THE FORMATION OF GLYCOGEN IN MUSCLE.

Plate I.

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It would appear from theoretical considerations that muscle tissue should be incapable of forming glycogen from a disaccharid. As has been shown by a number of observers, the amount of inverting substance present in muscle or blood is small, or is entirely absent. The injection experiments with saccharose, and perfusion experiments with the liver also tend to show that saccharose is quite incapable of being used by the organism as a direct glycogen former. Moreover, the direct transformation of a disaccharid to a polysaccharid without the preliminary inversion to a simple hexose has not, as far as we are aware, been shown to take place.

Nevertheless E. Külz, in a series of experiments undertaken to decide this question some years ago, obtained results which in effect showed that saccharose was indeed a direct former of glycogen in muscle.

It seems that the work of Külz, has never been repeated, although the anomalous character of the findings as recognized by him, and he asked that his observation be tested by other observers. On his authority the statement that saccharose is a direct former of glycogen has passed into the text-books, and Hammarsten, among others, mentions saccharose as an immediate antecedent in the formation of glycogen. On reference to Külz’s original experiments, it will be seen that the results on which he bases his statement are by no means unequivocal. Three experiments

1 Tebb: Journ. of Physiol., xv, p. 421, 1893.
2 Bernard: Leçons sur le diabète.
3 Pfuger: Das Glykogen, p. 205; see also Jappelli and D’Errico: Atti Real. Accad. di Med. di Napoli, 1903, cited by Moscati, Zeitschr. f. physiol. Chem., I, p. 90, 1906. These authors believe that saccharose is transformed into glycogen by injection into the portal vein.
only are reported which confirm his conclusions, and these are not given with very great confidence. For this reason we have thought it advisable to repeat Külz's experiments with improved technique, and try if possible to obtain his somewhat anomalous results.

Through the exhaustive work of Pflüger\(^1\) we are now in possession of a method which permits the more accurate determination of glycogen than the Brücke-Külz\(^2\) method employed by Külz\(^3\), and using a method of perfusion which will be presently described, we believe we have been able to keep a muscle in a surviving condition for a period of time sufficient to decide the question. At the same time we have been able to fulfill certain experimental conditions which are necessary to render the results trustworthy.

The methods used by Külz were two. In the first, a limb was perfused with blood to which 0.1 per cent of saccharose had been added, and glycogen estimations were carried out in the limbs before and after perfusion, using the unperfused limb as a control. This method is faulty, in that one is never certain that the glycogen destruction which is continually taking place is going on more rapidly or more slowly than the glycogen formation by the sugar. It is quite possible to have a formation of glycogen which would be completely masked by the simultaneous destruction which is taking place. It will also be seen that the figures as presented by Külz do not differ markedly from the differences which Cramer\(^4\) obtained in his analysis of symmetrical muscles, using the Brücke-Külz method employed in the work under discussion. The objections to this method were realized by Külz and he accordingly changed his experimental conditions to meet the difficulty.

The second method which was used was the perfusion of two symmetrical limbs of the same animal, one with blood containing saccharose, the other with blood containing no foreign sugar. Symmetrical muscles were analyzed. It is the results obtained

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from this series of experiments on which Küllz bases his conclusions.

This method, suitably changed, appeared to fulfill more closely the experimental conditions, and was the one chosen by us.¹

There are two points which Küllz does not appear to have taken into consideration, and which may have some bearing on his results. The perfusion of a limb with blood which has not been properly arterialized does not conform to the state of affairs obtaining under normal conditions. The perfusing fluid was also run in at constant pressure. This while not directly affecting the results has unquestionably an influence on the metabolism of other organs, as Sollmar² has shown in the case of the kidney. For that reason, we have performed the experiment, using intermittent pressure. In using the lung itself to arterialized the blood instead of the less efficient mechanical oxygenation, one may be guided to some extent by the recent experiments of Riehl,³ who has shown that lung tissue is incapable of inverting lactose.

The method which Küllz used for analyzing the muscle is also very seriously open to question. This is especially the case as in the present instance where the amounts of glycogen are small. In the course of a large number of controls which were undertaken to test the accuracy of the Pflüger method, we convinced ourselves of its entire suitability for the estimation of small quantities of glycogen.

