Glucose metabolism and fructose synthesis in the diabetic rat lens

John F. R. Kuck, Jr.

A comparison of the uptake of glucose in vitro by lenses from normal and alloxan-diabetic rats involves the effect of secondary factors which may obscure any effect of diabetes itself. These factors have been investigated and their influence evaluated. A procedure has been developed for the incubation of lens dispersions which yields values for glucose uptake and lactate production within the normal range for intact lenses in Tyrode's solution. The results of this investigation show that the rate of uptake of glucose by dispersions of diabetic lenses is about 80 per cent of normal. Furthermore, it is shown that the amount of fructose produced by dispersions of diabetic lens is equivalent to 20 per cent of the glucose taken up, a fraction five times that for normal lens dispersions. Making the likely assumption that the depletion of the sorbitol pool in diabetic lenses is no greater than it is in normal lenses, the diabetic lenses would actually have available for energy production only about 80 per cent of the glucose taken up. On the basis of this reasoning the diabetic lenses obtain energy from the metabolism of glucose at a rate only 60 per cent of normal. It is possible that diabetic cataracts may in part be the result of decreased production of energy. The conversion of sorbitol to fructose leads to a diversion of DPN which quantitatively is equivalent to the 20 per cent depression of glucose uptake found in diabetic lenses. It is suggested that this depression of glucose uptake may be a result of the diversion of DPN from anaerobic glycolysis.

That the formation of diabetic cataract is due to a failure of the energy-producing mechanism of the lens is an attractive hypothesis, but the exact location or nature of this supposed metabolic lesion is still a matter of conjecture. Macintyre, Polt, and Patterson reported that lenses from alloxan-diabetic rats exhibit an uptake of glucose in vitro which is 45 per cent of that for normal lenses under the same conditions; a later paper by Farkas, Ivory, and Patterson gave a value of 25 per cent. Such a defect in glucose utilization is of great interest in the study of diabetic cataract because the lens derives most of its energy from the utilization of glucose and because in diabetes the general utilization of glucose is impaired.

Macintyre and co-workers incubated a lens in a small volume of Tyrode's solution and measured the glucose concentration of the medium after incubation. The amount of glucose lost was converted to the glucose uptake expressed as milligrams of glucose used per gram of lens per hour. In a later paper the procedure was modified to account for the appreciable glucose content of diabetic lenses. In the present investigation it soon became apparent that such studies must be interpreted in the light of four significant facts: (1) the lenses from

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severely diabetic rats contain so much glucose that no extracapsular supply is needed for a one hour incubation, (2) the sugar in diabetic lenses may consist of from 30 to 50 per cent fructose, \textsuperscript{a} which is derived from glucose via the sorbitol pathway\textsuperscript{a, c} and is not further metabolized, (3) diabetic lenses must be well cleaned of clinging aqueous and vitreous humors to avoid introducing an appreciable but indeterminate amount of sugar, and (4) lenses with slight capsular injuries deteriorate badly during incubation in Tyrode’s solution with a consequent depression in glucose uptake; the level of injury at which this depression occurs is easily produced by the careful cleaning necessary to remove extraneous sugar from around diabetic lenses.

In view of these facts the experiments were repeated with such variations in the experimental conditions that the difficulties outlined above could be overcome or minimized.

In addition, the same type of incubation was carried out with lens dispersions\textsuperscript{b} made by thoroughly crushing single lenses in the incubation medium.

**Methods**

The methods have been described previously.\textsuperscript{c} In diabetic rats one lens was always taken as a control to determine on the protein-free extract of the lens the initial contents of glucose, fructose, and lactate. Tyrode’s solution was used as such and as a modified medium made by replacing 12.5 per cent of the sodium chloride (NaCl) by a mole equivalent amount of Tris-HCl solution of pH 7.4. A modified Krebs-Henseleit solution was made by replacing half the NaCl by Tris-HCl pH 7.4 and adding a mixture of trace elements.

In all experiments with intact lenses the medium after incubation was treated with zinc sulfate solution before crushing or removing the lens. The development of more than a trace of turbidity at this point was taken as an indication of a lens injury serious enough to allow protein to leak through the capsule and also enough to depress the glucose uptake. On this objective basis injured lenses were excluded from consideration.

