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-4-14C and fed constant amounts of cholesterol-1,2-2H 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-
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 extra-hepatic 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 synthesis that makes it difficult to demonstrate cholesterol feedback 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. 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 Kreb's-Ringer phosphate buffer, pH 7.0, blotted, weighed, and transferred to the outer well of 25-ml center-well flasks containing 2 ml of Kreb's-Ringer phosphate buffer, pH 7.0, and acetate-2-14C. The flasks were gassed with 95% O2: 5% CO2 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 H2SO4 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 14CO2 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 ethyl alcohol 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 digitanides were formed and washed as described by Sperry and Webb (24). The digitonides were dissolved in methanol.

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 blender 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, 2-14H was dissolved in the triolein before mixing.

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null, and 1-ml portions were added to 10 ml 0.5% 2,5-di-
diphenyloxazole in toluene and assayed for $^{14}C$ in a scintilla-
tion 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 ± 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-$^{14}C$
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 $2.5 \times 10^{-4}$ m to $1 \times 10^{-4}$ m and plateaued
thereafter even up to concentrations of acetate as high as
$3 \times 10^{-4}$ m. In all subsequent studies the acetate concen-
tration was $1 \times 10^{-4}$ m. Second, the relation between incubation
time and cholesterol synthesis was evaluated, and in both
liver and intestine, the incorporation of acetate-$^{14}C$ 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-$^{14}C$
incorporation/mg tissue per 2 hr at an acetate-$^{14}C$
concentration of $10^{-4}$ m.

It was also essential to determine whether needle biopsies
of liver and slices of ileum provide a representative sam-
ping of cholesterol synthesis within these tissues. There-
fore, 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 1). 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 measure-
ment 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 syn-
thesis 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-$^{2}C$ 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 ani-
mal. The methods for the assessment both of total cho-
lesterol production rates (PRA) and of endogenous cho-
lesterol production (synthesis) have been described in de-
tail (21). In brief, after 4 wk on a low cholesterol diet or a
high cholesterol-$^{3}H$ diet, each animal was given chole-
sterol-$^{4}C$ 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 ex-
tracted from serum and diet and analyzed for cholesterol
content and for $^{1}H$ and/or $^{3}C$. Cholesterol production rates
were estimated from analysis of the serum-$^{3}H$ die-away
curve as previously described (21) by the method of Good-
man 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 produc-
tion 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 quan-
tity 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-$^{1}H$ and-$^{3}C$ is plotted with

![Figure 1](https://example.com/figure1.png)

**Figure 1** Relation between acetate concentration and the
incorporation of acetate-$^{14}C$ 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-$^{14}C$. At the
end of the incubations the samples were processed as de-
scribed in the text.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Tissue</th>
<th>Site of biopsy</th>
<th>Acetate-$^{14}C$ conversion to cholesterol</th>
<th>Mean synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pmoles/mg per 2 hr</td>
<td>pmoles/mg per 2 hr</td>
</tr>
<tr>
<td>1</td>
<td>Liver</td>
<td>Left lobe</td>
<td>53, 50, 39</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle lobe</td>
<td>55, 39, 48</td>
<td>47</td>
</tr>
<tr>
<td>2</td>
<td>Liver</td>
<td>Right lobe</td>
<td>40, 66, 48</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left lobe</td>
<td>238, 280, 372</td>
<td>297</td>
</tr>
<tr>
<td>1</td>
<td>Ileum</td>
<td>Middle lobe</td>
<td>327, 256, 252</td>
<td>278</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right lobe</td>
<td>253, 255, 291</td>
<td>266</td>
</tr>
<tr>
<td>2</td>
<td>Ileum</td>
<td>Segment 1</td>
<td>112, 91, 75</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Segment 2</td>
<td>72, 70, 78</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Segment 3</td>
<td>78, 89, 91</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Segment 1</td>
<td>197, 160, 204</td>
<td>187</td>
</tr>
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<td></td>
<td></td>
<td>Segment 2</td>
<td>263, 168, 144</td>
<td>172</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Segment 3</td>
<td>160, 154, 221</td>
<td>178</td>
</tr>
</tbody>
</table>

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-$^{2}C$ (1 $\times 10^{-4}$ m). At
the end of the incubation period, the samples were processed as described
in the text.

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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-2-14C into cholesterol-14C 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 14CO2 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 cho-
lesterol synthesis observed in liver biopsies in the nor-
mal 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 possi-
ibility 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 
pxoles/mg per 2 hr) are in the same range as values 
observed in the squirrel monkey (27) and rat (29) un-
der 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 in-
creased synthesis of bile acids (30) and cholesterol (31), 
was increased to 973 pmoles/mg per 2 hr, a value also 
similar to that of the monkey (27) and rat (32) follow-
ing bile duct cannulation.

The variation in cholesterol synthesis in the liver 
biopsy samples obtained from animals fed high cho-
lesterol is more difficult to explain. In each of the 14 
theses, 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 sup-
pression 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 differ-
ces 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 mea-
sured in normal animals (Table III and Fig. 4). It can 
be seen that not until absorption reaches approximately

FIGURE 2 Change in serum cholesterol-1H and -14C specific activity in a baboon fed a low cholesterol diet and then a 
diet high in cholesterol-1H and injected with cholesterol-14C. The ratio of the serum cholesterol-1H specific activity to 
that of the diet is shown by the open circles. The time of the 
cholesterol-14C injection is indicated by the vertical ar-
rows at day 28, and the experimental values observed for 
the serum cholesterol-14C specific activities are represented 
by triangles. Extrapolation of the terminal linear portion 
of the 14C line back to the time of injection provides the 
intercept Cx (dashed line). Subtraction of this extrapolated 
line from the experimental 14C 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 Ca.

