THE MECHANISM RESPONSIBLE FOR THE HYPERCHOLESTEREMIA INDUCED BY TRITON WR-1339*

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The hyperlipemic and hypercholesteremic effect of triton A-20, a dilute solution of triton WR-1339,[1] was first observed in rabbits by Kellner, Correll, and Ladd (1, 2) and later in mice by Cornforth, Hart, Rees, and Stock (3). Both groups of workers found the effect of a single injection to be a prolonged one. Cornforth *et al.* (3) were able moreover to detect triton in the blood of mice for 4 days after the intravenous injection of 25 mg. The mechanism of the triton effect, has not been discovered, however, although Kellner *et al.* (1) suspected interference with the rate of synthesis or degradation of cholesterol.

Triton WR-1339 is only one of several substances with surface active properties whose injection is followed by hypercholesteremia. One of these substances has been found (4–7) by us to be cholic acid, a natural detergent occurring normally in the blood and bile of mammals. Since both triton and cholate are surface active and hypercholesteremic agents we thought it desirable to study the mechanism by which triton induces hypercholesteremia. The present study shows that triton achieves its hypercholesteremic effect in the rat by altering the physicochemical state in which cholesterol exists in plasma so that this lipid becomes less available for removal by the liver.

Methods

Long-Evans, male rats, approximately 200 gm. in weight, were fasted for 18 hours and then were injected intravenously with various doses of triton dissolved in 10 per cent concentration in half-strength McIlvaine's buffer, pH 7.2. After various intervals of time plasma, bile, feces, or tissue specimens were obtained. These samples were analyzed for one or more of the following substances: cholesterol and its esters, cholic acid, total lipid, phospholipid, and neutral fat (by difference).

Cholesterol and cholic acid were determined as described in previous studies (5, 8–11). Total lipid determinations of plasma were done by the method of Bragdon (21), and phospholipids by the method of Fiske and SubbaRow (22) as modified by Stewart and Hendry (23).

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[1] A polymeric *p*-isooctyl polyoxyethylene phenol, supplied by the Rohm and Haas Company, Philadelphia.

RESULTS

1. Hypercholesteremic Effect of Triton

The injection of triton produced a spectacularly rapid rise in the plasma cholesterol of normal fasted rats. Table I shows that the average total cholesterol of the plasma doubled within 4 hours after 50 mg. of triton was injected, and continued to increase with result that normal values were quadrupled in 24 hours. Even greater rises were obtained following the administration of 100 mg. of triton. A further increase in dosage, however, produced no further significant rise in plasma cholesterol.

TABLE I

The Rise in Plasma Cholesterol after Injection of Triton

Amount triton injected	No. of rats	Average weight	Average plasma cholesterol concentration				
			Before injection	4 hrs.	6 hrs.	12 hrs.	24 hrs.
mg.		*gm.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*
50	41	252	48 (32–76)	96 (78–109)	138 (92–212)	180 (136–216)	199 (118–270)
100	65	248	55 (37–71)	105 (84–136)	173 (108–302)	210 (155–258)	363 (243–480)
150	24	233	56 (39–74)	—	182 (160–209)	228 (182–257)	381 (306–550)

Numerals in parentheses indicate range of values.

The excess cholesterol appearing after triton injection is composed of approximately equal increments of free and ester components, so that the final free ester/cholesterol ratio is much higher than that of the normal rat. Thus the average free cholesterol in 10 rats given 100 mg. of triton rose from 8.9 mg. per 100 cc. plasma (range: 7.2 to 11.4 mg. per 100 cc.) to 158 mg. per 100 cc. (range: 136 to 168 mg. per 100 cc.) within 24 hours whereas esterified cholesterol increased from an average of 38.5 mg. per 100 cc. (range: 31.8 to 54.6 mg. per 100 cc.) to 191 mg. per 100 cc. (range: 181 to 225 mg. per 100 cc.).

The duration of response to a single injection of triton was determined on 65 rats by analyzing individual plasma samples obtained from the tail before injection of 100 mg. of triton and individual samples from each animal in groups of 10 or more animals at intervals of 24, 48, 72, 96, and 120 hours after injection. 2 ml. of blood was taken at each bleeding so that no rat was bled excessively. The average plasma cholesterol rose from 55 mg. per 100 cc. before injection to 363 and 456 mg. per 100 cc. at 24 and 48 hours, respectively. At 72 hours the average value declined to 236 mg. per 100 cc. and this decline con-

tinued at 96 and 120 hours, the corresponding average plasma cholesterol values being 119 and 71 mg. per 100 cc., respectively.

