Changes in Bacterial Composition and Enzymatic Activity in Ileostomy and Ileal Reservoir during Intermittent Occlusion: a Study Using Dogs

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Bacterial flora, activities of 10 potential mucus- and dietary polysaccharide-degrading enzymes, blood group antigenicity of the intestinal glycoproteins, and proteolytic activity in the output from experimentally colectomized dogs with conventional ileostomies and dogs with valveless ileal reservoirs (pouches) were determined. The ileostomies of dogs with conventional surgery (group II) and with pouches (group III) were occluded intermittently during a 6-week period. The duration of occlusion was progressively increased. Group I, five dogs with conventional ileostomies, served as a control group. After occlusion of the ileal pouch for 7 h, total numbers of bacteria increased threefold, glycosidase activity increased fivefold, and blood group antigenicity of the intestinal glycoproteins, which was high in the output from the nonoccluded pouch, was no longer detectable. Proteolytic activity was not influenced by occlusion of the pouch. Significantly lower numbers of bacteria, only minor glycosidase activity, high blood group antigenicities of the intestinal glycoproteins, and higher proteolytic activity were found in ileostomy effluents from groups I and II. Histopathological examination showed chronic inflammation and changes in crypt-villus ratio in all dogs with ileal reservoirs; the ileal mucosa from the dogs with conventional ileostomies did not show any abnormalities. Consequences of the flora-related enzyme activities for the ileal mucosa are discussed.

Until recently, colectomy with a permanent ileostomy was the only surgical treatment of chronic ulcerative colitis or familial polyposis. For many patients, a permanent ileostomy caused psychological and sexual disturbances as well as local complications. An attractive alternative was the introduction of the continent ileostomy or pelvic ileal pouch in which a reservoir is constructed from the terminal ileum, establishing continence for stool and gas for most patients.

The ileal mucosa of the pouch shows morphological changes arising from its reservoir function. Nonspecific chronic inflammation occurs in almost each patient studied and colonic metaplasia and villous atrophy occurs in nearly half of the patients, regardless of the functioning of the pouch. In conventional ileostomies, no villous atrophy and only occasional signs of inflammation were found (25, 28, 30).

Mechanisms that may explain these phenomena are poorly understood but may be associated with the flora in the pouch. The ileum reservoir is colonized with large numbers of bacteria that closely resemble the flora found in the colon (23, 24, 28). Microorganisms are known to be active producers of metabolites and toxins, and when localized in the ileostomy reservoir, bacterial products could injure the mucosal epithelial cells. Bacterial enzymes with the capacity to degrade intestinal mucus are also potentially dangerous to the ileal mucosa. Enzymes involved in the breakdown of oligosaccharide side chains of mucin glycoproteins are glycosidases. These enzymes are produced by several representatives of the colonic flora (4, 39–41). Breakdown of the polysaccharide moiety of the glycoprotein may result in impairment of the protective function of the mucus, which may become more permeable to toxic bacterial metabolites and host-derived enzymes such as proteases.

The aim of the present study was to investigate the possible role of the flora in the production of potential mucus-degrading enzymes and enzymes that might be involved in the release of toxic compounds from dietary products in an animal model. Experiments were performed on dogs with experimental conventional ileostomies and dogs with experimental valveless ileal reservoirs. Both types of ileostomies were occluded intermittently in a progressive way.

MATERIALS AND METHODS

Animals. Fifteen purebred beagles (Harlan, Zeist, The Netherlands), nine males and six females aged 2 to 3 years, were housed separately in regular open cages at the Erasmus University. Except for the period immediately after the operation (4 to 5 days), the animals were fed on a constant diet of standard animal feed (Hope Farms, Woerden, The Netherlands).

