

## TISSUE CHANGES IN EXPERIMENTAL CHRONIC ENDOGENOUS HYPERLIPEMIA

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### SUMMARY

Tissue changes produced by Triton-induced hyperlipemia were studied in dogs receiving a stock diet for 6–28 months:

(1) Atheromatous plaques or calcification were not observed in these animals.

(2) Mobilization of endogenous lipids resulted in diffuse deposition in the reticuloendothelial system, gallbladder and kidney.

(3) Serum lipid patterns differed from those following high-cholesterol feeding. Each doubling in normal cholesterol concentration was accompanied by a 3-fold increase in free cholesterol and a 9 to 10-fold elevation in triglyceride. The ratio of total cholesterol to phospholipid, however, did not change.

(4) Serum and other tissue fatty acid patterns were markedly changed. Cholesterol ester fatty acids were more affected than those of triglyceride or phospholipid. In general, a shift toward increased saturation of fatty acids took place in all tissues, except adipose. As a result of this change, fatty acid patterns approached those found in normal adipose tissue.

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### INTRODUCTION

Epidemiological data suggest that elevated serum lipid levels accelerate atherosclerosis<sup>1,2</sup>. Severe hyperlipemia can be readily produced in several animal species<sup>3–6</sup>, by intravenous injection of nonionic surface active agents, such as Triton WR-1339\*, without increasing dietary lipids.

In the present studies, the effect of a Triton-induced, endogenous increase in serum lipids on the production of experimental atheroma and on lipid changes in several tissues was investigated in chronically treated dogs. Changes in serum lipids and fatty acid distribution in ten of these animals were reported previously<sup>7</sup>.

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## METHODS

Hyperlipemia was produced according to a slight modification of the method by SCANU *et al.*<sup>5</sup>. A 20 % (w/v) solution of Triton WR-1339 was prepared in isotonic phosphate buffer pH 7.2 and injected into 14 adult mongrel dogs twice a week at levels ranging from 62 to 200 mg Triton/kg. One dog receiving 200 mg/kg died after 7 months while one receiving 125–200 mg/kg survived for 11 months. Two dogs were injected with 60–100 mg/kg for 28 months before Triton was withdrawn 6 weeks prior to the termination of the experiment. The remaining dogs were killed 2 weeks after Triton injections were ceased. Four control dogs received injections of phosphate buffer without Triton for 6 months.

Specimens for histological and cytochemical studies were obtained immediately following death and fixed at 4°C in Zenker's or Carnoy's fixatives. Frozen and cryostat sections were stained with Sudan IV, oil red O and by Schultz and Adams techniques. Matching sections, 4–6  $\mu$  thick, were prepared and stained with Masson's trichrome, Verhoeff's elastic, Lillie's silver oxide for reticulum, hematoxylin–eosin, PAS–alcian Blue following testicular hyaluronidase digestion for polysaccharides, and May–Grünwald–Giemsa stain for nucleic acids. Specimens for electron microscopy were fixed in a phosphate buffer containing 1 % of glutaraldehyde, postfixed in buffered osmium tetroxide, imbedded in Epon 812 mixtures and stained with uranyl acetate–lead hydroxide or citrate before examination with an Elmiskop 1 microscope.

Samples of serum, adipose tissue, aorta, liver, spleen, myocardium, kidney, and gallbladder were analyzed for total lipid, total cholesterol, free cholesterol, phospholipid, triglyceride, and fatty acid distribution of the cholesterol ester, triglyceride and phospholipid fractions, as in an earlier study<sup>7</sup>. Lipid values were calculated per g of fresh tissue and fatty acids expressed in per cent distribution. Triton-treated animals were compared to the control group and the differences in averages and standard deviations between these groups were expressed in *P* values for statistical comparison.

## RESULTS

*Serum*

Severe hyperlipemia developed in all dogs within 4–12 weeks. In general the degree of hyperlipemia was proportionate to the dosage and to the length of administration of the compound. Table 1 gives the averages, standard deviations, and

TABLE 1

AVERAGES, STANDARD DEVIATIONS, MAXIMUM AND MINIMUM VALUES OF SERUM LIPID FRACTIONS  
Values in mg/100 ml.

	<i>Average</i>	<i>S.D.</i>	<i>Min.</i>	<i>Max.</i>
Total cholesterol (TC)	827	592	147	2970
Free cholesterol (FC)	509	529	33	2450
Phospholipid (PL)	1122	846	270	3900
Triglyceride (TG)	1154	1593	14	6117

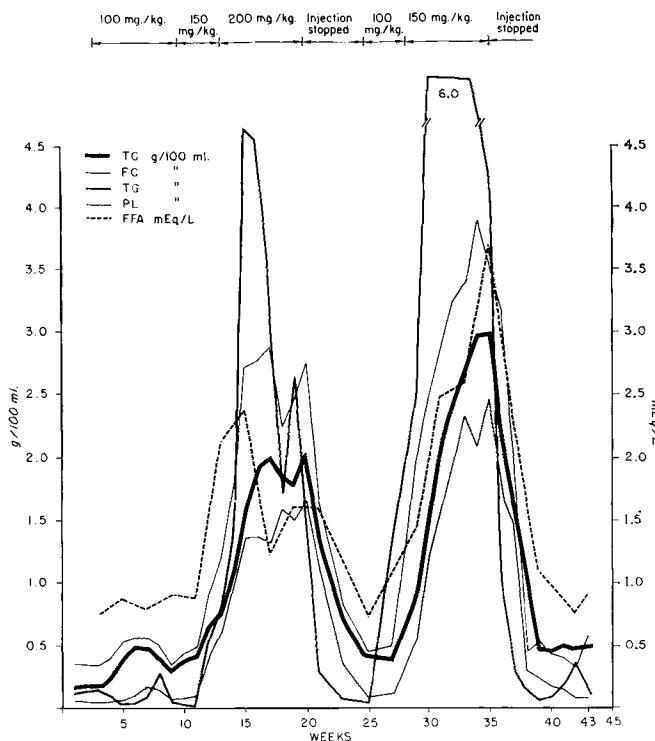


Fig. 1. Variations in average serum lipid levels ( $n = 8$ ) with time and Triton dosage. Note quick response to both withdrawal and resumption of Triton administration.

the minimum and maximum values for each lipid fraction in all animals. Fig. 1 depicts variations in average serum lipid levels with time and dosage. The rapid fall in lipid concentration after Triton withdrawal followed by the sharp rise when injections were resumed are also shown. During Triton administration all animals remained hyperlipemic.

