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Research Article

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The Relation between Cholesterol Absorption and Cholesterol Synthesis in the Baboon

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ABSTRACT To determine the relation between cholesterol absorption, total endogenous cholesterol synthesis, and hepatic cholesterol synthesis in a primate, cholesterol synthesis has been studied in biopsies of liver and ileum from normal baboons fed varying amounts of cholesterol and in biopsies of liver from baboons that had been subjected to ileal diversion. In addition, total cholesterol production rates, cholesterol absorption, and total endogenous cholesterol synthesis have been measured in these animals by a double isotope technique in which the animals were given a single injection of cholesterol-4-^{14}C and fed constant amounts of cholesterol-1,2-^{3}H for 4 months. From these studies, it has been concluded that on a low cholesterol intake cholesterol synthesis in the liver accounts for about three-fourths of total endogenous cholesterol production. The feeding of cholesterol produces complete inhibition of hepatic synthesis in the normal animal only when absorption approximates the amount synthesized by the liver when no cholesterol is fed, e.g., 400–500 mg/day. Finally, the intestine, which does not possess complete negative feedback control of cholesterol synthesis when cholesterol is fed, may be a significant site of nonhepatic cholesterol synthesis in these animals.

In studies of four baboons subjected to ileal diversion, it was concluded that the regulation of cholesterol synthesis is distinctly different when the enterohepatic circulation is interrupted. These animals did not exhibit negative feedback of hepatic cholesterol synthesis when cholesterol was fed, despite the fact that cholesterol absorption approximated that of normal animals fed similar diets. The inference has been drawn that bile acids may be involved directly or indirectly in the regulation of hepatic cholesterol synthesis in this species or that the ileum itself may modulate the hepatic negative feedback.

INTRODUCTION

As the result of studies in the dog and rat, it has been concluded that the liver is the major biosynthetic source of cholesterol (1–4) and that the rate of cholesterol synthesis in this tissue is regulated via a negative feedback system by the amount of dietary cholesterol (5–8). The net effect of this feedback control of cholesterol synthesis in these species is to tend to stabilize within certain limits the amount of cholesterol entering the miscible pools each day. In regard to the regulation of hepatic cholesterol synthesis in man, however, the situation is less clear; although virtually complete inhibition of hepatic cholesterol synthesis has been reported after the feeding of cholesterol to adults (9) and children (10), in one study it was possible to demonstrate only a partial inhibition of synthesis (11), and in another no inhibition of hepatic cholesterol synthesis was produced by cholesterol feeding (12).

In contrast to the dog (3) and rat (4), furthermore, the feeding of large amounts of radioactive cholesterol to man for long periods results in an apparent isotopic steady state in which only 20–40% of the circulating cholesterol is derived from the diet (3, 12, 13). Finally, in most in vivo studies in normal human subjects it has not been possible either as the result of balance studies (13, 14) or of deuterium feeding experiments (12) to demonstrate that the feeding of dietary cholesterol results in any decrease in the net endogenous production of cholesterol. However, it has been assumed on the basis of studies in which endogenous cholesterol synthesis increased following the feeding of plant sterols that such a feedback control system must be present in man (14), and Quintao, Grundy, and Ahrens have recently reported that total endogenous cholesterol synthesis in some hypercholesterolemic patients is inhibited by cho-

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lesterol feeding (15). If the hepatic feedback control of cholesterol synthesis in man were either absent or incomplete, it follows that any net dietary cholesterol absorption, no matter how small, would either have to be compensated for by some other mechanism or would produce indefinite expansion of the miscible pools.

There are at least three reasons why such a negative feedback system, even if present in human liver, might be difficult to demonstrate. First of all, the capacity of the human intestine to absorb cholesterol is limited in comparison with other species (13–16). Since the maximal absorption is less than total turnover per day, feeding a diet of high cholesterol content would be expected under most conditions to have little effect on hepatic synthesis and external balance, and the present methodology may be inadequate to demonstrate these changes either in in vitro synthesis or in cholesterol balance in man under circumstances in which small amounts are absorbed. Second, it is possible that, as the result of cholesterol ingestion over many years, the liver of man is partially inhibited and that the studies to date have not consistently provided sufficient time on a cholesterol-free diet to allow derepression of this control mechanism before the institution of a high cholesterol intake. Third, the possibility has been raised that in man the intestine, a known site for the synthesis of circulating cholesterol in the rat (17) and monkey (18), or some other extrahepatic tissue that does not contain a negative feedback system might be more important as sources for endogenously synthesized cholesterol than the liver itself (3, 13).