Three ileostomy groups were studied. In 10 dogs (groups I and II), a standard Brooke ileostomy was constructed (length of <25 mm) by subtotal colectomy. The ileum was transected 2 to 3 cm proximal to the ileocecal junction. In five dogs (group III), a valveless ileal reservoir (pouch) was fashioned by a side-to-side iso-antiperistaltic anastomosis over a distance of 20 cm extending from the level of the abdominal wall. The final capacities, measured with inflatable balloons, were about 10 ml for the ileostomy and 70 to 80 ml for the pouch. After a recovery period of 2 weeks, a schedule of increasing periods of occlusion was started with groups II (n = 5) and III. Group I (n = 5) served as a control group. The stoma was occluded with a Conseal colostomy...
plug (Coloplast, Amersfoort, The Netherlands), initially for 1 h daily. This period was increased weekly by 1 h. Occlusion was terminated when animals showed signs of discomfort or when expulsion of the occluding device occurred. A small part of the terminal ileum, proximal to the stoma (groups I and II) or a part of the pouch (group III) was removed for histopathological investigation. The dogs were then sacrificed.

At 2 and 8 weeks after the initial operation, ileostomy effluent from each dog from group I was studied. The maximum tolerable occlusion time (2.5 to 3.5 h) for group II was reached after 4 to 5 weeks; samples were collected 5 and 8 weeks after operation. Three samples from group III were processed: 2 (no occlusion had happened), 4 (4 h of occlusion), and 8 weeks after operation (7 h of occlusion). Stool samples (10 ml) were obtained using a sterile tube and transferred to the laboratory in completely filled vials to assure minimal contact with oxygen.

**Microbiology.** Within 1 to 2 h after collection, the stools were processed. The samples were thoroughly mixed and 10-fold dilutions were prepared in anaerobic dilution solution (36). Samples of appropriate dilutions were plated aerobically on MacConkey (Oxoid), Sabouraud (Oxoid), malt extract (Oxoid), Rogosa (Oxoid), azide blood (Oxoid), and blood agar plates. Anaerobes were cultured in anaerobic culture flasks filled with a 90% N₂ -10% CO₂ mixture as described before (47, 48), on Schaedler broth (Oxoid) supplemented with 2% agar (Difco), 0.002% resazurin (BDH), and 0.025% dithiothreitol (Sigma), the same medium with 0.00075% vancomycin (Lilly, Amsterdam, The Netherlands) and 0.002% garamycin (Schering Corp., Bloomfield, N.J.), and azide blood agar with 0.05% cysteine (Sigma) and 0.025% dithiothreitol. After 2 days of incubation at 37°C, colonies on the various media were counted. All colonies grown on the anaerobic flasks were tested for aerobic growth on blood agar plates. Members of the family *Enterobacteriaceae*, streptococci, and yeasts were classified by using API systems (BioMerieux, Marcy-l’Etoile, France). The other aerobes were identified by conventional methods. Anaerobic bacteria were identified by using an API system, a Minitek system (BBL Microbiology Systems, Cockeysville, Md.), and methods described by Holdeman et al. (17) with minor changes: for the analysis of end products of glucose fermentation, isolates were grown for 5 days on Schaedler broth instead of PYG medium. Alcohols and volatile and nonvolatile acids were analyzed by gas chromatography as described before (36). Formic acid was assayed colorimetrically (22).

**Enzyme assays.** Within 2 h of collection, the stool samples were stored at −20°C. Preliminary studies showed no changes in enzyme activities for at least 1 month of storage. Samples were diluted 1:25 in distilled water and homogenized (Stomacher Lab Blender 400) for 10 min. Coarse particles were removed by gauze filtration (Utermöhlen) (5 by 5 cm, refolded to two layers). Further samples were made in the appropriate buffer solutions.

