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The Effect of Bile and of Sodium Taurocholate on the Epithelial Cell Dynamics of the Rat Small Intestine (38943)

C. C. ROY, G. LAURENDEAU, G. DOYON, L. CHARTRAND, AND M. R. RIVEST
(Introduced by G. Jasmin)

Department of Pediatrics, Hôpital Sainte-Justine and the University of Montreal, Montreal, Canada

In animals and man, the small intestinal mucosa maintains a constant morphological appearance despite a very fast renewal rate. This is accomplished through the interplay of a basic dynamic process with a number of factors regulating enterocyte proliferation, differentiation, and loss. Physical trauma (1), gut flora (2), hormones (3), vitamin D (4), the state of nutrition (5), and the pattern of feeding (6) are some of the factors known to influence epithelial cell kinetics in the small intestine.

During the past decade, a large body of information has become available on the chemistry, physiology, and metabolism of bile and bile acids. However, their influence on intestinal mucosal cell turnover remains unexplored except for two reports showing mucosal hyperplasia following bile duct implantation into isolated ileal segments of rats (7) and increased turnover of ileal mucosal cells by cholic acid feeding in germ free mice (8). This study explores the regulatory role of bile and bile acids on epithelial cell dynamics in bile fistula rats after 2 days of biliary diversion and under a constant duodenal perfusion of Na taurocholate started with the creation of the bile fistula.

Material and Methods. [^{3}]*thymidine incorporation into DNA.* A bile fistula was established by inserting a cannula (Clay Adams PE-10) in the common duct 5 mm distal to the bifurcation of the hepatic ducts in 10 male Sprague–Dawley rats weighing between 150 and 200 g. A similar number of animals were sham operated. The animals were placed in restraining cages and offered a 5% sucrose solution containing 40 and 20 mEq/liter of Na and K, respectively. After 48 hr, [^{3}H]thymidine (New England Nuclear, 6.7 Ci/mmole) was injected iv at a dose of 1 μCi/g body wt. One hour later, the animals were sacrificed. Sections for autoradiography were taken 1 cm distal to the ligament of Treitz and 2 cm proximal to the ileocecal junction. After fixation in 10% formol–saline, the tissue embedded in paraffin, was cut at a thickness of 2.5 μm, colored with the standard Feulgen method, dipped in Eastman Kodak NTB_2 emulsion, and kept for 48 hr at 4° in a light tight box under a nitrogen atmosphere. The slides were then developed in Eastman Kodak D-170, fixed in hypo, washed in distilled water, and mounted. Background grains were negligible. If a cell had four grains or more over its nucleus, it was counted as labeled. The cellularity of crypts and the labeling index was calculated under oil immersion in eight crypts from each of two sections taken from the proximal and distal small intestine. The rest of the small intestine was rapidly removed and measured by attaching a constant weight to its distal end. Its mucosa was removed with a glass slide and weighed. The protein concentration was determined by the method of Lowry (9). The DNA content of the mucosa was extracted with cold TCA and ethanol at room temperature followed by hydrolysis in hot TCA according to the method of Schneider (10). The DNA was then estimated by the colorimetric method of Burton (11) as modified by Croft and Lubran (12). Highly polymerized calf thymus DNA (Sigma type 1, organic phosphorus content 6.1%) was used as standard. [^{3}H]thymidine activity in aliquots of DNA extracts was expressed as cpm $\times$ 10^3/mg of mucosal DNA.

DNA loss by cell exfoliation. Two groups of eight animals were studied 48 hr after a biliary diversion or a sham operation. Segments of polyvinyl chloride tubing 8F and 10F were tied separately at the duodenojejunal flexure and at the terminal ileum. The small intestine with the cannulae was returned to the peritoneal cavity and normal saline, warmed to 37°, was infused through

TABLE I. MORPHOLOGICAL FINDINGS AND PROTEIN CONTENT OF THE SMALL INTESTINE OF CONTROL AND BILE FISTULA RATS[a].