With the increase in serum lipids there was a change in the fatty acid distribution particularly in the cholesterol esters. Fatty acids of cholesterol esters of normal dogs contained 22 % oleic acid, and only 24 % when serum lipids increased to almost double their normal value ( $TC = 400-500 \text{ mg}/100 \text{ ml}$ ). However, there was a critical level above which a rapid rise in oleic acid occurred and in severe hyperlipidemia ( $TC = 1500 \pm 430 \text{ mg}/100 \text{ ml}$ ) this acid constituted almost half of the esterified fatty acid component.

Serum free fatty acids increased from  $512 \pm 142 \text{ mequiv/l}$  in control dogs to  $1482 \pm 635 \text{ mequiv/l}$  in Triton treated dogs ( $P < 0.001$ ). The injection of phosphate buffer solution did not change blood lipid levels or alter the fatty acid distribution in blood or other tissues in control animals. Chronic Triton injection did not interfere with eating habits and the weights of the animals remained unchanged ( $\pm 0.5 \text{ lb}$ ) during experimental period.

*Aorta*

*Histochemical changes.* Gross findings consisted of pinpoint, intimal lipid deposits demonstrable in "en face" preparations following surface staining with oil red O or Sudan IV-silver nitrate (Fig. 2). These lesions were less than 1 mm in dia-



Fig. 2. Pinpointed intimal lipid deposits (arrows) in thoracic aorta. Sudan IV-silver nitrate.  $2 \times$ .

meter, more common in the posterior aspect of the aorta and were not related to the areas of intimal thickening shown in some specimens as pearly elevations. Focal areas of intimal hyperplasia were also found in the anterior ascending coronary, iliac and carotid arteries in both Triton-treated (Fig. 3) and control dogs. Some animals showed localized areas of intimal changes in the thoracic and abdominal aorta with fracture and reduplication of the internal elastic membrane but large fatty streaks or lipid-rich atheromatous plaques were absent.

*Lipid fractions.* Total cholesterol concentration in the aorta of the control animals was  $5.4 \pm 1.6$  mg/g compared to  $3.8 \pm 2.09$  mg/g in the Triton treated group (Table 2). Triglyceride concentration was  $125 \pm 36.1$  and  $91 \pm 57.6$  mg/g respectively. Both total cholesterol and triglyceride reductions in the Triton dogs were not significant.

*Fatty acid per cent distribution.* (a) Cholesterol ester fatty acids: Linoleic acid was reduced in the aorta from  $16.0 \pm 3.2$  to  $8.7 \pm 4.29$  % ( $P < 0.01$ ) (Table 3). (b) Triglyceride fatty acids: No change (Table 4). (c) Phospholipid fatty acids: Stearic acid decreased from  $24.4 \pm 1.52$  to  $21.3 \pm 2.88$  % ( $P < 0.05$ ) and oleic acid rose from  $22.6 \pm 2.1$  to  $27.9 \pm 8.1$  % ( $P < 0.01$ ) (Table 5).

*Myocardium*

*Histochemical changes.* Coronary vessels of Triton treated dogs showed some

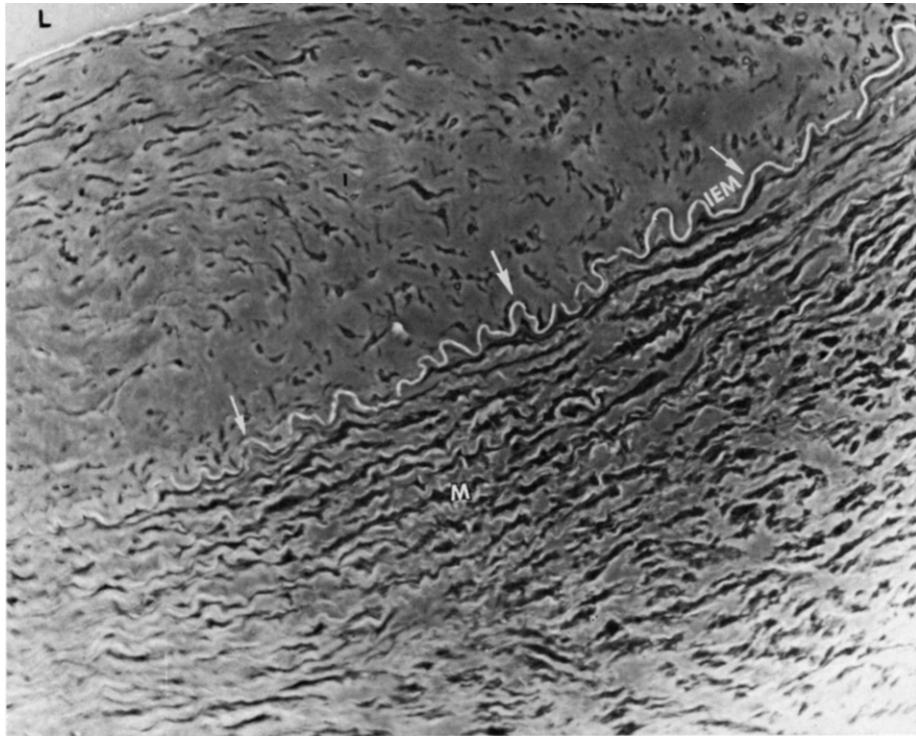


Fig. 3. Area of intimal hyperplasia (I) in iliac artery, Triton-treated dog. Note preservation of internal elastic membrane (IEM), lumen (L), media (M). Masson's trichrome staining, phase contrast microscopy.  $525 \times$ .

areas of intimal hyperplasia and mucopolysaccharide deposits, but no evidence of lipid infiltration. No myocardial scarring or focal necrosis was found, but several animals showed extensive fatty infiltrates in the wall of the right atrium.