The activities of α-L-fucosidase, α- and β-Nac-α-galactosaminidase, α- and β- and p-galactosidase, β-Nac-α-glucosaminidase, α-D-mannosidase, β-D-glucosidase, and β-D-glucuronidase were estimated by using their p-nitrophenyl glycoside substrates (Sigma and API) (37). One unit of enzyme activity was defined as the amount which released 1 μmol of p-nitrophenol per min at 37°C. Neuraminidase activity was measured with NAc-neuramin-lactose (Sigma) as substrate by the method of Warren (45). One unit of neuraminidase released 1 μmol of neuraminic acid per min at 37°C. The limit of detection in these assays was 0.01 U.

**Proteolytic activity was determined by using azocasein (Sigma) as the substrate (38). Proteolytic activity was expressed in milligrams of azocasein hydrolyzed in 1 h at 37°C. All enzyme activities were described per gram (dry weight).**

**Degradation of blood group antigens.** Dog intestinal glycoproteins were obtained by dissolving and homogenizing the intestinal samples in distilled water and centrifuging the samples 30 min at 10,000 × g. Clear supernatants were heated (10 min at 80°C) and lyophilized, and 5% solutions (wt/vol) in 0.1 M phosphate buffer (pH = 7.4) were prepared. The presence of blood group antigens in the solutions was estimated by determining titers of A and H (lectin) antigens in duplicate hemagglutination inhibition tests (39). The reciprocal value of the highest dilution of antigen glycoprotein that completely inhibited hemagglutination was defined as the antigen titer.

**Histology.** During the initial operation, a small part of the ileum was removed. At the end of the experiment, a part of the terminal ileum proximal to the stoma (groups I and II) and a part of the pouch (group III) was removed. The specimens were stained with hematoxylin and eosin. The abnormalities evaluated were infiltration of inflammatory cells, lymph follicles, crypt abscesses, erosion of epithelial cells, ulceration of lamina propria, villous atrophy, crypt proliferation, and number of mitoses.

**Statistics.** The Mann-Whitney U test was used to compare numbers of bacteria, glycosidases and proteolytic activity, obtained from ileostomy effluent with those obtained from the ileal pouch. The Wilcoxon signed-rank test was used to compare numbers of bacteria and glycosidases in the output from the nonoccluded ileal pouch with pouch output after occlusion. The coefficient of correlation was calculated on the basis of the least-squares criterion (15).

**RESULTS**

**Occlusion.** All dogs made a good recovery after operation. One dog in the ileal pouch group (group III) had to be sacrificed before the end of the experiment because of weight loss (>20% of body weight). Intermittent occlusion (up to 7 h) was tolerated very well in group III, and no signs of discomfort were shown. The liveness of one of the dogs prohibited occlusion for more than 4 to 5 h. The plug could be left significantly longer in situ in the pouch group (group III) than in the ileostomy group (group II), 5 to 7 h versus 2.5 to 3.5 h (P < 0.01).

**Flora.** Table 1 does not show any significant differences in flora composition between the control ileostomy group (group I) and the ileostomy group after maximal occlusion of 2.5 to 3.5 h (group II). Table 2 shows that total numbers of bacteria were 10 times higher in valueless ileal reservoirs (group III, no occlusion) than in ileostomy output (groups I and II, see table 1), (P < 0.01). This difference is entirely brought about by the anaerobes (P < 0.01); total numbers of aerobes did not differ between the groups. The aerobic-to-anaerobe ratios were 20 to 1 in ileostomy effluent and 1 to 3 in the ileal pouch. Composition of the aerobic flora was essentially the same in all groups. Only four anaerobic species, *Clostridium perfringens*, *Fusobacterium nucleatum*, *Fusobacterium necrophorum* were found more than once in ileostomy output. These species were found in a majority of samples from group III in high numbers; other predominant species...
from the pouch were *Bacteroides ruminicola* and *Gaffkia anae""""rob""""a*.

