# Cholesterol Synthesis in the Squirrel Monkey: Relative Rates of Synthesis in Various Tissues and Mechanisms of Control

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ABSTRACT Cholesterol synthesis has been extensively investigated in various tissues of lower mammals; however, there is little specific information concerning cholesterologenesis in the primate. Furthermore, experiments in whole animals suggest that important differences may exist in the features of cholesterologenesis in the dog and rat versus the monkey and man. Using the new world squirrel monkey, therefore, we performed the present studies to determine the rates of cholesterologenesis in various tissues per unit weight, to define the relative rates of whole organ synthesis, and to evaluate the operation of control mechanisms in these tissues.

In control animals fed a low cholesterol chow diet, the liver and ileum were the two most active sites for cholesterologenesis followed, in order, by the colon, esophagus, and proximal small bowel. Rates of synthesis in 10 other tissues tested were considerably lower than these found in the gastrointestinal tract. When rates of whole organ synthesis were calculated, three tissues, i.e. liver, bowel, and skin, accounted for 92% of the total demonstrable synthetic activity.

Following cholesterol feeding utilizing either a solid chow or liquid formula diet, marked suppression of hepatic cholesterologenesis occurred while synthesis in other organs remained essentially unaltered. Similarly, fasting animals for periods up to 96 hr resulted in suppression of synthesis in the liver, but not in various levels of the intestine. Finally, biliary diversion for 48 hr caused a twofold increase in hepatic cholesterologenesis and a six- to eightfold increase in sterol synthesis in the small but not the large intestine.

## INTRODUCTION

While virtually every mammalian tissue is capable of cholesterol synthesis (1, 2), several quantitative aspects of cholesterologenesis have never been elucidated. First, large differences in rates of synthesis exist between different organ systems. Such tissues as liver, skin, and intestine manifest active conversion of acetate into sterol, whereas at the other end of the spectrum, muscle and mature nervous tissue synthesize cholesterol at extremely low rates (1, 2). Second, several control mechanisms have been described which act in specific tissues to modulate the rate of sterol synthesis. Cholesterol feeding or fasting, for example, selectively depresses the rate of sterol synthesis in the liver of the rat (2–5), while biliary diversion in the same animal results in a striking enhancement of sterol synthesis by the small in-

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testine (6, 7). Third, not only do inherent rates of synthesis and control mechanisms vary among tissues, but in addition, it is necessary in considering over-all sterol economy in the intact animal to take into account the extreme variation in organ size. Thus, it is entirely possible that large tissues with low rates of cholesterol synthesis when expressed as a function of unit weight might, in fact, make major contributions to cholesterol balance in the whole animal. Fourth, a separate problem involves evaluation of the ultimate biosynthetic site(s) of the circulating cholesterol pool. Only two tissues have been shown thus far to contribute significantly to the circulating cholesterol pool. Several early investigators provided convincing evidence that the liver made an important contribution to the serum cholesterol (3, 8–10); more recently, data have been presented from this laboratory which indicate that sterol synthesized de novo in the gastrointestinal tract also reaches the circulation (11).

And, finally, in addition to consideration of organ size, synthetic rates and control mechanisms in different tissues, the relative importance of these various factors probably varies among species. For example, evidence suggests that in the rat and dog the liver is the principal endogenous source for circulating cholesterol (12, 13), whereas in man (13–15) and several other primate species (16, 17), other tissues such as the intestinal tract may serve as the major biosynthetic sites.

Despite the importance of knowledge of sterol synthetic rates and control mechanisms in problems of sterol metabolism, however, cholesterol synthesis has never been characterized in detail in tissues of a primate. Utilizing one species of new world primate, the squirrel monkey, we designed the present investigations to systematically compare the relative rates of cholesterol synthesis in various tissues per unit weight and the magnitude of whole organ synthesis both in normal circumstances and under conditions of altered metabolic control.

## METHODS

*Animal preparations.* Female *Samiris* or squirrel monkeys of undetermined strains, weighing 442–825 g, were used in these experiments.[1] All animals were housed in individual cages and quarantined for 1 wk during which time they were allowed access ad lib. to monkey chow (Ralston Purina Co., St. Louis, Mo.) and water containing tetracycline, 100 mg/liter. After this week of quarantine, no further antibiotics were administered. During the periods of study records of weights and diet consumption were kept. At the termination of these experiments, occasional animals were found to be infested with filaria; however, no difference was noted in experimental results in tissues obtained from infested, as compared with noninfested, animals.

