apy had been stopped. All symptoms and laboratory findings improved and patients gained physical activity. A few patients suffered from short-period attacks of bloody diarrhea after therapy had been ended. Complaints disappeared in nearly 10 days under a dose scheme of 3 × 3. 10^6 IU/week. Besides well-known side-effects, large ulcers and gross bleeding external hemorrhoids were observed in 4 patients.

**Conclusions:** The results of the study suggest that Interferon alfa-2a may be benefit to colon mucosa in patient with Ulcerative Colitis and therapeutical effect of drug results probably from its immunomodulatory and/or antiviral properties.

### 131 Hepatobiliary Complications of Ulcerative Colitis. A Study on Estonian Patients

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The aim of the study was to determine the prevalence of hepatobiliary complications in patients with ulcerative colitis (UC) attending Tartu University Hospital during a 20-year period. A computer search to identify all patients with UC for the years 1973–1992 was performed. The case records were carefully reviewed, and patients fulfilling the criteria of UC were included in the study. The diagnosis of primary sclerosing cholangitis (PSC) was based on typical findings on endoscopic retrograde cholangiography and liver biopsy. For the diagnosis of chronic autoimmune hepatitis (CAH) a liver biopsy compatible with CAH, anti-nuclear antibodies, and negative tests for hepatitis B were required.

The prevalence of UC was 31.0 per 10^5, the annual incidence was 1.5 per 10^5. At the time of diagnosis 59% of the patients had proctitis, 15% left-sided and 26% total colon. Among 98 patients with UC, 18 were found to have elevated alkaline phosphatase (ALP) and/or transaminases values. In 15 patients ALP values did not exceed the upper normal limit more than two times, and transaminases elevation was mild. Abnormal liver tests normalized when the patients were in remission. These patients were diagnosed as having their liver abnormalities on follow-up. Chronic liver disease was diagnosed in three patients. Among these patients, there were one female with CAH and two males with PSC. All of them had total colitis. The present study on Estonian UC patients leads us to two major findings: the prevalence of abnormal liver tests was about 18%, and the prevalence of CAH and PSC was 3%.

### 132 The Effect of Colectomy on Histological and Biochemical Liver Changes Associated with Ulcerative Colitis

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The effect of colectomy on the hepatobiliary changes associated with ulcerative colitis (UC) has been controversial. In the present follow-up study we included colectomized ulcerative colitis patients with even minor histological liver abnormalities found at preoperative liver biopsy in 1982–1987 to assess the effect of colectomy on histological and biochemical features of the liver in patients with UC. We were able to carry out a follow-up liver biopsy on 24 (71%) of the 34 patients with liver abnormalities together with 8 patients with initially normal liver biopsy. Liver function values, i.e. alkaline phosphatase, alanine aminotransferase and bilirubin were analysed before the initial liver biopsy at colectomy and before the follow-up biopsy. The mean follow-up period was 46 months.

In five out of 7 patients whose initial liver biopsy showed PSC-like cholangitis, the stage of the disease had remained unchanged or progressed. There were no statistically significant improvements in the liver function values. All but one of the eight patients with nonspecific reactive hepatitis (NRH) at initial biopsy showed normal liver histology at the follow-up biopsy. Seven of the nine patients with steatosis initially showed normal liver histology at follow-up. Two of the eight patients with normal liver biopsy initially showed abnormalities at follow-up, one moderate steatosis and the other lymphoid cholangitis.

There seems now to be convincing evidence that colectomy has no beneficial effects on PSC-like cholangitis in patients with UC. On the other hand, NRH and steatosis seem to disappear or be subdued after colectomy. Since, however, in nearly 10 days under a dose scheme of 3 × 10^6 IU/week, there is no additional indication for protocolecotomy in patients with UC. The disappearance of NRH after colectomy in most patients with UC suggests that it may be caused by toxins related to colitis.

