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PLASMA TRANSPORT OF LIPIDS AND LIPOPROTEIN PROTEINS IN DOGS TREATED WITH TRITON WR-1339*

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Production of hyperlipemia after administration of Triton[1] WR-1339, a non-ionic surface-active agent, has been described in rabbits (1), guinea pigs (1, 2), mice (3), and rats (4). A similar observation has been recently extended to dogs (5). These latter animals, after prolonged administration of the detergent in a dose of 250 mg per kg every fourth day, died within a period of 2 to 3 months (5). Pathological studies showed: *a*) large accumulation of lipids in the subintima of aorta and coronary arteries; *b*) marked lipid engulfment of the reticuloendothelial cells of liver, spleen, and lymph nodes; and *c*) diffuse depletion of fat stores. According to the plasma lipid levels, the course of the disease could be divided into a normolipemic stage of 2 to 3 weeks' duration, followed by a hyperlipemic stage of 4 to 8 weeks' duration, characterized by a progressive rise of all plasma lipids until death of the animals.

In vitro studies from this laboratory (6) have furnished evidence that, after addition of Triton to canine serum lipoproteins, the detergent combines preferentially with their lipid moiety, weakening the association between protein and lipid. Similar alterations were produced in chylomicrons, where Triton was able to displace part of their protein moiety from its association with lipids (6).

In dogs treated with Triton the establishment of the hyperlipemia was accompanied by profound changes in the ultracentrifugal lipoprotein pattern with disappearance of the high-density class and accumulation of lipids in the very low-density lipoproteins (5).

On the basis of the above observations and in an attempt to better define the type of lipoprotein abnormality in the plasma of dogs given Triton, canine serum lipoproteins, separated by ultracentrifugation, were isotopically labeled in each of their components, and their plasma transport and distribution among the various ultracentrifugal lipoprotein classes was studied in dogs treated with Triton WR-1339. The results of these studies are presented herein.

MATERIALS AND METHODS

Male mongrel dogs weighing from 9 to 14 kg were used. They were fed a commercial diet (5). Hyperlipemia was produced by intravenous administration of Triton (250 mg per kg every fourth day) according to the method of Scanu and co-workers (5). Another group of dogs was kept as a control.

Ultracentrifugal methods of separation and purification of serum lipoproteins were the same as previously described (7). We will refer to lipoproteins of density (D) < 1.006 as chylomicrons, D 1.006–1.063 as β-lipoproteins, and D 1.063–1.21 as α_1-lipoprotein.

Preparation of labeled materials for injection into recipient dogs. In vitro labeling with I^{131} of the protein moiety of both βLP[2] and αLP was performed according to McFarlane (8). Free iodide was removed by passing the radioiodinated protein three times through an anion exchange resin, Ioresin (Abbott Labs., North Chicago, Ill.). We estimated that the labeled protein had about 1 atom of iodine per molecule.

C^{14}-labeling of lipoprotein cholesterol was performed according to Avigan (9) by incorporating *in vitro* cholesterol-4-C^{14} into the ultracentrifugally separated canine serum lipoproteins.[3] In these experiments 10 μc of labeled cholesterol per mg of lipoprotein protein was used.

P^{32}-labeling[4] of the lipoprotein phospholipids was performed *in vivo*. Normal fasting dogs were each given

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[1] Triton WR-1339, Winthrop and Stearns, New York, N. Y.

[2] The abbreviations used in the text are: βLP = β-lipoprotein, αLP = α_1-lipoprotein, and αP = the protein of αLP after removal of the lipid.

[3] Cholesterol-4-C^{14} purchased from Volk Radiochemical Co., Chicago, Ill.

[4] P^{32} obtained from Abbott Laboratories, North Chicago, Ill.

intravenously 10 mg of P^{32}, and approximately 12 hours later 500 ml of venous blood was withdrawn. The sera were ultracentrifuged at D 1.21 to float all classes of lipoproteins. These lipoproteins were washed twice in a medium of D 1.21 each time for 24 hours and the final product dialyzed against large volumes of 0.15 M NaCl.

To obtain C^{14}-labeled triglycerides, the technique of Havel and Fredrickson (10) was followed. Normal dogs, receiving a saline infusion, had their thoracic duct cannulated (11) and were given 100 μc of the sodium soap of palmitic-1-C^{14} acid [8] mixed in 50 ml of commercial 30 per cent cream through a stomach tube. Lymph was collected for 6 hours; chylomicrons were separated as was done for serum chylomicrons (12).

Experiments were also conducted by using a commercial product of radioiodinated triolein, Raolein, I.V.[5] Before intravenous use the content of each vial (triolein, 287 mg; radioactivity, 75 μc) was mixed with 2 ml of 1 per cent solution in saline of Lipomul-Oral.[6] A fine emulsion was thus obtained.

