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# The Accumulation of Tissue Cholesterol and Its Relationship to Bile Acid and Sterol Turnover

## **Authors**

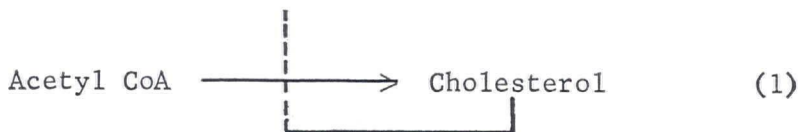
W. T. Beher, B. Rao, M. E. Beher, G. Semenuk, J. Bertasius, and N. Vulpetti

## The Accumulation of Tissue Cholesterol and Its Relationship to Bile Acid and Sterol Turnover\*†

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*Normal and hypophysectomized rats were divided into equal homogeneous groups to determine the effect of cholestyramine, corn oil and cholesterol on the excretion of fecal bile acids and sterols. Bile acid turnover rates, pool sizes, and spectrums were studied and compared.*

The homeostatic control mechanisms which determine tissue cholesterol concentrations are varied and highly complex. Although most of the processes which determine the rate of transfer of cholesterol among the various tissue pools are unknown, there is considerable information on the origin and elimination of this sterol. There are two main courses of tissue cholesterol, the diet and biosynthesis. Dietary cholesterol when absorbed via the small intestine, enters the general cholesterol pool and becomes indistinguishable from that synthesized by the organism. Synthesis of cholesterol from acetyl Co A occurs at various rates in all tissues except blood; however, most is synthesized by the liver. The rate of synthesis of liver cholesterol is inversely related to the concentration of cholesterol in this tissue's pool. Synthesis in other tissue pools apparently is not controlled by this mechanism. Therefore it is obvious that the amount of cholesterol synthesized by the liver plus that of dietary origin will normally determine the rate of cholesterol synthesis. This feedback reaction may be written:



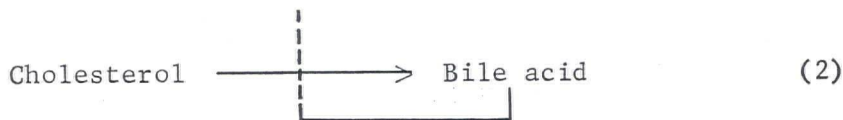
Tissue cholesterol is eliminated from the organism via two major pathways: that is via bile acid excretion and sterol excretion.

The bile acids, after synthesis from cholesterol in the liver, are conjugated with glycine or taurine, enter the small intestine via the common bile duct, are distributed

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along the small intestine and caecum, and are in part reabsorbed and returned via the portal blood to the liver. This constantly repeating cycle constitutes the enterohepatic recirculating pool. Free sterols excreted from the liver are recirculated similarly. With each cycle a certain fraction of the bile acids and sterols passes on to the large intestine, where absorption is virtually nil, and is eliminated via feces. The rate of synthesis of bile acids from cholesterol is controlled by the concentration of bile acids in the recirculating pool, and like cholesterol synthesis is a feedback reaction:



It is thus obvious that the rate of elimination of bile acids from their pool can in part determine the rate of cholesterol synthesis. By combining 1 and 2, we arrive at:



Since tissue cholesterol is eliminated from the organism via two major pathways—i.e., by bile acid excretion and by sterol excretion—to get a complete picture of the dynamics of the process, it is important to determine the elimination by both these routes.

An animal fed a diet containing cholesterol accumulates varying amounts of cholesterol in serum and liver. Among the important factors determining the amounts accumulated are the rate of conversion of cholesterol to bile acids, the rate of the subsequent elimination of the bile acids, and the rate of fecal sterol excretion. Study of all these factors may have a practical application in understanding cholesterol metabolism in humans, since diets normally contain varying amounts of cholesterol.

In the present experiments we studied: (a) The effects of cholesterol and of corn oil on the rate of synthesis and elimination of bile acids; (b) Bile acid and sterol elimination in hypophysectomized rats, animals which easily accumulate large concentrations of blood and liver cholesterol when fed diets containing cholesterol; and (c) The effects of cholestyramine (MK-135, Cuemid\*) on the rates of elimination of bile acids and of accumulation of tissue cholesterol in rats. MK-135 is a bile acid-sequestering anion exchange resin.

