SHORT ARTICLES

EFFECTS OF BILIARY DIVERSION ON INTESTINAL MICROFLORA IN THE RAT

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IN man, the upper small bowel is normally relatively sterile. Factors limiting growth of bacteria in the jejunum are complex and include gastric acidity (Drasar, Shiner and McLeod, 1969), intestinal motility (Dixon, 1960) and the ileo-caecal valve which limits contamination by organisms from the large bowel (Griffen, Richardson and Medley, 1971). Mucus (Goldsworthy and Florey, 1936), products of bacterial metabolism (Meynell, 1963) and immunoglobulins (Brown *et al.*, 1972) probably also contribute to the control of intestinal flora. In 1947, Stacey and Webb reported the antibacterial properties of bile acids and this observation has now been confirmed by the use of bacteriological techniques adequate for the culture of anaerobes (Floch *et al.*, 1971). That study showed the growth of intestinal anaerobic bacteria to be inhibited *in vitro* by unconjugated bile acids; the authors suggested that this might be a further mechanism limiting the growth of intestinal bacteria but presented no evidence of its role in the intact animal.

The present investigation reports the effects of biliary diversion on gastrointestinal flora of the rat.

MATERIALS AND METHODS

Adult Wistar rats weighing c. 200 g were fed a standard laboratory diet. They were caged on wire mesh to minimise coprophagy, and were fasted overnight before samples for bacteriological studies were obtained.

Surgical procedure. Under open ether anaesthesia, the bile duct was cannulated with polyethylene tubing [(PE 10, Intramedic (R), USA: internal diameter 0.28 mm, external diameter 0.61 mm)] 0.5 cm distal to the bifurcation of the hepatic ducts. A piece of soft polyvinyl tubing c. 1.5 cm long (internal diameter 0.33 mm, external diameter 0.98 mm) was tied to the distal end of this tubing and introduced into the urinary bladder through a small incision which was then closed with a purse-string suture. Pancreatic flow was not obstructed by this procedure. The animals were allowed food and water *ad libitum* after operation. They are referred to as bile-fistula animals.

Two types of control animals were used. Both were "sham-operated"; that is, a cannula was placed in the peritoneal cavity under open-ether anaesthesia. The first group had unlimited access to the laboratory diet; the diet of the second group was restricted to the same amount of food as was eaten by the bile-fistula animals in each experimental group.

After 5 days of dietary restriction, or 5 days after operation, the rats were fasted overnight and then killed with anaesthetic ether. Intestinal content (0.2 ml) was withdrawn from stomach, mid-jejunum and caecum, and each sample was added to 1.8 ml of sterile saline.

Microbiological methods. After serial 10-fold dilution, specimens were plated on horse-blood agar (aerobic and anaerobic), MacConkey's agar, Sabouraud's dextrose agar and the medium of de Man, Rogosa and Sharpe (1960). Strict anaerobic conditions were achieved

Received 29 July 1976; accepted 10 Sept. 1976.

TABLE I
*Organisms isolated from stomach**

Organisms	Mean log ₁₀ number of colonies per ml (and confidence limits of the counts) from		
	controls	pair-fed controls	bile-fistula rats
Coliform bacilli	1.26 (0.06, 2.46)	5.01† (3.36, 6.66)	2.59‡ (0.34, 4.84)
Lactobacilli	0.87 (0.10, 1.64)	4.01† (2.05, 5.97)	3.45‡ (1.07, 5.83)
<i>Proteus</i> sp.	0.57 (0.01, 1.13)	3.33‡ (0.39, 6.27)	1.45‡ (0.05, 2.85)
Total anaerobes	7.08 (6.05, 8.11)	5.75 (4.11, 7.39)	5.59 (4.47, 6.71)
Staphylococci	3.35 (1.94, 5.16)	4.05 (2.05, 6.05)	5.02 (4.37, 5.67)
Total organisms	5.32 (5.03, 5.61)	6.32 (4.61, 8.03)	5.66 (4.95, 6.37)

* Results are expressed as logarithmic means, with lower (L1) and upper (L2) confidence limits at the 5% level shown in brackets; $n = 6$ in each category.

