CHEMICAL STUDIES OF MUSCLE CONTRACTURE.

III. THE CHANGE IN GLYCOGEN DURING SHORTENING PRODUCED BY TETANUS TOXIN.

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In a preceding paper (2) negative findings were reported for any change in the lactic acid content of mammalian muscle during contracture produced by tetanus toxin. The study has been extended to glycogen and although definite changes were found, a simple interpretation of the changes could not be made on the basis of muscle shortening.

Analyses made by Ishizaka (which were reported by Fröhlich and Meyer (4)) showed that the glycogen content of gastrocnemii of cats was higher in muscles shortened through the agency of tetanus toxin than in untreated control muscles from the opposite side of the animal. The maximum amount found in the tetanus muscles was 0.37 per cent. Amounts in the control muscles were lower and in one instance only 0.01 per cent was found. These values are lower than those usually found in normal animals and suggest that some change in glycogen metabolism takes place during poisoning with tetanus toxin.

Although the mechanism of the formation of a contracture by tetanus toxin has not been satisfactorily explained, Ranson and Morris (10) and Ranson and Sams (11) have shown that the phenomenon can be divided into two stages. During the first stage, the muscle is kept in a shortened state by nerve impulses which reach it through the motor nerve. During the second stage the contracture persists after profound general anesthesia or section of the motor nerve and is no longer dependent upon nerve impulses.

Experiments by Wertheimer (13), Hoffman and Wertheimer (6),
and Embden and Habs (3) lead to the conclusion that the glycogen metabolism of striped muscle is intimately dependent upon its motor innervation. In Wertheimer's experiments, animals were fasted and given phlorhizin after one sciatic nerve was cut, and even though the glycogen in the innervated side fell to a low level, the amount found in the denervated muscles was comparable to that found in normal animals. Both Hoffman and Wertheimer and Embden and Habs found that the amount of glycogen in muscle could be increased by faradic treatment.

If in the establishment of a tetanus contracture, motor impulses are present to an extent greater than normal, one might expect a reduction in glycogen on the affected side. This result (opposite from Ishizaka's findings) was reported by Wertheimer in 1928 (14), although a reduction was not invariably present. Cutting the sciatic nerve of the uninjected limb did not cause a change in results. This last report appeared during the time our work was in progress so we have abridged our review of the literature and refer the reader to Wertheimer's article for a more complete discussion of the theories involved.

A complete review of the subject of local tetanus will be found in the article listed under reference (9).

EXPERIMENTAL.

Guinea pigs, white rats, and rabbits were used for experiments with tetanus toxin, and cats, for three experiments in which contractures were produced by cutting the dorsal roots of spinal nerves.

The toxin\(^1\) was injected aseptically into the popliteal space of one limb, and after the contracture had developed, both gastrocnemii were removed and prepared for glycogen determinations. The soleus muscle was usually removed with the gastrocnemius. Muscles were frozen in the same manner as for lactic acid determination (1). After removal from the animal they were cut while frozen into 0.1 to 0.2 mm. cross sections and the slices put into ice-cold 30 per cent sodium hydroxide solution. About 5 cc.


\(^2\) The tetanus toxin for this work was furnished by Parke, Davis and Company.
of the strong alkali solution were used for each gm. of muscle. Weights were obtained by weighing the flask before and after it received the sample.

The analyses for glycogen were carried out according to the principles established by Pfüger, and sugar was determined after acid hydrolysis by Somogyi's (12) modification of the Shaffer-Hartmann procedure.

Since amytal was used for the anesthetic, it seems appropriate to review the status of our knowledge concerning its effect on the metabolism of muscle glycogen. Long (7) found that general anesthesia (ether or amytal) caused a decrease in glycogen. Hinsey and Davenport (5) were not able to confirm this observation but found (unpublished data) in agreement with Long, that if a muscle is stimulated during amytal anesthesia and the glycogen thereby reduced, the original resting glycogen level is not restored for a period of 2 to 3 hours. It would seem that amytal would not affect the findings in tetanus, for the glycogen would be
neither reduced from a resting value nor increased from a decreased amount.