## Methods

### 1. General Procedures.

a. *Determination of bile acid turnover rates.* Rats injected intraperitoneally with 5  $\mu\text{C}$  cholic acid-24-C<sup>14</sup> (material of high specific activity was injected so that the size of the bile acid pool would not be altered) were placed in metabolism cages and feces collected daily for 9 to 11 days. The rats were then killed and small intestines, caecums, large intestines (all plus contents), and livers removed. All feces and tissue samples

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were dried by lyophilization. The dried material was finely ground, and small aliquots combusted by the Schöniger oxygen-flask method. The radioactivity of the combusted material was determined by absorption of  $C^{14}O_2$  in ethanolic ethanolamine, followed by scintillation counting. The  $-\log(1 - \frac{u^t}{u^{max}})$  was plotted against the time in days. The point where  $u^t/u^{max}$  is 0.5 marks the half-life of the bile acid pool.  $u^t$  = accumulated total bile acid- $C^{14}$  (counts/min.), excreted up to and including a given day;  $u^{max}$  = total bile acid- $C^{14}$  (counts/min.) accounted for in tissue plus feces.

*b. Bile acid pool size determination.* The ground small intestine plus contents was exhaustively extracted with boiling ethanol. The extract was evaporated and the lipids saponified with 6 N sodium hydroxide for three hours at 15 psi. The sterols were then extracted with petroleum ether. The aqueous residue was acidified and exhaustively extracted first with petroleum ether to remove fatty acids, and then with ethyl ether to recover the bile acid fraction. The bile acids were separated by thin-layer chromatography, and determined by densitometry.<sup>1,2</sup> Since the amount and specific activity of bile acids in the small intestinal pool are known, and the bile acid- $C^{14}$  counts/min. is known for the other pool sites, the total amount of bile acid in the recirculating pool can be calculated.<sup>3</sup> Ninety to 95% of the bile acid pool is in the small intestine plus content.

### 2. Experimental Studies.

#### *a. Effect of cholesterol and corn oil on bile acid synthesis in the rat.*

Thirty-two adult female albino rats were divided into four equal groups and fed the following diets *ad lib* for three weeks: Group I (controls), Rockland Rat Diet (RRD); Group II, RRD + 3% corn oil; Group III, RRD + 1% cholesterol; Group IV, RRD + 1% cholesterol + 3% corn oil. The animals then received intraperitoneal injections of cholic acid-24- $C^{14}$ . Bile acid turnover and pool sizes were determined by methods *a* and *b*.

#### *b. Comparative study of the accumulation of blood and liver cholesterol and of bile acid and sterol excretion rates in normal and hypophysectomized rats.*

Twenty normal, along with 20 hypophysectomized adult female albino rats were divided into equal control and test groups. The following diets were fed *ad lib*: control groups, RRD; test groups, RRD + 1% cholesterol + 3% corn oil. The animals were sacrificed after three weeks. Serum cholesterol was determined according to Sperry and Webb;<sup>4</sup> liver cholesterol by the method of Abell *et al.*<sup>5</sup> Bile acid turnover and pool size were determined as described in *a* and *b* respectively. Aliquots of the non-saponifiable fraction isolated from feces by the method outlined in procedure *b*, were used for the determination of fecal sterol output by gas-liquid chromatography.<sup>6</sup>

#### *c. Effect of MK-135 on the accumulation of blood and liver cholesterol and on bile acid excretion in the rat.*

Thirty normal and 30 hypophysectomized adult female albino rats were each divided into three equal homogeneous groups. One group of normals and one of hypo-

physectomized rats were fed RRD; a second group of each received RRD + 1% cholesterol + 3% corn oil. The third group consumed RRD + 1% cholesterol + 3% corn oil + 1% MK-135. The animals were maintained on these diets for three weeks *ad lib*. They were then sacrificed and liver and blood cholesterol determined as in the preceding study.

For the bile acid excretion studies, 16 normal rats were divided into two groups. The first group was fed RRD, the second RRD + 2% MK-135. After three weeks each animal received an intraperitoneal injection of cholic acid-24-C<sup>14</sup>. Bile acid turnover rates and pool sizes were determined by procedures *a* and *b*. Sixteen hypophysectomized rats were treated in exactly the same way.