### 133 Serum Anti-Neutrophil Cytoplasmic Antibodies in Ulcerative Colitis: Clinical Utility

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Antineutrophil cytoplasmic antibodies (ANCA) are useful for diagnosis and follow-up of patients with systemic vasculitis. These antibodies have also been detected in the serum of patients with inflammatory bowel disease (IBD), especially in cases of ulcerative colitis (UC). The aim of the present study is to determine the presence of ANCA using indirect immunofluorescence (IF) in patients with IBD and some other illnesses that give rise to diarrhea and to evaluate their possible use as a serological marker and their relation to other clinical parameters, both analytical and therapeutic.

**Methods:** Using IF, the serum of 91 consecutive patients with IBD was evaluated, 46 ulcerous colitis (UC) and 45 Crohn's disease (CD), together with that from 10 patients with other diseases giving rise to diarrhea (3 cases of salmonella enteritis, 1 shigella, 4 of unknown etiology and 2 cases of irritable bowel syndrome).

**Results:** ANCA was detected in 26 of the 46 patients with UC (60.8%) but in only 3 of the 45 patients with CD (6.6%) (p < 0.01) and in no other illnesses that cause diarrhea. The pattern of predominant staining was perinuclear in 62% of cases of UC (23/38), and in 3 cases of CD. Thus, the sensitivity of the test IF of perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) in the diagnosis of UC attained 50%, with a 93% rate of specificity. In patients with UC no correlation was found between a positive test result and age, sex, stage of development, location, extrapolimentary manifestations or the treatment that followed. A certain relation between the presence of ANCA and the activity of UC was found, although this did not attain statistical significance (p = 0.05).

**Conclusions:** (1) The presence of ANCA as detected by IF is associated mainly with UC. (2) In patients with UC the predominant staining pattern is perinuclear (p-ANCA). (3) No correlation has been found between a positive test result and clinical features or treatment in patients with UC. (4) Screening IBD patients for ANCA can be helpful in distinguishing UC from CD patients as well as for other diseases which cause diarrhea.

### 134 Bone Mineral Density in Children with Inflammatory Bowel Disease

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Bone mineral density (BMD) was measured by Lunar DPX densitometer in 27 children (M-14, F-13) aged 7.8-18.3 years with Inflammatory Bowel Disease (IBD). Crohn Disease (CD) was diagnosed in 8 pts, Ulcerative Colitis (UC) in 15 pts and Nonspecific Colitis (NS) in 4 pts. The reference BMD was calculated for age, height and body weight of 318 healthy children (M-164, F-154) aged 5-18 yrs.

The height of 7 (26%) pts and body weight of 8 (30%) was below 10 percentile. No differences between CD and UC was noted.

Z-score of BMD was calculated for age, height and body weight. Z-score for body weight was below −2 in 3 (37%) pts with CD (mean −1.82), 2 (13%) pts with UC (mean −0.78) and in none with NS. Z-score for height below −2 was found in 2 (25%) pts with CD (mean −0.37), 1 (7%) pts with UC (mean −0.22) and none with NS. Z-score for body weight below −2 was found in 3 (37%) pts with CD (mean −0.54), 2 (13%) pts with UC (mean −0.35) and none with NS.

The duration of disease in children with CD and z-score for age below −1 was 5.9 yrs and in children with CD and z-score above −1 was 1.9 yrs. The duration of disease in children with UC and NS had no significant meaning for BMD. The previous steroid treatment didn't significantly influence BMD.

**Conclusions:** (1) BMD is lower in children with CD and UC compared with children with NS. (2) The duration of CD is an important factor influencing BMD. (3) The steroid treatment didn't significantly influence BMD of children with CD and UC.

### 135 NADPH Diaphorase Activity in Ulcerative Colitis

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Evidence has been presented that there is increased nitric oxide (NO) production in ulcerative colitis (UC) and that the enzyme NO synthase (NOS) is induced. Neuronal NADPH diaphorase (NADPHD) activity and NOS co-localised and are homogenous on purification. NADPH diaphorase was investigated in colonic biopsies from patients with acute UC. 24 snap frozen mucosal biopsies stored before analysis at −80°C were incubated with 2.5 mM NADPH, 1 mM nitro blue tetrazolinium and 10% dimethyl sulphoxide for 30 minutes at 37°C. L-Immonomethylarginine (LNNMA) 300 μM was used as an inhibitor of
NOS and rat cerebellum served as a positive control. Omission of NADH resulted in no staining. Colonic epithelial cells showed positive formazan staining. Surface staining was similar in both controls and in UC but increased staining was observed in UC. No correlation was seen between NADPH-D activity and leucocyte infiltration. LNMNA had no effect on the intensity of staining in controls, UC or rat cerebellum.

