EFFECT OF INSULIN AND MUSCLE TISSUE ON GLUCOSE IN VITRO.

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INTRODUCTION.

Whereas no doubt exists as to the excellent therapeutic effect of insulin it is still an open question as to how insulin affects the carbohydrate metabolism. The decrease in the blood sugar which is the main immediate result of injection of insulin may be explained in different ways. Some investigators think that insulin increases the burning of sugar, others consider an increased glycogenesis the most probable explanation, and still others see the main effect of insulin in a diminution of the sugar production in the liver. Too few experiments are as yet at hand to decide this question. An important part of the problem of the effect of insulin on the sugar metabolism is to find out what is the immediate result of the effect of insulin on the sugar molecule and in what manner the effect is brought about.

Glucose shows the well known phenomenon of mutarotation. Immediately after pure glucose is dissolved in water the solution shows a specific rotatory power \([\alpha]^{20}_D > 105^\circ\). This value decreases gradually after from 6 to 24 hours at room temperature to 52.5°. If the solution is heated or if small amounts of alkali are added, this value is reached at once. This phenomenon is interpreted by the assumption that pure glucose in the dry state and immediately after it is dissolved is present in the \(\alpha\) form, the specific rotatory power of which is \(> 105^\circ\). In solution a part of the \(\alpha\)-glucose is transformed into a form with a lower specific rotatory power, \(\beta\)-glucose. This transformation goes on until an equilibrium is established between the \(\alpha\) and \(\beta\) forms of the glucose.
β-Glucose was first isolated by Tanret in 1895. He determined that its specific rotatory power was 22.5°. An aqueous solution of β-glucose shows increasing specific rotatory power until it reaches 52.5°, because it is partly transformed into α-glucose until an equilibrium between α- and β-glucose is established.

In later years the existence of still another form of glucose—a γ form—has been assumed. This γ form has not been isolated so far. Only substitution products of it, such as the methylglucoside, have been prepared. This hypothetical γ-glucose is thought to be chemically highly active, to be unstable, and to have a specific rotatory power of less than 22.5°.

In 1920 Hewitt and Pryde showed that glucose in aqueous solution, when in contact with living intestinal mucosa, became changed into a chemically more active form, which they assumed to be the γ form of glucose. They advanced the hypothesis that in normal blood an enzyme is present which transforms ordinary glucose (α, β-glucose) into the γ form, whereas it was lacking in the blood of diabetics.

Clark showed in 1917 that the optical rotation of fluid containing glucose was changed if the fluid was perfused through the pancreas. He therefore assumed that the pancreas contained an enzyme capable of converting ordinary glucose to another form which the body could metabolize.

In 1922 Winter and Smith made a comparative study of the reducing and rotatory power of the blood sugar. They found that in blood of animals and of healthy individuals the rotatory power of the sugar, if compared with the reducing power, was always lower than that of the ordinary α, β mixture and sometimes even lower than that of the β-glucose alone.

No such discrepancy was found in blood sugar prepared from diabetics. From these experiments they drew the conclusion that, in normal individuals, the blood sugar is in the form of γ-glucose, whereas in diabetics it is present as α, β-glucose. They furthermore assumed that the action of insulin is to convert α, β-glucose to γ-glucose, which form they considered the diabetic organism able to metabolize.

Eadie repeated Winter and Smith's experiments and obtained identical results. He, however, did not consider the experiments

1 Tanret named it γ-glucose.
sufficient proof for the presence of the \( \gamma \)-glucose in the blood of normal individuals.

Hewitt expresses doubt as to the reliability of the procedure used by Winter and Smith in order to obtain blood sugar in a state suitable for polarimetry. Winter and Smith, however, have since found that insulin affected pure solutions of \( \alpha, \beta \)-glucose, changing a certain fraction into \( \gamma \)-glucose. Slosse found that pure solutions of \( \alpha, \beta \)-glucose to which insulin was added showed a decrease in rotatory power compared with the reducing power. The solutions were kept at 37\(^\circ\) for 24 hours.

Recently Irvine (1923) in a review has discussed the chemical evidence for the presence of a \( \gamma \) form of glucose. He concludes that even if an unsubstituted \( \gamma \)-glucose has not been isolated, the experiments already at hand point to the possibility that such a form is produced in the body as an intermediary product during the glucose metabolism.

In this paper we shall report a series of experiments which we have made on the action of insulin on glucose under various conditions.