### Results and Discussion

#### *a. The effects of corn oil and cholesterol on the excretion of bile acids in the rat.*

When an animal increases its intake of dietary cholesterol, certain defense mechanisms become active in an attempt to maintain normal tissue cholesterol concentrations. It is well-known that as the liver cholesterol concentration rises, synthesis of this sterol is blocked by feedback, thus eliminating this source of tissue cholesterol. If the intake of dietary cholesterol is too high, this mechanism may not be able to limit cholesterol accumulation. Recently Jean Wilson<sup>7,8</sup> has presented balance data which suggests that a second defense mechanism may be active in certain species: — an increase in intake of exogenous cholesterol increases the rate of conversion of liver sterols to bile acids.

In the experiments reported here, we studied the effects of dietary cholesterol on the bile acid turnover rate, pool size, and pool spectrum of rats. In Figure 1, a log function of the excretion of bile acid-24-C<sup>14</sup> is plotted against time; the dotted vertical line cuts the curves at the bile acid half-life (the time needed to eliminate one-half of the bile acid pool). The bile acid half-life in control rats was about three days. When the rat diet was supplemented with 1% cholesterol and 3% corn oil, the half-life was reduced to about one and one-quarter days. Since this effect could have been due to the cholesterol or the corn oil, further studies were necessary.

When the diet was supplemented with corn oil alone, the half-life remained at the control rate. However when the diet was supplemented with 1% cholesterol, the half-life decreased to one and one-quarter days. Therefore we must conclude that the enhanced bile acid turnover rate in rats fed a diet supplemented with corn oil and cholesterol is entirely due to the cholesterol content of the diet. This is of interest since corn oil enhances the rate of absorption of cholesterol and aggravates the accumulation of blood and liver cholesterol in the rat.

The bile acid pool sizes are shown in Table I. None of the diets had any significant effect on the total bile acid pool size. However, when the diets were supplemented with cholesterol, there was a significant decrease in cholic acid and a compensating increase in chenodeoxycholic acid in the bile acid pool. Since the total bile acid pool size

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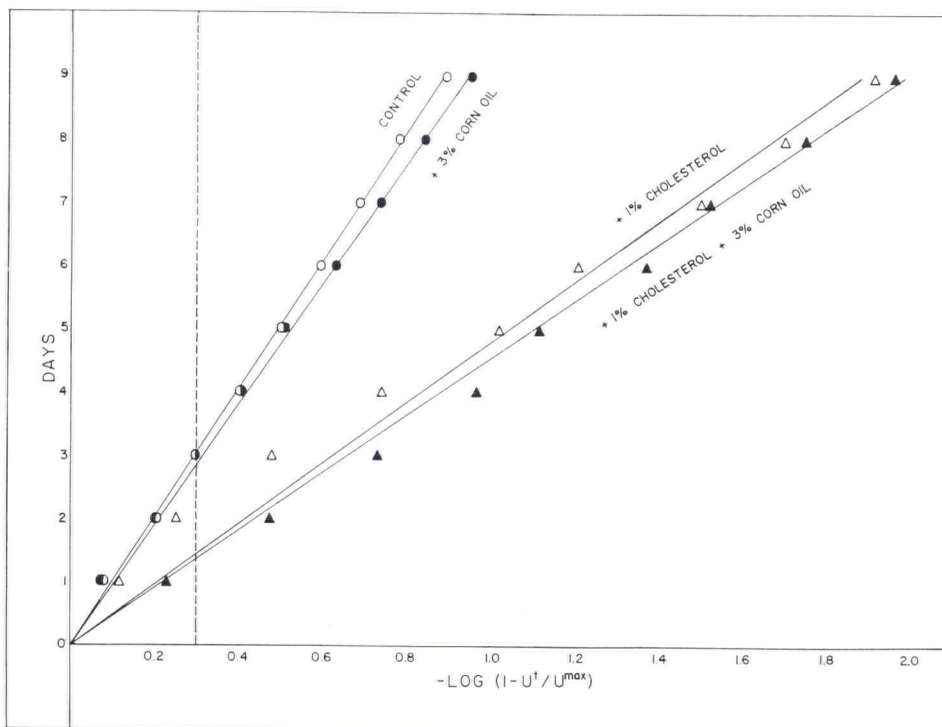


Figure 1

Effect of cholesterol and corn oil on the turnover of the cholic acid pools in rats. The broken vertical line cuts the curves at the half-life of the pools.