2. Hyperlipemic Effect of Triton Injection

Following the injection of 100 mg. of triton, the average plasma total lipid of 10 rats rose from 159 mg. per 100 cc. (range: 131 to 218 mg. per 100 cc.) to 2346 mg. per 100 cc. (range: 1915 to 2790 mg. per 100 cc.) within 24 hours. Similarly, the average plasma phospholipid rose from 81 mg. per 100 cc. (range: 67 to 111 mg. per 100 cc.) to 1200 mg. per 100 cc. (range: 785 to 1450 mg. per 100 cc.) in the same period of time. The average plasma neutral fat in these 10 rats increased from 26 mg. per 100 cc. (range: 8 to 32 mg. per 100 cc.) to 769 mg. per 100 cc. (range: 515 to 1290 mg. per 100 cc.). These observations indicate that the hyperlipemic effect of triton is far more pronounced than its hypercholesteremic effect.

3. Hypercholatemic Effect of Triton Injection

Triton also induced a rise in plasma cholate. Following the injection of 100 mg. of triton, the average plasma cholate of 59 rats rose from 6 mg. per 100 cc. (range: 1.0 to 12 mg. per 100 cc.) to 21 mg. per 100 cc. (range: 12.8 to 28.6 mg. per 100 cc.) within 4 hours. No additional rise, however, was observed and the value reached at 4 hours was maintained at 6, 12, and 24 hours.

4. The Effect of Triton Injection upon the Organs of the Rat

The liver, kidney, adrenal, heart, lung, and intestines of 5 rats were examined carefully 24, 48, and 72 hours after the injection of 100 mg. of triton. Sections also were taken of the liver, kidney, adrenal, lung, and aorta of 3 rats, 48 hours after the injection of 100 mg. of triton, stained with both sudan III and hematoxylin and eosin and examined microscopically. No abnormality was detected in any organ or tissue.

5. Possible Relationship of Hypercholatemia to Hypercholesteremia after Triton Injection

In view of our previous observations (4–7) that hypercholatemia itself was capable of inducing hypercholesteremia, it was considered necessary to determine whether triton effected its hypercholesteremic effect by causing an accumulation of cholate in plasma.

Effect of Cholate Feeding upon Triton-Induced Hypercholesteremia.—

15 rats were given 100 mg. of triton and food was withheld thereafter. 8 of these animals also received 100 mg. of sodium cholate per day by stomach tube. At the end of 72 hours, plasma samples were analyzed for total cholesterol and cholate.

The ingestion of the cholate did not induce either an additional hypercholesteremic or hypercholatemic effect. Thus the 8 rats fed cholate showed an

average plasma cholesterol of 229 mg. per 100 cc. (range: 180 to 300 mg. per 100 cc.) and an average plasma cholate of 8.6 mg. per 100 cc. (range: 4.0 to 13.5 mg. per 100 cc.). The 7 control rats injected only with triton had an average plasma cholesterol of 251 mg. per 100 cc. (range: 135 to 450 mg. per 100 cc.) and an average plasma cholate of 8.9 mg. per 100 cc. (range: 5.5 to 16 mg. per 100 cc.). The plasma cholate values of the two groups moreover, at 72 hours, were far too low, according to previous data (5), to account in themselves for the hypercholesteremia observed.

Effect of Biliary Obstruction and of Bile Loss upon Triton-Induced Hypercholesteremia.—In further experiments, advantage was taken of the fact that retention of bile acids follows upon bile duct ligation, whereas biliary cannulation results in loss of bile acids. If the triton hypercholesteremic effect were brought about because of bile acid accumulation, the plasma cholesterol values should differ markedly between a group of rats receiving triton and subjected to enforced bile acid retention and a group receiving triton and subjected to loss of bile acid by cannulation of the bile duct.