To differentiate among these possibilities, the relation between dietary cholesterol and endogenous production has been evaluated in the baboon, a higher primate which, like man, shows very little change in serum cholesterol in response to cholesterol feeding (19). 36 studies of in vitro cholesterol synthesis by liver biopsies and 25 studies of cholesterol production and absorption have been performed in 14 normal baboons and 4 baboons subjected to ileal diversion under circumstances in which measured quantities of high and low cholesterol diets were fed. In addition, cholesterol synthesis in liver and ileum has been compared in eight baboons that were fed the low cholesterol diet for 1 month before study. As the result of these studies the conclusion has been drawn that the liver is the major source of endogenously synthesized cholesterol in this species, that hepatic synthesis and endogenous production in the normal animal are regulated by the amount of cholesterol absorbed, that a limitation of absorption is probably the major factor back in man, and finally that hepatic cholesterol syn- that makes it difficult to demonstrate cholesterol feed- thesis must be subject to regulation by factors in addition to the cholesterol absorbed into the enterohepatic circulation.

METHODS

Treatment of the animals. Mature male baboons of a variety of *Papio* species (20) were obtained from the Southwest Foundation for Research and Education. Each baboon was maintained in an individual squeeze cage, allowed free access to water, and fed twice daily.

The animals were fed measured quantities of low and high cholesterol diets for varying periods.[1] This quantity of diet (272 or 275 g dry weight) was chosen since the animals ate the entire portion and had very little weight change on this regimen (21). Blood was drawn from the antecubital vein under phencyclidine anesthesia, and in some studies Menghini needle biopsies of liver were obtained. In several instances the abdominal cavity was opened under combined phencyclidine and pentobarbital anesthesia, and wedge biopsies were taken from the ileum, 30 cm proximal to the ileocecal valve. Finally, in four experiments, the terminal third of the small intestine was diverted into a self-emptying blind pouch as described by Buchwald and Varco (22) before the start of the feeding studies.

In vitro incubation studies. The miniaturized assay of cholesterol synthesis described by Bhattathiry and Siperstein (9) was adapted for these experiments. Samples of liver were obtained with the aid of a Menghini needle, and full thickness slices of ileal wall, approximately 0.5 mm thick, were prepared by hand. The samples were rinsed in cold Krebs-Ringer phosphate buffer, pH 7.0, blotted, weighed, and transferred to the outer well of 25-ml center-well flasks containing 2 ml of Krebs-Ringer phosphate buffer, pH 7.0, and acetate-2-^{14}C. The flasks were gassed with 95% O_2: 5% CO_2, capped, and incubated with shaking at 37°C. The incubations were terminated by the addition through the stopper of 0.8 ml 1 N NaOH to the center well and 0.2 ml 10 N H_2SO_4 to the outer well of the incubation flask. The flasks were then placed in an incubator and shaken for 30 min at 5°C.

For the analysis of $^{14}CO_2$, the contents of the center well were transferred quantitatively to 10-ml volumetric flasks; 1-ml portions were assayed for radioactivity by scintillation spectrometry (23). 0.5 ml of 10 N KOH and 1.0 mg of carrier cholesterol were then added to the outer well of the flask. Each flask was autoclaved for 30 min at 15 lb. pressure and 237°F, and the flask contents were then washed with 15 ml ethanol into a 250 ml Erlenmeyer flask and taken to dryness on a steam bath. The residue was suspended in 20 ml ethanol: water (1:1) and extracted twice by shaking with 100 ml petroleum ether. The petroleum ether fractions were combined and taken to dryness, and the residue was transferred into centrifuge tubes with three 2-ml washes of acetone: ethanol (1:1). Sterol digitonides were formed and washed as described by Sperry and Webb (24). The digitonides were dissolved in metha-

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[1] The low cholesterol diet was prepared by pouring 22 g of triolein (ICN Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio) into a Waring blendor in which had been mixed 250 g of Purina Monkey Chow and 400 ml water. After the mixture had been blended, it was allowed to dry overnight in room air until it reached a semisolid form in which it was fed to the animals. Each day's diet contained 34 g of fat, 38 g of protein, and, as determined by direct analysis, 32 mg of cholesterol and 42 mg of plant sterol. The high cholesterol diet was identical except that 3 g of cholesterol-1, 2-^{3}H was dissolved in the triolein before mixing.

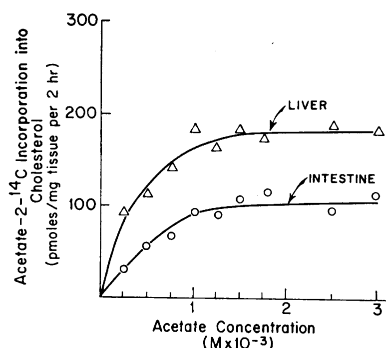

FIGURE 1 Relation between acetate concentration and the incorporation of acetate-2-^{14}C into cholesterol by slices of baboon liver and ileum. Slices (25 mg) were incubated for 2 hr at 37°C in 2 ml Krebs-Ringer phosphate buffer, pH 7.0, containing varying amounts of acetate-2-^{14}C. At the end of the incubations the samples were processed as described in the text.

nol, and 1-ml portions were added to 10 ml 0.5% 2,5-diphenyloxazole in toluene and assayed for ^{14}C in a scintillation spectrometer. In 30 instances, cholesterol content was also assayed on portions of the methanol solutions and the recovery of the added cholesterol averaged 90.8±SEM 3.4%.