For studies of plasma free fatty acids (FFA) an albumin-C^{14} palmitate complex was used. This was prepared by mixing 100 μc of palmitic-1-C^{14} acid (1.95 mc per mmole) after neutralization with 0.1 N NaOH, and approximately 1 g of canine serum albumin.[7]

Labeling of Triton with I^{131} was performed as previously described (6).

Procedure of in vivo experiments. Approximately 1 month before administration of the labeled material, plasma volume was determined in each dog, according to the isotope dilution principle, 10 minutes after intravenous injection of I^{131}-labeled canine serum albumin (15 μc). At the same time the hematocrit value was determined. After injection of one of the labeled materials, hematocrit readings were used to assess possible changes in plasma volume. No significant changes were observed in either normal or Triton-treated animals.

All radioactive materials were injected intravenously, usually in doses of 100 μc, and blood samples were taken at intervals to determine their rate of disappearance from circulation. The distribution of radioactivity among the various lipoproteins, ultracentrifugally separated, was determined. The dogs injected with I^{131}-labeled lipoprotein protein or I^{131}-Triton received 10 drops of Lugol's solution in their drinking water daily to prevent radioiodine uptake by the thyroid. The urinary excretion of radioactivity was also determined. The radioactivity was found related to free iodine; in fact, when urines were filtered through an anion exchange resin (Ioresin), which selectively retains unbound iodine, the effluent from the column was practically free of radioactivity.

Chemical analyses. Protein was measured according to Lowry, Rosebrough, Farr and Randall (13), cholesterol according to Abell, Levy, Brodie and Kendall (14), and triglycerides according to Van Handel and Zilversmit (15). Phospholipids were calculated as 25 times the value of the lipid phosphorus obtained by the method of Fiske and Subbarow (16). The method of Dole (17) was used for plasma free fatty acids. In the analysis of plasmas from both normal and Triton-treated dogs, the factor of error of this method was around 1 per cent.

Electrophoretic studies. We performed these on both filter paper according to Durrum (18) and starch-gel according to Smithies (19), using the discontinuous system of buffers of Poulik (20). Details of the techniques and staining procedures have been previously reported (7).

Measurements of radioactivity. Serum samples (1 ml), obtained from dogs injected with I^{131}-labeled materials, were counted in a sodium-crystal scintillation detector (Tracer-Lab) with a counting efficiency of approximately 33 per cent. C^{14}-labeled FFA and triglycerides, after solvent extraction from plasma, were plated on lens paper-covered stainless steel planchets after addition of palmitic acid to a final weight of 15 mg per planchet (21). Cholesterol-4-C^{14} was plated on aluminum planchets as digitonide and counted at infinite thickness. Lipid extracts containing P^{32}-phospholipids were mixed with 100 mg of commercial lecithin and plated on stainless steel planchets. Counting of C^{14}-cholesterol and P^{32}-phospholipids was performed in a windowless gas-flow GM tube (Tracer-Lab) with a background of about 22 cpm. The counting error was $\pm$ 3 per cent.

RESULTS

1. *Normolipemia*

The duration of the normolipemic stage being only 2 to 3 weeks (5), long-term studies with labeled lipoproteins could not be performed. The rates of disappearance of intravenously injected C^{14}-labeled triglycerides, I^{131}-triolein, and albumin-bound palmitate-1-C^{14} were all within the limits of the values reported in the following section for normal dogs. The distribution of radioactivity among the various lipoprotein classes was normal.

2. *Hyperlipemia (4 to 6 weeks' duration)*

The rates of disappearance from circulation and plasma distribution of various labeled lipids injected intravenously are summarized in Table I. For each study two normal and two Triton-treated dogs were used.

Cholesterol-4-C^{14} (Figure 1) was removed from the plasma of normal animals with half-times of

[5] Raolein, I. V., Abbott Labs., North Chicago, Ill. We are deeply indebted to Dr. B. Eberle for the generous supply of the radioiodinated material. Each vial contained 287 mg triolein, 1,657 mg glycerol, and 55 mg lecithin. A chromatographic analysis of this product has been reported by Lakshminarayana and co-workers (Fed. Proc. 1959, **18**, 269) and found to contain mainly triglycerides and appreciable proportions of mono- and diglycerides.

[6] Upjohn Co., Kalamazoo, Mich.

[7] Pentex Co., Kankakee, Ill.

