Hepatitis coma occurred in 16 (8%) patients in whom the sclerotherapy was performed once or twice. Eleven (68%) died. Another 13 pts died because of hemorrhagic shock, so the total mortality in this seria is 12%. Balloon tamponade was placed in 19 unsuccessful sclerotherapies. It was successful in 52%. Following complications have occurred: in 15 (7.6%) of pts: 1 fibrinolysis and 3 coagulum aspiration which were fatal.

1286 Massive Arterial Bleeding of Gastroduodenum-Sclerotherapy: Treatment of Choice

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Materials: In 287 patients with massive arterial bleeding of gastroduodenum, proved with urgent gastroscopy, athiologically were found 272 (94%) gastroduodenal ulcers and 15 (6.4%) ulcer "dieulafoy" with mean age of 53.2 years. In 166 (58%) were found Forrest Ia lesions, and in 121 (42%) Forrest II (fixed coagulum or visible vessel). Temporary hemostasis was done with infiltration of adrenalin, and definitive one with Aetoxysclerol 1%.

Results: Successful definitive hemostasis was obtained in 166 (91%) of 182 Forrest Ia lesions, and in 16 (9%) it was unsuccessful, and these patients had to be surgically urgently treated. After the operation, 9 pts were definitively cured, and 7 (4%) died (2 of them because of necrosis of duodenal wall). Out of 105 pts with Forrest II lesions, successful definitive hemostasis was obtained in 97 (92%), and the hemostasis was unsuccessful in 8 (8%) pts and they had to be surgically treated: 5 of them died (3 postoperatively and 2 from hemorrhagic shock), so the mortality rate in this group is 5%. Rebleeding occurred in 14 (9%) from both groups, and 2 of them were surgically treated (2 pts died postoperatively because of surgical complications). In total, definitive hemostasis in the whole serial, except the rebleedings, was obtained in 249 (87%) of patients. Together with the rebleedings, urgently were surgically treated 33 (11.5%) pts, with total mortality (pre- and postoperative) of 5%. The causes for death were: necrosis of duodenal wall in 2 pts, hemorrhagic shock in 6, postoperative complications in 2, and cardiopulmopathies in 4 patients.

1287 Palliative Endoscopic Treatment of Oesophagogastric Cancer

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Endoscopic intubation is specific indication for palliative treatment of extensive oesophagogastric cancer. The aim of the treatment is to relieve the dysphagia of the patients with obstructive tumors of the upper digestive tract. Sixty patients with unresectable malignant oesophago- and gastric strictures were managed by endoscopic intubation with plastic or metal endoprosthesis ("Cook", "Notingam", "Röche"). The success rate was 88% (51 from 60 pts) and the complication rate was 7% (4 from 60 pts; perforation – in 2 pts and bend of the prosthesis – in 2 pts). Occlusion of the endoprosthesis up to 1 month after its placement was observed in 3 cases (3.5%). No mortality there was for the follow-up period of 1 month after endoscopic procedure. The two types prosthesis were with similar efficacy, but the metal ones were easier for placement, significantly more atraumatic for the patients and may placement in severe strictures. We conclude that endoscopic placement of endoprosthesis is highly effective as palliative treatment of oesophago-gastric malignant lesions and strictures.

1288 Endoscopic Sphincterotomy (ES) for Common Bile Duct Stones in Patients with Gall Bladder in Situ

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Many patients with gall bladder and common bile duct stones are elderly and often have major medical problems. ES is a safe and effective method of postcholecystectomy common bile duct (CBD) stones. The aim of this study was to evaluate the place of ES in patients with CBD stones and gall bladder present. ES was performed in 690 pts with CBD stones: group A – 400 pts with cholecystomy and group B – 290 pts with intact gall bladder. The success rate of ES was 95% (380 pts) in group A and 91% (264 pts) in group B. Complications were significantly more frequent in patients with gall bladder in situ – 4.5% for group A and 13% for group B. The frequency of acute cholecystitis and cholangitis after ES was 10%. The complication and mortality rates were significantly higher ($p < 0.001$) in cases with acute cholecystitis or cholangitis before ES. In conclusion ES is indicated in elderly patients, in cases with high operative risk and without acute cholecystitis or cholangitis.