*Lipid fractions.* Free cholesterol was increased from  $0.9 \pm 0.06$  to  $1.9 \pm 1.26$  mg/g, but the large variability in Triton dogs made this change significant only to the 10 % level.

*Fatty acid per cent distribution.* Stearic acid in the triglyceride fraction was reduced from  $10.4 \pm 0.55$  to  $8.8 \pm 1.11$  % ( $P < 0.01$ ) and linoleic acid in the phospholipid fraction from  $20.1 \pm 2.5$  to  $15.2 \pm 4.6$  % ( $P < 0.05$ ) in Triton dogs.

#### *Liver*

*Histochemical changes.* Gross fatty infiltration in both lobes was found in all Triton-treated animals (Fig. 4). Most hepatocytes had large membrane-bound intracellular droplets containing neutral fats and both esterified and free cholesterol disrupting the cell ergastoplasm, particularly rough endoplasmic reticulum. These cells usually had short microvilli extending into spaces of Disse (Fig. 5). There were no morphological changes in parenchymal blood vessels or bile ducts. Kupffer's cells showed scattered lipid droplets. Endothelial cells in sinusoids were normal.

TABLE 2

TOTAL CHOLESTEROL (TC), FREE CHOLESTEROL (FC), PHOSPHOLIPID (PL) AND TRIGLYCERIDE (TG) CONCENTRATION IN mg/g OF WET TISSUE IN CONTROL ( $n = 4$ ) AND TRITON-TREATED DOGS ( $n = 8$ ).

C = control group,  $n = 4$ ; T = Triton treated group,  $n = 8$ ; p = difference between control and Triton-treated dogs in P value.

Lipid fraction	Animal group	Arteria	Myocardium	Liver	Spleen	Gallbladder	Kidney	Adipose	Serum <sup>a</sup>
TC	C	5.4 ± 1.6 <sup>b</sup>	1.7 ± 0.4	2.5 ± 0.6	3.8 ± 0.5	2.2 ± 0.2	3.6 ± 0.4	23.0 ± 4.0	188 ± 44.3
	T	3.8 ± 2.1	2.5 ± 1.7	5.5 ± 5.7	11.5 ± 9.8	3.1 ± 0.5	4.7 ± 3.3	13.0 ± 6.9	736 ± 163
	p	—	—	—	< 0.1	< 0.05	—	< 0.02	< 0.001
FC	C	1.3 ± 0.1	0.9 ± 0.1	1.5 ± 0.3	3.1 ± 0.4	1.5 ± 0.1	2.8 ± 0.9	3.3 ± 1.5	39 ± 3.7
	T	1.2 ± 0.7	1.9 ± 1.3	2.6 ± 2.3	5.9 ± 5.6	1.7 ± 0.2	4.3 ± 2.6	2.8 ± 2.0	432 ± 168
	p	—	< 0.1	—	—	—	—	—	< 0.001
PL	C	3.9 ± 0.9	16.8 ± 1.2	20.7 ± 5.7	11.2 ± 2.6	10.9 ± 0.7	17.5 ± 5.8	8.8 ± 5.6	370 ± 44.1
	T	3.4 ± 1.4	16.9 ± 3.9	18.4 ± 7.6	15.1 ± 8.0	8.5 ± 0.4	19.2 ± 2.5	3.7 ± 2.3	997 ± 272
	p	—	—	—	—	< 0.01	—	—	< 0.001
TG	C	125 ± 36.1	5.7 ± 2.0	2.9 ± 0.8	1.8 ± 0.8	4.4 ± 1.8	15.1 ± 14.5	525 ± 256	30 ± 11.6
	T	91 ± 57.6	6.4 ± 2.0	5.5 ± 3.4	5.7 ± 7.4	8.7 ± 0.5	6.8 ± 5.0	389 ± 179	953 ± 627
	p	—	—	< 0.1	—	< 0.05	—	—	< 0.005

<sup>a</sup> Serum values in mg/100 ml from last samples of blood drawn before tissue examination.

<sup>b</sup> Figures preceded by ± are standard deviations.

