

vein (9.0%). All 12 patients were operated after they had achieved good general health and nutritious status. All operations were successful, and TPN provides high degree of the nutritious status of the pts, contributes to the success of the op. itself and decreases the postop. morbidity and mortality.

1706 Intestinal Ischemia and Reperfusion Induces Increased Concentrations of Endothelin-1 in Portal Blood

E. Schlichting, T. Asplin¹, T. Grotmol², T. Lyberg¹. ¹ Department of Surgery and Research Forum, Oslo, Norway; ² Ullevaal Hospital, and Nycomed Imaging, Oslo, Norway

Episodes of splanchnic ischemia are common in critically ill patients. A significant degree of myocardial impairment during splanchnic ischemic shock is also often registered. Endothelin is a novel, potent, endogenous vasoconstrictor derived predominantly from endothelium and macrophages.

Methods: Release of endothelin-1 (ET-1) into the jugular and portal vein, carotid artery, thoracic duct lymph and ascitic fluid was determined by radioimmunoassay in pigs undergoing either a hemorrhagic (3 h) or superior mesenteric artery (SMA) occlusion (5 h) shock followed by reperfusion (90 min). Endotoxin was also measured in the same biological fluids. A control group was observed for 7 h. A Swan Ganz catheter was used for hemodynamic measurements.

Results: After surgery, there was a significant increase in ET-1 in jugular and carotid plasma, lymph and ascitic fluid in all three models. The portal plasma ET-1 level was significantly increased ($p < 0.05$) in both shock models, but no significant increase was noted in the control model. Very high portal ET-1 levels (26.5 fmol/ml) were found during the early reperfusion period in the SMA occlusion shock model. At the same time an almost immediate circulatory collapse took place. All the pigs in this group died within 100 min after reperfusion. In the hemorrhagic shock model, no such further increase in portal ET-1 was registered at reperfusion and the hemodynamic parameters normalised to presurgery levels. No circulating endotoxin was demonstrated in any of the models.

Conclusion: The splanchnic bed may be a considerable source of ET-1 production during ischemia and reperfusion after general and intestinal shock. Reperfusion of ischemic intestines results in a cardiovascular collapse that may be enhanced by an "intestinal factor" and/or by mediators produced by the gut or liver as a consequence of the high portal ET-1 levels.

1707 The Relationship Between Mucosal Injury and Permeability Changes After Intestinal Ischemia and Reperfusion in Pigs

E. Schlichting, T. Grotmol¹, H. Kähler², T. Lyberg². ² Department of Surgery and Research Forum, Oslo, Norway; ¹ Ullevaal Hospital, Nycomed Imaging, Oslo, Norway

Ischemia and reperfusion of the gut may be an important etiological factor in the development of multiple organ failure.

Methods: The pigs were divided into three experimental groups: 1. SMA occlusion shock for 5 h followed by 90 min reperfusion. 2. Hemorrhagic shock, where the mean arterial blood pressure was reduced to 50 mm Hg for 3 h by drawing blood to an external reservoir followed by reinfusion of the shed blood and 90 min observation. 3. Control model. Samples of terminal ileum, jejunum, colon, liver and lung were taken immediately after death for histological examination and scanning electron microscopy. 5-bromo-2'-deoxyuridine (BrdU) was used to assess the proliferative activity. Ussing chambers were used to determine mucosal permeability (probe molecules of different molecular weights). A tonometer was used to measure the intramucosal pH (pH_i). Blood samples were drawn from the jugular and portal vein and the carotid artery, while lymph was collected from a catheter in the thoracic duct. Bacterial examinations were made from the blood, lymph and ascitic fluid as well as from specimens from mesenteric lymph nodes, liver and lung.

Results: Mucosal ulceration and sloughing were found in the SMA shock model, while the morphological changes were less pronounced in the hemorrhagic shock model. pH_i was reduced in both shock models. BrdU incorporation in the enterocytes was almost completely lost from the jejunum to the rectum in both shock models. Augmented small molecular flux and loss of short circuit current were found in both shock models (most marked in the SMA shock model). No pathogenic bacteria or endotoxin could be detected in blood, lymph or tissue homogenates in any of the models.

Conclusion: Although the mucosa morphology was deranged and mucosal permeability increased after intestinal ischemia and reperfusion, no translocation of bacteria or endotoxin took place.

1708 Purified Plasma Lipoproteins Have no Neutralizing Effect on Endotoxin-Induced Monocyte Responses Related to Initiation of Coagulation and Fibrinolysis

E. Schlichting, T. Henriksen¹, T. Lyberg. Dept. of Surgery, Ullevaal Hospital, Oslo, Norway; ¹ Dept. of Obst. and Gyn., Aker Hospital, Oslo, Norway

There is overwhelming evidence that the cells of the monocyte/macrophage lineage play a role in the mediation of the biological effects of endotoxin (lipopolysaccharide, LPS). When stimulated by LPS, monocytes are able to modulate their production of tissue factor (TF) (procoagulant activity), plasminogen activator (PA) (fibrinolytic activity) or tumor necrosis factor (TNF). These factors may play a significant role in the development of disseminated intravascular coagulation (DIC) associated with endotoxemia. In several assay systems LPS bound to lipoprotein has been reported to be less active than unbound LPS in stimulating monocytes.

Methods: Human monocytes were isolated from buffy coats obtained from healthy blood donors. LDL, VLDL and HDL were isolated from fresh EDTA plasma by sequential ultracentrifugal flotation with KBr. TF activity was measured in a one stage clotting assay, TNF α by an EASIA technique and PA was determined using a chromogenic substrate assay. Adherent monocytes were cultured in RPMI 1640 medium containing 5 or 20% FCS for 6 (TF), 22 (TNF) or 64 (PA) hours. First, LPS (0.01 and 1 μ g/ml) were preincubated with the lipoproteins for 2 or 16 hours before addition to the monocytes. To investigate whether binding of LPS to monocytes was affected by preincubation of the cells with lipoproteins, we secondly performed another series of experiments where lipoproteins were added to monocytes for 2 or 16 hours before LPS (0.001–1 μ g/ml) were introduced.

Results: The LPS-induced enhancement of TF activity and reduction of PA production were not prevented by lipoproteins. The increased release of TNF was likewise not inhibited by lipoproteins.

Conclusion: Lipoproteins do not render LPS less effective in stimulating TNF release, procoagulant and fibrinolytic activities in human monocytes.

1709 Inexpensive and Palatable Substitutes for Commercial Liquid Foods

S. Shabib, F. Pillo-Blocka, S. Zlotkin. The Hospital for Sick Children, Toronto, Canada

A wide range of commercial liquid foods are available which contain whole macronutrients (standard formulations) or nutrients in their most elemental form (elemental formulation). These liquid foods are used for a wide range of medical/surgical indications from infancy through adolescence. It is our clinical impression that noncompliance with their use in the pediatric population is a common problem due to their high cost, poor accessibility, especially in remote or isolated communities and their limited palatability. The objective of this study was to describe an alternative liquid food supplement that was inexpensive, nutritionally complete, palatable and readily available even in remote or isolated communities. The nutrient content of five liquid food products – a commercial product, whole milk, evaporated milk (diluted 1:1 with water), whole milk plus 18% cream and diluted evaporated milk fortified with table sugar were compared. A volume of 1300 ml of the "pediatric" commercial liquid food was found to meet the recommended nutrient requirements of an average 3 year old child (Canadian RNI, 1990). The volumes of whole and evaporated milk needed to meet the energy requirements were too high (2031 and 1855 ml) as were the protein intakes. Cream-fortified whole milk (5:1 ratio) fed at 1500 ml met energy needs but was too high in fat and too low in iron and B vitamins. Diluted evaporated milk fortified with 60 g of sugar per L fed at 1500 ml/day met all nutrient needs with the exception of iron and some B vitamins. Ferrous sulfate (15 ml on alternate days) and daily multivitamin drops would complete the vitamin and mineral needs. The recommended nutrient requirements of children between 1 and 7 years could also be met with fortified evaporated milk. This alternate food has an osmolarity of 414 mOsm/kg, was found to be palatable by a taste panel of 12 adults, is available internationally, and most importantly is only 12% of the cost of the commercial liquid food (\$560/yr compared to the commercial liquid food at \$4680/yr). In conclusion: when commercial liquid food products are not an option, the use of evaporated milk diluted 1:1 with water and supplemented with 60 g table sugar per litre, together with ferrous sulfate and multi-vitamins, will supply adequate nutrients and volume to meet the needs of children 1 and 7 years of age.