After the determination of turbidity, barium hydroxide was added and the preparation of the protein-free filtrate completed. All determinations for glucose, fructose, and lactate were made on this filtrate. If the filtrate consisted of the medium plus the lens, the lactate determination was corrected for the initial lactate content of the lens (determined on the control diabetic lens or estimated for normal lenses).

In one experiment the sugar measurements were made on protein-free extracts of the whole eye. The control eye was separated into the clean lens and the remainder of the eye. The incubated lens included an amount of clinging humors, the remainder was heated with the zinc sulfate at once. Thus, in both cases the sugar content of the eye was determined, but only in the case of the control eye was the distribution of this sugar between the lens and the remainder known pre-

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Glucose uptake (mg./gram-hour)</th>
<th>Blood sugar (mg./100 mL)</th>
<th>No. of rats</th>
<th>Glucose uptake (mg./gram-hour)</th>
<th>Blood sugar (mg./100 mL)</th>
<th>Lens fructose (mg./100 Gm.)</th>
<th>No. of rats</th>
<th>p Value for glucose uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6 ± 0.3 (S.D.)</td>
<td>109 ± 4</td>
<td>7</td>
<td>1.5 ± 0.2</td>
<td>421 ± 120</td>
<td>250 ± 38</td>
<td>9</td>
<td>0.39</td>
</tr>
<tr>
<td>2</td>
<td>1.3 ± 0.2</td>
<td>131 ± 18</td>
<td>9</td>
<td>1.0 ± 0.1</td>
<td>374 ± 200</td>
<td>178 ± 54</td>
<td>8</td>
<td>0.13</td>
</tr>
<tr>
<td>3</td>
<td>1.1 ± 0.1</td>
<td>108 ± 9</td>
<td>5</td>
<td>0.9 ± 0.2</td>
<td>380 ± 130</td>
<td>196 ± 16</td>
<td>8</td>
<td>0.27</td>
</tr>
<tr>
<td>4</td>
<td>1.0 ± 0.2</td>
<td>80 ± 5</td>
<td>8</td>
<td>0.8 ± 0.1</td>
<td>411 ± 120</td>
<td>130 ± 36</td>
<td>5</td>
<td>0.22</td>
</tr>
<tr>
<td>5</td>
<td>1.5 ± 0.2</td>
<td>136 ± 11</td>
<td>4</td>
<td>1.3 ± 0.2</td>
<td>214 ± 53</td>
<td>44 ± 26</td>
<td>4</td>
<td>0.26</td>
</tr>
<tr>
<td>6</td>
<td>1.2 ± 0.2</td>
<td>103 ± 14</td>
<td>14</td>
<td>1.1 ± 0.1</td>
<td>319 ± 54</td>
<td>147 ± 29</td>
<td>5</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*In all experiments the lenses were incubated for 2 hours in a medium with an initial glucose concentration of 100 mg./100 mL.

1 Tyrode’s, initial pH 7.6; average lens weight, 23 mg.
2 Tyrode’s, pH not measured; average lens weight, 23 mg.
3 Tyrode’s, initial pH 7.6; average lens weight, 28 mg.
4 Krebs-Henseleit-Tris pH 7.4; average lens weight, 27 mg.
5 Whole eye experiment; Tris-Tyrode’s, pH 7.4; average lens weight, 27 mg.
6 Tris-Tyrode’s, pH 7.4; average lens weight, 28 mg.
Table II. Lactate production rates in normal and diabetic rat lenses

<table>
<thead>
<tr>
<th></th>
<th>Normal (14)</th>
<th>Diabetic (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate production rate, mg./gram-hour</td>
<td>1.05 ± 0.14*</td>
<td>1.02 ± 0.14*</td>
</tr>
<tr>
<td>Glucose uptake rate, mg./gram-hour</td>
<td>1.2 ± 0.2</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Blood sugar level, mg./100 ml.</td>
<td>103 ± 14</td>
<td>319 ± 54</td>
</tr>
<tr>
<td>Lens glucose level, mg./100 Gm.</td>
<td>4.0 ± 2.4</td>
<td>100 ± 32</td>
</tr>
<tr>
<td>Lens lactate level, mg./100 Gm.</td>
<td>100 ± 20</td>
<td>120 ± 20</td>
</tr>
</tbody>
</table>

*p, 0.37.

cisely. The purpose of this procedure was to prepare a lens for incubation without blotting it and without adding an unknown amount of sugar in the diabetic humors clinging to it.