Increased staining in the crypts of patients with UC suggests patchy induction of NOS. Changes in the crypts may represent an early stage in the pathogenesis of UC.

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136 **125**-Interleukin 2 (IL2) for in Vivo Detection of Activated Lymphocytes in Crohn's Disease (CD)

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Increased activated intestinal lymphocytes capable of binding IL2 are present in CD. Aim of our study was to evaluate "in vivo" the presence and the extent of intestinal lymphocytic infiltration in CD by using 125I-labelled IL2. 125I is a radiopharmaceutical that binds specifically to activated T lymphocytes homing in the infiltrated tissues, allowing their external localization by γ-camera imaging. Methods: We studied 8 healthy volunteers (NS) and 6 CD (3 inactive). CD involved the ileum in 5 and ileum-colon in 1, as assessed by radiology. No patients were under immunosuppressive or steroid therapy. Two mix of 125I-IL2 (10 μg) were injected i.v. and tomographic γ-camera images (SPET) acquired after 1 hr. Results: No intestinal activity was found in NS, while all CD had accumulation of 125I-IL2 of various degree and extent. This was also observed in CD without clinical and hematological evidence of activity. Area of interest were drawn in the right, middle and left part of this section and the bowl/bone radioactivity ratio calculated for each area. We found a significant difference between CD and NS in all regions, as shown:

<table>
<thead>
<tr>
<th>Area</th>
<th>Right</th>
<th>Middle</th>
<th>Left</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>NS CD</td>
<td>NS CD</td>
<td>NS CD</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td>0.011</td>
<td>0.011</td>
<td>0.003</td>
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</tbody>
</table>

Conclusions: This study demonstrates for the first time that an active lymphocytic infiltration is visualized "in vivo" in the bowel of both active and inactive CD and this may have relevant therapeutic implications. Our technique is non-invasive and may also be used for therapy follow-up in CD.

137 Perinuclear Neutrophil Autoantibodies (p-ANCA) in Unaffected Relatives of Patients with Ulcerative Colitis (UC). Suggestions Against Familial Aggregation

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Background: Evidence is accumulating to show that p-ANCA are strongly associated with UC reflecting immune disregulation in these patients. Such a strong association also suggested that p-ANCA may represent an indicator of genetic susceptibility to UC.

Aim: to further examine whether p-ANCA may serve as a genetic marker of UC. Specific aim was to determine the frequency of p-ANCA in UC patients and their unaffected family members from a defined geographic area.

Methods: 79 patients with UC diagnosed according the usual clinical, endoscopic and histological criteria were enrolled and a total of 80 unaffected family members (first or second degree relatives) were studied. Sera of 10 spouses of UC patients were also tested. The sera of 50 patients with Crohn's disease (CD) and of 24 unaffected relatives were studied. 35 patients with Irritable bowel syndrome (IBS) and 20 family members were included as further control groups. All patients and relatives were born, grown and living in the same locality (Provincia di Catanzaro, Province of Catanzaro, Province of Catanzaro, Italy). Serum samples were tested for p-ANCA by ELISA and indirect immunofluorescence. ELISA was performed using fixed normal neutrophils as the source of antigen and alkaline-phosphatase conjugated anti-human IgG as second antibody. Positive ELISA was confirmed by immunofluorescence defining the pattern of positive reaction. For the purpose of this study only the perinuclear pattern was considered.

Results: p-ANCA were detected in 38 probands (48%). There was no relation between p-ANCA and a number of clinical variables including activity, extent, treatment, duration, extraintestinal manifestations. Only 5 of the 80 unaffected relatives were positive for p-ANCA (6%) and two of these were from the same family. Overall in only 3 out of a total of 30 families the proband and at least one unaffected relative were positive for p-ANCA. 650 sera of CD patients (12%) were positive for ANCA. When CD subgroups were considered it was found that 516 patients with colonic CD were positive for ANCA (31%). None family member were positive in the CD family group and 1 was positive in the family group.