Oclusion of the pouch for 4 h did not cause a significant increase in total numbers of bacteria. However, after 7 h of occlusion, total numbers were 3 times higher than in the open pouch and 2.5 times higher than in the output from the 4-h occluded pouches (P = 0.05). No change in composition or number of aerobes was observed; the anaerobic flora accounted for the increase of total numbers. In order to find out whether the increase in anaerobes was due to a single species or to certain groups of bacteria, we compared their numbers before and after occlusion. None of the separate species showed significantly higher numbers. However, a significant increase was found in total numbers of *Bacteroides* species (P = 0.05), *Fusobacterium* species (P = 0.05), and the group of gram-positive anaerobes (P = 0.05). No differences were found in numbers of *Clostridium* species. Output from each dog was cultured 2, 6, and 8 weeks after operation, and over this period the overall composition of the flora of the individual dogs was found to be rather stable and persistent. The flora of two dogs consisted of about 75% (75, 77, and 80% for one dog and 81, 72, and 76% for the other dog) gram-negative rods (*Fusobacterium* and *Bacteroides* species); one dog had about equal percentages of gram-negative (50, 44, and 47%) and gram-positive anaerobes, and two dogs contained only a small percentage of gram-negative bacteria (0.1, 2, and 5% for one dog and 10 and 12% for the other dog).

Table 3 shows that the pH values of the samples from ileostomy effluent were 7.7 (group I) and 7.3 (group II), which is significantly higher than in the ileal pouch (pH = 6.6; P < 0.01); occlusion of the pouch did not cause any change in pH. Dry weight of the output from group II was significantly higher than the values for groups I and III (P = 0.01). No correlation was found between pH and total numbers of bacteria nor between dry weight and total numbers of bacteria.

**Enzymatic activity.** Table 4 shows that glycosidase activity was absent or low in ileostomy output; no difference between groups I and II was found. In the output from the nonoccluded pouch, all enzymes were detected and the activities of most enzymes were significantly higher than in ileostomy effluent.

As shown in Fig. 1, occlusion of the pouch for 4 and 7 h resulted in an increase in glycosidase activity, namely, 3 and 5 times higher, respectively, than in the nonoccluded pouch (P < 0.01 for both comparisons). A linear correlation was found between the total number of anaerobes cultured from the 13 samples of group III and the activity of each individual enzyme in the corresponding sample (r = 0.789 to 0.951 [P < 0.001]), except for α-D-galactosidase (r = 0.617, P = 0.02); α-D-mannosidase, which was determined in only 4 of 13 samples, was not considered.
mucosity in accordance with the dog blood group secretor status. No blood group antigens were detected in glycoproteins prepared from the pouch output after 4 or 7 h of occlusion (Table 5).

Figure 2 shows that proteolytic activity in ileostomy effluent from group I is two times higher than in pouch output \( P = 0.05 \). Group II occupies an intermediate position but is not significantly different from groups I and III. Occlusion of the pouch did not alter the activity in the pouch. In feces from healthy dogs, the proteolytic activity found was very low; the median value from five samples was 13 (range, 1 to 69) mg of hydrolyzed azocasein per g (dry weight).

**Histology.** Histologic examination of full-thickness biopsies, collected during the first (terminal ileum) and second operation (terminal ileum, proximal to the stoma) revealed no changes in ileostomy group I or II. In all pouches from group III, however, a mild (\( n = 3 \)) to moderate (\( n = 2 \)) chronic, locally active inflammation with slight mucosal atrophy (crypt-villus ratio of 1:1 to 1:2) and crypt proliferation could be demonstrated. No colonic metaplasia was detected. All stomas developed mucosal atrophy (crypt-villus ratio of 1:1) and a chronic, active and ulcerative inflammation at the mucocutaneal border as a result of mechanical damage.