The L.M.S.-Tris (Lens Mineral Solution-Tris) used for preparing lens dispersions has a pH of 7.4 and the following composition: K 0.014M, Na 0.0085M, Tris 0.050M, Ca 0.00040M, Mg 0.00062M, PO_4 0.031M, Cl 0.0021M, SO_4 0.00075M, CO_3 0.01M, and is the calculated osmotic equivalent of 0.074M NaCl. Its unusual characteristics as a medium are the high K/Na ratio, the relatively elevated level of phosphate, and the high buffer capacity due to the large amount of Tris present. The lens dispersions prepared with this medium used glucose and produced lactate at about the normal rates for intact lenses in Tyrode's solution, whereas dispersions prepared with Tyrode's solution exhibited a nearly complete failure of glycolysis.

Results

The rates of glucose uptake for normal and diabetic lenses are compared in Table I with blood sugar values and lens fructose levels. The variations in experimental conditions are noted. Diabetic lenses appear to have slightly lower glucose uptakes than normal but the difference is not significant.

Table II gives the lactate production rates for one set of experiments (No. 6 of Table I). There is no significant difference between the two groups of lenses.

The results from a series of incubations with lens dispersions are shown in Table III. Excluded from consideration were all diabetic lenses (1) having a fructose level below 100 mg. per 100 Gm. or (2) coming from rats with a blood sugar level below 300 mg. per 100 ml., or (3) showing visible opacities. The results for glucose uptake are much like those of Table I, but the difference here is highly significant. The standard error is 0.039 compared with the actual difference between the means of 0.39. The 22 per cent depression in glucose uptake confirms the value of 24 per cent found by Farkas and co-workers for intact lenses in Tyrode's solution.

Table IV gives the lactate production rate for the experiments of Table III. The 20 per cent depression found in diabetic lenses is close to the 22 per cent depression found for the rate of glucose uptake in the same experiments but the difference for lactate is not significant.

Also shown in Table IV are the fructose production rates and initial glucose levels of the dispersions. There is a fivefold increase in the rate of fructose production in diabetic lens dispersions compared with normal lens dispersions. This difference is to be compared with the fifteenfold difference in the fructose concentration between normal and diabetic lenses (Table III).

Discussion

Although diabetic lens dispersions take up about 80 per cent as much glucose as normal lens dispersions (Table III), the experiments with intact lenses (Tables I and II) only suggest that measurable differences exist; the differences are not significant.

Since the high concentration of glucose present indicates that there is no impairment of glucose transport through the capsule of a diabetic lens, the use of lens dispersions is not subject to the objection that such a preparation removes any effect of the capsule on penetration of glucose. Furthermore, reduction of the glucose concentration in the medium permitted a more accurate determination of the amount used. The glucose concentration of the fluid used for diabetic lenses was adjusted downward to make the concentration of glucose in both normal and diabetic lens dispersions approximately equal. With these modifications it became possible to compare...
diabetic and normal lenses under conditions in which all lenses were equally damaged (i.e., dispersed) in a medium containing glucose at a low enough level that changes could be accurately determined.

Although the difference in lactate production rates shown in Table IV is not significant, the difference is in the right direction to support the data on glucose uptake.

The rate of fructose production is markedly greater in dispersions of lenses from diabetic animals than in those from normal animals (Table IV). This difference seems to be an inherent characteristic of the diabetic lens since it occurs at the same initial glucose level as in normal lens dispersions, and is greater than would be expected from a consideration of the steady-state levels of fructose and glucose in vivo in which the average fructose level in the diabetic lens is at most only twice that of glucose. However, in vivo the fructose level would be lowered by continual loss to the aqueous humor. It must be remembered that the diabetic lens contains a rich supply of sorbitol, the immediate precursor of fructose. This factor may be the inherent characteristic of diabetic lenses leading to a high rate of fructose production since the concentration of sorbitol is highly important in regulating the rate of fructose production. It is also possible that other factors, such as the DPN concentration, may be involved in the regulation of fructose production.

