

Cholesterol Synthesis by the Gastrointestinal Tract: Localization and Mechanisms of Control *

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The synthesis of cholesterol has been demonstrated in virtually every tissue of the mammalian body with the important exception of the mature nervous system (1). Yet studies in this field have primarily centered about cholesterologenesis in the liver for at least two important reasons: first, early reports suggested that the liver was the sole endogenous source of circulating cholesterol (2), and secondly, the rate of hepatic cholesterol synthesis was shown to be controlled by a sensitive feedback mechanism of considerable practical and theoretical interest (3, 4). By contrast, little information is available concerning either the physiologic importance or the means of regulation of cholesterologenesis in the intestine. Yet the recent report of Lindsey and Wilson (5), which unequivocally demonstrates that the intestine contributes to the circulating cholesterol pool, emphasizes the need for a more thorough evaluation of cholesterol synthesis by the gastrointestinal tract. The present studies, therefore, were undertaken to investigate three aspects of intestinal sterol synthesis: 1) characterization of synthetic rates along the length of the gastrointestinal tract, 2) localization of the synthetic site within the small bowel wall, and 3) evaluation of mechanisms operative in the control of intestinal cholesterologenesis.

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Methods

Animal preparation. Female Sprague Dawley rats weighing 180 to 200 g were used in the present study. Preliminary experiments with male rats showed no significant difference in data obtained from either sex. All animals were allowed water and Purina rat chow ad libitum before being killed or operated upon. In some experiments groups of animals were given a ground rat diet to each 100 g of which was added 5 g of cholesterol dissolved in 5 g of oleic acid; the control diets for these studies contained an equivalent amount of oleic acid but no added cholesterol. Bile fistula animals were prepared under ether anesthesia by intubation of the common bile duct with a polyethylene catheter (o.d., 0.038 inch) that was exteriorized through a dorsal stab wound. Control animals were similarly operated upon except that the common duct was not entered. Both bile fistula and sham-operated animals were maintained in restraining cages postoperatively and allowed free access to rat chow diet and water unless otherwise specified in the Results section. There was no significant difference in the net food intake between the two types of animal.

Bile fistula and sham-operated animals were also prepared with indwelling intestinal catheters. Polyethylene tubing (o.d., 0.050 inch) was inserted about 1 cm into the intestinal lumen through a puncture wound in the antimesenteric border of the bowel and secured with a purse-string suture. The catheter was also sutured superficially to the serosa of the intestine to keep the tip directed caudally within the lumen, and the other end of the tube was brought to the outside of the animal through a dorsal stab wound. Perfusion of the intestinal lumen was performed through the indwelling catheter by a constant rate infusion pump¹ at a rate of 1.0 ml per hour.

Tissue preparation. Each animal was killed by decapitation and the gastrointestinal tract immediately excised and placed in cold Krebs bicarbonate buffer (6). Only the distal half (glandular portion) of the stomach was used in these experiments. The entire small bowel was divided into 10 equal parts of about 10 to 13 cm in length, and the intestinal contents were removed from each segment by flushing with cold buffer. These segments were numbered from one to ten, number one being the most proximal segment. The cecum and large bowel were also cleansed with buffer; the large bowel was separated from the cecum and divided into three equal segments that

¹ B. Braun, Melsungen, West Germany.

were numbered from one to three, number one again being the most proximal segment. One-millimeter thick slices were then prepared with a McIlwain tissue slicer from each portion of the gastrointestinal tract under study. Four hundred mg of slices was placed in incubation flasks that contained 5.0 ml of Krebs bicarbonate buffer (pH 7.4) previously gassed with 95% oxygen: 5% CO₂, 1.0 μ c of acetate-2-C¹⁴, and 10 μ moles of sodium acetate. The flasks were gassed with 95% oxygen: 5% CO₂, capped, and placed in a metabolic shaker at 37° C for 2 hours.

In order to compare the metabolism of various portions of the small bowel wall a scraping technique was devised that allowed the subdivision of the wall into relatively pure preparations of villi, crypts, and smooth muscle. After flushing a bowel segment with buffer, it was opened along the mesenteric border, laid with the mucosal side up on a glass plate, and kept moistened with cold buffer. Lightly scraping the mucosa with the edge of a glass microscope slide removed most of the villi. A more forceful scraping removed a second tissue layer that contained most of the intestinal crypts and left only the denuded smooth muscle layer. The villi and crypts were suspended directly in the buffer mixture while the smooth muscle layer was first sliced then incubated as described above. The histology of these intestinal layers was determined on formalin fixed, hematoxylin and eosin stained sections of each preparation.

Chemical methods. Methods for determining the incorporation of radioactive acetate into digitonin precipitable sterols, long chain fatty acids, and carbon dioxide have been previously described in detail (3). Briefly these procedures may be outlined as follows: After incubation, the contents of the flask were acidified with 1 N H₂SO₄, and the CO₂ evolved was trapped in 1 ml of 1 N NaOH previously placed in the center well of each flask. A sample of this alkali solution was counted in the scintillation fluid described by Bray (7). The flasks' contents were then saponified, made up to a 50% ethanolic solution, and extracted with petroleum ether to remove nonsaponifiable lipids. After acidification, the residue was next extracted with pentane to remove acidic lipids. These pentane extracts were backwashed with water, and a sample was counted in a scintillation fluid containing 0.3% diphenyloxazole and 0.015% *p*-bis-phenyloxazolybenzene in toluene (DPO-POPOP solution). Sterols were precipitated from the petroleum ether extracts as the digitonide, dehydrated with acetone, washed with diethyl ether, and dissolved in methanol. A sample of this solution was placed in DPO-POPOP scintillation fluid for C¹⁴ assay. All samples were counted in a Packard liquid scintillation counter, series 314E. The data are expressed as the millimicromoles of acetate-2-C¹⁴ incorporated into digitonin precipitable sterols (DPS), fatty acids (FA), and carbon dioxide (CO₂) per gram wet weight of tissue or, in some instances, as the C¹⁴ counts per minute found in each of these fractions.

For purposes of fractionation of the digitonin precipitable sterols by thin layer chromatography (TLC), the dried sterol digitonides were first dissolved in 1.0 ml of

ethanol that had been saturated with a 90% potassium hydroxide solution. The free sterol was then extracted four times with petroleum ether; each ether extract was filtered and taken to dryness under nitrogen. The sterol mixture was redissolved in a small volume of chloroform and spotted on 20- × 40-cm plates coated with either silica gel G² or a mixture of silica gel G and silver nitrate. Three different solvent systems were employed to develop the thin layer chromatograms. 1) Separation of lanosterol, Δ^7 - and Δ^8 -methostenol, Δ^7 -cholestenol, and an area containing both cholesterol and cholestanol was carried out on plates coated with silica gel G using benzene: ethyl acetate, 5:1 (8). 2) In instances in which resolution of Δ^7 -cholestenol from the cholesterol-cholestanol spot was inadequate, these areas were respotted on silica gel G coated plates and run in benzene: ethyl acetate, 20:1 (8). A pad of filter paper was clamped to the top of each of these plates to allow the developing fluid to ascend continuously for 36 hours. 3) Separation of cholestanol from cholesterol was accomplished on silver nitrate-silica gel G plates run in benzene: hexane, 15: 85 (9). Individual spots were scraped from the plates and counted directly in the DPO-POPOP scintillation fluid using an internal standard to correct for quenching. Duplicate spots were eluted with ethanol, dried, redissolved in chloroform, and analyzed by gas-liquid chromatography (GLC) using a 6-foot column packed with Gaschrom P coated with 0.75% neopentyl glycol succinate at 230° C with a gas flow of 100 ml per minute (10). Tentative identification of individual spots was made on the basis of a comparison of R_f values and retention times with standard sterols in both the TLC and GLC systems, respectively.

