TRITON HYPERLIPEMIA IN DOGS*

II. Atherosclerosis, Diffuse Lipidosis, and Depletion of Fat Stores Produced by Prolonged Administration of the Non-Ionic Surface-Active Agent

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Plates 30 to 32

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In a previous paper (1) it was shown that triton WR-1339,[1] a non-ionic surface-active agent, produces, *in vitro*, notable physical and chemical changes of serum lipoproteins and chylomicrons. It was assumed that if similar alterations occurred *in vivo*, they might be partially responsible for the hyperlipemia that had been described in rabbits (2), guinea pigs (2, 3), mice (4), and rats (5) following administration of the detergent. To test this assumption, 14 dogs were given prolonged intravenous treatment with triton. Overt hyperlipemia developed in all animals, and they died within 4 months after receiving the first injection of the detergent. The changes in plasma lipids and lipoproteins and the clinicopathologic findings will be described.

Methods and Procedures

Animals and Diet.—The animals used were male mongrel dogs, from 1 to 2 years of age, which weighed between 9 and 14 kg. They were kept in single cages and were consistently fed a diet that was 61 per cent carbohydrate, 32 per cent protein, and 7 per cent fat. The amount of food offered daily furnished about 1300 calories,[2] 195 calories or 15 per cent of which was due to fat. Blood samples, for chemical analyses, were drawn from the femoral vein weekly, after a fasting period of about 12 hours.

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[1] Winthrop Laboratories, New York.

[2] This diet was prepared by mixing 2 parts of Kibbled Biscuits (Central Kennel Supply, Dayton, Ohio), and 1 part of beef (Fromm, Dog Food, Federal Foods, Inc., Thiensville, Wis.)

Surface-Active Agent.—Triton WR-1339, a polyoxyethylene ether of an alkyl phenol, was prepared in two concentrations, as 12.5 and as 20 per cent solutions, in phosphate buffer pH 7.2, Γ/2 0.05, and then was autoclaved. The solutions were allowed to stand no longer than 1 week before use. In some of the experiments, I^{131}-triton was used. The technique of iodination was reported previously (1).

Laboratory Analyses.—Serum lipids were extracted in chloroform-methanol (2:1) according to Folch *et al.* (6). In aliquots of these extracts, triglycerides were determined by the method of Van Handel and Zilversmit (7), total cholesterol by the method of Abell *et al.* (8), and free cholesterol by that of Sperry and Webb (9). Phospholipids were calculated as 25 times the lipid phosphorus values obtained by the method of Fiske and Subbarow (10). Plasma-free fatty acids were determined by the Dole method (11). Protein concentration was determined according to the method of Lowry *et al.* (12). In hyperlipemic samples and in those samples containing triton, proteins were first precipitated with 5 per cent TCA, then washed twice with a 2:1 mixture of chloroform : methanol. The protein content was calculated by multiplying by the factor 6.25 the nitrogen values obtained by the micro-Kjeldahl procedure of Lange (13).

Lipoproteins were separated by ultracentrifugation at D 1.21 in a Spinco model L ultracentrifuge according to the technique of Lewis *et al.* (14). Analyses of these lipoproteins were performed in a Spinco model D ultracentrifuge at 52,640 RPM, at 20°C.

Starch gel electrophoretic analyses were performed following the technique described by Smithies (15) with the discontinuous system of buffers (tris-boric acid, pH 8.2) proposed by Poulik (16).

The radioactivity of the I^{131}-labeled samples was determined in a sodium iodide crystal scintillation detector (tracer-lab) with a counting efficiency of 33 per cent and an error of ±3 per cent.

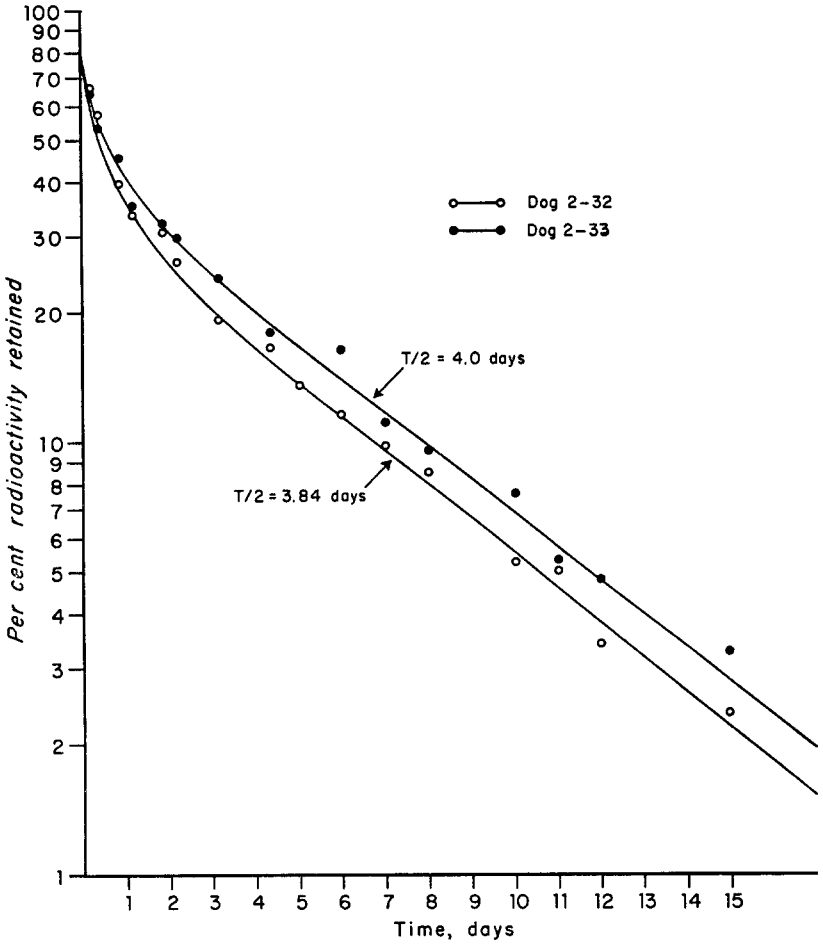
Necropsy was always performed within 4 hours after death. Samples from each organ were extracted with a 2:1 chloroform-methanol mixture according to Folch *et al.* (6). In these extracts cholesterol (8), phospholipids (10), and triglycerides (7) were determined. Other samples from each organ were fixed in Zenker's and in formalin solutions. The aorta was carefully dissected from its origin to the iliac bifurcation; one section was stained with Sudan IV according to Holman's method (17), and one section was put into fixative. For animals that received radioactive material, autoradiographs of the aorta were made on Kodak nuclear track plates, 2.5 x 7.6 cm. The time of contact was from 2 to 3 weeks, at 4°C. The plates were developed with ethol developer, simple mix/UFG (Plymouth Products Co., Inc., Chicago) and were fixed with Kodak fixer.

