Sorbitol pathway metabolites in the diabetic rabbit lens

John F. R. Kuck, Jr.

The lenses of diabetic rabbits with an average plasma glucose of 502 ± 119 mg per 100 ml. have 141 ± 44 mg per 100 Gm. glucose, 81 ± 37 sorbitol, and 94 ± 12 fructose. For a comparable level of lens glucose, the values for sorbitol and fructose in the diabetic rabbit lens are, respectively, one tenth and one half of the values found in the diabetic rat lens. This difference appears to depend primarily on relative deficiencies of TPNH and DPN in the rabbit lens, such deficiencies being enhanced by diabetes. Incubations of lens homogenates indicate a sluggish sorbitol pathway in the rabbit but such homogenates may not furnish the best evidence. Rabbit lenses develop peripheral vacuoles at much lower sorbitol levels than rat lenses, suggesting that in the diabetic rabbit lens a metabolic disturbance may be more important than osmotic effects.

It has been known for some time that the lens of the diabetic rabbit contains much fructose, but such lenses have not been studied as intensively as the rat lens. Preliminary experiments suggested that the effect of diabetes on the rabbit lens might be different from the effect on the rat lens. It is the purpose of this paper to report a study of lenses from normal and diabetic rabbits, to compare the results with those for the rat, and to explore possible explanations for any difference.

Methods

Rabbits (New Zealand White) weighing about 2 kilograms were rendered diabetic by the procedure of Reddy and Kinsey. After one week, the plasma glucose levels were measured and animals with values below 300 mg per 100 ml were excluded from this study. None of the diabetic lenses had central opacities but most of them showed peripheral opacities in the form of fine striations running perpendicular to the equator and extending as much as 2 to 3 mm. over the anterior face. These were observed grossly after lens extraction and appeared very similar to the opacities seen in diabetic and galactose-fed rats at an early stage preceding actual appearance of the nuclear cataract. The diabetic rats were similar to those reported previously; the duration of diabetes was only 2 weeks but the blood sugar levels were very high at the time of sacrifice. The lenses of both diabetic and normal animals were rinsed in saline a few seconds and dry blotted to remove traces of sugars in the aqueous and vitreous humors originally adhering to the lens. Then they were converted to Somogyi protein-free extracts which were analyzed for glucose (glucose oxidase), fructose (Roe’s resorcinol method), and sorbitol (Faulkner’s method) by modified procedures given in more detail in previous papers.

The incubation experiments were run using cell-free extracts centrifuged in the cold. Lenses were homogenized in a tube with a Teflon pestle in ten times their weight of medium having a composition based on the Lens Mineral Solution-Tris-PO,
used previously for rat lenses. The glucose concentration range was 0.0002 to 0.003M; TPNH, 3.75 to 7.2 × 10^{-5}M; DPN, 5 × 10^{-5}M; LiSO_{4}, 0.4M; (NH_{4})_{2}SO_{4}, 0.4M; pH, 6.8 to 8.5. Various combinations of these reagents and conditions were employed. Analyses were run on Somogyi extracts of the incubation media.

Results

The lens glucose level is directly proportional to plasma glucose as shown in Fig. 1. The normal pool value is represented by the large cross at a plasma glucose arbitrarily taken as 100 mg. per 100 ml. It should be noted that in the range of 300 to 400 mg. per 100 ml. plasma glucose the corresponding lens glucose levels were sometimes highly erratic. This was perhaps due to rapidly fluctuating plasma glucose levels characteristic of an unstable low-grade diabetes.

The lens fructose levels (open circles in Fig. 2) were much less erratic. They rose rapidly from a normal level of about 30 mg. per 100 Gm. lens to values approaching 100 mg. per 100 Gm. lens in the weakly diabetic rabbits and did not rise appreciably thereafter, even in severe diabetes. This leveling off of the lens fructose concentration is quite like the situation found in the rat lens, except that in the latter the maximum is about 200 mg. per 100 Gm.