Observation	Controls	Bile fistula	*P* value
Initial weight (g)	163.8 ± 4.2	169.2 ± 3.9	NS[b]
Weight loss (%)	8.2 ± 1.0	8.3 ± 1.4	NS
Intestinal length (cm)	117.1 ± 2.4	118.8 ± 2.2	NS
Weight of the mucosa (g)	2.1 ± .1	2.3 ± .06	NS
Proteins (mg/g of mucosa)	99.7 ± 4.5	109.0 ± 5.7	NS

[a] Results correspond to mean ± SE in two groups of ten animals studied 48 hr after biliary diversion or a sham operation.

[b] NS, not significant.

the proximal cannula at a rate of 2 ml/min for a period of 120 min following the technique described by Loehry *et al.* (13). The washings, collected in a polycarbonate bottle containing ice cold EDTA. 2 *M* to inactivate intracellular and pancreatic deoxyribonuclease, were added with 29 ml of 50% TCA at the end of the perfusion (14). Rapid freezing and thawing was used to ensure disruption of the exfoliated cells prior to measurement of total DNA content in aliquots of the washings. After sacrificing the animals, the perfused small bowel was removed, dissected free of its mesentery, and lyophilized to constant weight.

Cell migration under a perfusion of Na taurocholate. After creating a bile fistula, an intraduodenal cannula was positioned through a gastrostomy. The animals were restrained, fasted, and perfused with a 5% protein hydrolysate (Amigen, Baxter) and 10% sucrose solution containing 35 mEq/liter of Na and 19 of K. In one group, the amigen–sucrose solution contained in addition, repurified (15) sodium taurocholate NaTc Maybridge Research Chemicals, U.K.). The rate of delivery (1.6/hr) and the bile concentration (30 μM/ml) of the perfusate were close to the average bile flow (1.4 ml/hr) and bile salt concentration (30.7 μM/ml) obtained in this laboratory during the first 4 hr following the creation of a bile fistula in nine rats. The pH of the solutions was adjusted to 7.4. After 48 hr of perfusion, [^{3}H]thymidine (1 μCi/g body wt) was given iv and the duodenal perfusate was continued. Groups of four rats perfused with or without added taurocholate were sacrificed 1, 12, 24, and 48 hr following injection of the tritium label. Sections were taken 1 cm distal to the ligament of Treitz as well as 2 cm proximal to the ileocecal valve and prepared for autoradiography. Ten well-oriented crypts and villi were examined from each of two slides from the jejunum and ileum. Crypt depth and villus height were estimated by counting the right hand column of cells lining the crypt–villus units. The rate of epithelial cell migration was determined by identifying from an established reference point in the center of the bottom of crypts the cell position of the topmost labeled epithelial cells 1, 12, 24, and 48 hr after DNA labeling (16).

Results. Absence of bile. Table I shows that there was no difference between the initial weight of the animals, the percentage of weight loss during the 48-hr interval before DNA labeling, the intestinal length, the weight, and protein of the intestinal mucosal scrapings of the control and bile fistula rats. Mean DNA content/g of mucosa and DNA ^{3}H-TdR specific activity was higher in controls but the difference was significant only in the case of the sp act (Fig. 1). Total cell counts per crypt in the sections from the proximal jejunum and distal ileum of controls did not differ from those in the experimental animals. However, the labeling index (mean ± SE) in the distal ileum (5.8 ± .2) of animals who had had a biliary diversion was significantly lower ($P < .001$) than that (11.6 ± .8) of animals with a normal complement of intestinal bile. The percentage of labeled crypt cells in the proximal jejunum of the experimental group (12.2 ± .6) was the same as that (10.9 ± .7) of the control group. Table II summarizes

the study of cell loss by the 2-hr saline perfusions of the entire small intestine from bile fistula and control rats. The two groups of animals could not be separated on the basis of initial body weight, percentage of weight loss during the 48 hr before the DNA exfoliation study, and the dry weight of their mesentery free small bowel. DNA content of the intestinal washings was much greater in the control group than in the one with a 48-hr period of biliary diversion. Histological sections showed no evidence of appreciable trauma to villous structures.

