¹ · SEM; * p < 0.05, ** p < 0.01 by paired t-Test)

When infused into the small intestine PB was a poor substrate for nitrogen absorption. Obviously the small intestine is not capable of relevant N-assimilation from protein based diets. In contrast, the absorption of amino acids as single components occurs more efficient from PB than PFP. Neither PB nor PFP supplied glutamine in amounts sufficient for Gin-appearance in mesenteric venous perfusate.

992 HIV DNA Concentration is Substantially Higher in the Intestinal Mucosa than in the Peripheral Blood of AIDS Patients


The state of activation and differentiation of T cells in the intestinal mucosa differs from circulating T cells. These differences may affect infectibility by HIV as well as HIV replication. Therefore we investigated differences in HIV load between duodenal biopsies and peripheral blood of AIDS patients.

Methods: Total DNA was extracted from duodenal biopsies and simultaneously drawn venous blood of 21 male AIDS patients (age 44 (26-56) years). HIV gag-specific DNA was amplified by PCR and diluted three fold serial dilutions and detected (detection limit <15 copies) photometrically after hybridization using an enzyme-conjugated probe (Amplico®, Roche).

Results: HIV-DNA was detected in blood of 13 and in 16 biopsies of patients. Biopsies contained significantly more HIV-DNA than blood (mean titer 1.88 vs. 0.90, p = 0.028). Five patients without detectable HIV-DNA in the blood had very high HIV-DNA concentrations in biopsies (titer ≥ 1.100). No correlation was found between HIV-DNA content in blood and biopsies.

993 Interleukin-8 is Increased in the Colon Mucosa of Patients with Inflammatory Bowel Disease (IBD)


Background: The concentration of Interleukin-8 (IL-8) is enhanced in the intestinal mucosa of IBD patients. In the mucosa of patients with Crohn’s disease (CD) IL-8 is less increased than in the mucosa of patients with ulcerative colitis (UC). The cell type responsible for the production of IL-8 in the gut is not known.

Methods: We determined IL-8 protein by ELISA and focalized IL-8 mRNA by ISH-situ hybridization in 55 biopsies of colonic mucosa from 26 CD patients, 67 biopsies from 35 UC patients, 38 normal control patients with adenomas or irritable bowel syndrome and 8 inflammatory control patients with diverticulo- or infectious colitis.

Results: Compared to normal controls (median 4 pg IL-8/biopsy) IL-8 protein was significantly increased in macroscopically inflamed biopsies of UC (median 140 pg/biopsy; p < 0.001), CD (median 118 pg/biopsy; p < 0.0001) vs. adenoma/bowel syndrome and controls (median 5 pg/biopsy; p < 0.01). In 29 uninfamed UC biopsies (median 9 pg IL-8/biopsy; p = 0.3) IL-8 was also increased in unfaimed CD biopsies (median 46 pg/biopsy; p < 0.001). IL-8 mRNA was detected by in-situ hybridization in 31/65 biopsies of CD (56%), 38/67 biopsies of UC (67%), in 5/8 inflammatory controls (63%) and in 5/8 normal controls (13%). There was a correlation between IL-8 mRNA and IL-8 protein (r = 0.48; p < 0.001) and between IL-8 mRNA and the macroscopic aspect of inflammation (CD: r = 0.47; p < 0.001; UC: r = 0.60; p < 0.001).

Conclusion: IL-8 mRNA was only detected in inflammatory cells in the lamina propria but in none of the 168 biopsies in the mucosal epithelial cells.

994 Association of HLA-DR15, P-Anchor and IL-1 Receptor Antagonist Allele 2 with Ulcerative Colitis


Background: HLA-DR2, pANCA and recently IL-1ra allele 2 have been found to be genetic markers associated with ulcerative colitis.

Methods: DNA was extracted from peripheral blood cells of patients with Crohn’s disease (CD: n = 105), ulcerative colitis (UC: n = 70) or from patients with non-inflammatory bowel disease like irritable bowel syndrome (CO: n = 69). DNA was amplified with DR specific primers and HLADR genotypes were determined by reverse dot blot detection using allelespecific probes (Standard INNO, UPA HLA DRB). IL-1ra genotypes were analyzed by PCR. pANCA were determined by immunofluorescence, using ethanol fixed human PANK2 as a substrate.

Results: The frequency of pANCA was 64% (p = 0.00001) in the UC patients, 9% in the CD patients and 5% in CO patients. In patients with severe UC (frequent exacerbations, corticoid dependency), the frequency of pANCA...
was significantly (p < 0.01) higher (73%) than in patients with uncomplicated UC (50%). The frequency of the HLA-DR15(2) allele was also significantly increased in UC (48%, p < 0.01) compared to the patients with CD (22%) and the controls (26%). The frequency of IL-1α allele 2 was 28.6% in UC patients, 26.7% in CD patients and 20.3% in control patients (no significant difference). 50.0% of UC patients 45.7% of CD patients and 37.7% of control patients had at least one IL-1α allele 2 (n.s.d.). There was a trend to a higher frequency in patients with diarrhea and 26500 pg/ml inflammatory controls, IL-1β and proctitis (42%) (n.s.d.). In our population there was no difference in HLA-DR15 or HLA-DR2 frequency between pANCA positive and negative UC patients (p = 0.4). The IL-1α allele 2 was more common in the subgroup of HLA-DR15 positive UC patients than in HLA-DR2 negative UC patients (p = 0.015). pANCA positive and negative UC patients had similar frequencies of the IL-1α allele 2.

Conclusions: HLA-DR15 and pANCA are strongly and independently associated with ulcerative colitis. The IL-1α allele 2 is only very weakly associated. pANCA are associated with a severe course in ulcerative colitis (odds ratio 1.47).

995 Increased Secretion of Immunoglobulins A and G by Short-term Cultured Duodenal Biopsies in HIV Infection

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In contrast to abnormalities of systemic humoral immunity in HIV infection alterations in secretory mucosal immunity are not well characterized. Therefore we studied immunoglobulins secreted by cultured duodenal biopsies in correlation to saliva and serum.

Methods: Duodenal biopsies of 18 controls [2 f, 16 m; median age 44 years] and 40 HIV patients [11, 39; median age 42 years; 10 HIV+, 30 AIDS] were incubated for 48 h in culture medium. Supernatants (SN) and simultaneously obtained serum and saliva samples were assayed for immunoglobulin (Ig) A, IgG, and IgM by radial immunodiffusion.

Results: In both HIV-infected patients and controls lower proportions of IgG and higher proportions of IgA and IgM were found in SN and saliva samples compared with serum (Table). Compared with controls HIV patients had higher concentrations of all Ig classes in serum, of IgG and IgA in SN, and of IgG and IgM in saliva. In HIV patients weak correlations were observed between serum and SN in IgG concentration (r² = 0.11, p = 0.04), between SN and saliva in the proportions of IgG (r² = 0.20, p = 0.02) and IgM (r² = 0.19, p = 0.02). Other correlations were not found.

Conclusions: In HIV infection secretion of IgG and IgA is increased in the intestinal mucosal immune system. The lack of correlation between saliva and SN indicates that saliva does not adequately mirror intestinal secretory immunity. The specificity and function of the secreted antibodies remains to be determined.

Supported by grants III-0089-1 and 01 KI 9468 from the BMFT.

996 Imbalance Between Pro- and Antiinflammatory-Cytokines in the Colonic Mucosa in Inflammatory Bowel Diseases (IBD)


Background: The proinflammatory cytokines IL-1 and TNF are controlled by IL-1 receptor antagonist (IL-1ra) and the soluble TNF receptors p55 and p75. It is hypothesized that there is an imbalance between pro- and antiinflammatory cytokines in IBD.

Methods: 15 IBD (ReDo Systems, Minneapolis, MN), TNFα (Medgenix, Flemur, Belgium), sTNF-Rp55 and p75 (Hoffmann La-Roche, Basle, Switzerland) were measured by ELISA in homogenates from 35 colonic non-infected biopsies (CO) and from 8 inflamed biopsies (IBD) from 49 Crohn’s disease biopsies or from 53 ulcerative colitis biopsies.

Results: Interleukin-1β (IL-1β) and IL-1 receptor antagonist (IL-1ra), but not tumor necrosis factor α (TNF), and soluble TNF receptors (sTNF-R) are significantly increased in intractably inflamed colonic biopsies (CO) from 8 IBD patients compared to 11 healthy controls (CD). The median levels of IL-1β and IL-1α in normal controls were 11 pg/ml total protein and 6630 pg/mg respectively. They were 614 pg/mg and 15500 pg/mg respectively in severe and mild CD, 507 pg/mg and 40100 pg/mg in severely inflamed UC, and 1310 pg/mg and 26500 pg/mg respectively. It was significantly increased in uninfamed CD biopsies (median: 75 pg/mg). Mucosal IL-1β and IL-1α concentrations correlated significantly with macroscopic (IL-1β: r = 0.68, p < 0.001, IL-1α: r = 0.57, p < 0.001) and histologic

(1-IL-β: r = 0.62, p < 0.001, IL-1α: r = 0.62, p < 0.001) grading. IL-1α/IL-1β ratios were decreased in severely inflamed mucosa in IBD and in inflammatory controls from approximately 300 to 40. IL-1β, IL-1α, and TNFα mRNA were detected in situ hybridization predominantly inflammatory cells in the lamina propria.

Conclusion: There is a local imbalance between IL-1β and IL-1α in the inflamed colonic mucosa, which is not disease specific.

997 Distinct CD4 T Cell Depletion in the Small and Large Intestinal Mucosa and Blood of HIV-Infected Patients


There is increasing evidence that HIV is not equally distributed throughout the immune system. However, differences in immunological effects of HIV between lymphoid compartments have not been studied. Therefore we analyzed CD4 T cell depletion which is the hallmark of HIV infection in different compartments of the mucosal immune system and blood.

Methods: We studied 9 HIV-infected patients [1, 8 f; age 41 (27-57) years; 2 HIV +, 7 AIDS] undergoing both upper and lower endoscopy within a maximum of two days. Lymphocytes were isolated by gradient centrifugation from venous blood and from enzymatically digested biopsies of the duodenal and sigmoid colon. Cells were triple-stained with fluorochrome-conjugated monoclonal antibodies against CD3, CD4, and CD8 and analyzed by flow cytometry.

Results: The percentage of CD4+ T cells (%CD4) was lower in the duodenum compared with blood and sigmoid colon (p < 0.05). No correlation of %CD4 was found between the three compartments. Blood and sigmoid colon contained clearly more CD4 T cells than the other compartments each in two cases (Table).

Conclusions: The considerable differences in %CD4 between the three compartments studied may result from differences in HIV load and/or HIV replication as described for other immunological compartments. Findings in the peripheral blood are apparently not representative of the immunological effects of HIV infection. Local differences in susceptibility or inefficiency could be of major importance in the susceptibility of different parts of the intestine to opportunistic infections and malignancy in HIV infected patients.

Supported by grants III-0089-1 and 01 KI 9468 from the BMFT.

