Effect of Antibiotics on Bacterial Flora of Rats with Intestinal Blind Loops[1] (34149)

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The blind-loop syndrome is characterized by macrocytic anemia and/or steatorrhea associated with overgrowth of bacteria in the upper part of the intestinal tract (1). In man the syndrome has been documented in a variety of conditions such as jejunal diverticulosis, surgical blind loops, strictures, enteric fistulas, poorly functioning gastroenterostomies, and scleroderma (1). Although the syndrome was first produced experimentally in dogs in 1924 (2), more extensive experimental work followed the description of macrocytic anemia in rats with surgically created self-filling blind loops in the upper small intestine reported by Watson *et al.* (3) in 1948. In 1950 Aitken *et al.* (4) reported the occurrence of steatorrhea in the rat experimental model, and this was confirmed by later investigators (5–7). Studies of the flora in rats with blind loops have been limited in detail and quality. In 1963 Bishop (8) reported a profuse flora in rat blind loops but a normal flora proximal and distal to the loop, and Panish (5) found overgrowth in the blind loop in all animals, proximal to the loop in 3 of 5 animals, and a sterile small intestine distal to the loop. Hoet and Eyssen (6) reporting preliminary results of bacteriological findings in 1964 found a profuse flora in the blind loop and in the small intestine distal to it. Bacteroides were not recognized as a prominent component of the flora in these studies. In 1967 Donaldson (1) in a review paper presented quantitative counts of jejunal clutures in blind-loop rats which included consistently high counts of *Escherichia coli*, lactobacilli, anaerobic streptococci, and bacteroides. Bacterial overgrowth has been implicated as the cause of the streatorrhea in rats by demonstration of decreased fecal fat excretion following treatment with tetracycline (5, 6), virginiamycin (6), and neomycin (7), but the effect of the antibiotics on the rat flora has not been reported.

The purpose of this study was to carefully quantitate the flora in self-filling blind loops, small intestine, and cecum of rats, and to determine the changes in the flora after treatment with antibiotics in hopes of determining the types of bacteria responsible for the steatorrhea.

Materials and Methods. Male Simonsen strain rats weighting 200–310 g were housed individually and allowed rat chow and water *ad libitum*. Eight-cm long self-filling blind loops were created 20 cm distal to the ligament of Treitz by the method of Cameron *et al.* (9). The rats were weighed weekly. During the ninth week after operation one of each pair of blind loop animals was treated with antibiotics and the other received saline intragastrically. The two antibiotic regimens used were (i) neomycin sulfate, 20 mg; polymyxin B sulfate, 2 mg; and bacitracin 1000 units intragastrically twice daily with neomycin sulfate, 1.3 g/liter; polymyxin B sulfate, 60 mg/liter; and bacitracin, 30,000 units/liter in the drinking water; and (ii) lincomycin hydrochloride monohydrate, 25 mg intragastrically twice daily; and 500 mg/liter in the drinking water. Normal rats from the same shipments were used to compare growth rate and to determine the normal flora. Four day fat balance studies were done on blind-loop rats during the week prior to treatment and during the last 4 days of the treatment period. The rat chow contained

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4.1% fat. Food and feces were analyzed for total fat by the method of Saxon (10) and fecal fat excretion was expressed as percentage of intake.

After the 7-day treatment period the animals were killed by cervical dislocation. One-tenth-ml samples of blind loop and cecal content and a 12-cm segment of mid-small intestine below the loop were diluted 1:10 with sterile saline and ground using a sterile mortar and pestle. One-tenth-ml sample of 10-fold serial dilutions were spread on agar plates using a sterile bent glass rod. Media and incubation conditions were similar to those in our previous reports (11, 12). Blood agar, Sabouraud agar, and MacConkey agar plates were incubated aerobically at 37°F; SF agar, aerobically at 45°F; and blood agar, kanamycin–vancomycin blood agar, and lactobacillus agar, anaerobically at 37°F.

