OBSERVATIONS CONCERNING THE PRODUCTION AND EXCRETION OF CHOLESTEROL IN MAMMALS

IX. The Mechanism of the Hypercholesteremic Effect of Cholic Acid*

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Previous studies from this laboratory (1, 2) have indicated that when the cholate content of the rat's plasma is elevated, either by biliary obstruction or by the continuous intravenous injection of cholate into normal rats, a hypercholesteremia promptly appears. Moreover, excess cholate was found in the plasma of patients suffering from various states involving hypercholesteremia (3). These findings suggest that this "natural detergent" of the body may be concerned not only in the hypercholesteremia occurring in biliary obstruction but also in that of other conditions.

The mechanism by which cholate induces hypercholesteremia was not discovered in these studies, however, although it was found that the cholate-induced hypercholesteremia of the rat did not depend upon an increase in the intestinal absorption of cholesterol (2). More recently it also was found (4) that the mechanism did not involve the possible conversion of cholic acid into cholesterol; nor does it depend upon hemolysis.

The present report describes our more recent studies concerning the possible mechanism involved in cholate-induced hypercholesteremia. The evidence obtained indicates that cholate acts in the blood stream. Here it retards the exit of cholesterol from the plasma, leading to a surfeit of cholesterol in the plasma alone.

A Comparison of the Hypercholesteremia Produced by Biliary Obstruction with That Produced by Continuous Intravenous Injection of Cholate

8 Long-Evans strain, male rats, approximately 12 weeks old and weighing between 202 and 308 gm. were fasted for 20 hours but given water and then subjected to biliary obstruction as described in a previous report (5). 19 fasted rats treated in the same way were unilaterally nephrectomized and then given a continuous intravenous injection of 25 mg. of cholic acid per hour as sodium cholate in Tyrode solutions for 24 hours after an initial injection of 25 mg.,

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according to a previously described method (2). These latter rats were unilaterally nephrectomized to retard renal excretion of cholate. Plasma samples obtained before, and 24 hours after, the initiation of either procedure were analyzed for both cholesterol and cholic acid (1, 6). In regard to the determination of "cholic acid," it should be pointed out that bile acids other than cholic acid may be included in the analysis.

Table I indicates that both types of procedure elevated the plasma cholate and, as a consequence, also the cholesterol content of plasma. Moreover, in the two series, when concentrations of cholate were equal, the concentrations of cholesterol were also approximately equal. These data confirmed those of a previous study (7) in which the hypercholesteremia occurring after biliary obstruction could be duplicated in an unobstructed normal rat receiving cholate

TABLE I

Plasma Cholesterol Concentration in Hypercholatemic Rats

No. of rats	Average weight	Plasma cholesterol		Plasma cholate	
		Before experiment	24 hrs. after experiment	Before experiment	24 hrs. after experiment
	gm.	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*
		(*a*) *Rats with Biliary Obstruction*			
8	224	44	114	1.3	21.0
Range.....	202–240	36–55	83–147	0.3–2.6	13.5–36.0
		(*b*) *Rats Continuously Injected with Cholate*			
19	248	50	111	1.8	22
Range.....	202–308	39–73	79–140	0.8–2.2	7.4–39.0

if the plasma concentration of the latter substance in the normal rat could be raised to equal that found in the plasma of the ligated rat. In other words, the mechanism at work in the production of hypercholesteremia in the two types of experimental animals appeared to be one and the same; that is, an accumulation of cholate in the plasma.

The Distribution of Cholesterol in the Body of the Rat after Elevation of Plasma Cholate

The preceding data indicated that an excess of cholesterol appeared in the plasma of rats after their plasma cholate had been elevated either by biliary obstruction or by continuous injection of cholate. It was not known, however, whether the excess cholesterol was confined to the plasma or whether other parts of the body also contained excess cholesterol.

To investigate this point, a series of male rats of Long-Evans strain, about 12 weeks old, were divided into three groups. The first group of 11 rats were subjected to biliary obstruc-

tion after fasting as before. The second group of 10 rats were subjected to fasting and biliary obstruction and in addition were given 200 mg. of sodium cholate a day for 3 days by stomach tube. The third group of 12 rats served as controls. The rats were anesthetized with ether at the end of 3 days, bled as completely as possible, decapitated, and the total carcass less the head was analyzed for cholesterol by a method previously described for stools (8). Blood plasma samples obtained at the beginning and end of the experiment also were analyzed for cholesterol and cholate.