**EXPERIMENTAL.**

*Technique.—*In all our experiments we compared the rotatory and reducing power of solutions of glucose before and after the solutions had been subjected to the action of insulin under various conditions.

The rotatory power was determined in a Schmidt-Haensch polarimeter. The light was filtered through a saturated solution of potassium dichromate. The reading scale was divided into Ventzke's degrees. Each time, ten readings were made and the average figure was taken. It was found, in numerous controls, that the greatest variation in ten readings was 0.02\(^\circ\).

The reducing power was determined by the method of Hagedorn and Jensen (1923). Each solution of glucose was diluted so that the sugar content in each analysis was about 0.15 mg. of glucose. The determinations were made in quadruple and the average figure was taken.

Controls showed that, by this procedure, the sugar content could be determined without error. In a series of solutions of glucose of varying strengths, from 1.3 to 10 per cent, in which equilibrium
between α- and β-glucose was present, the concentrations of glucose were as calculated from the figures obtained by determining the rotatory and reducing powers respectively. Even in the weakest solutions the concentrations calculated in the two ways always showed differences smaller than 1 per cent of their value.

The insulin used was the Danish preparation of insulin, "Medicinalco" (also called "diasulin"). Ordinarily it contains traces of lactose. The insulin therefore had to be specially prepared. It contained no lactose and showed in solutions neither reducing nor rotatory power. All our experiments were performed at a temperature of 37°C.

Whereas the mixture of pure sugar solution and insulin was clear and suitable for polarimetry, this was not the case in our later experiments when blood or muscle tissue was added. It, therefore, soon became necessary to devise a special procedure in order to obtain a clear solution of glucose.

The method used by Winter and Smith is very elaborate. It takes 6 to 8 hours, and criticism has been brought forward (Hewitt) as to its reliability. We decided to use dialysis through collodion tubes, dried in 80 per cent alcohol. These tubes are impermeable to proteins but relatively easily permeable to sugar.

Our experiments with blood and muscle tissue differed somewhat with respect to technique. With blood we proceeded as follows: Into a collodion tube we introduced about 40 cc. of a 0.9 per cent solution of sodium chloride. The solution had a temperature of 37°C. To this, glucose in varying concentrations, insulin, and a little sodium citrate were added. Then from 4 to 20 cc. of blood from normal individuals were allowed to flow directly into the tube, which was placed in a suitable beaker containing about 40 cc. of a 0.9 per cent solution of sodium chloride. After varying lengths of time samples of the clear dialysate in the beaker were analyzed.

In our later work with muscle tissue the first part of the experiments was performed in bottles. Into a 300 cc. bottle containing 0.9 per cent sodium chloride solution, glucose, insulin, and muscle tissue were introduced. The bottle was rotated continuously in an incubator at 37°C. At various intervals samples of about 30 cc. were withdrawn, placed in collodion tubes, and dialyzed at room temperature. After 1/2 hours' dialysis, samples of the fluid in the beaker around the tube were analyzed. The muscle tissue used was from guinea pigs and mice. The animals were decapitated, and the muscles were immediately excised, cut into small pieces, and placed in the bottle with the solution. All our experiments were performed at 37°C.

2 For this we are indebted to cand. pharm. Gad-Andresen, the chief of the Insulin Laboratory of the Medicinalco Chemical Co. of Copenhagen.
RESULTS.

1. Effect of Insulin on Solutions of Glucose.

A considerable number of experiments were made. The concentration of glucose in the solution varied from 0.3 to 10 per cent. The amount of insulin added varied from 0.2 to 50 Toronto units per 10 cc. of the solution of glucose. The length of time for the experiments ranged from $\frac{1}{2}$ to 72 hours.

All the experiments showed similar results. The values of the concentration of glucose in the samples calculated from the reducing and rotatory power never deviated more than 1 per cent. There was, therefore, no evidence that insulin is able to change $\alpha, \beta$-glucose in pure solution into a form with lower specific rotatory power.

2. Effect of Insulin on Glucose When Blood Was Added.

A series of control experiments on solutions of sugar, salt, and blood alone was first made in order to determine whether or not the blood in itself contained substances which might influence the results. Such substances could be $\gamma$-glucose which, according to Winter and Smith, is present in normal blood, or they could be uric acid or creatinine which have a reducing but no rotatory power.

In none of a number of such control experiments did the values for the concentration of the glucose calculated on the basis of reducing and rotatory power deviate more than 1 per cent. Similar results were previously reported by Cooper and Walker.