1710 p53 Overexpression in Early and Advanced Gastric Cancer

AM Chiaravalli, M Cornaggia, M Salvatore, N Sonzini, C Capella. Dept. Human Pathology, University of Pavia at Varese, Italy

The tumor suppressor p53 gene encodes for a 53KD nuclear protein that can be immunohistochemically detected when mutations of the gene occur.

or, less frequently, when the p53 protein is complexed with viral or cellular proteins. Overexpression of p53 protein (p53-OE) has been reported in an increasing number of human malignancies, including gastric cancer and can be used to evaluate the presence of p53 mutations.

We analyzed immunohistochemically (ABC technique) the p53-OE in 122 advanced gastric cancers (AGC) and in 75 early gastric cancers (EGC) using the anti-p53 monoclonal antibody DO7 (Dako) on microwave treated, formalin fixed and paraffin embedded sections.

In AGC 53/122 cases (43%) showed p53 immunoreactivity (IR). P53-OE was observed in 49% of the glandular carcinomas (GLCs) (mean % of IR cells: 43%) and in 30% of diffuse carcinomas (DCs) (mean % of IR cells: 26%) ($p = 0.04$ Fishers' exact test). None of the 10 mucinous carcinomas was positive. Carcinomas of the mixed GL-D type displayed p53-OE in 30% of cases mainly concentrated in the glandular component. P53-OE was not associated with tumor grade or with prognosis (5-year survival rate). In EGC p53-OE was found in 31/75 (41%) cases: in 51% of GLCs (mean % of positive cancer cells: 46%) and in 36% of DCs (mean % of positive cancer cells: 7%).

These data seem to indicate that:

- (1) p53-OE occurs in the early stage of gastric cancer progression
- (2) p53-OE in AGC is not related to survival
- (3) p53 mutation could play a more relevant role in the development of glandular gastric cancers than in the diffuse ones.

1711 Bacterial Translocation is Reduced by Piperacillin in Colorectal Cancer

D. Lescut¹, P. Vincent², A. Cortot¹, I. Lescanne¹, P. Quandalle³, M. Vankemmel⁴, J.P. Triboulet⁴, N. Almar⁵, J.F. Colombel¹. ¹Service d'Hépatogastroentérologie, ²Service de Chirurgie Adulte Ouest, ³Service et Est, ⁴Service et Laboratoire de Bactériologie, Hôpital Huriez, CHRU Lille, France; ⁵Laboratoires Lederlé, France

We have reported that bacterial translocation (BT) occurred in 65% of patients operated on for colorectal cancer (CRC) regardless its location and Duke's stage [1]. *The aim of this study was to assess the efficacy of piperacillin in reducing BT during CRC surgery. Methods:* 20 patients (11 M, 9 F, mean age 62 yrs) operated on for CRC (rectum: 2, left colon: 14, right colon: 4) received a single 4 g i.v. dose of piperacillin before induction of anesthesia. Samples of pericolic lymph nodes, portal blood, peritoneum and liver were harvested at laparotomy prior to opening of the bowel lumen and studied for BT as previously described (1). Results were compared to those observed in 20 patients operated on for CRC in the same conditions but without piperacillin administration (1). Lymph nodes and fragments of colonic wall were also obtained and stored at -40°C. Piperacillin concentrations in tissues were further analyzed using HPLC.

Results: (1) Rates of BT in the 2 groups.

Samples	CRC without piperacillin	CRC with piperacillin
Total	13/20	6/20*
Lymph nodes	13/20	5/20*
Peritoneum	3/20	1/20
Liver	2/20	0/20
Portal blood	0/20	0/20

* $p < 0.05$ vs CRC without piperacillin.

(2) Piperacillin concentrations in colonic wall was superior to MIC 90 of *Enterococcus*, *E. coli* and *B. fragilis* in 100% of patients; concentrations in lymph nodes were superior to MIC 90 of *Enterococcus* and *B. fragilis* in all cases and of *E. coli* in 66% of patients. *Conclusion:* In CRC surgery, a single 4 g i.v. dose of piperacillin a) allowed tissue concentrations superior to MIC 90 of the main bacteria involved in post-operative infections; b) significantly reduces BT.

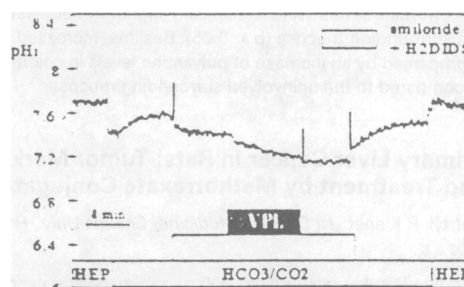
[1] J Infect Dis 1988; 158: 1395-6.

1712 Modulation of Multiple Drug Resistance with Verapamil, Cyclosporin A and Tamoxifen Decreases pHi in Human Colon Cancer Cell Lines

E.P. Cosentini, G. Hamilton, J. Zacherl, G. Bischof, B. Teleky, M. Riegler, W. Feil, R. Schiessel, E. Wenzl. *University Clinic of Surgery, A-1090 Vienna, Austria*

Reduced chemosensitivity of tumors in response to chemotherapeutic drugs such as anthracyclines, vinca alkaloids and podophylotoxins is mediated by the *mdr*-I-encoded ATPase-linked P-glycoprotein drug efflux pump. By reversing the MDR-phenotype with verapamil (VPM), cyclosporin A (CsA), certain hormones and other drugs an increase of intracellular drug accumulation can be achieved *in vitro*. Such a resistance-modification may be due to different mechanisms such as direct binding to P-glycoprotein and competitive inhibition of drug transport (VPM), nonspecific membrane alterations by lipophilic drugs and others. In our study we investigated the effect of VPM, CsA and tamoxifen (TMX) on pHi in different colon carcinoma cell lines (CaCo-2, HT-29,

SW 620). *Methods:* pHi was recorded by spectrofluorimetric monitoring of the pH-sensitive, fluorescent dye BCECF. Dye loaded cells were incubated either in Ringer-HEPES or Ringer-HCO₃ buffered solution (pH_o = 7.4, 25°C) and VPM (1 μM), TMX (2 μM) and CsA (1 μg/ml) were added. Within this concentration range resistance modification was demonstrated in chemosensitivity tests using doxorubicin in a thymidine incorporation assay. *Results:* Addition of VPM, CsA and TMX led to a reversible and dose-dependent decrease in pHi (0.1-0.3 units) within 10 min. for all colon carcinoma cell lines. Inhibition of the Na/H exchanger and the HCO₃-dependent transport systems with amiloride and H2DIDS showed that VPM, CsA and TMX do not interfere with these pH-regulating mechanisms, pointing to other mechanisms of acidification. *Conclusion:* Since colon carcinoma cells exhibit a higher resting pHi compared to normal colon crypt cells it is possible that intracellular acidification increases the sensitivity of the cell lines to chemotherapeutic agents. This is supported by a minimal cell survival at lower pHi in response to doxorubicin in the SW 620 cell line under tissue culture conditions at 15-20 mM HCO₃/5% CO₂. Alteration of pH-regulating mechanisms with specific ion channel inhibitors may enhance the modulating effect of MDR-targeted drugs and contribute to increased drug sensitivity.



1713 Chronology of p53 Protein Accumulation in Gastric Carcinogenesis

M.E. Craanen^{1,2}, P. Blok³, W. Dekker⁴, G.N.J. Tytgat¹. ¹Dept. of Gastroenterology, Academic Medical Center, Amsterdam; ²Dept. of Medical Oncology, The Netherlands Cancer Institute, Amsterdam; ³Dept. of Pathology, Westeinde Ziekenhuis, The Hague; ⁴Dept. of Internal Medicine, Kennemer Gasthuis, Haarlem, The Netherlands

Background: p53 Protein accumulation, reflecting p53 gene mutation, has been reported in up to 60% of advanced gastric carcinomas. However, the stages of gastric carcinogenesis, at which p53 gene mutation(s) can be found are still poorly addressed. We therefore examined p53 protein accumulation in early gastric carcinoma (EGC), dysplasia, chronic atrophic gastritis and subtypes of intestinal metaplasia (IM) to determine the chronology of p53 mutation in gastric carcinogenesis.