† $2\alpha = 0.01$ in comparison with the control group.

‡ $2\alpha = 0.01$ for difference between the pair-fed and the bile-fistula group.

TABLE II
*Organisms isolated from mid-jejunum**

Organisms	Mean log ₁₀ number of colonies per ml (and confidence limits of the counts) from		
	controls	pair-fed controls	bile-fistula rats
Coliform bacilli	0	3.91† (1.53, 6.29)	2.42 (0.26, 4.58)
Lactobacilli	1.56 (0.24, 2.89)	4.82† (2.19, 7.45)	1.28‡ (0.04, 2.52)
<i>Proteus</i> sp.	1.82 (0.50, 3.12)	2.25† (0.04, 4.46)	5.71† (5.42, 6.00)
Total anaerobes	5.60 (4.70, 6.50)	3.66 (1.93, 5.39)	4.71 (3.68, 5.74)
Staphylococci	3.28 (1.97, 4.59)	2.16 (0.27, 4.05)	4.59 (3.98, 5.20)
Total organisms	4.70 (4.20, 5.20)	6.13 (4.41, 7.85)	5.72 (5.28, 6.16)

* See footnote to table I.

TABLE III
*Organisms isolated from caecum**

Organisms	Mean log ₁₀ number of colonies per ml (and confidence limits of counts) from		
	controls	pair-fed controls	bile-fistula rats
Coliform bacilli	5.63 (4.67, 6.59)	7.19 (5.50, 8.88)	6.26 (5.66, 6.86)
Lactobacilli	7.59 (7.09, 8.09)	4.86 (2.13, 7.59)	5.97 (3.69, 8.35)
<i>Proteus</i> sp.	1.83 (0.99, 2.67)	4.15† (1.30, 6.96)	4.85† (2.70, 7.00)
Total anaerobes	6.32 (5.60, 8.22)	6.23 (5.41, 7.05)	6.66 (5.96, 7.36)
Staphylococci	5.48 (3.30, 7.66)	5.14 (3.96, 7.32)	6.03 (5.63, 6.43)
Total organisms	8.27 (7.82, 8.72)	8.18 (7.22, 9.14)	7.47 (7.24, 7.70)

* See footnote to table I.

by repeated evacuation of a modified McIntosh and Fildes Jar (Baird and Tatlock, London) containing a cold catalyst; the flushing gas was dry hydrogen. The hydrogen was then pumped out and replaced with hydrogen plus 10% carbon dioxide and the carbon dioxide concentration maintained throughout the culture period by a culture of coliform bacilli growing in glucose broth in an open-ended container in the jar. The adequacy of the anaerobic environment was checked by including a plate seeded with *Clostridium tetani* in each anaerobic jar. Anaerobic blood-agar plates were incubated at 37°C for 6 days. Organisms so isolated were subcultured on aerobic blood-agar plates to exclude facultative anaerobes.

Expression of results. Results are calculated as mean colony counts per ml of intestinal content. Results are given as the logarithmic mean with upper and lower limits at the 5% level of confidence. Differences between populations were assessed with Wilcoxon's rank sum method by a 2-tailed test; $2\alpha = 0.01$ was considered significant.

RESULTS

Data are given only for anaerobes and organisms in which changes were found or have been reported by others (Sacquet, Raibaud and Garnier, 1971). Anaerobic organisms were identified but, since no changes were found to be limited to specific types of anaerobes, results are given as the total number of anaerobes isolated from each specimen.

Mean daily intake of food in bile-fistula animals was only 9 g per rat during the 5 days after operation, in comparison with 15 g in normal rats. The pair-fed controls were therefore semi-starved animals.