In the experiments in which insulin was added the experimental conditions varied as follows: The glucose concentration ranged from 0.5 to 5 per cent. The amount of insulin added varied from 0.2 to 30 Toronto units per 10 cc. of the solution; the amount of blood, from 10 to 50 per cent of the solution to which it was added. The duration of the experiments ranged from 1 to 24 hours.

In none of the experiments did the concentration of sugar calculated on the basis of the reducing and rotatory power respectively vary more than 1 per cent. The results of Winter and Smith and of Slosse could therefore not be corroborated either for pure glucose solution or for glucose plus blood.
3. Effect of Insulin on Glucose When Muscle Tissue Was Added.

In a series of control experiments made on a mixture of glucose and muscle without insulin, no effect was observed. When insulin was added to a glucose-sodium chloride solution containing muscle tissue, a more or less marked change was found: in all the experiments the rotatory power was smaller than the reducing power when determinations were made on the fluid dialyzed through celloidin tubes at room temperature for 1½ hours.

Determinations of the content of glucose (by means of the reducing power) were made at the beginning, during, and at the end of the experiments. No change was found. It could therefore be excluded that the sugar was affected in any other way (for instance burned or transformed into glycogen) than changed to another optical form with the same reducing and a lower rotatory power. A further proof of this explanation was the fact that when such a solution showing a discrepancy between reducing and rotatory power was allowed to stand, it steadily increased in its rotatory power until this again, after a certain lapse of time, became equivalent to the reducing power. This difference between the reducing and the rotatory power of a given solution to which insulin and muscle had been added varied considerably in our experiments. We undertook, therefore, to investigate the quantitative effect of a number of factors which evidently were responsible for the variations in our results.

A. Influence of Time on the Transformation of \( \alpha, \beta \)-Glucose.

In Table I and in Fig. 1 an experiment is reported in which samples were analyzed after different periods of time. All other conditions were constant. From the results it is seen that the greatest difference between reducing and rotatory power is found after 2 hours. From 3 to 24 hours after the beginning of the experiment this difference again decreases. The reason for this is undoubtedly that the transformation lasts for only 2 hours. After that time the process reverts in the direction of establishing the previous normal equilibrium between the \( \alpha \)-glucose and the \( \beta \)-glucose.

In a series of similar experiments with other concentrations of glucose, identical results were obtained. The concentration of transformed glucose was in all experiments at its maximum after 2 or 3 hours. After that time the concentration became lower again.
TABLE I.

Influence of Time on the Transformation of α, β-Glucose.

<table>
<thead>
<tr>
<th>Time after the beginning of the experiment (hrs.)</th>
<th>Difference in glucose equivalents between reducing and rotatory powers of dialysate (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.9</td>
</tr>
<tr>
<td>2</td>
<td>18.5</td>
</tr>
<tr>
<td>3</td>
<td>18.2</td>
</tr>
<tr>
<td>4</td>
<td>17.9</td>
</tr>
<tr>
<td>6</td>
<td>17.1</td>
</tr>
<tr>
<td>24</td>
<td>13.7</td>
</tr>
</tbody>
</table>

Fig. 1. Influence of time on the transformation of α, β-glucose.

**B. Which Factors Determine the Cessation of the Process?**

Several possibilities suggest themselves as responsible for the fact that the transformation of the α, β-glucose comes to a standstill after about 2 hours. Our first thought that it was a change in the reaction of the solution was easily excluded. We then proceeded to ascertain whether the insulin or the active substance (or principle) in the muscular tissue was used up or destroyed in the course of the first 2 hours of the experiment.
TABLE II.
Influence of Repeated Additions of Insulin or Muscle on the Transformation of α, β-Glucose.

<table>
<thead>
<tr>
<th>Time after the beginning of the experiment</th>
<th>Difference in glucose equivalents between reducing and rotatory powers of dialysate</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>hrs.</td>
<td>per cent</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10.5</td>
<td>After the 3rd hr. 50 units of insulin were added.</td>
</tr>
<tr>
<td>5</td>
<td>9.5</td>
<td>After the 5th hr. 15 gm. of muscle were added.</td>
</tr>
<tr>
<td>7</td>
<td>18.5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>15.1</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 2.** Influence of repeated additions of insulin and muscle tissue on the transformation of α, β-glucose.