Intestinal content, 0.3 ml, or colonies of selected organisms were innoculated into 10 ml of thioglycolate broth containing 10 mg of glycocholic acid and incubated for 4 or 24 hr at 37°F, and then frozen. Purity of the glycocholic acid was determined by gas liquid chromatography. A single cholic acid peak was recorded when the trifluoracetate of the saponified methylated acid was injected on to a QF-1 column. Control tubes contained broth and glycocholic acid and were either incubated without organisms or contained organisms and were not incubated. After thawing and extraction with chloroform-methanol at pH 2, free and conjugated cholic acid were separated using thin-layer chromatography by the method of Hofmann (13), developed using 15% phosphomolybdic acid, photographed, and the amount of deconjugation was estimated. Results were analyzed statistically by the Student's t test or Fisher's exact test.

Results. The weight of blind loop rats began to stabilize or drop 4 weeks after operation resulting in a net weight gain of 6 ± 32 (SD) g between the fourth and seventh weeks compared to a gain of 47 ± 17 g for normal rats ($p>0.001$). During the 1-week treatment period neomycin–polymyxin–bacitracin (NPB)-treated rats gained 27 ± 14 g compared to 3 ± 4 g for paired untreated blind-loop rats ($p<.05$ and lincomycin-treated rats gained 31 ± 21 g compared to −6 ± 3 g for paired untreated blind loop rats ($p<.05$). Fecal fat, expressed as percentage of intake, fell from 23 ± 9% before treatment to 11 ± 3% after treatment with NPB. This was not significant ($p<0.2$), probably because of the small number of animals. One blind-loop rat treated with lincomycin had a normal growth curve, and fecal fat did not fall with treatment. Fecal fat in the other 4 animals fell from pretreatment levels of 15 ± 5% to 8 ± 2% after treatment ($p<0.01$).

Table I presents the flora changes before and after treatment in blind-loop rats compared to controls. The mid-small intestinal and cecal flora in normal rats is similar to that in our previous reports (11, 12). The striking change in blind-loop rats was the increase in *E. coli* and bacteroides counts in the small intestine and cecum. The flora in the blind loop itself was indistinguishable, qualitatively and quantitatively, from that in the small intestine and cecum. Neither NPB or lincomycin significantly reduced the numbers of *E. coli* in the blind loop or small intestine, but NPB reduced or eliminated them from the cecum. Both treatments reduced bacteroides counts in the blind loop and small intestine; lincomycin eliminated them from the cecum, but not always from the loop or small intestine, while NPB eliminated them from the loop and small intestine, but not the cecum. NPB eliminated enterococci from all three sites and lactobacilli from the loop and small intestine. Lincomycin had an inconstant effect on lactobacilli. The significantly higher yeast counts in the cecum of treated rats may be artifactual, as yeasts are difficult to culture from the untreated animals because of overgrowth of other organisms. Other organisms occasionally cultured from blind-loop rats included clostridia, anaerobic streptococci, α-hemolytic streptococci and pseudomonads. Micrococci, staphylococci, and α-hemolytic streptococci were frequently isolated from normal rats, and pasteurella, herellea and neisseria were isolated from some animals.

Incubation of blind-loop content from un-

TABLE I. Cultures in Normal Rats and Blind-Loop Rats Treated with Saline, Neomycin–Polymyxin–Bacitracin or Lincomycin.