999 Detection of Adenovirus and Coronavirus in Stool is Associated with Diabetes in HIV-infected Patients


We examined the role of stool virus in the pathogenesis of diarrhea in HIV-infected patients. Methods: Intestinal biopsies and repeated stool samples of 256 HIV-infected patients (131, 243; age 19-68, median 40; 41 HIV+; 215 AIDS) undergoing endoscopy because of diarrhea (n = 136; 53%) or other gastrointestinal symptoms were examined for enteropathogens. Stool virus was detected by electron microscopy. Results: Adenovirus and coronavirus were found in 17 (6.6%) and 29 (11.3%) patients, respectively, two patients harbored both viruses. Other pathogens were detected in 102 (40%) patients and in 110 (47%) patients no infections were found. No significant differences were observed in sex, age, disease stage, risk group, and antiviral therapy between these groups. CD4 counts were low (mean 200 (p < 0.05) in patients harboring adenovirus (median 200(µl) or pathogens other than stool virus (225(µl) compared with patients harboring coronavirus (67(µl) or no pathogens (50(µl). In patients with diarrhea the prevalence of adenovirus and coronavirus was 10% and 15%, respectively. Diarrhea was more frequent in patients harboring adenovirus (13(17; 76%; p < 0.01), coronavirus (21(29; 72%; p < 0.01), or other pathogens (63(102; 62%; p < 0.01) compared with uninfected patients (41(110; 37%). In 14(17 (82%) and in 14(29 (48%) patients with adenovirus and coronavirus, respectively, additional pathogens were found, but the frequency of diarrhea was similar in patients with and without confections (adenovirus: 10(41 vs. 33%; coronavirus: 12(9 vs. 9(15%). In 30 (10%) of stool samples, adenovirus was detected more frequently in patients harboring adenovirus (14(17; 82%) than in patients without stool virus (10(212; 48%; p < 0.025).

Conclusions: Adenovirus and coronavirus were significantly associated with diarrhea indicating an opportunistic role of these agents in HIV infection. In addition, the high proportion of confections in patients with adenovirus suggests that these agent may facilitate infection by other pathogens. Supported by grants III-0089-1 and 01 KI 9468 from the BMFT.
1001 Immunochemical Study About the Innervation of the Gut-Associted Lymphoid Tissue
Krammer Heinz-Jürgen, IV. Department of Medicine (Gastroenterology), University Hospital of Heidelberg at Mannheim, Germany

The detailed mechanisms of intercommunication between the intestinal immune—and nervous systems are not yet clear. It was therefore decided to employ immunochemical techniques to investigate the structural organization of the ENS in the regions of the gut-associated lymphoid tissue (GALT), with special reference to the lymphoid follicles of the Peyers’ patches in the human and porcine intestine.

For that purpose we use polyclonal antibodies against the neuronal marker protein gene product 9.5 (PGP) and against the glial marker S-100 protein and glial fibrillary acidic protein (GFAP) for immunofluorescence and immunoperoxidase reaction in sections and whole mount preparations of the intestinal wall.

Due to the location of the lymphocytes in the epithelium and in the lamina propria mucosae they have a close relationship to the nerve fibers of the plexus mucosae. The lymphoid follicles, which extend over the tunica submucosa and tunica mucosa are in close topographical relationship with the two submucosal plexuses. The plexus submucosus externus is situated close to the base of the follicles. Nerve fibers run from its ganglia in the interfollicular area to the plexus submucosus internus. Nerve fibers of this plexus surround the lymphoid follicles and run to the tunica mucosae. An aganglionic plexus, which is of the laminae submucosae tunicae mucosae. Although we were unable to find nerve fibers in the follicle proper, many nerve fibers run together in the dome area of the tunica mucosae and form a dense network under the follicle-associated epithelium.

It seems that there is an intimate immunologic association between the compartments of the GALT and of the ENS. The results of the innervation patterns in intestinal lymphoid tissue provide a basis for the investigation of their changes under experimental conditions and in disease.

1003 Action of Intraluminal Amino Acids on Pancreatic Protein Secretion Before and After Cutting the Extrinsic Nerves of the Ileum in Dogs
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In different species, including cats, rats and humans pancreatic enzyme secretion is inhibited by intraluminal perfusion with fatty acids. In dogs the relationship between intraluminal nutrients and pancreatic exocrine secretion is greatly unknown. The Aim of the study was to elucidate the role of the ileum in the control of the hormonally stimulated pancreatic secretion. For this reason, we developed a model of total extrinsic denervation of the entire ileum by autotransplantation this segment of bowel in dogs. In four dogs the jejuno-ileal junction and the distal ileum were transected. Intestinal continuity was reestablished by two end-to-end anastomoses. In addition the superior mesenteric vessels and vein were denervated by resection of the mesenteric vessels and vein at the base of the mesentry. A Thomas-like cannula was placed into the stomach. duodenum (to collect pure pancreatic juice) and at the jeuno-ileal junction. Thus, ileal segments could only influence via hormones on exocrine mediators. Eight control dogs were operated only the three fistulas. After recovery, in both sets of dogs dose-response studies of the pancreatic protein response to perfusion of the extrinsically denervated or innervated ileum with trypsyn (TRP; 0.12–10.0 mmol/l) were performed, given against an intravenous (iv) background of secretin (S; 20.5 pmol/kg) and caerulein (C; 29.6 pmol/kg). After 1 h of S + C, perfusion of TRP into the ileum was begun, starting with the lowest load and trepling the load every 45 minutes. On separate days control experiments with intraluminal perfusion of 0.15 M NaCl were done. Results: Iv S + C significantly (p < 0.05) increased pancreatic protein output above basal in both sets of dogs. Intraluminal TRP caused a dose-dependent decrease in the pancreatic protein response to S + C. In the intact animals the highest loads of TRP significantly reduced the protein output by 47% and 54%, resp., as compared to the response to S + C infusion alone, whereas in the denervated animals low and high (0.37–10.0 mmol/l) loads of TRP reduced the protein output significantly by 56% to 59%.

In both sets of dogs the integrated protein response (IPR; mg/kg/min) to all loads of TRP was significantly lower than control (means ± SEM; control studies: a innervated dogs: 6475 ± 4606 mg; b denervated dogs: 115 ± 3732 mg. TRP: a innervated dogs: –1041 ± 6477 mg; b denervated dogs: –46420 ± 14093 mg) but the IPR was significantly lower in the denervated as compared to innervated animals. Conclusions: 1) Extrinsic denervation of the entire ileum is a valuable preparation to study the role of nerves in the control of the pancreatic secretion. 2) Even the highest loads of TRP significantly reduced the pancreatic protein output. 3) In the intact dogs the “ileal brake” exist of the hormonally stimulated pancreatic secretion for tryptophan; 3) the extrinsic nerves of the ileum are probably not the dominant mediators of the inhibitory action of ileal tryptophan on pancreatic output.

1005 Effect of a 28-day Therapy with the Proton Pump Inhibitor Pantoprazole and with the H2-receptor Antagonist Ranitidine on Intragastric PH in Healthy Human Subjects

A decrease of the inhibitory effect of different H2-receptor antagonists on gastric acid output after repetitive dosing has been reported. Whether this finding also occurs during a 28-day long therapy with the proton pump inhibitor pantoprazole is unknown. The Aim of this study was to compare the effect of a 28-day long-term therapy with pantoprazole (40 mg/die at 8:00 hr) with that of ranitidine (300 mg at 20:00 hr) on the intragastric (i.g.) pH. 24 hr i.g. pH-metry using combined glass electrodes, was performed on day 1, 7, 28 and between 8:00 and 8:00 hr for pantoprazole and between 20:00 hr and 20:00 hr for ranitidine in 20 healthy female (n = 11) and male (n = 9) volunteers. Standardized meals were given at 9:00, 14:00 and 21:00 hr. On separate days i.g. 24 hr pH-metries without any medication were performed as controls. Results: The daily oral therapy with pantoprazole significantly (p < 0.001) increased on day 1, 7 and 28 the i.g. median pH to 166%, 244% and 247%, resp., as compared to control (100%). After 7 days and 28 days of pantoprazole the median 24 hr pH was significantly (p < 0.001) higher than on day 1. There were no significant differences in the responses on day 7 and 28. The daily oral therapy with ranitidine significantly (p < 0.0007) increased on day 1, 7, 28 and 16412 the i.g., median pH to 160%, 133% and 127%, resp., as compared to control (100%). After 7 days and 28 days of ranitidine the median 24 hr pH was significantly (p < 0.0015) lower than on day 1. There were no significant differences in the responses on day 7 and 28. Pantoprazole vs ranitidine: On day 1, 7 and 28 the pH-metry was differentiated with pantoprazole as compared to ranitidine there was no significant difference in the 24 hr i.g. pH-metry. On day 7 and 28 during the therapy with the pantoprazole the i.g. median pH was significantly (p < 0.0014) higher than on day 1, 7 and 28 during the therapy with ranitidine.

Table: ig 24-hr median-pH in response to orally given pantoprazole (40 mg) and ranitidine (300 mg). Results are medians with the interquartile distances (1. n = 20)

<table>
<thead>
<tr>
<th>Pantoprazole</th>
<th>Ranitidine</th>
<th>Control</th>
</tr>
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<tbody>
<tr>
<td>Day 1:</td>
<td>2.5 (2.3–3.2)</td>
<td>2.4 (2.0–3.1)</td>
</tr>
<tr>
<td>Day 7:</td>
<td>3.6 (2.9-4.3)</td>
<td>3.0 (2.1-4.2)</td>
</tr>
<tr>
<td>Day 28:</td>
<td>3.7 (2.0-4.7)</td>
<td>2.0 (2.0-3.2)</td>
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</table>

Conclusions: (1) Pantoprazole (40 mg) is a more potent inhibitor of i.g. acidity than ranitidine (300 mg). (2) On the first day of a 28-day long-term therapy with pantoprazole, pantoprazole is as potent as the maximal inhibitory efficacy of ranitidine on i.g. acidity. (3) During a 29-day therapy with pantoprazole no tolerance is seen, but in contrast, after 7 days an increase of the efficacy of pantoprazole in inhibition of the i.g. acidity is soon remaining for the whole duration, (4) whereas tolerance of the i.g. acidity of ranitidine occurs within 7 days of treatment.

1009 Evaluation and Validation of a Crohn’s Disease Activity Index Reflecting Macroscopic Acuteness and Its Relevance Influencing Short and Long Term Outcome
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Activity of Crohn’s Disease (CD) can be assessed by means of clinical, biological, and morphological criteria; however, there is still lack of a general accepted indirect and objective parameter allowing reliable and easy prediction of acute flare up.

38 patients were included into a prospective study and underwent endoscopic investigation, 18 with clinically exacerbated disease and 18 with supposed remission after conservative therapy. The macroscopic findings were categorized to constitute dependent variable yielding two ordinal levels: acute active disease or remission. The extent of affected mucosal area was not taken into consideration. Macroscopic and histological findings were compared. The serum parameters α1-antitrypsin, α1-glycoprotein (AGP), C-reactive protein, salinic acids, prealbumin (PAB), and albumin were used as independent variables. In order to validate the developed index an analogous study was performed including 44 patients, 29 with active disease and 15 controls. Basing upon macroscopic findings resp. the validated index defining acute active macroscopic disease vs remission, the duration of acute phase treatment and consecutive remission time of 41 subjects prospectively followed (range 5 to 61 months) was calculated.

Endoscopic and histological degrees of inflammation were highly associated showing a contingency of 96% (p = 0.001). The following model was calculated by stepwise logistic regression analysis: If AGP (mg/dL) 4.2 x PAB (mg/dL) ≥ 0.8, then active disease will be predicted with a sensitivity of 100% and a specificity of 96% (p < 0.001). Predictive values of the parameters were lower, respectively. The validation study confirmed existence of the model showing again high values for sensitivity (93%) and specificity.
1011 Role of Specific Cytokine Antagonists (TNFR-p55, -p75, IFN-γR) in Patients with Alcohol-induced Liver Cirrhosis

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The pathogenetic role of cytokines in patients with alcohol induced liver cirrhosis is still unclear. Recent results demonstrate enhanced levels of proinflammatory cytokines (e.g. TNF-α) and Th-1 cytokines (e.g. IFN-γ) in hepatic tissue of cirrhotics. Naturally occurring antagonists (TNFRs, TNF-γRs, IL-10) regulate cytokine synthesis in vivo thus attenuating systemic cytokine effects. We therefore studied soluble receptor molecules (TNFRs p55, -p75, IL-10) and the Th-2 lymphokine IL-10 in patients with different stages of liver cirrhosis.