TABLE I

Rates of disappearance from circulation and plasma distribution of various labeled lipids after their intravenous injection into normal and Triton-treated dogs

Dog*	Wt	Plasma volume	Plasma† conc.	Plasma pool	$t_{\frac{1}{2}}$	Distribution of radioactivity				
						D <1.006	D 1.006–1.063	D 1.063–1.21	D >1.21	
	kg	*ml*	*mg/ml*	*mg*	*days*	%	%	%	%	
		Cholesterol					Cholesterol-4-C^{14}			
1, N	12	420	1.25	525	8.7	2.4	6.6	91.0		
2, N	10	400	1.87	748	7.8	2.0	7.9	90.1		
3, T	10	380	13.70	5,206	9.0	23.0	73.0	4.0		A
4, T	11	400	18.20	7,280	10.0	25.4	68.0	6.6		
		Phospholipids			*hrs*		P^{32}-phospholipids			
5, N	11	420	3.0	1,260	34	2.6	4.4	93.0		
6, N	12	450	3.2	1,440	38	1.6	5.7	92.7		
7, T	11	440	15.4	6,676	54	22.3	70.6	7.1		B
8, T	11	420	17.3	7,266	44	28.3	61.4	10.3		
		Triglycerides			*min*		I^{131}-triolein			
9, N	10	350	0.40	180	9	95.0	5.0			
10, N	10	460	0.46	211	11	97.0	3.0			
11, T	12	420	3.20	1,344	36	98.0	2.0			C
12, T	11	410	8.60	3,526	80	96.0	4.0			
		Free fatty acids‡			*min*		Na palmitate-1-C^{14}			
13, N	10	420	0.32	134	2.0	6.4		18.4	75.2	
14, N	11	435	0.27	117	2.0	8.7		19.1	72.4	
15, T	10	435	1.13	492	2.2	70.2			29.8	D
16, T	11	450	1.20	540	2.0	74.3			35.7	

* N = normal, T = Triton-treated.

† Because of the changes in plasma lipid levels produced by Triton, the lipid values reported in the table are an average of those collected within the experimental period: 16 days for A, 72 hours for B, 60 minutes for C, and 6 minutes for D.

‡ The values are expressed in μEq/ml.

8.7 and 7.8 days. In the Triton-treated animals the plasma disappearance of the injected labeled cholesterol was more difficult to evaluate because of the significant changes in blood cholesterol concentrations during the 16-day experimental period. By plotting values of specific radioactivity of plasma cholesterol against time, the curve shown in Figure 1 was obtained. From this curve we calculated half-time values of 9 and 10 days, considering only the tract of the curve (from experimental days 4 to 8) in which no significant changes in blood cholesterol were observed. When a correction factor was applied for the dilution in the plasma radioactivity produced by the rise in cholesterol, half-time values increased in the two dogs 5 and 10 per cent, respectively.

In Triton-treated dogs plasma cholesterol was about 10 times higher than normal and was partitioned among plasma lipoproteins, separated by ultracentrifugation, in a manner different from that in normal animals; in normal dogs most of the labeled cholesterol (91 per cent) was in the D 1.063–1.21 lipoprotein; in the Triton-treated dogs about 70 per cent was found in the D 1.006–1.063 and about 24 per cent in the D < 1.006 class (Table I, A).

P^{32}-phospholipids were removed from circulation (Figure 2) at a lower rate in Triton-treated animals ($t_{\frac{1}{2}}$, 54 and 44 hours) than in normal animals ($t_{\frac{1}{2}}$, 34 and 38 hours). In the relatively short experimental period no significant changes in plasma phospholipids were observed. The distribution of radioactivity (Table I, B) differed considerably from that of normal animals and was similar to that observed in the C^{14}-cholesterol experiments. Triton-treated dogs had a plasma pool of phospholipids about seven times higher than that in normal animals (Table I, B).

I^{131}-triolein plasma removal showed a major difference between the two groups of animals (Figure 3); it was considerably delayed in those given Triton ($t_{\frac{1}{2}}$, 36 and 80 minutes) as compared with normal ($t_{\frac{1}{2}}$, 9 and 11 minutes). The rate of removal of I^{131}-triolein was affected by each dose of

Triton. During the hours immediately after its injection the delay in egress of the labeled fat was five to six times greater than the values obtained 2 or 3 days later. Further, a greater delay of removal of I^{131}-triolein was observed with increasing severity of the hyperlipemia which, in turn, was proportional to the number of injections of the detergent.

In both normal and Triton-treated animals plasma radioactivity was confined mostly to the $D < 1.006$ lipoprotein class (Table I, C). This finding was of particular significance in the Triton-treated dogs, since most of the triglycerides carried by the D 1.006–1.063 lipoproteins failed to mix with the injected I^{131}-triolein.

For comparison similar experiments were conducted in both normal and Triton-treated dogs with C^{14}-labeled glycerides. A marked delay of these labeled lipids was observed in the Tritonized animals. After the first 20-minute period the curve of disappearance became more flat, probably related to the plasma recycling of some of the labeled molecules.