The lens sorbitol did not appear to be

Fig. 1. The relationship between plasma glucose levels and lens glucose levels in normal and alloxan diabetic rabbits.
directly dependent on the plasma glucose level (solid circles in Fig. 2), suggesting that its formation is influenced more by factors within the lens. Fig. 3 (solid circles) shows that one of these factors may be the lens glucose level, since the lenses with high levels of glucose nearly always have high levels of sorbitol. However, there are erratic points over the entire range of lens glucose levels.

When lens glucose levels are plotted against lens fructose levels (open circles in Fig. 3) it can be seen that the relation is much like that involving plasma glucose, i.e., with increasing lens glucose, the lens fructose rises rapidly to a maximum value of about 100 mg. per 100 Gm. A smooth transition might be expected between the normal pool value and the diabetic values as in the rat lens, but this is not proved by the results given here.

Fig. 4 illustrates the results for lenses from a group of severely diabetic rats with an average blood sugar of 670 (518 to 812) mg. per 100 ml. It appears that for this group of animals the lens fructose and glucose levels are independent of the lens sorbitol level. Similar plots (not given) show that the levels of glucose, sorbitol, and fructose in these lenses are mutually independent in the relatively narrow range of lens glucose values attained (161 to 233 mg. per 100 Gm.). The most striking features of this situation are the nearly constant values for lens glucose and fructose and the values for lens sorbitol which are not only extremely high but also vary over a wide range.

This situation in the diabetic rat lens is unlike that in the rabbit lens in some respects (Table I). In the diabetic rabbit lens, at a comparable level of lens glucose,
Fig. 3. The relationship between the glucose level and the levels of fructose and sorbitol in the lens of diabetic rabbits.

Fig. 4. The relationship between the lens sorbitol level in severely diabetic rats and the lens levels of glucose and fructose.
Table I. Average levels of sorbitol pathway metabolites in normal and diabetic lenses from rats and rabbits*

<table>
<thead>
<tr>
<th></th>
<th>Lens glucose</th>
<th>Lens sorbitol</th>
<th>Lens fructose</th>
<th>Blood or plasma glucose</th>
<th>Lens weight (grams)</th>
<th>Lens sorbitol/fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rabbit lenses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>14 Normal</td>
<td>8 ± 7</td>
<td>31 ± 15</td>
<td>35 ± 25</td>
<td>0.305 ± 0.020</td>
<td>0.89</td>
<td></td>
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<tr>
<td>13 Diabetic</td>
<td>141 ± 44</td>
<td>81 ± 37</td>
<td>94 ± 12</td>
<td>502 ± 119</td>
<td>132 ± 60</td>
<td>0.032 ± 0.006</td>
</tr>
<tr>
<td><strong>Rat lenses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 Normal</td>
<td>8 ± 7</td>
<td>27 ± 9</td>
<td>11 ± 6</td>
<td>0.030 ± 0.006</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>13 Diabetic</td>
<td>193 ± 9</td>
<td>901 ± 93</td>
<td>199 ± 10</td>
<td>667 ± 57</td>
<td>4.5</td>
<td></td>
</tr>
</tbody>
</table>

*All concentrations in this table are expressed as milligrams per 100 Gm. of fresh lens weight or milligrams per 100 ml. of rat blood or rabbit plasma.
†Standard deviation.

the lens sorbitol level is only 81 mg. per 100 Gm., about one tenth of the level in the diabetic rat lens. The lens fructose level in the rabbit lens is about one half of the level in the diabetic rat lens. It is clear that the lenticular levels of fructose and especially sorbitol are markedly higher in the diabetic rat than in the diabetic rabbit. Nevertheless, most of the diabetic rabbit lenses examined had visible equatorial opacities.