Perfusion of taurocholate. The duodenal infusion of the amigen–sucrose solution with added NaTc led to some increase of mucus in a few animals perfused for 72 or 96 hr. In the jejunum, the rate of new cell production assessed by the size of crypt columns was augmented but there was no change in villus height. The sections of ileum in these same animals failed to show a difference in crypt depth but there was a slight decrease in villus height. When these results were expressed as villus/crypt ratios, both the jejunum and ileum of bile salt perfused animals showed a drop in the relative proportion of differentiated nonproliferating villus cells and actively dividing crypt cells (Table III). Furthermore, progression of the labeled cells along the crypt–villus units was increased at 12, 24, and 48 hr after DNA labeling. The difference in the rate of cell migration estimated by the slope of the regression lines was more apparent in the ileum (Table IV).

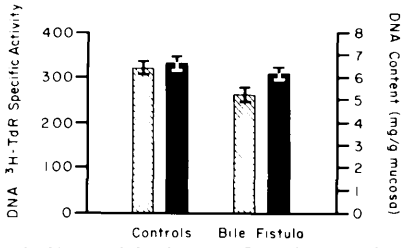

FIG. 1. Forty-eight hours after the creation of a bile fistula or a sham operation, [³H]thymidine was given and the animals were sacrificed 1 hr later. The means ± SE DNA ³H-TdR specific activity (hatched columns) of the small intestine mucosal scrapings expressed as cpm × 10^3/mg of DNA was greater ($P < .05$) in ten controls than in ten bile fistula rats. The difference between the DNA content (dark columns) was not significant.

Discussion. Results of the first part of this study show that the absence of bile from the lumen of the rat small intestine for a period of 48 hr led to a decrease in the proliferative activity of the enterocyte population and of its rate of loss. The evidence for an alteration in the proliferative cycle is based on a decrease of the mucosal scraping DNA specific activity which, although a rather crude technique, is thought to be a good index of the proliferative cell pool of the tissue (17). Autoradiographs showed that the decrease in cell production secondary to biliary diversion involved the distal small bowel since the labeling index, although unchanged in the jejunum, decreased by 50% in the ileum. Efflux of enterocytes from the villous epithelium, measured by DNA exfoliation under a perfusion of the intestinal lumen, is a valid method of comparing the rate of cell loss (13) and was found to be markedly lower in experimental animals than in controls. Because of identical dry weights of the saline washed small intestine, it is likely that the surface areas offered to the perfusate were identical in both groups of animals. The findings are, therefore, consistent with the interpretation that cell shedding was depressed in the absence of bile.

The bile component responsible for the changes in the proliferative cell pool and

TABLE II. CELL LOSS FROM THE SALINE PERFUSED SMALL INTESTINE OF CONTROL AND BILE FISTULA RATS[a].

Observation	Controls	Bile fistula	*P* value
Initial weight (g)	163.0 ± 2.8	168 ± 2.9	NS
Weight loss (%)	5.9 ± .8	5.2 ± .9	NS
Dry weight of intestine (g)	.78 ± .01	.77 ± .06	NS
DNA loss (μg)	143.0 ± 3.9	104.9 ± 7.2	<.001

[a] Forty-eight hours after biliary diversion or a sham operation in two groups of eight animals, the small intestine was perfused with saline for a period of 2 hr. Results are expressed as mean ± SE. NS, not significant.

TABLE III. CRYPT DEPTH AND VILLUS/CRYPT RATIOS IN BILE FISTULA ANIMALS PERFUSED WITH Na TAUROCHOLATE[a].

	Cell count per crypt column			Villus/cript ratios		
	−NaTc	+NaTc	*P* value	−NaTc	+NaTc	*P* value
Jejunum	17.6 ± .8	21.1 ± .9	<.01	4.7 ± .1	3.2 ± .1	<.01
Ileum	18.2 ± .6	19.4 ± .5	NS	3.2 ± .1	2.6 ± .2	<.01

[a] Pooled results (mean ± SE) from groups of four rats with biliary diversion perfused with Amigen–Sucrose solution (−NaTc) or with added Na taurocholate (+NaTc) and sacrificed serially after 48, 60, 72, and 96 hr of perfusion. NS, not significant.

TABLE IV. CELL MIGRATION AT VARIOUS TIMES AFTER [^{3}H]THYMIDINE[a].