As Table II illustrates, despite the excess of cholesterol found in the plasma of rats made hypercholatemic by biliary duct ligation alone, the carcasses con-

TABLE II

Cholesterol Concentration in the Carcass of Hypercholatemic Rats

No. of rats	Average weight	Plasma cholesterol		Plasma cholate	Carcass cholesterol (72 hrs.)		
		Before experiment	72 hrs. after experiment	72 hrs. after experiment	Weight		Total
	gm.	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*gm.*	*mg./100 gm.*	*mg.*
			(a) Normal Control Rats				
12	224	46	52	3.2	185	169	311
Range	189–248	36–51	38–71	2.1–4.4	155–208	149–187	272–353
S.E. mean						±3.7	±8.1
			(b) Rats with Biliary Obstruction				
11	215	50	135	34.0	165	173	282
Range	180–299	38–62	106–167	29–48	134–229	146–192	239–335
S.E. mean						±4.8	±9.0
			(c) Rats with Biliary Obstruction Injected with Cholate				
11	247	49	602	68	173	197	340
Range	169–357	41–68	270–840	43–96	157–194	182–216	300–363
S.E. mean						±3.6	±5.9

tained about the same cholesterol concentration (173 mg. per cent) as that found in the normal rat (169 mg. per cent). The average cholesterol content of the carcasses of the ligated rats also given cholate was 197 mg. per cent, which is significantly higher than the cholesterol content of the bodies of the animals of the other two groups. However, in these rats, as in the others, at least half of the blood volume (approximately 8.0 cc.) remained in the tissues analyzed for cholesterol. This amount of blood contained at least 24 mg. of cholesterol (4 cc. of plasma × 6.02 mg. of cholesterol/cc. of plasma) which, if subtracted from the average total cholesterol of the organs, would reduce the latter value to 316 mg. and the cholesterol concentration from 197 to about 182 mg. per cent, a value closer to that found in the normal rat. However interpreted, the

overwhelming portion of the excess cholesterol was in the blood and little or no excess was found in the remainder of the body.

These findings also indicate that the excess of cholesterol found in the plasma of the hypercholatemic rats was not derived from the *stored* cholesterol of any other portion of the body.

Biliary Excretion of Cholesterol after Cholate Injection

6 fasted, male, Long-Evans strain rats were subjected to biliary cannulation and their bile collected for 24 hours as described previously (9, 10). Four of these rats received 25 mg.

TABLE III

The Effect of Cholate Administration upon the Biliary Excretion of Cholesterol

Rat	Weight	Amount cholate injected	Bile (24 hrs.)				
			Volume	Cholesterol		Cholate	
	gm.	*mg./24 hrs.*	*cc.*	*mg./100 cc.*	*mg./24 hrs.*	*mg./100 cc.*	*mg./24 hrs.*
Normal Control Rats							
10	241	0	16.9	14.0	2.40	413	69.8
11	264	0	15.0	14.6	2.20	246	36.9
Average.....	253	0	16.0	14.3	2.3	330	53.4
Rats Given Cholate							
44	311	75	15.6	8.7	1.4	733	114.3
81	278	75	16.4	12.4	2.0	617	101.3
82	265	75	15.3	21.2	3.3	721	110.3
85	297	75	15.4	14.1	2.2	642	98.9
Average.....	288	75	15.7	14.1	2.2	678	106.2

of cholic acid as sodium cholate by intravenous injection every 2 hours for three injections. The bile was analyzed for cholesterol and bile acids by previously described methods (1, 6).

The injection of excess cholate was not found (see Table III) to alter the biliary excretion of cholesterol. However, the bile collected during the first 24 hours contained excess bile acid equivalent to two-thirds of the amount of cholate injected.