In separate experiments samples of I^{131}-triolein (287 mg), with a total radioactivity of 75 μc, were first mixed *in vitro* with various amounts of

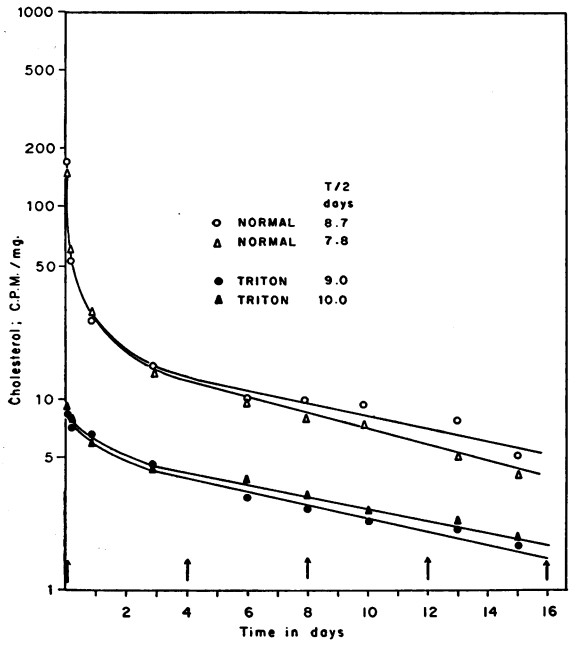

FIG. 1. DISAPPEARANCE FROM CIRCULATION OF CHOLESTEROL-4-C^{14} IN NORMAL AND TRITON-TREATED DOGS. Arrows indicate time of administration of Triton.

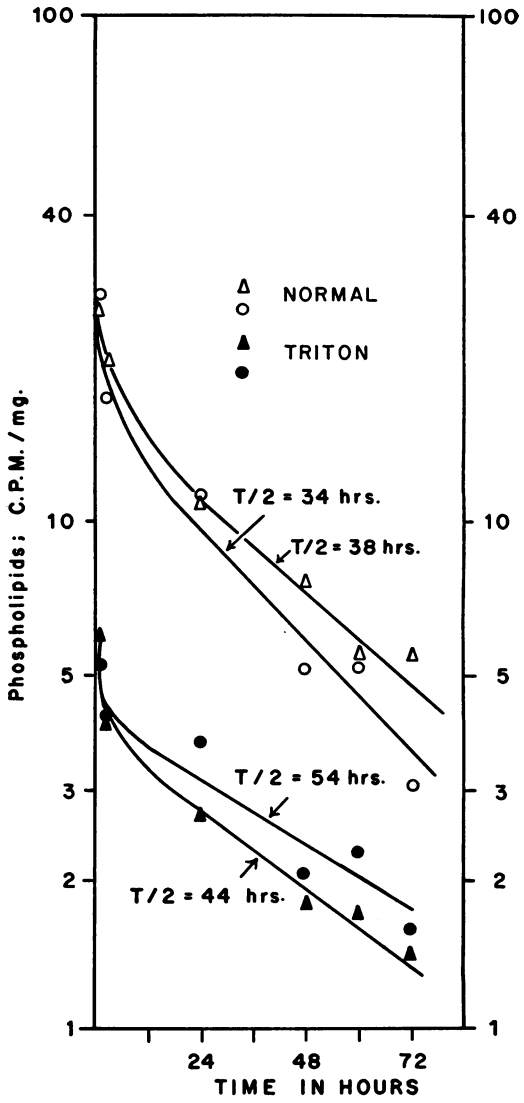

FIG. 2. DISAPPEARANCE FROM CIRCULATION OF P^{32}-PHOSPHOLIPIDS IN NORMAL AND TRITON-TREATED DOGS. Arrows indicate time of administration of Triton.

Triton (1 to 100 mg) and then injected intravenously into normal animals. It was found that samples containing the higher quantities of detergent were removed from circulation at lower rates.

Albumin-bound palmitate-1-C^{14} was injected intravenously into two normal and two Triton-treated dogs, and blood samples were taken after 0.5, 1, 1.5, 2, 3, 4, 5, and 8 minutes. All animals exhibited about the same plasma removal rate of approximately 2 minutes (Figure 4). A marked

difference between the two groups of animals was found in the size of the plasma FFA pool, which was about three times higher in the Triton-treated animals, and in the distribution of radioactivity among the plasma proteins (Table I, D). The radioactivity in the plasma of animals receiving Triton was mostly contained in the low-density lipoprotein class floating at D < 1.063, whereas in the normal animals it was found bound principally to the fraction of serum sedimenting at D 1.21 and better defined by paper and starch electrophoretic analysis as albumin.