In Table II and Fig. 2 are given the results of an experiment undertaken to throw light on this question. 200 cc. of a 2 per cent solution of glucose in a 0.9 per cent solution of sodium chloride were introduced into the bottle. 15 gm. of muscle tissue and 50 units of insulin were added at the beginning of the experiment.
In samples withdrawn after 2 and 3 hours the difference between the reducing and rotatory power was found to be 9.9 and 10.5 per cent of the reducing value respectively. Then 50 more units of insulin were added. This, however, did not increase the transformation of glucose, because in a sample withdrawn after 5 hours the difference was only 9.5 per cent. 15 gm. of muscle were now added. 2 hours later a fourth sample was withdrawn and dialyzed. In this sample the difference had risen to 18.5 per cent. 1 hour later the difference had decreased to 17.8 per cent, showing that the effect of the muscle tissue had lasted for only 2 hours. 20 hours after the beginning of the experiment the difference had gone down to 15.1 per cent.

The experiment shows clearly that the cessation of the process of transformation of the $\alpha$, $\beta$-glucose after 2 hours is not caused by lack of insulin but by lack of muscle tissue. It shows, furthermore, that enough insulin was added at the beginning of the experiment and that the action of the insulin lasts more than 5 hours.

The fact that the process stopped after 2 hours may be explained either by assuming that the 15 gm. of muscle added can transform only a certain quantity of $\alpha$, $\beta$-glucose or by assuming that the active principle (or substance) in the muscles can persist for only 2 hours. In order to decide between these two possibilities the following experiment was made.

15 gm. of muscle tissue were kept in a 0.9 per cent solution of sodium chloride at a constant temperature of 37° for 2 hours, after which glucose and insulin were added. After 2 and 3 hours, samples were taken and dialyzed. No difference was found between the rotatory and reducing value of the dialysate.

This experiment shows that the muscle tissue loses its activity in 2 hours under these conditions. It is therefore necessary to use the muscles immediately after the killing of the animals and to prepare and hash the muscles as quickly as possible.

C. Influence of the Concentration of Glucose on Its Transformation.

The results of five experiments are given in Table III. The concentration of glucose varied from 0.92 to 5.40 per cent. All the other experimental conditions were kept constant. The amount of solution in the bottle was 200 cc. and contained 0.9 per cent sodium chloride. 15 gm. of

* All the differences are in percentage of the highest; i.e., the reducing value.
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Muscle tissue and 50 units of insulin were added in each experiment. The duration of the experiment proper was 2 hours (at 37°), and of the dialysis, 1½ hours (at room temperature).

The figures in Table III show that after 2 hours the difference between the reducing and rotatory power is much higher (18.5 per cent) in the experiments with the lowest concentration of glucose than in those with the highest concentration (4.2 per cent).

Two possibilities suggest themselves as a cause of the decrease in the transformation of α, β-glucose with increasing concentration of glucose. One is that the higher concentration in itself in some way prevents the transformation, another is that only a certain quantity of α, β-glucose can be transformed under the conditions given. In order to decide between these two possibilities the following experiment was made.

**TABLE III.**

*Influence of the Concentration of Glucose on Its Transformation.*

<table>
<thead>
<tr>
<th>Concentration of glucose in the bottle</th>
<th>Difference in glucose equivalents between reducing and rotatory powers of dialysate.</th>
</tr>
</thead>
<tbody>
<tr>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>0.92</td>
<td>18.5</td>
</tr>
<tr>
<td>1.40</td>
<td>13.2</td>
</tr>
<tr>
<td>1.66</td>
<td>11.3</td>
</tr>
<tr>
<td>2.00</td>
<td>9.9</td>
</tr>
<tr>
<td>5.40</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Instead of using 200 cc. of a 2 per cent solution of glucose as in Experiment 4 in Table III, only 100 cc. were used. All the other conditions were the same in the two experiments. After 2 hours the rotatory power of the solution had decreased 19 per cent which is almost double the decrease found in the rotatory power in Experiment 4, Table III, where the concentration of glucose was the same (2 per cent), although the total amount of sugar was twice as large. It seems, therefore, justifiable to state that—within the conditions used in our experiments—the concentration of glucose is of no importance for the transformation and that only a certain quantity of glucose can be transformed.

**D. Influence of Amount of Muscle Tissue Added on the Transformation of Glucose.**

In Table IV and Fig. 3 there is reported a series of experiments on the relation between the transformation of glucose and the quantity of muscle tissue used. Apart from the variations in the amount of muscle tissue added, all the experimental conditions were kept constant. In the bottle at
the beginning there were 200 cc. of a solution containing 0.9 per cent sodium chloride and 2 per cent glucose. 50 units of insulin were added. The duration of all the experiments was 2 hours at 37° for the experiment proper and 1½ hours at room temperature for the dialysis.