Methods: 45 formalin fixed gastrectomy specimens from patients with EGC were retrieved from the files. The Lauren tumor type was reassessed. The presence and grade of dysplasia and the presence of chronic atrophic gastritis and IM in the gastric mucosa was noted. IM subtypes according to Filipe were defined using Alcian Blue pH 2.5/Periodic Acid Schiff and High-Iron-Diamine/Alcian Blue. p53 Immunoreactivity was semi-quantitatively assessed using the mouse monoclonal antibody DO-7. A tumor was judged as p53 positive in case at least 10% of the tumor cells showed p53 protein accumulation.

Results: p53 Protein accumulation was found in 27 (60%) early gastric carcinomas 14 out of 20 (70%) intestinal-type and in 13 out of 25 (52%) diffuse-type EGC (not significant). In nearly 80% of p53 positive intestinal-type EGC more than 60% of the tumor cells were p53 positive and showed strong immunoreactivity as compared to respectively 23% ($P < 0.01$) and 30.7% ($P < 0.05$) of p53 positive diffuse-type EGC. Out of 4 areas of gastric dysplasia (1 mild, 2 moderate, 1 severe) only severe dysplasia showed p53 protein accumulation. No p53 protein accumulation was observed in normal gastric mucosa, chronic atrophic gastritis and foci of IM subtypes (type I: 271 foci, type II: 189 foci and type III: 53 foci).

Conclusions: p53 Gene mutation is a late event in gastric carcinogenesis. Moreover, it is suggested that p53 gene mutation occurs in a final pathway of genetic alterations common to both intestinal- and diffuse-type EGC.

1714 EGF Receptors and Polyamine Levels in Human Colorectal Adenocarcinoma and Surrounding Mucosa

C. Messa, F. Russo, M. Linsalata, A. Cavallini, M. Notarnicola, P. Berloco, A. Di Leo. *Lab. di Biochimica, I.R.C.C.S. "S. de Bellis" Castellana G., Italy*

Epidermal Growth Factor (EGF) is a polypeptide with a growth-promoting capacity in a variety of *in vivo* and *in vitro* cell systems. The EGF activity is me-

diated by the specific membrane receptor (EGFR) whose existence has been demonstrated also in the normal and neoplastic gastrointestinal (GI) mucosa. Polyamines – putrescine, spermidine and spermine – have been suggested to play an important role in cellular differentiation and proliferation in GI mucosa. Studies on humans and laboratory animals indicate the existence of some interactions between EGF and polyamines. Therefore, *aim* of this study was to evaluate EGFR by an enzymatic immunoassay and polyamine levels (Put, Spd and Spm) by HPLC in neoplastic tissue and surrounding uninvolved mucosa samples taken from 20 patients (13 M and 7 F, age 65 ± 14 yrs) with histologically diagnosed colorectal adenocarcinoma. The *Results* were analyzed by Student's t test for paired data. Polyamines are expressed as nmol/gr wet tissue, EGFR as ng Ab.B/mg prot, M \pm DS)

	Tumour	Normal
EGFR	188.6 \pm 109.1	136.6 \pm 89.2
Put	32.3 \pm 30.4	13.4 \pm 11.1
Spd	285.8 \pm 107.9	152.5 \pm 60.4
Spm	659.7 \pm 288.6	535.3 \pm 137.8
tot	978.3 \pm 374.9	701.1 \pm 185.1
ratio	0.44 \pm 0.10	0.28 \pm 0.08

EGFR and Polyamine levels were statistically higher in neoplastic tissue than in normal surrounding mucosa ($p < 0.05$). Besides, increased levels of EGFR are accompanied by an increase of polyamine levels in colorectal neoplastic tissue compared to the uninvolved surrounding mucosa.

1715 Primary Liver Cancer in Rats: Tumor Markers and Treatment by Methotrexate Conjugates

V. Dufek, J. Petrtýl, P. Klener. *1st Dept. of Medicine, Charles Univ., Prague, Czech Republic*

In 28 male Sprague-Dawley rats (weight 300 g) a primary liver cancer (PLC) was induced by intraarterial methylcholantren (MECH) application. The PLC development was monitored both by tumor markers (AFP, CA 195, CA 19-9, DUPAN-2, CB-like) and US. After the end of carcinogenesis (70% of the liver affected by the tumor according to US), a chemotherapy by methotrexate (MTX, 300 mg/kg weight) or MTX-albumin conjugate has begun. *Results*: MECH induced PLC was observed in 26 rats (93% of animals) within 32 days. In the course of PLC development a significant elevation of AFP, CA 195, DUPAN-2 and CB-like was observed in 82% out of cases. In rats without a subsequent chemotherapy, the median survival was 27.6 days. In those treated by simple MTX, the median survival was 35.8 days. In rats receiving MTX-albumin in the same dose, the median survival reached 97.3 days. During such a treatment, the decline of above mentioned tumor markers was observed. We were also able to demonstrate a reduction of the tumor mass by US. *Conclusions*: MECH seemed to be a potent compound in the induction of PLC in rats. The development of PLC was accompanied by the increment of some tumor markers and again, by its decrement in the course of adequate chemotherapy. The MTX-albumin conjugate was significantly more potent in survival rate evaluation in comparison with MTX alone. The toxicity of the former was, even more lower, than that of the latter.

1716 Levels of Nitrite and Nitrate in Gastric Juice and Correlation with Gastric Histology

J. Esteve, M. Faisca, P. Borralho, P. Caldeira, T. Belo, H. Guerreiro, J.C. Silva, C. Carvalheira. *Dept of Gastroenterology, Hospital of Faro, Faro, Portugal; Dept of Clinical Pathology, Hospital of Faro, Faro, Portugal; Dept of Pathology Hospital Garcia de Orta, Faro, Portugal*

Nitrite had been implicated in gastric carcinogenesis, although the correlation between nitrite levels in gastric juice and premalignant lesions remains unclear. *Aims*: To assess nitrite and nitrate levels and pH in gastric juice, and to correlate these findings with the severity of gastric mucosal lesions. *Patients and methods*: Gastric juice, antral and body biopsies were obtained from 53 patients. Nitrite was measured after reaction of the diazotized sulphanilic acid with N-(1-naphthyl) ethylenediamine; nitrate was measured by a ultraviolet method (Boehringer Mannheim); pH was determined using a pH meter with a glass electrode. Statistical analysis was performed using ANOVA and linear regression. *Results*: Eight patients were normal or had non atrophic gastritis (N/NAG), 21 had antral atrophic gastritis (AAG), 18 had atrophic pangastritis (AP) and 6 had adenocarcinoma (ADC) of the antrum or body.

The table shows nitrite, nitrate and pH in each group:

	Nitrite ($\mu\text{mol/l}$)	Nitrate ($\mu\text{mol/l}$)	pH
N/NAG	1.4 (SD 4.1)	298.1 (SD 343.7)	2.9 (SD 1.7)
AAG	10.8 (SD 25.3)	462.3 (SD 368.3)	3.5 (SD 1.9)
AP	31.3 (SD 33.3)	491.1 (SD 455.3)	4.9 (SD 2.4)
ADC	60.6 (SD 29.4)	360.8 (SD 104.0)	7.0 (SD 0.5)
	$p < 0.001$	NS	$p = 0.001$

Patients with intestinal metaplasia presented a higher level of nitrite (25.2 vs 12.0 $\mu\text{mol/l}$, $p = 0.12$). There was a significant correlation between nitrite

and pH levels ($r = 0.79$, $p < 0.001$). No correlation was found between nitrate levels and nitrite levels or pH. *Conclusions*: Nitrite concentration and pH of gastric juice increased significantly with the degree of gastric atrophy strongly suggesting a role of these substances in gastric carcinogenesis.

