
Pathways of glucose metabolism in the lens

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In the lens the biological energy necessary for the maintenance of transparency, synthesis, and repair is supplied primarily by the reactions that metabolize glucose to lactic acid. The low levels of the enzymes associated with the aerobic oxidation of glucose restrict the lens metabolism mainly to anaerobic glycolysis. Even in the epithelium, anaerobic glycolysis appears to be the principal source of biological energy. The evidence for this is that the active transport mechanisms of the lens occur primarily in the epithelium and these energy-utilizing processes can be almost entirely supported by anaerobic glycolysis. Another unusual feature of lens glucose metabolism is the ability of the lens to synthesize sorbitol. It is the unusual set of circumstances of low hexokinase, relatively high aldose reductase, and high pentose shunt activities which render the lens a favorable site for sorbitol production.

Because of its rather peculiar structure and avascularity one gains the impression that the lens is a metabolically inert organ. A comparison of the rate of O₂ consumption and glucose utilization in the lens with those in other tissues would certainly support this conclusion. However, the lens must meet certain energy demands to maintain its viability. Obviously the main objective of the lens is to retain its transparency. The explanation of transparency of a tissue is far from being understood, but it is apparent that a normal state of hydration is important to maintain clarity. The volume of water in the lens is regulated primarily by the efficiency of energy-dependent mechanisms which control electrolyte levels. Furthermore, new fibers are continually laid down in the lens

so energy must be available for their synthesis. In meeting these and other energy demands the lens does so by a glucose metabolism restricted by the very nature of its function and environment. The aerobic phase of glucose metabolism would not be expected to play a prominent role in a tissue which is avascular and exists in an environment of low oxygen tension. Even if oxygen were readily available, since the lens must be not only a transparent but a colorless ocular medium, it cannot be richly endowed with pigmented oxidative enzymes of the cytochrome system or the riboflavin-containing group. Thus, in the lens, glucose is catabolized primarily to lactic acid, and is not appreciably combusted to CO₂ (Fig. 1).

Significance of aerobic and anaerobic phases of glucose metabolism in the lens

It is well established that the aerobic phase of glucose metabolism is quantitatively much more efficient than glycolysis in ATP synthesis so even though a limited amount of glucose is completely metabolized to CO₂ via the Krebs cycle (Fig.

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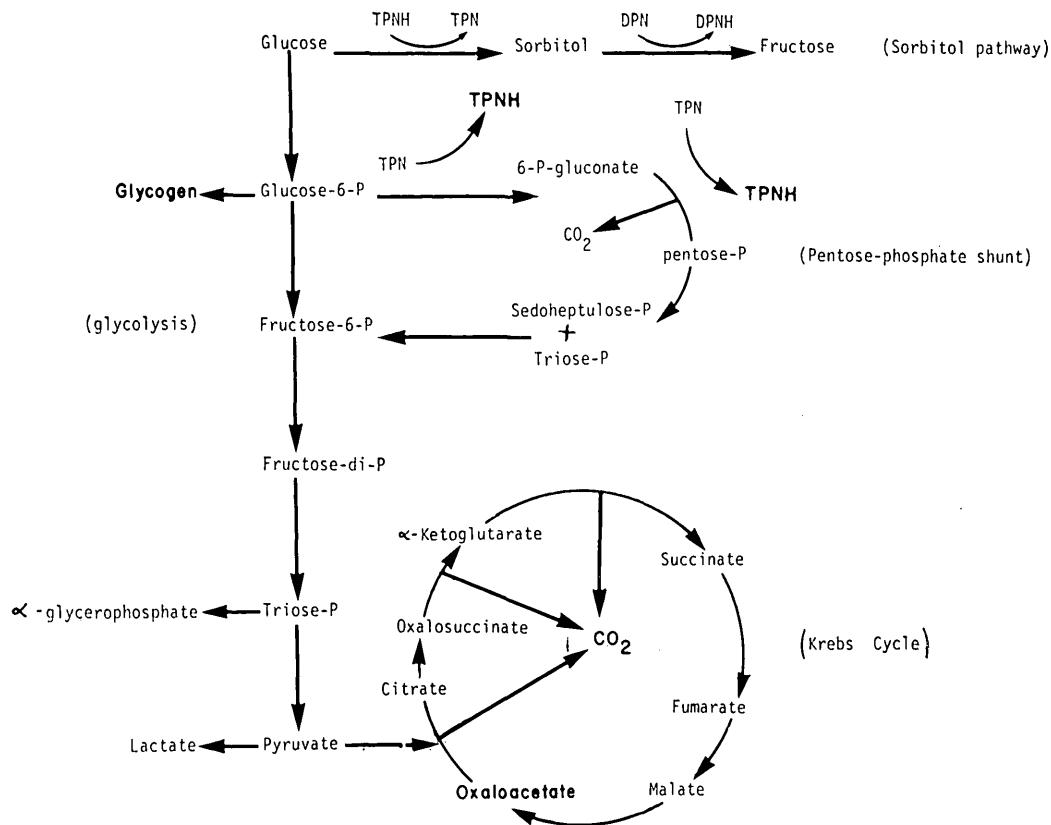