Fragility Test for Red Blood Cells.—8 ml of venous blood was collected in 2 ml of acid citrate dextrose (ACD) solution, and the plasma was separated by centrifugation at 2,000 RPM, at 4°C. After removal of the plasma, the red cells were washed three times in an isotonic phosphate buffer sodium chloride solution (NaCl) (18), pH 7.45, and the cells were then resuspended with the buffered NaCl solution to a final volume of 10 ml. Aliquots of 0.05 ml were suspended in 6 ml of the buffer, mixed with progressively larger amounts (0.2 to 20 mg) of triton, and were incubated at various intervals at 37°C. The degree of hemolysis was read at 520 mμ in a Coleman Jr. spectrophotometer, and was expressed in percentage of a standard solution, in which 100 per cent hemolysis was obtained by adding 0.05 ml of the oxygenated red cells to 8 ml of 0.04 per cent ammonia in water (19).

RESULTS

Rate of Disappearance of I^{131}-Triton from Blood.—Two normal dogs were used for these experiments. Starting three days before the injection of I^{131}-triton

and throughout the experiments, the dogs were given 10 drops of Lugol's solution daily in their drinking water to prevent uptake of the radioiodine. They were then injected with 25 mg. of I^{131}-triton of a specific activity of 1 μc per mg. Blood samples were drawn at intervals; the serum was separated by

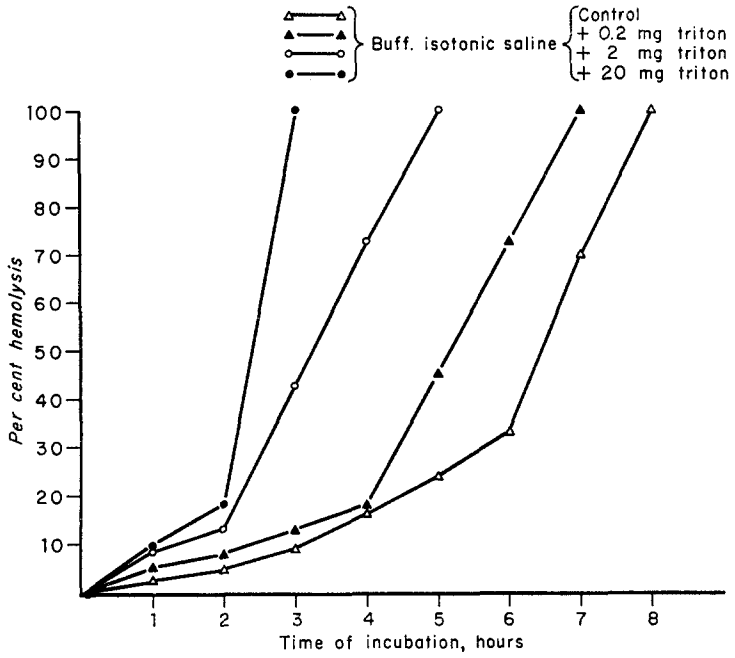


TEXT-FIG. 1. Graph showing the rate of disappearance of radioactivity from plasma of 2 dogs given intravenously 25 mg of I^{131}-triton (sp. act., 1 μc/mg).

centrifugation, and the radioactivity was determined in 1 ml aliquots. The curves of disappearance from plasma of the radioiodinated triton are shown in Text-fig. 1. Each value on the curves was corrected for background and physical decay. The values on the ordinate express the amount of radioactivity retained

in the plasma at each time interval, 100 per cent representing the serum radioactivity at zero time.

The value of half-time (3.84 and 4.0 days) was calculated from the slope of the exponential line of disappearance of the radioactivity from plasma. During these experiments daily total urinary excretion of radioactivity was recorded.



TEXT-FIG. 2. Graph showing the *in vitro* effect of triton on the osmotic fragility of canine red cells. For technical details, see text.

Twenty-one days after injection of I^{131}-triton, approximately 90 per cent of the radioactivity had been recovered in the urine.

That the radioactivity in the urine was due to free iodine was proved by the fact that when urine was filtered through an anion exchange resin ("ioresin", Abbott), which selectively retains free iodine, the effluent was practically free of radioactivity. When I^{131}-triton was added to normal canine urine and filtered through the column, only a small percentage of the radioactivity was retained.

From the values of half-time of I^{131}-triton it was calculated that the detergent should be administered every 4th day.

Early Effects of Triton Administration in Dogs.—In preliminary studies, dosages of detergent in the range of 250 to 400 mg/kg of body weight were chosen since they were of the order of magnitude previously reported to pro-

duce hyperlipemia in rabbits (2), guinea pigs (2, 3), and mice (4). Six dogs given triton in doses of 400 mg/kg at intervals of 4 days, died after the first or second injection. Two dogs that were given 250 mg/kg of triton every 3rd day died following the fourth injection. Ante mortem analysis of blood showed 40 to 60 per cent decrease in hemoglobin concentrations, great reduction in hematocrit readings, presence of hemoglobinemia and hemoglobinuria, all consistent with an acute intravascular hemolytic episode. Necropsy showed that the hepatic and splenic sinusoids were packed with erythrocytes, and that the collecting renal tubules were distended with eosinophilic masses resembling free hemoglobin. The morphologic changes were somewhat similar to those seen in phenylhydrazine poisoning.

To determine whether or not hemolysis was due to a direct effect of the detergent on red blood cells, *in vitro* experiments were performed in which various amounts of triton were added to washed canine red cells. The osmotic fragility of the red cells increased proportionally to the amount of triton employed (Text-fig. 2).