To establish conditions for the optimal assessment of cholesterol synthesis in vitro, Menghini needle biopsies of liver and slices of terminal ileum were obtained from freshly killed baboons, and the conversion of acetate-2-^{14}C to cholesterol-^{14}C by these tissues was assessed under a variety of conditions. First, the relation between acetate concentration and cholesterol synthesis was measured (Fig. 1). The rate of cholesterol synthesis in the samples of liver and intestine increased as the concentration of acetate was raised from 2.5×10^{-4} M to 1×10^{-3} M and plateaued thereafter even up to concentrations of acetate as high as 3×10^{-3} M. In all subsequent studies the acetate concentration was 1×10^{-3} M. Second, the relation between incubation time and cholesterol synthesis was evaluated, and in both liver and intestine, the incorporation of acetate-2-^{14}C to cholesterol-^{14}C was linear for 2 hr and tended to diminish thereafter. All subsequent incubations were terminated after 2 hr. Next, the relation between the weight of tissue slices and the rate of cholesterol synthesis was measured. Under the conditions of this assay the rate of incorporation was linear at all tissue weights studied between 10 and 50 mg. Therefore, the subsequent assays have been performed with tissues of a variety of weights within this range, and all results have been expressed as picomoles of acetate incorporation/mg tissue per 2 hr at an acetate-^{14}C concentration of 10^{-3} M.

It was also essential to determine whether needle biopsies of liver and slices of ileum provide a representative sampling of cholesterol synthesis within these tissues. Therefore, in two animals, cholesterol synthesis was assessed in triplicate samples of liver removed from the left, middle, and right lobes and in segments of ileum removed 10 cm apart (Table I). The agreement among individual lobes of the liver and among the various intestinal segments was a close one, and within a given tissue sample, variation was never greater than 30%. It was concluded that measurement of cholesterol synthesis in small biopsies of liver provides a reasonable assessment of cholesterol biosynthesis throughout the tissue. In the case of the intestine, of course, no such conclusion can be drawn; it is known that cholesterol synthesis varies significantly along the length of the small intestine (25). The site chosen for this study was the ileum, the site of maximal cholesterol synthesis in this tissue, and it can only be concluded that the rate of synthesis by ileal slices does not vary abruptly within the 20 cm segment chosen for study. Considered together, these results have been interpreted as evidence that a miniaturized system for the assessment of acetate-2-^{14}C incorporation into cholesterol probably gives as consistent an evaluation as procedures using larger amounts of tissue, provided that optimal conditions are met.

Assessment of cholesterol production rates in intact animals. The methods for the assessment both of total cholesterol production rates (PRA) and of endogenous cholesterol production (synthesis) have been described in detail (21). In brief, after 4 wk on a low cholesterol diet or a high cholesterol-^{3}H diet, each animal was given cholesterol-4-^{14}C intravenously. Blood was drawn at 1, 2, 4, 7, 11, and 14 days and weekly thereafter for a total of 90–100 days. At the end of the experiment, cholesterol was extracted from serum and diet and analyzed for cholesterol content and for ^{3}H and/or ^{14}C. Cholesterol production rates were estimated from analysis of the serum-^{14}C die-away curve as previously described (21) by the method of Goodman and Noble (26). In the case of animals fed a low cholesterol diet, cholesterol production rates were assumed to be equivalent to total endogenous cholesterol production and cholesterol absorption was assumed to approximate zero (32 mg/day or less). When high cholesterol diets were fed, the amount of cholesterol absorbed and the quantity produced endogenously were estimated by multiplying the production rate by the appropriate percentage factor as estimated from the serum-^{3}H data. This technique is illustrated in Fig. 2 in which the change in the specific activity of serum cholesterol-^{3}H and -^{14}C is plotted with

TABLE I
Regional Variation in Cholesterol Synthesis by Slices of Liver and Intestine

Animal	Tissue	Site of biopsy	Acetate-2-^{14}C conversion to cholesterol	Mean synthesis per segment or lobe
			pmoles/mg per 2 hr	*pmoles/mg per 2 hr*
		Left lobe	53, 50, 39	47
1	Liver	Middle lobe	55, 39, 48	47
		Right lobe	40, 66, 48	51
		Left lobe	238, 280, 372	297
2	Liver	Middle lobe	327, 256, 252	278
		Right lobe	253, 255, 291	266
		Segment 1	112, 91, 75	93
1	Ileum	Segment 2	72, 70, 78	73
		Segment 3	78, 89, 91	86
		Segment 1	197, 160, 204	187
2	Ileum	Segment 2	203, 168, 144	172
		Segment 3	160, 154, 221	178

Each sample was incubated for 2 hr at 37°C in 2 ml Krebs-Ringer phosphate buffer, pH 7.0 containing 4 μCi of acetate-2-^{14}C (1×10^{-3} M). At the end of the incubation period, the samples were processed as described in the text.

time for one animal. The details of the measurements of these various parameters for the first 5 animals who had in vivo studies during high cholesterol intake only have been published previously (21); the isotopic measurements for the remaining 13 animals are summarized in Table II.