Methods: 43 patients with alcohol-induced liver cirrhosis (age: 25-66 years; 33 men, Child-Pugh class A: n = 11; Child-Pugh B class: n = 18, Child-Pugh C class: n = 14) were included. 25 healthy persons (age 20-36) served as controls. Serum concentrations of TNF-α, IFN-γ, TNFR-p55, -p75 and IFN-γR were measured by enzyme-linked immunobinding assay (ELISA).

Results: Serum concentrations of the soluble cytokine receptors TNFR-p55, -p75 (p = 0.0001) and IFN-γR (p = 0.01) were strongly elevated in patients with liver cirrhosis compared to controls. Serum levels of both TNF-receptors reached maximum values in Child-Pugh class C, and were elevated compared to Child class A (p < 0.001). In contrast to TNF the IFN-γR levels declined during the progression of disease with significantly lower serum levels in Child-Pugh class C compared to class A (p < 0.05). TNF-α, IFN-γ and IL-10 reached maximum values in Child class C.

Summary: TNFR-p55 and -p75 are elevated in late stages of alcohol-induced liver cirrhosis, whereas in contrast highest levels of IFN-γR are found in lower Child stages A and B. Thus the pattern of soluble cytokine receptors in serum may characterize different stages of alcoholic liver disease. Evaluation of specific antagonists such as TNFRs and IFN-γR might modulate cytokine effects in vivo and contribute to the immunopathogenesis of alcoholic liver cirrhosis.
and IFN-γ might be involved in the immunopathogenesis of IBD by attenuating systemic cytokine effects.

**1016 Stromelysin 1 and 2 (MMP III and X) mRNA Expression in Inflammatory Bowel Disease**

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Introduction: Intraepithelial inflammation in Crohn’s disease (CD) is accompanied by excessive desquamation of crypts, whereas in other inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) fibrosis is less prominent. Previous investigations showed, that intestinal levels of mRNA transcripts for pro-collagens I, II, III and V increased in CD and UC compared to control. Therefore it was postulated that the amount of collagen deposition in IBD is also regulated by collagen degradation. We therefore showed that the mRNA expression of matrix metalloproteinases (MMPs) II and II is increased in UC but not in CD. Now we examined the mRNA expression of stromelysin 1 and 2 (MMP III and MMP X). These enzymes play an important role in the activation of MMP I, III and IX. Stromelysin 1 and 2 also digest various protein substrates such as proteoglycans, laminin, fibronectin, collagen type III, IV, V and gelsatin.

Methods: mRNA expression of stromelysin 1 and 2 was examined in colon tissue of specimens from 19 patients with UC (n = 6), CD (n = 7) and control (n = 6). On fresh frozen tissue sections in situ hybridization (ISH) was performed. For further characterization of the cells a combined immunohistochemical staining (IHS) and ISH was done with monoclonal antibodies against macrophages, leucocytes, desmin and smooth muscle actin.

Results: In areas with high inflammatory cell infiltration a large number of stromelysin mRNA producing cells was observed in both UC and CD. Stromelysin mRNA was also detected in areas of mucosal damage below the basement membrane and beneath normal epithelium in UC and CD. In histologically normal areas without cellular infiltration in CD and in controls only very few positive cells for stromelysin mRNA were detected. By combined IHS and ISH the stromelysin mRNA expressing cells were detected to be myofibroblasts and fibroblasts.

Conclusions: Transcripts for stromelysin 1 and 2 are significantly elevated in UC and CD compared to normal (control) intestine. The increased deposition of collagen in CD compared to UC thus may not be the result of a different mRNA expression but rather due to reassembled subepithelial collagens. Our findings suggest an increased mRNA expression of stromelysin in UC and CD combination with increased MMP I and II only in UC rather indicate an activation of collagen degradation in UC in contrast to CD.

**1018 Morphometric Analysis of MMP I mRNA Expression is a Predictive Parameter to Discriminate Between Ulcerative Colitis and Crohn’s Disease**

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Introduction: Discrimination between Crohn’s disease (CD) and ulcerative colitis (UC) sometimes remains a difficult task despite extensive histological examination. We have previously shown that matrix metalloprotease (MMP) I gene expression is differentially regulated in the active stages of CD and UC. The current study examines MMP I gene expression as a potential predictive parameter for classification in either entity of inflammatory bowel disease (IBD).

Methods: Biopsies of colon mucosa from patients with indeterminate colitis (n = 42) were investigated by mRNA expression of MMP I. Biopsies were classified as UC by ISH criteria if more than 60% of subepithelial myofibrobasts or less than 10% of the lamina propria were identified as positive for collagenase I transcripts. If these criteria were not fulfilled the biopsy was classified as CD. Statistical analysis was performed using the chi square test and positive and negative predictive values were analysed according to Bayes.

Results: All patients were followed up to 14 months. In 32 cases of the 42 patients it was possible by clinical and endoscopic criteria to identify the entity of IBD (CD = 18 cases; UC = 14 cases). In all biopsies examined by in situ hybridization, the classification to either CD or UC was prospectively correct using the defined criteria for MMP I mRNA transcript expression. These data reveal that ISH for MMP I has specificity and sensitivity of 1 for CD.

Conclusion: We therefore conclude, that in situ hybridization for MMP I mRNA transcripts in conjunction with morphometric analysis is a highly sensitive and specific approach to discriminate the two entities of IBD in cases of ambiguous histological results. We are currently substantiating these observations by examination of a large patient population to establish this method as an additional tool to conventional histological examination.

**1019 Value of Oesophageal Manometry in Different Indications**

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When organic reasons for dysphagia are excluded by endoscopy the oesophageal manometry is established for diagnostic evaluation in functional diseases of the oesophagus. Aim of our study was to determine the diagnostic value of manometry according to different indications and its consequences for therapy.

Methods: Over a 8-year period 1118 patients (565 male, 553 female; mean age 55 years) were examined by means of oesophageal manometry. Data of the manometries were analysed retrospectively for the indication, results of the manometric testing and following therapeutic consequences. Manometry was performed using a pneumohydraulic system and a 4-point sleeve in station-pull-through technique in all patients.

Results: Indication for manometry was dysphagia in 25% out of the patients (group A), 14% of dysphagia and 5% of non-ulcer dyspepsia in 36% (group B) and a primary motility disorder of the oesophagus was found in 36% and a secondary motility disorder in 20% of the patients. Manometry showed normal results in 44%. We found a substantial high positive correlation between the indication for manometry based on specific symptoms (e.g. dysphagia) and a pathological finding in manometry (group A: 76%; B: 58%; C: 45%). In 63% (395 out of 624) of the patients the manometric diagnosis led to a therapeutic consequence.

Conclusions: Oesophageal manometry is a well-established and important diagnostic tool in case of specific symptoms (e.g. dysphagia). But even in a lot of patients (45%) in our study examined for less specific complaints the manometry could detect pathological findings. In two third of our patients a pathological result in manometry was followed by a specific therapy.

**1021 Downregulation of IBD Mononuclear Phagocyte Activation In Vitro and In Vivo by Interleukin 10**

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Introduction: Active inflammatory bowel disease (IBD) has been shown to be associated with increased priming and activation of peripheral as well as intestinal monocytes/macrophages. The aim of the study was to investigate immunoregulatory properties of IL-10 in vitro and in vivo. Methods: Lamina propria mononuclear cells (LPMC) were isolated from colonic biopsies by collagenase digestion. The state of activity was determined by the capacity to secrete proinflammatory cytokines (TNF-α, IL-1β, IL-1α) and IL-1-α receptor antagonist (IL-1ra) or superoxide anions after stimulation with FMLP. TNF-α and IL-1-β gene transcription was studied by semiquantitative polymerase chain reaction using internal synthetic cytokine mRNA standards. In addition to in vitro studies three patients with ulcerative colitis received IL-10 enema treatment and proinflammatory cytokine release by LPMC/peripheral monocytes was studied.

Results: We confirm, that secretion of TNF-α, IL-1β and superoxide anions is increased in IBD intestinal LPMC as well as peripheral monocytes in comparison with normal controls. IL-10 downregulates IL-1β, TNF-α and superoxide anions in monocytes both in vitro and intestinal as well as peripheral mononuclear phagocytes in a dose dependent manner (see figure). In addition, secretion of IL-1ra is induced by IL-10. IL-10 induced downregulation of pro-inflammatory cytokine secretion in IBD mononuclear phagocytes is paralleled by inhibition of corresponding cytokine mRNA levels. Three patients with steroid refractory ulcerative colitis received IL-10 enema treatment once daily (100 μg/50 mL). LPMC as well as peripheral monocyte proinflammatory monocyte cytokine release was downregulated over 24 days. Moreover, in all patients clinical improvement (CAI, endoscopy) was seen.

Discussion: The increased levels of pro-inflammatory cytokine secretion (TNF-α, IL-1β, IL-1β) and superoxide release in IBD can be downregulated by IL-10. Downregulation of pro-inflammatory cytokine secretion by IL-10 is observed in vitro as well as in vivo. Most interestingly, topical treatment
**Impaired Deactivation of Intestinal Lamina Propria Macrophages by IL-4 in Inflammatory Bowel Disease: The Role of IL-4 Receptor Signal Transduction (IL-4 Stat)**


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Introduction: Active inflammatory bowel disease (IBD) has been shown to be associated with increased priming and activation of peripheral as well as intestinal monocytes/macrophages. Downregulation of macrophage activation by IL-4 is defective in IBD (Gastroenterology 1995; 108:21). We investigated whether formation of IL-4 STAT as the main event in IL-4 receptor signal transduction is altered in IBD. Methods: Lamina propria mononuclear cells were isolated from colonic biopsies by collagenase digestion. The state of activity was determined by the capacity to transcribe and secrete proinflammatory cytokines (TNF-α, IL-1β) or the IL-4 receptor antagonist (IL-1ra). Formation of IL-4 STAT was assessed by electrophoretic mobility shift assay. Results: IL-4 deactivates both IBD and normal intestinal macrophages in a dose dependent manner and specifically inhibits IL-1β and TNF-α mRNA formation and protein secretion. However, IBD macrophages require 50–100 fold greater amounts of IL-4 in comparison with normal controls to induce similar levels of inhibition. IL-4 induced downregulation of pro-inflammatory cytokine secretion in IBD macrophages is defective at the transcriptional level. Surface IL-4 receptor densities do not differ between IBD and normal monocytes. IL-4 induced formation of IL-4 STAT by the IL-4-R is identical between IBD and normal cells. Competitive inhibition-binding studies with recombinant IL-4 STAT-peptides and antibodies reveal no indication of structural differences between IBD IL-4 STAT and normal IL-4 STAT. Discussion: In comparison with normal cells, downregulation of IBD macrophage pro-inflammatory cytokine transcription and secretion is resistant against IL-4. IL-4 resistance is not due to a diminished number of IL-4 receptors or to an alteration of IL-4 receptor signal transduction (IL-4 STAT).