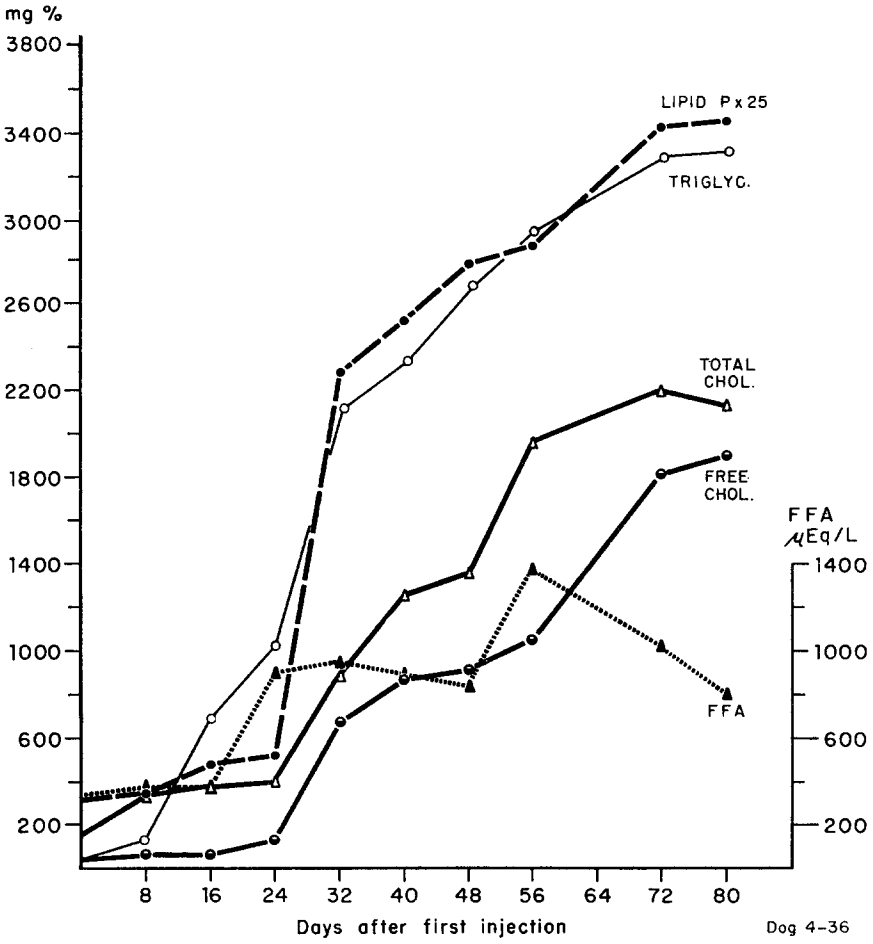
Group I. Dogs Receiving 250 Mg/Kg Doses of Triton.—

A. Physical findings: This dose was given intravenously every 4th day to 14 fasting dogs.

The course can be divided into 3 stages: *Stage 1* (2 to 3 weeks). Each animal appeared to be in good physical condition. The values of triglycerides, cholesterol, phospholipid, and lipoprotein were in normal ranges. *Stage 2* (4 to 12 weeks). The animals remained in good health without weight change. The serum became opalescent, then distinctly milky. The first lipid fractions to increase were the triglycerides, followed by cholesterol and phospholipids: about 80 per cent of the cholesterol was in the free form. The free fatty acid concentration increased from 2 to 3 times above normal and remained at this high value. A typical example of the changes in the serum lipid fractions caused by triton is shown in Text-fig. 3.

The hyperlipemia was accompanied by great changes in the serum lipoproteins. Analyses by the analytical ultracentrifuge showed an early disappearance of the D 1.063 to 1.21 lipoprotein class (Text-fig. 4). This was the fraction that in the normal animals contained approximately 90 per cent of the serum cholesterol and phospholipids. In the tritonized dogs, most of these lipid fractions (70 to 75 per cent of the total) were found in the D 1.006 to 1.063 lipoprotein (Table I, columns *b* and *c*), whereas the triglycerides, were almost evenly distributed between the D $<$ 1.006 and D 1.006 to 1.21 lipoproteins (Table I, column *d*). The large increase of the serum lipids produced by triton was not accompanied by a parallel rise in the serum lipoprotein proteins, which were distributed between the D 1.006 to 1.063 (80 per cent) and the D $<$ 1.006 (20 per cent) lipoprotein classes (Table I, column *a*).

Blood hemoglobin, hematocrit values, serum glucose, ketone bodies, and albumin were normal. Among the physical findings fundoscopy, blood pressure, and roentgenograms of the skull and long bones were all normal. *Stage 3* (1 to 3 weeks). Each animal, in spite of a regular food intake, lost from 2 to 3 kg of

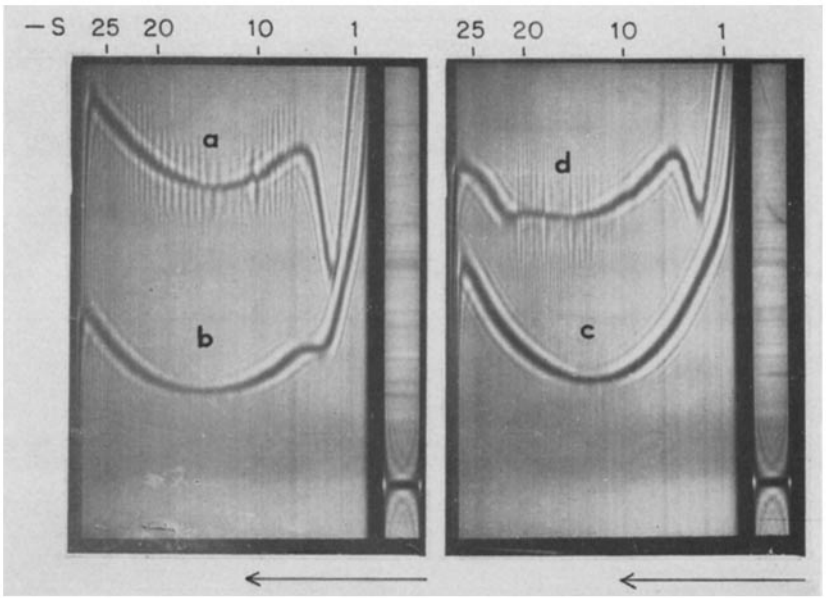


TEXT-FIG. 3. Variations of serum lipid fractions during the course of administration of triton.

weight. There were no gastrointestinal disturbances. Trophic ulcerations of the skin developed, especially over bony prominences. Serum lipid levels rose progressively, and were accompanied by changes of the lipoprotein pattern similar to those of stage 2, with exception of a greater concentration of the D $<$ 1.006 lipoprotein class.