To shed some light on reasons for this difference between the lenses of the two species, some incubation experiments were carried out to determine if increasing the level of TPNH in a normal lens homogenate containing glucose at a level of 150 to 540 mg. per 100 ml. would enhance sorbitol accumulation. Results were not conclusive because of the difficulty in determining small increases in the sorbitol concentration in the presence of high concentrations of glucose. However, there was no detectable accumulation of sorbitol at the lower glucose level and no enhancement of fructose accumulation under optimum conditions (i.e., added TPNH + DPN) at the higher glucose level. Under similar circumstances, rat lens dispersions accumulate sorbitol at a rate of 0.15 mg. per gram per hour and fructose at a rate of 0.40 mg. per gram per hour. Thus, it appears that the normal rabbit lens is less active than the normal rat lens in its capacity to convert glucose to sorbitol and this capacity is not enhanced by added TPNH.

Discussion

In general, the patterns of absolute and relative concentrations of the metabolites of the sorbitol pathway in the diabetic rabbit lens resemble those found in the diabetic rat lens. The most striking difference is in the maximum values for the levels of fructose and sorbitol, which are, respectively, about one half and one tenth of the value found in the rat.

In searching for an explanation for this difference, we must consider not only a comparison of the normal lens and its diabetic counterpart but also comparisons of normal lenses of the two species and diabetic lenses of the two species. The difference may be due to an inherent disparity between rabbit and rat lenses which is little affected by diabetes, to a greater response of one species to diabetes, or to a combination of the two. In view of the significant differences in the normal lens levels of sorbitol and fructose between the two species of animals at the same glucose level, it appears that an inherent species dissimilarity exists and should be considered first.

The difference in metabolite levels between the two lenses may be due to several factors: (1) interspecies variation in the level of initial substrate. (This explanation is excluded by the fact that both species of lenses have the same glucose level), (2) interspecies variations in levels of cofactors, (3) interspecies variations in enzyme activities, and (4) interspecies varia-
tion in rate of loss of metabolites due to size, metabolism, or permeability.

Let us first consider the cofactor levels. Bullard\textsuperscript{10} has recently reported on pyridine nucleotide levels in normal rats and rabbits. Results of interest from her paper are given in Table II along with results by Sippel\textsuperscript{11} for rats. It can be seen that the normal rabbit lens has 48 m\textsubscript{ug} per gram TPNH and 120 TPN, giving a TPNH/TPN of 0.40. For rats, the corresponding values are 36, 15, and 2.5. If cofactor concentration is limiting, the rabbit lens should have a slightly higher capacity for converting glucose to sorbitol. The TPNH/TPN ratio values, being quite different, indicate that the rabbit lens should have a significantly higher capacity for converting glucose to sorbitol. The steady state levels of sorbitol (Table I) are not significantly different for rabbits and rats, suggesting that normally TPNH is not rate limiting.

Table II also shows that the normal rabbit lens has 586 m\textsubscript{moles} per gram DPN, 363 DPNH, and a DPN/DPNH ratio of 1.6, as compared to corresponding values in the rat of 443, 29, and 15. If the DPN concentration is rate limiting in the oxidation of sorbitol (and there is evidence to suggest that it is not), the rabbit should be slightly more efficient in converting sorbitol to fructose. However, the DPN/DPNH ratio values indicate that the rabbit lens should have a significantly lower capacity for converting sorbitol to fructose.

With further regard to the availability of TPNH in the normal rabbit lens, Bullard and Pirie\textsuperscript{12} have found that the activity of glucose-6-phosphate dehydrogenase activity is only 20 per cent of that in the rat and the activity of 6-phosphate gluconate dehydrogenase is slightly lower in the rabbit. Thus, the evidence involving pyridine nucleotide levels is conflicting and the one definite conclusion which might be drawn, namely, that rabbit lenses should have lower levels of fructose, is in direct opposition to the greatest quantitative difference between lenses of the two species (Table I). The alternate conclusion is that in normal lenses the levels of pyridine nucleotides are not rate limiting.