Fig. 1. Pathways of glucose metabolism.

1), it may contribute significantly to the energy stores of the lens. To evaluate the relative importance of the aerobic and anaerobic phases of glucose metabolism as a source of biological energy in calf lens, the efficiency of a number of energy-utilizing mechanisms was studied under a variety of conditions.¹ As shown in Table I the most favorable conditions for the lens are achieved by incubating in a medium containing glucose in the presence of oxygen. Under these conditions of incubation, the lens remains completely transparent, it maintains normal levels of high energy phosphate bonds and cations, and it shows a high rate of arginine incorporation into protein. Under anaerobic conditions, as long as glucose is available, the lens does equally as well, as shown by its clarity and maintenance of normal levels of energy-related substances. Although the extent of arginine incorporation appears somewhat depressed, because

of the wide variation in the results, it is not statistically different from that observed under aerobic conditions. During the incubation in the absence of glucose, but in the presence of oxygen, the lens is not maintained as well as under the first two conditions. In this case, a haze is found in the lens, a drop in high energy phosphate level is observed, and changes in cation levels are apparent. A 50 per cent decrease in the incorporation of arginine into lens protein is also observed. However, without added glucose the lens does much better in oxygen than in nitrogen, for the most unfavorable condition for the lens is an anaerobic incubation in a medium without glucose. Here the incubation leads to the opacification of the lens, marked changes in cation levels, loss of labile phosphates, and no synthesis of protein.

These results indicate the calf lens is able to maintain its energy-requiring pro-

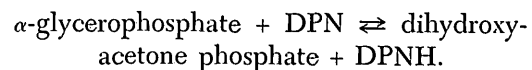
Table I. Effect of varying conditions of incubation on the levels of cations, ATP, transparency, and the extent of amino acid incorporation in calf lens

| Condition of incubation | | Trans- parency | Na ⁺ (mEq./Kg. HOH) | K ⁺ (mEq./Kg. HOH) | Labile P (μmoles/Gm. lens) | ¹⁴ C-arginine incorporation (c.p.m./Gm. lens/ 20 hr.) |
|-------------------------|---------|-------------------|--------------------------------------|-------------------------------------|----------------------------------|---|
| Gas phase | Glucose | | | | | |
| Air | + | 4+ | 16 | 128 | 4.9 | 7,990 ± 2,300 (S.D.) |
| N ₂ | + | 4+ | 16 | 127 | 4.6 | 6,880 ± 1,650 |
| Air | - | 2+ | 25 | 119 | 2.2 | 3,110 ± 740 |
| N ₂ | - | 0 | 66 | 82 | 0.2 | Negligible |
| Normal lens | | 4+ | 16 | 128 | 4.6 | |

The method of incubation and chemical procedures employed are those from Kinoshita, J. H., Kern, H. L., and Merola, L. O.: *Biochim. et biophys. acta* 47: 458, 1961.