1289 Platelet Activating Factor (PAF) in Combination with Interferon (IFN)-γ and Tumor Necrosis Factor (TNF)-α Inhibits In Vitro Growth of a Human Pancreatic Carcinoma Cell Line

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In mouse models, during cytokine-induced tumor inhibition, an intense cell to cell cross-talk between neoplastic cells, granulocytes, macrophages and lymphocytes constitutes a typical finding.

PAF is a phospholipid mediator of inflammation produced by and active on phagocytic cells known to infiltrate tumors. The possible role of PAF in cancer growth and development was assessed on human pancreatic carcinoma Capan-2 cell line. PAF, even at high concentrations, lacked direct cytotoxic and cytostatic activities. However, short preincubation with physiological dose of PAF (10^{-12} M) resulted in a remarkable inhibition of ^{3}H-Thymidine uptake by Capan-2 cells, when IFN-γ or TNF-α were added to the culture at a concentration (100 U/ml) unable by itself to exert an anti-proliferative activity. The cell growth inhibitory effect was almost blocked by specific PAF receptor antagonist WEB2170. The ability of PAF to modify the Capan-2 cell responsiveness to both cytokines seems related to the induction of IFN-γ and p55-TNF-α receptors on the cell surface, as demonstrated by the reactivity with specific monoclonal antibodies.

These preliminary results suggest that PAF may participate in the complex mechanisms of defense against tumors.

1290 The Distribution of N-CAM and N-Cadherin in Neuroendocrine Complex Fetal Stomach Cells and Small Intestine Cells of Human

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The purpose of the study is to investigate the expression of cell adhesion protein in neuroendocrine complex fetal stomach cell and small intestine cells human.

The samples from 12 fetuses (7–12 weeks) were collected at legal abortions. For light microscopic studies the tissues were fixed in Bouin's solution. Paraplast sections (5 mm) were stained by ABC complex with antisera to gastrin (G-17), somatostatin (SOM), bombesin (BOM), vasoactive intestinal peptide (VIP), neuron-specific elonase (NSE), neuronal cell adhesion molecule (N-CAM), N-cadherin.

The simultaneous presence of G-17, SOM, BOM, VIP in Auerbach's and Meissner's plexuses neurons, nerve fibres mucose of the stomach and small intestine of fetuses was shown. G-17 and SOM in endocrine cells of stomach and small intestine epithelium were also discovered. VIP, G-17 were shown in pancreas nerve tissue. The endocrine system was more developed in mucose of small intestine as compare as mucose of the stomach during early embryogenesis. N-CAM and N-Cadherin were simultaneously shown in neurons tissue of the stomach and neurons tissue of the small intestine. However, N-cadherin was only observed in a cytoplasm of the small intestine endocrine cells.

The observing colocalization of the neuropeptides in neurons tissue and N-CAM expression in neurons cells reflects need of neuroendocrine complex early embryogenesis in the stomach and small intestine of human.

1291 The Increase of Cytokines in the Endotoxicemia

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We measured the quantity of endotoxin, tumor necrosis factor (TNF), interleukin-1 (IL-1) and polymorphonuclear neutrophil (PMN)-elastase in 29 cases of endotoxin shock with high fever, a falling of blood pressure and leukocytosis. In these cases the relationship between cytokines and endotoxin was closely examined. Among these 29 cases, basal diseases were: 5 cases of hepatic cirrhosis, 3 cases of hepatoma, 3 cases of gastric cancer, 8 cases of pancreatic cancer, 3 cases of colon cancer, 1 case of pulmonary carcinoma, 3 cases of leukemia and 3 cases of pneumonia. A fixed quantity of plasma endotoxin in blood was measured in accordance with Endospecie. Human plasma IL-1, Human plasma TNF and plasma PMN-elastase were measured by ELLISA.

Endotoxin was observed in 20 of the 29 cases, TNF in 18 cases and IL-1 in 12 cases. A significant positive correlation was observed between the quantity of endotoxin and IL-1. PMN-elastase also increased during the state of endotoxin shock, showing a reciprocal positive correlation with the quantity of endotoxin. Thus, it was confirmed that TNF and IL-1 increased in the state of endotoxin shock.