**Growth Factors and Tissue Proliferation in Chronic Inflammatory Bowel Diseases and in Collagenous Colitis**


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Aim: Epidermal Growth Factor (EGF) and Transforming Growth Factor (TGF-α and -β) play a mostly unknown role in postinflammatory tissue repair processes in the colonic mucosa. We investigated the expression of these growth factors in colonic biopsy samples from patients with Chronic Inflammatory Bowel Diseases (IBD) and compared them to those of patients with Collagenous Colitis (CC) in order to evaluate possible similarities and differences. Methods: We used APAAP-technique and an absolute quantification of intramucosal immunoreactive signals to evaluate data about the expression level of the localisation of EGF and TGF-α and -β in colonic mucosa samples from 70 patients with CC and 50 patients with IBD. Furthermore, we investigated the expression of EGF-Receptor (EGF-R). Platelet Derived Growth Factor (PDGF) and of CD68, as an indicator for epithelial regeneration. We used biopsy samples from healthy persons as controls to compare data against. Results: Samples from CC-patients showed significantly higher EGF- and EGF-R-expression levels than samples from IBD patients, mostly located on cryptoblasts. However, IBD samples showed risen expression levels compared to healthy controls. TGF-α and TGF-β-expression levels were significantly risen in epithelium of IBD samples, compared to CC samples, but we did not find any differences between samples from CC patients and healthy controls. Similar to this, epithelial destruction marked by EGF was significantly risen in IBD samples, but there was no significant difference between CC patients and controls. We could not prove any difference in the expression of PDGF. Conclusions: The highly risen expression levels of EGF and EGF-R in CC might be related to an unphysiological chronic inflammatory stimulus in absence of any heavy epithelial destruction but dependent on risen EGF-R-activity, which consecutively leads to the typical CC-related subepithelial collagenous layer. This stimulation, may be caused by yet unknown bacteria. The strong epithelial expression of TGF-α and CD68 in IBD patients seems to be related to a balanced, TGF-α and -β dominated regular mucosal regeneration after epithelial destruction.

**Predictors of Relapse in IBD: Increased Secretion of Pro-inflammatory Cytokines by LPMNC**


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Background: It has been shown that LPMNC even from non-inflamed mucosa secrete increased amounts of pro-inflammatory cytokines (TNF-α, IL-1β) in active IBD (Clin Exp Immunol 1993; 174:181). The purpose of this study was to evaluate in vitro secretion of pro-inflammatory cytokines by isolated LPMNC as a predictor for relapse in quiescent IBD patients. Methods: Colon biopsies were obtained from 125 outpatients with Crohn's disease or ulcerative colitis in clinical remission without steroid medication and/or cytotoxic drugs (CDAI < 150, n = 78 or CAI < 4, n = 47, resp.). LPMNC were isolated by collagenase digestion. Short-term culture supernatant concentrations of TNF-α (PWM stimulated) and IL-1β (spontaneous) were determined by specific ELISA. In parallel serum levels of pro-inflammatory cytokines were assessed. Patients were enrolled in a prospective one year follow up program. A relapse was defined by a rise of the CDAI by more than 50 pts to values ≥ 150 or a CAI ≥ 4 with more than 50% bloody stools, resp.

Results: Secretion of more than 70 pg/ml of TNF-α in LPMNC supernatant concentrations (or more than 65 pg/ml of IL-1β, resp.) was associated with a significantly (p = 0.01) increased likelihood to suffer from a relapse within the next year (figure above). Moreover, an inverse correlation between remaining remission time and actual height of pro-inflammatory cytokine levels was seen. Serum levels of pro-inflammatory cytokines did neither correlate to the amounts secreted by isolated LPMNC nor had any predictive value for the occurrence of an acute relapse. Discussion: Even in clinically quiescent IBD...
Intrahepatic Expression of Interferon-Gamma in Chronic Hepatitis C and in Non-Viral Liver Cirrhosis

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Interferon-gamma is a cytokine with important immunoregulatory and antiviral effects and is involved in shifting T-cell responses towards cytotoxicity. We have studied the quantitative intrahepatic expression of interferon-gamma mRNA with reverse transcription/competitive polymerase chain reaction in 47 liver tissue specimens: 18 hepatitis C (including 2 cirrhosis), 16 non-viral liver cirrhosis (11 primary biliary cirrhosis (PBC), 2 primary sclerosing cholangitis, 2 autoimmune hepatitis, 1 hemochromatosis) and 13 control samples (5 donor livers at the time of transplantation, 8 liver resections). Equal levels of cDNA were amplified in presence of a heterologous competitor fragment containing primer binding sites for interferon-gamma (Platzer et al., Transplantation 58: 256-264 (1994)). Results: Very low levels of interferon-gamma mRNA were found in 4/13 samples from the control group. In contrast, all samples from hepatitis C (p < 0.001 vs control) and 8/16 samples from non-viral cirrhosis (p < 0.01 vs control, n.s. vs control) expressed interferon-gamma mRNA. Steady state mRNA levels were higher for chronic hepatitis C than for non-viral cirrhosis. Conclusion: Intrahepatic interferon-gamma mRNA is predominantly expressed in samples from chronic hepatitis C. This indicates ongoing intrahepatic cytotoxic T-cell responses which, however, are not sufficient in eliminating the viral infection.

Identification of 4 T Cell Epitopes in the C-Terminal Region of HCV NS4-Protein

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Recently, we have shown that patients with a benign course of HCV infection and without viral replication have stronger cellular immune response to HCV core and envelope epitopes than patients with chronic hepatitis (Hepatology 1996; 13: 290-3). It is unknown whether this holds true also for nonstructural proteins. Therefore, we studied T cell proliferative responses (3H-thymidine incorporation) in 21 patients with chronic hepatitis C and 6 subjects without viral replication (anti-HCV positive, without HCV-RNA). 15 peptides (25mers, 10 amino acids) corresponding to aa 1616-1773 of the NS4 protein were synthesized and used as stimulating antigens. Two epitopes at aa 1624-1648 (epitope 1) and aa 1724-1748 (epitope 2) were recognized by lymphocytes from both patient groups. In general, lymphocytes from patients without viral replication showed better proliferative responses than those without viral replication (epitope 1: 33% vs 14%; epitope 2: 50% vs 14%). Two more epitopes could be identified at aa 1664-1688 (epitope 3) and at aa 1754-1778 (epitope 4) which induced lymphocyte proliferation predominantly in patients without viral replication (epitope 3: 67% vs 14%, p = 0.024; epitope 4: 50% vs 5%, p = 0.025).

Our data suggest that immune recognition of viral peptides is generally stronger in patients, who have overcome HCV infection. In addition, lymphocytes from these patients respond to more peptides of the NS4 protein.

Polyomavirus Nuclear Cells as Sources of Pro-Inflammatory Cytokines in IBD

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Peripheral and intestinal mononuclear/macrophages have been shown to be highly activated in IBD. The intention of this study was to investigate the state of activation of polyomavirus nuclear cells (PMN) in IBD and the inhibitory properties of IL-10. Methods: Peripheral polyomavirus nuclear cells obtained from patients with IBD (n = 16) were treated with phorbol 12-myristate 13-acetate (PMA) and ionomycin and sequential cytokine secretion was measured by ELISA. The percent of viable cells was determined by propidium iodide (PI) staining of nuclei in a subpopulation of PMN. Results: Polyomavirus nuclear cells in IBD showed significantly higher IL-1ß and IFN-gamma production compared to PMN from healthy controls (p < 0.001). The IL-1ß production was significantly lower in patients with IBD than in those without inflammation (p < 0.05). Conclusions: Polyomavirus nuclear cells in IBD may represent an important source of proinflammatory cytokines.

Antineutrophil Cytoplasmic Antibodies in Hepatitis C Indicate a Poor Response to Interferon Therapy

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Hepatitis C virus infection (HCV) can be associated with autoantibody formation including antineutrophil cytoplasmic antibodies (ANCA). However, it is not known whether the presence of ANCA is associated with a worse outcome for chronic hepatitis C. Methods: Therefore we analyzed the outcome of interferon therapy in 30 patients with chronic hepatitis C virus infection who were tested for ANCA with a commercial polyclonal ANCA test (IFN: IFNb2: n = 15, dose 3 x 5 Mio IE/wk) regarding whether ANCA were present or not. ANCA were investigated by indirect immunofluorescence according to the guidelines of the first ANCA-workshop in Copenhagen 1988. After 6 months of therapy patients were classified as 'complete responder' (normal aminotransferases, HCV-RNA negative), 'partial responder' (only amino transferases normalized) and 'non-responders' (elevated aminotransferases and HCV-RNA positive). Results: In 9 of the 30 patients ANCA were found before interferon therapy (titers: 1:20-1:640). Two of these 9 patients were classified as 'complete responder'. In the group of patients with ANCA the rate of biochemical relapse (epitope 2: 50%) was not increased compared to patients without ANCA (p = 0.05; one-tailed Fisher's exact test). Conclusion: Our data suggest that the presence of ANCA in patients with chronic hepatitis C virus infection may indicate a poorer response to interferon therapy.

After Orthotopic Liver Transplantation (OLT), Bilateral Secretion of the Open T Tube Represents Total Bilirubin Secretion of the Graft

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Background: Bile physiology studies in patients after OLT are performed assuming that T tube bile represents total bile flow of the graft. Aim: The present scintigraphic study using 99mTc-BiRIDA was carried out to clarify hepatocellular uptake and secretion during the first 10 days after OLT and mainly to determine the fractions of bile flow appearing outside via the T tube and entering the duodenum. Method: We performed hepatobiliary scintigraphy in 10 patients (5 female, 5 male) 4 to 10 days after OLT. OLT had been performed because of HCV-cirrhosis (n = 4), alcoholic cirrhosis (n = 3), PBC (n = 2) and cystic degeneration (n = 1). Nine patients showed an uncomplicated postoperative course while one patient suffered from acute rejection and preservation damage. Each patient received 200-250 MBq of 99mTc-BiRIDA ([2,4,6 trimethyl-3-bromomioimidodiacetic acid] i.e. as hepatobiliary tracer. Results: Patients with uncomplicated postoperative course after OLT showed a significantly delayed hepatic uptake of the tracer within 23.9 ± 2.2 min as opposed to 10 min as control value. Similarly, bilirubin secretion of 99mTc-BiRIDA was significantly reduced to 25.9 ± 6.9% after 90 min as opposed to 50% after 30 min in controls. Acute allograft rejection resulted in almost no uptake and only minimal secretion of the tracer. Taking all patients together, the bile fraction appearing outside via the T tube represented 54.3 ± 7.3% of total dosage of injected 99mTc-BiRIDA. Acute rejection did not result in different bile flow pattern, despite an increase in extrahepatic flow. The ratio between extrahepatic and intrahepatic bile flow was constant. Discussion: Hepatobiliary scintigraphy can be used to estimate hepatobiliary function of transplanted livers in man. Furthermore, we demonstrate that bile emerging via the open T tube represents total bilirubin secretion of the graft. Therefore, it is justified and valid to perform bile physiology studies using T tube bile in patients after OLT.

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Do Intestinal Protein Losses Contribute to Hypoproteinaemia in Patients with AIDS?

