Cholesterol Synthesis in the Intestine of Man: Regional Differences and Control Mechanisms

JOHN M. DIETSCHY and WILLIAM G. GAMEL

From the Gastrointestinal-Liver Unit, Department of Internal Medicine, The University of Texas (Southwestern) Medical School at Dallas, Dallas, Texas 75235

ABSTRACT Cholesterol in the circulating serum pool is derived either from absorption of dietary cholesterol or from endogenous synthesis principally in the liver and gastrointestinal tract. While the control of intestinal cholesterogenesis has been elucidated in several lower animal species, no data currently are available in the case of man. In the present study using tissue specimens obtained by suction biopsy in 29 normal subjects, we have shown the rate of cholesterogenesis is low in the stomach (25 ±6 mμmoles/g per 2 hr) and rectum (40 ±8 mμmoles/g per 2 hr); in the small bowel the rate progressively decreases in the proximal duodenum (90 ±16 mμmoles/g per 2 hr); distal duodenum (80 ±11 mμmoles/g per 2 hr); and distal jejunum (35 ±5 mμmoles/g per 2 hr); but abruptly increases in the distal ileum (280 ±33 mμmoles/g per 2 hr). Indirect evidence is provided that the intestinal crypt epithelium is the main site of this sterol synthesis. Fasting for 48 hr suppressed the rate of cholesterogenesis in the distal duodenum from a control value of 80 ±11 mμmoles/g per 2 hr to 40 ±8 mμmoles/g per 2 hr while cholesterol feeding for 7 days did not alter the rate of cholesterol synthesis (75 ±12 mμmoles/g per 2 hr). This resistance to cholesterol feeding also was present in the distal ileum where control and cholesterol-fed subjects had comparable rates of cholesterogenesis (280 and 261 mμmoles/g per 2 hr, respectively). Interruption of the enterohepatic circulation, in contrast, resulted in greatly enhanced sterol synthesis with a mean rate of 259 ±29 mμmoles/g per 2 hr being found in the duodenum of four patients with biliary obstruction as compared with the rate of 80 ±11 mμmoles/g per 2 hr in control subjects. These studies indicate that the mechanisms of control of cholesterol synthesis by the human intestine are similar to those described for the intestine of lower animals; this also appears to be true for the human liver. Thus, the marked differences in over-all cholesterol metabolism between various lower mammalian species and man cannot be explained by fundamental differences in control mechanisms; rather, these differences must reflect variations in some other parameter of cholesterol metabolism.

Received for publication 19 October 1970 and in revised form 7 December 1970.

INTRODUCTION

The early studies of Srere, Chaikoff, Treitman, and Burstein provided the first evidence that several different mammalian tissues were capable of converting acetate into sterols (1). These observations have been confirmed and extended in recent publications from this laboratory, in which it was shown both in the rat (2) and in the squirrel monkey (3) that virtually every tissue is capable of at least some degree of cholesterogenesis. The important physiological and, presumably, clinical questions are which of these tissues contribute significantly to the circulating cholesterol pool and what are the mechanisms whereby sterol synthesis is controlled in those organs which do participate in maintenance of the serum cholesterol.

Initial studies designed to answer these questions suggested that only the liver contributed significant amounts of cholesterol to the serum pool (4, 5). This view, however, recently has been challenged, for in studies such as those reported by Lindsey and Wilson (6), it clearly has been demonstrated that cholesterol synthesized de novo in the intestinal mucosa becomes part of the rapidly miscible pool in equilibrium with blood cholesterol. Furthermore, it also was shown in these same studies that under experimental conditions in which hepatic cholesterogenesis was suppressed by cholesterol feeding and in which intestinal lymphatic diversion was performed, no radioactive cholesterol appeared in the blood following administration of acetate-^{14}C (6). Thus, in contrast to the older concept, these results indicate that the liver and gastrointestinal tract together are the ma-

jor if not the sole endogenous sources for the rapidly miscible cholesterol pool, which includes blood cholesterol.

The relative importance of these two organs in the monkey, as sites for the endogenous synthesis of serum sterols, has been shown in studies in which animals were fed a high cholesterol diet containing radiolabeled cholesterol for many weeks until the specific activity of the serum cholesterol reached a steady state. At that time, the specific activity of the serum cholesterol was found to equal only 50–65% of the specific activity of the dietary cholesterol, a finding which indicated that 35–50% of the circulating cholesterol arose from endogenous synthesis (7). Since in parallel experiments, direct in vitro assay demonstrated that hepatic cholesterogenesis was completely inhibited in these animals while, in contrast, intestinal cholesterogenesis continued at near control levels (3), this finding indicated that under the conditions of this experiment as much as 35–50% of the serum cholesterol ultimately may have been synthesized in the gastrointestinal tract.

Taylor, Patton, Yogi, and Cox (8) and Wilson and Lindsey (9) also have reported similar results in human subjects fed radiolabeled cholesterol until the isotopic steady state was attained. In both of these investigations it was determined that in subjects on a high cholesterol intake, from 60 to 80% of the serum cholesterol arose from de novo endogenous synthesis. If one assumes (*a*) that hepatic sterol synthesis in these human subjects was completely inhibited and (*b*) that the rate of intestinal synthesis was relatively unaffected by the high dietary cholesterol intake, then one can conclude from these experiments that in man the intestine may equal or even exceed the liver in importance as a source of circulating cholesterol. There are four reports, with somewhat conflicting results on the effectiveness of the negative cholesterol feedback mechanism in the liver of man (10–13), but there are no studies on the characteristics of the control mechanisms of cholesterogenesis in the human intestine. Absence of such data for the intestine therefore precludes precise interpretation of the suggestion of Taylor et al. (8) and of Wilson and Lindsey (9) that an extrahepatic site is a significant source of circulating cholesterol. Because of the need for such information in order to understand cholesterol metabolism in man, the present investigation was undertaken to characterize cholesterogenesis in the human gastrointestinal tract with respect both to the distribution of synthetic activity and to the mechanism of control.