*Experimental diets.* Two experimental diets were utilized in these studies: (*a*) a solid, chow diet (Purina Monkey chow) and (*b*) a liquid, formula diet (Lactum, Mead Johnson & Co., Evansville, Ind.). Low cholesterol chow diet was prepared by adding 7 g of triolein to each 100 g of diet and, by direct analysis, contained 0.04 mg of cholesterol/g of diet. High cholesterol chow diets were prepared by adding 7 g triolein in which was dissolved either 0.2, 0.5, or 1.0 g of cholesterol to 100 g of diet (0.2, 0.5, and 1.0% cholesterol chow diets, respectively). The liquid, formula diet was prepared by homogenizing in a food blender 300 g of Lactum powder, 300 ml of water, one tablet of Poly-vi-sol (Mead Johnson & Co.), and 100 mg of ferrous sulfate. This diet was used as the low cholesterol formula diet and by direct analysis contained 0.19 mg of cholesterol/ml. High cholesterol formula diet was prepared by homogenizing 1.0 g of cholesterol in 100 ml of the liquid diet (1.0% cholesterol formula diet). The feeding schedule and weight gains for each experimental period are given in the appropriate following sections.

*Tissue preparations.* At the termination of each experiment the monkeys were killed by a blow on the head. The various organs were dissected and washed in cold saline. Slices 1 mm thick of ovary, skin, striated thigh muscle, adrenal, and colon were prepared by hand, while a McIlwain (H. Mickle, Gomshall, Surrey, England) tissue slicer was utilized to make slices of the same thickness from liver, esophagus, stomach, small intestine,

TABLE I

*Rates of Incorporation of Acetate-2-$^{14}$C into Cholesterol as a Function of Varying Concentrations of Acetate in the Incubation Media*

| Final concentration of acetate-2-$^{14}$C in media | Conversion of acetate-2-$^{14}$C to cholesterol | | |
|---|---|---|---|
| | 0.55 | 1.10 | 2.20 |
| *μmoles/ml* | *mμmoles/g per 2 hr* | | |
| Liver | 332* | 486 | 558 |
| Small bowel | 29 | 48 | 53 |
| Colon | 80 | 104 | 109 |
| Skin | 16 | 18 | 16 |
| Kidney | 25 | 27 | 31 |

* Each value represents the mean of duplicate determinations.

[1] Animals obtained from Woodard Asiatic Animal Imports, Inc., San Francisco, Calif.

TABLE II

*Percentage Distribution of $^{14}C$ in Specific Sterols after Separation of the Total Digitonin Precipitable Fraction by Thin-Layer Chromatography*

| Tissue | Silica gel H system | | | | $AgNO_3$ system | |
|---|---|---|---|---|---|---|
| | Cholesterol + cholestanol | $\Delta^7$-Cholestenol | $\Delta^7$- and $\Delta^8$-Methostenols | Lanosterol | Cholestanol | Unsaturated sterols |
| | *% of counts recovered from plates* | | | | | |
| Liver | 94.2 | 0.8 | 0.7 | 1.2 | 0.5 | 99.5 |
| Esophagus | 85.6 | 5.9 | 0.4 | 0.4 | <0.1 | >99.9 |
| Stomach | 82.0 | 7.7 | <0.1 | 0.8 | <0.1 | >99.9 |
| Small intestine | 82.7 | 3.1 | 1.7 | 2.5 | 0.4 | 99.6 |
| Large intestine | 86.8 | 2.2 | 1.6 | 2.4 | 0.7 | 99.3 |
| Skin | 18.3 | 51.6 | 3.3 | 12.7 | 1.2 | 98.8 |

kidney, peritoneal adipose tissue, lung, spleen, and brain. Bone marrow was obtained from both femurs of each animal, weighed, and incubated without slicing. 400 mg of slices were used from all other tissues except the ovary and adrenal, which were sliced, weighed, and incubated in toto. All tissue slices were incubated in 5.0 ml of Krebs' bicarbonate buffer containing 1 $\mu$c of acetate-2-$^{14}C$ (1.0 $\mu$c/0.5 $\mu$mole) and 5 $\mu$moles of sodium acetate for 2 hr at 37°C in a metabolic shaker at 100 oscillations/min. In preliminary studies, as shown in Table I, it was determined that this concentration of acetate, 1.10 $\mu$mole/ml, was sufficiently high so that the incorporation rates were determined essentially under conditions of zero order kinetics.