TABLE 3  
CHOLESTEROL ESTER FATTY ACID PER CENT COMPOSITION

Fatty acid	Animal group	Aorta <sup>a</sup>	Myocardium	Liver	Spleen	Gallbladder	Kidney	Adipose	Serum <sup>b</sup>
14:0	C	4.0 ± 1.9	3.5 ± 1.2	3.1 ± 8.3	2.7 ± 1.4	2.5 ± 0.4	2.2 ± 1.6	2.9 ± 1.5	0.3 ± 0.2
	T	2.4 ± 1.4	3.2 ± 4.2	1.3 ± 0.7	0.6 ± 0.5	0.7 ± 0.4	1.4 ± 0.8	2.4 ± 2.0	0.3 ± 0.4
	P	—	—	< 0.01	< 0.002	< 0.005	—	—	—
16:0	C	19.0 ± 3.9	15.0 ± 2.0	16.5 ± 3.1	13.1 ± 3.0	15.3 ± 0.4	24.3 ± 8.2	17.7 ± 4.1	10.7 ± 1.5
	T	15.8 ± 1.6	17.8 ± 6.6	13.8 ± 2.1	8.7 ± 1.8	14.3 ± 3.6	29.9 ± 12.4	20.1 ± 4.9	13.8 ± 2.5
	P	—	—	—	< 0.05	—	—	—	< 0.05
16:1	C	6.3 ± 2.8	5.8 ± 1.5	7.2 ± 1.4	5.9 ± 2.1	6.1 ± 0.1	4.2 ± 1.6	4.9 ± 3.5	2.4 ± 0.6
	T	4.5 ± 1.6	4.5 ± 1.6	4.4 ± 1.0	3.0 ± 1.1	4.3 ± 1.2	4.9 ± 2.1	5.8 ± 2.9	4.4 ± 1.3
	P	—	—	< 0.05	< 0.05	< 0.02	—	—	< 0.001
18:0	C	16.8 ± 3.0	5.7 ± 2.7	11.5 ± 3.8	8.9 ± 1.8	5.2 ± 1.5	3.9 ± 1.5	19.8 ± 3.7	0.7 ± 0.7
	T	16.0 ± 4.1	8.3 ± 4.0	10.8 ± 1.4	6.7 ± 1.1	9.3 ± 2.7	5.9 ± 3.1	14.2 ± 4.4	5.7 ± 2.8
	P	—	—	—	0.1	0.1	—	0.05	< 0.001
18:1	C	35.5 ± 8.8	21.0 ± 3.2	31.1 ± 3.0	29.7 ± 6.3	45.4 ± 1.6	18.0 ± 4.9	45.3 ± 6.6	23.1 ± 2.4
	T	44.1 ± 4.7	27.8 ± 12.8	54.1 ± 3.3	45.5 ± 6.3	56.4 ± 2.6	24.9 ± 10.8	43.2 ± 3.7	38.5 ± 9.7
	P	< 0.1	—	< 0.001	< 0.01	< 0.005	—	—	< 0.001
18:2	C	16.0 ± 3.2	33.2 ± 5.2	23.0 ± 3.6	24.6 ± 3.2	18.5 ± 0.1	32.2 ± 7.3	8.0 ± 1.2	52.9 ± 5.0
	T	8.7 ± 4.3	17.6 ± 11.2	9.7 ± 2.5	9.4 ± 1.5	10.4 ± 2.1	15.9 ± 5.1	12.2 ± 7.3	29.1 ± 9.1
	P	< 0.01	—	< 0.001	< 0.001	< 0.001	< 0.01	—	< 0.001
20:4	C	2.7 ± 3.7	11.8 ± 4.0	6.4 ± 5.5	13.2 ± 3.5	4.6 ± 2.8	9.3 ± 7.1	0.2 ± 0.4	10.1 ± 3.5
	T	4.5 ± 3.4	7.6 ± 6.9	3.8 ± 2.4	20.5 ± 7.3	5.1 ± 9.1	7.9 ± 5.7	0	8.1 ± 5.3
	P	—	—	—	< 0.1	—	—	—	—

<sup>a</sup> Number of samples for all tissues: group C = 4, group T = 8.

<sup>b</sup> Number of samples for serum: group C = 14, group T = 8.

TABLE 4  
TRIGLYCERIDE FATTY ACID PER CENT COMPOSITION

Fatty acid	Animal group	Aorta <sup>a</sup>	Myocardium	Liver	Spleen	Gallbladder	Kidney	Adipose	Serum <sup>b</sup>
14:0	C	2.8 ± 0.8	1.9 ± 0.5	1.8 ± 0.3	2.1 ± 0.5	1.9 ± 0.4	2.5 ± 0.6	2.8 ± 0.9	1.0 ± 0.5
	T	3.4 ± 0.6	1.9 ± 0.3	1.4 ± 0.4	1.9 ± 0.0	1.9 ± 0.6	1.6 ± 0.5	3.3 ± 0.7	1.1 ± 0.1
	P	—	—	—	—	—	—	—	—
16:0	C	20.3 ± 1.9	19.7 ± 1.8	19.7 ± 2.7	20.3 ± 1.2	26.7 ± 1.4	21.3 ± 1.6	19.8 ± 1.1	18.7 ± 5.8
	T	22.6 ± 2.2	21.0 ± 1.7	23.9 ± 1.8	25.1 ± 1.8	23.9 ± 1.5	22.7 ± 2.3	21.6 ± 1.7	26.0 ± 1.4
	P	< 0.1	< 0.01	< 0.01	< 0.001	< 0.1	—	< 0.1	< 0.001
16:1	C	4.8 ± 1.4	5.3 ± 1.1	4.9 ± 0.8	5.2 ± 1.4	4.1 ± 0.8	4.8 ± 1.5	5.2 ± 2.0	8.6 ± 1.4
	T	5.9 ± 1.8	6.7 ± 1.4	4.5 ± 1.4	4.1 ± 0.9	5.4 ± 0.8	5.3 ± 1.1	6.1 ± 1.9	3.8 ± 0.2
	P	—	—	—	—	< 0.1	—	—	< 0.001
18:0	C	13.3 ± 1.5	10.4 ± 0.6	10.2 ± 2.3	13.4 ± 1.4	10.8 ± 1.6	10.6 ± 2.8	12.0 ± 2.2	6.5 ± 1.8
	T	11.8 ± 2.6	8.8 ± 1.1	11.3 ± 4.8	16.2 ± 6.1	10.4 ± 1.0	8.5 ± 2.3	11.5 ± 2.6	11.5 ± 1.9
	P	—	< 0.01	—	—	—	—	—	< 0.001
18:1	C	44.9 ± 3.4	44.9 ± 2.0	39.2 ± 4.1	41.9 ± 3.0	36.0 ± 0.6	42.3 ± 6.1	43.3 ± 3.7	48.7 ± 3.5
	T	43.5 ± 2.0	46.1 ± 4.3	41.7 ± 4.5	38.7 ± 5.6	46.5 ± 2.2	41.6 ± 3.8	46.2 ± 2.4	45.1 ± 1.6
	P	—	—	—	< 0.001	—	—	—	< 0.005
18:2	C	12.2 ± 1.8	15.0 ± 1.4	15.6 ± 1.8	11.8 ± 1.3	11.5 ± 1.4	13.8 ± 3.0	12.8 ± 2.2	13.2 ± 1.8
	T	11.4 ± 3.7	13.4 ± 3.4	14.5 ± 6.1	10.3 ± 6.1	12.0 ± 2.0	15.3 ± 2.2	10.8 ± 4.1	11.0 ± 1.3
	P	—	—	—	—	—	—	—	—
20:4	C	0      0	1.1 ± 0.2	6.5 ± 3.2	3.6 ± 2.6	3.5 ± 1.2	3.1 ± 3.0	0.4 ± 0.6	1.2 ± 0.55
	T	0.2 ± 0.4	1.0 ± 1.3	1.7 ± 1.4	1.8 ± 2.0	—	2.8 ± 1.7	0      0	2.0 ± 1.4
	P	—	—	< 0.001	—	< 0.1	—	—	—

<sup>a</sup> Number of samples for all tissues: group C = 4, group T = 8.