Results

Sterol synthesis along the length of the gastrointestinal tract. Initial studies were carried out to evaluate variations in the sterol synthetic activity at different levels of the gastrointestinal tract. Whole-wall-thickness slices were made from the esophagus, glandular stomach, and small and large bowel, and the ability of each of these tissues to incorporate acetate-2-C¹⁴ into digitonin precipitable sterols per gram wet weight of tissue was determined. As demonstrated in Figure 1, all portions of the gastrointestinal tract from the esophagus to the terminal large bowel were capable of incorporating acetate-2-C¹⁴ into digitonin precipitable sterols. The glandular stomach was approximately 2.5 times more active than the esophagus. Small bowel synthetic activity typically varied markedly at different levels along its length. The proximal half of the small intestine was consistently the least active area in the entire gut,

² Brinkman Instruments Inc., Westbury, N. Y.

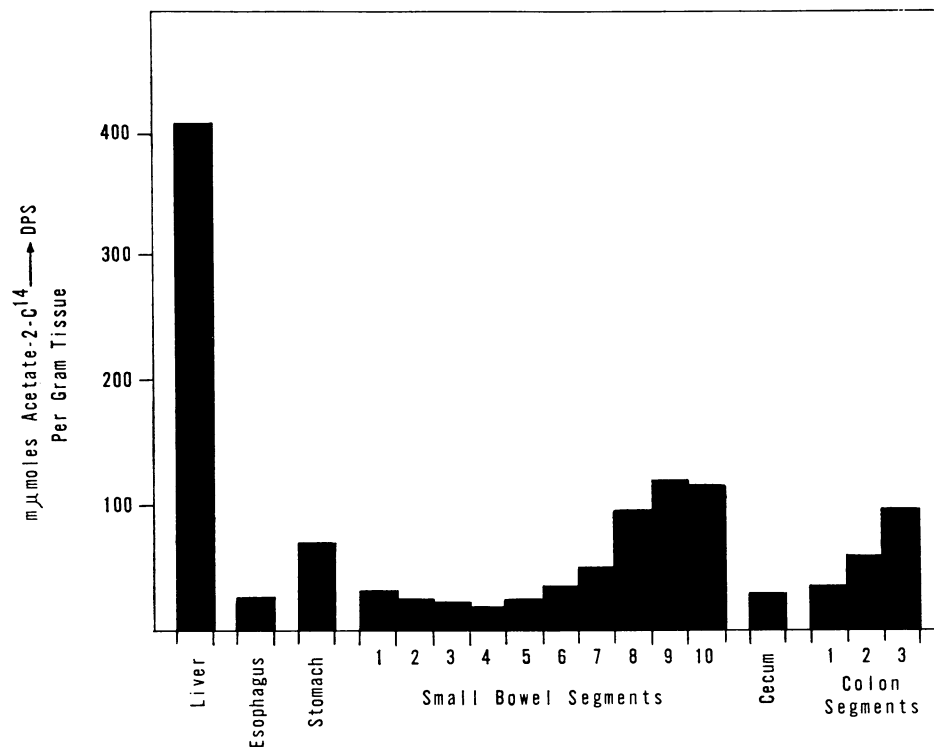


FIG. 1. CHOLESTEROL SYNTHESIS AT DIFFERENT LEVELS OF THE GASTROINTESTINAL TRACT. This diagram compares the ability of tissue slices prepared from different levels of the gastrointestinal tract to incorporate acetate-2-C¹⁴ into digitonin precipitable sterols (DPS) per gram wet weight of tissue during a 2-hour incubation period. The small and large bowels have been subdivided into 10 and 3 segments of equal length, respectively.

converting only 10 to 35 mμmoles of acetate-2-C¹⁴ to digitonin precipitable sterols per gram of tissue. Activity rapidly increased, however, in more distal segments, and maximal incorporation occurred in the terminal three ileal segments where 90 to 130 mμmoles of acetate-2-C¹⁴ was converted to digitonin precipitable sterols per gram of tissue. The cecum was relatively inactive, but, again, the rate of incorporation increased in more distal segments of the colon.

Sterol synthesis in different layers of the small bowel wall. In order to obtain tissue preparations of a more uniform cell type the intestinal wall was divided into several functionally distinct layers, and synthetic rates were determined in each of these different preparations. The data presented in Table I show the mean values of two experiments. Three contiguous six-cm long segments of mid-jejunum were cut from the intestine of a normal animal. One segment was sliced *in toto* and placed in an incubation flask (designated

“whole wall” in Table I). A second segment was opened along its mesentery border, and the villi were separated from the crypts and muscle; these two preparations were placed in incubation flasks and are designated as “villi” and “crypts and

TABLE I
Incorporation of acetate-2-C¹⁴ into carbon dioxide (CO₂), digitonin precipitable sterols (DPS), and fatty acids (FA) by different tissue fractions of the jejunal and ileal wall*

Tissue layer	Acetate-2-C ¹⁴ incorporated into CO ₂ , DPS, and FA per 2-hour incubation					
	Jejunum			Ileum		
	CO ₂	DPS	FA	CO ₂	DPS	FA
	<i>cpm</i> × 10 ⁻³					
Whole wall	684	22	27	554	40	29
Villi	92	0.03	0.04	36	< .01	0.25
Crypts and muscle	423	16	14	370	29	25
Muscle	56	0.12	0.36	69	0.04	0.23

* Each fraction represents the amount of tissue obtained from a 6-cm length of intestine.