RESULTS

Table III and Fig. 3 summarize the results of feeding a low cholesterol diet for varying periods and then a high cholesterol diet for 4 months on the incorporation of acetate-2-^{14}C into cholesterol-^{14}C by biopsy samples of liver from 14 normal baboons and 4 baboons subjected to ileal diversion. As has previously been reported in other species (8, 27), a high cholesterol intake had little effect on $^{14}CO_2$ production, which averaged 2491±294 and 1772±SEM 161 pmoles/mg per 2 hr for the normal animals and 1806±SEM 516 and 1976±SEM 541 pmoles/mg per 2 hr for the operated animals fed the low and high cholesterol diets respectively. The situation in the case of cholesterol synthesis in the liver is somewhat more complicated. The mean rate of cholesterol synthesis fell from 254±29 pmoles/mg per 2 hr in the normal group fed low cholesterol to 61±18 pmoles/mg per 2 hr after the feeding of a high cholesterol intake; however, within both groups the range of variation was wide, and in some animals fed a high cholesterol intake the rate of hepatic synthesis was clearly within the normal range. In the animals subjected to ileal diversion, there was no significant fall in hepatic cholesterol synthesis when high cholesterol was fed (783±SEM 136 pmoles/mg per 2 hr) as compared with the low cholesterol diet (973±SEM 36 pmoles/mg per 2 hr).

In regard to the wide variation in the rates of cholesterol synthesis observed in liver biopsies in the normal animals on a low cholesterol diet, it is likely that one important variable is the length of time the animals were fed the low cholesterol diet, for indeed the range was much narrower (206–340 pmoles/mg per 2 hr) in the four animals that had been fed the low cholesterol diet for 4 months (animals 6–9) than in the animals (1–5 and 10–14) fed such a diet for only 2 wk to 1 month before biopsy (71–435 pmoles/mg per 2 hr). This possibility is in keeping with the fact that baboons ingest large quantities of eggs and meat in the wild (28) and may consequently have suppressed hepatic synthesis for many years. Even with this variable in mind, however, it is interesting that the rates of hepatic cholesterol synthesis observed in these animals (averaging 254 pmoles/mg per 2 hr) are in the same range as values observed in the squirrel monkey (27) and rat (29) under similar conditions of assay. In addition, the rate of hepatic cholesterol synthesis in the animals subjected to ileal diversion (animal 15–18, Table II), a maneuver that interferes with bile acid absorption and causes increased synthesis of bile acids (30) and cholesterol (31),

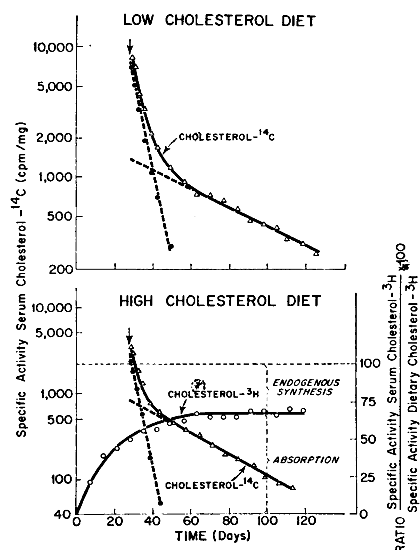

FIGURE 2 Change in serum cholesterol-^{3}H and -^{14}C specific activity in a baboon fed a low cholesterol diet and then a diet high in cholesterol-^{3}H and injected with cholesterol-^{14}C. The ratio of the serum cholesterol-^{3}H specific activity to that of the diet is shown by the open circles. The time of the cholesterol-^{14}C injection is indicated by the vertical arrows at day 28, and the experimental values observed for the serum cholesterol-^{14}C specific activities are represented by triangles. Extrapolation of the terminal linear portion of the ^{14}C line back to the time of injection provides the intercept C_B (dashed line). Subtraction of this extrapolated line from the experimental ^{14}C specific activity values provides the difference values shown as solid circles; the projection of the line drawn from these circles back to the time of injection provides the intercept C_A.

was increased to 973 pmoles/mg per 2 hr, a value also similar to that of the monkey (27) and rat (32) following bile duct cannulation.

The variation in cholesterol synthesis in the liver biopsy samples obtained from animals fed high cholesterol is more difficult to explain. In each of the 14 studies, the high cholesterol diet had been fed for 120 days, and the 3 g of cholesterol ingested is at least five times the normal cholesterol production rate. This amount of cholesterol per unit weight and the time of feeding are adequate to cause uniform and complete suppression of hepatic cholesterol synthesis in the squirrel monkey (27).

