per os (1 tablet 100 mg/twice/daily)]; (b) the patients of 2nd group received flucytosine (FCT) [150 mg/kg/daily per os (1 tablet 500 mg/twice/daily)]; (c) the patients of 3rd group received placebo (P) (1 tablet/twice/daily).

In order to evaluate the efficacy of pharmacological therapy, clinical examination was performed every week up to the end of follow-up (3 months); endoscopic examination was performed at the end of pharmacological treatment (5 weeks). All the patients selected for the study provided informed consent.

Results. After 5 weeks of treatment, complete remimission of endoscopic lesions was observed in 14 patients of F-group and in 4 patients of FCT-group ($p < 0.05$); partial remission of endoscopic lesions was observed in 6 patients of F-group and in 10 patients of FCT-group (p = n.s.), whereas 2 patients of P-group presented partial remission of esophageal lesions. No response was observed in 6 patients of FCT-group and in 18 patients of P-group, with a difference statistically significant in comparison with F-group ($p < 0.01$ vs FCT-group and $p < 0.001$ vs P-group).

As regards clinical symptomatology, complete remission was observed in 16 patients of F-group and in 12 patients of FCT-group (p = n.s.), with a difference statistically significant for both treatments in comparison with P-group ($p < 0.01$). Partial clinical remission was observed in 4 patients of F-group in 6 patients of FCT-group (p = n.s. vs F group) and in 6 patients of P-group. No clinical response was observed in 2 patients of FCT-group and in 14 patients of P-group ($p < 0.001$). No remarkable side-effect has been observed in the patients of both groups of treatment.

Conclusions. The results of this study have demonstrated that both F and FCT are efficacious in the treatment of esophageal candidiasis in AIDS patients with a difference statistically significant in comparison with P. F demonstrated a greater therapeutic efficacy than FCT, with a difference statistically significant, as regards both endoscopic and clinical response. Nevertheless, further controlled investigations are needed to improve our knowledges about the therapeutic action of antifungal drugs in the treatment of *Candida* esophagitis in HIV disease.

154 The Role of Colonoscopy in the Differential Diagnosis of Acute, Severe Haemorrhagic Colitis

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The diagnostic value of colonoscopy was assessed in 88 consecutive patients presenting with first attack of severe haemorrhagic colitis [sHC, ≥6 bloody bowel motions daily, fever and abdominal pain]. Blood tests and cultures, stool microscopy, parasitology and culture, abdominal films and sigmoidoscopy were routinely performed on admission. Colonoscopy was performed within 24 hours of admission (no later than 4 days after symptom onset) by a blinded endoscopist and biopsies were taken. Exclusion criteria were age over 65 years, regular use of NSAIDs and acute abdomen. The definite diagnosis of sHC was based on results of histology and stool culture. Acute self limiting colitis (ASLC) was diagnosed in 37, infectious colitis (IC) in 31 (salmonella 20, shigella 3, campylobacter 5, pseudomembranous colitis 2, yersinia 1), Crohn's colitis in 4 and UC in 16 patients. All patients were correctly classified as IC/ASLC or IBD by colonoscopy. Prominent endoscopic features in IC/ASLC were erythema, oedema, teleangiectasias, erosions, spontaneous bleeding and small aphthoid ulcers surrounded by a red halo. These lesions were patchily distributed; the sigmoid colon was always severely involved, followed by the rectum and the splenic flexure. The caecum was involved in only 31 IC/ASLC patients. Distal migration of lesions was seen in 15 IC/ASLC patients, who had rectal sparing on admission but subsequently showed severe rectal inflammation on colonoscopy. There were no complications. Repeat colonoscopies and biopsies every 6 months for 18 months confirmed that no IC/ASLC case had been initially mis-diagnosed. Thus, colonoscopy is invaluable in the early diagnosis and appropriate treatment of sHC.

155 Helicobacter Pylori Associated Gastritis in HIV Infection: Endoscopic and Histological Features in 32 Pts

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Helicobacter Pylori (HP) is the major etiologic agent associated with acute or chronic gastritis in immunocompetent patients. Moreover HP presence is demonstrated in 40–70% of biopsies of unselected patients undergoing upper endoscopy. Several recent studies have demonstrated low frequency of HP infection in HIV pts [1]. To verify the HP frequency in Italian HIV infected pts we have performed in the last three years, 220 symptomatic pts e.g. duodenal endoscopy with multiple antral biopsies (mean 6). In 32 pts (14.5%) the Giemsa staining was positif for HP. Endoscopic and histological features are showed in the table.