1717 Effect of Sulindac on Tumor Induction and Regression in Dimethylhydrazine Induced Murine Colonic Tumors

W. Fischbach, J. Rheinländer, Th. Papavassiliu. *Medizinische Poliklinik, University of Würzburg, Germany*

Sulindac, an inhibitor of prostaglandin synthesis, has been reported to cause regression of colon polyps chemically induced or in patients with familial polyposis. This study aims to examine the effect of Sulindac on the incidence and reduction of dimethylhydrazine (DMH) induced murine colon tumors. DMH was given by weekly intraperitoneal injections (20 mg/kg body weight) during 20 weeks. Starting with the carcinogen administration 64 male Sprague-Dawley rats were randomized to receive either Sulindac (10 mg/kg) or open-formula diet. In another 16 animals Sulindac was fed 20 weeks after the carcinogen exposure. All rats were necropsied, and tumor incidences and sizes were compared among the various groups at weeks 8, 16, 24, and 32. In all animals that consumed Sulindac there was a significant reduction in the number of tumors (1 vs. 5; 1 vs. 6; 6 vs. 9; 6 vs. 12), the tumors per animal (0.13 vs. 0.75; 0.1 vs. 0.8; 0.6 vs. 1.9; 1.1 vs. 2.8) and the size (mm) of the tumors (0.6 vs. 2.6; 0.7 vs. 2.7; 4.0 vs. 6.2; 3.7 vs. 8.1) at week 8, 16, 24, and 32, respectively. Similarly, animals that received sulindac 20 weeks after the start of DMH exposure significantly differed from the corresponding controls with respect to the number of tumors (6 vs. 8); the tumors per animal (1.2 vs. 2.3), and the tumor size (4.2 vs. 5.4 mm). The results of this study demonstrate that sulindac 1. inhibits DMH induced tumor incidence, and 2. causes tumor regression. (Grant by W. Sander-Stiftung)

1718 Impaired Glutamine Catabolism in Colonic Polyps

E. Goldin, J. Siguencia, E. Tsvang, L. Aptekar. *Gastroent. Dept., Hadassah University Hospital, Jerusalem, Israel*

Glutamine is an important aminoacid, having a key role in nitrogen metabolism. It is also known to be a major source of energy for colonic enterocytes. The *Aim* of our study was to investigate the process of glutamine catabolism in colonic mucosa of patients with adenomatous polyps of the sigmoid compared with those with normal colonic mucosa. *Methods*: The glutamine catabolism was studied in 13 and 21 patients with sigmoid polyps and normal colonic mucosa respectively. Biopsies were obtained both from the polyps themselves and also from the surrounding mucosa in the first group and from normal sigmoidal mucosa in the second group. Indications of glutamine catabolism were: free glutamine content and activity of two enzymes: phosphate-dependent glutaminase (EC 3.5.1.2) and glutamintransferase (EC 2.6.1.15). Level of glutamine was determined by colorimetric method with glutaminesynthetase and activity of the enzymes by measuring glutamine in incubating mixture.

Results:

	Glutamine $\mu\text{mol/mg}$ of protein	Phosphate-dependent glutaminase $\mu\text{mol/mg protein/2 h}$	Glutamintransferase $\mu\text{mol/mg protein/2 h}$
normal sigmoid	1.28 \pm 0.16*	0.91 \pm 0.25	1.72 \pm 0.45
sigmoid polyps	0.84 \pm 0.18	N	N
Surrounding mucosa	1.63 \pm 0.34*	1.09 \pm 0.50	1.25 \pm 0.57

N – the activity of enzyme was negligible; * $p < 0.05$ when compared with polyps

Free glutamine content was significantly decreased and the activity of its catabolic enzymes was absent in polyps when compared with surrounding tissue and normal colonic mucosa. *Conclusions*: (1) To our knowledge this is the first time that glutamintransferase has been examined in human colonic mucosa and found to be active. Our results also showed that glutamine may be degraded in colonic mucosa by two different enzymes. (2) The absence of activity of both catabolic enzymes in polyps may exert an important role in the pathogenesis and development of colonic polyps.

1719 DNA Measurement by Image Analysis in Surgically Treated Colorectal Carcinoma and its Prognostic Value

A. Guerra, F.J. Jiménez-Pérez, F. Borda, B. Larrinaga¹, J.M. Martínez-Peñuela¹. *Gastroenterology Dpt., Hospital de Navarra, Pamplona, Spain; ¹ Pathology Dpt., Hospital de Navarra, Pamplona, Spain*

Controversial results have been published about the prognostic value of DNA content in colorectal cancer in comparison with other clinical and patholog-

ical parameters. The aim of the present study is to discuss the prognostic implications of DNA measurement in patients with this malignancy.

Ploidy was determined in 108 colorectal adenocarcinomas treated surgically and with a 5-years survival period. Relationship between survival and Dukes staging, tumor location and pathological degree was evaluated. DNA measurement was performed by static cytometry (Visilog-software Texcan system). Kaplan-Meier and Logrank tests were used for survival and statistical analysis.

Results are shown in the following table:

	Diploid (n = 21)	Aneuploid (n = 87)	p
Overall	84.2 ± 8.9%	58.5 ± 5.3%	<0.02
Dukes A/B	92.8 ± 7.4%	69.8 ± 6.3%	<0.05
Dukes C	60 ± 2.6%	38.7 ± 9.5%	N.S.
Proximal	100%	60 ± 13.4%	N.S.
Distal	71.4 ± 19%	76.1 ± 7.3%	N.S.
Rectum	88.9 ± 13%	37.5 ± 8.2%	<0.02
Dis + Rec	81.2 ± 11%	58.1 ± 5.9%	<0.05
Degree I	83.3 ± 19%	73.3 ± 11%	N.S.
Degree II	90 ± 10%	63.1 ± 6.5%	<0.05
Degree III	66.6 ± 38%	26.7 ± 10%	N.S.

We conclude that: 1. In our series DNA measurement has prognostic value as survival is significantly higher in diploid tumors. 2. Prognostic significance keeps valid in Dukes A/B stages, rectal and moderately differentiated tumors. 3. DNA analysis is particularly useful in less advanced stages, improving prognosis evaluation.

1720 Histological, Immunohistochemical and Morphometric Studies of the Gastric Antral Mucosa in Normals and Gastric Cancer Patients

G.E. Holle, J. Riedel, R. Ringel, F. Holle, W. Eisenmenger, R. Siewert. *Gastroenterol, Research Labs, L.M. University, F.R. Germany; Dept. of Surgical Policlinic, L.M. University, F.R. Germany; Inst. of Forensic Medicine, F.R. Germany; Dpt. Surgery Techn, University Munich, F.R. Germany*

Cell count and morphometric examination of gastrin i.r. G-cells (GC), somatostatin i.r. D-cells (DC), 5-HT-i.r. cells (5-HT-C) and total gland cells were carried out in the total gastric antrum (70–100 tissue blocks per stomach) from 20 healthy persons (17–94 yrs) (forensic autopsy) and 9 patients (48–76 yrs) from total gastrectomy for carcinoma of the proximal part of the stomach. Results were summarized in the prox. (I), middle (II) and dist. (III) third of the antrum and in the major (A) and minor (B) curvature side.

In normals GC count amounts 2.52%; 4.06% and 4.77% of the total gland cells in A I, II and III respectively. Values in B were 10% lower.

DC count amounts 0.47%; 0.62% and 0.58% in A I, II and III. B I, II and III values were 10% lower.

In the cancer stomach antrum the GC count revealed a nonsign. 20–70%, the DC count a 35% lower value.

The 5-HT-C count amounts in normals of a representative age group 0.25%; 0.32% and 0.39% in A I, II and III respectively. The B values are not different from the A values.

The carcinoma antrum revealed a highly significant 200–400% increase in 5-HT-C, most pronounced in III.

Conclusion: 5-HT is known as a growth stimulant particularly for tumors (Tutton et al). The sign. increase of 5-HT-C in the cancer stomach may be a factor that contribute to the initial histologic changes in gastric tumor.

Supported by the DFG, Ho 936/1-3, Bonn-Bad Godesb.