1292 Growth Factors Inhibit Interleukin-1β Stimulated Xanthine Oxidase Activity in Culture of Regenerating Rat Liver Cells

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Interleukin-1β as a plurifunctional protein, mediates many biological processes, concerned with cell growth and differentiation. But one of the effects of IL-1β on the cell is the liberation of free oxygen radicals and tissue injury. It is well documented that growth factors can modulate proliferation of non-transformed as well as growing cells in vivo and in vitro. Current evidence suggests that *Liver cell growth factor (LCGF)* and *Hepatic stimulator substance (HSS)* are important promotores of liver regeneration. One of the key enzymes included in purine catabolic pathway is *Xanthine oxidase*. The reaction catalised by xanthine oxidase is a primary source of toxic oxygen radicals. An increasing body of evidence implicates the involvement of oxygen derived free radicals in the pathophysiology of tissue injury. The present work was the part of the study performed with the aim to examine the effect of cytokines and growth factors on the purine nucleotide metabolism, since purine nucleotides have a fundamental role in cell metabolism, especially during stimulated growth.

Cell cultures were prepared from collagenase digested regenerating rat liver cells, maintained in RPMI-1640 medium, supplemented with 10% FCS, 10 nM insulin, buffered with 20 nM HEPES. Cells were cultured at confluence for 10 days. After 7 days cultures were exposed for 72 h to the effect of growth factors (LCGF and HSS), IL-1β and their combination. Controls were received an equivalent volume of culture medium.

Results: IL-1β stimulates xanthine oxidase activity (3.083 ± 0.386***), compared with the control value (1.742 ± 0.430). LCGF decreases xanthine oxidase activity (1.683 ± 0.290), HSS also (1.174 ± 0.320*), as well as in their combination (1.279 ± 0.230***). In combination with LCGF, IL-1β has a smaller effect (2.332 ± 0.348*), as well as with HSS (2.181 ± 342*). (Expressed as IU/g p).

It is likely that the study of the interplay of growth factors and cytokines will help to explain how they create metabolic design of purine metabolism during liver regeneration and stimulated growth.

1293 The Influence of One Year Calcium Suppletion on Colorectal Epithelial Cell Proliferation in Colorectal Adenoma Patients

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Colorectal epithelial cell proliferation is increased in patients at risk for colorectal cancer. Medium-term calcium suppletion can decrease cellular proliferation, but long-term data are lacking. The effect of one year calcium suppletion in patients with colorectal adenoma was studied. After polypectomy of colorectal adenomas > 1 cm, 35 patients (mean age 57 y; 21 m, 14 f) were randomized to 1 g elementary calcium daily (n = 18) or no treatment (n = 17). At 0, 3, and 12 months, rectal biopsies were taken for bromoeoxyuridine (BrdU) and proliferating cell nuclear antigen (PCNA) immunohistochemistry. The epithelial cell proliferation index (PI) was defined as % immunoreactive nuclei per total number of colonic crypt nuclei counted. At the present, results are evaluable for 11 patients (6 on calcium, 5 controls).

PI(SD) BrdU	n = 7	baseline	3 months	1 year
controls	n = 4	3.3(1.2)	4.1(1.7)	6.5(1.4)
calcium	n = 3	4.7(1.9)	3.6(1.2)*	5.9(2.3)*
PI(SD) PCNA	**n = 9**	**baseline**	**3 months**	**1 year**
controls	n = 4	10.5(3.1)	9.6(2.4)#	9.4(4.8)
calcium	n = 5	6.9(4.5)	5.1(1.3)#	11.6(4.4)

*p = 0.009; # p = 0.02.

With both staining methods, in the calcium treated group there was a tendency to a decrease of PI at 3 months, which may be the result of the recent replacement of colonic contents and/or the given treatment.

1294 Azoxymethane (AOM) Activates Overall and Epidermal Growth Factor Receptor (EGF-R) Tyrosine Kinase (Tyr-k) in Isolated Rat Colonocytes

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Transforming Growth Factor-alpha (TGF-alpha), which is structurally and functionally similar to EGF has recently gained prominence as a potent mitogen for much of the GI tract, including the colon. Both TGF-alpha and EGF initiate their mitogenic action by activating Tyr-k of their common receptor, EGF-R. The aim of the presented study was to determine the significance of EGF and TGF-alpha in early phase of AOM induced carcinogenesis, as reflected by overall and EGF-R associated Tyr-k in isolated colonocytes. Furthermore, the possible role of PLC in signal transduction pathway was evaluated.