To determine whether the variation in the degree to which the negative feedback was demonstrable in the livers from the normal baboons might be due to differences in the rates of cholesterol absorption, the rates of cholesterol synthesis by the samples of liver have been plotted vs. the amount of cholesterol absorption for the 18 study periods in which production rates were measured in normal animals (Table III and Fig. 4). It can be seen that not until absorption reaches approximately

TABLE II

Isotopic Measurements in Baboons Fed Cholesterol-1,2-³H and Given Cholesterol-4-¹⁴C Intravenously

				Measurements of ¹⁴C disappearance					Measurements of cholesterol-³H	
					Specific activity of exponentials A and B at t = 0		$t_{1/2}$ of exponentials			
Animal	Operation	Diet	Serum cholesterol	R_A ¹⁴C injected	C_A	C_B	A	B	Specific activity of dietary cholesterol-³H	Specific activity of serum cholesterol during last 3 wk of study
			mg/100 ml ±SEM	*cpm*	*cpm/mg*	*cpm/mg*	*days*	*days*	*cpm/mg*	*cpm/mg*
6	None	Low cholesterol	112±2	6.37 × 10⁷	9000	1375	3.0	35	—	—
		High cholesterol	105±2	3.13 × 10⁷	4300	650	2.5	30	2332	1362
7	None	Low cholesterol	78±4	6.26 × 10⁷	7200	1425	4.0	40	—	—
		High cholesterol	115±3	3.06 × 10⁷	2850	850	2.5	25	1955	1325
8	None	Low cholesterol	115±2	6.27 × 10⁷	6000	1325	3.0	42	—	—
		High cholesterol	131±13	3.16 × 10⁷	2600	630	3.0	28	1841	1357
9	None	Low cholesterol	103±5	6.22 × 10⁷	11,500	1400	3.0	39	—	—
		High cholesterol	104±3	2.82 × 10⁷	3000	555	3.5	36	2378	1470
10	None	High cholesterol	109±5	2.00 × 10⁷	3200	480	3.0	33	1460	1038
11	None	High cholesterol	170±3	2.09 × 10⁷	1700	480	4.0	37	1256	1025
12	None	High cholesterol	111±4	2.11 × 10⁷	3400	400	3.0	37	1486	771
13	None	High cholesterol	154±4	2.08 × 10⁷	2200	480	3.5	34	1425	1032
14	None	High cholesterol	125±5	2.10 × 10⁷	2850	450	3.0	33.5	1497	1136
15	Ileal diversion	High cholesterol	—	5.33 × 10⁷	10,000	1475	2.0	21	2841	1018
16	Ileal diversion	Low cholesterol	97±6	2.04 × 10⁷	4000	500	1.5	18	—	—
		High cholesterol	101±3	6.86 × 10⁷	7000	610	3.0	26	1334	262
17	Ileal diversion	Low cholesterol	70±3	2.08 × 10⁷	3800	350	2.0	14	—	—
		High cholesterol	82±2	6.80 × 10⁷	8000	900	2.0	17.5	1284	247
18	Ileal diversion	Low cholesterol	118±6	2.16 × 10⁷	3250	250	2.0	14	—	—
		High cholesterol	126±3	6.81 × 10⁷	8000	235	2.5	30	1210	267

500 mg/day is the suppression of hepatic synthesis complete. Since the production rate in these 14 animals averaged 609±SEM 24 mg/day, it follows that cholesterol absorption must be equivalent to about three-fourths of the production rate before hepatic synthesis is completely suppressed. In the case of the ileal diversion, the situation is somewhat more complicated. Although absorption was within the normal range, hepatic synthesis was high, suggesting that in the steady state the acceleration of endogenous production after ileal diversion is not the consequence of impaired absorption of dietary cholesterol.

This relation is expressed even more clearly in Fig. 5 in which the endogenous production rate has been plotted vs. the rate of hepatic cholesterol synthesis for the normal animals and for the animals with ileal diversion. It can be seen that hepatic synthesis bears a significant relation to endogenous production through a wide range of activity. However, as hepatic synthesis falls, it approximates a value of zero when endogenous production rates are still about 100–150 mg/day. Since the liver is the only tissue that exhibits almost complete negative feedback control (*27, 29*), this relationship suggests that the liver is the major endogenous biosynthetic source for cholesterol and may account for as much as three-fourths of the production rate under circumstances of low cholesterol feeding (and potentially even more, of course, in the derepression that follows ileal diversion). Stated differently, hepatic synthesis is not suppressed completely until cholesterol absorption approximates the normal hepatic contribution. It also follows from this relationship that a fourth of the daily production of cholesterol or less may be derived from endogenous sources that do not possess negative feedback control of cholesterol synthesis.