In the stomach (table I), numbers of *Proteus* sp., coliform bacilli and lactobacilli were increased in bile-fistula and pair-fed controls in comparison with control rats fed an unrestricted diet. There was a significantly greater increase in numbers of *Proteus* sp. in pair-fed controls than in bile-fistula animals. A similar trend was found with coliform bacilli but this was not statistically significant. The number of anaerobes, staphylococci and total organisms was unchanged.

In the mid-jejunum (table II), numbers of *Proteus* sp. and coliform bacilli were increased in bile-fistula and pair-fed animals. In pair-fed animals, lactobacilli were increased; a decrease in numbers of staphylococci was not statistically significant.

In the caecum (table III), only the numbers of *Proteus* sp. were increased both in bile-fistula and pair-fed animals. There was no change in numbers of coliform bacilli, lactobacilli, staphylococci, anaerobes or total organisms.

DISCUSSION

Sham-operated control rats fed an unrestricted diet had intestinal flora similar to normal rats studied in this laboratory. There were changes in numbers of *Proteus* sp., lactobacilli and coliform bacilli in both bile-fistula rats and pair-fed controls and there were only minor differences between these two groups of animals. Pair-fed animals had greater numbers of *Proteus* sp. in the stomach and lactobacilli in the mid-jejunum with some decrease in numbers of staphylococci at the same level in comparison with bile-fistula rats. The inverse relationship between numbers of lactobacilli and staphylococci has previously been noted (Sacquet *et al.*, 1971). Coliform bacilli also tended to be more numerous in the pair-fed animals, but the difference between this group and bile-fistula animals was not significant. The trend to higher numbers of these faecal organisms in the semi-starved controls may be explained simply by more ingestion of faeces in these animals. Caging on mesh does not completely prevent coprophagy, and these control animals were hungry in contrast to the bile-fistula group in which appetite was very poor.

Although bile acids have antibacterial effects *in vitro* (Floch *et al.*, 1971), this investigation shows that proliferation of intestinal flora does not follow diversion of bile from the intestine in the rat *in vivo*. In particular, anaerobic organisms were unaffected. The pattern of changed intestinal flora in bile-fistula rats is almost identical with that seen in pair-fed

control animals. The findings do not suggest that deficiency of bile alone modifies the intestinal flora.

Sacquet *et al.* (1971) studied intestinal flora in rats 3 to 12 weeks after diversion of bile into the urinary bladder. Their results showed that the number of coliform bacilli was increased in the stomach, small intestine and caecum and of staphylococci in the small intestine and caecum. The total number of organisms was decreased in the stomach but unchanged in the small intestine and caecum. The authors suggested that this may be evidence of an effect of bile on intestinal flora. As the food intake of the animals and the way in which they were caged was not mentioned, it is difficult to comment on these results in relation to our own findings. Without this information it is not possible to decide whether an increase in coliform bacilli after 3–12 weeks of biliary diversion is caused by lack of bile or whether the increase is related to other factors, such as poor nutrition or coprophagy.

Our results are consistent with those of Williams, Showalter and Kern (1975) who found no relation between intraluminal bile-salt levels and numbers of intestinal anaerobes in man.

SUMMARY

Certain bile acids inhibit the growth *in vitro* of organisms commonly found in the intestine and, in particular, anaerobic bacteria. As the importance of this effect *in vivo* has not been demonstrated, we investigated changes in intestinal flora in the rat after diversion of bile to the urinary bladder.

Five days after operation there was an increase in numbers of *Proteus* sp. in the stomach, mid-jejunum and caecum and of coliform bacilli in the stomach and mid-jejunum. Lactobacilli were increased in the stomach. There was no change in numbers of anaerobic organisms. Similar changes in bacterial flora followed 5 days of pair feeding in control rats. Our results do not suggest a specific antibacterial effect of bile salts in the rat *in vivo*. The changes found are probably related to semi-starvation, because the food intake of the rats after operation was about half that of control rats having an unrestricted diet.

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