Because of the preferential association of the radiopalmitate with the low-density plasma lipoproteins of Triton-treated animals, experiments were performed to compare, after intravenous administration of albumin-bound C^{14}-palmitate, the rate of disappearance from circulation of radioactivity bound to albumin and that bound to lipoproteins. The values were essentially similar with an average half-time of 2 minutes.

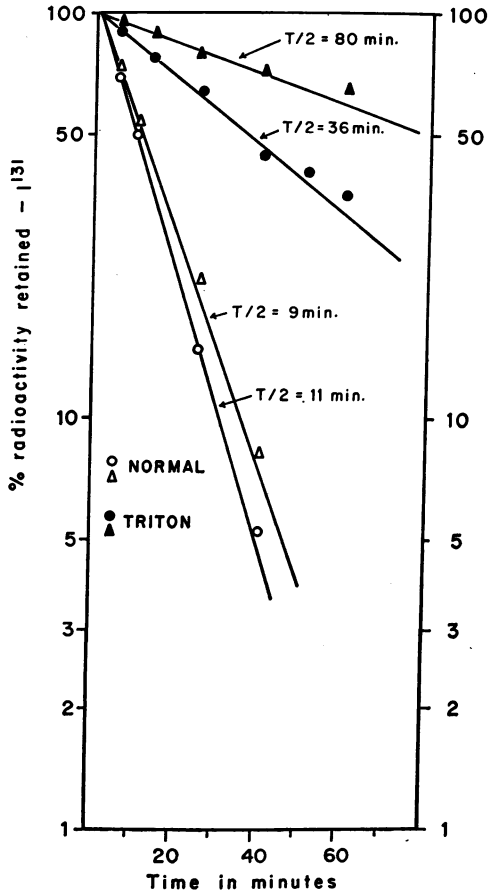

FIG. 3. DISAPPEARANCE FROM CIRCULATION OF I^{131}-TRIOLEIN IN NORMAL AND TRITON-TREATED DOGS.

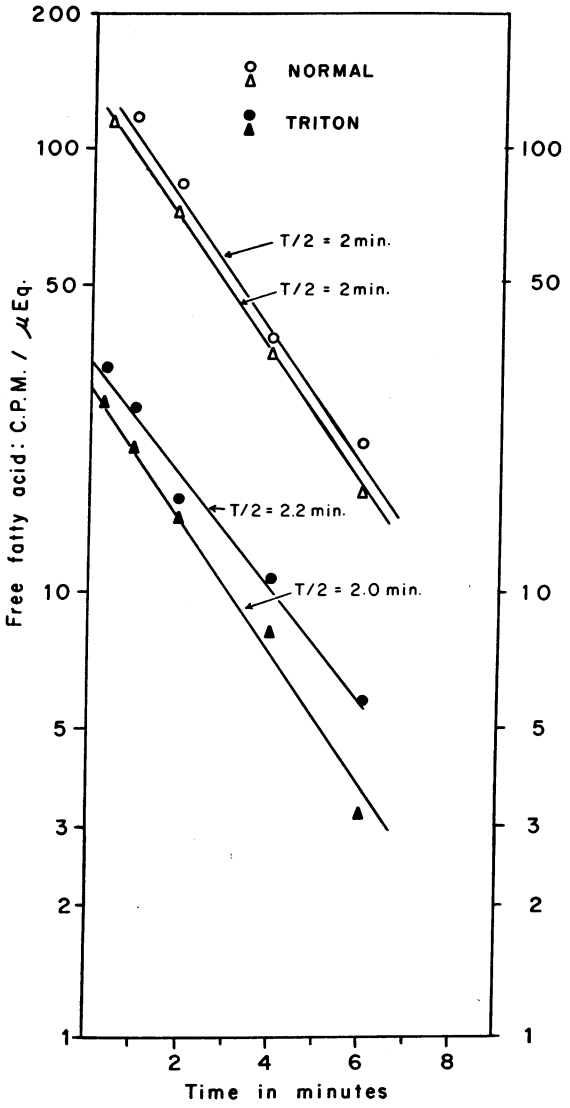

FIG. 4. DISAPPEARANCE FROM CIRCULATION OF ALBUMIN-BOUND PALMITATE-1-C^{14} IN NORMAL AND TRITON-TREATED DOGS.

When β-lipoprotein and α-lipoprotein labeled in the protein moiety with I^{131} were injected intravenously into Triton-treated animals, their rate of removal from plasma had an average half-time of 2.3 and 4.7 days, respectively (Figure 5). These figures were similar to those obtained in normal dogs (Table II). The size of the plasma pool of the lipoprotein proteins was also similar in the two groups of animals (Table II).