Colonocytes were isolated from 4 mo Fischer-344 rats acc to Roediger, 5 days after the animals were injected with either AOM (20 mg/kg) or saline.

Exposure of isolated colonocytes from control and AOM injected rats to EGF and TGF-alpha (10^{-8} M) for 2 min resulted with the stimulation of overall Tyr-k: in control rats by 20–40%, in AOM-treated animals-by 80–120%, when compared to corresponding basal levels. Those differences were especially pronounced in distal part of colon, as regards to the proximal one. In addition, those treatments activated EGF-R Tyr-K by 40–50% in controls and by 80–100% in AOM-treated animals. This was also associated with a 2–3 fold increase in 4 mucosal proteins tyrosine phosphorylation with Mw of 60, 115, 125 and 160 kDa. Incubations of colonocytes with EGF and TGF-alpha also stimulated PLC activity-in controls by 20–40% and in AOM injected rats-by 150–200%, as regards to corresponding basal levels. We conclude, that AOM enhances responsiveness of the colonocytes to EGf and TGF-alpha, which may be one of the mechanisms involved in colorectal carcinogenesis.

1295 RNA Content in the Gastric Juice

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RNA isolated from different tissue samples can be used to study the in vivo expression of different genes by the polymerase chain reaction (PCR). The gastric mucosa is characterized by constant shedding of epithelial and/or somatic cells, remnants of which accumulate in gastric juice. The purpose of the present study was to determine the feasibility of the isolation of RNA from gastric juice in view of its potential use for the PCR.

Five milliliters of gastric juice were collected during endoscopy from each of the group of 10 patients presenting with dyspeptic symptoms. The proteases in the specimen were inhibited using pepstatin (50 μg/ml), aprotinin (10 ug/ml) and leupeptin (10 μg/ml). The sample was centrifuged for 10 minutes at 1000g and a RNAse inhibitor, guanidinium thiocyanate solution as well as 0.2 M Tris-HCl buffer were added to the pellet. The extraction of RNA was performed according to the method of Chomczynski and Sacchi (Anal. Biochem. 1987; 162: 156), and the quantity of RNA was determined by measuring the absorption at 260 nm. The mean amount of RNA isolated was 2.04 ± 0.69 (range 0.96–3.12) μg (i.e.; 0.41 ± 0.14, range 0.19–0.62 μg/ml). However, on agarose gel electrophoresis, no characteristic bands for ribosomal RNA appeared, suggesting the RNA degradation.

We conclude that although the RNA is present in detectable quantity in gastric juice, its degradation, which probably occurs in vivo, excludes its use for the study of the gene expression by PCR.

1296 Voltage Dependent K^+ Channel in Guinea-Pig Isolated Hepatocytes

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While the various mechanisms of ion transport across the liver cell membrane, for example Na^+ -H^+ exchange or Na^+ -K^+ pump, have been studied, the voltage dependent ion channel as ion transport system in hepatocytes almost has been unknown. Therefore, we tried to study and clarify the voltage dependent membrane current in isolated guinea-pig hepatocytes with applying the patch clamp technique. Single hepatocytes were isolated by using the collagenase liver perfusion technique. In the large patch voltage clamp study, when the holding potential was depolarized from −20 mV to +20 mV, channel activity was observed, which was gradually inactivated. 20 μM Quinidine, a specific K^+ channel blocker, blocked this activity. The reversal potential for this current was almost equal to the theoretical Nernst potential for K^+ selective channel. In the single channel recording, two types of K^+ currents with different conductances were observed, and the single channel conductances were 24 pS and 80 pS, respectively.

These observations indicate that voltage dependent K^+ channel exists in guinea-pig hepatocytes and at least two types of K^+ channels are differentiated. Moreover, it is suggested that voltage dependent inactivation state exists besides open and closed state.