1721 Anticarcinogenic Role of the Selen in Gastric Mucosa. The Experimental Study

S. Jančić, K. Jaloul, D. Djurić, T. Jovanović, V. Katić, I. Andjelković. *School of Medicine, University Belgrade, Yugoslavia*

More and more people die from cancer in the World and carcinogenesis is yet unknown. The important anticarcinogenic role of the Selen (contained in glutation peroxidase) is suggested from same authors. To test the anticarcinogenic significance of the Selen we have treated the animals with 9, 10-Dimethyl-1, 2-Benzanthracene (DMBA) in drinking water 40 mg/l (group I) and with DMBA + Selen 1 mg/l (group II). The experiment lasted 2 months. Histologically, histochemically (on mucins) and immuno-histochemically (on G- and EC-cells) the antral biopsy specimens were studied. The following methods were used: HE, HID-AB, PAS and ABC with anti-gastrin and anti-serotonin.

First group of the animals showed characteristic severe dysplastic and metaplastic antral mucosal lesions, then qualitative and quantitative changes of mucins and strong G-cell and EC-cell hyperplasia. Antral gastric mucosa of the animals from second group showed only functional changes: hyposecretion of the acid mucins and focal, mild hyperplasia of G- and EC-cells.

The authors discuss the possible protective, anti-dysplastic, mechanisms of the Selen in gastric carcinogenesis.

1722 Collagenase Activity in the Follow Up of Surgically Treated Patients with Colorectal Cancer

J.R. Vidán, F.J. Jiménez-Pérez, A. Echarri, E. Jiménez, F. Borda, P. Liso. *Hospital de Navarra, Pamplona, Spain*

Increased collagenolytic activity has been demonstrated in serum of patients with colorectal cancer and an evident relationship between this increment and the stage of disease has also been found. The aim of the present study is to analyze the possible use of collagenase measurement in the biological follow up of surgically treated patients with this malignancy.

Collagenase activity was measured in a 1-year follow up period in 20 patients with colorectal cancer who underwent surgery for tumor resection and showed no evidence of remaining disease at hospital discharge. Measurements were performed the day before surgery, and 1 week, 1, 6 and 12 months after operation. Once follow up was over, patients were divided into two groups according to the presence (Group A) or absence (Group B) of clinical/pathological evidence of tumoral disease (local recurrence or metastases) and results in both groups were compared.

Results are shown in the table below (ng%). Collagenase activity decreased in both groups immediately after surgery but then its behaviour was different with statistical significance ($p < 0.01$) depending on tumor evolution.

	0	1w.	1m.	6m.	12m.
A	26.3 ± 0.6	20.1 ± 0.5	22.3 ± 0.7	24.5 ± 0.7	33.8 ± 0.9
B	22.1 ± 0.5	18.6 ± 0.3	18.3 ± 0.4	18.5 ± 0.5	18.2 ± 0.3

We conclude that collagenase activity measurement may be useful in the biological follow up of patients with colorectal cancer, with prognostic implications. Once again, these findings support the theory that tumoral cells are responsible of the increased synthesis and release of this protease.

1723 Collagenolytic Activity in Colorectal Cancer and Its Relationship with Tumor Spread

F.J. Jiménez Pérez, A. Echarri, E. Jiménez, J.R. Vidán, F. Borda. *Hospital de Navarra, Pamplona, Spain*

Increased collagenase activity has been demonstrated in colorectal tumors, both in tumor samples or cell culture and in serum of patients with this malignancy. The purpose of the present study is to investigate the relationship between collagenolytic activity in serum and the stage of disease.

Collagenase activity was measured in serum of 49 patients with colorectal cancer. Patients were divided into two groups according to Duke's stage as follows: Group A (n = 25) Duke's B; Group B (n = 24) Duke's D. Age and sex distribution was similar in both groups. A control group of 30 healthy volunteers was also evaluated. Collagenolytic activity was determined by R.I.A.

Significantly elevated activity of collagenase was found in tumoral patients compared with control group ($p < 0.01$). When results of groups A and B were compared between themselves collagenase activity was higher in direct relation with the more advanced stage and the presence of metastases ($p < 0.01$). Results are shown in the following table:

	Control	Group A	Group B
Collagenase (ng%)	19.6 ± 0.7	22.8 ± 0.8	26.1 ± 0.6

In conclusion, serum collagenase activity is raised in patients with colorectal cancer and an evident relationship between the level of activity and the stage of disease is demonstrated. These findings support the theory that tumoral cells are the source of this increased proteolytic activity as a possible mechanism of facilitating tumor invasion through extracellular matrix. Collagenase measurement might be a useful aid in the biological follow-up of patients with colorectal cancer.

1724 Proteolytic Activity in Serum of Patients with Colorectal Cancer

F.J. Jiménez-Pérez, E. Jiménez, A. Echarri, J.R. Vidán, F. Borda. *Hospital de Navarra, Pamplona, Spain*

Peptidases are proteolytic enzymes which have been involved in the process of invasion of tumor cells through the extracellular matrix. Although an increased activity in colorectal tumor tissue has been reported, no references regarding activity in serum have been found in the literature.

We measured the activity of trypsin, elastase, cathepsin D and B and collagenase in serum of 42 patients with colorectal cancer (mean age 64.2). Trypsin, cathepsin B and cathepsin D activities were determined fluorimetrically, whereas R.I.A. was used for elastase and collagenase measurements. A control group of 30 healthy volunteers was also evaluated.

Results are shown in the following table where statistical significance is also indicated

	Control	Tumor	p
Trypsin (U/l)	1.01 ± 0.13	7.47 ± 0.67	<0.01
Elastase (ng%)	196.48 ± 19.19	479.92 ± 56.62	<0.01
Cath.D (mU/ml)	32.78 ± 2.21	32.26 ± 1.41	N.S.
Cath.B (U/l)	1.88 ± 0.10	7.18 ± 0.12	<0.01
Collagenase (ng%)	19.66 ± 0.73	24.67 ± 0.59	<0.01

Proteolytic activities are increased in serum of patients with colorectal cancer. Tumoral cells are likely the source of these peptidases which surely play an important role in tumor invasion. The possibility of detecting these activities opens new expectatives in the search for tumoral markers.

1725 Experimental Endocavitary Irradiation in a Rat Rectum Model

H.O. Johannessen, D.R. Olsen, K. Tverå, K.E. Giercksky. *The Norwegian Radium Hospital, Oslo, Norway; Institute of Cancer Research, Oslo, Norway*

Local treatment of GIT cancers can be given using lasers and endocavitary radiotherapy. Using the radiotherapeutic option iridium sources are introduced by afterloading technique for the total radioprotection of the staff. In the rectum primary Dukes A-C cancers in high risk patients and intraluminal residual disease can be treated using a rectal probe. By destruction of the bulk of the tumor local symptoms are reduced alleviating the need for a diverting colostoma. Symptoms of chronic radiation injury are increasingly frequent after potentially curative doses of 60–80 Gy and limit the usefulness of external beam radiotherapy.

The experimental model was designed in order to study the normal tissue damage after endocavitary irradiation in the rat rectum. Concerning the clinical problem of therapeutic tolerance we have been particularly interested in optimization of endocavitary irradiation. The model was established using an afterloaded HDR (high dose rate) Ir-192 source for the endocavitary irradiation of the rectum of Fisher F344 male rats. Single doses of 4–30 Gy was given in the first study. Next we studied the effects of fractionated treatment of 2 and 3 fractions for the total dose of 8–45 Gy. The effects of lead shielding and reduction of the source length were compared in a separate study. The clinical appearance of ileus from rectal stenosis and the histological classification of the rectal injury were the main end-points in our studies.

Functional disturbances from radiation injury was only seen in the target organ, the rectum, and the testis. Azospermia was regular, but did not influence the relevance of the model. After single doses >20 Gy ileus caused by a stenosing rectal ulcer always developed. By fractionating this dose (3*7 Gy = 21 Gy) ileus never appeared, and triple fractionation of 3*9 Gy was tolerated by 30–40% of the animals. Latency time for the development of ileus increased with lower dose. Increased latency time was seen with shielding and reduced source length, but single doses >20 Gy always resulted in ileus from a chronic rectal ulcer.

The studies demonstrate the feasibility of simulating endocavitary irradiation in the rat. Ileus developing after single doses >20 Gy in contrast to the merely asymptomatic response after lower doses fits in with a steep sigmoid dose response curve. Fractionation moves the dose response curve to the right; i.e. higher total doses are tolerated. Lead shielding and reduced source length both reduce the volume of irradiated rectum, but so far total dose for the development of ileus is not increased by these means.