Endoscopic Features			
Gastric Ulcer 1 (3%)	Antral gastritis 15 (47%)	Duodenitis 8 (25%)	Normal 17 (53%)
Histologic Features	Total (32 pts)	HIV (18 pts)	AIDS (14 pts)
Superficial gastritis	3 (9.5%)	2 (11.5%)	1 (7%)
Chronic gastritis	13 (40.5%)	6 (33%)	7 (50%)
Chronic active gastritis	16 (50%)	10 (55%)	6 (43%)

Our data confirm the low frequency of HP infection in HIV infected pts even if symptomatic for epigastric pain, dyspepsia and vomiting. In all pts we observed various degrees of histologic gastritis even if endoscopic features were macroscopically normal. Not significant difference was registered in frequency and type of gastritis between HIV and AIDS pts. This low frequency of HP infection in HIV pts can be explained by hypochlorhydria typical in those patients [2] and by frequent antimicrobial therapy even if this suggestion requires subsequent studies to be confirmed [3].

[1] Edwards P.D. et al.: Am. J; Gastroenterology 1992; 12, 1761–4
[2] Lake-Bakaar G. et al.: Am. Int. Medicine 1988; 109, 502–4
[3] Blecker U. et al.: Am. J. Gastroenterology 1993; 88, 1294

156 The Enteropathogenic E. coli Strain RDEC-1 produces a new enterotoxin active on rabbit ileum in vitro

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RDEC-1 is known to possess biological features similar to human EPEC strains and to be diarrheogenic in the rabbit. We have found that RDEC-1 elaborates an enterotoxin inducing intestinal secretion in the small bowel in vitro.

Rabbit distal ileum and colon, stripped of the serosal and muscular layers were mounted in Ussing chambers where they were bathed by a Ringer solution at 37C and gassed with 95% O2/5% CO2.

In distal ileum both the whole culture and the supernatant from RDEC-1 induced a significant increase in potential difference (Delta PD) and short-circuit current (Delta Isc) that was abolished by substituting the chloride ion with sulphate in the bathing solution, by treatment with proteinase K and by heating the supernatant at 90C for 15 minutes. After fractionating the supernatant, the activity could be demonstrated in the 30–100 kDa fraction.

Curing RDEC-1 of a 42 MDa conjugative plasmid of previously unknown function (which we named pSR) eliminated the enterotoxic activity. Transforming pSR into E. coli HB101 conferred enterotoxin activity to native strain. This strongly suggests that the gene encoding for the toxin is located on pSR.

Preliminary attempts to establish the second messenger mediating the action of this new toxin showed no additive effect with maximal stimulation by theophylline, 8BrcGMP and calcium ionophore A23187.

In conclusion, our data suggest that RDEC-1 elaborates a new plasmid-encoded enterotoxin that we named RET for RDEC Entero Toxin.

157 Development of Retinoic Acid as a New Therapeutic Strategy for the Treatment of Pancreatic Cancer

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The goal of this study was to evaluate the potential role of retinoic acid (RA) in the treatment of human pancreatic adenocarcinoma. Six different human pancreatic carcinoma cell lines were characterized by cytokeratin phenotyping and found to express cytokeratins 8, 18 and 19 that are typically expressed in ductal pancreatic carcinomas. RA resulted in a time- and dose-dependent decrease of anchorage-dependent and -independent growth of all cell lines. In addition to growth inhibition, all cell lines demonstrated increased cellular differentiation as demonstrated by increased protein synthesis, decreased saturation density, decreased expression of protein kinase C, increased expression of ductal specific marker genes as well as morphological criteria. All-trans, 9-cis and 13-cis retinoic acid were found to be the most potent retinoids regarding growth inhibition and induction of differentiation. To evaluate the molecular basis of the RA action we used a combined approach of Western and Northern Blotting, immunoprecipitation and RT-PCR. In all cell lines tested we found a homogenous expression pattern of the intracellular RA effector molecules. All cell lines expressed the retinoic acid receptors (RAR) alpha, beta and gamma. Expression of the RAR gamma is usually restricted to epidermal cells, the most sensitive target organ of retinoids. In contrast, retinoic X receptor isoform (RXR) beta could not be detected. Of the cellular retinoid binding proteins we demonstrated the expression of CRABP and CRBP I but not CRBP II. To verify the expression of RAR in human pancreatic cancer we performed in situ hybridization of 25 human pancreatic carcino-

mas. In accordance to the in vitro data, we found significant expression of RAR alpha, beta and gamma in all pancreatic cancers. Furthermore, the grade of RAR gamma expression seems to directly correlate with the degree of tumor differentiation. These data therefore suggest, that retinoids by nuclear receptor mediated inhibition of growth and induction of differentiation represent a promising experimental strategy in the treatment of human pancreatic cancer.