TABLE III

*Rates of Cholesterologenesis per U Wt in Various Tissues of the Squirrel Monkey**

| Tissue | Low cholesterol chow diet | 0.5–1.0% Cholesterol chow diet |
|---|---|---|
| | *m$\mu$moles/g per 2 hr* | |
| Liver | 612.8 ± 176.3 | 24.9 ± 16.3 |
| Colon‡ | 71.5 ± 21.7 | 50.7 ± 13.5 |
| Small bowel‡ | 54.8 ± 13.9 | 26.2 ± 10.8 |
| Ovary | 14.6 ± 4.6 | 35.6 ± 12.8 |
| Skin | 10.3 ± 4.9 | 14.6 ± 3.2 |
| Kidney | 6.5 ± 1.8 | 3.3 ± 0.6 |
| Marrow | 5.7 ± 0.7 | 4.2 ± 0.2 |
| Adipose tissue | 5.4 ± 0.7 | 2.6 ± 0.2 |
| Lung | 3.5 ± 0.6 | 3.3 ± 1.1 |
| Spleen | 3.3 ± 0.6 | 2.3 ± 0.4 |
| Adrenal | 0.9 ± 0.2 | 0.5 ± 0.2 |
| Brain | 0.5 ± 0.3 | 0.1 ± 0.0 |
| Muscle | 0.3 ± 0.1 | 0.1 ± 0.0 |

* Animals were fed either the low cholesterol chow diet (n = 6), or the 0.5% (n = 2), or 1.0% (n = 4) cholesterol chow diet for 1 wk. Since no differences were noted in animals fed either of the high cholesterol diets, data from these six animals were combined. The tissues of the control animals are arranged in order of descending activity. Mean values ±1 SE are given.

‡ Mean rates of synthesis are given for colon and small bowel; rates at different levels of the gastrointestinal tract are shown in Fig. 2.

*Chemical methods.* The method for determining the incorporation of radioactive acetate into digitonin precipitable sterols has been described in detail (5). Briefly, this procedure may be outlined as follows: the contents of the incubation flasks were saponified, made up to a 50% ethanolic solution, and extracted with petroleum ether to remove nonsaponifiable lipids. The petroleum ether extracts were taken to dryness, and the residue was redissolved in ethanol: acetone, 1 : 1, 3$\beta$-hydroxy sterols were then precipitated as the digitonides, washed with acetone and diethyl ether, and dissolved in methanol. A sample of this solution was placed in 2,5-diphenyloxazole-1,4-bis[2-(5-phenyloxazolyl)] benzene (PPO-POPOP) scintillation fluid for $^{14}C$ assay as previously described (6). All samples were counted in a Packard liquid scintillation counter, series 314E. The data are expressed as the mean ± 1 SE of the m$\mu$moles of acetate-2-$^{14}C$ incorporated into digitonin precipitable sterols per g wet weight of tissue per 2 hr incubation.

*Chromatographic methods.* For purposes of fractionation of the digitonin precipitable sterols by thin-layer chromatography (TLC), the free sterols were first regenerated by the method of Sperry (18). In this procedure the precipitate of sterol digitonides was dissolved in pyridine, and the free sterols were extracted with diethyl ether. After drying in vacuo over concentrated sulfuric acid to remove the pyridine, the free sterols were redissolved in chloroform for chromatographic analysis.

TLC was carried out on 20 × 40 cm glass plates coated with a suspension of silica gel H (Brinkmann Instruments Inc., Westbury, N. Y.) in distilled water, 50 g/100 ml, and activated in an oven for 1 hr at 100°C. After standard and tissue sterols were applied, the plates were developed for 4–6 hr in benzene–ethyl acetate 5 : 1 until the solvent front had run a full 35 cm. These long plates gave good separation of four major groups of sterols;