**DISCUSSION**

The most important function of intestinal mucus is to protect the delicate mucosal epithelial cells from mechanical...
injury, the action of antigens and toxins, and the invasion of enteric bacteria. The chief organic constituent of the intestinal mucus secreted by goblet cells consists of a glycoprotein that contains up to 85% carbohydrates. Common parts of the carbohydrate are fucose, galactose, NAc-glucosamine, NAc-galactosamine, NAc-neuraminic acid, and mannose in α- or β-glycoside linkage (1, 2, 21). Intestinal mucus may be degraded and serve as an energy source for the bacterial flora (11). This degradation depends on the action of bacterial glycosidases, releasing monosaccharides from the polysaccharide chains that surround the protein core of the glycoprotein (43). Intestinal microorganisms with mucin-degrading properties have been isolated from human and animal sources (7, 34, 46, 49).

The healthy ilea of humans and animals contain low numbers of bacteria. Although bacteria in ileostomy effluent from colectomized patients (13, 20) are more numerous than in the ileum (8, 12), the activities of glycolytic enzymes were found to be absent or very low (35). This was confirmed by our current findings: in the ileostomy effluent of dogs, the total number of bacteria was between 5 × 10⁷ and 5 × 10⁹ per gram (dry weight) and the activity of glycolytic enzymes was low or not detectable. Furthermore, the crude glycoprotein fraction prepared from supernatants did possess high blood group antigenicity, indicating that no or only minor mucous breakdown takes place in the ileum. Histopathological findings revealed a complete normal ileal mucosa in both ileostomy groups, which is probably the result of adequate protection by the unconverted mucus. Although numbers of bacteria from the valveless ileal reservoir were at least 10 times higher than in ileostomy

| TABLE 3. pH and dry weight values for samples from ileostomy effluent and ileal pouch outputa |
|-----------------|-----------------|-----------------|
| Sample          | Median pH (range) | Median amt (mg [dry weight]/g of output (range) |
| Ileostomy       |                  |                  |
| No occlusion    | 7.7 (6.2–7.8)   | 126 (67–188)    |
| Occlusion for 2.5 to 3.5 h | 7.3 (6.9–7.9) | 184 (133–245)   |
| (group II)      |                  |                  |
| Ileal pouch (group III) |                  |                  |
| No occlusion    | 6.6 (6.1–7.0)   | 136 (116–159)   |
| Occlusion for 4 h | 6.6 (6.0–7.5)  | 129 (107–177)   |
| Occlusion for 7 h | 6.5 (5.5–7.2)  | 114 (69–122)    |

a From each dog from group I (n = 5), two samples were investigated 2 and 8 weeks after the initial operation; from each dog from group II (n = 5), two samples were collected after the maximal tolerable occlusion time (5 and 8 weeks after operation); from each dog from group III, one sample was investigated from the nonoccluded pouch (2 weeks after operation; n = 5), from the 4-h-occluded pouch (6 weeks after operation; n = 5), and from the 7-h-occluded pouch (8 weeks after operation; n = 3).

| FIG. 1. Glycosidase activities in ileostomy effluent and output from a nonoccluded ileal poucha |
|-----------------|-----------------|-----------------|
| Enzyme          | Median glycosidase activity (range) | Ileostomy       |
|                 |                  |                 |
|                 |                  | 2.3- to 3.5-h occlusion |
|                 |                  |                  |
| 1. α-L-Fucosidase | 0.09 (ND–0.36) | 0.25 (ND–0.27) | 0.22 (0.13–0.38) |
| 2. α-NAc-D-galactosaminidase | ND | ND | 0.05 (0.01–0.13) |
| 3. β-NAc-D-galactosaminidase | ND | ND | ND (ND–0.21) |
| 4. α-D-Galactosidase | 0.18 (ND–1.00) | 0.82 (0.21–1.46) | 1.44 (1.01–2.87) |
| 5. β-D-Galactosidase | 0.21 (ND–0.96) | 0.24 (0.07–1.53) | 0.93 (0.26–2.23) |
| 6. Neuraminidase | ND | ND | 0.22 (0.20–0.43) |
| 7. β-NAc-D-glucosaminidase | ND | 0.15 (ND–0.22) | 0.56 (0.36–1.07) |
| 8. α-D-Mannosidase | ND (ND–0.10) | ND (ND–0.17) | ND (ND–0.06) |
| 9. B-D-Glucosidase | 0.05 (ND–0.27) | 0.15 (ND–0.34) | 0.14 (0.11–0.37) |
| 10. B-D-Glucuronidase | ND | ND (ND–0.05) | 0.26 (0.21–0.54) |