Since we have found that, in the rat lens at least, fructose is consumed only in the absence of glucose, the fructose formed via the sorbitol pathway must be derived directly from sorbitol. Insofar as the amount of sorbitol in the system remains constant, the difference in the amount of fructose present before and after incubation represents glucose taken up but unavailable for production of energy. Thus, to estimate the relative utilization of glucose in normal and diabetic lenses which gives rise to potentially useful energy, the measured glucose uptake of 1.01 mg. per gram-hour for the diabetic lens should be corrected by subtracting from it the fruc-

Table III. Lens dispersion studies: Glucose uptake rate in normal and diabetic rat lenses

<table>
<thead>
<tr>
<th>Rats</th>
<th>Normal uptake</th>
<th>Blood sugar</th>
<th>Lens fructose</th>
<th>No. of rats</th>
<th>Diabetic uptake</th>
<th>Blood sugar</th>
<th>Lens fructose</th>
<th>No. of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose (mg./gram-hour)</td>
<td>mg./100 ml.</td>
<td>(mg./100 Gm.)</td>
<td>No. of rats</td>
<td>Glucose (mg./gram-hour)</td>
<td>mg./100 ml.</td>
<td>(mg./100 Gm.)</td>
<td>No. of rats</td>
</tr>
<tr>
<td>1</td>
<td>1.11 ± .07</td>
<td>114 ± 14</td>
<td>10 ± 2</td>
<td>7</td>
<td>1.05 ± .10</td>
<td>477 ± 104</td>
<td>144 ± 34</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1.33 ± .10</td>
<td>111 ± 6</td>
<td>9 ± 4</td>
<td>9</td>
<td>1.09 ± .12</td>
<td>403 ± 29</td>
<td>194 ± 19</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>1.33 ± .12</td>
<td>110 ± 6</td>
<td>13 ± 2</td>
<td>6</td>
<td>1.11 ± .06</td>
<td>380 ± 35</td>
<td>150 ± 11</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>1.43 ± .13</td>
<td>107 ± 3</td>
<td>14 ± 3</td>
<td>3</td>
<td>0.90 ± .04</td>
<td>519 ± 103</td>
<td>180 ± 12</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>1.38 ± .07</td>
<td>107 ± 10</td>
<td>11 ± 3</td>
<td>5</td>
<td>1.05 ± .20</td>
<td>439 ± 100</td>
<td>169 ± 15</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>1.39 ± .19</td>
<td>70 ± 21</td>
<td>3 ± 3</td>
<td>8</td>
<td>0.94 ± .26</td>
<td>470 ± 170</td>
<td>171 ± 41</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>1.25 ± .10</td>
<td>92 ± 14</td>
<td>12 ± 4</td>
<td>7</td>
<td>0.78 ± .05</td>
<td>387 ± 50</td>
<td>105 ± 40</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1.30 ± .14</td>
<td>101 ± 22</td>
<td>10 ± 4</td>
<td>47</td>
<td>1.01 ± .15</td>
<td>439 ± 93</td>
<td>156 ± 27</td>
<td>Total 22</td>
</tr>
</tbody>
</table>

*p < 0.0001.

Table IV. Lens dispersion studies: Lactate and fructose production rates in normal and diabetic rat lenses

<table>
<thead>
<tr>
<th>Rats</th>
<th>Normal (45)</th>
<th>Diabetic (22)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate production rate, mg./gram-hour</td>
<td>0.95 ± 0.35</td>
<td>0.76 ± 0.31</td>
<td>0.25</td>
</tr>
<tr>
<td>Fructose production rate, mg./gram-hour</td>
<td>0.05 ± 0.02</td>
<td>0.25 ± 0.06</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Blood glucose level, mg./100 ml.</td>
<td>101 ± 22</td>
<td>439 ± 93</td>
<td></td>
</tr>
<tr>
<td>Initial glucose in dispersion, mg./100 ml.</td>
<td>53</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Glucose uptake rate, mg./gram-hour</td>
<td>1.30 ± 0.14</td>
<td>1.01 ± 0.15</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
ose production: $1.01 - 0.25 = 0.76$. This value can then be compared with that for normal lens dispersions similarly corrected: $1.30 - 0.05 = 1.25$. The effective depression of glucose utilization as far as production of energy is concerned in diabetic lens dispersions is then 39 per cent.

A possible reason for the depression (22 per cent) in the amount of glucose uptake uncorrected for the conversion to fructose may lie in the influence of the sorbitol pathway\(^5\) in using DPN needed for the oxidation of glyceraldehyde phosphate, a step in anaerobic glycolysis. In normal lenses the rate of diversion is the equivalent of 0.05 mg. per gram-hour of fructose, but in the diabetic lenses it occurs at about five times this rate, and corresponds fairly well with the 22 per cent drop in glucose uptake ($0.22 \times 1.30 = 0.29$ mg. per gram-hour). Whether this increased rate of diversion of DPN is deleterious to the lens cannot be said. Sippe\(^6\) has implied that changes in the ratio of pyridine nucleotides rather than lack of high-energy phosphate may be a factor leading to lens opacity in diabetes. We have been able to show that glucose uptake and lactate production are depressed by the addition of DPNH, a treatment which is equivalent to diverting DPN. This effect is produced only by relatively large amounts of DPNH; the opposite effect is not obtained by the addition of DPN, perhaps because the endogenous rate of production of DPN is already high.