<sup>b</sup> Number of samples for serum: group C = 14, group T = 8.

TABLE 5  
PHOSPHOLIPID FATTY ACID PER CENT COMPOSITION

Fatty acid	Animal group	Aorta <sup>a</sup>	Myocardium	Liver	Spleen	Gallbladder	Kidney	Adipose	Serum <sup>b</sup>
14:0	C	1.1 ± 0.5	1.1 ± 0.6	0.3 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.2	2.1 ± 0.5	0.3 ± 0.4
	T	1.8 ± 0.8	1.0 ± 0.7	0.2 ± 0.2	0.4 ± 0.3	0.3 ± 0.2	0.5 ± 0.4	2.2 ± 1.0	0.1 ± 0.4
	P	—	—	—	—	—	—	—	—
16:0	C	21.0 ± 2.0	9.1 ± 0.9	14.6 ± 1.2	25.3 ± 1.1	24.6 ± 2.8	25.0 ± 1.6	20.0 ± 3.8	18.5 ± 1.6
	T	24.7 ± 1.9	12.1 ± 3.8	19.8 ± 4.5	28.9 ± 5.5	23.3 ± 3.3	30.1 ± 4.4	23.1 ± 1.9	28.8 ± 2.5
	P	—	< 0.1	< 0.05	—	< 0.05	—	—	< 0.001
16:1	C	2.0 ± 0.7	1.1 ± 0.2	1.4 ± 0.2	1.5 ± 0.5	2.4 ± 0.1	1.6 ± 0.9	3.8 ± 1.6	1.9 ± 0.6
	T	2.8 ± 1.2	1.6 ± 1.2	1.8 ± 1.1	1.2 ± 0.7	2.4 ± 1.5	0.9 ± 0.7	4.6 ± 1.5	1.8 ± 0.2
	P	—	—	—	—	—	—	—	—
18:0	C	24.4 ± 1.5	16.6 ± 0.6	30.7 ± 1.5	18.5 ± 0.8	17.2 ± 0.1	11.3 ± 1.1	19.2 ± 3.9	29.2 ± 1.6
	T	21.3 ± 2.9	20.9 ± 6.9	26.3 ± 8.2	23.2 ± 5.8	18.4 ± 4.8	12.4 ± 2.2	19.0 ± 3.6	15.8 ± 3.7
	P	< 0.05	—	< 0.1	< 0.1	—	—	—	< 0.001
18:1	C	22.6 ± 2.1	13.2 ± 1.5	10.9 ± 1.9	11.9 ± 6.9	15.5 ± 1.8	15.9 ± 1.7	32.5 ± 9.0	13.3 ± 1.6
	T	27.9 ± 8.1	17.6 ± 6.7	16.6 ± 2.9	17.8 ± 3.6	16.4 ± 0.9	15.6 ± 1.4	36.1 ± 7.5	13.5 ± 2.3
	P	< 0.01	—	—	—	—	—	—	—
18:2	C	8.1 ± 0.7	20.1 ± 2.5	12.8 ± 2.2	7.1 ± 5.9	15.1 ± 2.1	12.1 ± 2.3	12.5 ± 2.8	17.4 ± 2.5
	T	8.6 ± 4.6	15.2 ± 4.6	13.6 ± 5.9	10.0 ± 6.2	16.0 ± 2.7	11.8 ± 2.5	8.6 ± 4.9	21.3 ± 3.4
	P	—	< 0.05	—	—	—	—	—	< 0.005
20:4	C	12.5 ± 2.6	23.5 ± 1.8	26.1 ± 2.8	28.9 ± 1.4	20.0 ± 2.8	29.7 ± 3.7	5.3 ± 5.0	17.6 ± 3.1
	T	7.9 ± 7.3	22.3 ± 10.1	19.3 ± 8.9	16.2 ± 9.7	23.3 ± 6.1	26.0 ± 1.7	1.8 ± 3.4	17.3 ± 5.8
	P	—	—	—	< 0.01	—	< 0.1	—	—

<sup>a</sup> Number of samples for all tissues: Group C = 4, Group T = 8.

<sup>b</sup> Number of samples for serum: Group C = 14, Group T = 8.

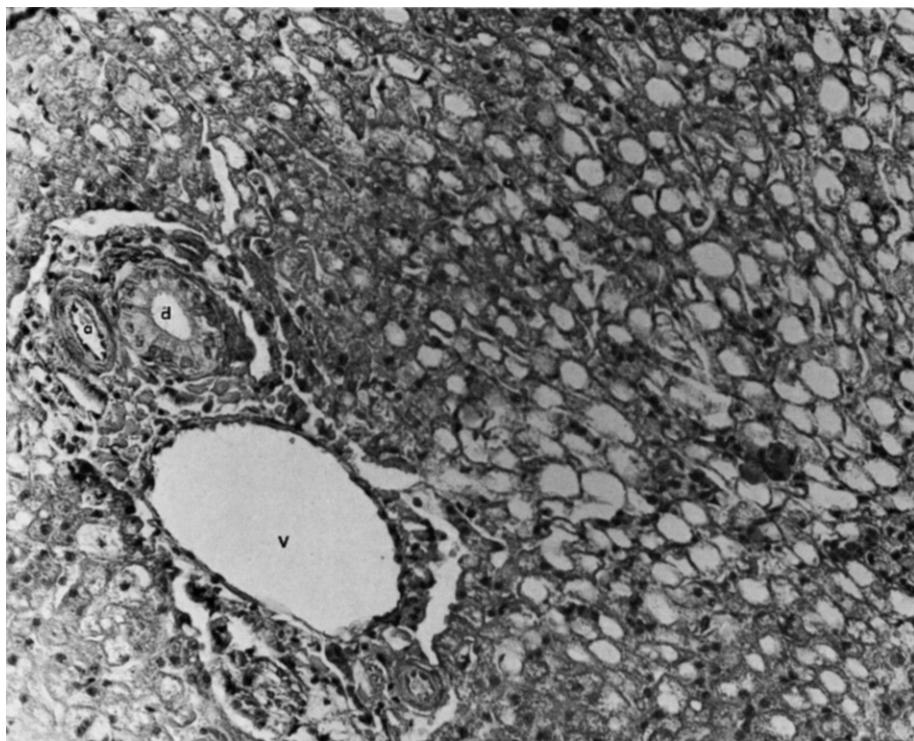


Fig. 4. Extensive fatty infiltrates in liver cells of a Triton-treated dog. Note lack of involvement of elements of the portal space: Bile duct (d), hepatic artery (a), or portal vein (v). Masson's trichrome. 525  $\times$ .

