OBSERVATIONS CONCERNING THE PRODUCTION AND EXCRETION OF CHOLESTEROL IN MAMMALS

X. Factors Affecting the Absorption and Fate of Ingested Cholesterol*

By MEYER FRIEDMAN, M.D., SANFORD O. BYERS, Ph.D., and EICHI SHIBATA

(From the Harold Brunn Institute, Mount Zion Hospital, San Francisco)

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Previous studies (1, 2) from this laboratory have indicated that the supply of endogenous cholesterol to the plasma of the rat is primarily a function of the liver. The rate of this supply may be increased by the administration of thyroid extract (3, 4) or decreased by administration of thiouracil (3, 4), by partial hepatectomy (5), and by carbon tetrachloride intoxication (5). Other studies (6, 7) have demonstrated that the removal of cholesterol from the plasma is also a function of the liver, which first stores the cholesterol (8), then converts most of it into bile acid and excretes it (8). The rate of removal of cholesterol from plasma has been found to be increased by administration of thyroid extract (3) and decreased by administration of thiouracil (3), accumulation of bile acid in plasma (9) and by injection of the surface active substance, triton WR-1339 (10).

Despite the importance of the above endogenous phases of cholesterol metabolism in the regulation of cholesterol in plasma, considerable evidence is available which suggests that the absorption of exogenously derived cholesterol may affect not only these same endogenous phases (11) but also may alter the plasma level of cholesterol of the rabbit (12) and, under certain circumstances, also that of the dog (13) and the rat (14).

Although absorbed cholesterol has been known to enter the intestinal lymph since demonstration of the fact by Mueller (15, 16), quantitative studies concerning the amount of cholesterol absorbed have been done by analyzing the feces of animals given known amounts of cholesterol (17–19). This latter method has been employed (despite the ambiguity possible because of the breakdown of cholesterol in the intestine, as well as its secretion therein) because of earlier technical difficulties associated with chronic cannulation of the lymph duct of experimental animals, and because of uncertainty as to the fraction of absorbed cholesterol which was carried in the lymph.

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However, the difficulties associated with cannulation of the thoracic duct have been overcome, in the rat, by the techniques introduced by both Reinhardt (20) and Bollman and Flock (21). Later, too, it was found in this laboratory (22) that the intestinal lymph serves as the *only* pathway for the absorption of cholesterol (22). In view of these recent discoveries then, the total amount of cholesterol absorbed from the gut can be estimated quantitatively by analysis of the complete collection of intestinal lymph alone.

Therefore, in the studies to be reported herein concerning the factors affecting the absorption and fate of ingested cholesterol, we have analyzed the intestinal lymph, as well as the feces, of several series of rats under various experimental conditions.

Methods

A. Determination of Intestinal Absorption of Cholesterol

1. Collection and Analysis of Feces.—The feces of individually caged male Long-Evans rats were separately collected for 72 hours. At the end of this time, the rats were anesthetized, the small and large intestines were removed and opened, and their contents added to the respective individual fecal collections. Each collection then was analyzed for (*a*) "total sterols;" *i.e.*, total digitonin precipitable substance, (*b*) total cholesterol–Lieberman-Burchard positive material, calculated as cholesterol, and (*c*) "non-cholesterol sterols," obtained by subtracting (*b*) from (*a*). The chemical procedures associated with this analysis have been published elsewhere (23).

All animals were allowed to ingest water and the sterol-free diet, *ad lib.* This latter diet consisted of dextrose 72 per cent, sodium caseinate 14 per cent, gluten 6 per cent, cellulose fiber 5 per cent, U.S.P. salt No. 2, 3 per cent, supplemented with methyl linoleate 60 mg. per 100 gm. of diet and litrison[1] vitamin mixture 200 mg. per 100 gm. of diet. When excess free cholesterol was given it was dissolved in 3 cc. of olive oil and administered by stomach intubation. When excess cholate was given, it was dissolved in the equivalent quantity of 1 N NaOH, diluted with water, and also administered by stomach tube. Cannulation of the bile duct was done according to previously described methods (24), except that the rats were confined in restraining cages so that the catheter could be brought out directly through the abdominal wall. This avoids the necessity of piercing the leg of the animal.