METHODS

Patients and experimental subjects. Four different groups of subjects were utilized in these studies for intestinal biopsy. (*a*) One group of patients had small intestinal biopsies performed in order to establish the diagnosis of idiopathic sprue. In this group, a portion of each biopsy was utilized for morphologic studies, while the remainder was incubated as described below. (*b*) The major number of studies was undertaken in healthy volunteers, both male and female, between the ages of 21 and 40 yr. (*c*) A third group of subjects consisted of patients with obstructive biliary disease. These patients were evaluated for the presence of clotting defects before biopsy and no patient was included in the study who manifested a defect in his clotting mechanisms. (*d*) Finally, biopsies of the terminal ileum were obtained in several patients who had had a total colectomy and ileostomy. These patients had had no significant ileal resection and had no steatorrhea. All subjects in groups *b*, *c*, and *d* were informed of the nature of these experimental studies and of the possible hazards of the biopsy procedures; each subject gave his written consent before being included in the study. Finally, human liver tissue was obtained from patients undergoing needle biopsy for diagnostic purposes. A portion of each biopsy was used for morphological studies while the remainder was assayed for cholesterogenic activity. Data were used only from those biopsies that ultimately proved to be morphologically normal.

Diets. Control, low cholesterol diets consisted of balanced meals selected by the subjects but devoid of foods containing large amounts of cholesterol. Each subject kept a daily record of his food intake from which a dietition estimated the total cholesterol intake during the experimental periods. By this means the cholesterol intake in subjects during the control periods was estimated to vary from 98 to 280 mg per day. During the experimental periods of high cholesterol intake, each subject added 15 large eggs each day to his usual diet. This resulted in an estimated cholesterol intake of from 3400 to 4000 mg per day.

Biopsy procedures. All subjects were biopsied between 7 and 10 a.m.; fluid and food were discontinued approximately 4–8 hr before the biopsy. In all instances, intestinal biopsies were obtained with a multipurpose suction biopsy tube [1] using a capsule with two holes so as to obtain two mucosal samples simultaneously. Using a fluoroscope with image intensifier, the biopsy tube was placed either at the level of the midstomach, the proximal duodenum, the duodenal-jejunal junction at the ligament of Treitz, or in the distal jejunum (180 cm from the nose). The distal ileum was biopsied in subjects with ileostomies by inserting the biopsy tube 10–15 cm into the stoma. Rectal mucosa was biopsied by inserting the biopsy tube 10-15 cm into the rectum through a sigmoidoscope. After the tube was placed at the desired location, one set of biopsies was obtained; the knife in the capsule was then reset, the tube was withdrawn 1–2 cm and a second set of biopsies was obtained. The entire biopsy tube was then quickly withdrawn from the subject and the mucosal biopsies were immediately placed in a large volume of cold Krebs bicarbonate buffer. By this means, from two to four separate pieces of mucosa weighing 2.5–10 mg each were obtained from each subject. Biopsies weighing more than 10 mg were divided into two separate specimens. There was no complication from this procedure in any subject or patient; specifically, there was no evidence of gastrointestinal bleeding or perforation. Liver biopsies were obtained in the usual manner after an overnight fast using a Menghini needle.

Incubation procedure. Immediately after the biopsy specimens were obtained they were washed in cold oxygenated buffer, blotted dry on filter paper, and individually weighed. Each specimen was incubated in a separate center-well

[1] Quinton Instruments, Seattle, Wash.

TABLE I

Standard Errors of the Mean Rates of Incorporation of Acetate-2-^{14}C Into Cholesterol, Fatty Acid, and CO_2 in Individual Subjects

Experimental subject	No. of Biopsy specimens	Incorporation of acetate-2-^{14}C into		
		Cholesterol	Fatty acid	CO_2
		±% *of mean values*		
A	4	±7.3	±6.6	±6.8
B	4	±10.0	±8.0	±6.4
C	4	±16.6	±4.9	±6.9
D	4	±8.3	±12.0	±13.9
E	4	±13.6	±13.2	±11.8
F	4	±14.5	±4.9	±8.0
G	5	±3.6	±7.5	±7.6
Average		±10.5	±8.1	±8.7

This table contains data from all subjects in whom four or more biopsy specimens were obtained during a single biopsy procedure. Each specimen was incubated separately and the mean rates of incorporation of acetate-2-^{14}C into cholesterol, fatty acid, and CO_2 were determined. The standard errors of these mean values were calculated and are expressed in this table as per cent of the mean.

flask containing 1.0 ml of Krebs bicarbonate buffer previously gassed with 95% oxygen–5% carbon dioxide and 5 μCi (2.5 μmoles) of acetate-2-^{14}C. The flasks were then flushed with the same gas mixture, capped, and placed in a metabolic shaker for 2 hr at 37°C.

