ACCELERATION OF HEPATIC CHOLESTEROL SYNTHESIS BY TRITON WR-1339*

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Previous work from this laboratory has provided evidence that the synthesis of cholesterol is increased in rats subjected to ligation of the common bile duct (1). Intravenous injection of the detergent triton WR-1339 has been shown to produce a rise in serum cholesterol even more pronounced than that which follows biliary obstruction (2). Since there is some reason to believe that these two stimuli act through similar mechanisms, it seemed desirable to investigate by isotopic methods the rate of cholesterol synthesis after injection of triton. The present experiments lend support to the idea that in this instance, also, cholesterol synthesis is increased. Indeed, any explanation of the phenomenon which does not postulate such an increase is subject to certain rather serious criticisms, as discussed below.

EXPERIMENTAL

The rats utilized were males from the Harvard colony, maintained on a diet of Purina laboratory chow. In some experiments 1 per cent cholesterol was added to this diet. The cholesterol was added in ether solution, the ether being allowed to evaporate. Triton WR-1339 was kindly supplied by Rohm and Haas Co. 1-C^{14}-sodium acetate was prepared by the Grignard reaction (3). Blood for determination of serum cholesterol by the Schoenheimer-Sperry method (4) was obtained from the tail. The rats were anesthetized with ether during this procedure. The incubation of liver slices, the isolation of cholesterol from them, the determination of liver cholesterol, and the calculation of results in terms of total nitrogen were carried out as previously described (5). Cholesterol was recovered from the digitonide by the method of Schoenheimer and Dam (6). Fieser's directions for passage of cholesterol through the dibromide were followed (7).

RESULTS

Cholesterol Synthesis in Vivo.—

Each of 6 rats was given an intravenous injection of 100 mg. of triton WR-1339 in 1 ml. of water. Each of 3 controls was given 1 ml. of distilled water, and 3 other controls were untreated.

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24 hours later each rat was given intraperitoneally 316 μg. of 1-C^{14}-sodium acetate containing 50 μc. or 7.3×10^6 C.P.M. (measured at the same efficiency as the cholesterol digitonide). After 1 hour the specific activity of the liver cholesterol was determined.

As shown in Table I, the average value for the rats which received triton exceeded by 3-fold that for the controls. Although the spread of the data was

TABLE I

Cholesterol Synthesis in Vivo in Rats 24 Hours after Intravenous Injection of 100 mg. of Triton

Rat	Body weight	Treatment*	Serum cholesterol	Liver cholesterol	Specific activity of liver cholesterol‡
	gm.		mg./100 ml.‡	mg./100 gm.‡	C.P.M./*mg.*
1	154	Triton	750	300	2695
2	166	Triton	706	271	1831
3	193	Triton	535	271	1177
4	150	Triton	603	293	2892
5	158	Triton	656	259	2631
6	149	Triton	656	236	2825
Mean.....	162		651	272	2342§
7	156	Water	76	218	669
8	171	Water	65	243	1053
9	155	Water	79	231	423
10	175	None	66	267	1146
11	150	None	65	273	567
12	171	None	52	245	686
Mean.....	163		67	246	757§

* Rats 1 through 6 were given intravenous injections of 100 mg. of triton WR-1339 dissolved in 1 ml. of water 24 hours prior to administration of the labelled acetate. Rats 7 through 9 were given intravenous injections of 1 ml. of sterile distilled water. The other controls were untreated.

‡ All figures represent the average of determinations in duplicate.

§ $p < 0.001$.

considerable, there was no overlap between the experimental animals and the controls.

Cholesterol Synthesis in Vitro.—Another group of 7 rats was treated similarly with triton. After 24 hours, the ability of slices from their livers to synthesize cholesterol from acetate was compared with the synthetic capacity of slices from control rats. The results, presented in Table II, confirm the impression obtained from the experiment *in vivo* that cholesterol synthesis is increased after injection of triton. The ratio between experimental animals and controls is approximately equal for the two methods. It is evident from Tables I and II

that the large increase in serum cholesterol produced by triton is accompanied by little if any rise in liver cholesterol 24 hours after the injection.