Intestinal Excretion of Cholesterol after Biliary Obstruction and after Biliary Obstruction Combined with Intravenous Injection of Cholate

A series of 10 normal male rats and 14 rats with biliary obstruction due to ligation of the bile duct were placed in Bollman restraining cages (10), fed a sterol-free diet (11), and their individual intestinal excretions were collected for 72 hours. 5 of the 14 obstructed rats also received 100 mg. of cholic acid as sodium cholate a day by intravenous injection. The stools

were analyzed for total cholesterol and total digitonin-precipitable sterols according to previously described methods (8). Plasma obtained at the beginning and at the end of the collection was analyzed for cholesterol.

As Table IV demonstrates, biliary obstruction, and biliary obstruction plus intravenous injection of cholate, increased the cholesterol and total sterol content of the stools of the rats. Since both of these procedures appear to induce hypercholesteremia by causing an accumulation of cholate in the plasma, it is clear that this latter accumulation did not produce the hypercholesteremia by

TABLE IV

Fecal Sterol Content of Hypercholatemic Rats

No. of rats	Average weight	Plasma cholesterol		Feces (72 hr. collection)						
		before experiment	72 hrs. after experiment	Dry weight	Cholesterol		Non-Cholesterol sterols		Total sterol	
	gm.	*mg./100 cc.*	*mg./100 cc.*	*gm.*	*mg./100 gm.*	*mg./72 hrs.*	*mg./100 gm.*	*mg./72 hrs.*	*mg./100 gm.*	*mg./72 hrs.*
					(a) Normal Control Rats					
10	250	61	62	3.68	381	14	467	17	848	31
Range	186–320	44–83	44–83	3.12–4.36	310–540	10–18	165–520	7–40	540–1580	17–53
					(b) Rats with Biliary Obstruction					
9	255	53	219	3.80	634	24	572	23	1207	47
Range.	202–322	37–69	188–290	2.78–5.69	510–780	19–32	220–990	13–56	870–1540	28–70
					(c) Rats with Biliary Obstruction Injected with Cholate					
5	284	47	402	3.42	788	27	1452	51	2240	78
Range.	273–298	39–60	280–480	2.78–4.16	660–870	21–34	940–2560	28–106	1650–3350	52–139

interfering with the normal rate of intestinal excretion of cholesterol. In other words, the etiology of cholate induced hypercholesteremia is not a decrease in the rate of intestinal excretion of cholesterol.

The Source of the Excess Cholesterol Occurring in Plasma after Elevation of Plasma Cholate

Previous studies from this laboratory (5, 12, 13) have demonstrated that the liver is the chief, perhaps the sole, source of the cholesterol found in the plasma of the rat. In order to determine whether the liver is also the source for the excess cholesterol appearing in plasma after hypercholatemia had been induced, the following experiments were done.

7 fasted, male, Long-Evans rats, each weighing approximately 285 gm., were subjected to bile duct ligation and then given 25 mg. of cholate intravenously every 3 hours, until 75 mg. had been given. A second series of 9 fasted rats were subjected to biliary obstruction and then to subtotal hepatectomy. These rats also received 75 mg. of cholate postoperatively. Plasma samples obtained before and 24 hours after the operative procedure were analyzed for cholesterol and cholate.

Table V illustrates that when 67 per cent of the liver was removed from the series of bile duct-ligated rats also receiving cholate, no hypercholesteremia occurred even though a hypercholatemia was present. The rats, however, whose livers were left intact exhibited a rise in their average plasma cholesterol from

TABLE V

The Role of the Liver in Cholate-Induced Hypercholesteremia

No. of rats	Average weight	Amount liver removed	Plasma cholesterol		Plasma cholate	
			Before experiment	24 hrs. after experiment	Before experiment	24 hrs. after experiment
	gm.	*per cent*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*
			(*a*) *Rats with Biliary Obstruction Injected with Cholate**			
7	285	0	52	171	2.5	41.0
Range....	246–301		46–60	129–214	1.0–3.1	25.2–64.0
			(*b*) *Rats with Biliary Obstruction and Partial Hepatectomy Injected with Cholate**			
9	188	67	54	57	2.6	22.2
Range....	142–255	61–75	48–64	50–68	0.8–4.6	10.0–27.0

* 75 mg. of cholate injected in 3 doses.