In preliminary experiments using similar size biopsies from the intestine and liver of the squirrel monkey, it was demonstrated that under these incubation conditions the rates of incorporation of acetate-2-^{14}C into cholesterol, fatty acids, and CO_2 were essentially linear with respect to time and tissue weight, and the concentration of acetate (2.5 μmoles/ml) was sufficient to achieve zero order kinetics. These validation studies were extended to human tissue in so far as was possible. Using two or three pieces of tissue obtained from the same patient, it was shown that incorporation rates were proportional to tissue weight and to the time of incubation but were independent of increases in acetate concentration in the range of 1.5–3.0 μmoles/ml, i.e., zero order kinetics obtained. The addition of glucose to the incubation medium did not alter the incubation rates.

Chemical methods. Following the incubation period 1 mg of carrier cholesterol was added to each flask and the amount of acetate-2-^{14}C incorporated into CO_2, long-chain fatty acids, and cholesterol was determined as described previously (14). The data are expressed as the mμmoles of acetate-2-^{14}C incorporated into cholesterol, fatty acids, and CO_2 per gram wet weight of tissue per 2 hr incubation.

Fractionation of the digitonin-precipitable sterols into specific sterol fractions was carried out by means of two thin-layer chromatographic procedures previously described (2). After regeneration of the free sterols, thin-layer chromatography was first carried out in a system that allowed separation of cholesterol, the Δ^7- and Δ^8-methostenols, Δ^7-cholestenol, and lanosterol; however, this system does not separate the saturated sterol cholestanol from cholesterol. Therefore, it was necessary to rechromatograph the sterols recovered from the cholesterol area in a second system utilizing silver nitrate–impregnated plates in order to determine the amount of labeling in cholestanol (2). The data are expressed as the per cent of the total ^{14}C recovered from the plate found in each specific sterol fraction.

Estimation of cell distribution in the biopsy specimens. In order to estimate the relative distribution of cell types in biopsies obtained from control subjects and patients with idiopathic sprue, a portion of each biopsy was used for routine histologic examination. Photomicrographs were taken of full thickness, perpendicular cuts of the biopsies and 11 by 14 inch photo enlargements were prepared. The areas of the photographs representing the epithelial surface of the villi, the crypt epithelium, and the submucosal connective tissue were cut out by hand and weighed. Uniform density of the various cells was assumed and the distribution of these three cell types in each specimen was expressed as a percentage of the total weight of the biopsy.

Calculations. In each subject from two to five tissue specimens were obtained during each biopsy procedure and these were assayed individually for their ability to incorporate acetate-2-^{14}C into cholesterol, fatty acids, and CO_2. The results obtained were then averaged to give a single set of values for each subject under a particular experimental condition.

RESULTS

Variance of incorporation rates determined in individual subjects. These studies necessarily were carried out using very small pieces of tissue so that problems in weighing the biopsies or variability in the relative amount of villi, crypts, and connective tissue present in each biopsy might result in significant artifactual variation in the calculated incorporation rates among individual tissue specimens. In order to estimate the degree of variance in the data due to these purely technical problems, the standard error of the mean incorporation rate measured in biopsy specimens obtained simultaneously from a given subject was determined as shown in Table I. As is evident in this table, the standard error of the incorporation of acetate into cholesterol, fatty acids, and CO_2 averaged ±10.5%, ±8.1%, and ±8.7%, respectively. These values compare favorably with results we have obtained using larger tissue samples in animal experiments and indicate that this assay procedure can be used to accurately quantitate sterol synthetic rates in man.

Fractionation of the digitonin-precipitable sterols. Other initial experiments were performed to determine if, under the conditions of these experiments, acetate-2-^{14}C actually was being incorporated predominantly into cholesterol and not into some other digitonin-precipitable sterol. When the labeled digitonin-precipitable sterols recovered after incubation of small intestinal mucosal specimens from 15 subjects were fractionated into specific sterols, as shown in Table II, this was found to be the case. Of the total radioactivity incorporated into sterols during the 2 hr incubation period, an average of 77.7% was found specifically in cholesterol. In subse-

quent sections, therefore, the incorporation of acetate-2-^{14}C into digitonin-precipitable sterols is considered to approximate the rate of cholesterogenesis.

The rate of cholesterol synthesis in the liver and variation in the rates of cholesterogenesis down the length of the gastrointestinal tract. As shown in Fig. 1, hepatic cholesterogenesis equalled 71 ±12 mμmoles/g per 2 hr. Synthesis in the proximal duodenum (90 ±16 mμmoles/g per 2 hr) and at the level of the ligament of Treitz (80 ±11 mμmoles/g per 2 hr) occurred at similar rates but a significantly lower acetate incorporation rate was found in mucosa obtained from the distal jejunum (35 ±5 mμmoles/g per 2 hr). In the distal ileum, however, cholesterogenesis abruptly increased reaching a mean rate (280 ±33 mμmoles/g per 2 hr) that was nearly four times higher than the rates found in the liver or proximal small intestine. It should be emphasized that these variations in sterol synthetic activity occurred despite the fact that, as shown in the two lower panels of Fig. 1, the rates of incorporation of acetate-2-^{14}C into fatty acids and CO_2 were relatively uniform down the length of the small intestine.

Cholesterogenesis occurred, but at considerably lower rates, in both the stomach (25 ±6 mμmoles/g per 2 hr) and rectum (40 ±8 mμmoles/g per 2 hr). Both of these tissues, however, also incorporated acetate into fatty acids and CO_2 at much lower rates than did the small intestine.