2. Collection and Analysis of Intestinal Lymph.—Intestinal lymph was collected from the abdominal portion of the thoracic duct just above the cysterna chyli according to previously described methods (22). All rats were allowed to ingest 0.45 per cent saline solution and sterol-free diet *ad lib.* In some of the experiments, lymph was collected daily for 72 hours. The total cholesterol, and in some experiments the free cholesterol, of the lymph samples was determined according to methods previously described for blood (25).

B. Determination of Cholesterol in Blood and Tissues

Cholesterol in blood was determined by previously described methods (25), and that in tissues was determined in the same manner as for feces (23).

I. THE INTESTINAL ABSORPTION OF CHOLESTEROL

A. Effect of Cholesterol in Diet

1. Rats Given No Cholesterol.—When rats are placed upon a diet containing no cholesterol, and when, in addition, all bile is diverted from the intestine,

[1] Provided through the courtesy of Hoffman-La Roche, Inc., Rahway, New Jersey.

the flow of lymph will contain a physiologic minimum of cholesterol. This cholesterol may possibly be derived directly from the blood stream (26). Thus (see Table I) the residual content of lymph cholesterol under these circumstances averaged 2.9 mg. per 24 hour collection. If bile is allowed to enter the

TABLE I

The Intestinal Absorption of Cholesterol in Rats Deprived of Bile Acids and in Rats Given Excess Cholate

	No. of rats	Average weight	Intestinal lymph					
			0–24 hrs.			24–48 hrs.		
			Vol.	Total cholesterol		Vol.	Total cholesterol	
		gm.	cc.	mg./100 cc.	mg./24 hrs.	cc.	mg./100 cc.	mg./24 hrs.
*A. Rats Deprived of Bile Acids**								
1. Given No Cholesterol								
Average	6	295	16.7	18	2.9	—	—	—
Range	—	219–324	11.5–27.5	12–22	1.7–3.7			
2. Given 100 Mg. of Cholesterol								
Average	14	299	26.7	23	5.5	51.9	13	5.9
Range	—	249–377	12.0–46.0	3.4–45	1.0–8.6	17.5–79.0	3.3–17	2.3–10
S.E. mean	—	—	±3.0	±8.8	±0.51	±10.4	±2.39	±1.59
B. Rats Given Excess Cholate‡								
1. Given No Cholesterol								
Average	9	276	44.0	38	15.3	—	—	—
Range	—	231–340	27.0–86.0	28–54	7.5–19.8	—	—	—
S.E. mean	—	—	±5.8	±3.82	±1.07	—	—	—
2. Given 100 Mg. of Cholesterol								
Average	7	254	52.0	79	39.9	—	—	—
Range	—	213–282	34.0–69.0	60–99	29.4–51.8	—	—	—
S.E. mean	—	—	—	—	—	—	—	—

* External bile fistula established.

‡ Sodium cholate equivalent to 50 mg. of cholic acid given daily by stomach tube.

lumen of the intestine, endogenous cholesterol, previously secreted into the intestinal contents, is reabsorbed. This reabsorption was sufficient to increase the cholesterol content of a 24 hour collection of intestinal lymph (see Table II) to an average of 11.4 mg. Approximately two-thirds of this was in esterified form. Therefore, the rat, although fed a cholesterol-free diet, nevertheless absorbs about 8.5 mg. of cholesterol per day. Furthermore, it excretes at least an additional 5.4 mg. per day in the feces, since Table III shows that 16.2 mg.

TABLE II

The Intestinal Absorption of Cholesterol in Rats after the Ingestion of Various Amounts of Cholesterol