A group of 10 rats therefore were each injected with 100 mg. of triton and subjected to biliary duct ligation under ether anesthesia as previously described (11). A second group of 9 rats were similarly injected but then were submitted to biliary duct cannulation. Two control groups also were used. The first control group received 100 mg. of triton, and the second control group did not receive triton but underwent bile duct ligation. Plasma samples for cholic acid analysis were obtained before, and at 6, and 24 hours after these four experimental procedures. Cholesterol analyses were done only on the plasma samples obtained before and 24 hours after the experimental procedure.

Table II shows clearly that any possible cholate effect was overwhelmed by the influence of triton; for although bile duct ligation of rats was of itself sufficient to more than double their plasma cholesterol within 24 hours and to quadruple plasma cholate, its combination with the injection of triton did not enhance the hypercholesteremic effect of triton. This equivalence of plasma cholesterol between the two experimental groups was maintained even though the ligated animals, which also received triton, had a higher plasma cholate level than those that received triton alone. It was also apparent from this experiment that cholate had far less influence to promote hypercholesteremia than 100 mg. of triton; for although the plasma cholate level of rats submitted only to bile duct ligation was similar to the cholate level in rats receiving triton only, the latter group had a plasma cholesterol value almost triple that of the duct-ligated group.

The loss of cholate by biliary cannulation was found not to alter significantly the hypercholesteremic effect of triton. The average plasma cholesterol of the cannulated rats injected with triton (see Table II) was 301 mg. per 100 cc. and that of normal rats injected with triton was 358 mg. per 100 cc., 24 hours after injection. Similarly the plasma cholate concentration of the cannulated

rats injected with triton also was about the same as that of the normal rats receiving the substance. This last finding suggested that triton could maintain a state of hypercholatemia despite the biliary loss of cholate.

Effect of Injection of Triton on Biliary Excretion of Cholate.—If the effects of triton injection were mediated exclusively by retention of bile salt in plasma, diminution in normal rate of excretion of bile salts in the bile might be expected.

TABLE II

The Results of (1) Ligation and (2) Cannulation of the Bile Duct on Triton Hypercholesteremic Effect

No. of rats	Average weight	Plasma cholesterol concentration		Plasma cholate concentration	
		Before injection	24 hrs. after injection	Before injection	24 hrs. after injection
	gm.	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*
		(a) Rats Ligated and Injected with Triton			
10	194	48 (31–59)	335 (300–390)	6.4 (5.1–7.7)	30 (15–71)
		(b) Control Rats Ligated			
9	183	48 (41–59)	125 (96–175)	6.4 (5.1–8.6)	26 (15–47)
		(c) Rats Cannulated and Injected with Triton			
9	211	54 (39–67)	301 (240–350)	6.5 (3.9–9.5)	27 (11–49)
		(d) Control Rats Injected with Triton Only			
11	209	57 (42–46)	358 (243–445)	4.8 (2.3–6.7)	23 (17–28)

Numerals in parentheses indicate range of values.

In order to investigate this possibility, the bile ducts of 15 rats were cannulated after which each rat was injected with 100 mg. of triton. The 24 hour bile output then was collected and analyzed for bile salts. The bile of 15 normal control rats was collected for the same period of time. Plasma samples also were obtained at the end of 24 hours and analyzed for bile salts.

The volume of bile and bile salt concentration was found (see Table III) to be essentially the same in the triton-injected and uninjected rats, even though the injected animals had four times the plasma bile acid concentration of the uninjected controls. It is evident therefore that the plasma cholate level after triton injection represents at most a very slight retention of cholate in the

plasma alone and is not indicative of any general tissue level of cholate. Thus a retention of only about 1.5 mg. of cholate in only the plasma of the rat would be sufficient to raise its concentration therein from a normal average value of 3.8 mg. per 100 cc. to an average of 16.2 mg. per 100 cc. (the value found in the triton-injected rats) and this slight amount would not be detected in analysis of a 24 hour bile sample. If however, the level of cholate were increased in all tissues as it was in the plasma of the triton-injected rat, at least 30 mg. of cholic acid would have had to be retained, and this amount of bile acid retained would have led to a significant, detectable, decrease of cholate in the bile.