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Introduction: Hypoproteinaemia and wasting are well known complications in patients with advanced stages of the acquired immune deficiency syndrome (AIDS). This may be due to impaired protein synthesis or intestinal absorption of nutrients. However, possible intestinal losses of endogenous protein as a cause of hypoproteinaemia in patients with AIDS have not yet been further elucidated. Therefore, it was the aim of the present study to differentiate these pathogenetic factors. Methods: We studied stool frequency (tid), wet and dry weight (ww, gw), oranoal transit time (OAT [hrs]: radiopaque marker), serum albumin (AAL), gastrin (ggl), D-xylose-test (Xy1 [g, g5 hrs urine excretion]; spectrophotometry), and serum-amin in 13 unselected patients (m; mean age 34.2 year, range 27–50) with AIDS CDC IV (CD4 T lymphocytes: 72 ± 24/μl; body mass index [BMI]: 17.8 ± 0.7; ± SEM) and in 6 patients with HIV infection CDC II (CD4 T lymphocytes: 202 ± 126; BMI: 22 ± 1.1). 15 healthy persons (HP) (m; mean age 30.7 year, range 24–56; BMI: 23.6 ± 0.6) served as controls. Results: In patients with HIV infection CDC IV and IV, A45 were significantly increased, whereas BMI, Xyl, OAT, albumin and stool characteristics were different (p < 0.05) only in patients with AIDS (tab.).

p < 0.05

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Conclusions: Intestinal protein loss appears to be an early event in HIV associated malnutrition. In advanced disease, malabsorption is an additional factor.

1039 Cytokine Differences in Patients with Steroid-Resistant Versus Steroid-Sensible Rejection After Liver Transplantation


Evaluation of the cytokine network after liver transplantation may give some insight in pathophysiologic mechanisms of rejection and may lead to detection of patients at high risk.

Fifty patients with split organ transplants were monitored for various cytokines on a daily basis between August 1993 and September 1994. Rejection was assayed by histology in combination with clinical signs of rejection and laboratory investigations.

During the first postoperative month, 28 patients (34.6%) developed rejection; 14 patients (17.3%) were successfully treated with methylprednisolone (steroid-resistant rejection), while further 14 patients required additional treatment with FK506 or OKT3 (steroid-resistant rejection). a-GST (glutathion-S-transferase) was increased significantly 2 days prior to rejection in patients with steroid-resistant (33 ± 6.5 nmol/ml) and steroid-sensible rejection (52 ± 7.1 nmol/ml) compared with patients with no rejection (12.6 ± 3.5 nmol/l; p < 0.01). In patients with steroid-sensible rejection, a-GST normalized completely within one week, while a persistent increase was observed in patients experiencing steroid-resistant rejection. Similar observations were made for bilirubin, while AST and ALT levels showed minor differences between groups. While sIL-2R and IFN-γ were virtually absent in patients with steroid-sensitive or no rejection, a significant increase was observed during the late course of steroid-resistant rejection. A significant increase in patients with steroid-resistant rejection was also observed for sIL-6, sIL-10 and ICAM-1. Although there was an elevation of stNF-α and sIL-2R in patients with acute rejection, differences between steroid-sensible and steroid-resistant were minor.

a-GST, sIL-2R and IFN-γ in conjunction with sIL-6, sIL-10 and ICAM-1 may be useful as indicators for severity of rejection. Soluble IL-2R and sTNF-Rf seem to be involved in the rejection process, but differences between groups were not significant.

1040 Cytokine Pattern in Patients with Infections After Liver Transplantation


Severe infections may compromise the outcome of liver transplantation. Determination of cytokines may increase the knowledge of pathophysiologic mechanisms and lead to early changes in post-operative therapeutic management of patients at risk.

Between August 1993 and September 1994, 81 patients with split transplants were monitored for cytokines on a daily basis. Serious infections (n = 11; 13.6%) included microbiologic evidence and at least 2 secondary organ dysfunctions. Patients with mild cholangitis (n = 25; 30.9%) or no infection referred as control groups.

1041 Northern Blot Analysis of Histaminergic and Muscarinic Receptors on Rat Parietal Cells

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Muscarinic agonists stimulate gastric evo- and endocrine cells, while histamine selectively stimulates parietal cells. However, the presence of appropriate receptors on gastric mucosal cells is controversial: While in situ hybridization failed to detect muscarinic and histaminergic receptors in epithelial cells of rat gastric mucosa (Science 1992; 258: 1662–5), PCR revealed the mRNA of M3 but not of other muscarinic receptor subtypes in partially enriched parietal cells and fundic glands of the rat stomach (Gastroenterology 1992; 103: 870–5). We used highly purified fractions of isolated rat gastric mucosal cells to study receptor mRNA expression. Enzymatically isolated rat gastric mucosal cells were separated by counterflow elution into 3 fractions (F3–F5) according to increasing cell diameter and parietal cell content (4, 27, 80%). Density gradient centrifugation of F4 yielded highly enriched chief cells (90% of F4; > 95% of total chief cells), with 5% of the density gradient centrifugation of F5 almost pure parietal cells (> 95% chief; chief cells < 5%); F7. Plasmid DNAs are as probes for the muscarinic receptor subtypes M1, M3, M4, M5 (rat) and M2 (human) (T1. Bonner, Bethesda) and plasmid DNA of the H2 receptor (rat, J. C. Schwarz, Paris) were used to prepare [32P]phlatable cDNAs as specific probes for Northern blot of total cellular RNA of F3–F7. Signals were analysed by quantitative determination of radioactivity. DNA fragments were cut from F3 to F5 and were normalized. In addition, relative mRNA expression levels in F3–F5: F4. F5: F6: F7 = 0.69: 0.79: 0.89: 0.47: 1.00). No signal was obtained for the M2, M3, M4 and M5 subtypes. On the other hand, the H2 receptor was detected in F5 and F7 and a weaker 1.2 kb band. Corresponding to the contribution of parietal cells, the H2 signal was detectable in F3 and F4, much more pronounced in F5, and maximal in F7 while it was totally absent from F6. We conclude that the results indicate the presence of M1 receptors in rat gastric parietal cells (103: 870–5) — in the rat gastric mucosa not only the M2, but also the M1 receptor mRNA is expressed. Expression of both muscarinic receptor messages was more pronounced in parietal cells (F5) than in chief (F6), mucus and endocrine cells (F3), while the H2 receptor message is confined to parietal cells.

1044 14CO2 Breath Tests for Dynamic Measurement of Liver Function in Children

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Dynamic liver function tests for the measurement of disturbed liver function in children with chronic liver disease are of increasing interest. In order to test the 14CO2 breath test (ABT) and the iodine metatobazole formation (MEGx-test), we introduced the 14C-phenylalanine and the 14C-caffeine breath test (PBT, CBT). The aim of the present study was to compare the results of all these tests with routine ABT (Dxylose, GPT, PCHE, albumin), MEGx-test (992 kBq PT, bile acids, bilirubin, Gamma-GT) and to prove their validity in follow-up investigations. In 22 patients, aged 2–18 years, ABT, PBT, CBT, and the MEGx-test were performed: (1) 13 children with liver cirrhosis (LC) and portal hypertension (PH), (2) 3 children with M. Wilson before and during therapy, and (3) 6 patients after liver transplantation (LTX). The cumulative 14C-elimination of 14C-CE in PBT (applied dose) 13C(1) after 2 h (mean: 0.7 %), 992 kBq PT (1.5 mg/kg bw p.o.) and 4 h for CBT (3 mg/kg bw p.o.) and the 30-min MEGx serum concentration (1 mg/kg bw iodine i.v.) were used to measure liver function. The results of these tests were compared with the above mentioned laboratory data.
1045 Regulation of Nitric Oxide Synthesis in Enteric Synaptosomes from Rat Ileum

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Nitric oxide is suggested to be a NANC transmitter in the gastrointestinal tract. We have previously shown that enteric synaptosomes contain NADPH diaphorase and are capable of generating nitric oxide (NO) from L-arginine. The aim of the present study was to investigate the regulation of NO at the subcellular level. Homogenized rat ileum was submitted to various steps of differential centrifugation. Specific binding of 3H-saxitoxin served as neuronal marker. NO synthesis was determined as the rate of conversion of H-L-arginine to H2-L-citrulline. Experiments revealed an increased NO activity in ileal synaptosomes compared to controls (0.3 ± 0.1 nM) and NADPH (2.4 ± 1.6 nM) to 10-3 mg protein) compared to basal (12.9 ± 2.9 dpm x 10-3 mg protein). Ca2+ removal by EDTA reduced NO activity markedly (13.9 ± 3.3 dpm x 10-3 mg protein). NO activity was significantly increased by addition of calmodulin (1 μM) and the cofactor tetrahydrobiopterine (THB) 0.1 μM (16 ± 6.9 dpm x 10-3 mg protein) and was sensitive to the inhibition by L-NAME (100 μM) (157 ± 4.6 dpm x 10-3 mg protein). In the presence of forskolin (10 μM) NO activity was significantly reduced to 3.4 dpm x 10-3 mg (addition of L-NAMe and Ca+2 removal by EDTA blocked NO activity to basal levels (14.9 ± 4.0 dpm x 10-3 mg protein). Carbachol (1 μM) reduced NO activity to the same extent (18.8 ± 2.8 dpm x 10-3 mg). In summary, enteric synaptosomes from rat ileum contain a Ca2+, calmodulin-dependent NO, which consists up to 80% of constitutive NO. Unspecific stimulation of adenylyl cyclase by forskolin blocked NO activity suggesting an inhibitory role of protein kinase A. There is also evidence for inhibition of NO by protein kinase C demonstrated by a remarkable decrease of NO in the presence of carbachol.

1046 Mechanisms of CGMP-dependent Inhibition in Rat Ileum


Nitric oxide, a major candidate for NANC inhibitory neurotransmission, causes activation of soluble guanylate cyclase, subsequent increase of cGMP-levels and activation of G-protein. The aim of the present study was to characterize the pathways of muscarinic receptor-dependent mechanisms, especially the role of intracellular Ca2+ stores. Isolated segments of rat ileum were pre-stimulated with carbachol (10-5 M) or the Ca2+ channel activator Bay-K-8644 (10-6 M) and the effect of G-protein activators B-8-CGMP and B-8-CGMP on isometric contraction was investigated. 8-BrcGMP (ED50: 7.4 ± 5.4 μM, n = 12) and B-8-CGMP-GMP (ED50: 8.1 ± 10-5 M), (n = 8) induced a dose-dependent relaxation of the precontracted strips, which was unaffected by neuronal blockade using tetrodotoxin. Cyclosporin A (CPA), a blocker of the sarcoplasmic Ca2+ ATPase, caused a contraction of the uninjected muscle strips, which could also be inhibited by B-8-CGMP and B-8-CGMP (inhibition 88.2% of CPA-induced contraction, n = 11). However, when the tissue was pretreated with CPA and the inhibitory effect of B-8-CGMP was blocked (inh., ~0.7% of precontraction, n = 6). After washout the inhibitory effect of B-8-CGMP on CPA- or CPA-induced contractions was restored. In a second series of experiments intracellular Ca2+ stores were emptied by incubation in Ca2+ free buffer and repetitive stimulation with CCH. Immediately after restoring extracellular Ca2+ the inhibitory effect of B-8-CGMP and B-8-CGMP on CPA was maintained. When intracellular Ca2+ stores were emptied in addition presence of CPA, the inhibitory effect on CCH + CPA was blocked or reduced, analogous to the protocol without emptying Ca2+ stores. These results demonstrate that stable membrane permeable analogues of cGMP, which cause an activation of G-protein, are able to inhibit contractions induced by activation of CCH by CPA, the presence of B-8-CGMP, by blockade of the sarcoplasmic Ca2+ ATPase and by carbachol, which is known to cause influx and release of Ca2+. However, when CCH is used after blockade of Ca2+ ATPase, the inhibitory effect of B-8-CGMP or B-8-CGMP is greatly reduced or blocked. Thus it is speculated that CGMP-dependent mechanisms might activate Ca2+ ATPase activity. Supported by DFG Ai 2458/1.