Site and organism	Mean[f] $\log_{10} \pm$ SD (rats with positive culture/total)			
	Normal rats; saline	Blind-loop rats; saline	Blind-loop rats; neomycin–polymyxin–bacitracin	Blind-loop rats; lincomycin
Blind loop				
E. coli		7.1 ± 1.2 (6/6)[a]	7.2 ± 0.3 (2/3)	7.8 ± 1.2 (5/5)
Proteus		5.5 ± 1.7 (5/6)	3.6 (1/3)	4.5 ± 0.7 (2/5)
Lactobacilli		6.9 ± 1.5 (6/6)	(0/3)[c]	6.7 ± 3.3 (2/5)
Bacteroides		9.0 ± 0.8 (6/6)[ab]	(0/3)[c]	5.8 ± 1.8 (2/5)[e]
Enterococci		4.6 ± 1.3 (5/6)	(0/3)[c]	5.3 ± 2.6 (3/5)
Yeasts		5.4 ± 2.1 (2/6)	7.2 ± 0.2 (3/3)	6.7 ± 1.1 (5/5)
Mid-small intestine				
E. coli	4.3 ± 2.1 (4/8)	6.8 ± 1.1 (6/6)[a]	6.4 ± 1.8 (2/3)	7.5 ± 0.9 (5/5)
Proteus	3.7 ± 1.0 (3/8)	5.5 ± 2.2 (4/6)	4.4 (1/3)	3.1 ± 1.1 (2/5)
Lactobacilli	7.2 ± 1.5 (8/8)	7.8 ± 0.9 (6/6)	(0/3)[c]	4.7 ± 1.1 (2/5)[e]
Bacteroides	6.5 ± 1.0 (2/8)	7.8 ± 0.9 (6/6)[ab]	(0/3)[c]	4.5 ± 0.4 (2/5)[e]
Enterococci	3.5 ± 0.4 (5/8)	4.0 ± 1.3 (6/6)	(0/3)[c]	4.9 ± 2.6 (4/5)
Yeasts	6.5 ± 0.9 (7/8)	6.5 ± 0.5 (4/6)	7.1 ± 0.9 (3/3)	7.1 ± 1.1 (5/5)
Cecum				
E. coli	5.0 ± 1.1 (8/8)	7.3 ± 0.6 (6/6)[a]	3.7 (1/3)	7.6 ± 0.6 (5/5)
Proteus	4.6 ± 0.8 (6/8)	5.5 ± 1.3 (5/6)	7.3 (1/3)	5.0 ± 1.4 (2/5)
Lactobacilli	8.4 ± 0.8 (8/8)	8.1 ± 0.4 (6/6)	7.7 ± 0.6 (2/3)	8.9 ± 0.9 (3/5)
Bacteroides	6.2 ± 1.5 (5/8)	9.3 ± 0.3 (6/6)[ab]	9.2 ± 0.2 (2/3)	(0/5)[c]
Enterococci	5.2 ± 0.4 (8/8)	5.2 ± 1.6 (6/6)	(0/3)[c]	4.9 ± 2.0 (4/5)
Yeasts	6.0 ± 0.6 (7/8)	5.7 ± 0.8 (3/6)	7.4 ± 0.2 (3/3)[d]	6.6 ± 0.5 (5/5)[d]

[a] Mean greater than normal ($p < 0.05$).

[b] Frequency of positive cultures greater than normal ($p < 0.05$).

[c] Frequency less than blind loop without antibiotics ($p < 0.05$).

[d] Mean greater than blind loop without antibiotics ($p < 0.05$).

[e] Mean less than blind loop without antibiotics ($p < 0.05$).

[f] Mean $\log_{10}$ per g of blind loop or cecal content or 12-cm length of small intestine based on those rats with positive cultures.

treated rats with glycocholic acid produced strong deconjugation in 3 of 4 instances; whereas, blind-loop content from treated rats produced moderate deconjugation in one instance and little or none in two instances. Small-intestinal content from normal rats produced little or no deconjugation in 3 instances. Bacteroides organisms isolated from untreated animals produced strong to moderate deconjugation in 7 of 9 instances where, *E. coli* produce only moderate deconjugation in 1 of 7 instances.

Discussion. In this study we found that *E. coli* and bacteroides, but not other organisms, were present in significantly greater numbers in rat self-filling upper small intestinal blind loops compared to the normal small intestinal content. In blind-loop rats these same organisms were significantly increased in the small intestine and cecum. Treatment with either neomycin–polymyxin–bacitracin or lincomycin resulted in a significant weight gain compared to controls, reduced or eliminated bacteroides but did not alter *E. coli* counts in the blind loop and small intestine, and reduced the ability of blind-loop content to deconjugate glycocholic acid *in vitro*. Cultural isolates of bacteroides produced more deconjugation of glycocholic acid than did *E. coli*.