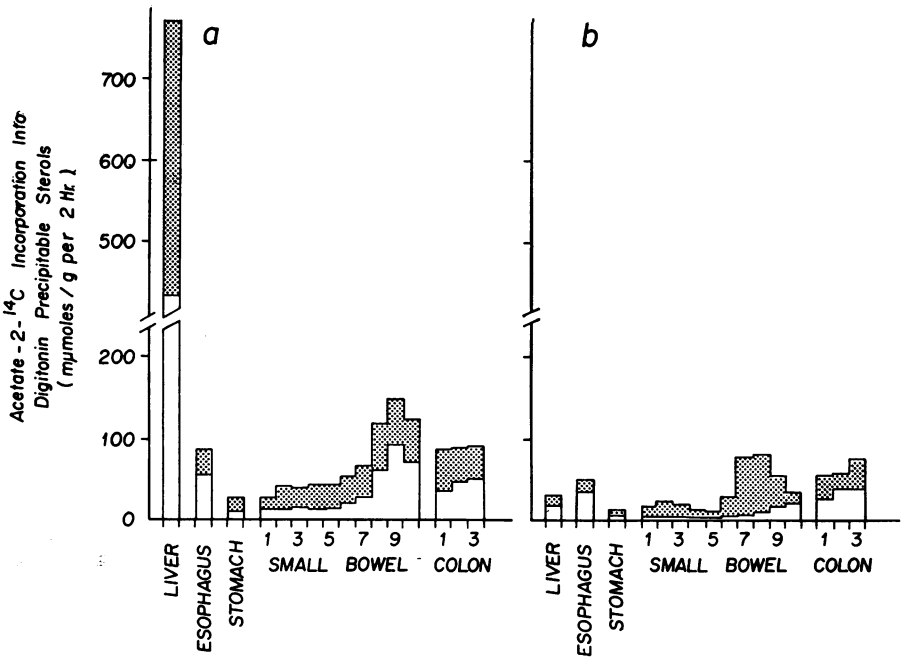


FIGURE 1 Rates of cholesterologenesis in the gastrointestinal tract of monkeys fed chow diet. Animals were fed either the (*a*) low cholesterol chow diet (n = 6), or (*b*) the 0.5% (n = 2), or 1.0% (n = 4) cholesterol chow diet for 1 wk. No difference was noted in the rates of synthesis in the animals fed either the 0.5 or 1.0% cholesterol diets so that data from these six animals were combined. The stippled areas represent mean values ± 1 SE. The small intestine was divided into 10 segments numbered 1–10, proximal to distal; the colon was similarly divided into three segments. The data are expressed as the mμmoles of acetate-2-$^{14}$C incorporated into digitonin precipitable sterols per g of tissue during a 2 hr incubation.

cholesterol plus cholestanol, lanosterol, $\Delta^7$-cholestenol, and $\Delta^7$- plus $\Delta^8$-methostenols. A second aliquot of the tissue sterols was chromatographed on 20 × 20 cm plates coated with a 50% suspension of silica gel G (Brinkmann Instruments Inc.) in 12% silver nitrate solution as previously described (2). This method allows separation of the faster running cholestanol from cholesterol and other unsaturated sterols.

After development the plates were dried, sprayed with a 0.005% rhodamine G (Allied Chemical Corp., New York) solution, and examined under ultraviolet light. For assay of the $^{14}$C content of each sterol, the individual spots were scraped from the plates and put directly into counting vials containing PPO-POPOP scintillation fluid. Quenching was corrected for by internal standardization.

As shown in Table II, the great majority of the $^{14}$C was found in cholesterol in all tissues except skin. Thus, of the total acetate-2-$^{14}$C incorporated into digitonin precipitable sterols, 82.0–86.8 and 94.2% were found specifically in cholesterol in the gastrointestinal tract and liver, respectively, whereas only 18.3% of the $^{14}$C from skin was in the cholesterol area. This finding correlates with the observation that of all the tissues in this species only in skin have significant amounts of sterols other than cholesterol been identified (17). It is clear that in the case of the liver and gastrointestinal tract, digitonin precipitable sterol-$^{14}$C primarily represents labeled cholesterol; since these tissues are quantitatively the most important sites of cholesterol synthesis and since they were the main subject of scrutiny in these studies, acetate-2-$^{14}$C incorporation into sterols is subsequently referred to as cholesterol synthesis.

## RESULTS

*Relative rates of cholesterologenesis in tissues of control animals.* Initial experiments were undertaken to determine the relative rates at which slices from various tissues of animals fed the low cholesterol chow diet incorporate acetate-2-$^{14}$C into digitonin precipitable sterols. Animals were fed the low cholesterol chow diet for 1 wk; the average food intake was 24 g/day, and the mean weight gain was 52 g/week. The results of these studies are shown in Table III and Fig. 1 *a*.