Localization of the cellular site of cholesterogenesis in the small intestine. In previous studies reported from this laboratory, a scraping technique was utilized to divide the small intestine of the rat into three separate layers that contained predominantly the intestinal villi, crypts, and smooth muscle. By this means it was found that virtually no cholesterogenesis occurred in the preparations of villi or smooth muscle whereas nearly all of the sterol synthetic activity found in the whole gut was recovered in the tissue layer containing the intestinal

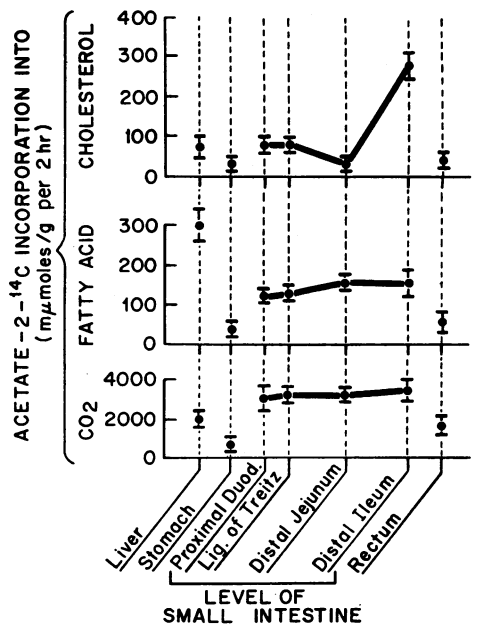

FIGURE 1 Rates of cholesterol synthesis at different levels of the gastrointestinal tract. Biopsies were obtained from the liver and from various levels of the gastrointestinal tract, as indicated at the bottom of the diagram, from subjects on a low cholesterol intake and assayed for their ability to incorporate acetate into cholesterol, long-chain fatty acids and CO_2. The results are expressed as the mean ±1 SE number of mμmoles of acetate-2-^{14}C incorporated into each of the products per gram of wet tissue weight during the 2 hr incubation period. The number of subjects biopsied in each group were as follows: stomach (3), proximal duodenum (3), ligament of Treitz (7), distal jejunum (4), distal ileum (4), and rectum (8).

TABLE II

Recovery of ^{14}C in Specific Sterol Fractions

Per cent of total radioactivity recovered from chromatographic plates found in each specific fraction				
Silica Gel H system				$AgNO_3$ system
Cholesterol + cholestanol	Δ^7- and Δ^8-methostenols	Δ^7-Cholestenol	Lanosterol	Cholestanol
81.0 ±3.5	10.0 ±1.2	6.2 ±2.1	2.7 ±0.2	3.3 ±0.4

Each value represents the mean percentage (±1 SE) of the radioactivity found in each specific sterol fraction after chromatographic separation of the ^{14}C-labeled total digitonin-precipitable sterols recovered after incubation of small intestinal biopsies from the duodenum and jejunum of 15 normal subjects. For purposes of thin-layer chromatography the sterols from three to four subjects were pooled into four samples that were then run in duplicate.

crypts (15). However, it was impossible to utilize this technique on the small biopsy samples obtained in this study. Advantage, therefore, was taken of the fact that patients with untreated idiopathic sprue have essentially no villi. Thus, it was possible to compare the rates of cholesterogenesis in control biopsies that contained mature villi, crypts and submucosal connective tissue with the rates found in biopsies from patients with untreated sprue that contained only a small amount of surface epithelium, hypertrophied crypts, and submucosal tissue. As shown in Table III, the mean rate of sterol synthesis at the level of the ligament of Treitz in eight control subjects equalled 80 ±11 mμmoles/g per 2 hr while the average in four patients with sprue equalled 122 ±35 mμmoles/g per 2 hr.

Histologic examination of representative sections of the biopsy specimens from the eight control subjects revealed that 36 ±5% and 24 ±2%, respectively, of the tissue weight was comprised of the surface epithelium of the mature villi and the crypt epithelium. The remaining 40 ±4% was accounted for by the connective tissue

TABLE III

Rates of Cholesterogenesis in the Small Intestine of Patients with Idiopathic Sprue

Subjects	Acetate-2-^{14}C Incorporation Into		
	Cholesterol	Fatty acid	CO_2
	mμmoles/g per 2 hr		
Normal controls (8)	80 ±11	137 ±11	3452 ±340
Idiopathic sprue (4)	122 ±35	65 ±10	2940 ±250

The mean rate (±1 SE) of small intestinal cholesterogenesis at the level of the ligament of Treitz in four patients with idiopathic sprue is compared with the mean rate found in eight control subjects. The data are expressed as the mμmoles of acetate-2-^{14}C incorporated into cholesterol, long-chain fatty acids, and CO_2 per gram of tissue during the 2 hr incubation period.

in the cores of the villi and the submucosal tissue. In contrast, in the four patients with idiopathic sprue, only approximately 5 ±2% of the tissue weight was comprised of surface epithelium on the flattened villi while the crypt epithelium accounted for 37 ±6% of the weight of the biopsies. Assuming that cholesterol synthesis occurs predominantly or exclusively in the crypt epithelium, these data can be used to normalize the rates of synthesis shown in Table III to a constant crypt weight. By this means, it can be calculated that the rate of cholesterogenesis in the crypt tissue from control subjects and patients with sprue theoretically equals approximately 340 mμmoles/g per 2 hr and 330 mμmoles/g per 2 hr, respectively.

Effect of fasting and cholesterol feeding on intestinal cholesterogenesis. The possible rate-controlling effects of fasting and of cholesterol feeding were next investigated, as shown in Table IV. Following a 48 hr period of fasting the rate of intestinal cholesterogenesis decreased from a value of 80 ±11 mμmoles/g per 2 hr at the level of the ligament of Treitz in control subjects (B) to 40 ±8 mμmoles/g per 2 hr (C). A decrease of similar magnitude also were found in the rates of acetate-2-^{14}C incorporation into fatty acids and CO_2.