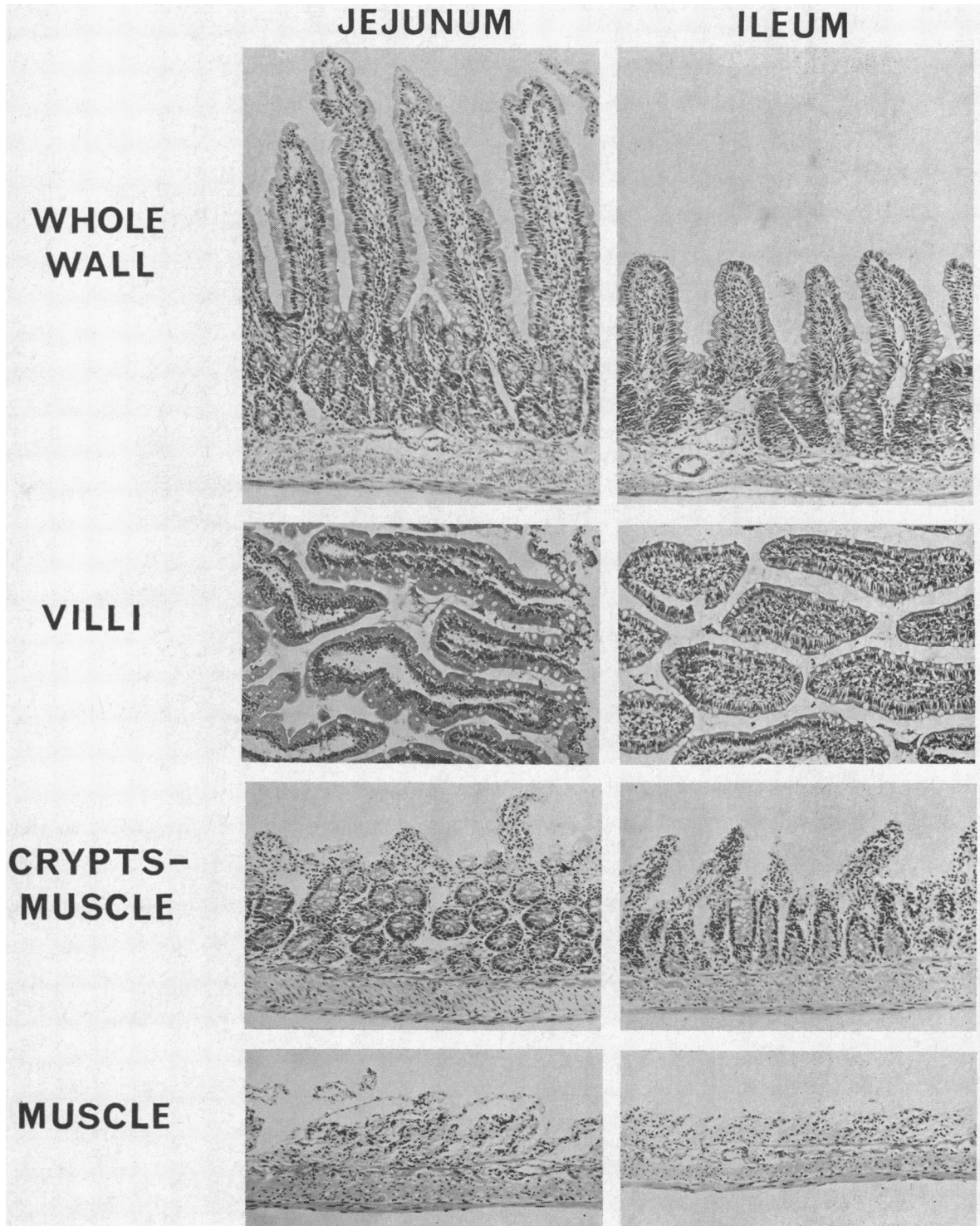


FIG. 2. PHOTOMICROGRAPHS OF TISSUE LAYER PREPARATIONS OF THE SMALL BOWEL WALL. These photomicrographs illustrate the histology of each of the tissue fractions prepared from the wall of both the jejunum and ileum as described in the text. The relative synthetic activity of each of these fractions is given in Table I.

TABLE II
Incorporation of acetate-2-C¹⁴ into digitonin precipitable sterols by jejunal and ileal villi, crypts, and smooth muscle*

		Acetate-2-C ¹⁴ incorporation into DPS	
		Experiment 1	Experiment 2
Jejunum	Villi	67	110
	Crypts	632	666
	Muscle	34	57
Ileum	Villi	158	24
	Crypts	4,640	4,550
	Muscle	22	41

* Each fraction represents the amount of tissue obtained from a 5-cm length of intestine.

muscle," respectively, in Table I. The third segment was similarly opened and vigorously scraped to remove all tissue except the smooth muscle. This was sliced for incubation and is designated as "muscle" in Table I. Similar preparations were made from three 6-cm segments of mid-ileum. A small portion of each tissue fraction was used for histologic examination (Figure 2), and the remainder was evaluated for its ability to incorporate acetate-2-C¹⁴ into digitonin precipitable sterols, carbon dioxide, and fatty acids.

As is evident from the data in Table I, all tissue fractions oxidized significant amounts of acetate to carbon dioxide, yet these same tissue layers were not all capable of active lipid synthesis. The villi preparations, which histologically contained virtually no extraneous tissue (Figure 2), did not incorporate acetate into either digitonin precipitable sterols or fatty acids to any significant degree. In contrast, crypt-muscle preparations actively synthesized labeled digitonin precipitable sterols and fatty acids, and, indeed, synthesis of these lipids approached the rates observed in the whole wall preparations. Histologically (Figure 2) roughly half of the tissue bulk of the crypt-muscle layer was made up of intestinal crypts while half was smooth muscle. That the intestinal smooth muscle alone incorporated little acetate into digitonin precipitable sterols or fatty acids is apparent from the data in the last line of Table I. These experiments strongly implied that the cells of the crypt layer of the intestinal wall were the site of incorporation of the major portion of acetate-2-C¹⁴ into digitonin precipitable sterols and fatty acids.

It was obviously desirable, therefore, to study sterol synthesis in isolated crypts. Table II shows the results of two experiments in which five-cm long pieces of jejunum and ileum were fractionated into villi, crypts, and smooth muscle and evaluated as to their synthetic ability. Of the three intestinal layers, the isolated crypts showed by far the highest rates of incorporation of acetate-2-C¹⁴ into digitonin precipitable sterols. In the case of the ileum, sterol synthesis in the crypt cells was at least 30 times that in the villi or the muscle layers. The results of this and the preceding experiments, therefore, clearly demonstrate that the major site of intestinal sterol synthesis lies in the crypt cell layer of the gut.

Influence of prolonged cholesterol feeding and fasting upon sterol synthesis by the bowel. Both fasting (11) and cholesterol feeding (12-15) are known to produce profound depressions of hepatic cholesterol synthesis, effects that imply important physiological mechanisms for over-all sterol balance within the intact organism. The effect of both of these dietary manipulations upon cholesterol synthesis in the intestine was therefore evaluated. Four animals were placed on a 5% cholesterol diet for 6 weeks while four other animals were placed on a control, cholesterol-free diet. A third group of four animals was deprived of food but allowed water for 48 hours before being killed. Representative sections were taken from each animal's duodenum, mid-jejunum, mid-ileum, transverse colon, and liver and tested for their ability to incorporate acetate-2-C¹⁴ into digitonin precipitable sterols. The mean results (± 1 SE of the mean) of these studies are shown in Table III. As expected, hepatic sterol synthesis was markedly de-

TABLE III
Effect of cholesterol feeding and of fasting upon digitonin precipitable sterol synthesis by different portions of the gastrointestinal tract

Tissue	Acetate-2-C ¹⁴ converted to DPS per gram tissue (mean 4 animals ± 1 SE)		
	Control	Cholesterol-fed 6 weeks	Fasted 48 hrs
	<i>m</i> moles		
Duodenum	17 \pm 4	16 \pm 4	17 \pm 3
Jejunum	15 \pm 10	8 \pm 5	7 \pm 2
Ileum	119 \pm 38	89 \pm 17	69 \pm 9
Colon	38 \pm 9	60 \pm 4	33 \pm 6
Liver	232 \pm 66	1.2 \pm 0.3	15 \pm 3

pressed by both cholesterol feeding (to 0.5% of control) and by fasting (to 6.5% of control); by contrast, in no instance was sterol synthesis by any portion of the bowel depressed to a similar degree by these dietary manipulations.

However, in the preceding experiment acetate-2- C^{14} incorporation was measured only in the total digitonin precipitable sterols; it was possible, therefore, that the specific synthesis of cholesterol was inhibited but that this effect was masked by the simultaneous accumulation or overproduction of another labeled 3- β -OH sterol. For this reason thin layer chromatography was used to determine the relative labeling of specific sterols in the digitonin precipitable fractions in each of the experimental groups. These data are reported in experiment 1 of Table IV. The incorporation of acetate-2- C^{14} into specific sterols was similar in the three groups of animals, and most importantly, cholesterol synthesis in the intestines of the cholesterol-fed and fasted rats was not significantly different from that in the intestine of the control animal. Thus, cholesterol synthesis by the intestine, in striking contrast to the situation in the liver, appears to be virtually insensitive to both prolonged cholesterol feeding and prolonged fasting.

Effects of bile upon intestinal sterol synthesis. During the course of studies on the feedback control of cholesterol synthesis it was noted that intestinal slices from animals with bile fistula were approximately 10 times more active in incorporating acetate-2- C^{14} in the digitonin precipitable sterols than were slices taken from animals with intact biliary systems. This finding was extended by comparing the mean rates of incorporation of acetate-2- C^{14} into digitonin precipitable sterols,

fatty acids, and carbon dioxide by intestinal slices from four sham-operated control animals and from four animals with biliary diversion of 48 hours duration. These data are shown in Figure 3; the striking stimulation of small bowel sterol synthesis produced by biliary diversion is apparent in every segment and amounted to as much as 13-fold in the middle segments. By contrast, neither the stomach nor colonic segments showed this effect. The increase of sterol synthesis produced by diversion of bile is clearly not due to a nonspecific stimulation of small bowel mucosal metabolism since, as shown in the lower two panels of Figure 3, fatty acid synthesis was actually somewhat depressed in the small intestine of the fistula animals, whereas the oxidation of acetate to carbon dioxide was nearly identical at all levels of the gastrointestinal tract in both experimental groups. When the digitonin precipitable sterols synthesized by the intestine of an animal with a bile fistula were fractionated into specific sterols by thin layer chromatography, it could be shown that the synthesis of all sterols examined was increased by bile diversion; however, the marked increase in cholesterol synthesis was clearly the major cause for the enhanced sterol synthetic activity (experiment 2, Table IV).