1726 Further Evidence of Gastrin as an Etiological Factor in the Colon Cancer Carcinogenesis (A Pilot-Study)

R. Meier, A. Pullwitt, G. Gamboni¹, L. Baselgia¹, C. Beglinger. *Gastroenterology Units, Kantonsspital Liestal, Switzerland; ¹ University Hospital Basel, Switzerland*

Several lines of evidence support an etiological role for gastrin (G) in colon cancer carcinogenesis. Recently it was shown that in some patients with colon cancer (CC) fasting and postprandial plasma G concentrations were increased compared to normal controls. Surgical resections of the tumor resulted in a fall in plasma G. In none of these studies the role of *Helicobacter pylori* (Hp) causing hypergastrinemia was assessed. The aim of this study was therefore to determine whether increased plasma levels of G could be demonstrated in colon cancer patients before and after surgery and to assess the role of the presence of Hp infection.

Methods: In 15 non-metastatic CC patients, mean age 72 years, and 13 patients without malignant disease (normal colonoscopy) (controls [C]), mean age 56 years, fasting plasma G and Hp infection were assessed. In 12 of the 15 CC patients fasting plasma and postprandial (after a 250 kcal Ensure®-meal) G were assessed before and after resection of the tumor. G concentrations (pg/ml) were measured by a specific RIA. Hp infection was determined by a second generation ELISA (F. Hoffmann-La Roche Ltd.).

Results: In 15 CC patients 9 were Hp positive and 6 negative. In the 13 C 5 were Hp positive and 8 negative. G data are given as mean ± SEM.

		N	Basal G before surgery	G delta increase after the meal	Basal G after surgery	G delta increase after the meal
C	Hp+	5	44 ± 11			
C	Hp-	8	44 ± 9			
CC	Hp+	9	56 ± 7*			
		8	57 ± 6*	17 ± 13	52 ± 5*	12 ± 6
CC	Hp-	6	34 ± 6			
		4	33 ± 6	8 ± 4	32 ± 6	8 ± 5

* p < 0.05; Hp+ vs Hp- (Wilcoxon signed-rank test).

Summary: (1) A higher number of CC patients were Hp positive compared to the C. (2) Only the Hp positive CC patients had increased G levels before and after surgery. (3) The postprandial G levels were not different. (4) There was no significant decrease of G levels after surgery of the tumor.

Conclusion: Hp may play an important role in hypergastrinemia in CC patients. These findings must be confirmed in a large controlled study.

1727 Genomic Changes and p53 Overexpression in Barrett's Epithelium and Adenocarcinoma

M.B.E. Menke-Pluyers, A. Hagemeyer, A.H. Mulder, K.K. Krishnadath, H.W. Tilanus. *University Hospital Rotterdam Dijkzigt, The Netherlands*

In order to study genomic changes in the process of malignant degeneration of Barrett's oesophagus, tissue specimens of 17 patients (10 men, 7 women) with an adenocarcinoma in Barrett's oesophagus were analyzed by flow-cytometric and cytogenetic methods and p53 was determined.

Results:

	Barrett's epithelium	Adenocarcinoma
Flowcytometry	N = 12	N = 15
- diploid	6 (50%)	1 (7%)
- aneuploid	6 (50%)	14 (93%)
Cytogenetic analysis	N = 18	N = 13
- normal karyotype	2	3
- numerical aberrations	3	5
- structural and numerical aberrations	1	1
- clonal aberrations	2	4
p53	N = 17	N = 17
- overexpression	10 (59%)	15 (88%)
- no overexpression	7 (41%)	2 (12%)

No cytogenetic marker common to all specimens of Barrett's epithelium or adenocarcinoma was found.

Conclusion: Neoplastic progression in Barrett's oesophagus is a multistep process of numerical and structural genomic changes. The lack of a common cytogenetic denominator indicates that a large number of genetic pathways must exist in the genesis of adenocarcinoma in Barrett's oesophagus.

1728 C-erbB-2 Expression in Lymph Node Negative Colorectal Cancer

H. Mulcahy, E. Kay, M. Leader, D.P. O'Donoghue. *Gastroenterology & Liver Unit, St. Vincent's Hospital, Dublin; Department of Pathology, Royal college of Surgeons in Ireland*

Purpose: Dukes' B disease accounts for over one third of all colorectal cancers. Outcome is especially variable in this group and additional indicators of disease progression would be of value. The c-erbB-2 oncogene encodes for a putative membranous growth factor receptor related to the EGF receptor. Membranous staining correlates with c-erbB-2 amplification, but while such staining is a common feature of breast carcinomas, it appears limited in colorectal cancer. Cytoplasmic c-erbB-2 staining also correlates with gene amplification in bladder, breast and colorectal cancer, though its biological and prognostic significance is uncertain. The aim was to examine the incidence and prognostic significance of c-erbB-2 protein expression in Dukes' B colorectal cancer. **Methods:** 164 consecutive Dukes' B patients admitted to a single institution (1983–1988) were studied. Follow-up: Mean 6.3 years; range, 3.5–10. Paraffin wax sections were examined with the monoclonal antibody NCL-CB11 (Novocastra) using the avidin-biotin immunoperoxidase technique. Slides were reviewed by a single pathologist blinded to clinical details and outcome. Membrane and cytoplasmic staining were assessed separately. **Results:** Membranous staining was not detected in any case. Cytoplasmic c-erbB-2 staining was seen in 55 cancers (34%). Cytoplasmic staining was unrelated to patient age (p = 0.31), sex (p = 0.69), tumour site (p = 0.69), size (p = 0.57) or histological grade (p = 0.42), but was weakly related to tumour ploidy status (p = 0.21) and found more frequently in obstructing cancers (p = 0.03). Five year survival estimated by the Kaplan-Meier life-table method was 47 per cent for those with cytoplasmic c-erbB-2 staining and 77 per cent for those without such staining (Logrank analysis; p < 0.0001). Stepwise regression analysis identified c-erbB-2 staining (Relative risk, 2.51;

$p = 0.0005$) and bowel obstruction (Relative risk, 1.99; $p = 0.015$) as the only independent predictors of outcome. **Conclusion:** These results suggest that genetic events may be involved in the progression of lymph node negative colorectal cancer and that cytoplasmic c-erbB-2 staining may provide a useful marker of tumour behaviour in the Dukes' B group.

1729 Urokinase-Type Plasminogen Activator (uPA) Predicts Outcome in Large Bowel Cancer

H. Mulcahy, D. Gibbons, P. McCarthy, M.J. Duffy, N.A. Parfrey, K. Sheahan, D.P. O'Donoghue. *Gastroenterology & Liver Unit, St. Vincent's Hospital, Dublin, Ireland; Department of Pathology, St. Vincent's Hospital, Dublin, Ireland; Department of Nuclear Medicine, St. Vincent's Hospital, Dublin, Ireland*

Purpose: Destruction of basement membrane is important during tumour spread and the progression from non-metastatic to metastatic colorectal cancer. Urokinase-type plasminogen activator (uPA) is a serine protease implicated in the breakdown of the extracellular matrix, and may be involved in the progression of human cancers. The prognostic significance of uPA measurements has not been determined in large bowel cancer. The aim of this study was to correlate uPA immunohistochemical staining with multiple clinical and pathological features, and survival in patients with non-metastatic colorectal cancer extending beyond the bowel wall (Dukes' B). **Methods:** 70 patients admitted to a single institution with Dukes' B colorectal cancer were studied. Mean follow-up time, 7.1 years (range, 4.9–10). Formalin-fixed paraffin embedded sections were stained for immunohistochemistry using a monoclonal antibody against the B-chain of uPA (American Diagnostica Inc., CT). Epithelial and stromal positivity were scored independently by a pathologist blinded to clinical details and patient outcome. Epithelial staining was graded as: (1) <2%; (2) 2–20%; (3) 21–50%; (4) >50%. Stromal staining was graded: (1) few cells; (2) focal cellular aggregates; (3) multifocal aggregates; (4) diffuse. **Results:** Grade 1 epithelial uPA reactivity was found in 0 cases, grade 2 in 5 (7%), grade 3 in 8 (12%) and grade 4 in 57 (81%). Grade 1 uPA stromal staining was seen in 16 patients (23%), grade 2 in 22 (31%), grade 3 in 19 (27%) and grade 4 in 13 (19%). A positive correlation between both forms of staining was seen ($p = 0.036$). No significant association was found between epithelial or stromal reactivity and patient age, sex, tumour site, tumour size, histological type, tumour grade, vascular invasion or perineural invasion. High levels of epithelial uPA (grade 4) correlated with tumour necrosis ($p = 0.03$). Five year survival estimated by the Kaplan-Meier lifetable method was 81% for patients with grade 1, 2 and 3 epithelial uPA positivity ($n = 13$) versus 58% for those with grade 4 ($n = 57$) (Logrank analysis; $p = 0.01$). Stromal reactivity was not significantly related to survival. Regression analysis identified epithelial uPA reactivity as an independent prognostic factor within the Dukes' B group (relative risk 4.25; $p = 0.04$). **Conclusion:** These results suggest that epithelial uPA reactivity may be a useful marker of tumour aggressiveness in Dukes' B colorectal cancer.