**Table I**  
THE EFFECTS OF DIETARY CHOLESTEROL AND CORN OIL  
ON THE BILE ACID POOL SIZES AND SPECTRA OF RATS

Group	Cholic Acid	Chenodeoxy- cholic Acid	Total Bile Acids
	mg/100 gm rat	mg/100 gm rat	mg/100 gm rat
Control	5.40 ± 0.26	0.83 ± 0.12	6.23
Corn Oil, 3%	5.79 ± 0.37	0.69 ± 0.20	6.48
Cholesterol, 0.5%	3.48 ± 1.20	1.77 ± 0.35	5.25
Cholesterol (0.5%) + Corn Oil (3%)	3.73 ± 0.63	1.70 ± 0.23	5.43
Cholesterol (1.0%) + Corn Oil (3%)	4.71 ± 0.35	1.86 ± 0.14	6.57

remained constant in all groups, the rate of bile acid synthesis is proportional to the bile acid half-life, and is at least doubled in animals receiving diet supplemented with cholesterol. Data recently obtained by our group has shown that chenodeoxycholic acid has a much more rapid turnover rate than cholic acid. Therefore the shift in the bile acid spectrum toward chenodeoxycholic acid in the cholesterol-treated rats indicates a further defense mechanism acting in this species.

*b. A comparative study of the bile acid and sterol excretion in normal and hypophysectomized rats.*

It has been known for some time that hypophysectomized rats exhibit extremely high blood and liver cholesterol concentrations when fed diets supplemented with cholesterol. Normal rats fed the same diets accumulate much less tissue cholesterol despite the fact that they consume approximately 30% more diet per day. The graphs shown in Figure 2 illustrate this effect. It should be noted that although hypophysec-

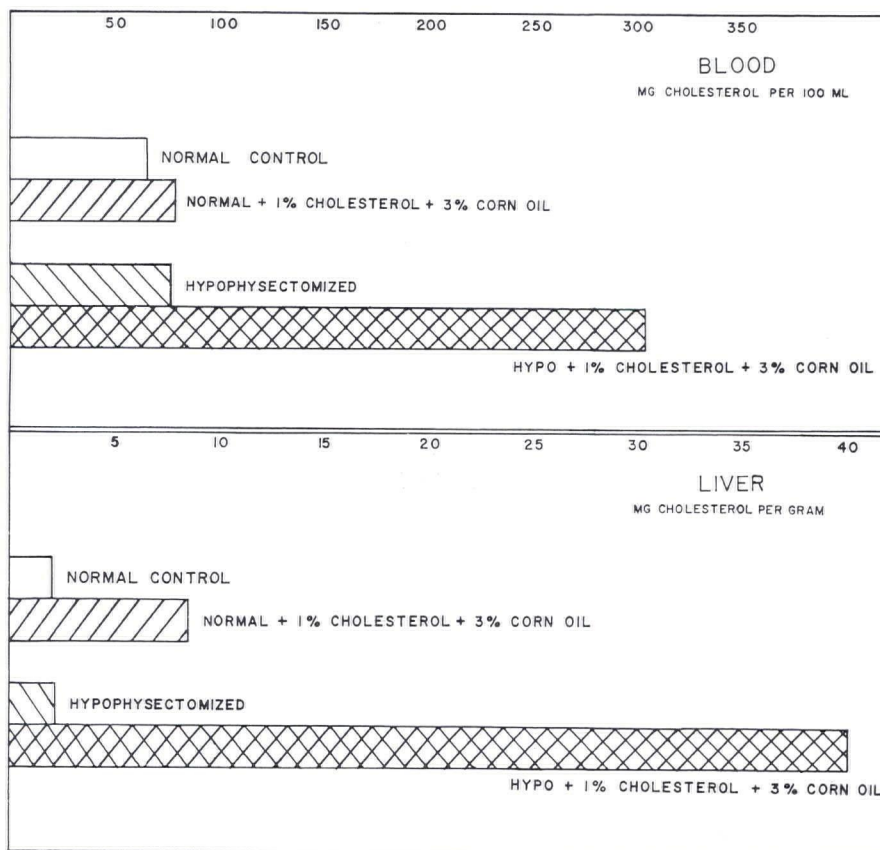


Figure 2

The accumulation of blood and liver cholesterol in normal and hypophysectomized rats fed diets supplemented with cholesterol and corn oil.