At the outset of this work, it was realized that a perfusion apparatus, fulfilling the conditions of efficient arterIALIZATION and certainty of action was absolutely necessary. A number of

¹ One of us, with Dr. B. J. Dryfuss, has attempted the perfusion, by quickly extirpating all the organs of the abdominal cavity. The liver was ligated off piece by piece. Practically no liver tissue was left. There was very little hemorrhage. One iliac artery was then ligated. Artificial respiration was employed, and the animal kept warm on a hot plate, additional heat being supplied by electric lamps. The animal survived over two hours. It was found that the amount of glycogen in the ligated leg was almost unweighable, showing the complete disappearance of the saccharid from ligation. Our principal object in this experiment was to obviate the difficulty of using defibrinated blood. See Barcroft and Brodie, Journ. of Physiol., xxxii, p. 19, 1904; Pfaff and Vejux-Terode: Arch. f. exp. Path. u. Pharm., xlix, p. 324, 1903.
preliminary experiments lead to the adoption of the apparatus described below, which has accomplished admirably the purpose for which it was designed. We also made experiments in mechanically perfusing one limb, using the unperfused limb as a control. It was soon found that the rapid destruction of glycogen in the unperfused limb which sometimes occurred with rigor did not permit of its use.

Kisch\(^1\) has recently examined the destruction of glycogen after death, and has shown that the process takes place with decreasing velocity, owing possibly to a using up of the glycogen-splitting ferment. In the course of this work, we have been able in part to confirm his results. We are also able to add that the destruction of glycogen seems to be dependent on factors of which very little can be conjectured. It was found that in experiments taking place under what appear to be identical conditions, one obtained a complete disappearance of glycogen from the ligated limb, while in the second experiment, the amount of glycogen from symmetrical muscles of perfused and unperfused limbs was identical. That this is not due to disappearance of glycogen in the perfused limb is shown by the high amount of glycogen which is often obtained under these conditions.

In designing this experiment, it was necessary to have an apparatus which possessed two completed systems for perfusing and arterializing each limb. The apparatus is a combination of that used by Embley and Martin\(^2\) with the chamber for containing the limbs and lungs and for keeping the blood and organs at constant temperature as designed by Brodie.\(^3\)

The accompanying plate (I) gives the general arrangement of the apparatus, while the following schematic diagram explains the individual parts of the apparatus.

**Description of Diagram.** The pumps representing the right and left hearts are formed from ordinary syringe bulbs, \(L, M, N, O\), with hard rubber seat valves. \(L\) and \(N\) are the right ventricles, pumping blood to the two pairs of lungs, \(C\) and \(D\), which are supplied with air through the tube \(E\). After the blood has been

2. Embley and Martin: *Journ. of Physiol.*, xxxii, p. 147, 1904.
3. We wish to acknowledge the great assistance which one of us received in the technique of perfusion from Prof. T. Gregor Brodie.
arterialized, it returns through $V$ and $W$ to glass receivers $G$ and $I$. Thence it is pumped by means of the left hearts $L$ and $M$ to the respective limbs $A$ and $B$. Returning, deprived of oxygen, it reaches the receivers $H$ and $J$, and again completes a cycle.

The coils $F$ are beneath the organs to be perfused, and are completely submersed in water at body temperature. By means of the connections between the reservoirs $G$ and $H$, and $I$ and $J$, the levels in the venous and arterial reservoirs are kept equal. At the same time the flow from one reservoir to another is insufficient to mix the two bloods appreciably. Between the heating coils and the organs small air traps are interposed, which are not shown in the figure.

The heating tank is 107 cm. long, 35 cm. wide and 41 cm. deep. The upper part contains two separate trays pierced at the bottom with four holes 2.5 cm. wide, flanged to admit of being closed with two holed rubber stoppers, through which the artery and vein for one organ pass. The water which fills the tank does not enter the trays, but is in contact with the bottom, and supplies the necessary heat to the organs. If the temperature is not sufficiently high in the chambers, additional heat is furnished by electric lamps, which are turned off and on at will.