a From each dog from group I (n = 5), two samples were investigated 2 and 8 weeks after the initial operation; from each dog from group II (n = 5), two samples were collected after the maximal tolerable occlusion time (5 and 8 weeks after operation); from each dog from group III (n = 5), one sample was investigated from the nonoccluded pouch (2 weeks after operation).

b All glycosidase activities were measured as micromoles of p-nitrophenol released per minute (units) per gram (dry weight), except for neuraminidase which was measured as micromoles of neuraminic acid per minute (units) per gram (dry weight). ND, not detected (<0.01 U).

c Statistically different from values obtained for groups I and II (P < 0.05).
TABLE 5. Blood group antigenicity of intestinal glycoproteins from ileostomy effluent and from ileal pouch output

<table>
<thead>
<tr>
<th>Blood group antigen</th>
<th>Ileostomy</th>
<th>Ileal pouch (group III)</th>
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<tbody>
<tr>
<td></td>
<td>Group I. 0 h</td>
<td>Group II. 2.5–3.5 h</td>
</tr>
<tr>
<td>A</td>
<td>2^{10} (2^{10–2^{11}})</td>
<td>2^{10} (2^{10–2^{12}})</td>
</tr>
<tr>
<td>H</td>
<td>2^9 (2^{9–2^{10}})</td>
<td>2^{10} (2^{9–2^{11}})</td>
</tr>
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</table>

* Intestinal glycoproteins were prepared from fecal supernatants, as described in Materials and Methods. From each dog from group I (n = 5), two samples were investigated 2 and 8 weeks after the initial operation; from each dog from group II (n = 5), two samples were collected after maximal tolerable occlusion time (5 and 8 weeks after operation); from each dog from group III, two samples were investigated from the nonoccluded pouch (2 weeks after operation; n = 5) and from the 4-h-occluded pouch (6 weeks after operation; n = 5) and from the 7-h-occluded pouch (8 weeks after operation; n = 3). The hemagglutination inhibition titer was defined as the reciprocal value of the highest dilution that completely inhibited hemagglutination.

...effluent and enzyme activity increased significantly, the intestinal glycoproteins still showed considerable blood group antigenicity. However, after occlusion of the pouch for 4 or 7 h, blood group antigenicity was lost. The enzymes responsible for the removal of the H and A antigenic determinants, α-L-fucosidase and α-D-galactosidase, showed a significant increase of activity on p-nitrophenol substrates. With occlusion of the pouch, a situation of stasis is created and bacteria get a chance to increase. Probably small amounts of simple sugars and disaccharides from the gut that escape absorption by the small intestine are largely sufficient for the ileostomy and the nonoccluded pouch flora to survive, but the flora has to resist food competition by producing glycosidases to split off sugars from dietary and endogenous material as mucous glycoproteins, which was confirmed by the loss of blood group antigens, after occlusion of the pouch. Blood group degrading properties have been described for anaerobes such as *Bacteroides* species, *Bifidobacterium* species (18), *Bacteroides vulgatus* (39) as well as of aerobic, facultative anaerobic intestinal bacteria such as *Shigella flexneri* (33).