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tomized rats easily accumulate large quantities of cholesterol, these animals maintain nearly normal blood and liver cholesterol concentrations when fed commercial rat rations low in this sterol. It is thus clear that the basic defect in these animals is inability to handle exogenous cholesterol. Although an increased rate of cholesterol absorption could account for this phenomena, it seems more likely that in the hypophysectomized rat the basic difficulty involves a decreased ability to eliminate absorbed cholesterol via the bile acid and/or fecal sterol pathways.

To investigate these possibilities, we made comparative studies of the bile acid turnover, pool sizes and spectrums of normal and hypophysectomized rats. Figure 3 shows the cholic-24-C<sup>14</sup> and chenodeoxycholic-24-C<sup>14</sup> turnover rates in such rats. The turnover rates of both bile acids were much slower in hypophysectomized than in normal rats. The curves also show that the turnover rate of chenodeoxycholic acid was more rapid than that of cholic acid in both normal and hypophysectomized rats.

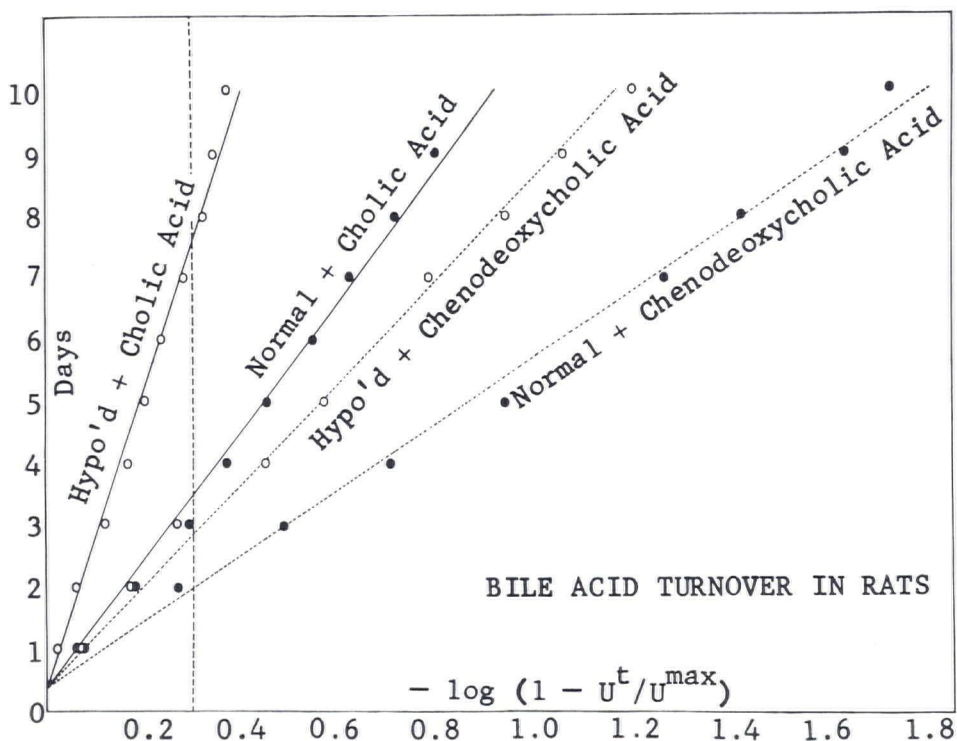


Figure 3

Cholic acid and chenodeoxycholic acid turnover rates in normal and hypophysectomized rats. The broken vertical line cuts the curves at the half-life of the bile acid pools.

Table II  
CHOLIC AND CHENODEOXYCHOLIC ACID POOL SIZES AND BILE ACID  
SYNTHESIS RATES IN NORMAL AND HYPOPHYSECTOMIZED RATS

Group	Cholic Acid	Chenodeoxy- cholic Acid	Total Bile Acids	Bile Acid Synthesis Rate
	mg/100 gm rat	mg/100 gm rat	mg/100 gm rat	mg/day/100 gm rat
Normal	7.04 ± 0.94	2.02 ± 0.61	9.06	1.39
Hypophysectomized	6.75 ± 1.20	0.05 ± 0.02	6.80	0.43

Table II shows the cholic and chenodeoxycholic acid pool sizes in the rats. The total bile acid pool was somewhat smaller in hypophysectomized rats than in normals, primarily because chenodeoxycholic acid almost entirely disappeared from the bile acid spectrum. Since in one half-life half the pool is excreted, we can calculate the daily bile acid excretion in these animals. Excretion in hypophysectomized rats was much lower than in normals. It is interesting to note that chenodeoxycholic acid, which has a higher turnover rate than cholic acid, has almost disappeared from the bile acid pool, adding to the difficulty in elimination of tissue cholesterol in hypophysectomized rats.