The system of cams, which is shown in the photograph, and also extremely well in the diagram in Embley and Martin's article, permit of a very accurate adjustment of the flow to any of the organs. It will be observed that instead of the two cams and compressors used by Embley and Martin, we have used four.

The Operation. An animal is killed by medulla puncture, and bled at once from the carotids. The blood is defibrinated, and filtered through glass wool. A second dog is killed in a similar manner, bled and the blood added to that of the first dog. The lungs and hearts are removed from both. The hearts are opened, and very large glass cannulas with heavy shoulders are introduced into the pulmonary arteries and veins through the right ventricles and left auricles. The cannulas are securely tied in, and the excess of heart muscle cut away. Three-way cannulas are placed in the tracheae. The lungs are then placed in warm saline solution till ready to be used.

The abdomen of the second dog is then opened, and cannulas introduced into the right and left iliac arteries and veins. In
order to prevent collateral circulation as much as possible, ligatures are tied around the profunda arteries and veins just beyond the origin of the iliacs. The limbs are severed from the rest of the body, the bladder tied off and bleeding points stopped with ligatures or with hæmostats. All the cut surface of muscle and skin are cauterized thoroughly, by brushing lightly with a blow-pipe flame. The spinal canal is plugged with modeling wax. The amount of capillary oozing which takes place from the limbs is small.

In some few instances we have been able to detect a certain amount of collateral anastomosis, but there were few of these anomalous cases.

After joining up the arterial system with the organ, being careful to exclude air, and removing clotted blood with a small feather, the pumps are separately set in motion by hand, and the blood flowing from the iliac veins and pulmonary arteries collected in basins. About 150 cc. of blood are washed through. This is defibrinated, filtered through glass wool and returned to the proper receiver. In this way the organs are washed out with defibrinated blood, and one has no further trouble from intravascular clotting.

The cams are adjusted, so that each heart makes about 70 beats per minute. The respiration pump inflates the lungs about 25 times per minute. By means of the adjusting screws, the flow to the organ is regulated so that limb and lung receive the same quantity of blood.

The sugar solutions added were isotonic with blood. In every case where sugar solutions were added, an equal amount of isotonic salt solution was added to the control side. In this way we believed we kept the conditions on the two sides more closely alike than if the control side was allowed to receive undiluted blood.

Perfusion was continued for two hours, after which symmetrical muscles were removed from the two limbs as quickly as possible for analysis.\(^1\)

\(^1\) It may be noted that it was found to be extremely difficult to filter the alkaline solution of muscle clear, and it was found preferable to let it stand a day or more for sedimentation to take place: this very greatly facilitates the subsequent portion of the analysis, and no destruction of glycogen occurs even on prolonged standing, of which we satisfied ourselves by experiments.
Formation of Glycogen in Muscle

Before undertaking this work it was necessary to repeat some of the experiments which had been performed to determine the difference which might exist in the glycogen content of symmetrical muscles. This question has been inquired into a number of times since Cramer took the matter up at the suggestion of Külz. The following are some of the result which we obtained.

We give but one of several sets of results which all show approximately the same difference for symmetrical limbs.\(^1\)

<table>
<thead>
<tr>
<th>Right</th>
<th>Left</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fore limb</td>
<td>0.523</td>
</tr>
<tr>
<td>Hind limb</td>
<td>0.570</td>
</tr>
<tr>
<td>Pectoral</td>
<td>0.533</td>
</tr>
</tbody>
</table>

The difference in the content of glycogen of symmetrical muscles would appear from our results to be much less than that observed by other investigators. Recent observations on the glycogen content of the liver tend to show that the difference in concentration is also less than that heretofore believed to be the case.

The Perfusion of Muscle with Blood Containing Saccharose.

The following table (\(\text{II}\)) gives the results which we have obtained by the method above described.

As will be seen, the perfusion of a muscle with a solution of saccharose in no case led to an increase in the glycogen content of the side through which the sugar solution was perfused. A number of experiments with a single perfusion, after ligation of one side are not detailed. The loss of glycogen on the ligated side during the two hours of the experiment is sometimes quite large. This is not always the case, however, and we have sometimes observed as great an amount of glycogen in the non-perfused side as in the perfused. The reason for the difference in individual experiments we are at a loss to explain.