TABLE III

The Cholate Content of Bile after Triton Injection

No. of rats	Average weight	Plasma cholate concentration		Bile		
		Before injected	24 hrs. after injected	Volume	Cholate	
	gm.	*mg./100 cc.*	*mg./100 cc.*	*cc./24 hrs.*	*mg./100 cc.*	*mg./24 hrs.*
			(a) Rats Injected with Triton			
15	228	6.2 (3.3–8.7)	16.2 (10–50)	15.5 (10.9–25.2)	180 (145–243)	28 (20–44)
			(b) Normal Control Rats			
15	226	6.2 (3.3–8.7)	3.8 (0–11)	16.2 (8.2–21.8)	184 (98–290)	29 (17–39)

Numerals in parentheses indicate range of values.

6. The Source of Excess Plasma Cholesterol after Triton Injection

Evidence has been obtained in the laboratory (12, 13) to show that the liver is the source of plasma cholesterol in both the normal and the bile duct ligated rat. This led us to believe that this organ was the source also of the excess cholesterol appearing in the plasma of the rat given triton. The following experiment substantiated this belief.

Rats were subjected to a complete functional "hepatectomy" in the following manner. The entire gastrointestinal tract, spleen, pancreas, and kidneys were removed from 5 rats and the hepatic artery was tied, depriving the liver of its blood supply, thus making the rat liverless from a functional standpoint. For control purposes, 5 rats were similarly eviscerated save that the hepatic artery was left as a blood supply to the liver and the bile duct was cannulated. Both groups of rats were then given 100 mg. of triton. Plasma samples, obtained before operation and 6 hours after triton administration were analyzed for cholesterol.

The plasma cholesterol of the control eviscerated rats with functioning liver tissue rose from 56 mg. per 100 cc. (range: 51 to 61 mg. per 100 cc.) to 95 mg.

per 100 cc. (range: 80 to 120 mg. per 100 cc.) within 6 hours. The plasma cholesterol of the animals without functioning liver tissue remained essentially unchanged, averaging 60 mg. per 100 cc. (range: 59 to 71 mg. per 100 cc.) before and 57 mg. per 100 cc. (range: 49 to 65 mg. per 100 cc.) 6 hours after the injection of triton. The liver thus appeared to be the source of the excess plasma cholesterol occurring after triton injection.

TABLE IV

The Cholesterol Content of Bile after Triton Injection

No. of rats	Average weight	Plasma cholesterol concentration		Bile		
		Before injection	24 hrs. after injection	Volume	Cholesterol	
	gm.	*mg./100 cc.*	*mg./100 cc.*	*cc.*	*mg./100 cc.*	*mg./24 hrs.*
			(a) Rats Injected with Single Dose of Triton			
15	226	55 (41–68)	279 (175–350)	15.4 (10.8–25.1)	11.7 (8.1–18.1)	1.85 (1.1–2.9)
			(b) Rats Repeatedly Injected with Triton			
2	340	60 (55–65)	2210 (1570–2850)	17.9 (17.2–18.6)	15.0 (12.7–17.3)	2.69 (2.4–2.98)
			(c) Normal Control Rats			
15	225	55 (42–69)	62 (42–75)	16.2 (8.2–21.8)	15.2 (8.3–21.2)	2.44 (1.4–3.9)

Numerals in parentheses indicate range of values.

7. *The Relation of Hepatic Rate of Synthesis of Cholesterol and Hypercholesteremic Effect of Triton Injection*

The rate of excretion of bile cholesterol has been found to depend upon the rate of synthesis of hepatic cholesterol (14, 15). Therefore by means of analysis of the daily rate of excretion of biliary cholesterol, the rate of hepatic synthesis of this steroid can be gauged.

The total 24 hour output of bile was collected: (*a*) from 15 rats which received a single injection of 100 mg. of triton immediately preceding cannulation of the bile duct, (*b*) from 2 rats which had received 100 mg. of triton twice a week for over 21 weeks, and (*c*) from 15 untreated control rats.

Table IV shows that the daily rate of excretion of bile cholesterol, and hence that the rate of hepatic cholesterol synthesis was not increased after triton administration, even though the usual hypercholesteremia occurred. Indeed,

the data can be interpreted to indicate a moderate decrease in the rate of cholesterol synthesis during the first 24 hour period following a single dose of 100 mg. of triton possibly due to acute liver injury. The two rats however which had been injected repeatedly with triton had almost the same rate of hepatic synthesis of cholesterol as the control rats.

The triton-induced hypercholesteremia therefore was not caused by an increase in the rate of hepatic synthesis of cholesterol.