Weakness progressed until death ensued. A few animals that had reached the terminal stage were given an intravenous injection of pentobarbital.

B. Pathologic findings: The subcutaneous, mesenteric, omental and retroperitoneal adipose tissues were atrophied. Microscopically, the fat cells appeared atrophic (Fig. 1). These changes resembled closely the so called "brown atrophy" of severe fasting.



TEXT-FIG. 4. Showing serum ultracentrifugal patterns of a dog (4-36) during administration of triton after withdrawal of the detergent. (*a*) Before administration of triton, (*b*) After 3 weeks of administration of triton, (*c*) After 3 months of administration of triton. Administration of the detergent was stopped on the 93rd day. Pattern *d* refers to the lipoprotein of a serum specimen taken 4 months after withdrawal of triton. All analyses were performed at 52,640 RPM for 36 minutes, at 26°C. The arrows represent the direction of flotation.

Livers and spleens were from 2 to 3 times larger than normal and had higher concentrations of cholesterol, phospholipids, and triglycerides than those found in the organs of normal animals (Table II). These organs showed major pathologic changes suggestive of an unusual type of storage disease. The Kupffer cells were distended by granular material that, in frozen sections, was intensely stained by Sudan IV, oil red, and Sudan black B. Numerous macrophages were also present in the sinusoids of the spleen (Fig. 2) and of the enlarged lymph nodes. The kidneys were 20 to 40 per cent heavier than normal. On section, the cortex appeared normal; the pyramids were increased in size and were white. The whiteness was caused by lipid-laden macrophages ac-

cumulated in large and small groups in the interstitial tissues among the renal tubules within the pyramids.

The aortas showed sudanophilic material when stained with Sudan IV, outlining points of attachment of the semilunar valves and extending for as far as 5 mm along the aorta. Sudanophilic material was also found in variably sized, linear streaks and patches along the distal aorta from the region of the

TABLE I

Chemical Composition of Serum Lipoproteins in 6 Normal and 6 Tritonized Dogs

Lipoprotein class D	Mg./100 ml of serum ± standard error							
	(a) Protein		(b) Cholesterol		(c) Phospholipid		(d) Triglycerides	
	N*	T‡	N*	T‡	N*	T‡	N*	T‡
<1.006	6 ± 3.1	75 ± 12	2 ± 1.2	267 ± 68	4 ± 1.6	540 ± 87	11.2 ± 4.1	231 ± 52
1.006–1.063	25 ± 7.1	170 ± 72	11 ± 3.9	859 ± 102	20 ± 7.8	1775 ± 315	25.3 ± 5.2	203 ± 61
1.063–1.21	271 ± 52	30 ± 6.2	129 ± 47	51 ± 13	330 ± 89	120 ± 46	7.17 ± 2.1	8.3 ± 3.4

* N, normal dogs.
‡ T, tritonized dogs.

TABLE II

Lipid Analyses of Liver and Spleen in Normal and Tritonized Dogs

Animals, total No.	Body weight	Liver				Spleen			
		Per cent of body weight	Cholesterol	Phospholipids	Triglycerides	Per cent of body weight	Cholesterol	Phospholipids	Triglycerides
	kg.		*mg/gm wet tissue*				*mg/gm wet tissue*		
Normal									
6	11.2 ± 2.1	4.2 ± 1.6	3.5 ± 0.9	19.3 ± 4.0	1.8 ± 0.6	0.36 ± 0.2	3.44 ± 1.2	12.8 ± 1.6	1.12 ± 0.6
Triton									
6	10.6 ± 2.4	9.6 ± 2.1	9.2 ± 1.3	28.2 ± 5.3	2.8 ± 1.0	0.87 ± 0.6	17.6 ± 6.2	25.0 ± 3.6	2.0 ± 0.9

renal arteries into both common iliac arteries (Fig. 3). Sudanophilic material was found in the thoracic portion of the aortic arch in only 1 dog. In 2 dogs that had reached the terminal stage, 10 mg of I^{131}-triton (sp. act., 4 μc/mg) was injected intravenously; 24 hours later the animals were given pentobarbital; autoradiographic studies were performed on strips of the aortas. The most intense areas of radioactivity corresponded to those with the greatest deposits of sudanophilic material. Microscopically, the sudanophilic material either was arranged in droplets of various sizes which penetrated the aorta up to half of its mural thickness (Fig. 4), or was engulfed in small clusters of macrophages. Occasionally some of these clusters caused the endothelium of the aorta to bulge.

The hearts, not enlarged, showed extensive atrophy of the epicardial fat. Many of the coronary arteries were thickened in the form of a club-shaped mass just prior to their penetration into the myocardium (Fig. 5). The earliest histopathologic change was the accumulation of small numbers of subendothelial macrophages (Fig. 6); in other areas these masses involved the full thickness of the coronary arteries. Many macrophages were found around the adventitial portion of the arteries. Lipid stain showed that these macrophages contained a sudanophilic material distributed in droplets of various sizes. Some of the droplets were outside of the macrophages.

In most dogs the right atrium was thickened and white, with extensive infiltration of macrophages filled with sudanophilic material. A large amount of sudanophilic material distributed in a patchy fashion was seen throughout the pulmonary artery, particularly in the area of attachment of the semilunar valves.

Group II. Dogs Receiving Triton and Heparin.—

Four dogs received 250 mg of triton intravenously every 4th day, and 5 mg/kg of heparin daily. The general course and autopsy findings were similar to those observed in animals receiving only triton (Group I), with the exception that the onset of the hyperlipemia occurred an average of 2 weeks later than in Group I. Group II died within 3 or 4 months.

Group III. Effects of Withdrawal of Triton after Establishment of Hyperlipemia.—

In five animals triton was withdrawn when cholesterol values were in the range of 3 to 4 gm per 100 ml. The animals were weak and had severe skin ulcerations. Discontinuance of triton was followed by steady improvement of physical conditions, healing of the ulcerations, and increase in body weight. Serum lipid fractions returned progressively to normal within 4 or 5 months. Four months after discontinuance of triton the D 1.063 to 1.21 lipoprotein reappeared as indicated in the ultracentrifugal pattern in Text-fig. 4 *d*. It can be seen that abnormalities were still present owing to an unusual lipoprotein fraction with a flotation coefficient between —S 10 and 20.