Localization of the bile effect upon intestinal cholesterogenesis. Although the experiments just described clearly imply that bile contained a potent inhibitor of intestinal cholesterogenesis, they did not distinguish whether this inhibitor acts locally upon the mucosal cells or whether it undergoes an enterohepatic circulation and so suppresses intestinal sterol synthesis by virtue of its presence in the blood. These two possibilities were exam-

TABLE IV

Incorporation of acetate-2- C^{14} into specific sterols by intestinal slices obtained from the ileum (segment 8) of a control, cholesterol-fed, and fasted animal (experiment 1) as well as from an animal with biliary diversion (experiment 2)

Exp.	Animal preparation	Acetate-2- C^{14} converted to specific sterols per gram tissue				
		Cholesterol	Methostenol (Δ^7 and Δ^8)	Cholestanol	Δ^7 -Cholestenol	Lanosterol
1	Control	73	3.6	<i>m</i> μ moles 0.9	1.8	3.6
	Cholesterol-fed, 4 weeks	90	2.5	1.2	1.2	3.7
	Fasted, 48 hrs	62	1.6	0.8	0.8	3.3
2	Bile fistula, 48 hrs	220.1	24.7	13.7	16.5	

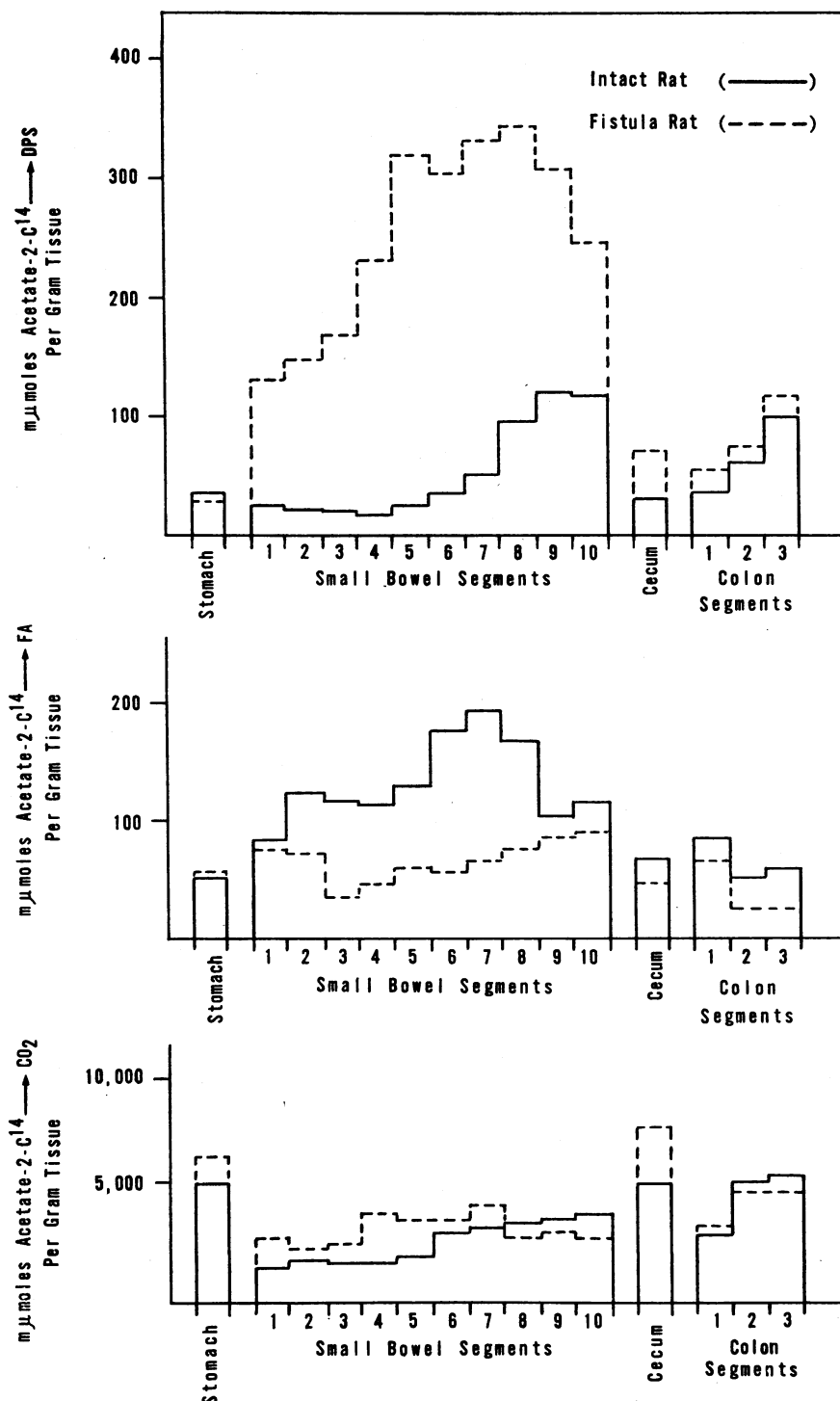


FIG. 3. EFFECT OF BILE UPON GASTROINTESTINAL STEROL AND FATTY ACID SYNTHESIS. The data in this diagram compare the ability of gastric and intestinal slices obtained from sham-operated rats and similar slices taken from animals that had biliary diversion for 48 hours to incorporate acetate-2-C¹⁴ into digitonin precipitable sterols (DPS), fatty acids (FA), and carbon dioxide (CO₂) per gram of tissue during a 2-hour incubation period.

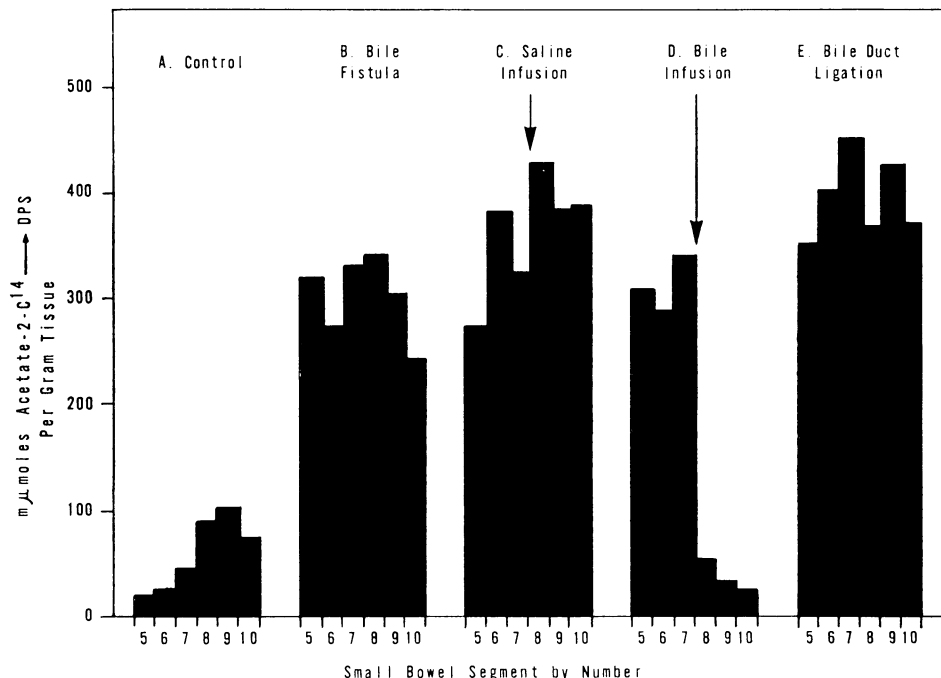


FIG. 4. LOCALIZATION OF THE INHIBITORY EFFECT OF BILE UPON SMALL BOWEL DIGITONIN PRECIPITABLE STEROL SYNTHESIS. These data show a comparison of the digitonin precipitable sterol (DPS) synthetic activity in the six terminal small bowel segments of animals in five different experimental situations as described in the text. In experiments C and D each animal had an indwelling intestinal catheter at the junction of segments seven and eight as indicated by the arrows.