TABLE V

The Cholesterol Content of Liver and Carcass after Triton Injection

No. of rats	Average weight	Plasma cholesterol Concentration		Liver			Decapitated carcass		
		Before injection	48 hrs. after injection	Dry weight	Cholesterol		Dry weight	Cholesterol	
	gm.	mg./100 cc.	mg./100 cc.	gm.	mg./100 cc.	mg./organ	gm.	mg./100 cc.	mg. carcass
				(a) *Rats Injected with Triton*					
8	230	43 (29–58)	484 (380–580)	2.49 (2.1–2.9)	882 (710–955)	22.1 (16.8–26.9)	58.0 (47–66)	550 (490–606)	317 (255–382)
				(b) *Normal Control Rats*					
5	247	45 (37–51)	47 (38–53)	2.85 (2.5–3.4)	815 (730–1000)	23.1 (18.7–25.7)	69.6 (63–76)	459 (445–480)	319 (303–338)

Numerals in parentheses indicate range of values.

8. *Relation of Liver and Tissue Cholesterol Content to Hypercholesteremic Effect of Triton Injection.*

The preceding observations while showing that the liver was the source of the excess cholesterol present in plasma after injection of triton also indicated that the hepatic tissue did not furnish this excess cholesterol by increasing its rate of synthesis of cholesterol. It therefore was thought advisable to determine whether an abnormal loss in the liver's *own* content of cholesterol took place after injection of triton. Similar studies also were done on the remainder of the carcass.

8 normal rats were injected with 100 mg. of triton and 48 hours later, they were bled as completely as possible and the liver was then separated from the remainder of the carcass. Cholesterol analyses of the plasma, liver, and carcass (minus the head) then were done. Similar analyses were made on 5 normal rats.

Table V shows that despite the fact that the plasma cholesterol content of the triton-injected rats was over 10 times as high as that of the controls, the

total cholesterol contents of the liver and carcass of the two groups of rats were approximately the same. The actual cholesterol concentration of the liver and carcass of the injected rats was slightly higher, owing we believe, to the relatively smaller dry weight of the tissues in the triton-injected rats. This latter phenomena, incidentally, has been observed consistently in tissues of rats given triton.

Calculation also makes it obvious that the excess plasma cholesterol could not have come from the liver's own stored cholesterol. Thus in the 48 hour period, the amount of cholesterol actually accumulated in the blood was about 39 mg. (plasma volume (approximately 8.0 cc.) multiplied by cholesterol concentration (4.84 mg. per cc.)), an amount almost double that found in the entire liver.

These results therefore show that the excess plasma cholesterol occurring after injection of triton could not have been due to a loss in the preformed cholesterol of either the liver or carcass. The results also demonstrated that the excess cholesterol appearing after administration of triton is a phenomenon limited to the plasma alone.

9. The Relation of Intestinal Excretion to the Hypercholesteremic Effect of Triton Injection

The foregoing data show that after injection of triton the liver continued to discharge cholesterol into the blood stream at either its usual or possibly at a slightly reduced rate. The accumulation of excess cholesterol in plasma after triton therefore must have resulted from some derangement in the removal of cholesterol.

Since intestinal excretion seems to remove some cholesterol from blood, this process was investigated.

The 72 hour stool collection of each of 14 rats was collected and analyzed for cholesterol and total digitonin-precipitable sterols by methods previously described (10). 7 of the 14 animals received 100 mg. of triton at the start of the collection period. The remaining animals served as controls.

Contrary to expectation, triton did not diminish the excretion of stool sterols. As Table VI demonstrates, intestinal excretion of both cholesterol and non-cholesterol digitonin-precipitable sterols was greater in the triton-injected animals than in the control group. These data proved that accumulation of excess cholesterol in plasma after administration of triton was not the result of a decreased intestinal excretion of cholesterol or its sterol conversion products.

10. Effect of Triton Injection upon the Cholesterol-Binding Power of Plasma

Review of all the foregoing data led, almost by a process of elimination, to the conclusion that triton must produce hypercholesteremia by interfering

with the normal process by which cholesterol is removed from the blood. Other studies from this laboratory have indicated (16) that cholesterol is removed from blood chiefly by the liver which converts it into other substances of which the chief one is cholic acid (17, 18). However, the above data also had shown that the hepatic excretion of cholate and the content of cholesterol in the liver were not changed after administration of triton. This indicated that triton did not interfere with cholesterol catabolism in the liver. These facts led inescapably to the concept that triton must induce hypercholesteremia not by altering the cholesterol metabolism of any organ but by altering the plasma alone.