Two of the 5 dogs in which triton was discontinued were killed 4 and 6 months after stopping the drug. There were no gross pathologic findings. Microscopic studies of the coronary arteries showed a slight degree of fibrosis most evident in the adventitia. A few macrophages were still present in the liver and spleen, where sudanophilic material was present in the animals that died because of administration of triton. The abdominal aorta of one animal killed 4 months after withdrawal of the drug, had a few patchy deposits of sudanophilic material within the media of the vessel; the aorta of the second animal was normal.

DISCUSSION

In brief, our experiments showed: (*a*) production of sustained hyperlipemia in dogs maintained on a regular diet and given intravenous injections of 250 mg/kg of triton every 4th day; (*b*) presence of notable lipid deposition in the aorta and the coronary and pulmonary arteries in all animals that received triton for from 3 to 4 months; (*c*) upon staining, accumulation of sudanophilic material in the cells of the reticuloendothelial cells of liver, spleen, and lymph nodes; (*d*) general depletion of the adipose fat stores; and (*e*) reversal of the sustained hyperlipemia, cardiovascular changes, and the macrophagic reaction following withdrawal of triton.

Hyperlipemia.—The development of hyperlipemia in dogs treated with triton corroborates and extends to another animal species the previous observations made on rabbits (2), guinea pigs (2, 3), mice (4), and rats (5). In all of these small animals, hyperlipemia promptly followed administration of triton. In the dogs, the increase in serum lipids occurred after 2 or 3 weeks, reaching a maximum only after 2 or 3 months. This time interval was favorable for studying the gradual progress of the lipemia and the concurrent changes of the lipid and the lipoprotein fractions of the serum.

The early increase in triglycerides observed in our dogs, was previously observed by Friedman and Byers (20) in rats given triton. Cholesterol and phospholipids showed elevation only at a later stage and increased at almost identical rates. These results are in agreement with those reported occurring in rats (20) and in rabbits (2, 21) treated with triton.

The preponderance of free cholesterol observed in the dogs, also corroborates previous results in rabbits by Kellner *et al.* (2), and Payne and Duff (21). This preponderance was probably caused by an inhibitory effect of triton on the cholesterol esterase activity of plasma. Inhibition of this esterase by surface-active agents, like bile salts, has been reported (22).

With the establishment of overt hyperlipemia there was an early disappearance of the high density D 1.063 to 1.21 lipoprotein class. In a previous study (1) evidence was presented for a direct action of triton on the lipid moiety of the serum lipoproteins, with preference for the high density, α-lipoprotein class. Those data suggested that a triton-lipid complex forms with consequent displacement of the lipid from its usual association with the lipoprotein protein. We postulate that if similar alteration of the α-lipoprotein occurs in the serum of animals receiving triton, it could be the cause of the disappearance of the D 1.063 to 1.21 lipoprotein class from circulation.

Cardiovascular lesions. Coronary and pulmonary arteries and aorta all showed unusual subintimal deposition of lipids either free or engulfed in macrophages as "foam cells." We believe that these lipids came from the plasma, being aided by their combination with triton to cross the endothelial lining of the arterial vessels. Another possibility is that triton may have altered

the permeability of the vascular endothelium through a direct combination of the detergent with the lipoprotein lipids of the cell membranes—a mechanism which was also probably responsible for the increased fragility observed in canine red blood cells exposed to large amounts of triton.

The lipids, deposited in the arterial vessels, appeared to be combined with triton, for when labeled triton was given to dogs having hyperlipemia, the areas of radioactivity in the aorta corresponded to those with accumulation of sudanophilic material. It is conceivable that these deposited lipids, because of their combination with I^{131}-triton, were unable to be filtered through the muscular and elastic walls of the arterial vessels and acted as foreign bodies to stimulate a local macrophagic reaction, resulting in the accumulation of "foam cells."

According to this concept, the formation of "foam cells" was the resultant of a reaction occurring within the arterial wall between triton-combined lipids of plasma origin and local mesenchymal cells with macrophage-like properties. This macrophage reaction to lipids represents an early stage of atherosclerosis. Possibly fibrotic changes and plaque formation could be produced in dogs given small doses of triton for longer periods.

The possibility of producing an early stage of atherosclerosis in dogs by administration of triton is of interest because: (*a*) experimental atherosclerosis is difficult to produce in dogs, (*b*) a regular diet was given to our animals, (*c*) triton was previously shown by Kellner *et al.* (23) to protect rabbits, fed cholesterol, against atherosclerosis.

The only example of which we are aware concerning the production of experimental atherosclerosis in dogs fed a regular diet is that reported by Lindsay *et al.* (24), in thyroidectomized and hypophysectomized dogs after 1 to 2 years. Fourteen of the 21 dogs showed aortic lesions; only 7 had lesions in the coronary arteries. In all our dogs, both aortic and coronary lesions developed.

In addition to the formation of subintimal foam cells in the aorta, and the coronary and pulmonary arteries of the dogs that had received triton from 3 to 4 months, large amounts of sudanophilic material appeared in the reticuloendothelial system of liver, spleen, and lymph nodes. This observation, corroborating a previous finding in rabbits given triton (21), suggests a possible participation of the reticuloendothelial system in the removal of the triton-bound lipids. The engulfment of lipids in the cells of the reticuloendothelial system was probably responsible for the increased lipid content and enlargement of liver, spleen, and lymph nodes.

Effect of Heparin on Triton Hyperlipemia.—Graham *et al.* (25) and Constantinides *et al.* (26) reported that heparin administration to cholesterol-fed rabbits significantly reduced the levels of serum cholesterol and low density lipoproteins, and also retarded the formation of atherosclerotic lesions. Similar

results were obtained by Horita and Loomis (27) in cholesterol-fed chicks. On the contrary, Horlick and Duff (28), and Schumaker (29), found no change in serum lipids in cholesterol-fed rabbits treated with heparin. Heparin is believed to clear alimentary lipemia (30) by promoting the release of lipoprotein lipase, a lipolytic enzyme (31, 32). This mechanism was used by Day *et al.* (33) to explain the reduction in serum concentrations of total fatty acids in cholesterol-fed rabbits treated with heparin.