	Duration of perfusion (hr)	Time after ^{3}H-TdR (hr)	Cell position of topmost labeled epithelial cells from center of bottom of crypts		
			−NaTc	+NaTc	*P* value
	48	1	4.1 ± .5	8.2 ± 1.3	NS[b]
Jejunum	60	12	12.5 ± .9	29.2 ± 1.1	<.05
	72	24	46.5 ± 2.2	63.9 ± 1.6	<.02
	96	48	90.9	*	—
			b = 1.94	b = 2.52	<.05
Ileum	48	1	3.3 ± .2	4.2 ± .2	NS
	60	12	8.7 ± .8	24.0 ± 2.7	<.05
	72	24	27.9 ± .9	49.6 ± 2.7	<.01
	96	48	43.4	69.7	—
			b = .89	*b* = 1.39	<.01

[a] Four bile fistula animals in each group and at each time (mean ± SE) except at 96 hr where autoradiography following the amigen–sucrose duodenal perfusions alone (−NaTc), or with added Na taurocholate (+NaTc) was carried out in only two experiments each. The slope of the regression lines (b) was calculated using the hours after the ^{3}H-TdR injection. The *P* value was derived from an *F* test of the coefficient of variation of the regression lines.

[b] NS, not significant. *, cell position not identifiable since the leading edge of labeled cells had reached the top of the 20 villi examined from each area of the small intestine.

cell loss is likely to be bile acids. The bile fistula animals, perfused with NaTc, showed accelerated cell migration. In contrast with the findings in the part of the study concerned with the absence of bile, the changes were evident in both the jejunum and ileum. However, the difference in migration rate was more striking in the ileum. In steady state conditions, an accelerated cell loss is accompanied by an increased cell production and migration rate provided the villus area remains constant (18). It is apparent that, in the acute experiments with NaTc, compensatory increases in proliferation did not quite keep up with the accelerated migration rate in the ileum since the decreased villus/crypt ratios were partly accounted for by minor decreases in villus height.

The effect of bile and NaTc on the epithelial cell turnover could be mediated by a change in pancreatic secretions. However, all perfusion experiments were carried out under a constant perfusion of protein hydrolysate known to be an important stimulus to pancreatic enzyme output (19) while bile acids are considered, at most, of minor importance in this regard (19). It is likely, therefore, that bile and bile acids exert a direct regulatory role on cell turnover. Because of its detergent properties, bile could influence epithelial cell dynamics by inducing topical alterations in the tenacity of mucus secreted by goblet cells and adsorbed to the surface of epithelial cells. It could also modify the glycocalyx or the plasma membrane itself. Conjugated bile salts do not give rise to the morphological and functional changes noted following perfusion or feeding of bile acids in their unconjugated form

(20, 21). However, physiologic concentrations of conjugated bile salts can alter the permeability of various biologic membranes (22). It is possible that the action of bile and bile acids is mediated through changes in the structure and composition of the epithelial cell membranes since they are thought to be important regulatory components of cell proliferation and differentiation (23).

The physiological and clinical implications of our findings need to be explored. Recent work (24) suggests that proximity of bile and pancreatic secretions is a major factor in the mucosal hyperplasia of the ileum which follows a proximal small bowel resection. In our study, the fact that the ileum appeared to be the segment of small intestine affected by the absence of bile is interesting in view of the fact that 70% of bile acids are absorbed in that area (25). There are few facts available on the relationship between cell turnover and absorption. When the turnover of cells is markedly increased such as in celiac disease (26), the dimunition in the number of differentiated absorptive cells is compounded by an abnormal shedding of cells leading to severe malabsorption. In clinical situations associated with the absence of bile, fat malabsorption is more modest (27) and could relate in part to the decreased endogenous losses of lipids associated with altered epithelial cell dynamics.

Summary. The absence of bile from the intestinal lumen of rats for a period of 48 hr led to: a decreased proliferative cell pool, reduced cell shedding, and a 50% decrease of the labeling index in the ileum. The constant duodenal perfusion of Na taurocholate for periods of 48, 60, 72, and 96 hr in animals with a biliary diversion was associated with: deepening of crypts and decreased crypt/villus ratios as well as with acceleration of the epithelial cell migration rate on the crypt–villus units. The data suggest that bile and bile acids constitute important regulatory factors influencing enterocyte proliferation, migration and loss.

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