TABLE VI

The Cholesterol and Sterol Digitonides in Feces after Triton Injection

No. of rats	Average weight	Average plasma cholesterol concentration		72 hr. intestinal collection						
		Before injection	72 hrs. after injection	Average dry weight	Cholesterol		Total sterol		Non-cholesterol 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) Rats Injected with Triton					
7	239	—	292 (227–339)	4.6 (3.4–4.9)	467 (380–500)	21.2 (18.0–25.5)	1036.0 (650–1350)	46.4 (32.5–63.0)	561.0 (230–800)	25.2 (13.5–37.5)
					(b) Normal Control Rats					
7	238	54 (37–78)	74 (64–97)	2.7 (1.9–4.0)	439 (390–540)	12.1 (9.4–15.5)	966 (560–1250)	27.0 (12.5–48.5)	609 (150–880)	17.3 (4.5–33.0)

If this last hypothesis were substantiated, then the transfer into a normal rat of blood from a rat previously injected with triton should not prevent the progressive rise in cholesterol which normally occurs in tritonized blood (See Table I for the rate at which cholesterol accumulates in plasma after triton injection). Conversely, removal of blood from a tritonized animal and its replacement with normal blood should prevent the development of the hypercholesteremia otherwise to be expected in this injected animal. Accordingly, a series of experiments were done in which the bloods of both triton-injected and uninjected rats were interchanged.

A series of triton-injected rats were bled as completely as possible 6 hours after the injection of 100 mg. of triton and the blood replaced with an equivalent amount of previously pooled heparinized normal rat's blood. This bleeding and replacement process was repeated twice, so that each rat received about 15 cc. of normal blood in the total process. A similar type of bleeding and replacement was done on uninjected normal rats except that they re-

ceived a quantity of previously pooled blood obtained from rats given 100 mg. of triton 6 hours before. Five successful experiments of each type were obtained. For further control purposes two triton-injected rats were cross-transfused twice 6 hours after injection. Blood samples were obtained immediately before and after the bleeding and replacement procedure and again 24 hours after the latter process. They were analyzed for plasma cholesterol. Table VII lists the results.

It can be seen that the 2 control triton-injected rats which were only cross-transfused, developed a progressive hypercholesteremia quite unaffected by the transfusion and entirely comparable with that of rats given triton and other-

TABLE VII

The Plasma Cholesterol Changes in (1) Triton-Injected Rats and (2) Normal Rats after Exchange of Their Bloods

No. of rats	Average weight	Average plasma cholesterol concentration			
		Before injection	Immediately* before exchange	Immediately after exchange	24 hrs. after exchange
	gm.	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*	*mg./100 cc.*
(a) Control Triton-Injected Rats Cross-Transfused					
2	318	50	171	150	377
(b) Triton-Injected Rats, Later Bled and Given Blood of Normal Rats					
5	305	50	180	84	72
(c) Normal Rats, Later Bled and Given Blood of Triton-Injected Rats					
5	310	—	47	104	223

* Bleeding and replacement done 6 hours after injection of Triton.

wise untreated. The transfusion alone, therefore, did not affect the ability of the rat to develop hypercholesteremia.

The 5 rats, however, whose blood was replaced with normal blood 6 hours after the rats had been injected with triton, failed to develop a significant hypercholesteremia, despite the fact that the organs and tissues of these rats had been exposed to the possible action of triton for the 6 hours prior to the time of transfusion. This strongly suggested that the triton action did not appear to reside in the fixed organs or tissues of these animals. On the other hand, the normal, uninjected rats after receipt of the blood from the triton-injected rats, promptly developed a progressive hypercholesteremia over the ensuing 24 hours. This, of course, demonstrated that the action of triton resided in the blood alone.

11. Plasma Lipoprotein Alteration in the Rat after Triton Injection

The mechanism of the action of triton on plasma was sought in a study of the low density lipoproteins in normal rats and in rats receiving triton.