In contrast to these results, and of particular importance, intake of a diet high in cholesterol content for 7 days had no suppressive effect upon intestinal cholesterogenesis. As shown in line D (Table IV) biopsies obtained from the level of the ligament of Treitz in cholesterol-fed subjects had a rate of cholesterol synthesis of 74 ±12 mμmoles/g per 2 hr as compared with a rate of 80 ±11 mμmoles/g per 2 hr in comparable control subjects fed a diet low in cholesterol content. The rates of fatty acid synthesis (111 ±12 mμmoles/g per 2 hr *versus* 137 ±11 mμmoles/g per 2 hr) and CO_2 production (3678 ±475 mμmoles/g per 2 hr *versus* 3452 ±340 mμmoles/g per 2 hr) also were essentially identical in subjects fed the high and low cholesterol diets.

These data, however, were obtained in the proximal small intestine at the level of the ligament of Treitz where, as shown in Fig. 1, the rate of cholesterogenesis is relatively low. In order to examine the possibility that a suppressive effect of cholesterol might be more evident if these experiments were carried out with tissue from the more active synthetic sites in the distal small intestine, biopsies were obtained from the distal ileum in two subjects fed a high cholesterol diet for 7 days. Cholesterogenesis averaged 261 mμmoles/g per 2 hr in these two individuals (F, Table IV) as compared with a rate of 280 ±33 mμmoles/g per 2 in four other subjects maintained on a low cholesterol intake (E). The rates of fatty acid synthesis and CO_2 production also were comparable in these two experimental groups.

TABLE IV

Effect of Fasting, Cholesterol Feeding, and Biliary Obstruction on Intestinal Cholesterogenesis

Tissue	Level of biopsy	Dietary regimen	Number of Subjects	Acetate-2-^{14}C incorporation into		
				Cholesterol	Fatty acids	CO_2
				mμmoles/g per 2 hr		
A. Liver	Right lobe	Low cholesterol	8	71 ±12	310 ±61	1957 ±157
B. Intestine	Ligament of Treitz	Low cholesterol	7	80 ±11	137 ±11	3452 ±340
C. Intestine	Ligament of Treitz	Fasting	5	40 ±8	76 ±10	2413 ±400
D. Intestine	Ligament of Treitz	High cholesterol	6	74 ±12	111 ±12	3678 ±475
E. Intestine	Distal ileum	Low cholesterol	4	280 ±33	159 ±40	3740 ±460
F. Intestine	Distal ileum	High cholesterol	2	261	162	3410
G. Intestine	Ligament of Treitz	Low cholesterol + biliary obstruction	4	259 ±29	133 ±20	3560 ±250

Before biopsy, each subject was on either a low cholesterol control diet or a high cholesterol diet for 7 days or else was fasted for 48 hr with noncaloric fluids allowed *ad libitum*. The patients in group G were judged to have complete biliary obstruction by clinical criteria and while they were placed on a low cholesterol diet, generally had a poor food intake. Data are mean values ±1 SE.

Thus, it is apparent that variations in dietary cholesterol content between very wide extremes do not alter the rate of cholesterol synthesis in the human intestine.

Effect of biliary obstruction on intestinal cholesterogenesis. Bile acids have been shown to suppress the rate of intestinal cholesterogenesis in animals (16); contrariwise the absence of bile acids in the intestinal lumen as occurs with biliary diversion and biliary obstruction is associated with marked enhancement of small intestinal sterol synthesis (15, 16). In order to evaluate the possible role of bile acids in controlling cholesterol synthesis in the human small intestine, the rate of cholesterogenesis was measured in the proximal small bowel of patients with biliary obstruction. As shown in line G of Table IV, the rate of acetate-2-^{14}C incorporation into cholesterol increased by more than 3-fold (259 ±29 mμmoles/g per 2 hr *versus* 80 ±11 mμmoles in control subjects) in the face of biliary obstruction. Furthermore, this increase appeared to be specific for cholesterogenesis since the rate of fatty acid synthesis and CO_2 production from acetate-2-^{14}C remained unchanged.

DISCUSSION

According to current concepts serum cholesterol arises either from the absorption of exogenous dietary cholesterol or from the de novo synthesis of cholesterol within the body (7–9, 17). Of the various possible tissue sites for the endogenous synthesis of sterols, recent experimental data indicate that only two, the liver and gastrointestinal tract, contribute significantly to the rapidly miscible pool which includes circulating cholesterol (6, 7). While sterol synthesis in these two organs has now been fairly well characterized in several mammalian species including lower primates (1–3, 15, 16, 18–25), little or no data are available in the case of hepatic and intestinal synthesis in man.

In lower animals as well as in the squirrel monkey, hepatic cholesterogenesis has been shown to be altered in a major way by three experimental manipulations. First, feeding a diet rich in cholesterol uniformly results in a marked reduction in hepatic sterol synthesis, often with rates of less than 1% of control levels, in every species which has been tested (2, 3, 14, 18, 20, 21, 23). Second, fasting for periods of 48–96 hr also produces marked inhibition of cholesterol synthesis in the liver although this inhibition usually appears less rapidly and is of a lesser degree than that seen following cholesterol feeding (2, 3, 19, 26). Third, hepatic cholesterogenesis is enhanced by any manipulation that interrupts the enterohepatic circulation of bile acids so that biliary diversion, biliary obstruction, or the administration of cholestyramine increases the rate of sterol synthesis by the liver (25, 27–29).