cesses equally as well anaerobically as aerobically as long as glucose is present. However, the results obtained from the aerobic incubation in the absence of glucose indicate the aerobic phase of metabolism is capable of providing energy to some degree. Apparently the Krebs cycle and the oxygen-utilizing mechanism are sufficiently active to consume enough endogenous substrates to support partially the energy-utilizing mechanisms. The endogenous substrates were apparently sufficient to maintain the aerobic metabolism functioning at a maximal rate, for there was no evidence that the addition of pyruvate or Krebs cycle intermediates further stimulated energy production. The nature of the endogenous substrates utilized is obscure. It may be that some of the lactate is converted to pyruvate and metabolized through the Krebs cycle. Pirie² recently made the observation that α-glycerophosphate is also one of the end products of lens metabolism. It is conceivable that α-glycerophosphate could be utilized in the mitochondria and consequently could be the endogenous substrate in question. The oxidation of this substance with DPN as the cofactor could channel its electrons directly into the electron transmitting system to produce energy without involving the Krebs cycle. α-Glycerophosphate is formed from intermediates of the glycolytic scheme by reduction of dihydroxyacetone phosphate, one of the triose phosphates produced in glycolysis (Fig. 1). This compound is thought to play an

interesting role in intermediary metabolism because α-glycerophosphate dehydrogenase is found in the soluble cytoplasm as well as in the mitochondria in other tissues.² Because of the distribution of its enzyme, α-glycerophosphate is thought to link glycolysis with the electron transmitting system of the mitochondria. α-Glycerophosphate, formed from the intermediates of the glycolytic scheme by the dehydrogenase in the soluble cytoplasm, gains access into the mitochondria. Here the dehydrogenase of the mitochondria catalyzes the transfer of electrons to form DPNH by the following reactions:



The DPNH is channeled into the oxidative phosphorylation mechanism to form ATP. The dihydroxyacetone phosphate then diffuses out into the soluble cytoplasm, interacts with the glycolytic intermediates by the reversal of the above reaction, and the cyclic mechanism is begun over again. That this type of electron transport system functions in the lens was proposed by Pirie,² who presented evidence that more α-glycerophosphate is formed anaerobically than aerobically in the lens. The lens is different from most tissues in that it contains fewer mitochondria to utilize the intermediates of glycolysis for aerobic oxidation. For lactate to serve as the substrate for aerobic oxidation it must be in close proximity to a mitochondrion along with DPN and lactic

dehydrogenase. The DPNH and pyruvate formed in the reaction would then have to diffuse into the mitochondrion to initiate the aerobic phase of oxidation. The main disadvantage of lactate as a substrate for aerobic oxidation, in contrast to α -glycerophosphate, is that lactic dehydrogenase is not found in the mitochondria. The unique features of the α -glycerophosphate cyclic mechanism as a link between glycolysis and aerobic oxidation may be ideally suited for the lens.

A number of other studies have indicated that it is primarily the glycolytic mechanism which supports the energy-requiring processes in the lens. The recovery of cations to normal levels after cold exposure could be achieved under anaerobic conditions in rabbit lens³ as well as in calf lens.¹ Another energy-expending process, the transport of amino acids into calf lens, could be supported entirely by anaerobic glycolysis as shown by Kern.⁴ Kinsey and Reddy⁵ reported that anaerobiosis depressed the uptake of α -aminoisobutyric acid in rabbit lenses by 27 per cent, but more recently⁶ could find no effect of lack of oxygen on uptake of this amino acid. Further evidence that cation transport in the lens is mainly supported by anaerobic glycolysis was shown by Becker,⁶ who found that the accumulation of rubidium was relatively little affected by anoxia, dinitrophenol, and cyanide but was very markedly affected by iodoacetate.

All of these experiments dealing with energy-expanding processes leave little doubt that in calf and rabbit lenses the Embden-Meyerhof pathway is the primary source of biological energy. The interesting aspect emerging from the transport studies is that although active uptake of cations and amino acids takes place mainly in the epithelium,⁷ the mechanisms are not dependent on aerobic metabolism. It would have been anticipated that the epithelium would be the segment of the

lens most dependent upon oxygen-utilizing mechanisms. Even though the lens epithelium contains mitochondria, apparently the energy gained from glycolysis exceeds that derived from the Krebs cycle and its associated oxidative phosphorylation mechanisms. Perhaps the lack of abundance and size of mitochondria in the lens epithelium relative to other tissues⁸ may in part explain these effects.

