A Study of the C\textsuperscript{14}-Glucose Metabolism of the Rabbit Lens*  

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Because of its low oxygen consumption, about 0.1 \text{ml./mg./hr.}, the ocular lens has been considered as a metabolically inert tissue. However, any interference in the carbohydrate metabolism results in the opacification of this normally transparent organ. Therefore, it appears that metabolism is essential for the viability of the lens. That a consumption of oxygen, even if low, is also necessary was evidenced by Harris et al. (1) who found that the lens is incapable of maintaining its intracellular ionic composition without the presence of oxygen.

Many attempts have been made to determine the pattern of glucose metabolism in the ocular lens. Studies with lens homogenates have shown the presence of enzymes of the Embden-Meyerhof pathway (2, 3), the citric acid cycle (4), and the phosphogluconate oxidation pathway (5, 6). Differences observed in the oxidation of glucose-1-C\textsuperscript{14} and glucose-6-C\textsuperscript{14} have suggested the participation of the phosphogluconate oxidation pathway in the production of CO\textsubscript{2} (6). To obtain this information by the conventional techniques in \textit{vitro}, considerable quantities of material had to be used, since the lens is a relatively inactive tissue. The development of the lens culture technique (7, 8) has made it possible to incubate the lens for a longer period of time and to allow a study of the metabolism of a single lens. The lens is particularly well suited for the organ culture procedure, since its habitat \textit{in situ} can be more easily duplicated than that of other organs which are dependent on a direct supply of blood. Being avascular, the lens must receive nutrients from the bathing medium and dispose of end products of metabolism into it.

This report deals with a study of the glucose metabolism of an intact organ in a system \textit{in vitro} by the use of the culture technique. An attempt is made to evaluate the pattern of C\textsuperscript{14}-glucose metabolism of the rabbit lens which is maintained for 24 hours in an environment closely simulating the physiological conditions which exist in the eye.

**EXPERIMENTAL.**

The apparatus used for the culture of lenses has been previously described in detail (7, 8). In the present study eight individual culture tubes, each to contain a lens, were attached to the arm of a mechanical rocking device and immersed in a water bath at 37°. Into these vessels 5 ml. of Kresge Eye Institute Medium No. 2 (8, 9) containing 10 mmoles per liter of inorganic composition without the presence of oxygen.

The procedures for C\textsuperscript{14} assay of CO\textsubscript{2}, glucose, and lactate, in addition to the experimental methods and materials used, have already been described (5, 10, 11). The only method used that was not described previously was the anthrone method for the determination of glucose (12).

**RESULTS.**

In Table I, the results are recorded from an experiment where in each case the lens of one eye of a rabbit was incubated for 24 hours in glucose-1-C\textsuperscript{14} and that from the other eye in glucose-6-C\textsuperscript{14}. In these experiments involving four pairs of lenses, the amount of glucose utilized, the lactic acid produced, and the C\textsuperscript{14} incorporated into lactic acid and CO\textsubscript{2} were determined. The recorded isotopic values have been corrected to an initial specific activity of glucose of 1000 c.p.m./pmole, although actually the glucose-6-C\textsuperscript{14} used had over twice this activity. From the amounts of C\textsuperscript{14}O\textsubscript{2} recovered, it was found that the lenses incubated with glucose-1-C\textsuperscript{14} in all cases yielded considerably more C\textsuperscript{14}O\textsubscript{2} than those incubated with glucose-6-C\textsuperscript{14}. On the average, 3000 c.p.m. of C\textsuperscript{14}O\textsubscript{2} were recovered from glucose-1-C\textsuperscript{14} as compared with only 57 c.p.m. from glucose-6-C\textsuperscript{14}. This indicates that the C-1 atom of the glucose molecule was oxidized at a rate 41 times that of the C-6. The preferential oxidation of the C-1 atom of glucose strongly suggests the participation of the phosphogluconate oxidation pathway.

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The experiments in which uniformly labeled glucose was used as the substrate are summarized in Table II. Upon 24 hours of incubation of the rabbit lens, the direct chemical measurements revealed the utilization of 27.6 μmoles of glucose, with the appearance of 42 μmoles of lactic acid. The isotopic assay showed that of the 27,600 c.p.m. of labeled glucose utilized, 82 per cent or 22,300 c.p.m. were recovered as lactic acid. These results indicated that a major part of the glucose metabolized was recovered as lactic acid. This conclusion was further supported by the small amount of CO₂ recovered from the uniformly labeled glucose. The 620 c.p.m. of CO₂ recovered as CO₂ was represented only 2.3 per cent of the total glucose utilized. Since the specific activity of the glucose carbon atom was 1000:6 or 167 c.p.m. per μmole of glucose carbon, the 620 c.p.m. of CO₂ indicate that only 3.7 μmoles of glucose carbon atoms were oxidized.

**DISCUSSION**

The results obtained in the C-1- and C-6-labeled glucose experiments are similar to those previously observed (5). In those preliminary experiments, however, a greater number of lenses were used per reaction flask, the incubation period was shorter, and the conditions were less physiological than those used in the present experiments. Nevertheless, the results are comparable for the CO₂ ratio from C-1 to C-6 glucose previously reported that varied from 35 to 50, and, in the current experiments, the values that ranged from 33 to 64. The greater proportion of glucose oxidized in the earlier study can be attributed to the concentration of oxygen present in the reaction vessel. A gas phase of 95 per cent oxygen was previously used, whereas in the study reported here the gas phase contained 7 per cent oxygen. The gas mixture with the low percentage of oxygen is thought to lead to an oxygen tension which is similar to that found in the intraocular fluids bathing the lens (13).