As is apparent from these data, the liver with a synthetic rate of 612.8 ± 176.3 mμmoles/g per 2

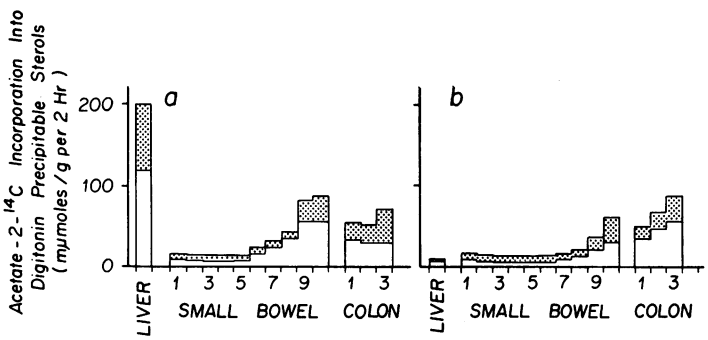


FIGURE 2 Rates of cholesterologenesis in the gastrointestinal tract of monkeys fed a liquid formula diet. Animals were fed by stomach tube either the (*a*) low cholesterol formula diet (n = 4) or the (*b*) 1.0% cholesterol formula diet (n = 4) for 1 wk. The rates of synthesis are shown at each of 10 different levels of the small bowel and of three levels of the colon. The stippled areas represent mean values ± 1 SE.

hr was by far the most active tissue. Various parts of the gastrointestinal tract manifested the next highest rates of cholesterologenesis; however, as demonstrated in Fig. 1, important variations in synthetic rates existed down the length of the alimentary tract. For example, synthesis was relatively high in the esophagus, terminal ileum, and colon, but was considerably lower in the stomach and proximal small bowel. It should be emphasized that the mean rate of acetate incorporation by slices from terminal ileum exceeded 100 mμmoles/g per 2 hr, establishing this tissue as the second most active site of cholesterologenesis found in the monkey. As shown in Table III, all other tissues tested had rates of sterol synthesis considerably lower than those of the liver and gastrointestinal tract; these varied from 14.6 ± 4.6 mμmoles/g per 2 hr in ovary to only 0.3 ± 0.1 mμmole/g per 2 hr in striated muscle.

TABLE IV

*Rates of Cholesterologenesis in Whole Organs of the Squirrel Monkey**

| Tissue | Mean organ weight (n = 4) | Low cholesterol chow diet | 0.5–1.0% Cholesterol chow diet |
|---|---|---|---|
| | *g* | *mμmoles/whole organ per/2 hr* | |
| Whole animal | 576.00 | — | — |
| Liver | 22.43 | 13,745 | 558 |
| Small bowel‡ | 25.12 | 1375 | 656 |
| Skin | 91.00 | 937 | 1329 |
| Colon‡ | 5.40 | 386 | 274 |
| Muscle§ | 284.00 | 85 | 28 |
| Stomach | 4.16 | 78 | 32 |
| Esophagus | 0.97 | 67 | 45 |
| Mesenteric fat | 5.45 | 30 | 14 |
| Kidney | 3.45 | 22 | 11 |
| Lungs | 5.12 | 18 | 17 |
| Brain | 22.50 | 11 | 2 |
| Spleen | 2.12 | 7 | 5 |
| Ovary | 0.21 | 3 | 7 |
| Adrenals | 0.25 | 0.2 | 0.1 |

* Tissues from animals fed the low cholesterol diet are arranged in descending order of synthetic activity.

‡ Mean values for the rates of synthesis at different levels of the small and large bowels were used in these calculations.

§ Muscle weight was determined by subtracting the weight of the bones from the weight of the dissected carcass containing both muscle and skeleton.

In order to evaluate the effects of a semisynthetic formula diet on gastrointestinal cholesterologenesis, we performed the experiments shown in Fig. 2 *a*. Animals were tube fed 40 ml/day of the low cholesterol formula diet; this resulted in an average weight gain of 14 g/week. While the liver again was the tissue with most active cholesterologenesis, the rate of cholesterol synthesis in this tissue (162.1 ± 45.5 mμmoles/g per 2 hr) was only about one-third of the rate found in the monkeys fed the solid chow diet. In contrast, however, cholesterol synthesis in the small and large intestine was similar in the animals fed the two diets with respect both to rates and to the distribution of activity down the length of the bowel.