	No. of rats	Average weight	Intestinal lymph													
			Volume	0–24 hrs						Volume	24–48 hrs.		Volume	48–72 hrs.	
				Cholesterol							Total cholesterol			Total cholesterol	
				Total		Free		Ester							
		gm.	*cc.*	*mg./100 cc.*	*mg./24 hrs.*	*mg./100 cc.*	*mg./24 hrs.*	*mg./100 cc.*	*mg./24 hrs.*	*cc.*	*mg./100 cc.*	*mg./24 hrs.*	*cc.*	*mg./100 cc.*	*mg./24 hrs.*
A. Rats Given No Cholesterol															
Average	26	290	36.0	31.5	11.4	9.4	3.2	22.1	7.4	—	—	—	—	—	—
Range	—	216–412	15.7–63.0	13–55	7.1–16	3.0–15	1.9–4.1	10–35	4.2–9.7	—	—	—	—	—	—
S.E. mean	—	—	±2.5	±2.1	±0.6	±0.8	±0.24	±2.3	±0.47	—	—	—	—	—	—
B. Rats Given 50 Mg. of Cholesterol															
Average	6	276	41.0	43.0	17.0	—	—	—	—	—	—	—	—	—	—
Range	—	248–335	26–58	31–73	11–25	—	—	—	—	—	—	—	—	—	—
S.E. mean	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
*C. Rats Given 100 Mg. of Cholesterol**															
Average	33	286	44.0	68.0	25.1	20	6.4	64	18.1	68	37	21.8	80	26	14.8
Range	—	215–327	17–107	25–104	15–39	7–34	3.7–10	30–110	11–29	30–128	16–70	14–32	39–134	9–50	8.0–23
S.E. mean	—	—	±4.0	±6.8	±1.23	±2	±0.6	±6.0	±1.7	±8.4	±4.2	±1.6	±14.7	±5.5	±1.51

* Analyses of free and esterified cholesterol done on only 12 of 33 rats.

of cholesterol was found in the 72 hour feces collection. The actual excretion may be greater, as some cholesterol presumably is converted to coprosterol before excretion. These data then demonstrate that the rat probably secretes at least 13.9 mg. of cholesterol (8.5 mg. + 5.4 mg.) into the lumen of his in-

TABLE III

The Intestinal Excretion of Cholesterol in Rats after the Ingestion of Various Amounts of Cholesterol

	No. of rats	Average weight	Intestinal collection—72 hrs.						Average total sterol retained (4)‖
			Total sterol (1)*		Non-cholesterol sterol (2)‡		Cholesterol (3)§		
		gm.	mg./100 gm.	mg./72 hrs.	mg./100 gm.	mg./72 hrs.	mg./100 gm.	mg./72 hrs.	mg./72 hrs.
A. Rats Given No Cholesterol									
Average	17	238	1302.0	37.6	677.0	21.5	567	16.2	—
Range	—	180–336	840–2230	18–53	310–1290	10–34	370–940	8.0–24	—
S.E. mean	—	—	±94.0	±2.45	±63.0	±1.8	±41.0	±1.1	—
B. Rats Given 50 Mg. of Cholesterol									
Average	10	204	2225	64.0	975.0	30.0	1250	36.0	23.6
Range	—	187–226	1750–2880	44–100	585–1785	22–48	950–1690	23–52	—
S.E. mean	—	—	±102.0	±4.8	±103.0	±2.8	±64.0	±2.76	—
C. Rats Given 100 Mg. of Cholesterol									
Average	15	265	2730	104.0	961.0	37.0	1769	67.0	33.6
Range	—	200–326	810–4250	83–130	300–1770	12–64	1050–2530	45–83	—
S.E. mean	—	—	±155.0	±3.2	±106.0	±3.9	±97	±2.1	—

* (1) equals total digitoxin-precipitable substance.

‡ (2) equals difference between (1) and (3).

§ (3) equals Lieberman-Burchardt positive substance.

‖ (4) equals (ingested cholesterol + average total sterol excreted of series *A* rats) − (average total sterol excreted by experimental series).

testine per day. No more than about 2.0 mg. of this is derived from bile (5).

2. Rats Given 50 Mg. of Cholesterol.—When six rats each were given 50 mg. of cholesterol in 3 cc. of olive oil and then placed upon the sterol-free diet, it was found (see Table II) that the cholesterol content of their lymph increased to an average of 17.0 mg. during the first 24 hours. Analyses of the feces of 10 rats similarly fed (see Table III) revealed that they apparently absorbed 23.6 mg. of exogenously derived cholesterol in 72 hours or about 47 per cent of the total amount given.