In considering point 3 (enzyme activities) for normal lenses, much evidence is available. However, it should be noted at the outset that the metabolic behavior of rabbit lenses appears to depend much more on the integrity of the lens than that of rat lenses. For instance, rat lenses carry on glycolysis and metabolism via the sorbitol pathway about as well after homogenization as before.\textsuperscript{7,4} Rabbit lenses, on the other hand, yield homogenates which are notoriously devoid of glycolytic activity unless fortified with hexokinase.\textsuperscript{13} Such homogenates in this laboratory have shown little capacity to metabolize via the sorbitol pathway. In view of this peculiarity of the rabbit lens, perhaps the most pertinent experiment bearing on aldose reductase activity is that of Kinoshita\textsuperscript{14} in which intact rabbit lenses, incubated for 21 hours in a solution containing 540 mg. per 100 ml. glucose, accumulated sorbitol to a level of over 500 mg. per 100 Gm. Rat lens homogenates incubated at an initial glucose concentration of 180 mg. per 100 ml. accumulate sorbitol over a 4 hour period at a rate of 0.16 mg. per gram per hour.\textsuperscript{9} If this were extrapolated to 21 hours, assuming no effect of the increasing sorbitol concentration on reaction rate, the final concentration would be 340 mg. per 100 Gm. Thus, within the limits of this calculation, the rat lens and the rabbit lens are equally capable of accumulating sorbitol.

Kinoshita\textsuperscript{15} purified aldose reductase

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|}
\hline
 & TPN & TPNH & DPN & DPNH \\
\hline
Rabbits\textsuperscript{*} & & & & \\
Normal & 120 & 48 & 586 & 363 \\
Diabetic & 145 & 5 & 380 & 410 \\
Rats\textsuperscript{+} & & & & \\
Normal & 15 & 36 & 443 & 29 \\
\hline
\end{tabular}
\caption{Pyridine nucleotide levels in lenses of normal rabbits and rats and diabetic rabbits in millimicromoles per gram}
\end{table}

\textsuperscript{*}Bullard.\textsuperscript{10} \textsuperscript{+}Sippel.\textsuperscript{11}
from both rabbit and rat lenses and found that the former was twice as active as the latter in an assay using glyceraldehyde in a medium of pH 6.2 containing 0.4M lithium sulfate. Our in vitro experiments using rabbit lens homogenates fortified with various combinations of TPNH, DPN, and lithium sulfate suggest that the rabbit lens has much less capacity than the rat lens to carry out the reduction of glucose to sorbitol.

Thus, the best evidence suggests that rabbit lenses suffer no lack of aldose reductase activity and may indeed be more capable than rat lenses of accumulating sorbitol to high concentrations. Why normal rat and rabbit lenses have about the same level of sorbitol is a question not likely to be answered by comparing aldose reductase activity.

The fourth point is that various factors may affect the rate of loss or metabolism of sorbitol and fructose to such an extent that normal lens levels are different in the two species. The rat lens, having a larger surface/volume ratio, might lose fructose to the aqueous humor faster than the larger rabbit lens and such a simple explanation may in part account for the fact that the fructose level in the rat lens is only a third of that in the rabbit. There is little evidence to indicate that either lens species can metabolize fructose to any great extent. The necessary enzyme, fructokinase, is not very active in the rabbit lens and rat lenses do not use fructose in the presence of glucose.

Variation in lens permeability between species has not been directly investigated, perhaps because so many other factors such as size, age, etc., would enter into the picture. The equivalent fructose levels in lenses of both species bathed in aqueous humor having about the same glucose concentration suggest that permeability differences are a minor factor (Table I).

In concluding this discussion of the comparison of normal rat and rabbit lenses, the following statements can be made:

1. The difference in sorbitol and fructose levels in rabbit and rat lenses appears not to be due to different substrate concentrations or the effects of different pyridine nucleotide concentrations.

2. There is no firm evidence that the rabbit lens has less capacity than the rat lens for metabolizing glucose via the sorbitol pathway.