The small intestine is of course a leading candidate for an important role for endogenous cholesterol synthesis which is nonsuppressible by cholesterol feeding; not only does this tissue synthesize cholesterol at a rate second only to liver (*27, 29*), but, in addition, it lacks complete feedback control to cholesterol feeding (*26, 27*) and contributes locally synthesized cholesterol to the circulation of the rat (*17*) and squirrel monkey (*18*) under circumstances in which the hepatic contribution is suppressed.

To determine whether the small intestine might play a similar role in the baboon, cholesterol synthesis has been measured in the terminal ileum in eight animals that were fed the low cholesterol diet for 1 month and in four baboons (animals 6–9, Table III) that had been fed cholesterol for 4 months. The results of these studies are tabulated in Table IV. Hepatic synthesis in the eight control animals was similar to the values observed

TABLE III

Relation between Cholesterol Synthesis by Baboon Liver Slices, Cholesterol Absorption, and Endogenous Cholesterol Production in the Baboon

				In vitro studies of liver slices			Measurement of cholesterol production in intact animals			
					Acetate-2-^{14}C conversion to					
Animal	Operation	Wt	Cholesterol content of diet	Wt of tissue	CO_2	cholesterol	Cholesterol production rate	Serum cholesterol-^{3}H / Dietary cholesterol-^{3}H $\times$ 100	Total endogenous cholesterol synthesis	Cholesterol absorption
		kg		*mg*	*pmoles/mg per 2 hr*	*pmoles/mg per 2 hr*	*mg/day*	*cpm/mg*	*mg/day*	*mg/day*
1	None	19.1	Low	14	1710	71	—	—	—	—
		20.4	High	10	1910	10	662	78.0	140	522
2	None	18.2	Low	8	1380	288	—	—	—	—
		20.7	High	10	2020	5	635	78.0	144	491
3	None	24.2	Low	12	2106	166	—	—	—	—
		25.5	High	15	2322	243	642	53.0	241	401
4	None	21.8	Low	19	2192	358	—	—	—	—
		24.3	High	18	2188	112	487	59.5	234	253
5	None	22.3	Low	11	3188	348	—	—	—	—
		25.0	High	19	2066	8	721	64.9	161	560
6	None	25.9	Low	19	2756	340	572	—	572	0
		25.9	High	17	2076	105	715	58.4	297	418
7	None	24.3	Low	16	2780	206	499	—	499	0
		25.0	High	25	1824	41	698	67.8	225	473
8	None	23.4	Low	13	2211	280	576	—	576	0
		24.1	High	24	2842	13	866	73.7	228	638
9	None	27.3	Low	24	2581	228	471	—	471	0
		27.7	High	16	2243	37	635	61.8	243	392
10	None	23.6	Low	25	2213	435	—	—	—	—
		25.0	High	19	1218	124	444	71.1	128	316
11	None	22.6	Low	24	2991	232	—	—	—	—
		23.8	High	22	1260	12	574	81.6	106	468
12	None	26.4	Low	22	1658	84	—	—	—	—
		27.2	High	18	821	60	571	51.9	275	296
13	None	23.6	Low	24	5758	158	—	—	—	—
		24.0	High	16	975	19	591	72.4	163	428
14	None	22.6	Low	25	1345	358	—	—	—	—
		23.4	High	21	1045	60	603	75.9	146	457
15	Ileal diversion	22.7	High	5	3480	706	1591	35.8	1034	557
		20.9	Low	7	3290	950	—	—	—	—
16	Ileal diversion	23.6	Low	22	912	892	935	—	935	0
		25.9	High	25	2041	1154	1276	19.6	1026	250
17	Ileal diversion	22.0	Low	14	1624	1065	1145	—	1145	0
		19.3	High	24	1257	503	1472	19.2	1189	283
18	Ileal diversion	23.2	Low	18	1400	987	1492	—	1492	0
		23.4	High	25	1125	769	1741	22.1	1356	385

The incubation conditions are described in Table I and the text. The details of the measurements of PRA and the serum cholesterol-^{3}H in animals 1–5 have been published (21); the measurements upon which these calculations have been made for animals 6–18 are given in Table II.

in the animals that had been fed the low cholesterol diet in the earlier part of the study (358±45 vs. 254±29 pmoles/mg tissue per 2 hr). Synthesis in the ileum of these animals was lower than that in the liver (241±14 pmoles/mg per 2 hr). In the animals fed a high cholesterol diet on the other hand, hepatic synthesis was suppressed to about a fourth of the control value (49±19 pmoles/mg per 2 hr) whereas synthesis in the ileum was more than half the normal rate (145±13 pmoles/mg per 2 hr). These results suggest that the small intestine of the baboon, like that of other animals, does not possess complete cholesterol negative feedback and may play an important role as a biosynthetic source for the fraction of the daily production which is not derived from liver or diet. Such findings do not exclude the possibility that other, more slowly turning over pools may also play an important role in this function (33).