158 Abstract: GP-3: A Newly Characterized Glycoprotein on the Inner Surface of the Zymogen Granule Membrane Undergoes Regulated Secretion

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We have recently reported the cloning of the rat zymogen granule membrane glycoprotein GP-3 and the related pancreatic secretory lipase (Wishart, M. J., et al. (1993) J. Biol. Chem. 268, 10303–10311). We have now generated specific anti-peptide antibodies against both GP-3 and secretory lipase and used these antibodies for the biochemical and physiological characterization of GP-3. Western blotting confirmed that GP-3 was found exclusively in zymogen granule membranes and was absent from zymogen granule content which contains the majority of secretory lipase. Extraction of zymogen granule membranes with Triton X-114 showed GP-3 to be significantly more hydrophobic than lipase. The GP-3 amino acid sequence contains one potential N-linked glycosylation site at Asn 336. The loss of concanavalin A labeling after both chemical deglycosylation with TFMS and enzymatic deglycosylation with N-glycanase showed GP-3 to possess a small N-linked oligosaccharide side chain. Digestion of intact and permeabilized zymogen granules with the nonspecific protease pronase further localized GP-3 to the inner surface of zymogen granule membranes. Since GP-3 is resident on the inner surface of the zymogen granule membrane, it should appear on the outer cellular surface after exocytosis. Although membrane attachment of GP-3 was resistant to treatment with phosphatidylinositol-specific phospholipase C, we observed that GP-3 is released into the pancreatic juice and that secretion of GP-3 was greatly enhanced by CCK.

159 Progression to High Grade Pancreatic Duct Adenocarcinomas Could Be Related to P53 Tumor Suppressor Gene Alteration

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K-ras gene activation by point mutation has been reported in 90% of human pancreatic duct cell adenocarcinomas (PDCAs) Similar activating mutations in K-Ras genes have been found in Syrian Golden Hamster induced PDCAs and related preneoplastic duct lesions suggesting that they act as an early event. Wild-type P53 gene is rate limiting for cellular proliferation, negatively regulating the cell cycle via the P53 protein and has been shown to inhibit oncogene-mediated transformation. Mutated protein cannot arrest the cell cycle in G1 phase to allow DNA repair. This suggest that lack of functioning P53 protein implies a genetic instability and rapid selection of neoplastic clones.

To confirm this model in pancreatic tumors we studied 98 PDCAs by immunohistochemical technique using P53 antibody. In addition 35 cases have been studied by the PCR and DGGE sequencing techniques. P53 hyperexpression was found in 46/98 PDCAs and P53 gene mutation in 18/35 PDCAs According to the histologic grade we found immunoreactivity in 5/14 (30%) G1, in 11/40 (27%) G2, in 30/44 (68%) G3 PDCAs; P53 gene mutation: in 4/13 (30%) G1, in 7/15 (46%) G2 and in 7/7 (100%) G3 PDCAs. In the 64 (48%) tumors with P53 alterations we found 9 (14%) G1, 18 (28%) G2 and 37 (57%) G3; in the 69 (51%) with no P53 alteration we found 18 (26%) G1, 37 (53%) G2 and 14 (20%) G3. No immunoreactivity was found in metaplastic and dysplastic pancreatic duct lesions we searched.

In conclusion our data suggest that P53 alteration is a late event not involved in the genesis of PDCAs but it seems to be implicated in tumor evolution from low grade to higher grade. This might be due to neoplastic cells unability to adequately repair damaged DNA, leading to genetic instability and to clonal expansion of P53 mutant cells because P53 mutated cells could have a selective advantage in the proliferation compared with cells without P53 mutations.

160 Medium-Chain Triglycerides are not as Well Absorbed in the Presence of Pancreatic Insufficiency as Many Would Believe

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Medium-chain triglycerides (MCT) are used in pancreatic insufficiency because they are more rapidly hydrolyzed than long-chain triglycerides (LCT) and can be absorbed as triglycerides. These assumptions are based on outdated reports. Moreover the decrease of steatorrhoea using MCT could be an analytical error if it was measured by the Van De Kamer's method which fails to extract completely MCT from feces. Aim of this study was to evaluate the absorption of MCT in the presence of pancreatic insufficiency using an accurate method for faecal fats.