TABLE II

Rates of disappearance from circulation and plasma distribution of lipoproteins labeled in the protein moiety with I^{131} after their intravenous injection into normal and Triton-treated dogs

Dog	Wt	Plasma volume	Plasma conc.	Plasma pool	$t_{\frac{1}{2}}$	Distribution of radioactivity			
						D <1.006	D 1.006-1.063	D 1.063-1.21	D >1.21
	kg	*ml*	*mg/ml*	*mg*	*days*	%	%	%	%
		LP proteins*					αLP-I^{131}		
17, N	11	380	2.92	1,109	3.8	2.0	8.0	90.0	
18, N	10	400	2.84	1,136	4.0	3.0	6.0	91.0	
19, T	12	410	2.80	1,148	3.5	27.2	62.8	10.0	
20, T	10	430	2.60	1,118	3.5	29.1	63.0	7.9	
		LP proteins*					αP-I^{131}		
21, N	11	400	2.75	1,100	3.8	1.9	5.9	92.2	
22, T	10	420	2.66	1,117	3.7	39.0	52.1	8.9	
		LP proteins*					βLP-I^{131}		
23, N	12	400	2.80	1,120	2.6	4.2	94.2	1.6	
24, N	11	380	2.73	1,037	2.3	4.6	93.6	2.0	
25, T	10	420	2.66	1,117	2.2	36.9	61.9	1.2	
26, T	11	400	2.90	1,160	2.2	41.2	57.2	1.6	

* The values refer to the protein moiety of all classes of serum lipoproteins floating at D 1.21.

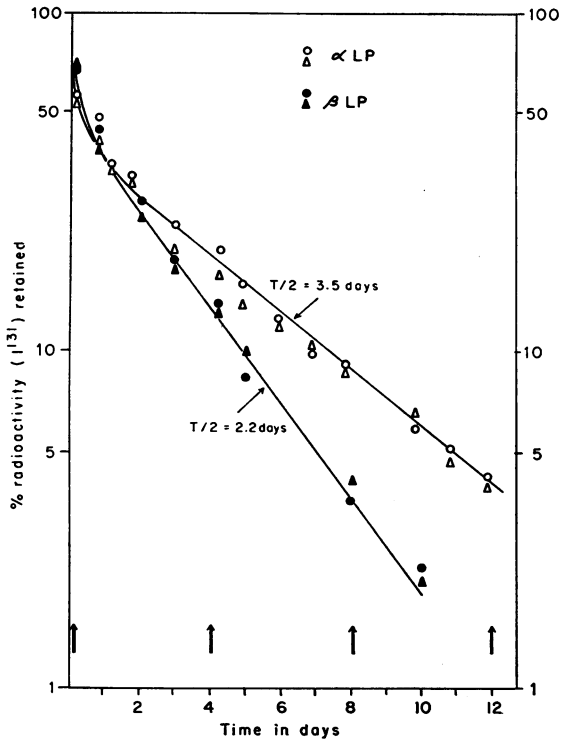

FIG. 5. DISAPPEARANCE FROM CIRCULATION OF βLP-I^{131} AND αLP-I^{131} IN NORMAL AND TRITON-TREATED DOGS. Arrows indicate time of administration of Triton.

Marked differences were observed in the distribution of plasma radioactivity. After the administration of either labeled βLP or αLP proteins, plasma radioactivity was confined in normal dogs to either the βLP or αLP class, respectively. In the Triton-treated dogs, regardless of which lipoprotein protein was injected, the radioactivity was found distributed between the low-density lipoproteins of D < 1.006 (average, 33 per cent) and D 1.006–1.063 (average, 60 per cent). The same type of radioactivity distribution was observed in the plasma of dogs given αP-I^{131} (Table II). When ultracentrifugally separated plasma lipoproteins from dogs treated with Triton were analyzed by starch-gel electrophoresis, lipid and protein moieties moved in the opposite directions.

TABLE III

Rates of disappearance from circulation of I^{131}-Triton after intravenous injection into normal and Triton-treated dogs

Dog	Wt	$t_{\frac{1}{2}}$	Distribution of radioactivity			
			D <1.006	D 1.006–1.063	D 1.063–1.21	D >1.21
	kg	*days*	%	%	%	%
1, N	10	4.2		9.0	91.0	
2, N	11	3.8		6.8	93.2	
3, T	11	2.8	35	62	3	
4, T	12	2.7	28	68	4	

In the normal dogs the two moieties moved together.

I^{131}-Triton, given intravenously to two normal dogs, left the circulation in an average half-time of 4 days. The radioactivity was mostly in the D 1.063–1.21 lipoprotein class. In two Triton-treated dogs (Table III), the radioactivity disappeared from plasma at a slightly faster rate (average, 2.75 days) and was distributed between the lipoproteins of D < 1.006 (33 per cent) and D 1.006–1.063 (67 per cent).