DISCUSSION

There are many difficulties in any attempt to correlate in vitro phenomena with events in the intact animal. One problem is whether the assessment of cholesterol synthesis in liver slices has any relation to hepatic synthesis in the living animal. In the present studies, it has been established that the incorporation of acetate-2-^{14}C into cholesterol by slices of ileum and liver was measured under optimal conditions. In addition, evidence has been obtained that biopsies of one lobe of the liver give

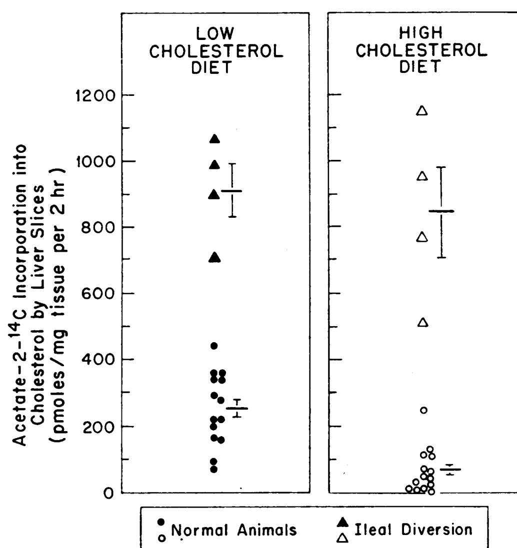

FIGURE 3 Effect of cholesterol feeding on the incorporation of acetate-2-^{14}C into cholesterol by slices of baboon liver. Slices were incubated for 2 hr at 37°C in 2 ml Krebs-Ringer phosphate buffer, pH 7.0, containing acetate-2-^{14}C (1×10^{-3} M) and processed as described in the text.

a reasonable sampling of cholesterol synthesis in the entire tissue. Under these conditions, the rate of cholesterol synthesis in the liver of baboons on a low cholesterol diet is similar to results obtained in rat (29) and monkey liver (27). Considered together, these findings suggest that when carefully performed, the assessment of cholesterol synthesis in liver biopsies may in fact reflect in vivo events despite the fact that it is not possible to extrapolate synthetic rates as measured in vitro directly to the intact animal.

It is of equal importance to know whether the estimates of cholesterol production, absorption, and synthesis in the intact animal are reasonably accurate. There are two types of evidence that analysis of the die-away curve in terms of the two pool model proposed by Goodman and Noble (26) provides a reasonable approximation of in vivo phenomena. First, estimates of miscible pool size as predicted by the model agree with estimates based on carcass analysis (21), and second, estimates of cholesterol production rates based on this formulation are in agreement with estimates made by balance techniques in man (34). The validity of the steady-state isotope ratio for determining cholesterol absorption and endogenous synthesis rates is less well established; it depends upon whether the animal is in fact in an isotopic steady state. Whereas absolute proof for an isotopic steady state would require many years of study, each of these animals was in a near isotopic steady state for at least the last month of the study, and, furthermore, even if the ratios were off 10%, it would make only slight differences in the various estimates. For these reasons, it has been concluded that the in vivo estimations of cholesterol production rates, absorption, and synthesis are soundly based.

Consequently, several conclusions appear warranted from these studies. First, in some but not all baboons, cholesterol feeding results in virtually complete inhibition of cholesterol synthesis by liver slices (as is true in man [9–12]). The degree of this feedback is an apparent linear function of the amount of cholesterol absorbed and is not complete until cholesterol absorption approximates the normal hepatic contribution to the daily production (e.g., about 500 mg). The effect of such a system is to provide a precise control mechanism by which on the one hand, absorbed cholesterol up to an amount equaling the hepatic contribution is compensated for by inhibiting synthesis and by which on the other hand, enhanced cholesterol synthesis in the liver ensues when cholesterol intake falls.

Second, the evidence presented here provides strong reinforcement for the concept that the liver is the predominant biosynthetic source for circulating cholesterol in the animal fed a low cholesterol diet, since approximately three-fourths of the daily production rate of cholesterol can be accounted for as coming from this source when no cholesterol is fed. The remainder of the endogenously produced cholesterol appears to arise from tissues that do not possess a complete negative feedback system; the small intestine may play a significant role in this regard and may be a more important site of cholesterol

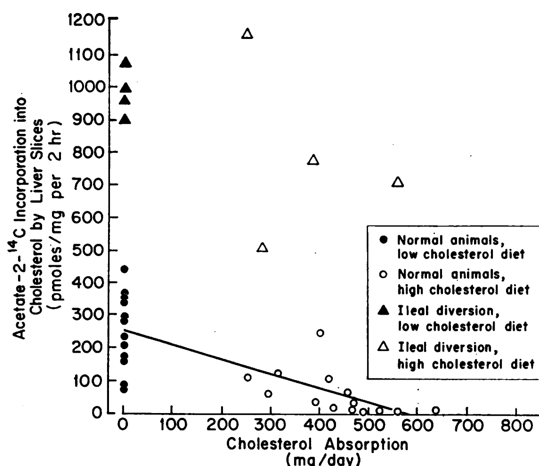

FIGURE 4 Relation between cholesterol absorption in the baboon and acetate-2-^{14}C incorporation into cholesterol by liver slices. The data in Table III for the 18 studies in normal animals in which both in vitro and in vivo measurements were made have been analyzed by the method of least squares; the values obtained from the animals with ileal diversion were excluded from this regression analysis. The regression line has a correlation coefficient of −0.76.

synthesis than the liver when high cholesterol diets are fed (27).