1297 Physiological and Pharmacological Regulation of Potassium Channels of Human Colonic Epithelium

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We previously characterised Na^+, K^+, Cl^- channels; a Cl^- : HCO_3-anion exchanger, a Na^+ : K^+ : Cl^- cotransporter and a Na^+ : K^+ pump in the sodium absorptive cells of the human colonic epithelium. In this study we examined regulation of K^+ recycling which is an important step in Na^+ absorption. Human colonic epithelium dissected off freshly resected colectomy specimens was mounted in Ussing chambers. The transepithelial voltage was clamped to 0 mV and the resultant short-circuit current (SCC) recorded. To study the basolateral membrane in isolation, the apical membrane was perforated by the ionophore nystatin (allows bi-directional passage of monovalent cations between the mucosal bath solution and the cytoplasm). A mucosa to serosa K^+ gradient was imposed. Since nystatin pores are also permeable to Cl^- and Ca^{2+} ions – these were reduced in the apical bath to 20 mM and 10 nM respectively.

Post addition of nystatin, the SCC stabilised at 50–70 $\mu A/cm^2$. 91% of the K^+ dependent SCC was inhibited by tolbutamide (100 μM) added to the serosal bath and the remaining basolateral K^+ current was inhibited by tetrapentylammonium (TPA) (inhibitors of ATP regulated K^+ channels (K_{ATP}) and Ca^{2+} activated K^+ channels (K_{Ca}) respectively). To determine how these channels are affected by intracellular pH and calcium, one was inhibited using either TPA or tolbutamide and the variation in the SCC produced by the other channel was monitored while varying pH or calcium concentration in the mucosal bath. Activity of the K_{ATP} channel was increased by intracellular alkalinisation and by a decrease in intracellular calcium. Current generated by K_{Ca} channels was increased by raising cytosolic Ca^{2+} and also showed maximum activity at pH 7.5. Diazoxide (100 μM) and aldosterone (100 nM) activate K_{ATP} channels in other tissues, and in colon both immediately increased the tolbutamide-sensitive serosal K^+ conductance (18% and 38% respectively). Addition of amiloride (100 μM which inhibits Na^+ : H^+ exchange) to the serosal bath prevented the rapid aldosterone effect.

The dominant electrogenic ion conductance in the serosal membrane is via K_{ATP} and K_{Ca} channels and because K^+ recycling is such an important step in Na^+ absorption, the clearer understanding of their regulation and pharmacological inhibition/up-regulation provided here may in the future allow for manipulation of fluid and electrolyte transfer across the colonic epithelium.

1298 Colonic Lamina Propria Lymphocyte' Subsets in Acromegalics with Intestinal Polyps

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There are few informations about immunological assessment of gut epithelium in acromegalic patients. We evaluated lymphocyte' subsets into colonic lamina propria (CLM) of patients with acromegaly.

Three months preliminary data are reported. Eight acromegalics (2 w, 6 m, age 27–65 years) underwent total colonoscopy to detect polyps. GH and IGF-I levels were 20.4 ± 17.5 and 328.3 ± 130 ng/ml in the serie. Only 1 patient showed intestinal symptoms. During endoscopy all polyps were resected and histologically analysed; biopsies were performed in the polips' surrounding areas to study CLM lymphocyte' subsets. Immunocompetent cells were detected by tissue digestion and cytophluorimetric assay as previously described [1].

We observed polyps in 7 patients (1 tubulo-villous adenoma, 5 tubular adenomas and 3 hyperplastic polyps). CLM lymphocytes from acromegalic patients (pts) and 20 control subjects (cts) were compared as shown in the table (median).

	CD3	CD19	CD4	CD8	3-DR+	3+DR+	CD16
pts	39.9	11.4	32.7	14.6	13.0	5.9	12.5
cts	52.0	8.2	37.6	13.5	8.6	5.9	10.0

	CD25	CD20	CD4+/8–	CD4+/8+	γ/δ
pts	6.9	11.4	9.6	8.9	7.9
cts	5.8	10.8	11.6	9.1	7.6

In conclusion no significant correlation between acromegalics and CLM lymphocyte' subsets was observed in this preliminary report, also regarding polyps in the colon.