1047 Secretion from Pancreatic, Neuroendocrine Cells Mediated by Small Synaptic Vesicle Analogues


Neuroendocrine cells synthesize two types of regulated vesicles, i.e. dense core vesicles (LDCV) and a vesicle type analogous to small synaptic vesicles of neurons (SSV-analogues). The function of dense core vesicles and their impact for the diagnosis and therapy of neuroendocrine tumor disease has been well elaborated. Properties of SSV-analogues in normal and transformed neuroendocrine cells are less understood. Here we present a biochemical and functional analysis of SSV-analogues in neuroendocrine tumor cell lines. By immunofluorescence microscopy and immunoreplica analysis, membrane proteins of neuronal synaptic vesicles, protein 25, synaptophysin and synaptobrevin can be detected in the cell lines AR42J, BON, RIN, and INR. In addition, neuronal proteins of the docking fusion complex involved in final exocytosis, syntaxin and SNAP25 are highly expressed in all four cell lines and also neuroendocrine tumor tissue (n = 8).

Transport of GABA by a low affinity plasma membrane transporter, different from the neuronal type is observed. GABA-uptake into AR42J cells is beta-sensitive whereas the uptake into BON, RIN, and INR cells is beta- or alpha-sensitive: With permeabilization, a 2-3 fold ATP-sensitive GABA uptake take into an intracellular compartment, probably the SSV-analogues is observed with all neuroendocrine cell lines. Using a antiseraum against the vesicular GABA-transporter this polypeptide can be detected in the four cell lines by immunofluorescence microscopy. In addition, 50 mM K+ or the calcium ionophore A23187 stimulate the release of GABA 1.5 fold from neuroendocrine cells.

To date data support the idea that pancreatic neuroendocrine tumor cells possess similar to neurons functional SSV-analogues which specifically take up, store and release GABA and probably other amino acid neurotransmitters.

1049 Association of Neuroendocrine Gastroenteropancreatic Tumor Disease with Multiple Endocrine Neoplasia Type I

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Multiple endocrine neoplasia type I (MEN I) is known to be associated with an increased risk of both bronchial and thymic neuroendocrine (NE) tumors as well as functional neuroendocrine tumors of the pancreas. Whether MEN I occurs more frequently in patients with neuroendocrine tumors of the intestine or with non-functional neuroendocrine tumors of the pancreas, has not yet been studied prospectively. We therefore started to screen patients presenting with NE tumor disease of the gastroenteropancreatic (GEP) system (apart from functional NE tumors of the pancreas) prospectively for MEN I. 94 patients (42 females/52 males; median age 54.2 years [range 17–90]) with histologically verified neuroendocrine tumors of the GEP system (stomach 5, pancreas 9, small intestine 1, colon 11, rectum 6, ovary 3, unknown primary 27) have so far been screened for additional endocrine tumor disease. The screening has comprised the medical history, the physical examination and as a minimum the determination of the serum calcium, the albumin and prolactin levels. Additional endocrine tumors have been found in 4 of the 94 patients. There were 3 proctacromomas and 3 primary hyperparathyroidism. The primary tumors of the 4 patients with additional endocrine tumors were localized in the stomach (n = 1), the pancreas (n = 2) and the stomach of the patient with the NE tumor of the stomach had a second NE tumor in her pancreas. 3 of the 4 patients with additional endocrine tumors had a positive family history for MEN I. Thus, patients with NE tumor disease of the GEP system, including functional NE tumors of the pancreas and NE tumors of the intestines, may have MEN I.

Early screening for NE tumor disease of the pancreas is warranted in MEN I patients. On the other hand, patients with NE tumor disease of the GEP system should undergo a limited screening for MEN I.

1050 Indeterminate Pancreatic Tumors: A Dilemma with Modern Diagnostic Methods


Background: In indeterminate pancreatic masses, histopathologic work-up of resection specimens is not a well-defined method of which to reliably differentiate between malignant and inflammatory tumors. We analyzed our experience with modern imaging methods such as ECRP endoscopic ultrasonography (EUS) and contrast-enhanced dynamic CT in providing this differential diagnosis.

Patients and Methods: All patients with Whipple procedures performed for pancreatic head tumors suspected to be malignant, who were operated on...
from 1989-1993, were retrospectively analyzed. Clinical data and laboratory values were noted. ERCP and CT images as well as EUS tapes were reviewed blindly by independent examiners.

Results: 40 patients (28 male, age 40-71 years) have been included so far; 11 of these had the initial histopathologic diagnosis of focal chronic pancreatitis based on work-up of resection specimens. However, on follow-up one of these patients had a pancreatic adenocarcinoma. Progression of abdominal ultrasound (US) and EUS in the diagnosis of malignancy were 73%/78% (CA-19-9, 80%/20% (ERCP), 81%/40% (CT) and 84%/16% (EUS). Preoperative biopsies (endoscopic, transpapillary, percutaneous) were performed in less than half of the patients, but were positive in only 55% of cancer cases.

Conclusion: Carcinoid tumors with modern imaging modalities and a reliable exclusion of malignancy in inflammatory pancreatic tumors is not possible (specificity < 50%), although sensitivity is usually over 80%. Imaging methods did not do significantly better than CA-19-9 determination. Follow-up of this patient cohort requires a preoperative histology is necessary to improve pa-
tient selection for surgery.

1052 Ultra-high-dose Lanreotide Treatment in Patients with Metastatic Neuroendocrine Gastroenteropancreatic Tumors

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Symptomatic control of functional neuroendocrine tumors (NET) of the gastroenteropancreatic (GEP) system can be achieved by somatostatin-analogues (octreotide, lanreotide). In addition, several studies reported about tumor regression. Assuming a dose dependent inhibitory effect of somatostatin and its analogues, we performed a study with 5 mg lanreotide s.c. three times a day in 30 patients with progressive gastroen-
teropancreatic NET.

Before entering the study, all patients (16 men, 14 female; mean age 57 years) had documented tumor progress as judged by computed tomography (CT) and/or abdominal ultrasound (US). All 30 patients with NE GEP tumors (7 foregut, 10 midgut, 5 hindgut, 8 unknown primary) underwent ultra-high-dose lanreotide therapy for one year. Tumor growth was evaluated at months 3, 6, 9 and 12 by abdominal CT. US chest X-rays. Serum chromogranin A, serum serotonin levels as well as urinary 5-HIAA levels were determined at 3-monthly intervals.

After one year of therapy, imaging procedures revealed a cessation of tumor growth in 13 patients (43%). One of these patients with a func-
tional midgut NET showed even a complete remission of liver metastases as demonstrated by CT scans. Another patient, ultra-high-dose lanreotide therapy led to a partial tumor regression. By contrast 13 patients (43%) had a tumor progression under this treatment protocol. 4 patients (14%) were excluded from the study after 1 (n = 1), 3 (n = 2) and 6 months due to progression of severe abdominal pain (n = 2) and toxicties (n = 1). Toxicties were observed in 6 patients (20%) and included initial mild abdomi-
nal pain (n = 4) and/or occasional discomfort at injection sites (n = 2). 70% of the patients with a functional NET showed a significant reduction of di-
arrhea; whereas fasting diminished in 50%. Elevated urinary 5-HIAA and serum serotonin-levels were significantly reduced after ultra-high-dose lan-
reotide therapy in 14 of 23 patients (61%).

Our data show that ultra-high-dose lanreotide treatment in patients with metastatic NET, progressive under previous therapies (e.g. low-dose somatostatin-analogues and/or interferon-alpha), can lead to an additional antitumor effect. Furthermore, toxicities were comparable to conventional dosages of somatostatin-analogues.

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1053 Is Luminal Somatostatin a Gastric Satiety Factor in Humans?

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In humans, the origin of satiety signals is primarily localized to the stomach. However, gastric hormones, i.e. gastrin and somatostatin, do not appear to act as circulating satiety signals at physiological concentrations. Recently, studies in pigs and rats showed that somatostatin within the lumen of the stom-
ach suppresses feeding. In humans, the effect of luminal somatostatin on food intake is yet unknown.

Methods: To address this issue, 8 healthy overnight fasted male volun-
tees received a 100 ml drink of water containing 0.3 or 6 mg somatostatin (Cramed®, 4 volunteers each received an additional dose of 12 mg somatostatin. All experiments were conducted on different days and according to a random-
ized crossover design. 10 min after the oral drink, the volunteers were offered standardized sandwich quarters (48 kcal; CHO:P:F = 40:20:40%) and mineral water. Food intake was subsequently recorded for 90 min. For measurement of peripheral venous somatostatin and gastrin, multiple blood samples were taken 15 and 5 min prior to the oral drink, 10 min after the drink (i.e. immedi-
ately before food availability) and 15, 30, 45, 60 and 90 min after initiation of eating.

Results: Food intake was essentially completed 45 min after initiation of eating and the amount of sandwich quarters consumed was 31 ± 2 g in control experiments (0 mg somatostatin). Following oral administration of somato-
statin, food intake 0-45 min was 30 ± 3 (mg), 31 ± 3 (mg) and 29 ± 2 (12 mg) sandwich quarters/month; in 12 patients, these data were compared to baseline levels that ranged between 25 and 30 pg/ml in all groups. 10 min after somatostatin ap-
plication, peripheral venous somatostatin levels were not different between groups (control: 27 pg/ml; 3 mg: 36 pg/ml; 6 mg: 28 pg/ml). Also postpran-
dial somatostatin increments above baseline were comparable in all groups. Basal gastrin levels (between 28 and 35 pg/ml) were not altered by oral so-
matostatin administration and reached a postprandial maximum 30 min after initia-
tion of eating in all groups (control: 102 pg/ml; 3 mg: 91 pg/ml; 6 mg: 98 pg/ml).

Conclusion: Thus, orally administered somatostatin did not alter human eating behavior suggesting that somatostatin acting locally within the gas-
tric/intestinal lumen does not induce satiety in humans as a single fac-
tor.

1054 No Synthase in the Human Gastrointestinal Tract

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Nitric oxide (NO) exhibits multiple physiological roles throughout the body. NO producing enzymes (NO synthases) are involved in the "non adrenergic, non cholinergic"-NANC-regulation of intestinal motility (neuronal NO synthase), regulation of vascular tone (endothelial NO synthase). Further NO synthase activities were reported to involve in immune defense mechanisms (inducible NO syn-
thesis). Using antibodies against the neuronal NO synthase on cryostat sections, we investigated the distribution of this enzyme in the human gastro-intestinal tract by means of immunofluorescence microscopy and the APAAP method.

For comparison, antibodies against classical marker proteins for cholinergic (synaptophysin, cholinacetyltransferase) neurons, adrenergic (tyrosine hy-
droxylase) neurons and neuroendocrine cells (synaptophysin) were applied. Antibodies against PGF 9.5 were used to detect all neural perikarya in the plexus myentericus.

Neither neuronal NO synthase immunoreactivity was observed in a sub-
population of intestinal neurons and neuroendocrine cells throughout the gastrintestinal tract. The enzyme occurred in 50 to 90% of the nerve cell bodies in the plexus myentericus. In addition, the neuronal NO synthase was detected in neuromuscular endings of the circular and in a lesser extend of the longitudinal muscular layer. The number of NO synthase positive neuro-
muscular endings was approximately twice as high in the circular muscula-
ture as compared to the longitudinal muscle layer.

