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Extensive Small Gut Resection in the Rat

Microbiological studies with a strict anaerobic technique

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ABSTRACT

The small intestinal microflora of the rat was examined 6 w after resection of the proximal half of the ileum. A comparison was made with animals where the ileum had only been transected and re-sutured. The study included a strict anaerobic culturing and an investigation of bacterial metabolites with a gas chromatography technique. In both groups the flora was found to be mainly facultative and there was no difference in the total number of bacteria. There was no difference between the groups or between the single animals in the proportional of aerobes-anaerobes. In spite of numerous adhesions between the ileal loops causing impaired intestinal motility and intraluminal stasis, there was no colonization of the small intestinal flora and the concentration of bacteria was too low to give a positive response for gas chromatography. It is stated that the low amount of intestinal bacterial flora is not likely to be a significant factor causing adaptive mucosal hyperplasia after gut resection.

INTRODUCTION

The normal small intestine usually harbours a sparse microflora. This situation is maintained unless the physiological and/or morphological integrity of the gastrointestinal tract is deranged (14). Abnormal bacterial growth in the small intestine can thus be seen with achlorhydria. It is also frequently observed in the presence of ileocolic fistulas, after massive intestinal resection, ileocecal resection or by-pass (9) or when the normal function of the gut is otherwise disturbed, e.g. by chronic inflammation. Conditions causing intraluminal stasis within the small intestine, such as narrow enteroanastomoses and adhesions after gut resection, also contribute to an abnormal bacterial growth.

The objective of this study was to evaluate whether a large proximal ileal resection would derange the normal ileal flora, resulting in an increase of faecal microorganisms (14). This would support the theory that mucosal hyperplasia, seen after resection (2,4,5), might have a bacteriological background. A strict anaerobic technique as well as a conventional aerobe technique was

used for culture. A group of resected animals was compared to a control group in which the gut was simply divided and reanastomosed end-to-end.

MATERIAL AND METHODS

Sprague-Dawley male rats weighing approximately 300 grams were used. They were fed a standardized grain diet (Ewos), and fasted one day before operation, but had free access to water.

Ileal resection was performed on 22 animals and 19 animals were used as controls.

Operative procedures

The animals were laparotomized by a mid-line abdominal incision under ether anesthesia. In the experimental group the proximal half of the ileum was resected, i.e. to a point between the second and third vessel arcades from the ileocaecal valve. Anastomosis between proximal and distal resection rims was made with interrupted 5/0 silk sutures.

In the control group the ileum was divided between the second and third vessel archades proximal to the ileocaecal valve and immediately reanastomosed by the technique described above.

In all animals 10 ml of physiological saline was deposited in the abdominal cavity before closure of the abdomen.

The animals were given another 10 ml of physiological saline subcutaneously on the first postoperative day. Free access was given to food and water from the second postoperative day.

The animals were sacrificed after 6 weeks and after one day of starvation. All of the small intestine was removed at this time. The vessel archades of the mesentery were divided as well as any postoperative adhesions, and the gut length distal to the anastomosis was measured.

Quantitative studies of the bacterial flora

The animals were sacrificed in the anaerobe-laboratory. A 15 cm long segment situated 15 cm proximal to the anastomosis was closed at both ends. It was washed through 10 times with 10 ml of PRASS-bouillon (prereduced - anaerobic - sterilized) from a closed, sterile syringe. The syringe was transferred into a glove-box anaerobic chamber. The washed fluid was diluted in 10-fold steps with pre-reduced bouillon. After mixing, 8 serial dilutions were inoculated into fresh blood-agar plates, enriched with hemin and vitamin K (11). The plates were packed in anaerobic jars (Gas-Pack^{R}) which were furnished with gas generators and closed inside the glove box.

The dilution series were thereafter removed from the glove-box. After renewed mixing, the dilution steps were cultured aerobically on blood-agar plates.