52 to 171 mg. per 100 cc. The liver therefore appeared essential for the production of cholate-induced hypercholesteremia.

The Rate of Synthesis of Cholesterol in Cholate-Induced Hypercholesteremia

In view of the immediately preceding observations, the possibility existed that elevation of plasma cholate induced hypercholesteremia by altering the rate of hepatic synthesis of cholesterol. This possibility was therefore investigated.

The Rate of Hepatic Cholesterol Synthesis after Cholate Administration:

As Table III indicates, the intravenous injection of 75 mg. of cholic acid in a period of 24 hours did not change the rate of biliary excretion of cholesterol of the rat during this same period. The average daily biliary excretion of cholesterol in 4 cholate-injected rats was 2.2 mg., and was 2.3 mg. in the 2 control

rats. These observations suggested that cholate administration had no effect upon the rate of hepatic synthesis of cholesterol, since this latter rate has been found to be reflected in the rate of excretion of bile cholesterol (11).

The Rate of Synthesis of Cholesterol in the Rat after Elevation of Plasma Cholate, as Measured by Rate of Incorporation of Tritium into Visceral Cholesterol

If accumulation of excess cholate in the plasma of the rat induces a hypercholesteremia by increasing the rate of cholesterol manufacture, then under such circumstances an animal should utilize cholesterol precursors more rapidly than an untreated animal. It follows that if a tracer substance were administered as one of such precursors, the cholesterol in the organs and blood of the cholate-injected rat should contain more of this tracer substance than the normal rat *during the first few days* after its injection. Since tritium water is incorporated in the cholesterol formed by the rat (14) tritium was employed as a precursor label to study the rate of cholesterol synthesis in this animal after injection of cholate.

12 male rats of the Long-Evans strain weighing from 243 to 387 gm. and approximately 20 weeks old were selected. These animals were separated into two groups of 6 each. The bile ducts of the rats of both groups were ligated, without a preceding fast and food and water were withheld during the 72 hours of the experiment.

The body water of each animal was labelled with tritium water as follows. Immediately following surgery, each animal received an intravenous injection of 0.5 cc. of H_2^3O per 100 gm. of body weight. The specific activity of the tritium water administered at this time was 1.73 mc./cc. Beginning 24 hours after surgery, the animals each received 5 cc. of tritium-labelled Ringer's solution intravenously per day in five divided doses at approximately 5 hour intervals. The specific activity of this solution was 14.4 μc./cc. The tritium water dosage was calculated to give a specific activity to the body water of approximately 12.0 μc./cc. for the 3 day period of the experiment.

Sodium cholate was included in the Ringer's solution administered intravenously to group II. Each rat of this group received 60 mg. of sodium cholate the 1st day and 100 mg. on day 2 and again on day 3. The cholate was given in divided doses of 20 mg. each.

Exactly 72 hours after the experiment was begun, all the animals were sacrificed. The total organ contents of the abdominal and thoracic cavities and all blood (except 3 cc. withdrawn for analytical purposes) of each rat were collected and refluxed in alcoholic KOH and the non-saponifiable material was extracted with ether. A cholesterol-digitonide precipitate was prepared from the extract and used for measurement of tritium-specific activity as previously described (4). Plasma cholesterol and cholate values also were determined at the beginning and termination of the experiment.

As demonstrated in Table VI, the plasma and the organ-blood mixture of ligated rats that had received cholate contained more cholesterol than comparable samples from control rats. The specific activity of this cholesterol in the cholate-injected animals was 1.38 μc./gm. (S.E. mean = $\pm$0.10) as compared to a comparable value of 1.33 μc./gm. (S.E. mean = $\pm$0.13) found in the cholesterol of the control ligated rats. Inasmuch as under the conditions

of the experiment the specific activity of the total visceral cholesterol analyzed is strongly dependent on the rate of cholesterol synthesis and much less a function of the rate of cholesterol destruction or removal, the data indicate that ligated, cholate-injected rats synthesized cholesterol at essentially the same rate as the control ligated rats.