**TABLE IV.**

*Influence of Amount of Muscle Tissue Added on the Transformation of Glucose.*

<table>
<thead>
<tr>
<th>Muscle tissue added (gms.)</th>
<th>Difference in glucose equivalents between reducing and rotatory powers of dialysate (per cent)</th>
<th>Difference in glucose equivalents between reducing and rotatory powers of dialysate per 5 gms. of muscle tissue added (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4.1</td>
<td>4.1</td>
</tr>
<tr>
<td>10</td>
<td>7.2</td>
<td>3.6</td>
</tr>
<tr>
<td>15</td>
<td>9.9</td>
<td>3.3</td>
</tr>
<tr>
<td>20</td>
<td>11.7</td>
<td>2.93</td>
</tr>
<tr>
<td>50</td>
<td>16.9</td>
<td>1.69</td>
</tr>
</tbody>
</table>

**FIG. 3.** Influence of amount of muscle tissue added on the transformation of glucose.

The results show that the amount of glucose transformed increases with increasing quantity of muscle tissue added. This increase is, however, not strictly proportional to the increase in the amount of muscle tissue, since relatively more glucose is trans-
formed in the experiments with the smallest amount of muscle tissue, as shown in Fig. 3.

**TABLE V.**

Influence of Amount of Insulin Added on the Transformation of $\alpha, \beta$-Glucose.

<table>
<thead>
<tr>
<th>Amount of insulin added (units)</th>
<th>Difference in glucose equivalents between reducing and rotatory powers of dialysate (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>9.9</td>
</tr>
<tr>
<td>20</td>
<td>9.9</td>
</tr>
<tr>
<td>10</td>
<td>10.5</td>
</tr>
<tr>
<td>5</td>
<td>9.3</td>
</tr>
<tr>
<td>2</td>
<td>4.9</td>
</tr>
</tbody>
</table>

![Graph](image)

**Fig. 4.** Influence of amount of insulin added on the transformation of $\alpha, \beta$-glucose.

**E. Influence of Amount of Insulin Added on the Transformation of $\alpha, \beta$-Glucose.**

In a series of experiments given in Table V and Fig. 4 the amount of insulin added varied from 50 to 2 units. The other experimental conditions were kept constant and were as follows: 200 cc. of 0.9 per cent solution of
sodium chloride with 2 per cent glucose. 15 gm. of muscle tissue were used in each experiment. The duration was 2 hours at 37° for the experiment proper and 1½ hours at room temperature for the dialysis.

The results show that 10 or possibly 5 units of insulin suffice to produce a maximum effect under the experimental conditions used. This suggests that the first part of the curve in Fig. 4 could be used as a means of standardizing insulin. Experiments are in progress to study this point.

F. Influence of Temperature on the Process.

An experiment was performed at 20°. Apart from the temperature, the experimental conditions were as usual. After 2 hours no decrease was observed in the rotatory power of the solution. Then the temperature was raised to 37° and 15 gm. of muscle tissue were again added. After 2 hours the usual decrease in the rotatory power was found, whereas the reducing power remained constant as usual.

TABLE VI.

Experiments to Show How Far the Transformation of Glucose Can Be Pushed by Repeated Additions of Muscle Tissue and Insulin.

<table>
<thead>
<tr>
<th>Time after the beginning of the experiment</th>
<th>Difference in glucose equivalents between reducing and rotatory powers of dialysate.</th>
<th>Remarks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>hrs.</td>
<td>per cent</td>
<td>Every other hr. 15 gm. of muscle and 10 units of insulin were added.</td>
</tr>
<tr>
<td>2</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>24.2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>34.2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>43.8</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>43.6</td>
<td></td>
</tr>
</tbody>
</table>

G. How Far Can the Transformation of Glucose Be Pushed?

This problem is of considerable theoretical as well as practical importance. In Table VI an experiment is reported in which it was possible by repeated addition of muscle tissue and insulin to increase the transformation considerably. The experimental conditions were the following.