*Calculated rates of cholesterologenesis in whole organs.* Whereas these experiments allow a comparison of the relative rates of cholesterologenesis per unit weight of various tissues, equally important is knowledge of the over-all rate of sterol synthesis per whole organ. In order to calculate such data for monkeys fed the low cholesterol chow diet, we multiplied the mean rate of acetate-2-$^{14}$C incorporation into sterols times the appropriate mean organ weight for each tissue as shown in Table IV. Clearly three tissues account for over 98% of the total sterol synthetic activity detected by this in vitro study; liver, combined small and large bowel, and skin demonstrated

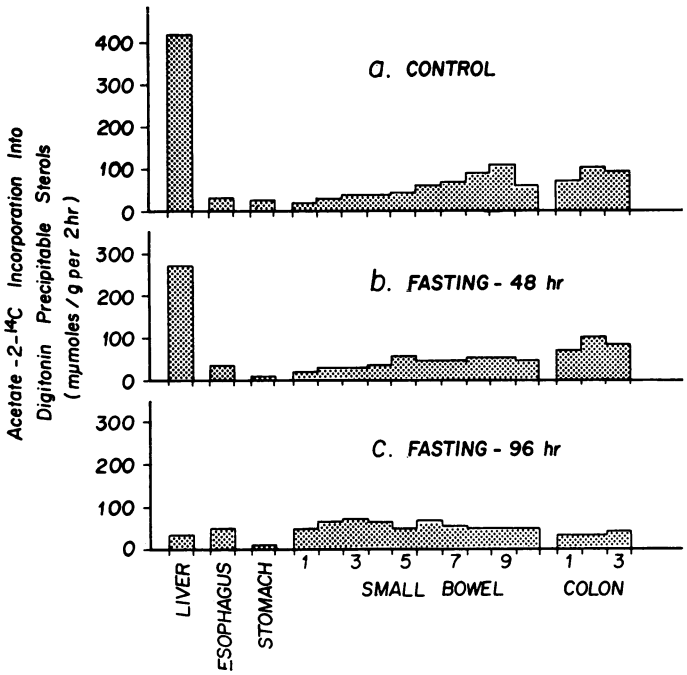


FIGURE 3 Effect of fasting on cholesterologenesis in the gastrointestinal tract of the monkey. Animals of similar weight either were maintained on (*a*) low cholesterol chow diet (control, n = 2), or were (*b*) fasted for 48 hr (n = 2), or (*c*) 96 hr (n = 2) at the end of which time the rates of sterol synthesis in the liver and gastrointestinal tract were assayed. Each value represents the mean of the rates determined in two animals.

whole organ acetate-2-$^{14}$C incorporation rates of 13,745, 1761, and 937 mμmoles/2 hr, respectively. The entire amount of synthesis detected in all other organs combined accounted for less than 2% of the total. Furthermore, if one takes into account the fact that only 18% of the sterol synthetic activity of skin represents the synthesis of cholesterol, then two tissues, the liver and gastrointestinal tract, account for at least 97% of the total cholesterol synthesized under these conditions. It is noteworthy that muscle, the largest tissue mass in the body, contributes little to the total assayable sterol synthetic activity (0.5%).

*Effects of cholesterol feeding on rates of cholesterologenesis in various tissues of the monkey.* The effects of feeding a high cholesterol chow diet for 1 wk on the rates of sterol synthesis in various organs is shown in Table III and Fig. 1 *b*. The average food intake in these animals equaled 29 g/day, and the mean weight gain was 33 g/week. The liver of the monkey was clearly responsive to cholesterol feeding, the rate of hepatic cholesterologenesis decreased from a control value of 612.8 ± 176.3 mμmoles/g per 2 hr to only 24.9 ± 16.3 mμmoles/g per 2 hr after cholesterol ingestion. Since no difference in the degree of inhibition was seen with the feeding of either the 0.5 or 1.0% cholesterol chow diet, data from these two groups of animals were combined. Feeding a 0.2% cholesterol chow diet, however, resulted in incomplete inhibition of hepatic cholesterologenesis to a mean rate of 126 mμmoles/g per 2 hr. In striking contrast to the findings in liver, cholesterol feeding did not result in a significant alteration in the rate of cholesterologenesis in any other tissue assayed; thus the liver of the monkey, like that of the rat, is the only tissue in which the rate of cholesterol synthesis is controlled by a feedback mechanism responsive to exogenous cholesterol.

As shown in Fig. 2, a similar finding was demonstrated in animals fed the 1.0% cholesterol formula diet. The average weight gain in these animals was 21 g/week. While the control value for hepatic cholesterologenesis in the animals fed the low cholesterol formula diet was only 162.1 ± 45 mμmoles/g per 2 hr, the effect of cholesterol feeding in these animals was on an average more marked than in the case of the chow diet (8.2 ± 2.3 vs. 24.9 ± 16.3 mμmoles/g per 2 hr). Again, no inhibitory effect upon intestinal cholesterologenesis was evident. These studies provide further evidence for the exclusive responsiveness of the liver of the monkey to exogenous cholesterol.