**Lipid fractions.** The large variability of lipid levels between animals especially in the Triton group obscured the statistical significance of the lipid increases.

**Fatty acid per cent distribution.** (a) Cholesterol esters: Only oleic acid was increased in the Triton group, showing a change from  $31.1 \pm 3.0$  to  $54.1 \pm 3.3\%$  ( $P < 0.001$ ) while myristic, palmitoleic and linoleic acids were reduced. Linoleic acid had the largest reduction ( $23.0 \pm 3.61$  to  $9.7 \pm 2.5\%$ ,  $P < 0.001$ ). (b) Triglyceride: Palmitic acid increased ( $P < 0.02$ ) and arachidonic decreased ( $P < 0.02$ ) (Table 4). (c) Phospholipid: Oleic and palmitic acids were elevated ( $P < 0.001$ ) (Table 5).

#### Spleen

**Histochemical changes.** Severe fatty infiltration was noted in all Triton animals. Lipid-laden macrophages containing neutral fats and cholesterol were present between Billroth cords of the red pulp, disrupting reticular fibres. No changes were found in the splenic artery or its branches.

**Lipid fractions.** All lipids were elevated, but the changes were not statistically significant because of the large variation in Triton-treated dogs. The greatest change was with total cholesterol which increased from  $3.8 \pm 0.48$  to  $11.5 \pm 9.77$  mg/g.

**Fatty acid per cent distribution.** (a) Cholesterol esters: There were reductions of

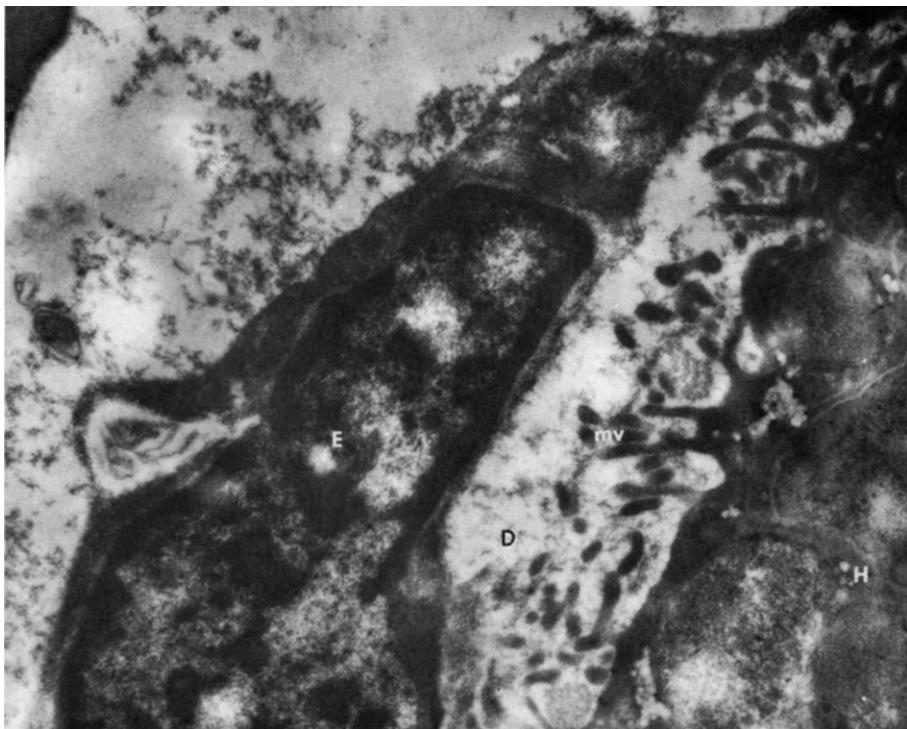


Fig. 5. Electron micrograph of liver showing lipid-laden hepatocyte (H) with short and thin microvilli (mv) extending into space of Disse (D) beneath endothelial cell (E). 42,000  $\times$ .

myristic ( $P < 0.002$ ), stearic ( $P < 0.05$ ), palmitoleic ( $P < 0.05$ ), and linoleic acids ( $P < 0.001$ ). Oleic acid, however, was increased from  $29.7 \pm 6.27$  to  $45.5 \pm 6.34$  ( $P < 0.01$ ) (Table 3). (b) Triglyceride: Palmitic acid increased from  $20.3 \pm 1.17$  to  $25.1 \pm 1.82$  % ( $P < 0.001$ ). (c) Phospholipid: Arachidonic acid fell from  $28.9 \pm 1.43$  to  $16.2 \pm 9.65$  % ( $P < 0.01$ ).

#### Gallbladder

*Histochemical changes.* The gallbladder was usually distended in Triton-treated animals and the congested mucosa suggested advanced lipid infiltration. Large accumulation of lipid deposits were found in the epithelial cells (Fig. 6) with abundant lipid-laden macrophages in the mucosa. No gallstones were present in any of the Triton-treated animals.

*Lipid fractions.* Total cholesterol increased from  $2.2 \pm 0.22$  to  $3.1 \pm 0.53$  mg/g ( $P < 0.05$ ) and triglyceride from  $4.4 \pm 1.84$  to  $8.7 \pm 0.54$  mg/g ( $P < 0.05$ ). Phospholipid was reduced from  $10.9 \pm 0.71$  to  $8.5 \pm 0.44$  mg/g ( $P < 0.01$ ).