When the experiment was started, 2.8 gm. of glucose were added to 200 cc. of a solution of 0.9 per cent sodium chloride. The concentration of glucose was, therefore, at the beginning 1.4 per cent. Then 10 units of insulin and 15 gm. of muscle tissue were added. The bottle was rotated
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continuously at 37°. After 2 hours a sample of 20 cc. was withdrawn for dialysis. 10 units of insulin and 15 gm. of muscle were again added. After the 4th hour, the second sample was withdrawn, etc. In this way five samples were withdrawn in 10 hours.

The samples were subjected to dialysis for 1 1/2 hours at room temperature. Then the difference between the reducing and rotatory power was determined. The figures in Table VI show that more and more glucose is transformed during the experiment. After the 8th hour the difference between the reducing and rotatory power is 43.8 per cent (of the reducing value) which corresponds to a specific rotatory power of 29.5°. After 10 hours the same value was found as after the 8th hour, and the experiment was stopped.

Whether the transformation is a formation of γ-glucose or simply a change in the equilibrium between the α and the β form at the cost of the concentration of the α form we are—as previously mentioned—as yet unable to say. However, in either case it appears that not all the glucose is transformed because the specific rotatory power of the solution does not decrease below 22.5°. This is most likely due to the reversibility of the process, which goes back during the dialysis.

H. Influence of Gastric Juice on the Speed of the Reverse Process.

In one experiment normal gastric juice was added to a solution containing a certain amount of transformed glucose. It was found that this did not increase the rate of the reverse process.

DISCUSSION.

The results obtained in these experiments seem to be of importance for our understanding of the carbohydrate metabolism. They lend support to the theory that the first step in the carbohydrate metabolism is a transformation of the ordinary α, β-glucose (equilibrium glucose, Tanret's β-glucose) into a form with a lower specific rotatory power but with the same reducing power. This transformation is produced by means of insulin and a principle or substance present in living muscle tissue. Insulin alone or this substance alone cannot produce this effect. Whether or not other tissues possess the same property we do not yet know.
On first consideration our results might seem to support the theory of Winter and Smith regarding the existence of γ-glucose in the blood of normal individuals. However, in none of our experiments did the specific rotatory power of the sugar solutions decrease below 22.5°.

Our results may therefore be explained either by assuming a formation of γ-glucose or simply by a change in the equilibrium between the α- and β-glucose in such a way that the concentration of β-glucose is increased at the cost of the α form. According to either view the decrease in the rotatory power must go parallel with the amount of glucose transformed. The observed difference between the observed reduction power and rotatory power must, therefore, be a quantitative expression of the amount of glucose transformed by the combined action of insulin + muscle tissue under the conditions given.

**SUMMARY.**

1. A procedure is described for obtaining a clear sugar-containing fluid from mixtures of glucose with insulin, blood, or muscle tissue. The method consists in dialyzing through collodion tubes, dried in 80 per cent alcohol.

2. When insulin was added to solutions with or without blood from normal individuals, no change took place in the rotatory power of the dialysate. This suggests that if the blood sugar in normal individuals is changed from the ordinary form (α, β-glucose) to a form with less optical rotatory power, the change probably takes place extravascularly.

3. When insulin and muscle tissue were added to solutions of ordinary glucose (α, β-glucose, equilibrium glucose, Tanret's β-glucose), a decrease in the optical rotatory power of the dialysate took place without any change in the reducing power. Insulin alone, or muscle tissue alone, could not produce this change.

According to our results this change may be due either to a change in the relative amount of α- and β-glucose in the mixture with formation of β-glucose at the cost of α-glucose, or it may be due to formation of a variety of glucose with a specific rotatory power lower than that of the β-glucose (perhaps γ-glucose). This transformation cannot be produced by insulin alone or by muscle tissue alone.
4. The influence on this process of a number of factors (concentration of glucose, amount of insulin, amount of muscle tissue, temperature) was studied quantitatively.

5. It was found that muscle tissue was active for only 2 hours. Muscles kept for 2 hours at 37° in 0.9 per cent solution of sodium chloride were inactive. The active principle or substance in the muscles is, therefore, presumably connected with vital processes in the cells.

6. Under the conditions of these experiments the process by which glucose is transformed into a form with lower rotatory power is reversible. The reverse process is, however, relatively slow. For this reason absorption and utilization of transformed glucose from the intestines may be possible. This question is being studied further.

7. Addition of normal gastric juice to a dialysate containing transformed glucose did not increase the speed of the reverse process.

8. In none of our experiments did we find any evidence that insulin (alone or with blood or muscle tissue) is able either to burn sugar or to transform it into glycogen.

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