The effect of cholesterol feeding in terms of whole organ synthesis is shown in the third column

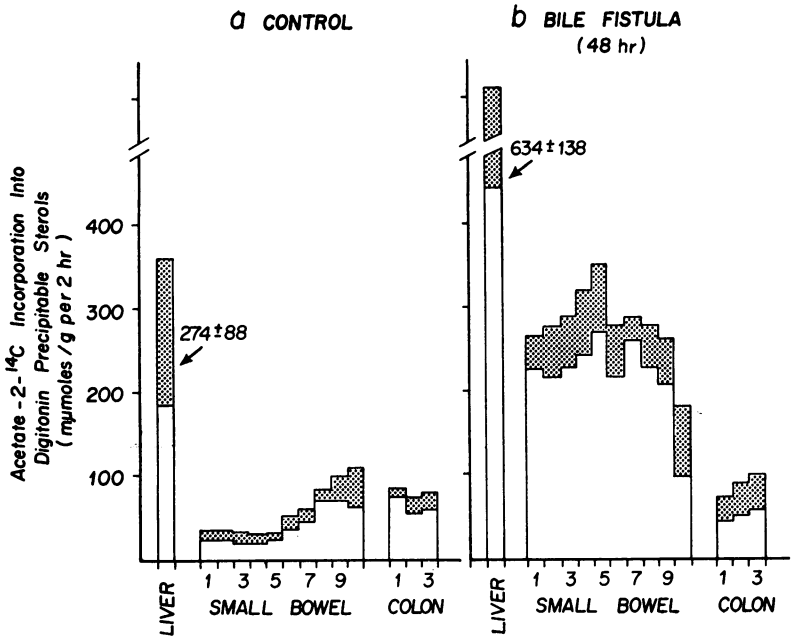


FIGURE 4 Effect of biliary diversion on cholesterologenesis in the gastrointestinal tract of the monkey. (*a*) Control animals (n = 4) were sham operated while external biliary diversion was performed on a (*b*) second group of animals (n = 4) 48 hr before assaying synthetic activity. Both groups were tube fed identical amounts of the low cholesterol formula diet during this experimental period. The stippled areas represent mean values ± 1 SE.

of Table IV. With marked suppression of hepatic cholesterologenesis the relative importance of synthesis in the combined small and large bowel and skin increases considerably, so that synthesis in these two tissues accounts for 31 and 44%, respectively, of the total detectable sterol synthetic activity. Again, taking into consideration that of the synthetic activity determined in skin only 18.3% represents cholesterol synthesis, then it can be further calculated that cholesterologenesis in the gastrointestinal tract accounts for at least 49% of the total detectable cholesterol synthetic activity.

*Effect of fasting on rates of cholesterologenesis in the liver and gastrointestinal tract.* In order to determine the effects of fasting on hepatic and intestinal cholesterologenesis, three groups of two animals each were either placed on a low cholesterol chow diet or were fasted for 48 or 96 hr. The two animals fasted 48 hr lost an average of 72 g in weight, while the two animals fasted for 96 hr lost an average of 133 g in weight.

As shown in Fig. 3, fasting for 48 hr resulted in a decrease in the rate of hepatic cholesterologenesis from a mean value of 418 mμmoles/g per 2 hr in control animals to 273 mμmoles/g per 2 hr. However, after a 96 hr fast hepatic sterol synthesis was profoundly depressed to a mean value of 36 mμmoles/g per 2 hr. In contrast to this tenfold depression of synthesis in the liver, cholesterologenesis in both the large and small bowel was, at most, depressed by fasting to 50% of control values.

*Effects of biliary diversion on cholesterologenesis in the liver and gastrointestinal tract.* In order to determine whether cholesterologenesis in the gastrointestinal tract in the monkey was under feedback control by bile, four control animals were sham operated, while the common duct was cannulated below the level of the cystic duct in four additional monkeys. The catheter was exteriorized through a dorsal stab wound, and bile was allowed to drain freely to the outside. Neither group was fed the night after surgery, but during the subsequent 36 hr they were tube fed 20 ml of the low cholesterol formula diet three times. 48 hr after surgery the animals were killed, and the synthetic activity in the intestine and liver was assayed.

As shown in Fig. 4 synthesis in the liver increased about twofold from 274 ± 88 mμmoles/g per 2 hr to 634 ± 138 mμmoles/g per 2 hr after biliary diversion. Synthesis in the small intestine increased much more dramatically, reaching values in the animals with biliary diversion which were nearly eightfold greater than those found in control animals. In contrast to the situation in the liver and small bowel, however, biliary diversion did not alter the rate of sterol synthesis in the colon.