In conclusion, it was shown that the measured (or apparent) uptake of glucose is depressed by 22 per cent in dispersions of diabetic rat lenses and that as much as 20 per cent of the glucose taken up may be converted to fructose, thus becoming unavailable for energy production. It is possible that a defect of this magnitude (40 per cent) may be an important factor in the production of diabetic cataract.

I wish to thank Dr. V. Everett Kinsey for suggesting this problem and for his continuing interest and encouragement during the investigation. I also wish to thank Donna Resch, Trudy Gregory, and Dianne Kokowicz for their technical assistance.

REFERENCES

7. Unpublished work.

Discussion

**Dr. Zacharias Dische, New York, N. Y.** Dr. Kuck's report contains several observations of great interest, and, like every good investigation, raises several intriguing questions. The first point of interest is the marked effect of injury on the glucose uptake of the whole lens in vitro. The effective factor in this injury apparently is the leak of protein from the lens. Sometime ago we observed in our laboratory that the breakdown of glucose in beef lenses in cortical as well as nuclear parts shows very great variation between individual lenses and it seems possible that these observations which apparently involve the glycolytic rather than oxidative metabolism have been due to injury under insufficiently controlled conditions. This effect of injury on the metabolism of the lens seems surprising because injury to animal tissues usually causes an increase in the breakdown of sugar by decreasing the Pasteur effect, among other reasons. The second important observation is the fact that homogenates of normal lenses show as high a glucose uptake as the intact lens itself. This, at first sight, appears puzzling since obviously the injury to the lens by homogenization is much more pronounced than...
the injury due simply to handling the intact lens although the latter tends to depress the glucose intake. This raises the question whether the type of metabolic reactions observed in homogenates is the same as that in intact lenses. We should remember that in various organs, particularly in the heart muscle, injury was shown not to depress but to increase the oxygen uptake. But the utilization of this uptake for functional purposes is always much lower in the damaged tissue than in the normal tissue. One of the facts responsible for this lowering of the efficiency of the metabolic processes may be a defect in the coupling of the oxidative processes and the synthesis of ATP. It is well known that the association of such a coupling may lead to an increased oxygen consumption if the availability of ADP is the limiting factor determining the over-all rate of oxidative processes. It therefore appears necessary to consider how far such factors, such as uncoupling of phosphorylation, may play a part in the high glucose uptake in homogenates. The third important observation, namely, the decrease in glucose uptake in diabetic lenses which is significant only after homogenization, leads to the question of whether the secondary oxidative processes which appear after homogenization are not defective in diabetic lenses. Finally, I should like to ask whether the very high fructose level found in dispersions in these experiments and the agreement between the 22 per cent depression of this concentration, a condition which may balance the effect of substances which do not readily penetrate the capsule. The possibility that the measured defect in glucose uptake of diabetic lens dispersions may be an artifact of the preparation is not to be considered lightly, yet the close agreement between the 22 per cent depression found in dispersions in these experiments and the 24 per cent depression found by Farkas, Ivory, and Patterson for intact lenses in Tyrode's solution suggests that the defect is a real one and that it involves chiefly glycolysis. Whether the defect involves oxidative processes at all will, I hope, be determined by Dr. Sippel for intact lenses. At present his elegant method for measuring oxygen uptake cannot readily be used for lens dispersions. We have no evidence that fructose levels in lens dispersions have any effect on glucose uptake. Involving chiefly glycolysis. We have shown conclusively that sorbitol at levels up to 250 mg. per 100 ml. has little effect on glucose uptake of lens dispersions although there is a significant accumulation of fructose at this concentration. Finally, it should be pointed out that the fructose level in the dispersion, because of the dilution by the medium, is much lower than in the intact diabetic lens. The amount produced in a 2 hour incubation is only about one third of the original content, and the final fructose concentration in the dispersion is rare above 40 mg. per 100 ml.