We studied 7 patients (1 protein-calorie malnutrition, 6 chronic pancreatitis; 5 with >20 g/day steatorrhea) on a low fat diet. For periods of 5 days, butter (60 g/day) or MCT (55 g/day) without or with pancreatin (LCT or MCT, LCT + P or MCT + P respectively) were added to the diet. In the last 3 days of each period, feces were collected, weighed and assayed for fat and nitrogen contents by the Jeejeebhoy's method and the Kjeldahl's one, respectively.

Results: A) Faecal weight, fat and nitrogen outputs of the patients with severe steatorrhea are shown in the table (x ± SE). Faecal weight, fat and nitrogen losses were significantly decreased using LCT with pancreatin (LCT vs LCT + P, p = 0.009, 0.034 and 0.013 respectively, t-test for paired data). The use of MCT caused a no significant decrease of fat losses (LCT vs MCT, P = 0.072). A further decrease of steatorrhoea was obtained when MCT were used with pancreatin (MCT vs MCT + P, P = 0.037). B) The 2 patients with mild steatorrhea passed much more feces using MCT (LCT vs MCT vs MCT + P: 135 ± 30.5 vs 300 ± 58 vs 495 ± 102.2, NS). Faecal fats were also increased with MCT (LCT vs MCT vs MCT + P: 8.1 ± 1.5 vs 10.5 ± 2.3 vs 15.2 ± 2.8, NS).

In conclusions, pancreatic extracts are necessary for an optimal absorption of MCT. MCT or MC fatty acids can determine a cathartic effect.

Table:	LCT	LCT + P	MCT	MCT + P
Weight (g/day)	456 ± 88	309 ± 64	497 ± 151	294 ± 88
Fat (g/day)	54.9 ± 10.3	22.0 ± 5.1	38.6 ± 11.2	22.6 ± 8.2
Nitrogen (g/day)	7.3 ± 0.6	5.5 ± 0.4	7.5 ± 1.3	4.2 ± 0.8

161 Octreotide in Acute Necrotizing Pancreatitis: Results of a Prospective Case-Controlled Study

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Background: Acute severe necrotizing pancreatitis is associated with a significant mortality. To date no successful treatment is established. The purpose of our prospective open case-controlled study was to assess the efficiency of high doses of octreotide in the treatment of patients with acute severe necrotizing pancreatitis.

Methods: 77 patients with severe acute necrotizing pancreatitis were studied. In all of them surgical intervention had been necessary and local (abscess, necrosis) or systemic (sepsis, pulmonary or renal failure, shock) complications developed under conservative treatment. 31 patients received 100 μg octreotide tid intravenously for 10 days in addition to the standard intensive care therapy. The outcome was compared with that of 46 case-controlled matched patients with acute pancreatitis who had not been treated with octreotide. Patients and controls were followed up until death or discharge from the hospital (maximum 70 days).

Results: The groups (Octreotide-group, control-group) were highly comparable with regard to age (mean age: 53, 49 years), sex, severity of illness (APACHE II-Score: 26.4, 27), ethiology of pancreatitis, and pretreatment at the time of admission to the intensive care unit. Mortality within 70 days was 30% (9 of 31) in the octreotide group and 50% (25 of 46) in the control group ($p < 0.05$).

Conclusion: The results of our case-control study showed a beneficial effect of octreotide in patients with severe acute necrotizing pancreatitis. Based on these data a prospective, double-blind, placebo controlled study should be performed to reconsider these results.

162 Signal Transduction of Acidic Fibroblast Growth Factor in Rat Pancreatic Acinar Cells

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Fibroblast growth factor (FGF) receptors contain an intrinsic tyrosine kinase domain that is activated upon receptor occupation and phosphorylates target proteins such as the γ-isoenzyme of phospholipase C. By contrast, heptahelical receptors like the CCK receptor activate β-isoenzymes of phospholi-