Conclusions: As reported in other geographic areas p-ANCA are detected in approximately one half of the patients with UC. p-ANCA were also relatively frequent in CD colitis suggesting that p-ANCA may be related to colonic involvement in UC. In the group of families recruited for this study p-ANCA were uncommon in unaffected relatives of UC patients suggesting that at least in the geographic area considered for this study p-ANCA not represent a marker of genetic susceptibility to UC.

138 Competitive RT-PCR of Th1-/Th2-Like Cytokine mRNAs in Patients with IBD

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It is widely accepted that a disturbance in the mucosal immune response participates in the pathogenesis of inflammatory bowel disease (IBD). Cytokines play an important role in the mediation and regulation of immunological processes and small changes in the pattern of lymphokines produced by intestinal T cells might contribute to the disease. Therefore, we performed a competitive reverse transcribed (RT)- PCR to determine the amount of cytokine mRNAs in T cells derived from mucosal biopsies of patients with IBD compared to normal controls. Methods: Quantitation of mRNAs specific for Th1-, IL2, IFNγ, Th2, IL4, IL10 like T cells was performed using synthetic mRNAs, containing a small intron but the same primer template sequence, as an internal control. Total cellular RNA of polyclonally activated intestinal T cells was extracted and mixtures of target RNA with known amounts of synthetic mRNAs were reverse transcribed and co-amplified in the same tube. Size differences allowed easy electron microscopic inspection of the resulting PCR products. Results: Quantitation of cytokine mRNAs showed an approximately 2-fold reduced transcription of IL2, IL4 and IL10 mRNA in T cells derived from inflamed tissue of patients with IBD (n = 13, 7 with 31% ulcerative colitis (UC) and 6 with Crohn's disease (CD)) compared to T cells derived from uninfamed mucosa of patients without IBD (n = 24). Such decrease was also observed in the supernatant of cell cultures of IBD patients. In conclusion, 2-fold reduced expression of Th1-like cytokines in mucosal biopsies might contribute to the mucosal inflammation.

139 Diosmectite Treatment Delays Colonic Water Secretion and Reduces Increase in Intestinal Permeability Induced by Clostridium difficile Toxins in Rats


Among the deleterious effects of Clostridium difficile toxins there are intestinal fluid secretion and increase in intestinal permeability. On the other hand, the natural aluminosilicate diosmectite exerts a protective action on digestive mucosa and reduces diarrhoea and alterations of intestinal permeability in humans as well as in different animal models. This study was aimed to evaluate the effects of a treatment with diosmectite on colonic hypersecretion and increase in intestinal permeability induced by C. difficile toxins. Firstly, net water flux was determined in a 5 cm colonic loop in 2 groups of 6 urethane anaesthetized rats infused with a Tyrode solution containing 14C-polymethylene glycol 4000 as a nonabsorbed marker. The colonic effluent was collected at 15 min intervals for 6 hours. Colonic C. difficile toxins (50 ng) were added in the Tyrode solution during the 3rd hour of infusion. A group of rats was orally treated by diosmectite (IPSEN, Paris; 500 mg/kg/day) for one week before water flux measurement. The control group received water. Secondly, intestinal permeability was assessed in 12 rats by the excretion in 24 h urine of 51-Cr-EDTA orally administered. Each animal received orally, at 2 week intervals, 50 ng of crude C. difficile toxins before Cr-EDTA and were treated by diosmectite or water for the preceding week. Controls were a diosmectite or water treatment for a week, without toxin administration. Colonic basal net absorption of water (±4 ± 1 ml/cm h) was reversed into an intense secretion (±65 ± 3 ml/cm h) from normal animals (C. difficile toxins). Then a net water absorption returned to values similar to controls (P > 0.05). In diosmectite treated animals, the toxin-induced hypersecretion had the same amplitude (±74 ± 44 ml/cm h) but was delayed, appearing during the hour following the toxin infusion. In controls, intestinal permeability corresponded to an urine excretion of 3.7 ± 1.4% of Cr-EDTA orally administered. C. difficile toxins increased the permeability, inducing a Cr-EDTA excretion of 6.8 ± 2.4% which was significantly reduced (P < 0.05) to 4.5 ± 1.1% after diosmectite treatment. Diosmectite treatment per se had no effect on basal net water absorption nor on intestinal permeability.
Microvascular Endothelium from Human Intestine is Constitutively and Functionally Different from Human Umbilical Vein Endothelial Cells