## DISCUSSION

Since the initial observation by Bloch, Borek, and Rittenberg in 1946 that rat liver could incorporate acetate into digitonin precipitable sterols (19), virtually every tissue in the mammal has been shown to be capable of cholesterologenesis (1, 2). In addition, a number of studies have been directed at the elucidation of mechanisms whereby control of cholesterologenesis in several of these organ systems is achieved. Virtually all of these investigations, however, have been undertaken in lower animals, particularly the rat, guinea pig, and dog, and only a very limited number of studies in the primate have been published. Yet several observations (13, 14, 16, 17) suggest that important differences exist in sterol metabolism between these lower mammals and the monkey and man. This study was undertaken, therefore, to investigate systematically cholesterol synthesis in the monkey with respect both to the relative rates of synthesis in various organ systems and to the mechanisms by which control of sterol synthesis is achieved.

From the results presented in this study, several conclusions are warranted. First, as in the rat (2), of all tissues examined liver manifests the highest rate of synthesis followed by various portions of the gastrointestinal tract, most strikingly the terminal ileum. Second, the importance of liver and intestine as biosynthetic sites for cholesterol becomes even more striking when organ weight is taken into consideration; thus, in the animals fed the low cholesterol diet, these tissues account for 97% of total cholesterol synthesis, while in the cholesterol-fed animals they account for 79%. When the gastrointestinal tract is considered alone, it accounts for 11% of cholesterol synthetic activity in control animals and 49% of total synthetic activity in animals fed a high cholesterol diet. Third, as previously reported by Cox et al. (20) and MacNintch et al. (21), there is an extremely effective cholesterol negative feedback mechanism in the liver of the monkey; hepatic cholesterologenesis is depressed 25-fold after only one wk of feeding a high cholesterol diet. Furthermore, as has been previously reported in lower animals (2–5) and the monkey (20), the liver appears to be unique in the possession of this feedback mechanism, no other tissue in this study having demonstrated a significant inhibition of cholesterol synthesis after this dietary maneuver. Fourth, cholesterol synthesis in the liver also appears to be very sensitive to the effects of fasting, since the mean hepatic sterol synthetic rate falls to about 8% of control values after 96 hr of deprivation under conditions in which synthesis at various levels of the gastrointestinal tract was not strikingly altered. Finally, after diversion of biliary contents from the intestine, there was a marked increase in sterol synthesis both in the liver and at all levels of the small intestine.

From these data two generalizations seem justified. First, in regard to absolute rates, the relative contributions of various organs and metabolic control, cholesterol synthesis in this species of monkey appears to be almost identical to the findings in the rat. While such extensive chemical dissection has not yet been reported for any species other than rat and monkey, fragmentary data in other animals as well as in man (22, 23) suggest that the important features of sterol synthesis are similar in all species. At any rate, it is clear that the quantitative differences which have been reported between rat and monkey in the relative importance of endogenous and exogenous contributions to the circulating serum cholesterol (13, 14, 16, 17) cannot be the result of differences in the control of cholesterol synthesis but must in fact be the consequence of differences either in cholesterol absorption, cholesterol turnover or excretion, or some other parameter of cholesterol metabolism.

Second, while there are definite limitations to the extrapolation to the whole animal of in vitro biosynthetic data, the results of the present study emphasize anew both the relative and absolute importance of the gastrointestinal tract as a biosynthetic site of cholesterol in the monkey under circumstances of both high and low cholesterol feeding. This is particularly true if these data are taken together with other recent studies of this problem. Not only does the intestine of the monkey contribute locally synthetized cholesterol into the circulation, but more importantly, acetate-$^{14}$C conversion into circulating cholesterol for at least 48 hr is almost entirely inhibited in the cholesterol-fed monkey in which the intestinal contribution is diverted from the circulation by intestinal lymphatic cannulation (17). In addition, utilizing a double isotopic technique, we have estimated the contribution of the intestine into the circulation of

this species of monkey to be constant regardless of the level of dietary intake of cholesterol (17). And, since the degree of equilibration between skin cholesterol and that of the circulation is limited both because of the epithelial barrier of the skin and because of continuous desquamation of hair, epithelial cells, and sebum, it is entirely possible that liver and intestine are the only significant sites for the biosynthesis of circulating cholesterol.

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