*Fatty acid per cent distribution.* (a) Cholesterol esters: Oleic acid was increased while linoleic, myristic and palmitoleic were reduced. These changes were significant at 0.5, 0.1, 0.5, and 2 % levels, respectively (Table 3). Oleic acid esterified with tri-



Fig. 6. Cross-section of gallbladder mucosa of Triton-treated dog showing extensive lipid infiltrates in epithelial cells. Frozen section, stained with Sudan IV. 600  $\times$ .

glyceride was increased ( $P < 0.001$ ) and there was no change in the phospholipid fatty acids (Tables 4 and 5).

#### *Kidney*

*Histochemical changes.* The kidney cortex showed extensive lipid infiltration with sudanophilic droplets accumulated in the epithelium of both proximal and distal convoluted tubules and in glomerular tufts (Fig. 7).

*Lipid fractions.* Both total and free cholesterol concentrations were increased while triglyceride was reduced in Triton animals, but the large variability of lipid levels between the animals obscured the statistical significance of these changes.

*Fatty acid per cent distribution.* Linoleic acid esterified with cholesterol ester dropped from  $32.9 \pm 7.3$  to  $15.9 \pm 5.1\%$  ( $P < 0.01$ ). No changes in triglyceride fatty acid and palmitic acid esterified with phospholipids increased from  $25.0 \pm 1.6$  to  $30.1 \pm 4.4\%$  ( $P < 0.05$ ).

#### *Adipose tissue*

*Histochemical changes.* All Triton-injected animals showed significant reductions of mesenteric and mediastinal adipose tissue. Remaining adipose tissue cells were morphologically normal both by light and electron microscopy.

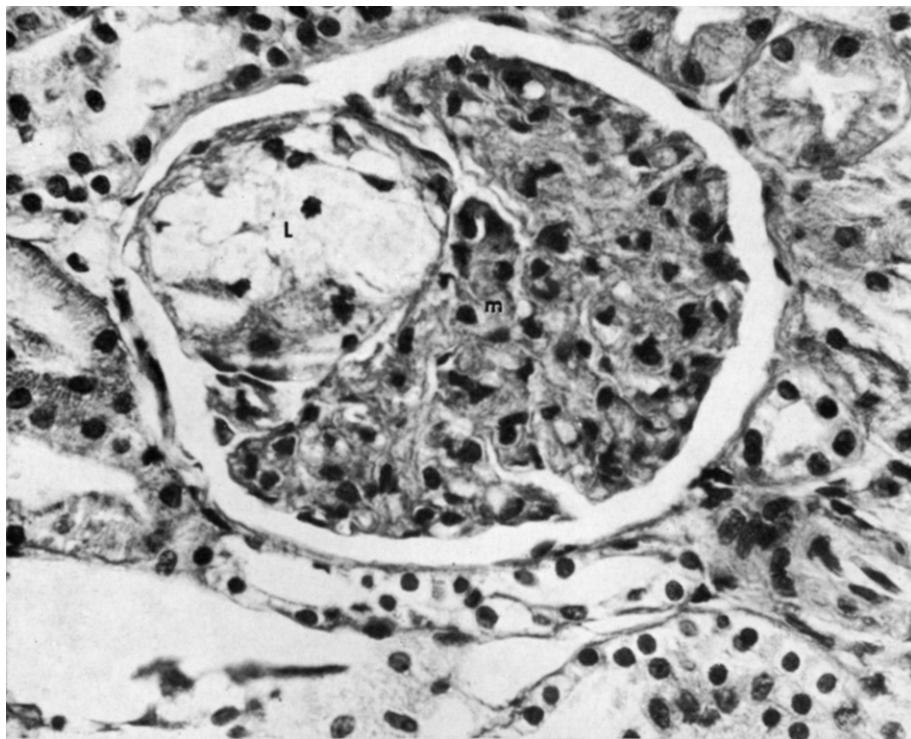


Fig. 7. Glomerular lesion in Triton-treated dog. Note large infiltrates within glomerular tuft (L), and hyperplasia of mesangial cells (m). Hematoxylin-eosin stain.  $1400 \times$ .

*Lipid fractions.* Total cholesterol was reduced from  $23.0 \pm 3.97$  to  $13.0 \pm 6.94$  mg/g ( $P < 0.02$ ) and triglyceride from  $525 \pm 256$  to  $389 \pm 179$  mg/g.

*Fatty acid per cent distribution.* Only stearic acid esterified with cholesterol esters was reduced from  $19.8 \pm 3.71$  to  $14.2 \pm 4.4$  % ( $P < 0.05$ ).

#### DISCUSSION

A reduction of total cholesterol concentration in adipose tissue of Triton-treated dogs indicated its mobilization from lipid stores by Triton. These dogs showed extensive fatty infiltration of the reticuloendothelial cells, particularly those in the liver and spleen. A large number of lipid-laden cells in tubules of kidney cortex and gallbladder mucosa were found suggesting that lipid deposition occurred preferentially in organs normally involved in cholesterol storage or excretion.

Severe hyperlipemia can be readily produced by intravenous Triton injection in the dog, but this increase in serum lipids appears to be different from one induced by atherogenic diets. In dietary hypercholesterolemia in the rabbit the normal cholesterol to phospholipid ratio is reversed due to a disproportionate increase in total cholesterol concentration<sup>8</sup>. In Triton-induced hyperlipemia (TC  $1577 \pm 430$  mg/100 ml) 90 % or more of the increase in serum cholesterol is due to elevation of free cholesterol and the

ratio of total cholesterol to phospholipid is unaffected<sup>5,7</sup>. The disproportionate increase in total cholesterol over phospholipid resulting from cholesterol feeding has been suggested to be the reason for the atherogenic effect of this treatment<sup>3</sup>.