Third, deductions can be drawn as to the reasons that some (11–14) but not all (9, 10, 15) investigators have found it difficult to demonstrate negative feedback control of cholesterol synthesis in the liver of man. It is clear that the ability to absorb cholesterol by the human intestine is limited in comparison with lower animals (13–15); provided that the liver of man contributes as in the baboon about 75% of the daily production when no cholesterol is fed, hepatic synthesis would not be expected to be inhibited completely until absorption reaches 500–750 mg/day (13). It is quite clear that no deduction can be drawn about the importance of the hepatic negative feedback or of the magnitude of the hepatic contribution to endogenous production from isotopic steady-state data alone (3, 12), from balance studies (12–16), or from in vitro assessment of cholesterol synthesis by liver biopsies from man (9–12) unless the amount of cholesterol actually absorbed is taken into account. In view of the fact that it has been possible to demonstrate negative feedback in human liver under some conditions (9–11, 15), it is likely that the liver of normal man possesses a negative feedback system capable of compensating for as much cholesterol as can be absorbed from the residual diet.

Finally, it is of interest that the enhanced hepatic synthesis of cholesterol that followed ileal diversion in the baboon was not suppressed even to the normal range by the high cholesterol diet, despite the fact that cholesterol absorption approximated normal values

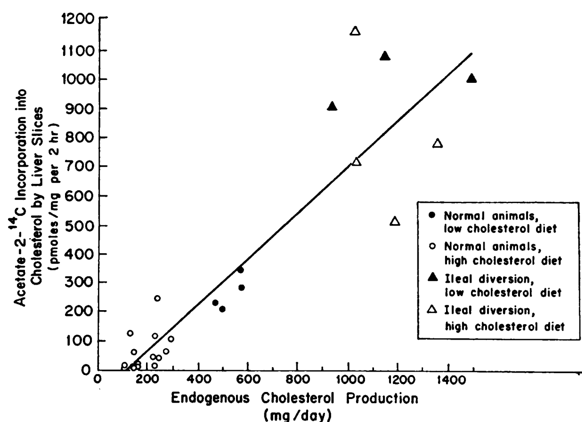

FIGURE 5 Relation between endogenous cholesterol production in the baboon and acetate-2-^{14}C incorporation into cholesterol by liver slices. The data in Table III for studies in 14 normal baboons and 4 animals subjected to ileal diversion in which both in vitro and in vivo measurements were made have been analyzed by the method of least squares. The regression line has a correlation coefficient of 0.91.

TABLE IV
Lack of Effect of High Cholesterol Intake on Cholesterol Synthesis by Slices of Baboon Ileum

Tissue	Diet	Number of animals studied	Acetate-2-^{14}C conversion to	
			$^{14}CO_2$	Cholesterol-^{14}C
			pmoles/mg per 2 hr ±SEM	
Liver	Low cholesterol	8	2,587±478	358±45
	High cholesterol	4	2,250±213	49±19
Ileum	Low cholesterol	8	12,138±557	241±14
	High cholesterol	4	14,460±1809	145±13

The eight animals designated "low cholesterol" were fed the low cholesterol-triolein diet for 1 month and were then biopsied under anesthesia. The four animals designated "high cholesterol" are animals 6–9 of Table II in which biopsies of ileum and liver were obtained after feeding cholesterol for 4 months. The incubation conditions are described in Table I.

in these animals. These results are strikingly similar to studies in man reported by Grundy, Ahrens, and Salen (35). This finding does not mean that cholesterol absorption would have been normal if the ileal resection had been more extensive, but it does imply that cholesterol absorption is not the only means by which cholesterol synthesis is regulated in the liver. This finding is in apparent contradiction to the thesis that bile acids exert a modulating influence on hepatic cholesterol synthesis only by regulating the amount of cholesterol absorbed into the enterohepatic circulation (32). Three possibilities may account for this finding. First, it is possible that bile acids play some direct regulatory role in mediating hepatic cholesterol synthesis. If this is indeed the case, this role must be remarkably sensitive since these animals did produce sufficient bile acids in the steady state to allow cholesterol absorption. Second, it is possible that physiological amounts of bile acids in the enterohepatic circulation are required for the entry into the lymph of critical lipoprotein complexes that are the real mediators of the cholesterol negative feedback. Third, it is conceivable that the ileum plays a vital role in the absorption or synthesis of some factor involved in this feedback. At any rate, it is clear that some factor(s) other than the absorption of exogenous cholesterol must play a role in regulating hepatic cholesterol synthesis.

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