The greater amount of cholesterol in the organ-blood collection of the cholate-injected rats was probably due, as explained previously, to the greater amount of cholesterol present in the blood increment of the collection. In

TABLE VI

The Effect of Cholate Administration upon the Specific Activity of Visceral Cholesterol in Rats with Biliary Obstruction

No. of rats	Average weight	Plasma cholesterol		Plasma cholate		Visceral cholesterol	
		Before experiment	72 hrs. after experiment	Before experiment	72 hrs. after experiment	Total cholesterol	Specific activity of cholesterol
	gm.	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*mg.*	*μc./gm.*
				*(a) Rats with Biliary Obstruction Given Cholate**			
6	305	44	752	3.7	79	75	1.38
Range	243–325	40–48	650–890	2.0–6.0	50–150	56–95	1.20–1.55
S.E. mean							±0.10
				(b) Control Rats with Biliary Obstruction Alone			
6	292	47	400	5.0	36	59	1.33
Range	259–387	44–52	330–450	3.0–6.0	23–59	46–72	1.19–1.43
S.E. mean							±0.13

* Each rat received a total of 260 mg. of cholate in the 72 hour period.

other words, if both groups of organs had been cleared of blood before analysis, it is probable that their cholesterol content would have been the same.

The Hepatic Content of Cholesterol in Cholate-Induced Hypercholesteremia

Although the rate of cholesterol synthesis is not changed by cholate, it was still considered possible that the excess cholesterol in the plasma might be derived from previously stored cholesterol in the liver.

The livers of 4 normal rats and the livers of 6 rats subjected to ligation and oral feeding of 200 mg. of cholate daily for 72 hours were analyzed for their cholesterol content by a method previously described for stools (8). Plasma samples also were obtained and analyzed for cholesterol.

Table VII indicates that the livers of the ligated, injected rats contained an average of 28.9 mg. of cholesterol as compared to the 22.9 mg. found in the

liver of the normal rat. The observed difference of 6 mg. may well be due to the presence of some hypercholesteremic blood still remaining in the livers of the ligated rats. For instance, if a single cubic centimeter of plasma remained distributed throughout an entire liver, the amount of cholesterol contained in this plasma (*i.e.* 5.49 mg.) would almost account for the difference observed in the two series. At any event, the excess cholesterol noted in the plasma of the ligated rats could not have been due to any loss of tissue cholesterol from their own livers.

TABLE VII

Liver Cholesterol in Hypercholatemia

No. of rats	Average weight	Plasma cholesterol		Liver		
		Before experiment	72 hrs. after experiment	Dry weight	Cholesterol	
						Total
	gm.	*mg./100 cc.*	*mg./100 cc.*	*gm.*	*mg./100 cc.*	*mg.*
*(a) Rats with Biliary Obstruction Given Cholate**						
6	241	50	549	8.7	1297	28.9
Range.....	220–270	41–68	270–770	7.8–10.2	1170–1430	27.0–33.5
(b) Normal Rats						
5	230	45	55	9.0	860	22.9
Range.....	220–238	38–49	38–67	7.8–10.6	750–940	21.5–26.0

* Rats were given 200 mg. of sodium cholate a day for 3 days by stomach intubation.

The Rate of Disappearance of Cholesterol from the Blood in Hypercholatemic States

The preceding data indicated that although the liver was necessary for the occurrence of cholate-induced hypercholesteremia, no essential change was observed in the rate of manufacture of cholesterol by this organ nor any diminution in its own cholesterol content. The facts suggested, therefore, that the hypercholesteremia must result from some interference with the ability of the liver to remove from plasma, cholesterol which previously had been formed by the liver and secreted into the plasma. The following experiments were done to investigate this possibility.

Rate of Disappearance of Cholesterol from the Normal Rat Receiving Cholate

18 male rats approximately 16 weeks old, were given 2 cc. of rat hypercholesteremic serum containing 12 mg. of cholesterol, by intravenous injection. The hypercholesteremic serum was prepared by feeding cholate to rats with a biliary obstruction and later bleeding them (15). 8 of these 18 rats also received a continuous intravenous injection of cholate (equivalent to

25 mg. of cholic acid per hour). An additional control series of 6 rats also were continuously injected with this same amount of cholate. Cholesterol and cholate analyses were done on plasma samples obtained before, and immediately and 6 hours after the beginning of the experiment.