3. The anomalous low-fructose level in the rat lens may be due to the ease with which it can escape from a smaller lens having a larger surface/volume ratio. Evidence for this is yet to be developed.

The following section of the discussion will be concerned with the effects of diabetes on the rat lens, which shows rapid enhancement of the sorbitol pathway following any procedure which increases the glucose level in the aqueous humor. The early effects of the accumulation of sorbitol pathway metabolites seem to be the osmotic injury described by Kinoshita for galactose-fed rats. This injury is accompanied by increases in permeability and later metabolic alterations of various types including depressed activities of certain enzymes and other constituents. Most of these changes are secondary but, nevertheless, are a real part of the course of development of diabetic cataract. Therefore, it is a mistake to believe that incubating lenses in a glucose-rich solution for a few hours will do any more than simulate the hyperglycemia of diabetes and produce the initial osmotic changes.

Data on pyridine nucleotide levels for normal rat lenses are sometimes conflicting and those for diabetic lenses are incomplete. Table III summarizes values taken from the papers of Bullard and Sippel. The data for rats rendered diabetic for 2 weeks show that in the lens the DPN + TPN/DPNH + TPNH ratio is 4.4 as compared to 7.7 for the normal rat lens. Corresponding values for the rabbit are 1.3 and 1.7. Thus, the change in ratio is less marked in the rabbit, indicating less effect of diabetes on the sorbitol pathway in the rabbit lens.

There is no reason to doubt that diabetes of a few days' duration affects some enzyme
Table III. Pyridine nucleotide ratios in lenses of normal and diabetic rabbits and rats

<table>
<thead>
<tr>
<th></th>
<th>Rabbits</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Diabetic</td>
</tr>
<tr>
<td>TPN + DPN</td>
<td>1.7</td>
<td>1.3</td>
</tr>
<tr>
<td>TPNH + DPNH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Bullord,10  
*Sippel.11

activities in the lens. Such is the case for the diabetic rat lens where a depression in the activity of the hexosemonophosphate shunt was recorded by both Lerman25 and Leveri and associates,22 whereas simply incubating normal rat lenses in a glucose-rich solution enhances the shunt.23 The in vivo result is the response of a normal lens to glucose loading; the two experiments are quite independent and in this case the responses are quite opposite.

For the rabbit, similar information comparing normal and diabetic lenses is less extensive. Diabetes has been shown to affect amino acid transport.24 Incubation of normal rabbit lenses in glucose-rich solutions enhances the shunt25 but this is not evidence that the shunt is enhanced in the diabetic lens. Bullard10 has found that in diabetic rabbits the levels of pyridine nucleotides show interesting alterations (Table II). The TPNH is reduced from 48 μmoles per gram to 5.0 and the TPNH/TPN ratio is decreased from 0.4 to 0.034 (changes which could hardly fail to depress sorbitol formation). The increase of TPN from 120 to 145 suggests that the capacity of the shunt to produce TPNH is impaired, perhaps directly by this shortage of TPN as Kinoshita25 believes. In diabetes lens, DPN is reduced to 65 per cent of the normal value and, because DPNH is slightly increased, the DPN/DPNH ratio falls from 1.6 to 0.90, a situation which could depress fructose formation. In this respect, it must be remembered that both steps of the sorbitol pathway are cofactor-requiring, reversible dehydrogenations, a type of reaction always highly affected by cofactor concentration and the ratio of the two forms. Thus, the altered cofactor levels in the diabetic lens might be enough to affect the levels of sorbitol and fructose without postulating changes in enzyme activity.

Permeability of the diabetic rabbit lens has not been studied as well as it has in the rat but Pirie and van Heyningen26 have shown that the rabbit lens with x-ray cataract has more glucose and less inositol than normal; the glucose has leaked in from the aqueous humor and the inositol has leaked from the lens. There is certainly reason to think that the diabetic rabbit lens is more permeable to sugars, a state which might not allow fructose to accumulate to very high levels.