The dissimilarity between Triton- and diet-induced hyperlipemia is apparent from their effects on circulating blood cells<sup>9</sup> and on serum<sup>7</sup> and tissue fatty acid patterns. The observed changes in fatty acid distribution towards increased saturation in blood and all other tissues differs from those results reported for experimental exogenous hyperlipemia in which palmitic<sup>10</sup> and stearic<sup>11</sup> are decreased while linoleic is slightly increased. The hypothesis that Triton hyperlipemia, at least in part, may be due to an endogenous solubilization of lipid from different depots is reinforced by the demonstration that as serum lipids rose, the fatty acid distribution gradually approached that of normal adipose tissue lipid<sup>12</sup>.

In agreement with previous observations in the guinea pig<sup>13</sup>, the present study suggests that lipid deposits in the arterial wall do not necessarily induce atheromatous plaque formation or calcification following chronic Triton treatment. However, when higher doses of Triton are administered to dogs, both SCANU *et al.*<sup>5</sup> and VIDONE *et al.*<sup>14</sup> have shown vascular lesions with abundant lipid deposits. The intimal changes due to Triton-induced lipemia are characterized by the presence of abundant foamy macrophages rather than smooth muscle cells<sup>15</sup> or atherophils<sup>16</sup>, the latter being a prominent feature of plaque formation in naturally occurring atherosclerosis. The differences between our observations and those previously reported<sup>5,14</sup> suggest that Triton-induced lipid rich vascular lesions might be reversible<sup>5</sup> after discontinuing treatment.

It is concluded that repeated injection of Triton results in the mobilization of lipids producing endogenous hyperlipemia followed by lipid accumulation in the reticuloendothelial system, gallbladder mucosa, and kidneys. In contrast to results usually found in experimental animals on high-lipid diets, Triton-induced hyperlipemia maintained for over two years failed to produce atheromatous plaques when the surface active agent was discontinued 2–6 weeks prior to the termination of the experiment.

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#### REFERENCES

- 1 KANNEL, W. B., T. R. DAWBER, G. D. FRIEDMAN, W. E. GLENNON AND P. M. McNAMARA, Risk factors in coronary heart disease. An evaluation of several serum lipids as predictors of coronary heart disease. The Framingham study, *Ann. intern. Med.*, 1964, **61**: 888.

- <sup>2</sup> EPSTEIN, F. H., W. D. BLOCK, E. A. HAND AND T. FRANCIS JR., Familial hypercholesterolemia, xanthomatosis and coronary heart disease, *Amer. J. Med.*, 1959, **26**: 39.
- <sup>3</sup> KELLNER, A., J. W. CORRELL AND A. T. LADD, The influence of intravenously administered surface-active agents on the development of experimental atherosclerosis in rabbits, *J. exp. Med.*, 1951, **93**: 385.
- <sup>4</sup> HIRSCH, R. L. AND A. KELLNER, The pathogenesis of hyperlipemia induced by means of surface-active agents. Part 2 (Failure of exchange of cholesterol between the plasma and the liver in rabbits given Triton WR-1339), *J. exp. Med.*, 1956, **104**: 1.
- <sup>5</sup> SCANU, A., P. ORIENTE, J. M. SZAJEWSKI, L. J. McCORMACK AND I. H. PAGE, Triton hyperlipemia in dogs. Part 2 (Atherosclerosis, diffuse lipidosis and depletion of fat stores produced by prolonged administration of the non-ionic surface active agent), *J. exp. Med.*, 1961, **114**: 279.
- <sup>6</sup> YAMAOA, K., F. KUZUYA, T. OGURI, M. KUNO AND M. KITAGAWA, Species difference in hypercholesterolemia induced by a surface-active agent (Triton WR-1339), *J. Atheroscler. Res.*, 1966, **6**: 299.
- <sup>7</sup> BUTKUS, A. AND J. N. BERRETTONI, Quantitative and qualitative lipid correlation in experimental endogenous hyperlipemia, *Lipids*, 1967, **2**: 212.
- <sup>8</sup> EVRAD, E., J. VAN DEN BOSCH, J. V. JOOSSENS AND P. DE SOMER, Fatty acid composition of plasma lipids of normal, Triton-treated and cholesterol-fed rabbits, *Amer. J. clin. Nutr.*, 1962, **10**: 240.
- <sup>9</sup> EHRHART, L. A., A. BUTKUS, A. L. ROBERTSON JR. AND I. H. PAGE, Effects of experimental endogenous hyperlipemia on circulating leukocytes and erythrocytes, *Lipids*, 1968, **3**: 84.
- <sup>10</sup> SWELL, L. AND C. R. TREADWELL, Tissue cholesterol ester and triglyceride fatty acid composition of rabbits fed cholesterol diets high and low in linoleic acid, *J. Nutr.*, 1962, **76**: 429.
- <sup>11</sup> BLOMSTRAND, R. AND S. CHRISTENSEN, Fatty acid pattern in the aorta lipids of cockerels in the initial stage of cholesterol- or stilboestrol-induced atherosclerosis, *Nature (Lond.)*, 1961, **189**: 376.
- <sup>12</sup> SCOTT, R. F., K. T. LEE, D. N. KIM, E. S. MORISON AND F. GOODALE, Fatty acids of serum and adipose tissue in six groups of eating natural diets containing 7 to 40 percent fat, *Amer. J. clin. Nutr.*, 1964, **14**: 280.
- <sup>13</sup> ROSSI, G. B., P. ORIENTE, M. CUZZUPOLI, A. VECCIONE AND M. MANCINI, Failure to induce atherosclerosis in "Triton" hyperlipemic guinea pigs, *Nature (Lond.)*, 1964, **203**: 416.
- <sup>14</sup> VIDONE, R. A., R. M. LOWMAN, P. H. HUKILL, C. M. BLOOR AND F. A. HIPONA, Experimental atherosclerosis in dogs: the effects of Triton-induced hyperlipemia, *Angiology*, 1967, **18**: 204.
- <sup>15</sup> GEER, J. C., Fine structure of human aortic intimal thickening and fatty streaks, *Lab. Invest.*, 1965, **14**: 1764.
- <sup>16</sup> ROBERTSON JR., A. L., Metabolism and ultrastructure of the arterial wall in atherosclerosis, *Cleveland Clin. Quart.*, 1965, **32**: 99.