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Background: Recent work has demonstrated several differences between large and small vessel endothelial cells in situ and in vitro. We therefore established a method for isolation and long-term culture of human intestinal microvascular endothelial cells (HIMEC) and compared them with human umbilical vein endothelial cells (HUVEC). Expression and regulation of the cell adhesion molecules CD36, E-Selectin, VCAM-1, and ICAM-1 were studied. Methods: In situ phenotyping of endothelial cells was performed by double immunofluorescence staining. In vitro antigen expression was examined by a cell ELISA. Results: Only the smallest vessels in the lamina propria expressed CD36 in situ. The endothelial cells isolated from the intestine continued to express CD36 in vitro, whereas HUVEC lacked CD36 in culture. E-Selectin, ICAM-1 and ICAM-1 were upregulated in a dose-dependent fashion on HIMEC after stimulation with LPS (0-1 μg/ml), IL-1α, or TNFα (both 0-1000 U/ml) and displayed characteristic time curves comparable to HUVEC. However, IL-4 appeared to have only a negligible effect on HIMEC. Thus, both VCAM-1 and ICAM-1 were upregulated on HIMEC (IL-4, 0-1000 U/ml) but only weak responses were observed on HIMEC. E-selectin was not influenced by IL-4. Conclusion: Our data suggest that leukocyte-endothelial interactions are differently regulated on HIMEC compared with HUVEC. HIMEC are therefore a more relevant test system for studies of endothelial cell involvement in inflammatory bowel disease.

Right-Sided and Left-Sided Colorectal Cancers Have Alternative Genetic Changes


The development of colorectal cancer (CRC) needs several genetic changes accumulated in one mucosal cell. Tumor suppressor genes are frequently inactivated as an essential step in colorectal carcinogenesis, through loss and/or mutation of both gene-alleles. We have earlier reported loss of the tumor suppressor gene TP53 in 68% of CRC, and mutation of TP53 is known to occur in approximately half of the CRC.

A genetic change called "RER" (DNA replication- or repair-error) has recently been discovered, and is thought to be caused by an inherited defect in one or more DNA repair enzyme(s). We and others have reported RER to be associated with CRC. Furthermore, we have also reported RER to be significantly associated with several clinicalopathological variables in 252 CRC: right-sided cancer, DNA diploid tumor, poorly differentiated tumor, and a better prognosis. So far, no association has been reported between RER and other genetic changes in CRC cells.

Methods: We have analyzed 156 “sporadic” cases of CRC for RER and TP53 changes. We used PCR (for RER, 7 random loci analyzed), PCR in combination with CGE (for TP53 mutations), and Southern technic (two loci near TP53 analyzed for loss, the losses detected are supposed to comprise TP53 in most of the cases).

Results: Loss of one TP53-allele was found in 68% (106/156), mutation of one allele in 44% (69/156). Nearly all (63/69) tumors with mutation of one allele also had loss of the remaining allele.

Rel. between loc, RER, and TP53 (loss = l. and mutation = m.):

<table>
<thead>
<tr>
<th>RER+</th>
<th>TP53 l. and m.</th>
<th>l. or m.</th>
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<tbody>
<tr>
<td>l. or m.</td>
<td>12/15 = 0%</td>
<td>31/20 = 61%</td>
</tr>
<tr>
<td>l. or m.</td>
<td>7/15 = 50%</td>
<td>81/1000 = 81%</td>
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1. RER was demonstrated in 106% of CRC, occurring in 30% of right-sided and 10% of left-sided CRC (p < 0.001).