\(^1\) These analyses (for which we are indebted to Dr. B. J. Dryfuss) were made by a method similar to that described by Ivar Bang (Festschrift für Olof Hammarsten, 1906), in which a centrifuge is used to separate out the glycogen. This method will be found much more convenient than the usual procedure of filtration and washing. Duplicate analyses are very satisfactory.
**TABLE II.**

<table>
<thead>
<tr>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.16</td>
<td>0.17</td>
<td>−0.01</td>
<td>−5.8</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>II</td>
<td>0.42</td>
<td>0.50</td>
<td>−0.08</td>
<td>−19.0</td>
<td>normal</td>
<td>normal</td>
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<tr>
<td>III</td>
<td>0.35</td>
<td>0.40</td>
<td>−0.05</td>
<td>−14.3</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>IV</td>
<td>0.48</td>
<td>0.48</td>
<td>0.00</td>
<td>0.0</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>V</td>
<td>0.71</td>
<td>0.70</td>
<td>+0.01</td>
<td>+1.4</td>
<td>partial rigor</td>
<td>partial rigor</td>
</tr>
<tr>
<td>VI*</td>
<td>0.27</td>
<td>0.27</td>
<td>0.00</td>
<td>0.0</td>
<td>normal</td>
<td>normal</td>
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<tr>
<td>VII</td>
<td>0.06</td>
<td>0.06</td>
<td>+0.02</td>
<td>+50.0</td>
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<tr>
<td>VIII</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.0</td>
<td>rigor</td>
<td>rigor</td>
</tr>
<tr>
<td>IX</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.0</td>
<td>rigor</td>
<td>rigor</td>
</tr>
<tr>
<td>X</td>
<td>0.56</td>
<td>0.51</td>
<td>0.05</td>
<td>+9.8</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>XI</td>
<td>0.71</td>
<td>0.58</td>
<td>+0.13</td>
<td>+22.8</td>
<td>normal</td>
<td>normal</td>
</tr>
</tbody>
</table>

*In the case of Experiment VII the difference which is calculated to amount to 50.0 per cent increase is one which depends on a difference in weighing of a fraction of a milligram. We give the determination in this way in order that our results may appear in the same way as the others. As a matter of fact, the amounts of glycogen in the two limbs were identical.*
In order to test the conclusion that saccharose is not a direct glycogen former in muscle, several animals were made glycogen-free by starvation and the administration of sub-maximal doses of strychnin. Where an animal was succumbing to the poison, artificial respiration was resorted to. The animal was finally killed by pithing, and the limbs used for perfusion. In the case of saccharose no glycogen was formed (Experiments VII and IX). What is perhaps more remarkable is that in the case of glucose no glycogen was also formed (Experiment VIII).

This may be explained in one of two ways. Either the rigor induced interferes with the normal physiological processes, or the glycogen is consumed as fast as it is formed. In support of the latter view two experiments may be cited.

<table>
<thead>
<tr>
<th>Muscles</th>
<th>Condition</th>
<th>Glycogen</th>
<th>Difference Per Cent.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>normal</td>
<td>0.55</td>
<td>+24.4</td>
</tr>
<tr>
<td>Left</td>
<td>rigor</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>normal</td>
<td>0.28</td>
<td>+55.6</td>
</tr>
<tr>
<td>Left</td>
<td>rigor</td>
<td>0.18</td>
<td></td>
</tr>
</tbody>
</table>

In an experiment in which both limbs were in rigor we found a complete disappearance of the glycogen from both limbs. The production of heat and the formation of carbon dioxide during rigor also goes to show that glycogen is used up during the process.¹

On the other hand when glucose was added to the blood, there was a distinct formation of glycogen amounting in the one case to 9.8 per cent, in the second to 22.8 per cent.

**SUMMARY.**

Glycogen is not formed in the perfusion of muscle by blood containing saccharose. Muscles rendered free from glycogen by starvation and strychnin do not form glycogen either from glucose or from saccharose. Glucose does form glycogen in muscle. The content of glycogen in symmetrical muscles is practically alike.

THE FORMATION OF GLYCOGEN IN MUSCLE
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J. Biol. Chem. 1907, 3:25-34.

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