3. Rats Given 100 Mg. of Cholesterol.—Rats given 100 mg. of cholesterol in 3 cc. olive oil, showed an average of 25.1 mg. of cholesterol (see Table II) in the lymph collected during the first 24 hours. If this quantity is compared with that found in lymph when no cholesterol was given (11.4 mg., see Table II), it can be seen that an increase of 13.7 mg. (25.1 − 11.4) attributable to the administered cholesterol, was absorbed the 1st day. Again, as Table II indicates, the major portion of the absorbed cholesterol was in the esterified form. Analyses of the lymph on subsequent days (see Table II) indicate that increased absorption also occurred during the 2nd and 3rd days. If the same type of calculation is made as used above to determine the excess amount of cholesterol absorbed, apparently 27.5 mg. of the 100 mg. of cholesterol administered was absorbed over the 72 hour period. This is probably a minimum amount, because it is unlikely that the basic cholesterol content of the intestinal lymph of the rat (on a cholesterol-free diet) will continue to be as much as 11.4 mg. daily, after the first 24 hour collection, as assumed in making this calculation.

The feces of 15 rats fed 100 mg. of cholesterol were analyzed for total sterol and for cholesterol. As Table III indicates, 33.6 mg. of this cholesterol was considered to be absorbed over the 72 hour period. The 18 per cent discrepancy between the value of 27.5 mg. obtained in direct recovery from lymph and that of 33.6 mg. calculated from the deficit in the feces could be due to various factors; the most likely one is our assumption that the irreducible minimum of cholesterol in lymph of the rat fed a cholesterol-free diet will continue to be 11.4 mg. of cholesterol daily even after the first 24 hours, which of course may not be true. The lymph content of cholesterol probably decreases with time on a sterol-free diet.

B. Effect of Absence of Bile

1. Rats Given No Cholesterol.—Six normal rats were deprived of intestinal bile by cannulation of their bile ducts; the thoracic ducts were cannulated and then the rats were placed upon a cholesterol-free diet. As can be seen from comparison of the figures for cholesterol found in the 24 hour lymph collection of a normal rat (Table II) with that found for the bile duct cannulated animal (Table I), a marked reduction occurred in the quantity of cholesterol found in the intestinal lymph. Apparently then, the absence of bile seriously interferes with the reabsorption of cholesterol excreted by the intestinal wall. The residual lymph content of cholesterol even in absence of bile may represent a direct contribution from the blood (26).

2. Rats Given 100 Mg. of Cholesterol.—Intestinal lymph, collected daily for 48 hours from bile duct cannulated rats fed 100 mg. of cholesterol, also showed (see Table I) a drastic reduction in cholesterol content as compared (see Table II) with that of lymph collected from intact rats receiving the same quantity of cholesterol.

C. *Effect of Excess Cholate*

1. Rats Given No Cholesterol.—The oral administration of 50 mg. of cholate to rats fed a sterol-free diet increased the lymphatic absorption of cholesterol. The average cholesterol found in the lymph of the first 24 hours was 15.3 mg. (see Table I) which, when compared to the 11.4 mg. found in the untreated rat (see Table II) represents an increase of 24 per cent.

TABLE IV

The Intestinal Excretion of Cholesterol in Rats Given Excess Cholate (50 Mg. per Day)

	No. of rats	Average weight	Intestinal collection—72 hrs						Average total sterol retained
			Total sterol		Non-cholesterol sterol		Cholesterol		
		gm.	*mg./100 gm.*	*mg./72 hrs.*	*mg./100 gm.*	*mg./72 hrs.*	*mg./100 gm.*	*mg./72 hrs.*	*mg./72 hrs.*
A. Rats Given No Cholesterol									
Average	10	286	1176	37.0	614	19.2	562	17.9	—
Range	—	245–336	905–1460	27–44	431–924	13–29	453–728	10–29	—
S.E. mean	—	—	±55	±2.1	±47	±1.5	±27	±1.38	—
B. Rats Given 100 Mg. Cholesterol									
Average	10	258	3392	88.7	1209	32.0	2183	56.7	48.9
Range	—	210–299	2390–3960	69–134	505–1760	13–61	1530–2500	44–72	—
S.E. mean	—	—	±49	±5.7	±120	±4.2	±122	±2.56	—

2. Rats Given 100 Mg. of Cholesterol.—The feeding of excess cholate to rats also getting 100 mg. of cholesterol markedly increased the intestinal absorption of cholesterol. The average cholesterol in the lymph of 7 rats so treated (see Table I) was 39.9 mg.,—an increase of 59 per cent over the control value of 25.1 mg. shown in Table II. Analyses of the feces of 10 rats similarly fed excess cholate showed (see Table IV) less fecal sterol than was found in rats not receiving cholate supplementation (Table III), and therefore an increase of 45 per cent in the apparent absorption of cholesterol.