As Table VIII illustrates, the 8 rats that received a continuous injection of cholate were unable to rid their plasma of injected excess cholesterol as rapidly as the 8 control rats. Thus the average plasma cholesterol of the 10 cholate injected rats, which was 153 mg. per 100 cc. immediately after the adminis-

TABLE VIII

The Effect of Continous Injection of Cholate upon the Rate of Disappearance of Injected Hypercholesteremic Serum in Rats

No. of rats	Average weight	Plasma cholesterol			Plasma cholate		
		Before injection	Immediately after injection	6 hrs. after injection	Before injection	Immediately after injection	6 hrs. after injection
	gm.	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*
			(*a*) *Rats Injected with Hypercholesteremic Serum* and Cholate*				
10	300	45	153	116	5.3	4.1	16.3
Range.....	249–327	40–58	130–190	95–150	1.9–7.5	2.4–5.1	10.0–27.6
			(*b*) *Rats Injected with Hypercholesteremic Serum*				
8	318	43	156	76	5.5	7.2	6.1
Range.....	269–362	26–54	130–185	65–92	2.8–7.5	4.2–11.0	5.0–6.1
			(*c*) *Rats Injected with Cholate*				
6	294	46	—	51	3.8	—	13.7
Range.....	252–347	43–53		39–59	3.0–4.4		10.3–18.8

* Rats given 2 cc. of serum containing 600 mg. of cholesterol per 100 cc.

tration of the hypercholesteremic serum, fell to 116 mg. per 100 cc., 6 hours later, a decrease of about 24 per cent. The average plasma cholesterol of the untreated rats fell from 156 mg. per 100 cc. to 76 mg. per 100 cc. in the same time interval, a fall of 51 per cent. The 6 additional control rats that received the same amount of cholate but no cholesterol showed little change in their plasma cholesterol indicating that the retardation in the disappearance of excess cholesterol noted in the series above was not due to a *new* increment of cholesterol furnished to the blood by the amount of cholate injected.

The Effect of Cholate on the Lipoproteins of Rat's Blood

The possible effect of cholate on the lipoprotein characteristics of rat serum was studied.

7 rats were subjected to biliary obstruction and then given 200 mg. of cholic acid as sodium cholate per day by stomach intubation for 3 days, at the end of which time blood samples were obtained. A part of the sample was analyzed for its cholesterol content and the remainder was submitted to analytic ultracentrifugation.[1] The serum of 3 normal rats served as controls.

The serum of the control rats contained only a very low concentration of a lipoprotein having an S_f rate of 6 units. The serum of the experimental rats was not only hypercholesteremic (average serum concentration: 600 mg. per 100 cc.) and hypercholatemic (average serum concentration: 101 mg. per 100 cc.) but its lipoprotein spectrum was altered in a consistent and characteristic fashion. Two major peaks were observed, the slower having an S_f rate of approximately 6 units; the faster, approximately 11 S_f units. None of the samples contained lipoproteins having S_f rates above 15 units.

DISCUSSION

Considered in its entirety, the foregoing data throw considerable light upon the mechanism by which a rise in plasma cholate induces a retention of cholesterol in the animal body.

It is clear that the retention of excess cholesterol is a phenomenon occurring only in the plasma of the cholate-treated rat. All other organs and tissues examined at the height of the hypercholesteremia showed either no change in cholesterol content or a slight increase which undoubtedly was due to the presence of residual hypercholesteremic blood. The excess then, of cholesterol in the plasma was neither part of a *generalized* cholesterol excess nor was it the result of a *redistribution* of cholesterol in the animal body.

The data indicated that this plasma excess of cholesterol did not result from any diminution in the rate of either intestinal or biliary excretion of cholesterol. Furthermore, the hypercholesteremia could not have been due to an increased intestinal absorption of cholesterol, for the animals were fasted. Also, the phenomenon occurred as readily after parenteral administration of cholate as after oral administration.