ined in the experiments designated A through E in Figure 4. For purposes of comparison experiment A demonstrates the level of incorporation of acetate-2-C¹⁴ into digitonin precipitable sterols in the terminal six segments of the small bowel of a sham-operated control rat with an intact biliary system. In this and the following experiments only the distal six segments of the small intestine were used, since in the animal with a bile fistula synthetic activity was maximal and almost uniform in these segments (segments 5 to 10). The animal in experiment B had a biliary fistula for 48 hours before being killed and demonstrates the expected increase in synthetic activity. Animals C and D both had biliary fistulae but, in addition, indwelling intestinal catheters were placed so as to enter the bowel at the junction of small bowel segments 7 and 8. By means of these catheters, isotonic saline (in C) and rat whole bile (in D) were infused into the lumen of the terminal three segments of the small bowel, leaving the proximal segments (5 to 7) free of bile. The rate of infusion was 1.0

ml per hour, and each infusion was continued for 48 hours before sacrificing the animal. The incorporation of acetate-2-C¹⁴ into digitonin precipitable sterols by the small bowel of animal C was at the usual high level observed in bile fistula animals; this experiment demonstrated that the trauma of an intestinal catheter and saline infusion in no way influenced the enhanced synthesis of the terminal three segments. In contrast, however, the infusion of whole bile into the bowel in experiment D resulted in a marked suppression of synthetic activity in the terminal three segments (segments 8, 9, and 10), whereas the three segments proximal to the point of infusion (segments 5, 6, and 7) incorporated acetate-2-C¹⁴ into digitonin precipitable sterols at the uninhibited rate. These results suggested that the inhibitory effect of bile involves a direct action upon the cells of the mucosa which synthesize the digitonin precipitable sterols.

However, to evaluate further a possible systemic effect of the biliary inhibitor of sterol synthesis, a fifth animal had a simple bile duct ligation 5 days

before being sacrificed (experiment E). All tissues of this animal including the gastrointestinal tract were grossly bile stained, yet the terminal six segments of small bowel incorporated approximately 350 m μ moles of acetate-2-C¹⁴ into digitonin precipitable sterols per gram of tissue, a rate equal to that found in the intestine of an animal with external biliary drainage. These data strongly supported the concept that the inhibitor in bile has no systemic action, but is effective only when directly in contact with the mucosal surface of the small bowel.

In animals with biliary fistula, inhibition of sterol synthesis at any level of the small bowel could be produced by the infusion of whole bile for 48 hours. Three such experiments are shown in Figure 5. In animals A and C whole bile was infused through indwelling intestinal catheters at the junction of small bowel segments 1 and 2 and

7 and 8, respectively. In experiment B a short polyethylene catheter drained bile from the animal's common duct into its small bowel at the junction of segments 5 and 6, thus leaving segments 1 to 4 free of bile. The results in all three experiments were identical; proximal to the point of infusion there was enhanced sterol synthesis, whereas distally, in the segments perfused with bile, there was marked suppression of acetate-2-C¹⁴ incorporation into digitonin precipitable sterols. In segments proximal to the infusion point the synthesis of digitonin precipitable sterols became elevated to levels characteristic of the synthetic activity of these segments in animals with total biliary drainage alone, viz., the gradient of synthetic activity along the length of the small bowel of animals with bile fistula noted in Figure 3 was again observed.

Nature has provided an experimental situation

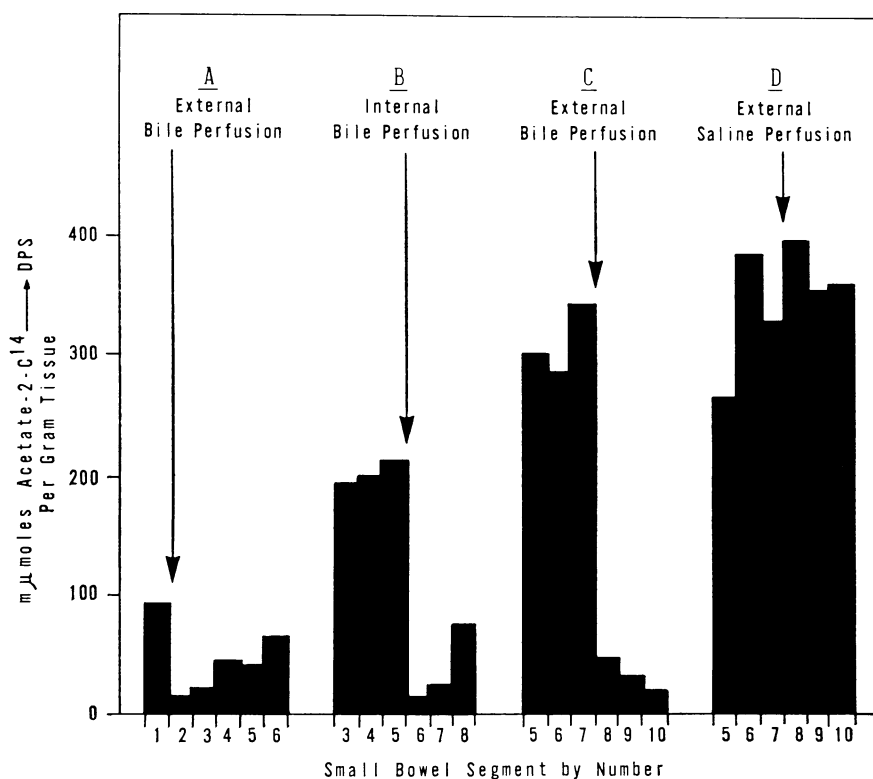


FIG. 5. DEPRESSIVE EFFECT OF BILE UPON DIGITONIN PRECIPITABLE STEROL SYNTHESIS AT DIFFERENT LEVELS OF THE SMALL BOWEL. In experiments A and C, bile was infused at two different levels of the small bowel by indwelling intestinal catheters, whereas in experiment B bile was allowed to flow from the animal's common duct to its mid-small bowel by an internal plastic fistula. The placement of the intestinal catheters with regard to the small bowel segments is indicated by the arrows.

similar to that described above in that the common bile duct enters the small bowel slightly distal to the gastroduodenal junction. That short initial segment of small bowel proximal to the ampulla of Vater should not be perfused with so high a concentration of bile as those portions of the duodenum immediately distal to the entrance of the common duct. In two normal animals this 1.2-cm segment of duodenum between the gastroduodenal junction and the ampulla of Vater was carefully dissected out. The rate of incorporation of acetate- 2-C^{14} into digitonin precipitable sterols was compared between slices from this proximal segment (segment A) and slices obtained from three contiguous segments of duodenum of similar length taken just distal to the entrance to the common duct (segments B, C, and D). As shown in Figure 6, in both experiments the proximal segment had 15 to 20 times the synthetic activity as did any of the next three segments. This observation adds further evidence for a direct inhibitory effect

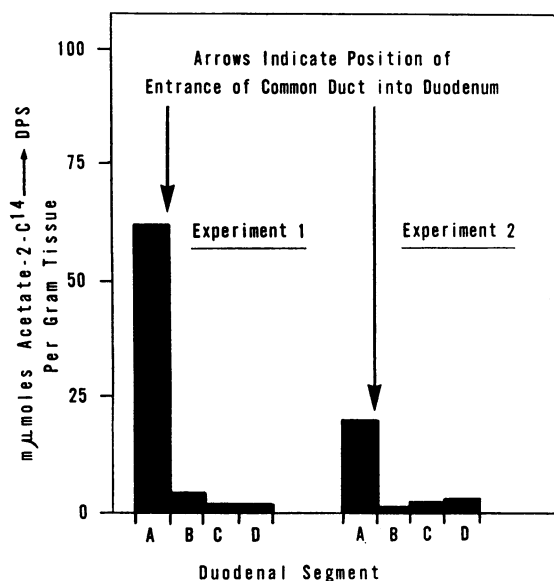


FIG. 6. DIGITONIN PRECIPITABLE STEROL SYNTHESIS IN SPECIFIC SEGMENTS OF THE DUODENUM. In two separate experiments the duodenum was divided into four segments, each approximately 1.2 cm long. The most proximal segment (segment A) was that portion of the duodenum between the gastroduodenal junction and the ampulla of Vater, and the next three sections (segments B, C, and D) comprised the remainder of the duodenum between the entrance of the common duct and the duodenal-jejunal junction.

of bile upon the sterol synthetic activity of the small bowel mucosa.