DISCUSSION

Studies in mice and rabbits by Hirsch and Kellner (3, 22) and in rats by Friedman and Byers (4, 23), supported the evidence for the hypothesis that Triton hyperlipemia is caused by primary physical or chemical changes produced in the plasma lipids by the administration of the detergent, leading in turn to their accumulation in the blood stream. This concept seems to apply to plasma glycerides in view of the experimental finding that Triton-treated rats have a delayed removal of injected I^{131}-triolein (24) and C^{14}-labeled chylomicrons (25), results now corroborated by our studies in dogs. According to Friedman and Byers (23) the primary accumulation of plasma triglycerides would lead to a rise of the plasma levels of cholesterol and phospholipids by favoring their mobilization from hepatic and extrahepatic sources and sequestration in the circulating plasma. Experimental work by Frantz and Hinkelman (26) has clearly shown that rats given Triton have a marked increase of the hepatic cholesterol synthesis, a factor likely to be largely responsible for the hypercholesterolemia in these animals. Our findings in dogs treated with Triton and injected with labeled cholesterol and phospholipids do not seem to indicate that these lipids are locked in the circulating plasma but tend to support the hypothesis that the hypercholesterolemia and hyperphospholipidemia of Tritonized dogs are secondary to an imbalance between their rate of synthesis and their rate of removal from circulation, the former exceeding the latter.

The observation that Triton-treated dogs maintain a normal level of circulating lipoprotein proteins, in spite of the large rise of all plasma lipids, is of interest. This finding and the fact that the rate of plasma removal of these proteins from circulation was similar in both normal and Triton-treated animals seem to suggest that their metabolism was not affected by the administration of the detergent. This assumption seems corroborated by recent studies by Radding and Steinberg (27) showing that rats given Triton have a normal hepatic synthesis of lipoprotein protein, in contrast to the marked increase in the rate of cholesterol synthesis.

Previous studies from this laboratory have shown that Triton induces a dissociation of the protein and lipid moieties of plasma lipoproteins *in vitro* (6) and produces *in vivo* a profound change in the spectrum of circulating lipoproteins with accumulation of the low-density classes unusually rich in lipids (5). In the present studies, when the labeled proteins of either βLP or αLP were injected into animals receiving Triton, the radioactivity was found distributed between lipoprotein fractions floating at solvent densities of < 1.006 and 1.006–1.063. In normal animals each of the injected labeled proteins traveled with its own lipoprotein class (Table II). Thus, in Triton-treated dogs, plasma lipoprotein proteins appear to have lost the property of combining specifically with their own lipoprotein class to become loosely associated with the main bulk of plasma lipids. This interpretation seems validated by the observation that in Triton-treated dogs, protein and lipid moieties of ultracentrifugally prepared plasma lipoproteins migrated differently in an electrical field. Further, when labeled Triton was injected into Triton-treated dogs the radioactivity was contained only in the plasma fractions very rich in lipids.

In fasting, the major source of FFA is in adipose tissue (28). The fact that fasting plasma FFA levels were elevated in Triton-treated dogs led us previously to postulate (5) that this increase was an expression of an enhanced lipid mobilization from fat depots. This hypothesis seems corroborated by recent studies of Byers, Cady and Friedman (29) and by our present experiments conducted with albumin-bound palmitate-1-C^{14}. If we assume that in both of these experiments a steady state existed during the period of rapid disappearance from circulation of the la-

beled palmitate, it could be calculated [8] that Triton-treated animals had a turnover rate of FFA two to three times larger than normal animals had. This leads us to postulate that animals receiving Triton have an enhanced metabolic activity in the adipose tissue which may explain why dogs chronically treated with Triton ultimately exhibited a diffuse depletion of their fat stores (5).

Our present studies have confirmed previous findings (30) that in Triton-treated dogs there are two plasma FFA compartments, one bound to albumin, the other (the major one) bound to lipoproteins. It now appears, from the radioisotopic data, that plasma FFA removal from each of these compartments is very rapid, indicating that association of FFA with albumin is not essential for their plasma transport and that lipoproteins can act as their major carriers whenever conditions interfere with their combination with albumin. Plasma lipoproteins are the major carriers of FFA in patients with the nephrotic syndrome due to lack of circulating plasma albumin (31). In the Triton-treated dogs, however, the plasma levels of albumin are normal (5).