In the dogs given triton and concurrent daily injections of heparin, there was a significant delay in the onset of the hyperlipemia. After this delay, however, serum lipids increased to values equal to those observed in animals receiving the surface-active agent alone. The cardiovascular changes produced by triton did not seem to be affected by administration of heparin. Lipoprotein lipase activity following injection of heparin either was absent or was largely inhibited in the dogs given triton;[3] this finding is in agreement with that, *in vitro*, reported by Schotz, Scanu, and Page (34) and by Scanu and Oriente (1).

Depletion of Fat Stores.—The depletion of fat stores was a regular occurrence in the terminal phase of triton disease induced in spite of adequate food intake. This depletion was probably responsible for the trophic cutaneous changes observed in the dogs, although a possible deficiency in liposoluble vitamins or essential fatty acids, secondary to an alteration of the lipid transport mechanism, may have contributed to the dermal changes. Depletion of fat stores was probably related to increased mobilization of lipids (indicated by the high levels of circulating free fatty acids) as an attempt to supply energy to the parenchymatous tissues in a state of "lipid starvation," in turn due to the preferential uptake of lipids by the reticuloendothelial system.

Effect of Withdrawal of Triton on Hyperlipemia.—An interesting aspect of triton hyperlipemia was that it could be reversed to normal upon discontinuing the detergent. The animals killed at the stage in which normolipemic levels were reestablished showed repletion of fat stores and minimal lipid deposition in the reticuloendothelial cells of liver, spleen, and lymph nodes. These data indicate that triton did not produce permanent damage of the structure of the various organs including the arteries, which appeared capable of disposing of the deposited lipids with no apparent residual damage.

SUMMARY

Fourteen dogs, fed a regular diet and given 250 mg/kg of triton (a non-ionic surface-active agent) intravenously every 4th day, exhibited a progressively severe hyperlipemia. Serum triglycerides were the first to increase. Cholesterol, mostly in the free form, and phospholipids showed elevation only at a later

[3] J. M. Szajewski, and A. Scanu, unpublished observation.

stage and increased at almost identical rates. The plasma-free fatty acid concentration was from 2 to 3 times above normal.

With establishment of sustained hyperlipemia, there was reduction, followed by total disappearance, of the high density D 1.063 to 1.21 lipoprotein. Most of the cholesterol and phospholipids (70 to 75 per cent of the total) were found in the D 1.006 to 1.063 lipoprotein class, the remainder in the D $<$ 1.006 class. Triglycerides were almost evenly distributed between these two classes. The concentration of the serum lipoprotein proteins was within normal limits.

All of the animals died within from 4 to 5 months after receiving the first injection of triton. Autopsy findings consistently showed: (*a*) numerous lipid-laden macrophages in the liver, spleen, and lymph nodes; (*b*) significant depletion of all fat stores; (*c*) presence of lipids, either free or engulfed in macrophages (foam cells), in the subintima of the coronary arteries, aorta, and pulmonary arteries, indicating an early stage of atherosclerosis.

Concurrent daily administration of heparin (5 mg per kilogram of body weight) did not substantially change the course of the disease.

Withdrawal of triton from animals that had been receiving the detergent for from 3 to 4 months, elicited a slow return to normal of the lipid pattern. In two dogs killed when normolipemia was reestablished, all tissues were normal with the minor exception of a few hepatic macrophages still laden with sudanophilic material.

It is postulated that the primary action of the injected triton was on the lipid moieties of plasma lipoproteins with formation of complexes, which, as foreign bodies, were preferentially taken up by the cells of the reticuloendothelial system. Depletion of fat stores was probably secondary to increased lipid mobilization, as an attempt by these tissues to supply energy to the parenchymal cells unable to utilize triton-bound lipids.

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BIBLIOGRAPHY

1. Scanu, A., and Oriente, P., Triton hyperlipemia in dogs. I. *In vitro* effects of the detergent on serum lipoproteins and chylomicrons, *J. Exp. Med.*, 1961, **113**, 735.
2. Kellner, A., Correll, J. W., and Ladd, A. T., Sustained hyperlipemia induced in rabbits by means of intravenously injected surface active agents, *J. Exp. Med.*, 1951, **93**, 373.
3. Patnode, R. A., Hudgins, P. C., and Janicki, B. W., Studies on the effect of triton (WR-1339) on guinea pig tissues, *J. Exp. Med.*, 1958, **107**, 33.
4. Hirsch, R. L., and Kellner, A., The pathogenesis of hyperlipemia induced by means of surface-active agents. I. Increased total body cholesterol in mice given triton WR-1339 parenterally, *J. Exp. Med.*, 1956, **104**, 1.