NO synthase immunoreactivity in nerve cell bodies and neuromuscular endings colocalized with synaptophysin and cholinacetyltransferase sug-
ferring that cholinergic neurons are also NANC neurons. Interestingly, neu-
roendocrine cells of the gastroenteropancreatic system characterized by synaptophysin immunoreactivity did not contain neuronal NO synthase. How-
ever, co-localization experiments using various, specific marker molecules have not been performed so far.

Moreover, our data shows that neuronal NO synthase is expressed in a large number of intestinal cholinergic neurons. Neuronal NO synthase is expressed — at least — in a subpopulation of gastroenteropancreatic neuro-
dendrocell.

1055 Modulation of CMAR-mRNA Expression Does Not Influence Adhesion of HT29 Colon Carcinoma Cells

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Background: The expression and function of integrin adhesion molecules have been shown to affect epithelial differentiation, carcinogenesis and metastasis. We have previously demonstrated, that adhesion of colon carci-

oma derived HT29 cells to the extracellular matrix (ECM) components is low in fibronectin and FN collagen coated assays by integrins. Expres-
sion of these adhesion molecules is diminished in undifferentiated colorectal carcinomas in vivo (Gut 1992; 33(3): 342-8). Recently Pullman and Bodmer described a new gene named CMAR (cell-matrix adhesion regulator) isolated from a CDNA library of a differentiated colon cancer cell line, that, transfected into an undifferentiated cell line, increased the integrin-mediated adhesion of these cells (Nature 1992; 365: 529-532). The authors speculated that CMAR might be involved in the morphogenesis of colon carcinoma cells. We therefore investigated, whether, CMAR gene expression also regulates integrin func-
tion in HT29 cells. Methods: By RT-PCR a CDNA containing the whole coding sequence of CMAR was amplified from HT29 cells. This PCR-product was cloned in sense and antisense orientation in an eukaryotic expression vector

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Results: In presence of the antigen the infusion of gastrin-17 (15 pmol/kg/h) in addition to vagal stimulation elevated plasma gastrin levels to levels not different from those during vagal stimulation alone. Moreover, with identical plasma gastrin levels the antagonistic had no effect on vagally stimulated acid secretion (88.3 ± 10.7 M vs. 99.4 ± 9.9 µeq/20 min in the controls, n=5).

In conclusion the present data demonstrate for the first time that in rats in vivo endogenous bombesin-peptides contribute to vagal stimulation of gastric release and gastric acid secretion. Furthermore, the action of endogenous bombesin-peptides on parietal cell function is mediated only by an increase of gastric release under the experimental conditions employed.

1059 Antisecretory Effect of Loperamide in Human Colon Epithelial Cells (HT-29/B6)


Besides its well established action on intestinal motility, loperamide exerts antisecretory effects, the mediation pathway of which is a matter of debate. A naloxone-sensitive inhibitory action on neurons of the ENS (Ahasan, Br J Pharmac 1987) was opposed to a primary action on enterocytes without involvement of opiate receptors (Zavecz, Eur J Pharmacol 1982). In order to characterize the effect of loperamide we studied the effect of loperamide on stimulated CF secretion of cultured, highly differ-

ented colon epithelial cells (HT-29/B6). CF secretion was determined as HCl$\textit{P}$ of HT-29/B6 monolayers mounted in modified Ussing chambers. Well characterized secretagogues served to stimulate CF secretion as secretin kinase A (PKA), protein kinase C (PKC) or Ca$^{2+}$-dependent Cl$^-$ secretion. Stimulation of adenylate cyclase by forskolin (FSK, 10$^{-5}$M) resulted in a rapid and constant stimulation of HCl$\textit{P}$ with maximal values of 134±46 nmol/cm$^2$/h. Application of the calcium channel blocker Ca$^{2+}$-Nifedipine (1 µM) or Ca$^{2+}$-free media resulted in a rapid increase of HCl$\textit{P}$, followed by a 2-fold decline almost back to base levels. Thus, the respective stimulation of the main signal transduction systems involved in intestinal Cl$^-$ secretion results in characteristic HCl$\textit{P}$ time courses. Addition of loperamide (10$^{-6}$M) after FSK or addition of loperamide 30 min before CCh or PMA, strongly inhibited the secretory effects of these agents. FSK stimulated HCl$\textit{P}$ was inhibited by loperamide by 80% (p < 0.001). PMA stimulated HCl$\textit{P}$ by 79% (p < 0.001) and CCh stimulated HCl$\textit{P}$ by 43% (p < 0.05). Loperamide was also actively effluxed into the mucosal to the basolateral side. Only its antisecretory action was not influenced by preincubation with nalox- ne (10$^{-10}$M) and was mimicked by the calmodulin antagonist trifluoperazine (10$^{-5}$ M). Conclusion: We demonstrate a direct antisecretory action of loperamide in human colon epithelial cells. This antisecretory effect is not mediated by opiate receptors and can be mimicked by the calmodulin antagonist trifluoperazine.
Expression of Tissue Inhibitors of Metalloproteinases (TIMP)-1 and -2 in Normal and Fibrotic Liver

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Fibrotic liver displays characteristic changes in extracellular matrix (ECM) content and composition which are partly due to altered ECM degradation by matrix-metalloproteinases (MMP). MMP activity is controlled by tissue inhibitors (TIMP-1 and -2 as well as LIMP (large inhibitor of metalloproteinases, a complex of TIMP-2 and MMP-2). TIMP-2 blocks gelatine (MMP-2). TIMP-1 and LIMP are inhibitors for interstitial collagenase (MMP-1). MMP-2 substrates are basement membrane proteins and denatured collagens (gelatine), whereas native collagen type I is almost exclusively degraded by MMP-1.

We studied the cellular localization and kinetics of TIMP-1, -2 and MMP-2 expression by isotopic in situ hybridization combined with immunohistology for cell-type specific markers in normal and fibrotic rat liver (different time points following a single or repeated CCl4 administrations, or bile fibrosis induced by bile duct ligation), in primary rat stellate cell cultures with and without addition of TGF-β1, and human fibroblastic/cirrhotic livers.

TIMP-1 and -2 RNA transcripts were detectable in all fibrotic livers and in rat liver as early as 3 hours after CCl4-intoxication, pointing to a role in protecting tissue from accidental MMP-activation. The distribution of TIMP-2 RNA in mesenchymal cells was largely superimposable to the pattern of TIMP-2 expression suggesting formation of LIMP isolated rat hepatic stellate cells continuously expressed TIMP-1 and -2 as well as MMP-2 RNA, addition of TGF-β1 increased. TIMP-1 and MMP-2 transcript levels whereas TIMP-2 expression was reduced.

MMP-2 seems to be produced in relative excess over TIMP-2 in fibrotic livers, raising the possibility of free MMP-2 generation. TIMP-und MMP-2 expression patterns suggest inhibition of MMP-1 activity by TIMP-1 and LIMP in the presence of MMP-2 activity. The previously described quantitative and qualitative alterations of ECM in fibrotic liver; in particular the excessive accumulation of collagen type I, is thus likely to be caused by continuing fibrogenesis paralleled by altered fibrolysis.

Modulation of Enterochromaffinlike Cell-Function by Interleukin 1-beta and Transforming Growth Factor Beta

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The enterochromaffinlike (ECL) cells are histamine-containing endocrine cells in the gastric epithelium which play a central role in the peripheral regulation of acid secretion. Gastric inflammation can modulate the responses of secretory cells and can be associated with increased levels of proinflammatory cytokines or growth factors. The pro-inflammatory cytokine interleukin (IL) 1-beta and Transforming Growth Factor (TGF) beta have been shown to affect acid secretion and/or histamine release in vivo, but cellular mechanisms remain unclear. The present study has investigated the effects of these cytokines on histamine release from ECL cells in vitro. ECL cells were isolated from 0.1% acetic acid mucosa by a combination of enzymatic digestion, elutriation, density gradient centrifugation and short term culture to a purity of 80-90%. Purified ECL cells were cultured in the presence of 2% fetal bovine serum without CCK for 2-3 days in primary culture, histamine release was determined under basal or gastric-stimulated conditions with or without the addition of interleukin 1 and TGF beta. Interleukin 1-beta increased basal histamine secretion 2-fold at 2-20 U/ml (corresponding to 0.01-0.1 ng/ml) after 60 min of incubation. Calcium-signals of single ECL cells in response to IL 1 could not be detected. The IL 1-induced histamine release was small compared to gastrin (1 nm) or adenrenergic (epinephrine at 10 μM) substances which enhanced histamine secretion 3-5-fold. More prominent effects were obtained when ECL cells were preincubated with IL 1-beta (0.02-20 U/ml) and gastric-stimulated histamine release (3-fold of basal secretion) was determined. Pre-incubation with IL 1-beta at 2-20 U/ml largely inhibited the gastric-stimulated histamine secretion which may reflect the potent anti-secretory action of IL 1-beta in vivo. In contrast, transforming growth factor beta did not exert very prominent acute effects on histamine secretion at physiological concentrations. TGF beta stimulated basal histamine secretion 1.5-fold at 2-20 ng/ml after 60 min of incubation. Gastrin stimulated histamine release was augmented at 2 ng/ml, but only 40% inhibition was achieved. When ECL cells were cultured in the presence of TGF beta (0.2 ng/ml) and gastrin (1 μM) without the addition of fetal bovine serum to the culture medium, basal histamine release increased over 4 days of culture and gastrin-stimulated histamine release was maintained which may be explained by TGF-beta effects on cellular proliferation. Conclusion. Our data support evidence for functionally important interleukin 1 and TGF-beta receptors on rat gastric ECL cells.

Expression of Platelet-derived Growth Factor (PDGF) A- and B-chains and PDGF-receptors in Normal and Fibrotic Liver

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Liver regeneration and fibrosis is influenced by several growth factors including transforming growth factor (TGF-β1 and PDGF. Whereas TGF-β1 predominantly stimulates fibrogenesis and collagen synthesis, PDGF supports proliferation of stellate (Ito) cells in vivo. PDGF is a hetero- or homodimeric polypeptide composed of two chains, A and B, binding to two different PDGF receptors, types A and B.

We studied the cellular localization and kinetics of PDGF and PDGF-receptor expression by isotopic in situ hybridization combined with immunohistology for the detection of cell-type specific markers in normal and fibrotic rat liver (different time points following a single or repeated CCl4 administrations, or bile fibrosis induced by bile duct ligation), in primary rat stellate cell cultures, and human fibroblastic/cirrhotic livers.

Weak expression of PDGF-B was observed in few mesenchymal cells of some human liver biopsies. PDGF-A RNA was regularly detected in hepatocytes of all fibrotic livers, and in rat liver as early as one hour and with highest levels around 6 hours after CCl4 application. Proliferating bile duct epithelial cells also expressed PDGF-A. Both PDGF receptors were expressed by mesenchymal cells, mainly stellate cells, in all fibrotic livers, beginning 3 hours following CCl4 administration. PDGF-A represents the predominant hepatic form of PDGF, whereas local synthesis of PDGF-B seems to have a limited role. Expression of PDGF-A by hepatocytes and of PDGF-B receptors by mesenchymal cells suggests a role for epithelial-mesenchymal paracrine regulatory loops. Expression of PDGF receptors prior to the onset of TGF-β1 expression points to a TGF-β1-independent initial stimulation of these genes after toxic injury. The continuative expression of PDGF-receptors in fibrotic and cirrhotic liver, on the other hand, may well be influenced by TGF-β1 which is known to induce PDGF-receptor expression. Blocking of PDGF-receptors may provide a strategy to selectively inhibit the proliferation of mesenchymal cells and thus reduce the number of cells participating in fibrogenic processes.