The results reported in the liver of man are less complete. Taylor, Cox, Nelson, Davis, and Hass in 1955, reported in an abstract (10) that the synthesis of cholesterol was "only slightly lower" in liver biopsies obtained at surgery from patients given large doses of cholesterol dissolved in cream than in those obtained from patients on a conventional diet. However, these results obtained in patients subjected to preoperative fasting and to anesthesia must be viewed with some reservation. In a more extensive study utilizing liver tissue obtained by needle biopsy, Bhattathiry and Siperstein (11) demonstrated that the rate of incorporation of acetate into cholesterol was inversely proportional to the dietary cholesterol intake. These results clearly suggest that the liver of man, as is the case in lower animals, is responsive to cholesterol-negative feedback inhibition. These results have been recently confirmed both in the adult by Pawliger and Shipp (13) and in the infant by Fujiwara, Hirono, and Arakawa (12). It appears safe, therefore, to conclude that hepatic cholesterogenesis in man is inhibited by cholesterol feeding. Data, however, are not available in man concerning the effects of fasting or of interruption of the enterohepatic circulation on sterol synthesis by the liver.

Cholesterogenesis in the gastrointestinal tract of lower animals, in particular the rat and squirrel monkey, has been characterized in considerable detail. First, in both species a consistent pattern is present in the relative rates of synthesis along the length of the intestine with maximum rates of cholesterogenesis being found in the terminal ileum (3, 15, 16). Second, sterol synthesis at all levels of the intestine is essentially unaffected by cholesterol feeding and is only slightly depressed by fasting, a finding which is in sharp contrast to the effects of these two dietary manipulations upon hepatic cholesterogenesis (2, 3, 15). Third, biliary diversion and biliary obstruction result in a marked enhancement of cholesterol synthetic rates at all levels of the small intestine but not in the colon or stomach (3, 15, 16); the effect, therefore, of interruption of the enterohepatic circulation of bile acids is similar in the small bowel and in the liver.

Virtually no data are available concerning the characteristics of cholesterol synthesis in the intestine of man. In the present study, therefore, cholesterol synthesis in the human gastrointestinal tract was characterized in detail using tissue samples obtained by a multipurpose intestinal biopsy tube. One technical aspect of this study warrants emphasis. The tissue specimens were very small so that variation in tissue composition might significantly effect apparent incorporation rates. As shown in Table I, however, variability in the incorporation rates among different biopsies from a single subject due to these

purely technical problems was acceptably small averaging only ±10.5% in the case of cholesterol synthesis.

Initial studies on the distribution of sterol synthetic activity along the length of the gastrointestinal tract in man, as illustrated in Fig. 1, showed a pattern very similar to that described in both the rat (15, 16) and the monkey (3) with maximum rates of synthesis being found in the terminal ileum. The fact that the incorporation of acetate into long-chain fatty acids and CO_2 was relatively uniform at all levels of the small intestine makes it very unlikely that the differences in the rate of acetate incorporation into sterols are due to differences in cellular permeability, acetate activation or intracellular acetate pool size at these different intestinal levels; rather, these data strongly suggest that the variations in the rates of incorporation of acetate into cholesterol shown in Fig. 1 reflect differences in the inherent cholesterogenic activity of the intestine at these different levels.

Not only is the distribution of cholesterol synthetic activity along the length of the small intestine in man similar to that described in animals, but, in addition, the cellular site within the intestinal wall also probably is identical. As shown in Table II the mean rate of cholesterol synthesis is higher in biopsies containing no intestinal villi from patients with untreated idiopathic sprue than in biopsies from control subjects. This is in contrast to the commonly encountered situation where enzyme systems known to be localized to the mature epithelium of the villi show very low activity in patients with sprue. Furthermore, when these rates of cholesterogenesis are normalized to the amounts of crypt tissue present in these two sets of biopsies nearly identical rates of synthesis are found. Thus, while these findings provide only indirect evidence as to the site of sterol synthesis in the human intestine, these data are consistent with the observation in the rat that sterol biosynthesis appears to occur predominantly if not exclusively in the crypt cell layer (15, 16).

Another point worthy of emphasis in these studies is the relative rate of cholesterol synthesis in the liver and the various levels of the gastrointestinal tract. In the rat and monkey, the rate of incorporation of acetate-2-^{14}C into cholesterol by liver commonly is 10- to 12-fold higher than the rate found in the proximal small intestine (3, 15, 16). In this study in man the rate of hepatic cholesterogenesis (71 ±12 mμmoles/g per 2 hr) just equals the rate found in the proximal small intestine while sterol synthesis in the ileum is over 4-fold greater than that found in the liver when expressed per unit wet weight. There are several possible explanations for this apparent difference. The animal data were obtained using whole intestinal slices while these studies in man were undertaken using biopsy specimens that contained no muscle. The weight contribution of muscle to intestinal slices is small, however, and cannot possibly account for the relatively higher rates of synthesis found in the intestine of man. Since the liver biopsies were performed in patients who were fasted overnight, it is also possible that hepatic cholesterogenesis was partially surpassed in these studies secondary to this deprivation. This also seems unlikely since, as shown in Table IV, the synthesis of longchain fatty acids in these same liver biopsies occurred at a relatively high rate. Since fatty acid synthesis usually is suppressed to near zero following significant starvation, it is unlikely that the relatively low rate of hepatic sterol synthesis observed in these subjects could be attributed to the overnight fast before the biopsies. Finally, it is conceivable that the liver of man constantly is partially inhibited as a result of the small amounts of cholesterol present even in a low cholesterol diet. Evaluation of this point would require measuring the rate of hepatic cholesterogenesis in patients maintained for long periods of time on a formula diet free of any cholesterol. Whatever the cause, however, it should be emphasized that the rate of hepatic cholesterogenesis relative to the rate of intestinal cholesterogenesis is much lower in man than the rate described previously in animal experiments.