5 rats were injected with 150 mg. of triton and blood samples were obtained 24 hours later. A part of the sample was analyzed for its cholesterol content and the remainder was submitted to analytic ultracentrifugation.[2] The serum of 3 normal rats served as controls.

The serum of the 3 normal rats was found to contain only a single lipoprotein species at the density studied (NaCl: 1.063 gm. per cc.) and it had a flotation rate (S_f) of 6 units. The serum of the triton-injected rats was not only hypercholesteremic (average serum cholesterol concentration: 532 mg. per 100 cc.) but its lipoprotein spectrum was altered radically. The usual lipoprotein species, *i.e.* one with a flotation rate of 6 units, was almost absent and new species having flotation rates of 100 to 500 were in preponderance. The turbidity of the serum samples appeared to be due to the high concentration of these newly formed lipoproteins of greater flotation rate.

12. Inhibition of Hypercholesteremic Effect of Triton Injection by Heparin

Heparin is known to cause changes in plasma lipoprotein species opposite to those ascribed to triton in the preceding section, in that its injection causes the disappearance of lipoproteins of high S_f rate (19, 20). If, then, triton effects hypercholesteremia by changing the character of plasma lipoproteins so that the plasma is able to bind cholesterol in a form which is not easily available for removal by the liver, then heparin conceivably might counteract this effect by reversing the changes in the lipoprotein spectrum of plasma and allowing cholesterol once again to be removed by the liver.

In order to determine this point, 7 rats were injected with 50 mg. of triton and 100 mg. of heparin. 7 additional rats were injected with triton alone, for control purposes. Blood samples were obtained 6 hours later and analyzed for plasma cholesterol concentration.

The injection of heparin markedly interfered with the cholesteremic effect of triton. The average plasma cholesterol of the rats injected with triton alone rose from 63 mg. (range: 60 to 65 mg. per 100 cc.) to 168 mg. per 100 cc. (range: 139 to 190 mg. per 100 cc.) whereas the average plasma cholesterol of the rats injected with both triton and heparin rose from 59 mg. (range: 54 to 63 mg. per 100 cc.) to 100 mg. per 100 cc. (range: 66 to 137 mg. per 100 cc.).

DISCUSSION

The preceding data indicate that the hypercholesteremic effect of triton is not caused by any change in the function of either the liver or intestine. Thus the liver was found unchanged in its ability to synthesize, discharge, and later

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

destroy cholesterol and the intestine actually increased its rate of excretion of cholesterol.

On the other hand, triton was found to alter the blood itself so that it appeared to retain excessive amounts of cholesterol which in the normal animal would have left the blood stream to be destroyed eventually by the liver. A change in the adsorptive properties of the plasma proteins, effected by injection of triton appeared to be the physicochemical basis for the greater retention of cholesterol observed. In other words, triton interferes with the normal rate of disappearance of cholesterol from blood, not by altering any of the functions of the organs concerned with cholesterol metabolism but by altering the lipoproteins of blood so that they are capable of carrying and retaining more cholesterol. It would appear that the rise of the other lipids in plasma is brought about in the same manner. The fact deserves emphasis also that the excess of cholesterol is confined to the blood alone.

The explanation here given of how triton achieves its hypercholesteremic effect points to a new mechanism for the genesis of hypercholesteremia and also suggests the possibility that other types of hypercholesteremia may result from a change in the blood, instead of from changes in some tissue(s) or organ(s) to which they have hitherto been referred.

SUMMARY

Injection of triton WR-1339 into rats leads to a rapid increase in the cholesterol, cholate, and various lipid fractions of their blood. The increase in cholesterol is confined to the blood itself.

The cholesteremic effect of triton was not dependent upon a prior accumulation of cholate in plasma.

The liver was found to be the source of the excess cholesterol but the rate of cholesterol synthesis, the excretion of cholate, and the cholesterol content of the liver were not changed by injection of triton.

The cholesteremic effect of triton is not due to alteration in the intestinal excretion of cholesterol.

Transfer of blood between "tritonized" and normal rats leads to a disappearance of the hypercholesteremia in the former and its appearance in the latter animals.

The plasma proteins of rats injected with triton are markedly changed qualitatively.

Heparin was found to inhibit the hypercholesteremic effect of triton.

The hypercholesteremia following triton injection appears to be due to a fundamental physicochemical change in the plasma proteins produced by injection of this detergent.

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