In order to find out which species or group of bacteria might be responsible for the increase of glycolytic enzymes, we carefully identified the ileostomy and pouch flora. The composition of the flora that we cultured from the pouch did in fact resemble the fecal flora (3, 5) more than ileal flora of dogs (9). The most striking difference with the fecal flora was the large numbers of fusobacteria in each sample. No single species was found to be responsible for the increase in total numbers after occlusion. But combining species to genera or groups showed an increase in each group except for *Clostridium* species. Although most bacteria were common to the flora of all dogs, each dog had different groups of microorganisms predominant in its flora, which was consistent over time. For all these individual differences in bacterial composition, the activities of enzymes increased significantly in each dog after occlusion. From this result, we concluded that the production of potential mucus-degrading enzymes is probably not restricted to a few species but is a widespread ability among bacteria in the pouch.

Chronic mucosal inflammation occurs in all patients with ileal reservoirs and is not correlated with the functioning of the pouch (30, 42). This is in line with our finding of chronic inflammation and changes in crypt-villus ratio in each of the dogs of group III. It is not very likely that the glycolytic activity we measured is of neutrophilic origin because (i) cellular enzymes are lysosomal or membrane bound and can only be liberated by disruption of the cells and (ii) most of the cellular enzymes show acid activity (27, 31). Kane and Vincenti (19) compared the activities of *N*-acetyl-β-glucosaminidase in neutrophil granulocyte-infiltrated mucosa of patients with ulcerative colitis with activities in noninflamed mucosa; no difference in enzyme activity was found.

By the construction of an ileal pouch, an ecological niche favorable to intestinal anaerobes is introduced in an organ that normally does not harbor numerous fast-growing bacteria. When numbers of bacteria increase, the production of glycolytic enzymes starts in order to supply the bacterial population of monosaccharides. After removal of the blood group determinants L-fucose and D-galactose from the intestinal glycoprotein, other carbohydrates such as N-acetyl-glucosamine, N-acetylgalactosamine, D-mannose, and N-acetylneuraminic acid might be removed by bacterial enzymes. Esposito et al. (10) showed that in vivo treatment of rat jejunal mucosa with a mixture of glycolytic enzymes, β-glucosidase, β-N-acetylglucosaminidase, α-mannosidase, β-glucosidase, sulfatase, and β-glucuronidase resulted in an increased permeability.

Degradation of the mucus may be continued when the protein core of the glycoprotein is no longer protected from action of proteolytic enzymes after the removal of the oligosaccharide side chains (32, 44). Proteolytic activity, likely originating from pancreas, liver, and brush border, was found to be high in pouches as well as in ileostomy effluent. Proteolytic activity in ileostomy effluent of dogs appeared to be the same as in humans (14, 35). Increased inactivation by the more numerous flora might be an explanation for the reduction in proteolytic activity in the ileal pouch compared with ileostomy. An increased degradation of the mucus layer in the ileal pouch by the combined action
of glycosidases and proteases may breach the protection of the underlying mucosa. The epithelial cells will be exposed more intensively to luminal contents, which contains several potential toxic products of bacterial metabolism. This study reveals a considerable activity of β-glucosidase and β-glucuronidase in the pouch. Recent studies showed that these enzymes are involved in the release of potential mutagenic and carcinogenic products from dietary glycosides (26). Considerable bacterial deconjugation of bile acids in ileal pouches has also been recorded; one such secondary bile acid, deoxycholic acid, has toxic properties (29).

After the loss of the mucous layer, the epithelial cells may become accessible to the action of host-derived proteases. It has been postulated that pancreatic proteolytic enzymes have an inhibitory effect on intestinal healing processes (16) and promote progress of hemorrhagic intestinal necrosis (6). Furthermore, the pouch content is a large reservoir of bacterial antigens, which may stimulate the immunological system and cause a local inflammatory reaction, when the integrity of the mucosa is affected.

In conclusion, this study shows that the ileal pouch flora when becoming numerous during stagnation, produces an increasing glycolytic activity which is able to degrade blood group antigen determinants from intestinal mucous glycoproteins. As a result, the protection of the underlying epithelial cells may be impaired and a situation is created in which luminal components may call an inflammatory response.

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