The cats included in the series had received no tetanus toxin but showed well defined contractures which did not relax under anesthesia. The contractures were a sequel to the cutting (intra-durally) of the dorsal roots of the spinal nerves from the third lumbar to the third sacral segments. In order to favor the development of contracture a small nick was made in the dorsal portion of the posterior funiculus of the cord at the level of the third lumbar segment and on the same side as the divided roots. A description of contractures following operative procedures of similar type has been reported (8).

Fig. 1 shows that there was a wide variation in the difference in glycogen content between the two sides. The guinea pigs and rabbits exhibited the largest variations while the rats showed very little. The maximum variation (with intact innervation) occurred in Guinea Pig 2 where the glycogen on the tetanus side was only 37 per cent of that on the uninjected side. Rabbits 22 and 23 showed a reversal of the usual order in that there was less glycogen in the flaccid muscles than in those in contracture. These two animals had refused to eat for several days and the glycogen content of both sides was below normal. Perhaps the glycogen was exhausted, during the general depletion of carbohydrate stores, more rapidly from the legs used in moving about than from those which were too stiff from tetanus to be exercised. This phenomenon appears to be possible in light of Wertheimer's (13) findings that a denervated (therefore unused) muscle retains its glycogen during fasting. Rat 32 showed low glycogen also but it was equal on the two sides, hence the above explanation may not be adequate.

If the reduction in glycogen had been uniformly present in all species and in both tetanus contracture and the contracture following dorsal root section with spinal cord injury, one would be led to the conclusion that it was directly related to the contracture. Wertheimer (14) expressed the belief that the variableness and relatively small amount of reduction in glycogen indicated that the loss of glycogen had very little to do with the contracture. This irrelevancy seemed even more likely since the contractures of longer duration showed less reduction and were at the same
time associated with some atrophy of the muscle. Our results are in agreement with his conclusions, and the interpretation is further complicated by the findings in Rabbits 25 and 26. These two animals were kept for 42 and 58 days respectively and even though function of the tetanus limbs was not regained, their gastrocnemii contained more glycogen than the functioning gastrocnemii on the opposite sides.

Guinea Pigs 9 and 11 had both sciatic nerves cut at the same time that toxin was injected into only one limb. These animals show that motor innervation is an essential feature of the glycogen change. The denervated tetanus muscles contained the same amount of glycogen as the controls but coincidentally developed no contractures. Guinea Pigs 10 and 12 had only one sciatic nerve cut and tetanus toxin injected into both limbs. Here the decrease in glycogen is seen only on the innervated side. Samples IT and IC of Specimen 11 F represent opposite quadriceps femoris muscles of Guinea Pig 11, and since these muscles were innervated on both sides and toxin was present in only one limb, the innervated tetanus muscle contains less glycogen than the other, while the denervated gastrocnemii (Specimen 11 G) show no change.

The negative findings in contracture produced by dorsal root section and cord injury show that contracture can occur without significant change in glycogen in the affected muscle when the functioning companion muscle is used as a basis for comparison. Values of 0.47 and 0.45; 0.49 and 0.50; and 0.60 and 0.56 per cent were obtained for functioning and contracted muscles respectively.

**SUMMARY.**

1. The glycogen content of guinea pig and rabbit gastrocnemii is usually reduced by tetanus toxin during the early stages of contracture.
2. In rats the glycogen shows little change under the same treatment.
3. Contractures resulting from dorsal root section of spinal nerves showed no change in glycogen.
4. The variability in reaction of the glycogen content among different species and during different stages of tetanus contracture
indicates that it does not have a cause and effect relationship to the contracture.

5. Tetanus toxin causes no change in the glycogen content of denervated muscles.

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