Time sequence for bile inhibition of intestinal digitonin precipitable sterol synthesis. In order to determine the rapidity with which bile infusion inhibited intestinal sterol synthesis, seven rats were prepared with bile fistula and indwelling intestinal catheters placed at the junction of intestinal segments 7 and 8. After 48 hours of biliary drainage, when the small bowel of each animal was presumed to be synthesizing sterols at its uninhibited rate, infusions were begun through the intestinal catheters at a rate of 1.0 ml per hour. Initially, all animals were begun on infusions of isotonic saline, but at different times during the subsequent 72 hours, rat whole bile was substituted for the salt solution. Thus, when the animals were sacrificed all had had their terminal three small bowel segments perfused for a total of 72 hours; in individual animals the infusate was whole bile for a duration of 0, 3, 6, 12, 24, 48, and 72 hours before termination of the experiment. The small bowel of each animal was removed, and the three segments proximal and distal to the infusion point were sliced and incubated with acetate- 2-C^{14} . The per cent of inhibition of synthetic activity in the perfused segments was determined by comparing the mean values of the millimicromoles of acetate- 2-C^{14} incorporated into digitonin precipitable sterols by the terminal three segments (segments 8, 9, and 10) with the mean values of the millimicromoles of acetate- 2-C^{14} incorporated by the three proximal segments (segments 5, 6, and 7). These data are plotted in Figure 7. No inhibition occurred with bile infusions of 6 hours or less; at times greater than this inhibition became manifest reaching apparent maximal values at about 48 hours.

Possible mechanisms of bile inhibition of sterol synthesis. When bile was diverted from the gastrointestinal tract, the experimental animals absorbed fat poorly and developed semisolid, bulky stools. It was possible, therefore, that the stimulation of digitonin precipitable sterol synthesis was only an indirect consequence of this malabsorption. Alternatively, the absence of bile and presence of excess fat in the gut lumen could allow abnormal bacterial overgrowth, and this in turn might enhance sterol synthesis by the intestinal mucosa.

In order to test both of these possibilities the

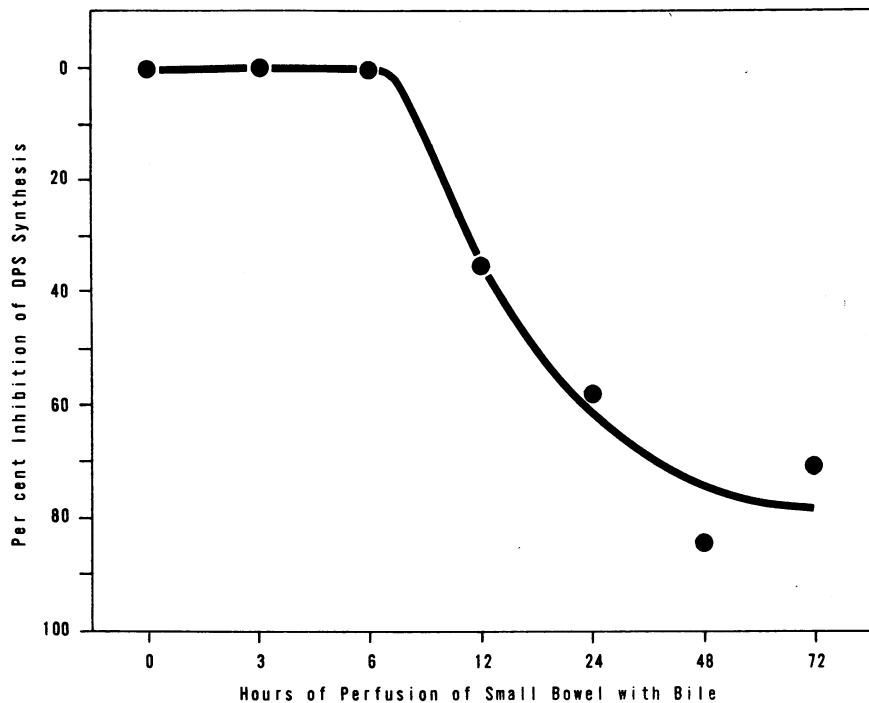


FIG. 7. TIME COURSE FOR THE INHIBITION OF INTESTINAL DIGITONIN PRECIPITABLE STEROL SYNTHESIS BY BILE. Bile was infused into the small bowel of rats via indwelling intestinal catheters (at the junction of segments seven and eight) for varying periods of time. Then a comparison was made of the DPS synthetic activity in segments proximal and distal to the infusion point. Any decrease in the rate of incorporation of acetate-2-C¹⁴ into DPS by the perfused distal segments was expressed as a per cent of the synthetic rate in the uninhibited proximal segments.

experiments illustrated in Figure 8 were carried out. After a 24-hour fast two animals were operated upon: a bile fistula was placed in one while the second was sham operated. Both were placed in restraining cages and allowed only water for 48 hours, at the end of which time they were sacrificed and intestinal slices were prepared. The influence of intestinal bile on the incorporation of acetate-2-C¹⁴ into digitonin precipitable sterols is still apparent in such fasted rats (Figure 8A) even though the bowels of these animals were essentially empty throughout the experimental period. It seemed unlikely, then, that any product of the maldigestion of dietary fat acted as a stimulating agent for sterol synthesis.

In order to test the second possibility, two animals were begun on oral antibiotics (polymixin B, 8 mg, and bacitracin, 5,000 U, per 24 hours) for 24 hours before being operated upon. Biliary diversion was then performed in one animal while the second was sham operated. Both animals were

allowed food and water ad libitum for 48 hours, and in addition, both were given their daily dose of antibiotics in the drinking water. The animals were sacrificed at the end of 48 hours, and sterol synthesis in the intestine was assayed. Quantitative bacterial counts were carried out on the intestinal contents of the sixth intestinal segment. For comparison bacterial counts were also made of the contents of the sixth intestinal segment of a sham-operated and fistula-containing rat not treated with antibiotics. The data in Figure 8B

TABLE V
Bacterial counts in the sixth small bowel segment of control and antibiotic treated rats

	Log ₁₀ bacteria per cm of intestine	
	Control	Antibiotic treated
Sham-operated animals	7.40	3.78
Biliary fistula animals	8.08	3.25