It appears obvious that the large difference in the rates of the C-1 and C-6 oxidation of glucose is not caused by any unusually high activity of the phosphogluconate oxidation pathway but rather to the relative inactivity of the citric acid cycle. This must be the case in view of the demonstration that most of the glucose is metabolized via the Embden-Meyerhof pathway.

One method which has been used to contrast the relative rates of the Embden-Meyerhof and the phosphogluconate pathways is to compare the CO₂ incorporation of C-1- and C-6-labeled glucose into lactic acid (10, 14). In this method it is assumed that the amount of CO₂ recovered into lactic acid from glucose-1-C₁₄ results solely from the Embden-Meyerhof activity. The ratio of the amount of CO₂ recovered from C-1 to C-6 glucose is thought to give the fraction of the amount of glucose metabolized by the glycolytic mechanism. When this calculation was applied to the results of the experiments from three rabbits (Table I), the percentage of glucose metabolized via the glycolytic pathway was found to be 94 per cent, 77 per cent, and 87 per cent. Thus an average of 86 per cent of glucose was metabolized via the Embden-Meyerhof pathway and presumably 14 per cent by the phosphogluconate oxidation pathway.

Another method by which this estimate can be made is to calculate what fraction of the total glucose utilized is oxidized via the phosphogluconate pathway (14). It seems that almost all of the CO₂ produced from the glucose-1-C₁₄ was derived via the direct oxidative route. The amount of CO₂ contributed by the citric acid cycle appeared negligible as reflected by the small amount of CO₂ recovered when glucose-6-C₁₄ was the substrate. From the data of Table I, after correcting for the amount of CO₂ formed via the citric acid cycle, it can be estimated that there were 2.3 μmoles of glucose oxidized by the phosphogluconate oxidation route. This indicated that 8.8 per cent of the 26.1 μmoles of glucose utilized was metabolized by the alternate pathway. Therefore, the percentage of glucose metabolized via the phosphogluconate oxidation pathway, as
Table III
Comparison of glucose-C\(^{14}\) oxidation by rat liver and rabbit lens

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Label</th>
<th>Glucose-C(^{14})</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Utilized</td>
<td>Oxidized</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\mu)moles/gm./hr.</td>
<td>(\mu)moles/gm./hr.</td>
<td></td>
</tr>
<tr>
<td>Rat liver*</td>
<td>1-C(^{14})</td>
<td>46.5</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>6-C(^{14})</td>
<td>37.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Rabbit lens</td>
<td>1-C(^{14})</td>
<td>3.6</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>6-C(^{14})</td>
<td>3.8</td>
<td>0.008</td>
</tr>
</tbody>
</table>

* Data from Ashmore et al. (14).

estimated by the C\(^{14}\) incorporated into lactic acid, was 14 per cent, whereas the results from the experiments with C-1 glucose oxidation indicated a value close to 9 per cent.

In the experiments from C-1- and C-6-labeled glucose, 90 per cent of the radioactivity disappearing from the glucose fraction could be accounted for by either lactic acid or CO\(_2\). On the other hand, in the experiments from the uniformly labeled glucose a total of 83 per cent radioactivity utilized was recovered in these two fractions. Therefore, it appears that 10 to 17 per cent of the radioactivity disappearing from the glucose is oxidized via the phosphogluconate oxidation pathway, a large percentage of CO\(_2\) seems to be produced by the alternate pathway. Perhaps a more striking way of illustrating the prominent role played by the phosphogluconate pathway to produce CO\(_2\) in the lens is by a comparison with the glucose metabolism of rat liver. This comparison is made in Table III. In rat liver (14) it was shown that 8.9 per cent of the C-1 glucose and 7.2 per cent of the C-6 glucose were oxidized to CO\(_3\)\(^{14}\). In the lens, although a much smaller amount of glucose is utilized, about the same percentage of C-1 of glucose was converted to CO\(_2\) as in the liver, whereas only 0.2 per cent of the C-6 glucose was oxidized. This means that if the lens were capable of utilizing glucose to the same extent as liver, then 4.2 \(\mu\)moles/gm./hr. of the C-1 of glucose would be converted to CO\(_2\), and only 0.1 \(\mu\)moles of the C-6 would be oxidized.

It is somewhat misleading to gauge the metabolism of the lens by expressing it on a weight basis. Unlike the liver, the rate of glucose metabolism is not uniform throughout the lens. The main bulk of the lens is acellular and is probably metabolically inactive. Only a small fraction of the lens can be considered capable of active metabolism. This fraction consists mainly of a sheet of epithelium confined to the undersurface of the anterior segment of the capsule and the newly formed lens fibers elaborated from those cells. If the glucose metabolism is mainly confined to this part of the lens, it may prove to be as active as, or perhaps more active than the other tissues.

Summary

A study was made of the glucose metabolism of rabbit lens maintained for 24 hours under conditions which are similar to those normally existing in the eye. Under these conditions it was found that most of the glucose utilized was converted to lactic acid. The presence of the phosphogluconate oxidation pathway was suggested by the preferential oxidation of the C-1 atom in contrast to the C-6 of glucose. However, it has been calculated that only about 10 per cent of the glucose metabolism is by this route and 90 per cent by the Embden-Meyerhof scheme. Of the small amount of glucose oxidized in the lens, the phosphogluconate pathway appeared to contribute significantly to the CO\(_2\) produced.

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