5. Friedman, M., and Byers, S. O., The mechanism responsible for the hypercholesterolemia induced by triton WR-1339, *J. Exp. Med.*, 1953, **97,** 117.
6. Folch, J., Lees, M., and Stoane Stanley, G. H., A simple method for preparation of total pure lipide extracts from brain, *Fed. Proc.*, 1954, **13,** 209.
7. Van Handel, E., and Zilversmit, D. B., Micromethod for direct determination of serum triglycerides, *J. Lab. and Clin. Med.*, 1957, **50,** 152.
8. Abell, L. L., Levy, B. B., Brodie, B. B., and Kendall, F. E., A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity, *J. Biol. Chem.*, 1952, **195,** 357.
9. Sperry, W. M., and Webb, M., A revision of the Schoenheimer-Sperry method for cholesterol determination, *J. Biol. Chem.*, 1950, **187,** 97.
10. Fiske, C. H., and SubbaRow, Y. J., The colorimetric determination of phosphorus, *J. Biol. Chem.*, 1925, **66,** 375.
11. Dole, V. P., A relation between non-esterified fatty acids in plasma and the metabolism of glucose, *J. Clin. Inv.*, 1956, **35,** 150.
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., Protein measurement with the Folin phenol reagent, *J. Biol. Chem.*, 1951, **193,** 265.
13. Lange, C., Single micro-determination of Kjeldahl nitrogen in biological materials, *Analyt. Chem.*, 1958, **30,** 1692.
14. Lewis, L. A., Green, A. A., and Page, I. H., Ultracentrifuge lipoprotein pattern of serum of normal, hypertensive and hypothyroid animals, *Am. J. Physiol.*, 1952, **171,** 391.
15. Smithies, O., Zone electrophoresis in starch gel group variations in the serum proteins of normal human adults, *Biochem. J.*, 1955, **61,** 629.
16. Poulik, M. P., Starch gel electrophoresis in a discontinuous system of buffers, *Nature*, 1957, **180,** 1477.
17. Holman, R. L., McGill, H. C., Jr., Strong, J. P., and Geer, J. C., Techniques for studying atherosclerotic lesions, *Lab. Inv.*, 1958, **7,** 62.
18. Parport, A. K., Lorenz, P. B., Parport, E. R., Gregg, J. R. and Chase, A. M., The osmotic resistance (fragility) of human red cells, *J. Clin. Inv.*, 1947, **26,** 636.
19. Darmady, E. M. and Davenport, S. G. T., Hematological technique, Grune and Stratton, Inc., New York, 2nd edition, 1958, 89.
20. Friedman, M., and Byers, S. O., Mechanism underlying hypercholesterolemia induced by triton WR-1339, *J. Exp. Med.*, 1957, **190,** 439.
21. Payne, T. B. P., and Duff, G. L., Effect of Tween 80 on the serum lipids and the tissues of cholesterol-fed rabbits, *Arch. Path.*, 1951, **51,** 379.
22. Sperry, W. M., and Stoyanoff, V. A., The influence of sodium glucocholate in the enzymatic synthesis and hydrolysis of cholesterol esters in blood serum, *J. Biol. Chem.*, 1937, **117,** 525.
23. Kellner, A., Correll, J. W., and Ladd, A. T., The influence of intravenously administered surface-active agents on the development of experimental atherosclerosis in rabbits, *J. Exp. Med.*, 1951, **93,** 385.
24. Lindsay, S., Feinberg, H., Chaikoff, I. L., Entenman, C., and Reichert, F. L., Arteriosclerosis in the dog. II. Aortic cardiac and other vascular lesions in thyroidectomized-hypophysectomized dogs, *Arch. Path.*, 1952, **54,** 573.
25. Graham, D. M., Lyon, T. P., Gofman, J. W., Jones, H. B., Yankley, A., Simon-

ton, J., and White, S., Blood lipids and human atherosclerosis. II. The influence of heparin upon lipoprotein metabolism, *Circulation*, 1951, **4**, 666.

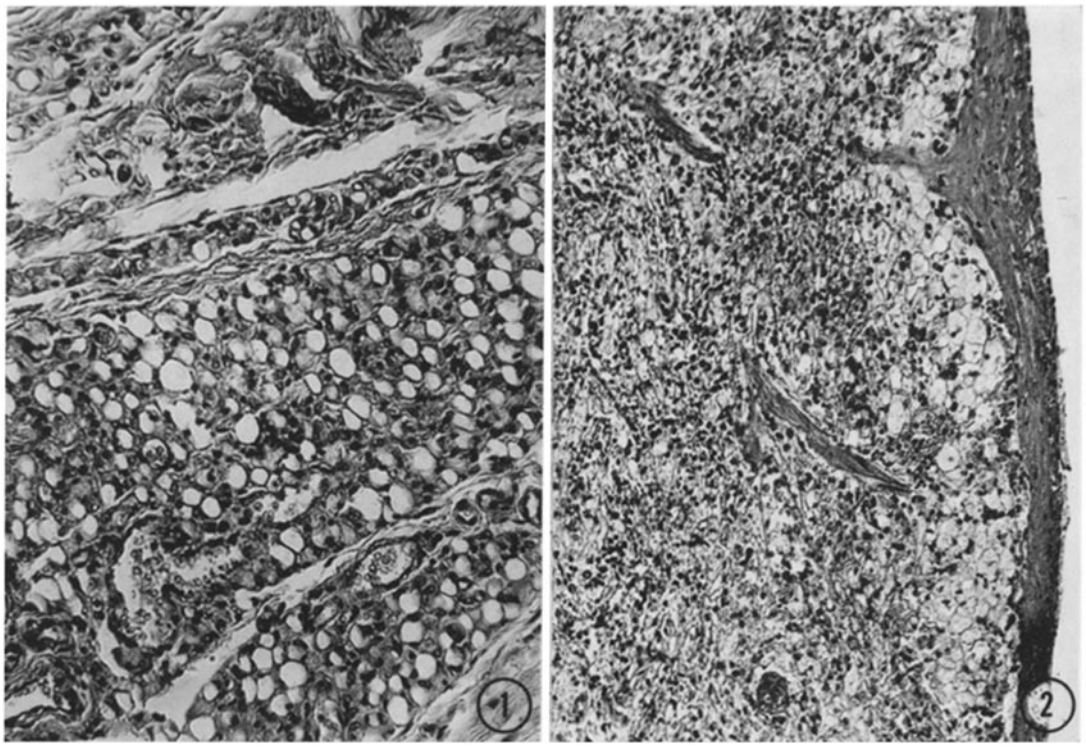
26. Constantinides, P., Szasz, G., and Harder, F., Retardation of atherosclerosis and adrenal enlargement by heparin in rabbits, *Arch. Path.*, 1953, **56**, 36.
27. Horita, A., and Loomis, T. A., The influence of anticoagulant on the formation and regression of experimental atherosclerosis, *J. Exp. Med.*, 1954, **100**, 381.
28. Horlick, J., and Duff, G. L., Heparin in experimental cholesterol atherosclerosis in the rabbit. I. The effect of heparin on the serum lipids and development, *Arch. Path.*, 1954, **57**, 417.
29. Schumaker, V. N., Cholesterolemic rabbit lipoproteins. Serum lipoproteins of the cholesterolemic rabbit, *Am. J. Physiol.*, 1956, **184**, 35.
30. Hahn, P. F., Abolishment of alimentary lipemia following injection of heparin, *Science*, 1943, **98**, 19.
31. Anderson, N. G., and Fawcett, B., Antichylomicronemic substance produced by heparin injection, *Proc. Soc. Exp. Biol. and Med.*, 1950, **74**, 768.
32. Brown, R. K., Boyle, E., and Anfinsen, C. B., The enzymatic transformation of lipoproteins, *J. Biol. Chem.*, 1953, **204**, 423.
33. Day, A. J., Wilkinson, G. K., Schwartz, C. J., and Peters, J. A., Changes in serum lipids and in aortic atherosclerosis following toluidine blue and heparin administration to cholesterol fed rabbits, *Australian J. Exp. Biol. and Med.*, 1957, **35**, 277.
34. Schotz, M. C., Scanu, A., and Page, I. H., Effect of triton on lipoprotein lipase of rat plasma, *Am. J. Physiol.*, 1957, **188**, 399.