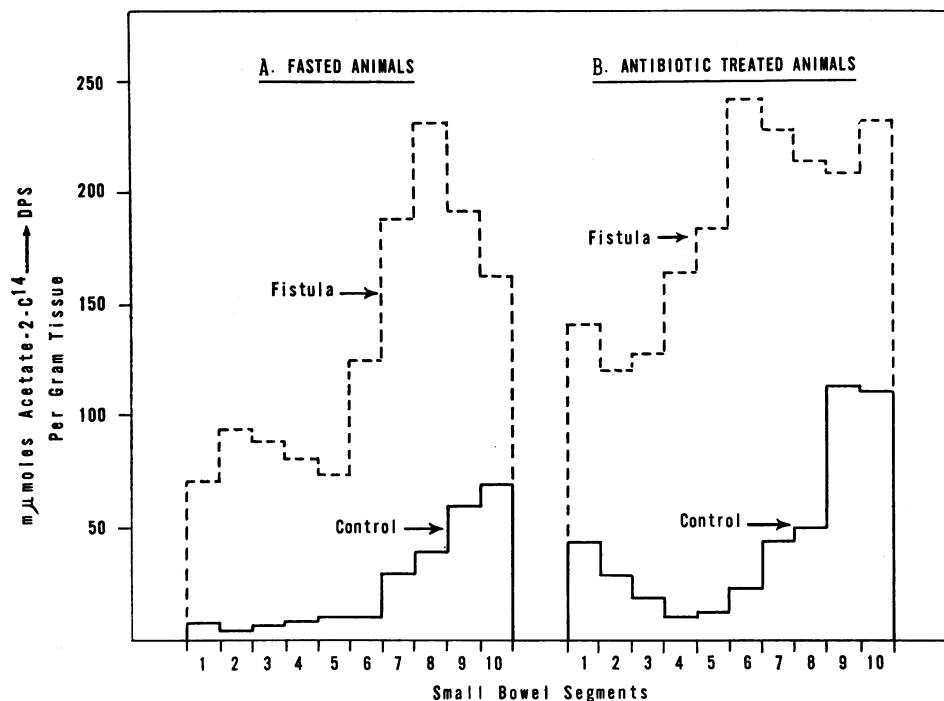


FIG. 8. EFFECTS OF BILIARY DIVERSION UPON INTESTINAL DIGITONIN PRECIPITABLE STEROL SYNTHESIS IN FASTED AND ANTIBIOTIC TREATED ANIMALS. These data show the effects of fasting and antibiotic treatment upon the enhancement of small intestinal DPS synthesis produced by biliary diversion. In experiment A the animals were fasted for 24 hours before operation and throughout the 48-hour experimental period. Similarly, in experiment B, each animal was begun on antibiotics 24 hours before being operated on, and the same dosage of drugs was given throughout the 48-hour experimental period.

show that the small intestine from the animal with biliary diversion still had enhanced labeling of the digitonin precipitable sterol even though antibiotic treatment had reduced the bacterial count of its mid-small bowel by a factor of nearly 10^5 below that found in a similar animal with no antibiotic treatment (Table V); furthermore, the bacterial counts in both the fistula-containing and sham-operated animals were nearly identical. This experiment demonstrates that, at least in these studies, bacterial overgrowth plays no significant role in the control of sterol synthesis, and it would, therefore, appear that the presence or absence of bile per se determines the rate of sterol synthesis in the intestine.

Discussion

Since the initial studies of Srere, Chaikoff, Treitman, and Burstein in 1950, a number of investigators have clearly demonstrated that the

small intestine is capable of actively incorporating C^{14} -labeled acetate into cholesterol (1, 3, 16, 17); however, very little is known about either the specific anatomical sites of such sterol synthesis or the factors that may control cholesterologenesis within the gut. For these reasons the investigations reported here were undertaken: first, to determine the ability of the various specific portions of the gastrointestinal tract, from the esophagus to the terminal colon, to carry out sterol synthesis; second, to examine further the anatomical sites of sterol synthesis by evaluating the rates of digitonin precipitable sterol synthesis in the three layers of the gut wall; and third, to attempt to determine what physiological conditions might normally regulate sterol synthesis in the intestinal tract.

It is apparent from the results of the present study that there are striking differences in sterol synthesis at various levels of the gastrointestinal

tract. The esophagus had a low rate of synthetic activity, whereas the glandular stomach manifested approximately 2.5 times the activity of the esophagus. The initial portion of the duodenum between the gastroduodenal junction and the ampulla of Vater synthesized labeled sterols at an active rate, but immediately distal to the entrance of the common duct synthesis was almost totally depressed. Synthetic activity remained low in the jejunum, but increased in a regular manner in the ileum reaching maximal rates in the last 30 cm of the small intestine. In the large bowel, the cecum usually had the least active rate of sterol synthesis, and, as was found in the small bowel, more distal colon segments had more active acetate incorporation rates. Such marked differences in synthetic activity along the length of the gastrointestinal tract not only implied anatomic variation in the distribution of cells responsible for sterol synthesis, but also raised the possibility of the presence of local control mechanisms as a possible explanation for these variations.

When the small bowel wall was divided into three tissue layers consisting of villi, crypts, and smooth muscle, the major portion of the sterol synthetic activity was clearly localized to the intestinal crypts. Since histologically the crypts account for approximately one-fourth of the tissue mass of the gut wall, one can calculate that in the ileum from 400 to 450 $m\mu$ moles of acetate-2- C^{14} is incorporated into digitonin precipitable sterols per gram of crypt tissue; this is a rate of sterol synthesis which equals or exceeds that found in the liver. Surprisingly, the mature epithelial cells of the villi consistently manifested less than 1% of the digitonin precipitable sterol synthetic activity found in the whole-wall preparation even though under the conditions of the experiment this tissue layer actively oxidized acetate-2- C^{14} to carbon dioxide.

This pattern of distribution for sterol synthetic activity transversely through the bowel wall is particularly interesting when considered in the context of our present knowledge of epithelial cell generation and turnover. It is now well established that cells of the mucosal surface of the gastrointestinal tract are being continuously replaced (18). The renewal rate is so rapid (32 to 38 hours for the rat) that at any one time in the small bowel of the rat approximately 3% of the epi-

thelial cells are undergoing mitosis (19). These dividing cells are found almost exclusively in the intestinal crypts and under normal conditions are not seen higher up on the intestinal villi. It is known that the epithelial cells newly formed in the crypts continuously migrate upward to provide the mucosal cells of the upper villi. By histochemical techniques Padykula has shown that these cells achieve enzymatic maturity only after they have moved up out of the crypt zone into the base of the villus (20). During this migration structural changes also occur as the blunt, immature microvilli of the crypt cells gradually become the slender, elongated microvilli typical of cells near the apex of the villus (21). Hence, cells are formed in the intestinal crypt, move upward and mature into the functionally competent absorptive cells of the villus, and finally, are sloughed into the intestinal lumen from the villus tip at the extrusion zone.

Our observation that cholesterol synthesis is most active in the area of epithelial cell generation (the crypts) and is virtually absent in the region of mature epithelial cells (the villi) would suggest that sterol synthesis in the gastrointestinal tract may serve the function of providing structural cholesterol for the newly forming cells.

Although several previous studies have demonstrated that dietary cholesterol- C^{14} collected in the thoracic duct lymph is diluted by endogenous cholesterol (22, 23), there has been until recently no unequivocal evidence that cholesterol synthesized locally in the intestinal wall contributes to blood cholesterol. The recent studies of Lindsay and Wilson have now amply demonstrated that cholesterol synthesized in the gut wall does enter the lymph and eventually becomes part of the circulating cholesterol pool (5). Precise details of how this process occurs are not known. If, as is postulated above, newly synthesized intestinal cholesterol is primarily used for structural purposes, then the cells containing the sterol must migrate up the villi, be sloughed into the intestinal lumen, and be digested before the cholesterol moiety can be absorbed and incorporated into chylomicrons. Alternatively, newly formed cells may contain a cholesterol pool that can be transferred directly into the chylomicrons of the central lacteal as the cell moves upward out of the crypt. Which of these possibilities is correct remains to be elucidated.

Three possible conditions that might influence intestinal cholesterologenesis were examined in the present study; these were the feeding of a high cholesterol diet for 6 weeks, fasting for 48 hours, and diversion of bile out of the gastrointestinal tract. Both cholesterol feeding and fasting are known to cause prompt suppression of cholesterol synthesis in the liver. Much data have accrued in this laboratory to demonstrate that cholesterol feeding inhibits hepatic cholesterologenesis by a feedback mechanism operating at the point of conversion of β -hydroxy- β -methylglutarate to mevalonic acid (3, 24). Inhibition of this same step in cholesterol synthesis has been identified as the probable cause of the depressed hepatic cholesterol synthesis in the fasting animal (25).

In contrast to these findings in the liver, feedback control of cholesterologenesis is absent in the intestine. In 1953, Gould and associates demonstrated in dogs that the feeding of a 1% cholesterol diet for seven to eleven days suppressed hepatic but not intestinal cholesterologenesis (16). Similarly, Cox, Nelson, Wood, and Taylor could demonstrate no inhibition of small bowel cholesterol synthesis in monkeys fed cholesterol in cream for 4 to 6 weeks (17), and a report from this laboratory showed that in the rat intestine cholesterologenesis was not suppressed by feeding a 5% cholesterol diet for as long as 2 weeks (3). Data reported in the present study substantiate these findings since the rate of incorporation of acetate- 2-C^{14} into digitonin precipitable sterols by the small bowel is not depressed by feeding a 5% cholesterol diet for as long as 6 weeks. In addition, however, the findings presented here also demonstrate that, unlike liver, the rate of intestinal sterol synthesis is also unaffected by fasting for as long as 48 hours.