Two right-sided cancers with RER have no complete TP53 inactivation, whereas

3. left-sided RER-positive cancers have similar TP53 inactivation as RER-negative CRC (p < 0.05).

One hypothesis is that right-sided CRCs have more inherited genetic changes/defects, and thus need fewer acquired genetic alterations in order to become malignant, whereas in left-sided CRC the situation is opposite. Our results agree with this.

Prognostic Significance of Retinoblastoma Gene Product Expression in Colorectal Cancer

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Loss or inactivation of the retinoblastoma (Rb) gene has been demonstrated in several non-GI human malignancies. Recently, altered Rb gene expression has been reported in colorectal cancer (CRC) but the prognostic significance of this finding has not been determined. The aim of this study therefore was to correlate Rb gene product expression with longterm survival in CRC.

Patients and Methods: CRC samples from 76 patients collected over 3 years (1984-1988) were analysed for Rb gene product nuclear protein expression by immunohistochemistry using the monoclonal antibody PMG3.245. Unlabelled CRC tissue and bladder cancer tissue were used as negative and positive controls, respectively. Prognostic significance was established from Kaplan-Meier curves which were generated from actuarial disease free survival, with differences in survival being compared by means of logrank analysis.

Results: Positive Rb expression of >80% in tumour cells was observed in 39/76 (51%) patients. Without Rb expression, it was present in the remaining 37/76 (47%). Patients with Rb expression of >80% had significantly better outcome (p = 0.03).

Conclusion: These findings suggest that the Rb gene is of prognostic significance in CRC and has an oncogenic-like function, in contrast to its reported tumour suppression activity in other human malignancies.

Overexpression of p53 in Relation to Long-Term Prognosis in Gastric Carcinoma

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Overexpression of p53 protein in the cell nucleus as a result of mutation is very common in human neoplasia, but the relationship of this phenomenon to prognosis remains to be defined. We investigated the relationship between p53 expression in gastric cancer and the outcome of the disease. The expression of p53 protein was analysed immunohistochemically in 170 gastric carcinomas by use of CM 1 antibody and routinely fixed tissue.

A significant association was found between p53 overexpression in cancer tissue and poor prognosis. The proportions in cancer tissue and poor prognosis. The proportions of patients with 5-year survival with and without p53 overexpression tumours was 4% and 49%, respectively (p < 0.001).

In a multivariate analysis against 10 established prognostic factors, p53 overexpression was the strongest predictor of poor prognosis followed by metastases to the regional lymph nodes (p < 0.001), RR = 6.7 and p = 0.03, RR = 2.66 respectively.

The results suggest that in gastric cancer an immunohistochemical detection of p53 can be helpful to identify patients at high risk of tumour recurrence and death for whom the use of an aggressive surgery and adjuvant chemotherapy should be considered.

Programmed Cell Death Throughout the Colorectal Tumor Sequence

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Homeostasis of the renewal of normal tissue, progression and regression of tumor lesions are regarded as the result of the interactions between cell proliferation and programmed cell death (PCD). There is abundant evidence that cell proliferation changes in histologically normal intestinal mucosa are early events directly and closely associated with colorectal carcinogenesis, whereas little is known about the role of PCD.

In this investigation PCD was evaluated in intestinal mucosa of control subjects (n = 10), in histologically normal mucosa with cell proliferation abnormalities from subjects with colorectal neoplasia (n = 10), in colorectal adenomas (n = 20) and adenocarcinomas (n = 10). Cells engaged in PCD were identified through immunohistochemical detection of DNA strand breaks that follow activation of endogenous endonuclease. The method is based on nick-end labeling with biotinylated polydeoxyuridine introduced by terminal deoxynucleotidyl transferase, and then stained with avidin-conjugated peroxidase. PCD Index was calculated as the percentage ratio between labeled and total cells.

In normal mucosa, most PCD-positive cells were found at the mouth of the crypt and in the surface epithelium, in sectors where the physiological shedding of the epithelium takes place, though sporadic activity was present at

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