pase C in a G-protein-dependent fashion. In the present study we investigated acidic fibroblast growth factor (aFGF)-induced inositol 1,4,5-trisphosphate (IP_3)-production and amylase secretion in isolated pancreatic acini and the role of G-proteins in this process. *Methods:* Pancreatic acini were isolated from rat pancreas by collagenase digestion and were permeabilized with 10 μg/ml of digitonin. IP_3-production and amylase release were measured using a radioreceptor assay and a colorimetric assay, respectively. Pertussis toxin-induced ADP-ribosylation was performed in isolated membranes from aFGF- or CCK-prestimulated acini and unstimulated control acini. *Results:* Incubation of the acini with aFGF caused a biphasic increase of both amylase release and IP_3-production. The maxima were observed at a growth factor concentration of 0.1 pM. The dose-response curve for CCK-stimulated amylase release was also biphasic with a maximum at 0.1 nM. However, different from aFGF, the downstroke of CCK-induced IP_3-production at supramaximal CCK concentrations was accompanied by a further increase in IP_3-production. In digitonin-permeabilized cells, guanosine 5'-(3-O-thio)triphosphate (GTP[S]) shifted the dose-response curve for aFGF-induced IP_3-production and amylase to higher growth factor concentrations. Pertussis toxin-catalyzed ADP-ribosylated of isolated membranes with [^{32}P]NAD^+, led to a specific labeling of a 40/41 kDa band, representing α-subunits of the G-proteins G_i1, G_i2 and G_i3. In membranes from aFGF- or CCK-preincubated acini ADP-ribosylation of this band was decreased by 53% and 45% as compared to control membranes. *Conclusion:* In pancreatic acini aFGF is a potent stimulator of amylase secretion. Interestingly, different from CCK the dose-response curve for aFGF-induced IP_3-production declined from the maximal response at supramaximal aFGF concentrations. Furthermore, this study shows for the first time that FGF receptors interact with pertussis toxin-sensitive G-proteins.

163 A Simple $^{13}CO_2$ Breath-Test for Assessing Pancreatic Exocrine Insufficiency

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A noninvasive test for assessment of fat digestion has been developed, which is based on the intraluminal hydrolysis of cholesteryl-[1-^{13}C]octanoate by pancreatic cholesterol esterase. We performed this test in 10 patients with pancreatic disease (9 with chronic pancreatitis, 1 with resected ampullary carcinoma), 6 with nonpancreatic disease and in 8 healthy volunteers. The substrate (500 mg of cholesteryl-[1-^{13}C]octanoate) was administered in a liquid meal together with 5 g of xylose to exclude any influence of gastric emptying. Breath samples were taken prior to ingestion of the meal and thereafter every 15 min for 6 hours. The ^{13}C was measured using an automated mass spectrometer (Europe Scientific, GB). *Results.*. Gastric emptying time of the test meal did not differ significantly among the 3 groups. In healthy subjects the median cumulative recovery of $^{13}CO_2$ at 6 hours was 32% (range 20–49). The pattern of $^{13}CO_2$ excretion in patients with pancreatic disease varied widely: the pattern was similar to that of controls in 2 patients, but excretion was delayed in 5, and was virtually absent in the remaining 3 patients. The median cumulative recovery at 6 hours was 13% (range 1–37). Statistically significant differences ($p < 0.05$) in hourly recovery of $^{13}CO_2$ were found between these patients and controls. In patients with nonpancreatic disease the median cumulative recovery of $^{13}CO_2$ at 6 hours was 17% (range 9–34), this was statistically significant ($p < 0.05$) when compared with controls. The 1-hr recovery of $^{13}CO_2$ was less than normal in 6 patients with pancreatic disease and in 2 patients with nonpancreatic disease. The 3 patients with severe steatorrhoea showed the lowest $^{13}CO_2$ excretion; in 2 of these, the test was repeated after pancreatic enzyme supplementation, which produced a significant rise in $^{13}CO_2$ recovery. There was a significant correlation ($p < 0.05$) between $^{13}CO_2$ excretion and the results of the fluorescein dilaurate test. *Conclusions.* This study indicates that severe pancreatic exocrine impairment can be easily detected with this simple and harmless breath test; these preliminary results also suggest that it has potential as a tubeless pancreatic function test in chronic pancreatitis.

164 Inhibition of CCK-Induced Pancreatic Growth by Dexloxiglumide, A New CCK-A Receptor Antagonist

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Dexloxiglumide (Dex; compound coded CR-2017) is a new selective CCK-A receptor antagonist which has been carefully characterized by *in vitro* and *in vivo* investigations. Previous studies from our laboratory have shown Dex to be capable of inhibiting CCK-induced pancreatic secretion in a dose-dependent manner. In the present investigation the effect of Dex (gift of Dr. L. Rovati, Rotta Research Laboratorium, Monza, Italy) on gastric and pancreatic adaptation in response to both exogenous and endogenous CCK was studied in rats. Caerulein (1 μg/kg, s.c., three times daily) was used as CCK agonist whereas camostate (200 mg/kg, i.g., once daily), a potent trypsin inhibitor was employed as endogenous CCK releaser. These compounds were administered to rats alone or in combination with Dex (25 mg/kg s.c., 20 min before each stimulation) for one week. Rats were then sacrificed, gastric corpus and antrum, as well as pancreas were excised, weighted and analyzed for tissue DNA and protein content.