1730 Levamisole Induces the Production of Pro-Inflammatory Cytokines *In Vitro*

J.M. Reimund^{1,2}, S. Dumont², Ch. Muller Ch.², Ph. Poindron², B. Duclos¹, R. Baumann¹. ¹ *Dept. of Gastroenterology and Hepatology, CHRU Hautepierre, 67098 Strasbourg Cédex, France;* ² *Dept. of Immunology, Immunopharmacology and Pathology, Pharmacological Research Center, 67400 Illkirch-Graffenstaden, France*

Combined Levamisole-5 Fluorouracil chemotherapy has a proven efficacy in the adjuvant treatment of colorectal cancer. The mechanisms of action for Levamisole (L) are not clearly understood and therefore, regarding the potential interest for cytokines in cancer management, we studied the effect of Levamisole on the *in vitro* human monocyte tumor necrosis factor α (TNF), interleukin 1β (IL1), and 6 (IL6) production.

Methods: blood monocytes were obtained after cytopheresis and elutriation in healthy donors. After a 24 hour incubation (10^6 cells/ml) they were cultured 24 hours later in presence of growing concentrations of Levamisole (100, 250, 500, 750, 1000 μ Mol/ml) with or without interferon γ (IFN: 500 U/ml) and lipopolysaccharides (LPS: 1 μ g/ml). Interleukin 1, IL6 and TNF were measured in the culture supernatants by mean of an ELISA using monoclonal antibodies.

Results: Levamisole alone stimulate TNF, IL1 and 6 production with a peak for concentrations between 250 and 750 μ Mol/ml (cytokine concentrations are expressed in pg/ml).

L μ Mol/ml	Controls (con)	100	250	500	750	1000	p vs con
IL1	0	78 \pm 10	482 \pm 3	454 \pm 6	298 \pm 12	371 \pm 71	0.01
TNF	0	0	47 \pm 19	150 \pm 19	431 \pm 26	213 \pm 15	0.01
IL6	74 \pm 47	ND	102 \pm 44	482 \pm 73	864 \pm 13	216 \pm 13	0.01

The TNF and IL1 production were higher in all cases with Levamisole alone than with IFN/LPS alone ($p < 0.01$) and the costimulation L-IFN/LFS did not

potentiate the stimulation compared to Levamisole alone. Interleukin 6 production by IFN/LPS alone was better than the stimulation by Levamisole alone ($p < 0.05$), both being more potent than costimulation by L-IFN/LPS ($p < 0.05$).

Conclusions: (1) Levamisole alone was shown to induce TNF, IL1 and IL6 production by human blood monocytes *in vitro*. (2) This effect may be of interest in combined anti-cancer therapy and may be potentially used in a larger extent. (3) The heterogeneous response of monocytes to L-IFN/LPS costimulation – in terms of cytokine production – remains unexplained.

1731 Allelic Abnormalities in Chromosome 17 and p53 Mutations in Gastrointestinal Carcinomas: A Molecular Study in Archival Material

A. Karameris, T. Rokkas. *401 Army General Hospital, Athens, Greece*

The p53 gene located on chromosome 17 at band p13.1, is frequently mutated or inactivated in several human tumors. Absence of wild-type p53 in tumor cells is often achieved by loss of one allele and point mutation of the remaining allele. Mutant p53 expression has been implicated in the pathogenesis of a large variety of tumors, including malignancies of lung, breast, colon and brain as well as sarcomas and leukemias. Germline abnormalities of the p53 gene have also been associated with the development of familial tumors in the rare Li-Fraumeni syndrome. Both hereditary and acquired mutations in p53 have been found to cluster in four "hotspots" in the highly conserved region of the p53 gene, encoding amino acids 110–370. We have screened for the presence of p53 gene mutations in formalin-fixed, paraffin-embedded cases of carcinomas of the stomach and the colon, using the new PCR/HydroLink mutation detection enhancement method. The PCR was used to amplify the most frequently mutated regions, including exons 5, 6, 7, 8 and 9. Abnormalities detected by PCR/HydroLink method were confirmed by direct sequencing of the amplified DNA. Of carcinomas examined, we found point mutations in 2/10 carcinomas of the stomach and in 4/10 carcinomas of the colon. All the mutations observed, were correlated with a wide depth of invasion and lymph node metastases. Furthermore, by using the multiplex PCR method we found loss of heterozygosity in 4/10 carcinomas of the colon and in 5/10 carcinomas of the stomach. The majority of the sites of mutation in the p53 gene were found in Exons 5–6 and 8–9. The observed mutations seems to be specific, with respect to the nature of the nucleotide change. All but one of the observed point mutations were base transitions (G:C-A:T transitions). These findings prompted us to postulate that mutations in the coding region of p53 and especially in exons mentioned above, are involved in tumorigenesis of carcinomas of the gastrointestinal tract especially those that showed nodal metastases and wide depth of infiltration. We also propose a genetic model for carcinogenesis, common to gastric and colorectal cancer.

1732 Phenotypic, Morphological and Cell Kinetic Modifications of Gastrointestinal Cancer Cell Lines in Various Culture Environments

J. Sakamoto¹, T. Ichihara², Y. Yamamura¹, M. Horisawa², T. Kito¹. ¹ *Aichi Cancer Center, Nagoya, Japan;* ² *Nagoya National Hospital, Nagoya, Japan*

Three gastric cancer cell lines and 13 colorectal cancer cell lines established from 148 gastric and colorectal cancer resections, were utilized to estimate their alteration of phenotype, morphology and cell kinetics under different culture conditions.

The expression of 12 gastrointestinal cell associated antigens and 11 blood group related antigens on these cell lines were examined by the mixed hemadsorption assay and by the enzyme immunoassay using monoclonal antibodies, and their reactivities were compared with the results of immunohistochemistry on frozen sections obtained from the original fresh tumor specimens. In 6 of these cell lines, deletions of either Lewis^b, Lewis^x, i, CEA or gp115 antigen were recognized and anomalous expression of sialy-Lewis^a, A-1 and A-2 were detected.

A colon cancer cell line, HT-29, was selected to estimate the influence of culture environment to established cell lines. The HT-29 cells were cultured in media containing different lots of fetal calf serum (FCS), in different type of serum-free media and in glucose-free medium. The cell line was also cultured on bovine corneal endothelial extracellular matrix (BCE-ECM) coated culture plates.

The HT-29 cells showed variable alteration of antigen expression and morphology, depending on the culture conditions. Cells cultured in 3 out of 4 serum-free media for 15 passages lost their ability to express Lewis^y antigen, which was originally positive in conventional FCS containing medium. Also, the HT-29 cells cultured in glucose-free medium or on BCE-ECM plate, demonstrated an unusual "dome formation" which is characteristic of cells with enterocytic differentiation accompanying brush border formation.

Also, HT-29 cells were irradiated and cultured under 8-Azaguanine-containing media. After 20 passages, survived cells retained 100 fold stronger resistance to 8-Azaguanine compared to the original cell lines.

All these results suggest that these established cancer cell lines might

alter their phenotype, morphology, growth pattern and drug sensitivity depending on their culture environment, and these evidences and informations would be essential and exploitable in utilizing these cancer cell lines to clinical or pathophysiological evaluations and researches.