The major point of interest in this study concerns the possible mechanisms of control for intestinal cholesterogenesis in man; the experimental manipulations which were investigated include (*a*) fasting for 48 hr, (*b*) the administration of a diet high in cholesterol content, and (*c*) interruption of the enterohepatic circulation.

The amount of suppression of intestinal cholesterogenesis produced in man by fasting is somewhat greater than that seen during a comparable period of deprivation in animals. In the squirrel monkey (3), for example, the rate of cholesterol synthesis in the proximal small intestine decreased by only 7% after a 48-hr period of fasting, whereas in this study an average decrease of 50% in sterol synthetic activity was observed.

As shown in Table IV, cholesterol synthesis in the small bowel of man, as is true in the rat, dog, and monkey (2, 3, 20), is not inhibited by cholesterol feeding. The difference between this finding in the intestine and the results described by Bhattathiry and Siperstein (11) in the human liver is of considerable physiologic importance and warrants emphasis; these investigators showed that a cholesterol intake of approximately 3.5 g per day markedly suppressed hepatic cholesterogenesis, whereas in the present study this same dietary regimen did not significantly alter the rate of intestinal cholesterogenesis.

Thus, in the subjects reported by Wilson and Lindsey (9) who had a cholesterol intake of 2.28 and 2.78 g/day,

it is very likely that endogenous sterol synthesis by the liver was markedly suppressed, whereas synthesis by the intestine was unaffected. Since there are no data in animals or in man to indicate that any tissue other than liver and gastrointestinal tract contributes to the circulating sterol pool, the finding that 60–80% of serum cholesterol in these subjects was derived from endogenous synthesis i.e. presumably intestine, strongly suggests that the gastrointestinal tract is of major importance for the biosynthesis of cholesterol entering the rapidly miscible sterol pool.

The final control mechanism in the present investigation was studied to determine the relationship of the rate of intestinal cholesterogenesis in man to the functional state of the bile acid enterohepatic circulation. It is apparent that when bile acids are totally excluded from the gastrointestinal tract, as was the case in the four patients in Table IV, cholesterol synthesis increases markedly as it does in the intestine of the rat and monkey (3, 15, 16). Thus, it is likely on the basis of these data that man possesses the same bile acid–negative feedback on small bowel cholesterogenesis that has been described in lower animals (16).

In a preliminary report Salen, Ahrens, and Grundy reached a similar conclusion using a different experimental technique (30). In this study patients were maintained on a zero cholesterol intake and injected with ^{14}C-cholesterol. The specific activity of the cholesterol in biopsies of the intestinal mucosa was then determined and used to gauge indirectly the rate of intestinal synthesis. These authors demonstrated that the ratio of the specific activity of mucosal to plasma cholesterol decreased after the administration of the bile acid sequestrant, cholestyramine. If one assumes that total sterol flux across the intestine was unaffected by cholestyramine administration then this result also is consistent with the view that local intestinal synthesis increases when the effective activity of bile acid in the intestinal lumen is reduced.

In summary, in so far as data are available it appears that the mechanisms of control of cholesterol synthesis in both the liver and the intestine are the same in man as in lower animals; in particular, hepatic cholesterogenesis is markedly suppressed by cholesterol feeding whereas intestinal cholesterogenesis is not. Thus, it is unlikely that the differences in regulation of over-all cholesterol metabolism in these various species are due to fundamental differences in the regulation of cholesterol biosynthesis at the tissue level. Rather, it is likely that differences in some other process, e.g. cholesterol absorption or cholesterol degradation, account for the striking differences in the response of various animal species to cholesterol feeding (7–9).

ACKNOWLEDGMENTS

This work was supported by U. S. Public Health Service Training Grant T1-AM-5490 and Research Grant T1-HE-09610 and by a grant from the John and Mary Markle Foundation. During these studies Dr. Gamel was a postdoctoral fellow supported by U. S. Public Health Service Training Grant T1-AM-5490. Dr. Dietschy is a Markle Scholar in Academic Medicine. The cholestyramine used in these studies was generously supplied by the Mead-Johnson Company, Indianapolis, Ind.