II. THE FATE OF ABSORBED CHOLESTEROL

A. *The Plasma Cholesterol during and after the Absorption of Cholesterol*

The plasma concentration of cholesterol was not found to change significantly following the administration of 100 mg. of cholesterol, even if cholate also was given. The average plasma cholesterol of 10 rats given 100 mg. of

cholesterol plus the sterol-free diet was 61, 60, 62, and 64 mg. per 100 cc., before, and 24, 48, and 72 hours, respectively, after the ingestion of the cholesterol. The average plasma cholesterol of 8 rats given 100 mg. of cholate in addition to the 100 mg. of cholesterol was 57 and 61 mg. per 100 cc., before and 72 hours after, respectively, the ingestion of the two substances.

B. The Deposition of Cholesterol in the Liver and Viscera

A previous study (9) had shown that when excess cholesterol, in the form of hypercholesteremic serum (27), was injected intravenously into rats, its disap-

TABLE V

The Deposition of Intestinally Absorbed Cholesterol

No. of rats	Weight	Plasma cholesterol		Liver			Viscera*		
		Before experiment	72 hrs. after exp.	Dry weight	Cholesterol	Total organ	Dry weight	Cholesterol	Total organ
	gm.			*gm.*	*mg./100 gm.*	*mg.*	*gm.*	*mg./100 gm.*	*mg.*
A. Normal Control Rats on Stock Diet									
5	181	51	—	2.31	893	20.7	4.87	1190	57.3
Range	166–200	50–53	—	1.96–3.16	715–1100	17–28	4.23–5.80	1060–1380	54–62
B. Rats Given High Cholesterol Diet for 72 Hrs.									
9	180	51	61	1.88	2150	39.1	4.80	1276	61.1
Range	146–220	49–73	49–87	1.47–2.53	1450–3230	28–54	4.65–5.07	1180–1350	58–66

* Viscera include the entire contents of both peritoneal and thoracic cavities with the exception of the liver.

pearance from the blood proceeded *pari passu* with its deposition in the liver. The other tissues of the body, however, showed no significant increase in cholesterol following this type of cholesterol administration. Furthermore, although the ingestion of a high cholesterol diet (containing 2 per cent cholesterol) by the rabbit led to an immediate hypercholesteremia within 72 hours, no excess cholesterol was found in any tissue except the liver (28).

In view of these findings, the livers of young rats, and those of young rats fed for 72 hours on a diet containing 2 per cent cholesterol, were analyzed for cholesterol. Similar analyses were made of the combined extrahepatic organs (intestines, kidney, adrenal glands, testes, heart, and lungs) after similar feeding of the high cholesterol diet.

The liver content of cholesterol in the 9 rats given a high cholesterol diet for 72 hours increased markedly (see Table V) as compared to the values found in

the control rats. This average increase of approximately 19 mg. of cholesterol, representing an increase of almost 100 per cent, of course accounts for only a fraction of the amount of cholesterol absorbed but it must be remembered that undoubtedly a considerable amount of cholesterol also was converted into and excreted as cholic acid in these young rats, as previously demonstrated (29).

Despite the marked increase in the cholesterol content of the livers of rats given a cholesterol-rich diet, there was only a slight increase in the cholesterol content of the extraheptaic organs. Thus the viscera of 9 rats (see Table V) contained an average of 61.1 mg. of cholesterol as compared to the control value (see Table V) of 57.3 mg.,—an increase of only 7 per cent. Apparently then, only the liver plays a prominent part in removing dietary cholesterol from the blood.

