evidence available indicates this is due to an effect on Ca^{++} permeability(3).

Summary. Tetraethylammonium chloride was found to increase greatly the amplitude and duration of cardiac trabecular and Purkinje fibers. It also produced an acceleration of firing in spontaneously active Purkinje tissue and the appearance of spontaneous activity in trabecular muscle.

1. Fatt, P., Katz, B., *J. Physiol.*, 1953, v120, 171.
2. Koketsu, K., Cerf, J. D., Nishi S., *J. Neurophysiol.*, 1959, v22, 178.
3. Fatt, P., Ginsborg, B. L., *J. Physiol.*, 1958, v142, 516.
4. Ushiyama, J., Brooks, C. McC., *Am. J. Physiol.*, 1961, v200, 718.
5. ———, *ibid.*, in press.
6. Shanes, A. M., *Pharmacol. Rev.*, 1958, v10, 59.
7. Macfarlane, W. V., *Circulation Res.*, 1960, v8, 47.

Received July 28, 1961. P.S.E.B.M., 1962, v109.

Effect of Triton WR-1339 on Lipoproteins and Lipoprotein Lipase of Guinea Pig Plasma. (27250)

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The hyperlipemia induced by Triton WR-1339, a non-ionic surface-active detergent, may be attributed to an alteration of plasma lipoproteins which results in a decrease in normal lipid catabolism(1). Although the precise nature of the alteration is unknown, the view suggested by Schotz and his coworkers(2) that some change in plasma lipoproteins occurs which interferes with lipid-clearing enzyme has received considerable attention. They found that plasma collected shortly after injection of Triton in rats inhibited the activity of lipoprotein lipase on a coconut oil emulsion. The inhibitory effect of Triton was shown to be a result of alteration of the substrate. Recently, Hirsch and his associates(3) reported that lipemic plasma from Triton-treated rabbits was not cleared by lipoprotein lipase. The purpose of the present study was to investigate the effect of Triton on plasma lipoproteins and lipoprotein lipase in the guinea pig.

Material and methods. Normal, male, albino guinea pigs (400-500 g) were maintained on a stock diet of commercial pellets supplemented with fresh cabbage. Guinea pig plasma used as a source of lipoprotein lipase was obtained from normal animals 30 minutes after intracardiac injection of heparin (7.5 U.S.P. units/kg). The animals were bled by cardiac puncture under sodium pentobarbital anesthesia using 3.8% sodium citrate as an anticoagulant(3). Blood samples were centrifuged at 600 $\times$ *g* and 5°C for 20 minutes; the plasma was removed, recentrifuged, and stored at 5°C. Lipemic plasma used as substrate was obtained from guinea pigs which were maintained on a diet prepared by mixing 8 g of corn oil with 92 g of commercial pellets. With the exception of heparin treatment, plasma was obtained and separated as described above. The Triton-treated animals were injected subcutaneously with a sterile 12.5% solution of Triton WR-1339 (lot #N328PA, Winthrop Labs., New York) in 0.16 M sodium chloride in a dosage of 300 mg/kg. Control animals received an equal volume of sterile saline.

Clearing of lipemic plasma by lipoprotein lipase was measured turbidometrically. Duplicate determinations were performed; each sample contained 2 ml of lipemic plasma, 2 ml of phosphate-buffered saline (pH 7.2), and 1 ml of post-heparin plasma. Substrate controls which contained 2 ml of lipemic plasma and 3 ml of buffered saline also were prepared. The optical density at 500 mμ was read initially and after 30 minutes' incubation at 37°C. The instrument was standardized with a blank tube which contained 5 ml of buffered saline. Lipolytic activity, expressed as % clearing, was calculated by di-

TABLE I. Effect of Lipoprotein Lipase on Lipemic Plasma of Control and Triton-Treated Guinea Pigs.

Group	% clearing*
Control	21.8 ±8.1
Triton-treated	.7 ±1.2

TABLE II. Effect of Incubation of Lipemic Plasma with Triton on Clearing by Lipoprotein Lipase.

Plasma	% clearing*
Untreated	23.1 ±3.3
Triton-treated	3.6 ±4.9

TABLE III. Lipoprotein Lipase Activity of Plasma from Control and Triton-Treated Guinea Pigs.

Group	% clearing*
Control	3.3 ±1.1
Triton-treated	15.7 ±1.7

* Values represent mean ± S.D.

viding the difference between initial and final optical densities by the initial optical density. A correction was made for clearing of the substrate during incubation in the absence of lipoprotein lipase by subtracting the % clearing for substrate controls from that obtained with samples to which lipoprotein lipase was added.

Results. The lipolytic activity of guinea pig lipoprotein lipase on plasma lipoproteins of control and Triton-treated guinea pigs was studied in a series of experiments. The results of a typical experiment are shown in Table I. The animals were fed the corn oil diet for 7 days; on the 5th day, 9 animals were injected with Triton and an equal number received saline. On the 7th day all animals were bled for plasma to be used as substrate. The post-heparin plasma from 3 normal guinea pigs was pooled and used as a source of enzyme. The results of such experiments indicted that lipemic plasma from the Triton-treated guinea pig was significantly ($P<0.01$) less susceptible to clearing by lipoprotein lipase than that of the control animal.