Neither exogenous nor endogenous CCK affected growth of the corpus and the antrum of the stomach but both caerulein and camostate treatment resulted in pancreatic hypertrophy and hyperplasia. Dex suppressed both caerulein- and camostate-induced increases in pancreatic weight, DNA and protein contents (Table).

Treatment	pancreas weight	pancreas DNA	pancreas protein
caerulein	125 ± 5#*	124 ± 6*	151 ± 9*
caerulein + Dex	101 ± 6	108 ± 7	108 ± 5
camostate	159 ± 6*	126 ± 8*	173 ± 7*
camostate + Dex	112 ± 6	100 ± 9	128 ± 6*

% of control; *p < 0.01 versus control (ANOVA test)

These results demonstrate the ability of Dex to antagonize the growth-promoting effects of both exogenous and endogenous CCK on the pancreas, and confirm that CCK induces pancreatic growth through activation of CCK-A receptors.

165 Ultrasound Guided Extracorporeal Shock Wave Lithotripsy (ESWL) of Pancreatic Duct Stones

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Introduction. Formation of stones in the pancreatic duct can lead to obstruction and maintain obstructive chronic pancreatitis. It is known that removal of the obstruction in the pancreatic duct produces immediate pain relief. We report our experience with ultrasound guided ESWL of symptomatic pancreatic duct stones, which were not primarily extractable by endoscopy.

Method. In 23 patients suffering from chronical calcifying pancreatitis ESWL of symptomatic duct stones was performed in combination with endoscopic sphincterotomy. Only 5 patients had solitary stones. The average diameter of the largest stone in each case was 11 (5–18) mm. The dilated pancreatic duct measured on average 8 (5–10) mm. Strictures of the pancreatic duct were present in 11 patients. For fragmentation of the pancreatic duct stones up to 2000 ECG-triggered shock waves (MPL 9000, Dornier/Munich) were delivered per session. Average shock wave energy was 18 (14–22) kV. After ESWL the fragments were extracted and/or their spontaneous passage documented.

Results. Disintegration of obstructive calculi was possible in all cases. Completely stonefree ducts were achieved in 7 patients, some stone material remained in 16; pancreatic obstruction, however, could be resolved in all cases. 8 patients became completely asymptomatic, 11 reported a marked reduction of their pain. No major complications were observed.

Discussion. ESWL combined with endoscopic sphincterotomy is a successful non-operative new treatment option in pancreatic stone disease. It should be performed as soon as possible after manifestation of clinically relevant symptoms in order to prevent parenchymal atrophy and consecutive exocrine and endocrine dysfunction.

166 Clinical Interest of Identification of KI-*ras* Mutations in Pure Pancreatic Juice

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Most human pancreatic adenocarcinoma are associated with mutational activation of Ki-ras at the 12th codon. In order to improve the earlier diagnosis of pancreatic cancer, we developed a two steps test based on amplification of DNA samples obtained during ERCP. *Patients:* endoscopic ductal aspiration for cells or brush cytology were performed to 87 patients who underwent ERCP for diagnostic or therapeutic reasons. Seventy nine samples could be amplified (91%), the 79 corresponding patients were classified in 3 groups according to standard tests: group 1 (n = 37): patients free of any pancreatic diseases or presenting non tumoral pancreatic disease (acute or chronic pancreatitis); group 2 (n = 23) pancreatic tumor; group 3 (n = 19) pancreatic diseases with unknown etiology by standard tests. *Methods:* ERCP samples were subjected to polymerase chain reaction (PCR) amplification of Ki-ras gene and codon 12 analysis was performed by PCR mediated restriction fragment length polymorphism (RFLP) analysis. As a complementary analysis, the DNA fragment was sequenced. *Results:* Results of the molecular blind study were evaluated by comparison with the clinical follow-up of patients. In group 1, 35 patients, were normal by PCR-mediated RFLP and direct sequencing. Two false positive cases by PCR-mediated RFLP in group 1 were invalidated

by direct sequencing showing a normal Ki-ras 12th codon. In group 2, 11/14 patients with pancreatic carcinoma, 2/2 cystadenocarcinoma, 2/3 ampullary tumors, 1/3 cholangiocarcinoma, and 0/3 islet cell tumors showed punctual mutation of Ki-ras. In group 3, 4/18 showed a mutation of Ki-ras at the 12th codon. *Conclusion:* Endoscopic retrograde intraductal catheter aspiration is a simple technique to analyse cell samples. Specificity of PCR mediated RFLP was 94.5, and sensitivity for pancreatic carcinoma was 81%. Identification of Ki-ras mutation could distinguish pancreatic carcinoma from islet cell tumors, but not from other tumors. Clinical follow-up patients from group 3 will found out the real value of this early diagnostic test.