The fundamental importance of the liver in removing the newly absorbed cholesterol from the blood was shown more strikingly by the following experiments. 5 of 10 rats were subjected to partial hepatectomy (approximately 48 per cent of the total liver was removed) and then all the rats were given 100 mg. of cholesterol in 3 cc. olive oil daily by stomach tube. The average plasma cholesterol of the operated rats rose from 56 mg. per 100 cc. (range: 49 to 62 mg./100 cc.) before, to 115 mg. per 100 cc. (range: 97 to 130 mg./100 cc.) 48 hours after, the partial hepatectomy. The plasma cholesterol of the control rats on the other hand did not increase, being 66 before and 51 mg. per 100 cc., 48 hours after the beginning of the cholesterol feeding.

DISCUSSION

The preceding studies indicate that the intestine of the rat is capable of excreting at least 13.9 mg. of cholesterol per day and that a portion of this secretion is reabsorbed to enter the blood by way of the intestinal lymph.

Following the ingestion of measured amounts of cholesterol, the rat was found to absorb a fraction inversely proportional to the dose. Thus, it appeared capable of absorbing about 47 per cent of a 50 mg. dose, but only about 34 per cent of a 100 mg. dose. The preponderance of the cholesterol was in the esterified form, as previously noted by Mueller (15), and by Bollman and Flock (21).

At least 82 per cent of the amount of cholesterol, calculated from the results of fecal analysis to have been absorbed over a period of 72 hours, was actually recovered in the lymph collected over the same period. Considering the assumptions upon which these calculations were based, the discrepancy of 18 per cent s understandable. Since, however, it was found in an earlier study (22) that all cholesterol absorbed from the intestines enters the general circulation *via* the intestinal lymph, the calculations based upon the lymph studies appear to be the most reliable.

The importance of bile in the absorption of cholesterol, first observed by

Mueller (15), and later also by various investigators (30, 31), was confirmed in these studies. The diversion of bile from the intestine led to a marked decrease in the intestinal lymph cholesterol both in animals fed a diet free of cholesterol and in those given 100 mg. of cholesterol. However, contrary to the findings of Bloom *et al.* (32), we found a slight absorption of cholesterol even in the absence of bile. The feeding of bile salt in the form of cholate increased the absorption of cholesterol previously excreted by the intestine itself, and it markedly increased the total amount of absorption of exogenously derived cholesterol.

From these findings concerning the intestinal activity of cholate, it becomes apparent that cholate acts in at least two ways in respect to cholesterol metabolism. First, it promotes the intestinal absorption of cholesterol. Secondly, when in excess in the plasma, it produces hypercholesteremia (33) and it seems to do so by preventing the egress of cholesterol from the blood (9). Whether both of these effects of cholate are due to its surface-active properties has not been determined.

Following the ingestion of cholesterol, neither the blood nor the various extrahepatic tissues exhibited a significant acute rise in cholesterol. However, qualitative changes occur in the blood cholesterol, since as has been shown by Biggs *et al.* (12), ingested labelled cholesterol quickly interchanges with blood cholesterol. More recently also, Page *et al.* (26), have demonstrated that the lipoproteins of intestinal lymph change following ingestion of cholesterol and with the drainage of such lipoproteins into the blood stream it seems most likely that for a while at least, the plasma cholesterol is altered qualitatively.

Following the intravenous administration of excess cholesterol, the blood is rid of it primarily by the ability of the liver to first store the cholesterol, then convert it and excrete it into bile as cholic acid (6, 8). The present studies indicate that the liver also functions in the same manner to rid the blood of cholesterol derived from exogenous sources. The liver then appears to occupy a pivotal position in the control of plasma cholesterol regardless of whether such plasma cholesterol arises *de novo* from the liver (1, 2), or is absorbed from the diet. Nevertheless, certain changes of the plasma itself have been shown (9) to be capable of influencing at least the rate of hepatic removal of plasma cholesterol.

SUMMARY

The absorption of oral cholesterol by rats was followed directly by analyses of the thoracic lymph and indirectly by calculating the deficit of fecal cholesterol under the amount fed. The two methods checked within about 20 per cent, with fecal analyses indicating the greater absorption. The absorbed cholesterol was found to be deposited in the liver, only very minor quantities being found in other organs or plasma.

Bile is necessary for normal absorption of cholesterol, although very small amounts are absorbed even in its absence. Excess oral cholic acid increases the absorption of cholesterol by otherwise normal animals. The rat absorbs about 47 per cent of a single 50 mg. dose of cholesterol and about 34 per cent of a 100 mg. dose.

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