It seemed of interest to study the situation at subsequent times, as the effect of the detergent began to subside. 48 hours after the dose was adminis-

TABLE II

Cholesterol Synthesis in Vitro in Rats 24 Hours after Intravenous Injection of 100 mg. of Triton

Rat	Body weight	Treatment	Cholesterol		Conversion*,‡	Relative isotope concentration§,‡
			Serum	Liver		
	gm.		*mg. per 100 ml.*	*mg. per 100 gm.*	*per cent*	*per cent*
1	118	Triton	391	326	6.3	3.4
2	125	Triton	505	282	9.3	5.7
3	167	Triton	560	244	9.8	7.0
4	164	Triton	630	241	10.1	7.4
5	223	Triton	486	237	6.7	5.3
6	202	Triton	503	250	6.2	4.4
7	179	Triton	533	274	8.9	5.7
Mean. ...	168		515	265	8.2‖	5.6‖
8	166	None	85	257	3.6	2.5
9	142	None	63	209	2.0	1.7
10	175	Water	72	238	3.7	2.9
11	150	Water	74	231	3.4	2.6
12	217	Water	68	218	2.4	1.9
13	192	Water	83	248	1.6	1.1
14	134	Water	65	285	1.6	1.0
Mean.....	168		73	241	2.6‖	2.0‖

Each vessel contained 3.7 cc. of Krebs-Ringer phosphate solution, 2.5 mg. of C^{14}-carboxyl-labelled sodium acetate (182,000 C.P.M. if counted at a thickness of 4 mg. spread over an area of 3.7 sq. cm.), and 0.5 gm. of liver slices, approximately 0.5 mm. in thickness. Temperature 37°; pH 7.4; time of incubation 3 hours; shaking rate 120 oscillations per minute; atmosphere 100 per cent oxygen.

* Percentage of the radiocarbon added which was recovered as cholesterol.

‡ All values represent the average from incubations in duplicate.

§ (Counts per minute per milligram of recovered cholesterol carbon × 100)/(counts per minute per milligram of added sodium acetate carbon).

‖ $p < 0.001$.

tered, the rate of hepatic cholesterol synthesis did not differ greatly from that of the controls. After 72 hours, however, a rebound phenomenon appeared. As seen in Table III, the serum cholesterol had begun to return to normal, the liver cholesterol was definitely elevated, and the ability of liver slices to convert acetate to cholesterol was depressed. This observation is in accord with our

earlier finding of a reciprocal relationship between cholesterol concentration and synthesis (5). It may be interpreted as indicating the return of newly synthesized cholesterol from the blood to the liver.

Effect of Triton on Cholesterol-Fed Rats.—We have also carried out experiments to determine the effect on cholesterol synthesis of triton and of biliary obstruction in rats in which synthesis had been depressed by cholesterol feeding. The rats in this series were fasted for 48 hours prior to sacrifice, to eliminate

TABLE III

Cholesterol Synthesis in Vitro in Rats 72 Hours after Intravenous Injection of 100 mg. of Triton

Rat	Body weight	Treatment	Cholesterol		Conversion*,‡	Relative isotope concentration§,‡
			Serum	Liver		
	gm.		*mg. per 100 ml.*	*mg. per 100 gm.*	*per cent*	*per cent*
1	137	Triton	422	471	0.31	0.11
2	172	Triton	342	585	0.27	0.08
3	152	Triton	326	445	1.14	0.45
Mean.....	154		363	500	0.57‖	0.22‖
4	142	None	87	240	4.1	2.9
5	180	None	80	241	4.6	3.3
6	150	None	97	234	4.6	3.5
Mean.....	157		88	238	4.4‖	3.2‖

See footnotes under Table II for experimental details.

* Percentage of the radiocarbon added which was recovered as cholesterol.

‡ All values represent the average from incubations in duplicate.

§ (Counts per minute per milligram of recovered cholesterol carbon × 100)/(counts per minute per milligram of added sodium acetate carbon).

‖ $p < 0.001$.

the complicating factor of diminished food intake following ligation of the common bile duct. This circumstance probably explains the fact that the rates of cholesterol synthesis observed were generally lower than in the other experiments described above (also see references 8 and 9).