Since significant quantities of several sterols other than cholesterol are present in the intestine, it was conceivable that the previous conditions might depress cholesterol synthesis per se but that such an effect would be obscured by compensatory synthesis of other $3\text{-}\beta\text{-OH}$ sterols. Labeled digitonin precipitable sterols, synthesized by the intestine of fasted and cholesterol-fed rats, were therefore fractionated into constituent sterols by thin layer chromatography, and the C^{14} content of the purified cholesterol was shown to be normal. In this way it was unequivocally demonstrated that the intestinal synthesis of cholesterol itself con-

tinues at an unaltered rate in the face of either cholesterol feeding or fasting.

In striking contrast to the apparent insensitivity of the intestinal sterol synthetic pathway to dietary manipulations, the presence or absence of bile within the gut lumen was shown to have a marked influence on the rate of cholesterologenesis by the small bowel. When bile was diverted from the gastrointestinal tract, the synthesis of digitonin precipitable sterols from acetate- 2-C^{14} increased from 2- to 13-fold at different levels of the small bowel whereas synthetic activity in the stomach and colon remained essentially unchanged. These findings indicate that bile must contain a potent inhibitor of intestinal sterol synthesis.

Several lines of evidence would suggest that this biliary inhibitor acts locally upon the mucosal cells rather than systemically by absorption from the gut into the circulation. When bile is infused into various sites along the small bowel of an animal with a biliary fistula, sterol synthesis is enhanced proximal to the infusion point but depressed distally. This is true of infusion at any level of the bowel, even as high as the beginning of the jejunum. If the inhibitor effected mucosal cell synthesis by virtue of its absorption into the blood, sterol synthesis in the intestinal segments proximal to the point of bile infusion should be as depressed as segments distal to the infusion point. In fact, in such experiments acetate incorporation into digitonin precipitable sterols in the unperfused proximal segments of the intestine always increased to levels found in similar small bowel segments of animals with only biliary diversion. Additional support for this argument came from the experiment in which simple bile duct ligation was performed. Despite the resultant biliary regurgitation, the small bowel of these rats incorporated acetate- 2-C^{14} into digitonin precipitable sterols at a markedly accelerated rate. This study would strongly suggest that the biliary regulation of intestinal sterol synthesis must be exerted through a local rather than a systemic action of bile.

When bile is diverted from the gastrointestinal tract, a defect in fat digestion and absorption is produced since bile salts are necessary for emulsification and micellar solubilization of the fatty constituents of chyme. Such a disruption of the absorptive physiology might indirectly alter sterol

synthesis by the small bowel either as a result of the maldigestion and malabsorption of fat *per se* or because of the bacterial overgrowth in the small bowel that may occur in malabsorption syndromes. In view of the experimental results shown in Figure 8, neither of these factors appears important in regulating small bowel sterol synthesis. Biliary diversion enhanced digonin precipitable sterol synthesis by the bowel wall even though the animals were fasted throughout the experimental period, or even when bacterial counts were reduced 10⁹-fold by antibiotics administration. These results suggest a direct depressive effect of bile acting locally upon the mucosal cells to inhibit intestinal sterol synthesis.

In view of the variations in sterol synthetic activity noted in various segments of the small bowel of the intact rat, it is likely that the presence of bile in the intestine plays a major physiologic role in regulating the rate of intestinal sterol synthesis. The experimental demonstration that 1) proximal to the entrance of the common duct the duodenum has very active synthesis of labeled sterol (Figure 6); 2) distal to the ampulla of Vater jejunal synthetic activity is very low, but can be enhanced over 10-fold by biliary diversion (Figure 3); and 3) the relatively active areas of sterol synthesis in the terminal ileum can be markedly inhibited by the introduction of bile directly at this level (Figure 4) clearly show the overwhelming importance of bile in determining variations in the synthesis of cholesterol along the length of the small bowel. Apparently there is little regurgitation of bile into the most proximal portion of the duodenum in the nonfasting rat so that synthesis is relatively active in this area. Below the level of the ampulla of Vater in the distal duodenum and proximal jejunum one finds the highest concentration of bile and the lowest rate of incorporation of acetate-2-C¹⁴ into labeled sterols by the bowel wall. More distally there is a progressive rise in synthetic activity suggesting that the concentration of the inhibitory constituent of bile is being gradually reduced, either by absorption out of the gut lumen or by inactivation by bacteria of the lower bowel.

In the animal that has had the normal depressive effect of bile eliminated by biliary diversion, however, there persist both the inherent variations in synthetic activity down the length of the small bowel as well as the differences between the small

bowel and the other portions of the gastrointestinal tract. Several possibilities may explain these variations: 1) If, as had been postulated, the synthesis of sterol is primarily related to new cell formation, then the rate of acetate-2-C¹⁴ incorporation into digonin precipitable sterols for a given area of bowel may be a reflection of the cell renewal rate for that area. 2) Since the intestinal crypt appeared to be the anatomic site for sterol synthesis, at least in the small intestine, variations in the absolute crypt cell mass could account for differences in synthetic activity at different levels of the small bowel. 3) Sterol synthesis in this study was expressed as a function of whole-wall weight; therefore, differences in the ratio of actively synthesizing cells to the synthetically inert portion of the bowel wall could effect the apparent synthetic activity calculated for different tissues of the gastrointestinal tract. Which of these factors is most important cannot be determined on the basis of present knowledge; however, it is noteworthy that there is a good correlation among the relative sterol synthetic rates in the esophagus, stomach, colon, and rectum observed in the present study and the cell turnover rates determined for these same areas in the rat gastrointestinal tract by Bertalanffy (26). Furthermore, Leblond and Stevens showed that in the duodenum 41% of the intestinal epithelial cells are located in the crypts as opposed to 65% in the terminal ileum and that the cell turnover times in these two areas were approximately 38 and 32 hours, respectively (19). Thus, there is a general correlation between a proportionately smaller number of epithelial cells in the crypts, slower cell turnover, and lower sterol synthetic activity in the proximal small bowel and a greater proportion of epithelial cells in the crypts, a more rapid cell turnover, and more active sterol synthesis in the terminal small bowel.

The clinical importance of the observation that bile normally contains a potent inhibitor of intestinal cholesterol synthesis remains to be elucidated. Since a portion of the circulating cholesterol pool in the intact individual is presumably of intestinal origin, it is possible that the enhanced synthetic activity produced by obstruction of the common bile duct may result in an increased delivery of cholesterol into the circulation. Such a process could play a role in the development of the hypercholesterolemia commonly associated with pro-

longed biliary obstruction. The clinical significance of this control mechanism is now under investigation.

Summary

These studies provide information concerning three aspects of sterol synthesis by the gastrointestinal tract of the rat: 1) the relative synthetic rates along the length of esophagus, stomach, and small and large bowel, 2) localization of the site of cholesterologenesis within the wall of the small intestine, and 3) possible mechanisms responsible for physiologic control of intestinal sterol synthesis.

1) The synthesis of digitonin precipitable sterols occurred at every level of the gastrointestinal tract; however, the synthetic rate in the esophagus, proximal small bowel, and proximal colon was relatively low when compared to the active rates of synthesis found in the stomach, ileum, and distal colon.

2) When the wall of the small intestine was subdivided into three tissue layers, sterol synthetic activity was almost exclusively found in the tissue preparation containing the intestinal crypts; by contrast, the intestinal villi and smooth muscle were essentially devoid of synthetic activity.

3) Neither cholesterol feeding nor fasting significantly altered the rate of intestinal sterol synthesis. Bile, however, was shown to contain a potent inhibitor that acts directly upon the intestinal mucosa to suppress cholesterologenesis. The importance of this inhibitor as a determinant of the normal variation in the rate of sterol synthesis along the length of the small bowel is discussed.

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