REFERENCES

1. Srere, P. A., I. L. Chaikoff, S. S. Treitman, and L. S. Burstein. 1950. The extrahepatic synthesis of cholesterol. *J. Biol. Chem.* **182**: 629.
2. Dietschy, J. M., and M. D. Siperstein. 1967. Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat. *J. Lipid Res.* **8**: 97.
3. Dietschy, J. M., and J. D. Wilson. 1968. Cholesterol synthesis in the squirrel monkey: Relative rates of synthesis in various tissues and mechanisms of control. *J. Clin. Invest.* **47**: 166.
4. Friedman, M., S. O. Byers, and F. Michaelis. 1951. Production and excretion of cholesterol in mammals. IV. Role of liver in restoration of plasma cholesterol after experimentally induced hypocholesteremia. *Amer. J. Physiol.* **164**: 789.
5. Hotta, S., and I. L. Chaikoff. 1955. The role of the liver in the turnover of plasma cholesterol. *Arch. Biochem. Biophys.* **56**: 28.
6. Lindsey, C. A., Jr., and J. D. Wilson. 1965. Evidence for a contribution by the intestinal wall to the serum cholesterol of the rat. *J. Lipid Res.* **6**: 173.
7. Wilson, J. D. 1968. Biosynthetic origin of serum cholesterol in the squirrel monkey: Evidence for a contribution by the intestinal wall. *J. Clin. Invest.* **47**: 175.
8. Taylor, C. B., D. Patton, N. Yogi, and G. E. Cox. 1960. Diet as source of serum cholesterol in man. *Proc. Soc. Exp. Biol. Med.* **103**: 768.
9. Wilson, J. D., and C. A. Lindsey, Jr. 1965. Studies on the influence of dietary cholesterol on cholesterol metabolism in the isotopic steady state in man. *J. Clin. Invest.* **44**: 1805.
10. Taylor, C. B., G. E. Cox, L. G. Nelson, C. B. Davis, Jr., and G. M. Hass. 1955. In vitro studies on human hepatic cholesterol synthesis. *Circulation* **12**: 489. (Abstr.)
11. Bhattathiry, E. P. M., and M. D. Siperstein. 1963. Feedback control of cholesterol synthesis in man. *J. Clin. Invest.* **42**: 1613.
12. Fujiwara, T., H. Hirono, and T. Arakawa. 1965. Idiopathic hypercholesterolemia: Demonstration of an impaired feedback control of cholesterol synthesis *in vivo*. *Tohoku J. Exp. Med.* **87**: 155.
13. Pawliger, D. F., and J. C. Shipp. 1968. Familial hypercholesteremia: Effect of exogenous cholesterol on cholesterol biosynthesis *in vivo* and by liver *in vitro*. *Clin. Res.* **16**: 51. (Abstr.)
14. Siperstein, M. D., and M. J. Guest. 1960. Studies on the site of the feedback control of cholesterol synthesis. *J. Clin. Invest.* **39**: 642.
15. Dietschy, J. M., and M. D. Siperstein. 1965. Cholesterol synthesis by the gastrointestinal tract: Localization and mechanisms of control. *J. Clin. Invest.* **44**: 1311.

16. Dietschy, J. M. 1968. The role of bile salts in controlling the rate of intestinal cholesterogenesis. *J. Clin. Invest.* **47**: 286.
17. Morris, M. D., I. L. Chaikoff, J. M. Felts, S. Abraham, and N. O. Fansah. 1957. The origin of serum cholesterol in the rat: Diet versus synthesis. *J. Biol. Chem.* **224**: 1039.
18. Gould, R. G. 1951. Lipid metabolism and atherosclerosis. *Amer. J. Med.* **11**: 209.
19. Tomkins, B. M., and I. L. Chaikoff. 1952. Cholesterol synthesis by liver. I. Influence of fasting and of diet. *J. Biol. Chem.* **196**: 569.
20. Gould, R. G., C. B. Taylor, J. S. Hagerman, I. Warner, and D. J. Campbell. 1953. Cholesterol metabolism. I. Effect of dietary cholesterol on the synthesis of cholesterol in dog tissue in vitro. *J. Biol. Chem.* **201**: 519.
21. Cox, G. E., L. G. Nelson, W. B. Wood, and C. B. Taylor. 1954. Effect of dietary cholesterol on cholesterol synthesis in monkeys' tissue in vitro. *Fed. Proc.* **13**: 31. (Abstr.)
22. Taylor, C. B., L. Nelson, M. Stumpe, G. Cox, and R. Tamura. 1956. Hepatic cholesterol synthesis: its depression by dietary cholesterol and subsequent recovery on a cholesterol-free diet. *Fed. Proc.* **15**: 534. (Abstr.)
23. Siperstein, M. D. 1960. The homeostatic control of cholesterol synthesis in liver. *Amer. J. Clin. Nutr.* **8**: 645.
24. Siperstein, M. D., and V. M. Fagan. 1966. Feedback control of mevalonate synthesis by dietary cholesterol. *J. Biol. Chem.* **241**: 602.
25. Weis, H. J., and J. M. Dietschy. 1969. Failure of bile acids to control hepatic cholesterogenesis: Evidence for Endogenous Cholesterol Feedback. *J. Clin. Invest.* **48**: 2398.
26. Bucher, N. L. R., K. McGarrahan, E. Gould, and A. V. Loud. 1959. Cholesterol biosynthesis in preparations of liver from normal, fasting, x-irradiated, cholesterol-fed, Triton, or Δ^4-cholesten-3-one-treated rats. *J. Biol. Chem.* **234**: 262.
27. Frederickson, D. S., A. V. Loud, B. T. Hinkelman, H. S. Schneider, and I. D. Frantz, Jr. 1954. The effect of ligation of the common bile duct on cholesterol synthesis in the rat. *J. Exp. Med.* **99**: 43.
28. Myant, N. B., and H. A. Eder. 1961. The effect of biliary drainage upon the synthesis of cholesterol in the liver. *J. Lipid Res.* **2**: 363.
29. Danielsson, H., K. Einarsson, and G. Johansson. 1967. Effect of biliary drainage on individual reactions in the conversion of cholesterol to taurocholic acid. *Eur. J. Biochem.* **2**: 44.
30. Salen, G., E. H. Ahrens, Jr., and S. M. Grundy. 1970. Regulation of intestinal synthesis of cholesterol in patients with hyperlipidemia. *J. Clin. Invest.* **49**: 84a. (Abstr.)