EXPLANATION OF PLATES

PLATE 30

FIG. 1. Photomicrograph of periadrenal fat showing atrophy of the adipose tissue. Hematoxylin and eosin stain, × 130.

FIG. 2. Photomicrograph of a spleen, showing the distended macrophages with watery cytoplasm within the splenic pulp. Hematoxylin and eosin stain, × 170.

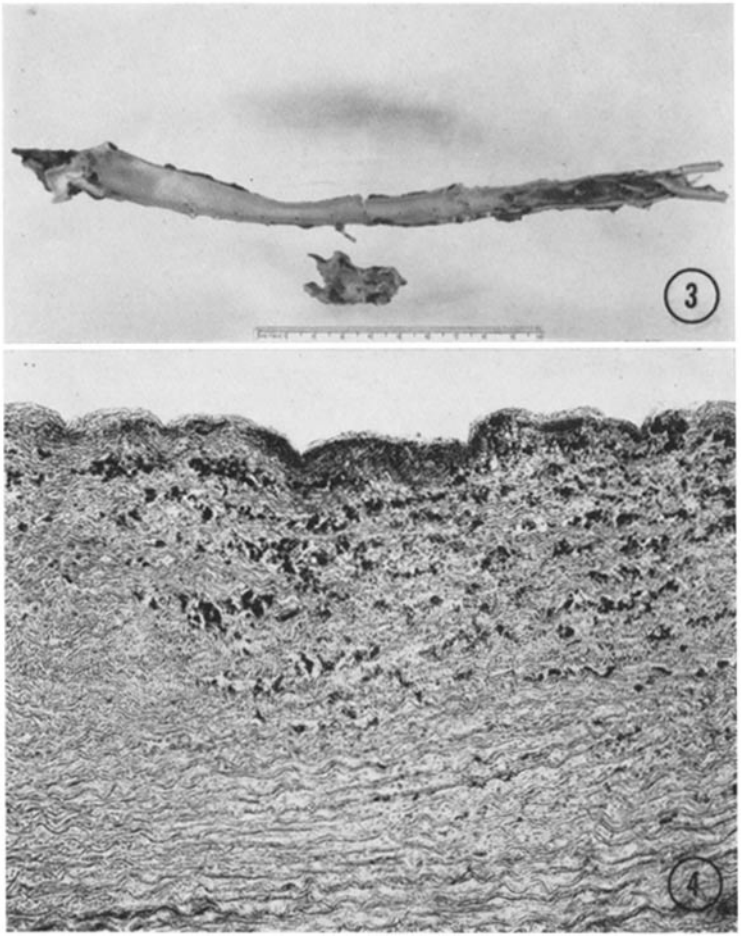


(Scanu *et al.*: Triton hyperlipemia. II)

PLATE 31

FIG. 3. Photograph of a gross specimen of the aorta and the pulmonary artery, showing a patchy deposition in the distal portion of the arch and a rather large deposition in the aorta distal to the superior mesenteric and renal arteries and extending into the common iliac arteries. The pulmonary artery shows a similar deposition. Sudan IV stain.

FIG. 4. Photomicrograph of an aorta, showing intimal and superficial medial accumulation of sudanophilic material, some of which is free and some engulfed by macrophages. Frozen section; Sudan IV stain, × 130.

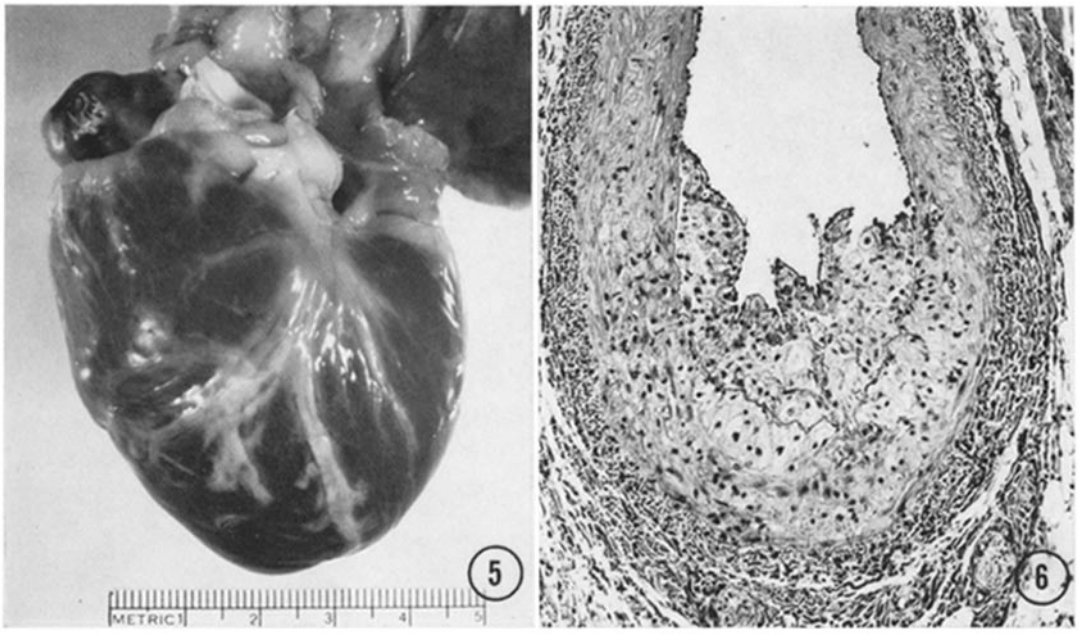


(Scanu *et al.*: Triton hyperlipemia. II)

PLATE 32

FIG. 5. Photograph of a heart, showing the widening and thickening of the coronary arteries in their distal distribution before penetrating the myocardium. Note the atrophy of the epicardial fat.

FIG. 6. Photomicrograph of a coronary artery, showing a focal subendothelial accumulation of macrophages, with some penetration and involvement of the media. The intima has been deflected into papillary folds. Hematoxylin and eosin stain, × 130.



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