Six rats were maintained on a 1 per cent cholesterol diet for 21 days prior to the period of fast. Six other rats received a normal diet. Two rats in each group were subjected to ligation of the common bile duct 48 hours before preparation of liver slices. Two rats in each group received 100 mg. of triton 24 hours before preparation of liver slices, and two rats were untreated.

It appears from the results in Table IV that the depressant effect of cholesterol feeding was completely overcome by either of the opposite stimuli. After

ligation of the bile duct or administration of triton, the rate of synthesis in cholesterol-fed rats was elevated well above that in normally fed, as well as in cholesterol-fed, controls, and nearly to the level of normally fed controls subjected to similar procedures.

Equilibration of Cholesterol between Serum and Liver.—Friedman and Byers have suggested that the rise in serum cholesterol that follows administration

TABLE IV

Cholesterol Synthesis in Vitro in Cholesterol-Fed Rats 24 Hours after Intravenous Injection of 100 mg. of Triton, or 48 Hours after Ligation of the Common Bile Duct

Rat	Body weight	Treatment	Diet*	Cholesterol		Conversion‡,§	Relative isotope concentration‖,§
				Serum	Liver		
	gm.			*mg. per 100 ml.*	*mg. per 100 gm.*	*per cent*	*per cent*
1	238	None	Normal	59	256	0.25	0.17
2	199	None	Normal	61	284	0.22	0.13
3	167	None	Cholesterol	101	416	0.007	0.003
4	213	None	Cholesterol	72	332	0.013	0.007
5	192	Triton	Normal	440	230	2.7	2.0
6	211	Triton	Normal	388	230	2.5	1.9
7	207	Triton	Cholesterol	424	230	2.0	1.5
8	192	Triton	Cholesterol	653	330	2.7	1.4
9	219	Ligated	Normal	255	260	2.5	1.7
10	244	Ligated	Normal	242	276	2.9	1.9
11	178	Ligated	Cholesterol	318	370	2.1	1.0
12	193	Ligated	Cholesterol	196	322	1.9	1.0

See footnotes under Table II for experimental details.

* All rats in this table were fasted for 48 hours prior to sacrifice.

‡ Percentage of the radiocarbon added which was recovered as cholesterol.

§ All values represent the average from incubations in duplicate.

‖ (Counts per minute per milligram of recovered cholesterol carbon × 100)/(counts per minute per milligram of added sodium acetate carbon).

of triton is due to retention in the serum of cholesterol normally marked for destruction in the liver (2). We have carried out experiments to determine whether, in the tritonized animal, the passage of cholesterol from serum to liver is slowed.

Rat 1 was given 100 mg. of triton intravenously. 18 hours later, this rat was given 115 μc. of labelled sodium acetate intraperitoneally. At the same time, rat 2, previously untreated, was given 300 μc. of labeled sodium acetate intraperitoneally. 24 hours later, the blood was drained from each of these rats. Rats 3 and 4, which had received 100 mg. of triton intravenously 24 hours earlier, were given intravenous injections of serum from No. 1. Rats 5 and 6, previously untreated, were given intravenous injections of serum from No. 2. 3 hours later, rats 3, 4, 5, and 6 were sacrificed, and the specific activities of their serum and liver cholesterol were determined.

The results are presented in Table V. At the time interval chosen, the equilibration of serum and liver cholesterol was only partially complete in all the animals. It is evident from the last column that, in the tritonized rats, the passage of labelled molecules from serum to liver was not delayed but rather was accelerated.