1733 A Comparison of Two Different Approaches to Quantifying Cell Proliferation in Colonic Epithelium

A. van 't Hof, K. Gilissen, A.L. Thornley, R.J. Cohen, L. Taylor, Z. Haffajee, I. Segal. *Department of Gastroenterology, Baragwanath Hospital, Johannesburg, South Africa; Department of Zoology and Histo-Pathology, University of Witwatersrand, Johannesburg, South Africa*

One of the confounding factors responsible for inconsistent results in studies of epithelial cell proliferation is the lack of an objective method for quantifying cell proliferation.

We compared manual cell counting with a computerized image analysis system in order to obtain better accuracy and standardization and less inter-observer variation.

35 different colonic crypts from 35 different patients were evaluated by 2 independent observers. Proliferating cells were detected using the Ki-67 antigen.

Variation in measured labelling indices between the two observers was slightly less in favor of the computerized method. However, both quantification methods showed unacceptable interobserver variation (limits of agreement: computer: 0.00 ± 0.10 , manual: 0.01 ± 0.12 , $p = \text{ns}$). Computerized counting resulted, for both observers, in a significant lower mean labelling index (LI) in comparison with manual counting (LI obs 1, computer: 0.17 ± 0.12 , manual: 0.28 ± 0.14 , $p = 0.0002$; LI obs 2, computer: 0.17 ± 0.10 , manual: 0.26 ± 0.13 , $p = 0.002$).

We conclude that both quantification methods are not reproducible between two observers. It is suggested that quantification of cell proliferation should be done by only one observer. Furthermore computerized counting is probably the best method for large scale studies.

1734 The Diagnostic Significance of p53 Overexpression in Preoperative Diagnosis of Gastric Cancer

T. Starzyńska. *Department of Gastroenterology, Medical Pomeranian Academy, Szczecin, Poland*

The alterations of p53 protein which lead to its inactivation and overexpression in the cell nucleus are an almost universal event in human cancer, but diagnostic significance of this phenomenon remains to be defined. The objective of the present study was to investigate the value of immunohistochemical detection of p53 in preoperative diagnosis of gastric cancer.

The expression of p53 was analysed immunohistochemically in 170 gastric carcinomas, 274 tissue samples from benign gastric disorders and 56 from normal gastric tissue. Routinely fixed material and CM 1 antibody were used. The p53 has been detected only in cancer tissue (42% of tumours) and has not been identified in any samples with benign gastric disorders and normal gastric epithelium. Immunohistochemical detection of p53 in endoscopic biopsies used in addition to conventional histology improved accuracy of preoperative diagnosis of gastric cancer from 86.2% to 93.5%.

The results demonstrate that the presence of p53 overexpression in gastric tissue is a marker of malignancy. A simple and inexpensive method as immunohistochemical detection of p53 provides useful conformating data in preoperative diagnosis of gastric cancer. Its greatest potential is for use instead of repeating endoscopies in cases where results of conventional morphological analysis are either unclear or possibly false negative.

1735 Successful Management of Hospitalized Persistent Diarrhoea Children in Bangladesh by Using Inexpensive Diets

H. Ashraf, A.K. Mitra, S. Ahmed, D. Mahalanabis. *International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh; Med. Outpatient Clinic Kantonsspital, Basel, Switzerland*

107 hospitalized persistent diarrhoea patients were treated with two simple diets used sequentially. These diets were prepared from inexpensive locally available ingredients. The main purpose of the study was to estimate the rate of success with these diets. The criteria for success was defined as the body weight on day 7 more than admission weight with less than or equal to 2 liquid stools per day. Identification of risk factors of failure to the initial diet was another purpose of this study.

All the 107 patients aged 4–23 months were treated with an initial diet prepared from milk powder, khai powder (popped rice), soya oil and sugar. The children who failed to improve with this diet were given another diet based

on khai powder, egg white, soya oil and glucose. The treatment of associated systemic and gut infections along with vitamin/mineral supplementation were done during hospital stay. 103 patients were either 2nd or 3rd degree malnourished.

65 patients (61%) were successfully treated with the initial low lactose milk cereal diet. Of the remaining 42 patients who failed to improve with the initial diet, 34 patients (32%) recovered from diarrhoea within next 7 days with subsequent lower carbohydrate lactose free diet. So, a total of 99 (93%) were successfully treated with two simple diets based on locally available, culturally acceptable ingredients.

We, therefore, conclude that persistent diarrhoea in children can be successfully managed by using simple, inexpensive diets prepared from locally available ingredients.

1736 Development of a New Test System for Studying Protein Binding to Small Intestinal Brush Border Membranes

G. Bolte, M. Knauss, I. Metzdorf, M. Stern. *University Children's Hospital, Tuebingen, FRG*

Enterocytes have been shown to process and present food antigens. This might have implications for small intestinal disorders like coeliac disease and cow's milk allergies. In order to study the interaction of food proteins with the first cellular part of the mucosal barrier, we developed a test system using rat intestinal brush border membranes (BBM).

Methods: BBM vesicles were obtained using a divalent cation precipitation technique. Solubilization of peripheral membrane proteins was performed with papain. Intact BBM and native peripheral membrane proteins were used in dot blots, denatured membrane proteins after SDS-PAGE in Western blots for binding experiments. Detection was performed with biotinylated proteins, peroxidase-conjugated streptavidin and a chemiluminescence system.

Results: Coeliac active gliadin fragments and cow's milk proteins were bound to intact BBM and denatured membrane proteins. There was no similarity between food protein and lectin binding patterns in Western blots. Furthermore sialylation of BBM interfered with food protein binding. Papain-solubilization of membrane proteins did not reverse their binding characteristics. Differential maturational changes were observed in dot and Western blots: Denaturation of membrane proteins of newborn rats reduced food protein binding (Western blot), whereas no uniform pattern in binding intensities in dot blots was seen comparing newborn and adult BBM.

In conclusion, BBM and membrane proteins applied to nitrocellulose membranes are suitable for studying interactions with food proteins. Using the described test system it is possible to investigate the contribution of protein modifications like glycosylation to binding. Our results indicate that native peripheral membrane proteins influence BBM binding of food proteins. Further experiments have to be done to clarify the role of BBM proteins in uptake and processing of food antigens by enterocytes.

1737 The Effect of Diet in Patients with Fructosemalabsorption

P. Born, T. Vierling¹, F. Paul¹. *II. Med. Klinik, Klinikum r.d. Isar, TU München; ¹ II. Med. Klinik, Klinikum Ingolstadt, Germany*

Fructosemalabsorption (fm) is often detected in patients suffering from unspecific abdominal complaints, but also in healthy controls. The importance is still controversially discussed. To further evaluate the clinical role of fm we investigated the effect of dietary restriction of fructose intake in symptomatic patients. Interviews were performed following a standardized questionnaire.

81 patients (36 m, 45 f, age 46.2 ys) were interviewed retrospectively 3–6 months after the diagnosis of fm was established. 46 patients (17 m, 29 f, 41, 4 ys) were followed prospectively for 3–6 months. These groups were further subdivided as for the existence of additional findings and the patients' compliance (graduated strict = s, moderate = m, poor = p). Fm was diagnosed by H₂-exhalation tests.

Success rates according to compliance were:

Retrospective with further findings (n = 46): 73.7% (s), 62.5% (m), 45.4% (p); without (n = 35): 100% (s), 62.5% (m), 33.3% (p).

Prospective with further findings (n = 23): 87.5% (s), 55.5% (m), 50% (p); without: 100% (s), 71.4% (m), 37.5% (p).

Recurrence of complaints was reported after dietary mistakes in 50–100%. Moreover it is important that 90% of all fructose malabsorbers were malabsorbers of sorbitol.

Our data demonstrate the beneficial effect of diet in patients with unspecific abdominal complaints and fm. The influence of the compliance is a further indicator of the importance of fm. After we also could show in another study that patients with symptomatic fm differ from patients with asymptomatic fm significantly by their stool bacteria capacity to metabolize fructose we think that fm must no longer be neglected as differential diagnosis in patients with unspecific abdominal complaints. Diet is a sufficient therapy.