In summary, the difference between diabetic rabbit and rat lenses with respect to sorbitol and fructose levels appears to be due chiefly to depressed levels of TPNH and DPN with concomitant changes in the ratios of oxidized to reduced forms, all of which would combine to inhibit the sorbitol pathway in the rabbit lens in spite of an increased initial substrate (glucose) level. This inhibition is relatively greater in the rabbit lens, perhaps because the rabbit lens has less reserve capacity for maintaining normal pyridine nucleotide levels and ratios. The possibility cannot be excluded that diabetes in the rabbit has a relatively greater effect in depressing activities of aldose reductase and sorbitol dehydrogenase.

The comparison of chemical analysis of the lenses and the morphology of the cataracts suggests that a comparable grade of diabetes in the two species (as measured by blood sugar levels) has less effect on the rabbit lens. The peripheral vacuoles in some of the rabbit lenses noted here are accompanied by an accumulation of total sorbitol pathway metabolites which is considerably lower than that found in the rat lens. Thus, unless we postulate that this accumulation is confined to the immediate region of the vacuoles, it appears that such
vacuole formation does not have primarily an osmotic explanation. According to Kinoshita, rabbit lenses incubated with aldoses accumulate sugar alcohols principally at the equator and anterior surface. Cotlier and Becker found that in the lenses of galactose-fed rats the concentration of dulcitol in the cortex is between two and three times the level in the nucleus. The distribution in the diabetic rabbit lens is yet to be determined.

**REFERENCES**


**Discussion**

Dr. John W. Patterson, Hartford, Conn. The major observation of this study relates to the species variation in the accumulation of sorbitol in the lenses of rabbits and rats with equally severe diabetes. On the basis of other data in the litera-
ture it is noted that this is associated with a striking decrease in the level of TPNH which occurs with diabetes. Since TPNH is a component of the reaction producing sorbitol this offers a reasonable explanation.

It is also noted that lenticular vacuoles occur in each species in spite of a tenfold difference in the levels of sorbitol. This is an interesting observation and will require further work to determine its full significance.

It is well established that the time required for the formation of mature cataracts varies with the nature of the cataractogenic agent and the age of the animal. Since such studies are pertinent to the subject under discussion, it might be helpful to summarize some results that are being published elsewhere.

Similar groups of rats fed 35 per cent galactose and 50 per cent xylose developed mature cataracts in a median time of 18 and 21 days respectively. The wet weights of the lenses of sacrificed animals were similar. However, the polyol levels were about 1200 mg per 100 Gm. for dulcitol and 900 mg per 100 Gm. for xylitol. This suggested a correlation of cataract formation with swelling rather than the exact level of polyol.

The results with galactose fed rats are shown in the accompanying table. Lens swelling and dulcitol levels were determined after the animals had been on their diets for 16 days. Cataract formation is shown as the mean time for the appearance of mature cataracts. Lens swelling represents the difference in weight between the wet weight and the weight calculated from the dry weight on the basis of a normal hydration index.

It will be noted that the amount of swelling does not follow the concentration of dulcitol. Nor does it follow the absolute amount of dulcitol per lens. Under these conditions, the time required for mature cataract formation seems to have an inverse relationship to the degree of swelling.

Dr. Kuck's paper again brings out the point that the eventual explanation of cataractogenesis must take into account all situations—species, age, and degree of provocation.

<table>
<thead>
<tr>
<th>Lens swelling (mg. H₂O)</th>
<th>Dulcitol (mg./100 Gm.)</th>
<th>Cataract formation (days)</th>
</tr>
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<tbody>
<tr>
<td>9.9</td>
<td>1270</td>
<td>18</td>
</tr>
<tr>
<td>6.0</td>
<td>1240</td>
<td>30</td>
</tr>
<tr>
<td>4.1</td>
<td>1160</td>
<td>42</td>
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75 Gm. rats, 35 per cent galactose

250 Gm. rats, 50 per cent galactose

250 Gm. rats, 35 per cent galactose