Table III  
THE EXCRETION OF FECAL STEROLS IN  
NORMAL AND HYPOPHYSECTOMIZED RATS

Group	Coprostanol Excreted	Cholesterol Excreted	Total Sterol Excretion
	mg/24 hr	mg/24 hr	mg/24 hr
Normal	4.79 ± 0.74	5.16 ± 0.78	9.95
Hypophysectomized	2.48 ± 0.58	3.05 ± 0.48	5.53

Data on excretion of fecal sterols are shown in Table III. Here we see that the rate of cholesterol and coprostanol excretion was greatly decreased in the hypophysectomized rats. Thus the rate of elimination of tissue cholesterol by both major pathways is greatly retarded in hypophysectomized rats. We may therefore conclude that these rats easily accumulate large quantities of dietary cholesterol because they are unable to eliminate absorbed cholesterol at the normal rate. Hypophysectomized rats fed normal diets can handle endogenously synthesized cholesterol and maintain normal tissue cholesterol concentrations. Studies<sup>9</sup> have shown that liver cholesterol synthesis rates are very slow in these animals.

*c. Effects of cholestyramine on the rates of bile acid elimination and the accumulation of tissue cholesterol in normal and hypophysectomized rats.*

Cholestyramine (MK-135), a nonabsorbable,<sup>10</sup> bile acid-binding, anion exchange resin has been shown to lower blood cholesterol concentrations effectively in a number of species.<sup>11</sup> It is believed that cholestyramine lowers blood cholesterol by increasing

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Table IV  
BILE ACID POOL SIZES AND SYNTHESIS RATES IN NORMAL AND HYPOPHYSECTOMIZED RATS TREATED WITH CHOLESTYRAMINE (MK-135)

Group	Cholic Acid mg/100 gm rat	Chenodeoxy- cholic Acid mg/100 gm rat	Total Bile Acid Pool mg/100 gm rat	Bile Acid Synthesis Rate mg/day/100 gm rat
Normal	7.04 ± 0.94	2.02 ± 0.61	9.06	1.39
" + 2% MK-135	7.00 ± 0.50	3.21 ± 0.85	10.21	6.81
Hypophysec- tomized	6.75 ± 1.20	0.05 ± 0.02	6.80	0.43
Hypophysec- tomized + 2% MK-135	5.82 ± 1.54	0.46 ± 0.10	6.28	2.09

the rate of elimination of fecal bile acids and sterols to the point where endogenous synthesis of cholesterol cannot maintain tissue sterol concentrations. If this is a true picture of the mechanism of action of this substance, then MK-135 should be effective in limiting the accumulation of tissue cholesterol in both normal and hypophysectomized rats fed diets containing this sterol. We therefore investigated the effectiveness of MK-135 in limiting the accumulation of exogenous cholesterol in normal and hypophysectomized rats, and its effect on the quantitative aspects of bile acid metabolism in these animals.