These findings suggest that bacteroides are the principal organisms responsible for the

steatorrhea in the blind-loop syndrome of the rat. Early cultural studies in rat blind loops failed to recognize bacteroides as a principal component of the bacterial overgrowth (5, 6, 8). The findings of Donaldson (1), however, closely approximate ours. Although previous studies have demonstrated reduced fecal fat excretion following antibiotic treatment of rats with blind loops (5–7), the effect of the antibiotics on the composition of the flora has not been previously demonstrated.

Although our findings suggest that bacteroides is the most important organism in the rat blind-loop syndrome, this does not fit with the finding of Donaldson (7) that neomycin reduces fecal fat excretion in rats with blind loops. Neomycin is not effective against bacteriodes, and, in fact, is often used in selective media for these organisms. In preliminary experiments with four blind-loop rats we found that neomycin did not alter bacteroides counts in the blind loop and resulted in weight gain in two rats and weight loss in the other two. We chose neomycin–polymyxin–bacitracin because we had previously found this combination effective in preventing bacterial overgrowth in the small intestine (11, 12). It is interesting that this combination eliminates bacteroides from the blind loop, and not the cecum, while *E. coli* are reduced in the cecum and not in the blind loop. The effect of this combination on the cecal flora is substantiated by our previous study (11). Lincomycin had a much more selective effect than neomycin–polymyxin–bacitracin. It reduced or eliminated bacteroides and to a lesser extent lactobacilli. Lincomycin was more effective against bacteroides in the cecum. Lincomycin is partially absorbed by rat small intestine, but nearly half of an oral dose reaches the colon by 4 hr and remains in high concentration for at least 12 hr (14). It is disturbing that we were not able to recover bacteroides from the cecum of all normal rats in this and our previous report (11), but the fact that they were easily recovered in all untreated blind-loop rats cultured under the same conditions suggests that this is more than a methodological error.

In humans, only recently, with the advent of more careful culture techniques, have bacteroides been recognized as important organisms in the human blind-loop syndrome (15). In some patients with the blind-loop syndrome coliform counts have remained unchanged in the small intestine after antibiotic treatment in spite of reduction in fecal fat excretion (16, 17). Hill and Drasar (18) found that bacteroides and other anaerobic bacteria isolated from the human intestinal tract deconjugated bile salts *in vitro* and Rosenburg *et al.* (17) found that mixed bacterial cultures from jejunal fluid deconjugated bile salts *in vitro*. The demonstration of free bile acids in the small intestine of rats (7), dogs (19), and man (7, 17, 20, 21) with the blind-loop syndrome further supports the concept that alteration of bile salts is important in producing steatorrhea. The reduction of fecal fat excretion in dogs (19) and man (21) with the blind-loop syndrome by orally administered sodium taurocholate and the failure to demonstrate morphologic damage to the intestinal mucosa by electron microscopy in rats (22) and man (21) with the syndrome suggests that defective micelle formation is more important than a toxic effect of altered bile salts in producing the steatorrhea.

Summary. Rats with weight loss resulting from surgically created self-filling upper small intestinal blind loops had increased numbers of *Escherichia coli* and bacteroides in the blind loop, mid-small intestine, and cecum. Counts of other organisms were not significantly elevated. Treatment with neomycin–polymyxin–bacitracin or lincomycin resulted in weight gain, reduced fecal fat excretion, and reduction of bacteroides but not *E. coli* counts in the blind loop and small intestine. Mixed cultures from blind loops of untreated rats and isolates of bacteroides deconjugated glycocholic acid *in vitro*, while mixed cultures from blind loops of treated rats or small intestine of normal rats and isolates of *E. coli* did not. The findings suggest that bacteroides are the important organisms producing steatorrhea in the rat blind-loop syndrome.

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