Finally, for clostridium studies, the dilution series were heated in a water bath (70^{o}, 10 min) and egg plates were inoculated from the 4 most concentrated dilution steps.

The plates were analyzed 3 days after inoculation. Recognition of the predominant flora was made by colony morphology and Gram stain. Further biochemical subspeciation was not performed.

Gas chromatography

The remaining 15 cm long segment between the removed segment and the anastomosis was washed through with 10 ml of acid physiological saline (water-soluble H_2SO_4 added to pH 2). The wash procedure was performed as previously described. Of the washed-out solution, 2 ml was carefully mixed with 2 ml of ether, and the water-phase was frozen. The ether extract was analyzed for volatile bacterial metabolites, by a gas chromatography technique, in accordance with that used for determination of anaerobic cultures (3).

RESULTS

Four of the resected animals, and 1 of the control animals, died of postoperative peritonitis within the first few days. In the remaining rats there were several adhesions between the ileal loops in the resected group. In the control group postoperative adhesions were few. The mean length of the resected ileum was 49.3 ± 5 cm and the mean length of the remaining ileum was 38.2 ± 6.7 cm.

For bacteriological studies, 18 animals were submitted in each group, but due to technical errors some specimens were excluded (Tables 1-5).

We did not find a significant difference in the flora between resected and non-resected rats. The predominant flora consisted of coliforms, anaerobic G- rods (bacteroides and fusibacterium), aerobic and anaerobic cocci, lactobacilli and other G+ bacilli.

Table 1. Anaerobic quantification of the small intestinal flora in resected and non-resected animals. Bacterial concentration expressed as log 10/ml of wash fluid. n = number of examined animals

Bact. conc. log 10/ml	10^1	2	3	4	5	6	7	n
resected		1	1	2	4	5	4	17
non-resected		1		1	4	6	6	18

Quantification with anaerobic technique showed that there was a variation from log 2 - log 7 in both the resected and non-resected groups. Most animals

had a concentration between log 5 and log 7 and there was no difference between the groups (Table 1). The findings with aerobic technique gave similar results (Table 2). Table 3 shows the anaerobic technique compared to the aerobic technique for resected and non-resected groups.

Table 2. Aerobic quantification of the small intestinal flora in resected and non-resected animals. Bacterial concentration expressed as log 10/ml of wash fluid. n = number of examined animals

Bact. conc. log 10/ml	10^1	2	3	4	5	6	7	n
resected			1	1	4	3	6	15
non-resected			1		4	7	6	18

Table 3. A quantitative comparison between aerobic and anaerobic bacteria in the remaining small intestine 6 w after resection of half the gut. Concentration expressed as log 10/ml of wash fluid. n = number of examined animals

Bact. conc. log 10/ml	10^1	2	3	4	5	6	7	n
aerobic			1	1	4	3	6	15
anaerobic		1	1	2	4	5	4	17

A comparison of the bacterial concentrations in resected and non-resected animals gave a good correlation between aerobic and anaerobic growth in the two groups. respectively (Figs. 1 + 2). As the concentrations of total anaerobes and aerobes (coliforms) were similar, it suggests that the microflora was mainly facultative.

The concentration of lecitinase-positive Clostridias are given in Table 5.

Table 4. A quantitative comparison between aerobic and anaerobic bacteria in the small intestine 6 w after transection of the gut without resection. Concentration expressed as log 10/ml of wash fluid.