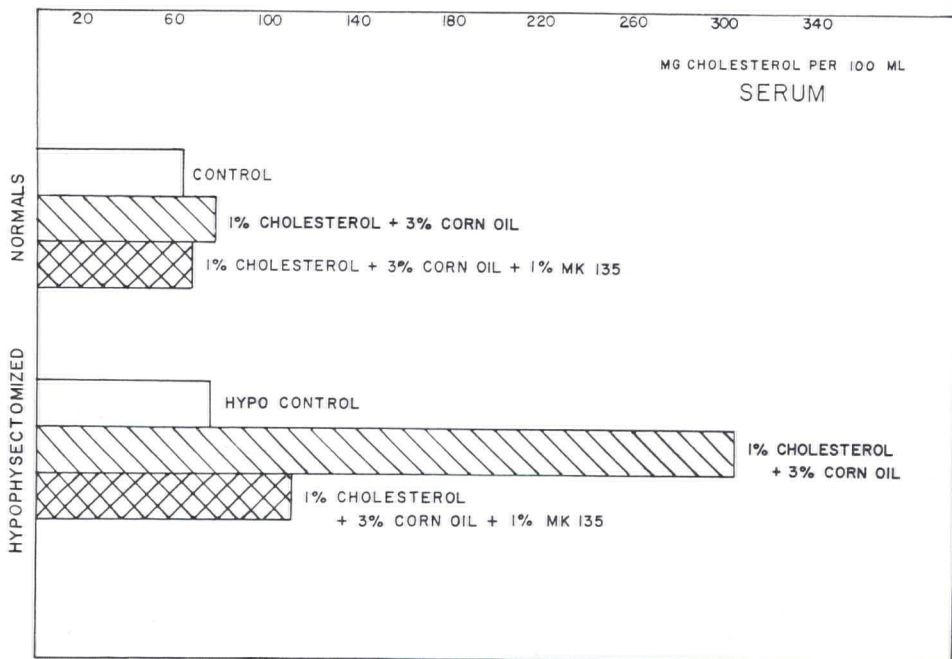


Figure 4

The effect of cholestyramine (MK-135) on the accumulation of serum cholesterol in normal and hypophysectomized rats fed cholesterol-containing diet.

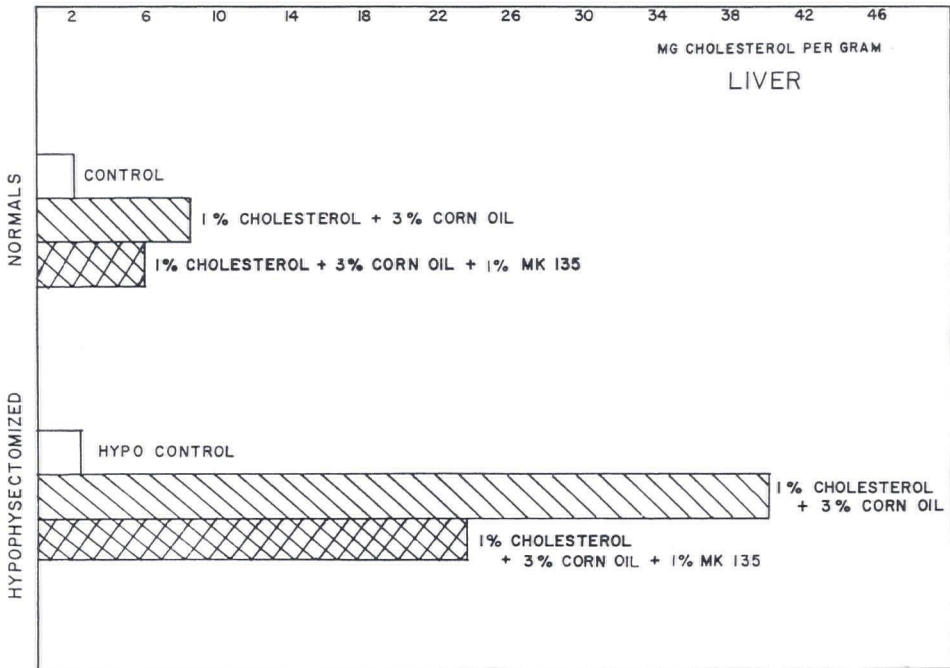


Figure 5

The effect of cholestyramine (MK-135) on the accumulation of liver cholesterol in normal and hypophysectomized rats fed cholesterol-containing diet.