Experiments also were performed to determine if the clearing of lipemic plasma from untreated animals can be inhibited by treatment of the plasma *in vitro* with Triton. In such an experiment, 6 samples, each containing 2 ml of plasma from a corn oil-fed guinea pig, were prepared. Two ml of buffered saline which contained 5 mg of Triton were added to each of 2 samples; 2 other samples received 2 ml of buffered saline, and the remaining 2 samples received 3 ml of buffered saline to serve as substrate controls. All samples were incubated for 1 hour at 37°C and then 1 ml of pooled post-heparin plasma was added to each of the first 4 tubes. The results obtained with plasma from 5 animals are summarized in Table II which demonstrates that *in vitro* treatment with Triton significantly ($P<0.01$) altered guinea pig plasma lipoproteins so that they were not cleared by lipoprotein lipase.

The effect of Triton on plasma levels of lipoprotein lipase also was investigated. In this case, 11 guinea pigs were used; 5 were injected with Triton and 6 received saline. Two days later, plasma was obtained from each animal, without prior heparin treatment, to be used as enzyme. Pooled plasma from 10 corn oil-fed guinea pigs was used as substrate for enzyme assays. The results (Table III) demonstrated that Triton treatment induced higher levels of enzyme. Comparison of enzyme activity in the plasma of the Triton group with that of the control group showed a highly significant ($P<0.01$) difference.

Discussion. These results suggest that the lipolysis of plasma lipoproteins by lipoprotein lipase is inhibited by Triton WR-1339 in the guinea pig in the same manner as in other animals(2,3). The view(2) that inhibition of lipolysis reflects an alteration of the plasma lipoprotein substrate appears to be supported by the observations that lipemic plasma from treated animals was not cleared by lipoprotein lipase (Table I) and that plasma lipoproteins from untreated animals may be rendered resistant to the action of lipoprotein lipase by incubation with Triton *in vitro* (Table II). It does not seem likely that the inhibition of lipolysis involves inactivation of lipoprotein lipase directly since the plasma of Triton-treated animals contained active lipolytic enzyme (Table III).

Some support for the belief(4) that Triton becomes irreversibly bound to the substrate may be gained from the present study. It was found that plasma from Triton-treated

animals exhibited marked lipoprotein lipase activity (Table III). The concentration of Triton in the plasma of such animals may be estimated at 1 mg/ml(5). If this amount of Triton were free in the plasma, it would have been sufficient to inhibit lipolysis as demonstrated in the experiment presented in Table II. On this basis, it would appear that Triton was bound to the plasma lipoproteins of treated animals. It is of interest to mention that the binding of Triton does not seem to occur immediately *in vivo* since Schotz and his coworkers(2) found that plasma collected from rats 30 minutes after intravenous injection of Triton inhibited the clearing of substrate by lipoprotein lipase. It would appear in this case that sufficient free Triton was available in the plasma of treated rats to inhibit their system. Additional evidence for the binding of Triton to plasma lipoproteins has been provided recently by Scanu and Oriente(6).

The findings of the present study also are of interest in view of the work of Engelberg. His studies have indicated that although lipoprotein lipase may be elevated during the post-alimentary hyperlipemia induced by fat diets(7,8) the enzyme level is significantly reduced during clearing *in vivo*. He has offered evidence(9) that lipoprotein lipase actually became inactivated during lipolysis. If this is the case, the elevated levels of lipoprotein lipase in treated animals (Table III) would seem to indicate a lack of *in vivo* lipolysis and thus support the current view that the Triton-induced hyperlipemia reflects decreased lipid utilization caused by inhibition of enzymatic lipolysis.

Summary. Administration of Triton WR-1339 to guinea pigs (300 mg/kg) altered plasma lipoproteins so that they were not cleared by guinea pig lipoprotein lipase. Plasma lipoproteins of normal guinea pigs similarly were rendered resistant to lipoprotein lipase by incubation with Triton *in vitro*. Comparison of plasma lipoprotein lipase levels in Triton-treated and control animals showed that the former group possessed significantly higher levels of enzyme. The mechanism responsible for the Triton-induced hyperlipemia was discussed in view of these findings.

We gratefully acknowledge the technical assistance of Mrs. Olha O. Holowecky.

1. Hirsch, R. L., Kellner, A., *J. Exp. Med.*, 1956, v104, 1.

2. Schotz, M. C., Scanu, A., Page, I. H., *Am. J. Physiol.*, 1957, v188, 399.

3. Hirsch, R. L., Kellner, A., Ireland, R., *J. Exp. Med.*, 1960, v112, 699.

4. Brown, R. K., Boyle, E., Anfinsen, C. B., *J. Biol. Chem.*, 1953, v204, 423.

5. Patnode, R. A., Hudgins, P. C., *J. Exp. Med.*, 1958, v107, 55.

6. Scanu, A., Oriente, P., *ibid.*, 1961, v113, 735.

7. Engelberg, H., *J. Appl. Physiol.*, 1957, v11, 155.

8. ———, *ibid.*, 1958, v12, 292.

9. ———, PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 489.

Received September 5, 1961. P.S.E.B.M., 1962, v109.

Extractable Collagen in the Fracture Callus of Normal and Aminoacetonitrile-Treated Rats.* (27251)

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It was reported(1) that the hexosamine content in a 2-week-old fracture callus of aminoacetonitrile-treated (AAN) rats is about half the amount found in the fracture callus of normal animals. On the other hand no significant difference was found in content of total hydroxyproline in fracture callus of experimental and control animals. If we consider hexosamine as an index of mucopolysaccharide content, and hydroxyproline as an index of collagen, it may be assumed that the mucopolysaccharides in the fracture cal-

* This work was aided by grant from Nat. Inst. of Health.