Bact. conc. log 10/ml	10^1	2	3	4	5	6	7	n
aerobic			1		4	7	6	18
anaerobic		1		1	4	6	6	18

Table 5. The concentration of lecitinase positive Clostridias in the resected and non-resected small intestine of the rat 6 w after operation. Concentration given as log 10/ml of wash fluid. n = number of examined animals

Bact. conc.					
log 10/ml	10^1	2	3	4	n
resected		3	3	3	9
non-resected	6	1	1	4	12

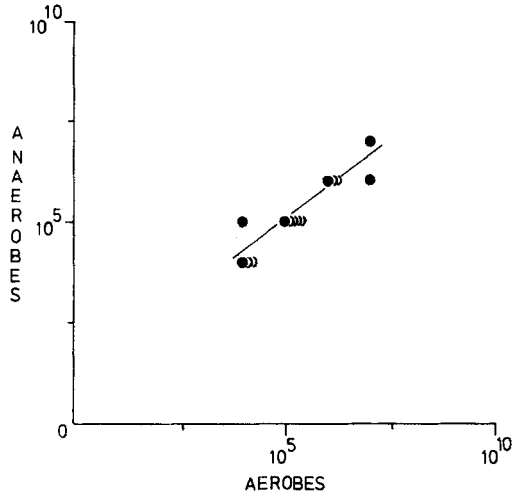

Fig. 1. An individual comparison of aerobic and anaerobic bacterial growth of the small intestinal flora 6 w after transection of the ileum. Bacterial concentration = log 10/ml of wash fluid. n = 14, Y = 0.791 X + 1.083, r = 0.93

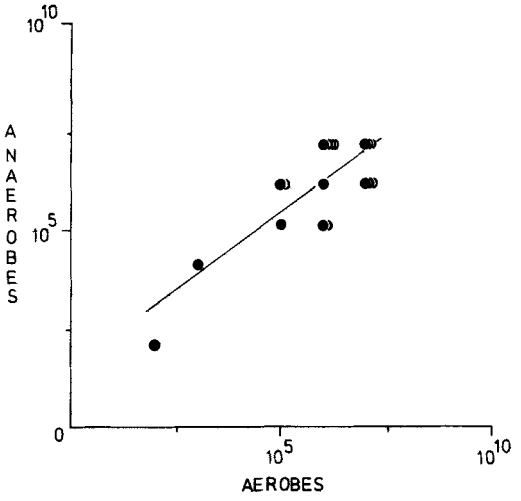

Fig. 2. An individual comparison of aerobic and anaerobic bacterial growth of the small intestinal flora 6 w after transection of the ileum. Bacterial concentration = log 10/ml of wash fluid. n = 18, Y = 0.771 X + 1.429, r = 0.81

Gas chromatography

In a few cases within the two groups, peaks were found for acetic acid and propionic acid, but not frequently enough for statistical evaluation. Analyses of colonic content for controls gave characteristic peaks for acetic acid, propionic acid, isobutyric acid, butyric acid, isovalerianic acid and valerianic acid.

DISCUSSION

It has been found that an adaptive hyperplasia occurs in the remaining small bowel after intestinal resection (4). The etiology for this hyperplasia is still unknown. Humoral, as well as intraluminal factors, have been discussed (2,5,6,8,16,17). The character of the intraluminal environment of the intestine might reasonably be expected to influence the life cycle of mucosal cells and the bacterial flora is a significant component of this environment (1).

It has been stated (13) that the usual mucosa can be described as one of physiological inflammation. With this in mind, we wanted to investigate whether there was a difference between resected and transected gut, concerning the microflora. Because the main interest was focused on an explanation for adaptive hyperplasia we did not study non-operated rats.

The number of total anaerobes and coliforms found was low in both resected and non-resected rats and corroborates the findings by Drasar et al. (7). The one day of fasting before sacrificing may quantitatively have influenced the results of both groups (1). The concentration was obviously not high enough to be accurately estimated by the gas chromatography analyses. A low concentration of bacteria was found in spite of fastidious bacteriological technique. These findings are in accordance with Weinstein et al. (15), whose results were obtained from studies on rats which were also fed a grain diet. With meat-fed rats, a higher concentration of anaerobes would possibly have been found.

In conclusion, resection of half the length of the ileum in the rat did not change the ileal microbial flora, as compared to rats where the ileum was simply divided and reanastomosed. The findings suggest that the bacterial flora does not significantly contribute to the adaptive small intestinal hyperplasia seen after resection.

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