The observations, on the other hand, demonstrated that the presence of the liver was necessary if cholate-induced hypercholesteremia were to occur. This can be understood when it is remembered that previous studies (5, 12, 13) have indicated that all or almost all cholesterol coming to the blood was derived from the liver.

Despite the necessity for the presence of functioning liver tissue for the genesis of cholate-induced hypercholesteremia, there was no increase in the rate of this organ's manufacture or discharge of cholesterol when the hypercholesteremia of the rat with bile duct ligation was intensified by the administration of excess cholate. Furthermore, Landon and Greenberg (16) recently have shown that the rate of hepatic synthesis of cholesterol in the rat

[1] We are grateful to John Gofman for the performance and analysis of these studies.

is not altered either by ligation or by cannulation of the bile duct. These observations indicate that there is no change in the liver synthesis of cholesterol after cholate accumulation in plasma.

These facts admit of but one conclusion: namely, that the excess of cholesterol present in plasma after cholate accumulation is due to some failure either in the normal removal of cholesterol from the blood or in its destruction by the liver (17). If there were some derangement in the latter mechanism, then a great excess of cholesterol should accumulate first in the liver and only later "spill back" into the plasma. No such accumulation of cholesterol in the liver was found in the present experiments, when the volume of blood in the liver is taken into account. This volume may amount to as much as ⅓ the blood volume of the body, when hepatic vessels are maximally distended (19). Since the hepatic vessels were not maximally distended in the present experiment, the volume of blood in the liver may be presumed to be less than this figure. However, if only a single cubic centimeter of plasma, or 1/7 of the total plasma volume of the rat, remained distributed throughout the entire liver, the amount of cholesterol contained in this plasma (*i.e.* 5.49 mg.) would nearly account for the difference observed in the two series.

On the other hand, the removal of cholesterol from the blood was found to be retarded when a state of hypercholatemia was induced. Therefore it appears that cholate in excess in the plasma induced hypercholesteremia by impeding the usual and normal rate of exit of cholesterol from the plasma into the liver (17). This leads to a retention of cholesterol primarily in the plasma, and probably only in the plasma. The manner in which cholate causes the plasma to hold on to a greater amount of cholesterol, is possibly connected with the surface-active properties of cholate and the effects of the latter upon those globulins of the plasma capable of carrying cholesterol. In other words, cholate probably allows or causes a greater degree of cholesterol binding by those plasma globulins concerned with the adsorption or combination with cholesterol. The change induced in the ultracentrifugal flotation characteristics of the lipoprotein spectrum by cholate excess in plasma strongly suggests such a mechanism.

The similarity in the modes of action of triton WR-1339 and of cholate is striking. The former substance, also surface-active but differing from cholate in its ability to remain in the blood for days (18), was found (13) to induce hypercholesteremia in the rat solely by altering the adsorption properties of its plasma. The actual difference then, in the action of these substances, may be a quantitative, not a qualitative one.

The elucidation of the mode by which cholate induces hypercholesteremia when conjoined with the observation that the plasma "cholate" (bile acid) is elevated in various states of clinical hypercholesteremia brings up for consideration the possibility that many states of hypercholesteremia, heretofore

considered as secondary to some specific defect in the body's ability to metabolize cholesterol, may well be due to an initial derangement in the ability of plasma proteins to retain cholesterol, a derangement characterized by an increase in the proportion of cholesterol adsorbed per molecule of lipoprotein globulin in the plasma. This mechanism, it should be stressed, would be independent of, hence not remediable by, any change in any organ or tissue of the body unless such a change was concerned with the cholesterol adsorptive properties of the plasma proteins themselves.

SUMMARY

Accumulation of cholate in plasma is the immediate cause of hypercholesteremia in the rat with bile duct ligation and in the normal rat given intravenous sodium cholate.

The hypercholesteremia induced by cholate administration does not appear to be dependent upon any preceding change in the rates of absorption, excretion, synthesis, or redistribution of cholesterol in the tissues of the animal.

Cholate administration seems to induce hypercholesteremia by impeding the normal rate of passage of cholesterol from the plasma into the liver; this impedance is probably due to an alteration of the cholesterol-binding power of plasma proteins induced by cholate. The chemical and physiological implications of this finding are discussed.

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