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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-fours 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

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**Table II**

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

<table>
<thead>
<tr>
<th>Animal</th>
<th>Operation</th>
<th>Diet</th>
<th>Serum cholesterol</th>
<th>[14C] Injected</th>
<th>Specific activity of exponentials at t = 0</th>
<th>Specific activity of dietary cholesterol-H during last 3 wk of study</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mg/100 m/SEM</td>
<td>cpm</td>
<td>cpm/mg</td>
<td>cpm/mg</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>Low cholesterol</td>
<td>112±2</td>
<td>6.37 × 10⁶</td>
<td>9000</td>
<td>3.0</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td>High cholesterol</td>
<td>105±2</td>
<td>3.13 × 10⁶</td>
<td>4300</td>
<td>2.5</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>Low cholesterol</td>
<td>78±2</td>
<td>6.26 × 10⁶</td>
<td>7200</td>
<td>4.0</td>
</tr>
<tr>
<td>9</td>
<td>None</td>
<td>High cholesterol</td>
<td>115±3</td>
<td>3.06 × 10⁶</td>
<td>2850</td>
<td>2.5</td>
</tr>
<tr>
<td>10</td>
<td>None</td>
<td>Low cholesterol</td>
<td>103±5</td>
<td>6.22 × 10⁶</td>
<td>11,500</td>
<td>3.0</td>
</tr>
<tr>
<td>11</td>
<td>None</td>
<td>High cholesterol</td>
<td>104±3</td>
<td>2.82 × 10⁶</td>
<td>3000</td>
<td>3.0</td>
</tr>
<tr>
<td>12</td>
<td>None</td>
<td>High cholesterol</td>
<td>111±2</td>
<td>2.11 × 10⁶</td>
<td>3400</td>
<td>3.0</td>
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<tr>
<td>13</td>
<td>None</td>
<td>High cholesterol</td>
<td>154±4</td>
<td>2.08 × 10⁶</td>
<td>2200</td>
<td>3.5</td>
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<tr>
<td>14</td>
<td>None</td>
<td>High cholesterol</td>
<td>125±5</td>
<td>2.10 × 10⁶</td>
<td>2850</td>
<td>3.0</td>
</tr>
<tr>
<td>15</td>
<td>Ileal diversion</td>
<td>High cholesterol</td>
<td>170±3</td>
<td>2.09 × 10⁶</td>
<td>1700</td>
<td>4.0</td>
</tr>
<tr>
<td>16</td>
<td>Ileal diversion</td>
<td>Low cholesterol</td>
<td>97±6</td>
<td>2.04 × 10⁶</td>
<td>4000</td>
<td>1.5</td>
</tr>
<tr>
<td>17</td>
<td>Ileal diversion</td>
<td>Low cholesterol</td>
<td>101±3</td>
<td>6.86 × 10⁶</td>
<td>7000</td>
<td>3.0</td>
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<tr>
<td>18</td>
<td>Ileal diversion</td>
<td>Low cholesterol</td>
<td>70±3</td>
<td>2.08 × 10⁶</td>
<td>3800</td>
<td>2.0</td>
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<tr>
<td></td>
<td></td>
<td>High cholesterol</td>
<td>82±2</td>
<td>6.80 × 10⁶</td>
<td>8000</td>
<td>2.0</td>
</tr>
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</table>
### Table III

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

<table>
<thead>
<tr>
<th>Animal</th>
<th>Operation</th>
<th>Wt</th>
<th>Acetate-2(^{14}C) conversion to cholesterol</th>
<th>CO(_2)</th>
<th>cholesterol</th>
<th>Acetate-2(^{14}C) production rate</th>
<th>Serum cholesterol(^{14}H)</th>
<th>Total endogenous cholesterol synthesis</th>
<th>Dietary cholesterol(^{14}H)</th>
<th>Cholesterol absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mg pmoles/mg per 2 hr</td>
<td>pmoles/mg per 2 hr</td>
<td>mg/day</td>
<td>cpm/mg</td>
<td>mg/day</td>
<td>mg/day</td>
<td>mg/day</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>19.1</td>
<td>Low 14</td>
<td>1710</td>
<td>71</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>20.4</td>
<td>High 10</td>
<td>1910</td>
<td>10</td>
<td>662</td>
<td>78.0</td>
<td>140</td>
<td>522</td>
<td></td>
</tr>
<tr>
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The incubation conditions are described in Table I and the text. The details of the measurements of PRA and the serum cholesterol\(^{14}H\) 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 biosyntheses of one lobe of the liver give

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in the study, and, furthermore, even if the ratios of cholesterol production, 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 absorption.

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.

**Figure 3** Effect of cholesterol feeding on the incorporation of acetate-2-14C 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-2-14C (1 × 10^-3 m) and processed as described in the text.

![Figure 3](image.png)

**Figure 4** Relation between cholesterol absorption in the baboon and acetate-2-14C 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.

![Figure 4](image.png)
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](image_url)

**Figure 5** Relation between endogenous cholesterol production in the baboon and acetate-2-¹⁴C 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.

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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.

**ACKNOWLEDGMENTS**

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REFERENCES