Gastrointestinal Neoplasms in Patients with AIDS

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AIDS-Associated non-Hodgkin's Lymphomas (NHL) are the second most common malignant complication of AIDS.

The gastrointestinal tract (GIT) is the most frequent extranodal site of lymphomas, almost exclusively B-cell derived neoplasms.

For a period of the development, we investigated the gastrointestinal involvement in 647 patients with AIDS. Gastrointestinal involvement with NHL was seen in 17/647 patients (2.6%) with AIDS during this period. Gastrointestinal KS was detected in 70/647 patients (10.8%). 308/647 patients had died at the time of data analysis. This subset we found in 10/606 patients (3.3%) gastrointestinal NHL, all of them high-grade B-cell lymphomas. Gastrointestinal Kaposi's sarcoma (KS) was seen in 49/306 Patients (16%).

In a prospective analysis for the period of 20 months) we found in 93 endoscopically examined AIDS-patients gastrointestinal KS in 21 patients (22%), NHL of the GIT in 7 patients (7.5%).

Treatment with combination chemotherapy has resulted in remission rates of more than 50% for NHL, remission rates of more than 90% for KS treated with liposomal doxorubicin, paralleled by resolution of symptoms.

These results are a strong argument for early endoscopic screening of the GIT in HIV-infected patients, especially in symptomatic patients, in order to start early with specific systemic chemotherapy.

Collagen I, III and VI Sequester Hepatocyte Growth Factor (HGF) in the Hepatic Extracellular Matrix (ECM)

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Aims: HGF is considered the most important mitogen for hepatocytes. HGF binds to heparan sulfate (HS) in the ECM, and interaction with membrane-bound HS is necessary for effective hepatocyte stimulation. HGF binds to its receptor that is encoded by the c-met protooncogene. Since we found immunoreactive HGF primarily in the interstitial ECM, a compartment that contains only little HS, we studied the interaction of HGF with several collagens and noncollagenous ECM proteins.

Methods: Radiolabeled HGF was incubated with collagens I-VI, denaturated collagen chains, collagen CBG-peptides, fibronectin, laminin, undulin and fibrogin that were either immobilized on polystyrene microtiter wells or blotted to nitrocellulose after SDS-PAGE. Inhibition assays were performed with...
soluble ECM proteins and heparin, and bound HGF was analyzed by gamma-counting and autoradiography. Dissociation constants were obtained from Scatchard analysis.

Results: HGF bound with high affinity (KD between 10^-8 and 10^-9 mol/l) to immobilized collagens in the order: collagen VI > III > I > V > IV, whereas the noncollagenous proteins were much less effective. This binding could be inhibited by: 1) single collagen chains, with α2(I), α1(I), and the single chains of collagen VI being the most potent inhibitors. Cross-inhibition studies and fragmentation with CNBr indicated that collagenous consensus sequences mediated the interaction. Heparin, when compared to the collagen chains, was an about tenfold (i.e.) more potent inhibitor of the interaction of HGF with native collagens.

Conclusion: 1. HGF binds to collagens, preferentially to the intestinal types I, III and VI. 2. this interaction is mediated by collagenous consensus sequences. 3. collagens type I, III and VI may serve as major molecular stores for HGF in the intestinal ECM.

**1067**

Effect of Loperamide on Bethanechol Stimulated Gallbladder Contraction and Pancreatic Polypeptide Release in Man

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Loperamide (L, Imodium®, a µ-opiate receptor agonist, suppresses cholcecytostasis in both isolated and intact contraction and pancreatic polypeptide (PP) release. Cholinergic mechanisms, however, are involved in gallbladder contraction and PP release, as well. The intention of the study was to show whether bethanechol (B), an acetylcholine analogue, stimulates gallbladder contraction and PP release and whether these effects may be influenced by L.

In six male subjects (21-38 years), B was infused intravenously in increasing doses (12.5, 25, 50 µg/kg in 40 min) into 5 adenomatous polyps of the colon, after a bolus application of 16 mg L or a appropriate placebo, respectively. At regular intervals, gallbladder volume was determined using ultrasound, and plasma samples were drawn for radioimmunologic measurement of PP and CCK.

The low dose of B already caused a significant reduction of gallbladder volume, which was further reduced by the second dose of B (from 27 ± 2 ml to 15 ± 2 ml). Upon the high dose, no further contraction was observed. L completely prevented B-stimulated gallbladder contraction (26 ± 2 ml vs. 25 ± 1 ml). Basal plasma PP levels were significantly decreased by L (from 16 ± 3 ± 1 ± pmol/l, three hours after application). B significantly and dose-dependently stimulated PP release after placebo (from 15 ± 1 ± max. 36 ± 2 pmol/l) and L, (from 6 ± 1 ± max. 26 ± 6 pmol/l), as well. Though plasma PP levels after L remained below those after placebo, incremental integrated plasma PP release after B was not significantly different in the two series.

Plasma CCK levels showed a decreasing tendency during B infusion with or without L.

The results show that B, an acetylcholine analogue acting on muscarinic receptors, caused a gallbladder contraction and a PP release, as well. CCK could be excluded as a mediator, as no increase of plasma CCK levels was observed. B-stimulated gallbladder contraction, whereas incremental integrated PP release was not affected. Therefore, L may influence B-induced gallbladder contraction mainly via its property as µ-opiate receptor agonist and not as a peripheral anticholinergic. On the other hand, the decreased basal PP levels observed after L may be due to an inhibition of the vagal-anticholinergic tone.

**1068**

Collagen Peptides (PIILIN CVI) in Serum in Patients with Porphyria Cutanea Tarda


Background: Porphyria cutanea tarda (PCT) is frequently associated with liver fibrosis. Therefore, it is of interest to know serum markers of hepatic fibrogenesis (aminoterminal procollagen III peptide, PIILIN and fibrosis (collagen VI, CVI) in PCT.

Methods: PIILIN was determined by radioimmunoassay (Behring RoAgnot) and CVI by ELSA.

Results:

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PCT Overt (n = 25)</th>
<th>PCT in remission (n = 51)</th>
<th>PCT release (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIILIN</td>
<td>14 ± 0.6</td>
<td>11 ± 0.4</td>
<td>11 ± 0.4</td>
</tr>
<tr>
<td>CVI</td>
<td>161 ± 156</td>
<td>198 ± 193</td>
<td>196 ± 193</td>
</tr>
</tbody>
</table>

Mean ± SD; normal values: PIILIN < 11 UI, CVI < 100 µg/l; P< 0.05

Conclusion: Whereas the elevation of serum PIILIN in overt disease appears to reflect active fibrogenesis, the elevated CVI levels indicate a simultaneously enhanced collagen degradation in all clinical stages of PCT.

**1070**

Influence of TNFa on Barrier Function of HT-29/B6 Human Colon Cells

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Increased levels of TNFa are found in the intestinal wall of patients with inflammatory bowel disease and HIV infection. Previously we have shown that TNFa provokes ion secretion in human distal colum (Schmitz et al., Gastroenterol. 106. A295). It is unknown so far, whether TNFa alters the paracellular intestinal ion transport. We studied the effect of TNFa on barrier function of highly differentiated HT-29/B6 colon cells.

Serosal addition of TNFa dose-dependently decreased transepithelial resistance (Rt) to 194 ± 3.2% of the initial resistance of 376 ± 26 Ω · cm². The highest effective concentration was 100 nmol/l. The addition of TNFa to the apical side was ineffective. Cell deterioration was not detected by LD measurement in supernatants of TNFa-treated cells. Flux studies showed that 22Na s-to-m flux increased from 3.5 ± 0.3 to 10.2 ± 1.0 µmol · h⁻¹ · cm⁻². The highest effective concentration was 100 nmol/l. The addition of TNFa to the apical side was ineffective. Cell deterioration was not detected by LD measurement in supernatants of TNFa-treated cells. Flux studies showed that 22Na s-to-m flux increased from 3.5 ± 0.3 to 10.2 ± 1.0 µmol · h⁻¹ · cm⁻². Since the increase of the H₂-mannitol flux correlated with the increase of 22Na flux, enhanced flux rates fully accounted for increased paracellular resistance.

The effect of TNFa was enhanced by interferon-γ (IFNγ), but not by interleukin-1 (IL-1). Neither IFNγ nor IL-1 alone had an effect on Rt. Furthermore, inhibited protein synthesis by cycloheximide (100 µg/ml) enhanced the effect of TNFa, preventing stabilization of Rₜ at 19.4 ± 3.2% of the initial resistance 8 h after addition of TNFa. TNFa action was completely blocking by the tyrosine kinase inhibitor genestin (50 µg/ml).

Conclusion: 1. TNFa dose-dependently enhanced paracellular permeability of HT-29/B6 cells. 2. TNFa action relies on receptors reserved to the paracellular membrane of polarized cells. 3. TNFa action is mediated by the tyrosine kinase pathway. 4. Patients required to maintain low level resistance after TNFa action has peaked (after 8 h). 5. Interferon-γ enhanced the effect of TNFa on transepithelial resistance.

In this manner TNFa could contribute to diarrhea in various intestinal diseases by the deterioration of intestinal barrier function.

**1071**

Differential CD44-variant Expression in Intestinal- and Diffuse-type Gastric Carcinomas — A Role for H. pylori?

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The expression of CD44 variants may play an important role in tumor growth and metastasis. Recent data have shown differential expression of CD44 variants in intestinal- and diffuse-type gastric carcinomas (v5, v8). We investigated CD44 splice products-standard (without variants), v3, v5, v6, and v7 — in samples of intestinal — (n = 5) or diffuse-type (n = 2) gastric carcinomas and tissues obtained after resection for peptic ulcer disease (n = 1).

Expression of CD44 variants in normal mucosa was correlated to H. pylori associated gastritis. Methods: Total mRNA was extracted and transcribed to cdNA. CD44 variants were amplified with exon-specific primers and separated on agarose gels (RT-PCR). Results: CD44 standard transcripts were detected in all tissues. In diffuse-type carcinomas a typical band of 350 bp was found. In contrast, 5/6 intestinal-type carcinomas showed the additional expression of 2-4 CD44 variants. V3 and V5 transcripts were negative, v6 and v7 variants were weakly detected in samples obtained from diffuse-type carcinoma (2/2) and in chronic H. pylori-associated peptic lesions. All carcinomas of the intestinal type (5/6) showed v31, v5, v6 and v7 transcripts, while in the adjacent mucosa and in diffuse-type carcinoma only few variants were detected. CD44 variant expression in the adenocarcinoma was not related to the degree of H. pylori gastritis. Conclusion: The expression of additional CD44 variants is mainly detected in gastric carcinomas of the intestinal type, indicating a different pathogenetic origin. H. pylori associated gastritis is not associated with alterations in CD44 splice control mechanisms.

**1072**

Urea Breath Test in H. Pylori: Diagnosis: Origin of “False” Results and Influence of Food Intake


We studied the origin of “false” 13C-urea breath test (UBT) results in H. pylori diagnosis and the influence of non-fasting on the UBT. UBT was performed using 75 mg 13C-urea as 2 point analysis of exhaled breath by isotope ratio mass spectrometer (Optima, Fisons). Evaluation was performed in comparison to histology (Warthin starry staining) in 125 consecutive patients undergoing gastroscopy (2 antrum and 2 corpus biopsies). In any case of mismatching results re-gastroscopy was performed.

UBT was correlated with the density of antral H. Pylori colonization. 74 of 77 patients with positive histology were detected (sensitivity 96%). Two of the false negative UBT results were due to very low colonization densities during spontaneous elimination of H. Pylori and to pyloric obstruction.