Our present results and the knowledge that dogs receiving Triton for a prolonged period of time ultimately die, showing diffuse lipidosis and depletion of fat stores (5), have led us to formulate a mechanism for the Triton disease in dogs as summarized in Figure 6. According to current views (32), triglycerides bound to low-density β-lipoproteins are the main form of transport of fatty acids moving from the liver to fat depots and other peripheral tissues, while FFA, bound mostly to albumin, represent the main form of transport of fatty acids moving in the opposite direction. When Triton is administered to dogs and appropriate plasma levels are reached, the detergent, by combining preferentially with the lipoprotein lipids (glycerides, cholesterol, and phospholipids), modifies their surface property, transforming them into such foreign bodies as will be removed by the reticuloendothelial cells. The establishment of a reticuloendothelial channeling in the removal of lipids from circulation results in lack of their uptake by the parenchymal cells of liver and adipose tissue. The liver then

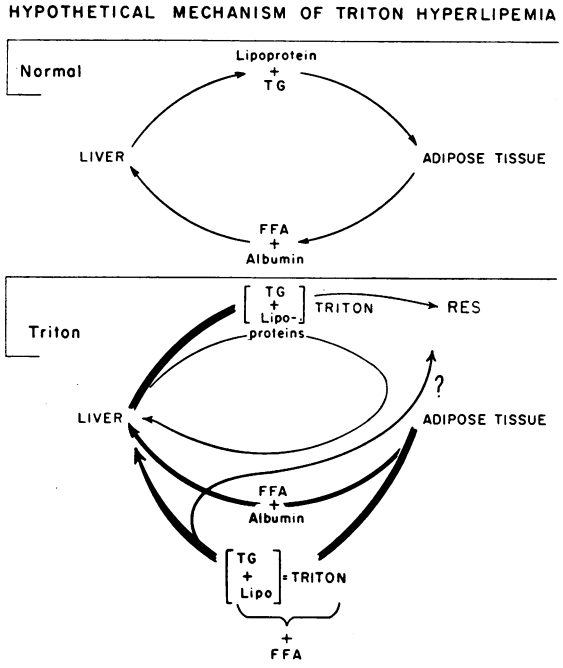

FIG. 6. HYPOTHETICAL MECHANISM OF TRITON HYPERLIPEMIA.

responds by increasing their production and the adipose tissue by mobilizing more FFA to supply energy to the parenchymal cells unable to utilize the other class of lipids. Lipoprotein proteins, not affected by Triton, maintain a normal metabolism. Glucose, not apparently affected in its metabolism by Triton administration (5), may provide the necessary energy source for fatty acid oxidation and lipogenesis.

SUMMARY

1. Dogs fed a regular diet were given 250 mg of Triton (a non-ionic surface-active agent) intravenously every fourth day. When sustained hyperlipemia developed within a period of 3 to 4 weeks, the animals were injected intravenously with one of the following materials: lipoproteins labeled with either cholesterol-4-C^{14} or P^{32}-phospholipids, C^{14}-labeled chylomicron triglycerides, I^{131}-triolein, albumin-palmitate -1-C^{14}, I^{131}-Triton, or lipoprotein labeled in the protein moiety with I^{131}. Normal dogs served as controls.

2. In Triton-treated dogs, the rate of removal from circulation of the labeled triglycerides was markedly delayed. Such evidence was not obtained for labeled cholesterol and phospholipids,

[8] Plasma turnover rate = FFA (in μEq/ml) $\times$ $1/T_t$, where T_t = half-time $\times$ 1.44.

leading to the belief that an increase of their hepatic production was likely responsible for the hypercholesterolemia and hyperphospholipidemia of dogs given Triton.

3. After administration of C^{14}-palmitate, labeled plasma free fatty acids (FFA) exhibited an abnormal partition, being partly bound to albumin but mostly associated with the low-density class of lipoproteins. From both compartments the rate of removal from plasma of FFA was approximately 2 minutes. It was estimated that Triton-treated animals had a plasma turnover rate of FFA two to three times higher than had normal animals.

4. Triton-treated dogs had normal levels of plasma lipoprotein proteins, which were removed from circulation at normal rates. These proteins were associated with lipids floating at low and very low densities.

5. I^{131}-Triton, injected into Triton-treated dogs, was removed from circulation at a slightly higher rate than in normal animals. In normal dogs the plasma radioactivity was contained in the high-density (D 1.063–1.21) class. In Triton-treated dogs, where this lipoprotein class is lacking, most of the radioactivity was in the low-density lipoproteins.

6. On the basis of the radioisotope experiments and the knowledge that Triton-treated dogs exhibit diffuse lipidosis and depletion of fat stores, it is postulated that the primary action of Triton is on the lipids of plasma lipoproteins with formation of complexes which, acting as foreign bodies, are preferentially taken up by the reticuloendothelial cells (RES). As a consequence of this RES channeling of plasma lipid removal, the liver increases its production of plasma lipids, while the adipose tissue increases the mobilization of the stored fats in the form of FFA to supply energy to the parenchymal cells.

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