167 Endogenous Nitric Oxide in the Control of Esophageal Motility in Humans

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Recent animal studies have suggested that nitric oxide (NO) plays an important role in the regulation of esophageal motility, being partly responsible for latency period and latency gradient between the onset of a swallow and contractions of esophageal circular smooth muscles. The aim of this study was to evaluate whether endogenous NO is responsible for physiological timing of the forthcoming contractions in the human esophageal body after swallowing. Five male volunteers (age 21–25 years, weight 67–72 kg) were involved in this placebo controlled study on the effects of increasing doses of the NO synthase blocker, N^G-monomethyl-L-arginine (L-NMMA 1.0–4.0 μmol/min i.v.) and/or l-arginine (L-arg) (30 μmol/kg-min i.v.) on the peristalsis of esophageal body in response to wet swallows (5 ml of water) and lower esophageal sphincter (LES) resting pressure. The esophageal motor activity was determined manometrically using 3-channel Konigsberg catheter (Konigsberg, Pasadena, USA) and Microdigitrapper (Synectics, Stockholm, Sweden). The motility patterns and statistics were analysed using specially developed software (Gastrosoft, Irvine, USA). Significance was accepted with p values less than 0.05. Additionally, during all examinations arterial blood pressure (BP) was measured every 5 min. L-NMMA resulted in a significant and dose dependent reduction of the latency period between the swallows and the onset of contractions which was mostly pronounced in the distal esophagus (control: 7.07 ± 0.74 s vs. L-NMMA 4.0 μmol/min: 5.87 ± 0.57 s), and this effect was partially reversed after addition of L-arg to the L-NMMA infusion (6.91 ± 0.62 s). The overall duration (4.07 ± 0.15 s) and the onset propagation (3.93 ± 0.82 cm/s) of esophageal contractions were significantly reduced (3.63 ± 0.21 s and 3.37 ± 0.40 cm/s) while the amplitude remained unchanged during L-NMMA infusion and again, those effects were reversed during simultaneous infusion of L-arg. The resting tone of LES increased significantly during infusion of L-NMMA (control 27.6 ± 8.3 vs. L-NMMA 4.0 μmol/min: 42.2 ± 11.3 mm Hg) and these effects were reversed by addition of L-arg. The mean BP significantly increased during infusion of L-NMMA (control 97.0 ± 5.7 vs. L-NMMA 4.0 μmol/min: 116.4 ± 3.1 mm Hg) and this was reversed by L-arg. We conclude that in humans endogenous NO is involved, at least in part, in the physiological regulation of motility pattern of distal portion of the esophageal body and LES.

168 High Dose Effects of Dietary Fat on Postprandial Gastrointestinal Motility are Reversed by a Specific Cholecystokinin (CCK)-A Antagonist

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Dietary fats evoke a dose-dependent inhibitory effect on postprandial antral motor activity. CCK is potentially involved in mediating nutrient-induced changes in gastrointestinal (GI) motility. CCK effects can be specifically blocked by the CCK-A antagonist loxiglumide (L). This study was designed to evaluate whether L affects the response of GI motility to fat during the postprandial period. A total of 16 manometric tests were performed in 4 healthy controls (2 M, 2 F; 19–25 yrs). Each subject was studied in 4 separate occasions, at least one week apart. Manometry was carried out by using a low compliance perfusion system attached to an 8-lumen probe which was positioned with the side-openings across the antroduodenal junction (5: 1 cm apart) and in the proximal small bowel (3: 10 cm apart). Motility was recorded for 3 hours after ingestion of a low-fat mixed meal (513 KCal; 9% fat). Twenty minutes before meal ingestion each subject received an i.v. infusion of either saline (S) as placebo or L (5 mg/kg × 10 min bolus followed by 10 mg/kg/h × 180 min). Ten minutes after the beginning of meal ingestion different emulsions were infused in the stomach via a 9th catheter assembled with the manometric probe: control emulsion (CE: bovine albumin 3 g in S up to 150 cc) or fat emulsion (FE: bovine albumin 3 g and 80 g corn oil in S up to 150 cc). The effects of the two emulsions were separately tested in the presence of S and L i.v. infusion. Fat emulsions and i.v. infusions were randomly administered. Antral (A) and descending duodenum (D) postprandial motility were analyzed and the results expressed as mean motility index (MI) (±SD) at 10 min intervals for 180 min:

	CE + S	FE + S	CE + L	FE + L
A MI	39.9 ± 18.9	20.3 ± 20.1*	31.6 ± 20.8	32.5 ± 13.4°
D MI	131.2 ± 87.7	186.0 ± 85.0	32.4 ± 31.8#	30.9 ± 47.9#

A MI: *$p < 0.001$ vs CE+S; °$p < 0.001$ vs FE+S; D MI: #$p < 0.001$ vs CE+S and FE+S; (ANOVA).

In conclusion, L reverses the inhibitory effect exerted by dietary fat on antral motility. L reverses the intestinal motor response to meal ingestion regardless the amount of fat.

169 Electrogastrography (EGG) in Chronic Pseudo-obstruction (CIP): A Noninvasive Test which Correlates with Pathology

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CIP is a panenteric disorder of intestinal motor function which may be caused by primary disease of enteric nerves (visceral neuropathy) or enteric smooth muscle (visceral myopathy). Intestinal motility studies may be technically difficult because of hypomotility and patients often need laparotomy for a tissue diagnosis. This study aimed to determine if surface EGG would accurately diagnose the presence and type of pathologically proven CIP.

After an overnight fast we assessed gastric electrical control activity for 1 hour in the fasting and fed state by cutaneous surface EGG in 14 adults (range 20–63 years) with proven CIP. Electrical activity was recorded from four pairs of silver/silver chloride bipolar skin electrodes, the captured signal amplified and digitalised and running spectral analysis performed. The dominant frequency and power of spectrum were calculated using a sequence of computerised algorithms and displayed as a pseudo three dimensional plot. Results were correlated with the known pathological diagnoses (visceral myopathy (M) n = 7, visceral neuropathy (N) n = 4, undifferentiated (U) n = 3).

Dysrhythmias were present in 13 of 14 patients (1) A neuropathic pattern of tachygastria (electrical control activity frequency >5 cycles/minute) was documented in 5 patients (N = 4, U = 1). (2) Myopathic patterns of irregular continuous activity (no dominant frequency) or bradyarrhythmia were found in 6 patients (M = 5, U = 1). In all 6 patients there was abnormal electrical response activity (ERA) to food. (3) Mixed abnormalities were noted in 2 patients (M = 1, U = 1) and (4) normal activity with a clear dominant frequency of 3 cycles/minute was present in only one patient (M = 1).

This non-invasive technique is both sensitive and specific in providing evidence of a dysrhythmia in patients with CIP and discriminates between primary pathologies. EGG should prove diagnostically valuable, in addition to providing insights about the disturbance of electrical control activity.

170 Continuous In-Vivo Manometry of the Human Gallbladder

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Continuous in-vivo manometry of the gallbladder was conducted in 6 patients who had undergone percutaneous cholecystolithotomy for symptomatic gallbladder stones ten days previously and had a foley catheter inserted into the gallbladder after the procedure. A solid state transducer was inserted into the lumen of the foley catheter and connected to a portable 24-hour data-logger which constantly recorded intracholecystic pressure. Patients were asked to note the type and timing of any oral intake during the recording and these were correlated with gallbladder activity. The gallbladder pressure response to intravenous CCK was also recorded.

Results:

Event/stimulus	Gallbladder pressure (mbar) (mean ± SEM)
Basal (Nocturnal):	18.4 ± 1.6
Basal (Diurnal):	17.4 ± 1.3
Cup of tea:	35.2 ± 1.5*
Breakfast:	29.9 ± 2.0
Lunch:	26.2 ± 1.7
Dinner:	38.7 ± 2.0*
Intravenous infusion of CCK (0.02 mcg/kg):	39.9 ± 1.4*

*$p < 0.05$ vs Basal (Nocturnal) pressure: Wilcoxon signed rank test

Our early experience has shown that continuous direct in-vivo gallbladder manometry in humans is possible and the validity of the technique may be confirmed by recording pressure rise after intravenous infusion of CCK. This new technique may help to elucidate patterns of gallbladder activity and clarify the role of various diets in gallbladder emptying.