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.

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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.

lus of AAN-treated rats are greatly decreased in comparison with normal animals, whereas collagen content appears similar in both groups.

However, changes in the collagen fibers were surmised from previous histological and histochemical studies(2,3) and recently Gross and Levene(4) have found that a great amount of newly synthetized collagen of lathyric animals is soluble in NaCl M solution.

In fracture healing there is a large production of new connective tissue. Therefore, changes in solubility of collagen in the fracture callus of lathyric animals similar to those observed in the collagen of young animals by Gross and Levene could be expected.

Method. Eighty Sprague Dawley male rats weighing an average of 155 g were used in 2 separate but identical experiments. Fracture in the middle third of both tibiae was produced manually in every rat under ether anesthesia by bending the leg over the dull edge of a large knife held in a vise. The fractures were not immobilized. Half of the animals received Purina chow and served as controls. The other half received ground Purina chow containing 0.075% AAN throughout the experiment.

Five, 10, 15 and 20 days after the fracture 5 animals in each group were sacrificed with ether, the posterior legs were dissected, the callus was obtained and placed in a lusteroid tube in dry ice. Femoral shafts of both legs were also dissected free of muscles and periosteum and placed in dry ice. The callus from 5 animals and the femurs were pooled and weighed separately and homogenized in Virtis "45" in cold sodium chloride M 1:10 (w/vol.) during 10 minutes (setting at 40). Octanol was added as preservative and extraction was started by stirring at 4°C during 24 hours. At the end of extraction the heavier particles were removed by low speed centrifugation at 2500 rpm. The supernatant was ultracentrifuged according to Gross at 50,000 g for one hour(4). After filtration 5 ml of the supernatant were lyophilized, then hydrolized in HCl 4N by reflux for 15 hours. The hydrolysate was dried in a flash evaporator. The residue was dissolved in distilled water, filtered and the final volume made up to 25 ml. Hydroxyproline analysis was performed on 1 ml of this solution according to the method of Neuman and Logan(5) modified by Leach(6).

TABLE I. μg of Hydroxyproline/ml in Supernatant.*

Days after fracture	From callus		From bone shaft	
	Exp.	Controls	Exp.	Controls
5	31.9	13.8	23.1	18.5
10	56.35	18.05	62.5	22.5
15	61.7	19	54.5	26
20	71.25	22.5	46.30	17.05

* Collagen = hydroxyproline × 7.46.

Results. The results can be seen in Table I. The content of hydroxyproline in the hydrolisate of a supernatant obtained by ultracentrifugation of extracts of fracture callus was greatly increased in the AAN-treated animals after 10 days of fracture. Some increase of hydroxyproline was also observed in extracts of femoral shafts of these animals.

Discussion. The data indicate that the large proportion of the collagen fibrils formed in the tibial fracture callus of the AAN-treated animals were altered so that they became soluble in cold saline. The increase of extractable collagen in the femoral shafts of these animals is not clear. The bone marrow of the femurs was not removed but it is unlikely that all the altered collagen was derived from the endosteum and marrow.

Summary. A great increase in amount of extractable collagen was observed in the fracture callus of AAN-treated mature rats in relation to a control group of animals. Also, some increase of extractable collagen was observed in the shaft of the femurs of the experimental rats.

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