[1] A. Balzano Europ. J. Gastroenterol & Hepatol 1993 (in press)

1299 Phenotypic Analysis of Peripheral Blood Lymphocytes in Inflammatory Bowel Diseases

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Several literature data suggest the immune system involvement in the Inflammatory Bowel Diseases (IBD) as documented by accumulation of activated lymphocytes in intestinal mucosa. Moreover an oligoclonality of Vβ TCR repertoire and an increased number of circulating gamma/delta TCR+ lymphocytes have been found in IBD patients. It is well documented that TCR heterodimers may be involved in MHC- and non-MHC restricted cytotoxic activities. The aim of our study was to examine the level of peripheral blood gamma/delta TCR+ lymphocytes and CD56+ cells recognizing NK phenotype in patients suffering from IBD. 43 patients, 25 Ulcerative Colitis (UC) and 18 Crohn's Disease (CD), were compared to 11 normal controls.

Our results demonstrated a significant increase of gamma/delta TCR+ cells in UC (6.9 ± 4.5% vs. 2.8 ± 0.8; p = 0.005) and in CD (6.4 ± 4.6; p = 0.017), with a similar rise of the absolute number in UC (92 ± 70 vs. 42 ± 19; p = 0.028) and in CD (75 ± 43; p = 0.024). CD56+ cell percentages were equally increased in UC (23.5 ± 11.4 vs. 15 ± 5; p = 0.025) and in CD (25.3 ± 10.3; p = 0.005). In UC the absolute number was 435 ± 219 vs. 252 ± 98 (p = 0.012), and in CD was 424 ± 167 (p = 0.005). No differences were found between the two diseases. When results were analyzed according to the disease activity, no significant differences were observed between acute and remission phase.

We suggest that the expansion of potentially cytotoxic cells in peripheral blood during IBD may derive, at least in part, from inflammed intestinal mucosa and may contribute to tissue damage.

1300 Gliadin Allergy with Chronic Urticaria and Atopic Eczema

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Introduction: The prevalence of allergic diseases with dermatological manifestations, as well as atopic eczema (A.E.) and chronic urticaria (Ch.U.) is gradually enhancing. Different food allergens can play important role in the pathogenesis of these diseases. Milk, egg, fish and cereal proteins are the most frequent food-allergens. The putative role of the most important cereal protein (gliadin) is poorly investigated.

Aim of the study: to examine the putative role of gliadin in the pathogenesis of A.E. and Ch.U.

Patients and methods: 10 patients with A.E., Ch.U. underwent detailed dermatological and immunological examinations. The local treatment of the cutan lesions and the oral administration of antihistamines remained unsuccessful in these patients. The following immunological parameters were measured in the blood: (1) gliadin specific IgG antibodies, (2) serum IgE level and/or Prick epicutan skin test, (3) the gliadin specific leukocyte migration inhibition (LMI) test. The mucosal mast cells' number (MMC) was analysed from endoscopic biopsies of the duodenal mucosa.

Results:

Patients	LMI	IgG-AGA	IgE	Prick-test	MMC
10 A.E.	7 +/10	4 ↑ /8	6 ↑ /7	3 +/7	7 ↑ /8
10 Ch.U	8 +/10	4 ↑ /10	3 ↑ /3	5 +/10	7 ↑ /10

These results show that in the majority of the patients gliadin sensitized lymphocytes are present in the blood, while the level of the antigen specific antibodies remains less enhanced. The increased number of MMC in the intestinal lamina propria may represent a local immunoregulatory defect in the patients with A.E. and Ch.U. Six month later after the gliadin elimination from the diet the intensity of the skin lesions abated or disappeared.

Conclusion: The gliadin – similarly to other nutritive allergens – can play an important role in the pathogenesis of A.E. and Ch.U. The LMI-test is more reliable test for the diagnosis of gliadin sensitivity. In atopic cases the conventional dermatologic therapy may be ineffective without the elimination of gliadin from the diet.

1301 Circulating Bacterial Lipopolysaccharides (LPS) and Evidence of Peripheral T Cell and Phagocyte Alterations in Patients with Inflammatory Bowel Diseases (IBD)

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LPS are the main components of the cell-wall from gram-negative bacteria endowed with different immunological activities. Since in patients with ulcer-