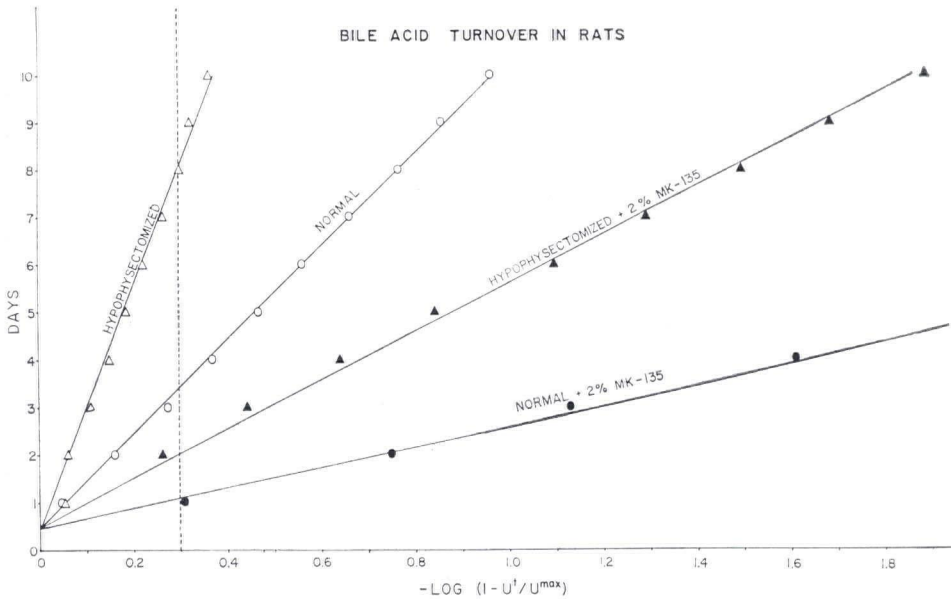


Figure 6

The effect of cholestyramine (MK-135) on the cholic acid turnover in normal and hypophysectomized rats.

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Figures 4 and 5 show the effect of MK-135 on the accumulation of serum and liver cholesterol in normal and hypophysectomized rats fed cholesterol-containing diets. Quantitatively MK-135 had a smaller effect on the accumulation of liver cholesterol than on the serum cholesterol concentration. This was to be expected since it has been shown<sup>12</sup> that liver cholesterol must rise to a certain concentration before serum cholesterol begins to accumulate. Turning to Figure 6, we see the effect of 2% MK-135 on cholic acid half-life in the bile acid pools of normal and hypophysectomized rats. In both cases, MK-135 greatly increased the turnover rate. Table IV contains the data on the effects of MK-135 on the bile acid pool sizes in normal and hypophysectomized rats. As can be seen, MK-135 had no significant effect on the pool sizes of either cholic or chenodeoxycholic acid in normal or hypophysectomized rats. However, it did greatly increase the output of bile acid in both types of rats. Thus, the faster bile acid synthesis and excretion rates in MK-135-treated animals are due to an increased rate of elimination of bile acids from their pools. Since the bile acid pool sizes did not change in the hypophysectomized rats, we can conclude that the defect in sterol metabolism in these animals is concerned not with the conversion of liver sterols to bile acids, but with the rate of elimination of bile acids from their pools.

### Summary

Several factors that significantly influence the accumulation of tissue cholesterol were studied to determine their effects on the quantitative aspects of bile acid and sterol metabolism.

a. A study of the effects of dietary cholesterol and corn oil revealed that cholesterol greatly increases the rate of conversion of liver sterols to bile acids by increasing the rate of elimination of bile acids from their pool. In addition, while cholesterol had little effect on the size of the bile acid pool, it increased the proportion of chenodeoxycholic acid. Corn oil had no effect on bile acid metabolism under the conditions of this experiment. The increased output of bile acids as influenced by dietary cholesterol constitutes a defense mechanism, which protects the rat from excess dietary cholesterol.

b. A comparative study of the accumulation of tissue cholesterol of dietary origin in normal and hypophysectomized rats demonstrated that hypophysectomized rats accumulate much more blood and liver cholesterol than normal rats when fed cholesterol-containing diets. Further experiments showed that the bile acid turnover rate is much slower in hypophysectomized rats than in normals. Since these animals had relatively normal bile acid pool sizes, bile acid synthesis was much reduced. The bile acid pool of hypophysectomized rats contained much less chenodeoxycholic acid than the pools of normal rats. A comparative study of cholesterol and coprostanol excretion revealed that hypophysectomized rats excrete much smaller quantities of these fecal sterols than normal rats. Thus, the hypophysectomized rat was shown to be relatively unable to handle excess body sterols.

c. Cholestyramine, an insoluble bile acid-binding polymer, was effective in preventing accumulation of tissue cholesterol in normal and hypophysectomized rats, when it was fed along with atherogenic diets. This resin was shown to greatly increase the turnover rate of bile acids and their synthesis in both types of rats. It had no effect on the size of the bile acid pools.

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