Purification of Cholesterol through the Dibromide.—In order to guard against the possibility that the apparently increased conversion of acetate to cholesterol after triton was due to a contaminant, liver cholesterol from rat 1 in Table I was recovered from the digitonide and passed twice through the dibromide. The results of duplicate determinations of specific activity, in C.P.M./mg., were as follows: Before passage through the dibromide, 43, 40;

TABLE V

Equilibration of Cholesterol between Serum and Liver 3 Hours after Intravenous Injection of Serum Containing Radioactive Cholesterol

Rat	Body weight	Treatment	Serum cholesterol	Radioactive cholesterol injected		Specific activity of liver cholesterol	Specific activity of serum cholesterol	Liver S.A.* / Serum S.A.
	gm.		*mg./100 ml.*	*mg.*	C.P.M.	C.P.M./*mg.*	C.P.M./*mg.*	
3	164	Triton	558	6.4	55,700	367	761	0.48
4	150	Triton	597	5.6	48,200	412	597	0.69
5	154	None	80	0.61	9,140	166	763	0.22
6	171	None	71	0.61	9,140	146	833	0.18

* S.A., specific activity.

after one passage through the dibromide, 40, 40; after two passages through the dibromide, 41, 40.

DISCUSSION

On the basis of current ideas about the rate of turnover of cholesterol (1, 10), the precipitous rise in serum cholesterol that follows administration of triton cannot be attributed only to the slowing of cholesterol excretion and destruction. This conclusion can be reached entirely apart from the evidence for increased synthesis presented above, as follows:—

Suppose we attempt to calculate a half-time for serum plus liver cholesterol in the normal animal on the assumptions that after injection of triton cholesterol synthesis is unchanged and that the rise in serum cholesterol is due entirely to cessation of excretion and destruction. Our typical control rat (Tables I and II) weighed 166 gm. Its liver weight was 8.22 gm., and its serum volume may be assumed to be 5.0 ml. (11). The cholesterol content of its liver was 20.0 mg. and of its serum 3.5 mg., making a total pool of 23.5 mg. 24 hours after injection of triton the liver cholesterol content rose slightly or remained con-

stant, and the serum cholesterol rose to 28.9 mg., making a total pool of at least 48.9 mg. A total of 25.4 mg. of cholesterol was added to the pool in 24 hours; therefore the time required for synthesis of as much cholesterol as was present originally was $\frac{23.5}{25.4} = 0.93$ day. If the same rate of synthesis prevails in the normal rat, this same time interval must represent the mean life of liver plus serum cholesterol in the steady state. The half-time, therefore, would appear to be $0.93 \times 0.693 = 0.64$ day. This value, however, is far below the values of 4 to 6 days obtained by other methods (1, 10), and it provides reason to doubt the initial assumption of unchanged synthesis, apart from the positive experimental evidence presented above.

Isotopic experiments have led to the conclusion that cholesterol synthesis is increased after ligation of the common bile duct (1) and after administration of triton, and that it is decreased after the feeding of cholesterol (12, 13). On the other hand, if one uses the biliary excretion of cholesterol as an index of synthesis in these conditions, it appears that the synthesis of cholesterol is unchanged (2, 14, 15). We feel that under these circumstances, the reliability of the latter index should be regarded as open to question.

The suggestion of Friedman and Byers (2), that the primary cause of a rise in serum cholesterol after injection of triton is a change in the physico-chemical state of the blood cholesterol, is compatible with our results. Although the passage of individual cholesterol molecules between serum and liver seems to be unimpaired, the partition ratio may still be altered. It appears that surface-active agents may favor removal of cholesterol from the liver, when the deficiency thus created is continually made up by increased synthesis.

In three of our rats (Nos. 8, 11, and 12 in Table IV) the rate of cholesterol synthesis was well above that of the controls, in spite of a definite elevation in liver cholesterol concentration. This apparent violation of the reciprocal relationship previously observed (5) may be explained if it is assumed that surface-active agents, by their stabilizing action, reduce the effective concentration of cholesterol, as far as inhibition of cholesterol synthesis is concerned.

SUMMARY

Synthesis of cholesterol from acetate in the rat was studied after injection of triton WR-1339, both in the whole animal and by the liver slice technique. Synthesis appeared to be increased 3-fold 24 hours after the injection. It was depressed after 72 hours, concurrently with a rise in the cholesterol concentration in the liver and its fall towards normal in the blood. When triton was injected into cholesterol-fed animals, or when their bile ducts were ligated, cholesterol synthesis was faster than in the untreated, normally fed controls, despite, in some instances, an elevated concentration of cholesterol in the liver.

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