There are, however, species differences to consider. The high sensitivity of chicken lens to dinitrophenol, probably attributable to the unusual annular pad of epithelium, suggests that this lens is primarily an aerobic tissue.⁹ The human lens is also susceptible to dinitrophenol, as evidenced by the dinitrophenol cataracts, and it suggests the active participation of the aerobic phase of glucose metabolism.⁹ Further studies, however, are required before these tentative conclusions can be established as facts.

Embden-Meyerhof glycolytic pathway

The enzyme hexokinase seems to be a key enzyme in the glucose metabolism of the lens. Earlier studies by Green and co-workers¹⁰ had indicated that the rate of glycolysis is increased in rabbit lens extract when hexokinase is added to the reaction mixture or when glucose is re-

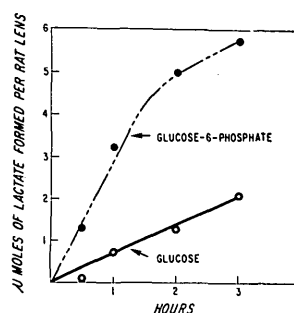


Fig. 2. Rat lens glycolysis. The reaction mixture consisted of glucose or glucose-6-phosphate (20 mmoles), ATP (4 mmoles), AMP (2 mmoles), DPN (0.5 mmole), Mg^{++} (5 mmoles), phosphate (20 mmoles), rat lens extract (85 mg. wet weight), and 0.2M Tris to make final volume 3.1 ml. Aliquots were taken at various intervals, deproteinized, and lactate measured.¹⁴

⁹Personal communication.

placed by glucose-6-phosphate as the substrate. These means of bypassing the "bottleneck" in lens glycolysis result in a marked increase in lactate production. In contrast to the earlier studies, a recent finding in rat lens extract indicated that under optimal conditions not only was the rate of lactate formation from glucose equal to that from glucose-6-phosphate but it approached that from pyruvate.¹¹ Because of these conflicting results, this aspect of lens glycolysis was reinvestigated with calf, rabbit, and rat lenses. As shown in Fig. 2, under conditions in which all the cofactors were present in optimal concentrations, lactate production from glucose-6-phosphate was substantially greater than that from glucose in rat lens extracts. We have conducted these incubations under conditions where the supplements were either absent or present in varying concentrations, and under all circumstances the lactate production was much greater from glucose-6-phosphate than from glucose. The same effect was also observed in calf and rabbit lens extracts as well.

It therefore appears that the rate-limiting factor in lens glycolysis is the activity of the hexokinase enzyme. In fact, it is presumably by this mechanism that the lens governs its rate of glucose metabolism. Since glucose is the principal substrate and lactate the main end product of metabolism, if the metabolic rate were not regulated, lactate might accumulate to an unfavorable level. Since the Krebs

cycle is relatively inactive in the lens, there is no effective mechanism to metabolize lactic acid. Thus diffusion into the intraocular fluids is the only other means to eliminate lactic acid. A regulating mechanism is essential so that the rate of glucose metabolism is sufficiently rapid to meet the energy demands imposed on the lens, but not so rapid that lactate accumulates to a level where the lens buffer capacity is exceeded.

Sorbitol pathway

Because of the low hexokinase activity, another pathway of glucose metabolism assumes a prominent role in the lens. The sorbitol pathway was demonstrated in the lens by van Heyningen,¹² who showed that

Table II. The conversion of glucose to sorbitol in calf lens

| Initial specific activity (c.p.m./ μ mole) | Final specific activity (c.p.m./ μ mole) | | | |
|--|--|----------|----------|----------|
| | Glucose | Sorbitol | Fructose | Inositol |
| 1,050 | 717 | 820 | 753 | |
| 1,050 | 740 | 765 | 630 | 83 |
| 1,050 | 783 | 763 | 753 | 65 |

Calf lenses were incubated for 24 hours with U-¹⁴C-glucose (10 mM.) in 10 ml. of balanced salt solution. From Kinoshita, J. H., et al.: *Biochim. et biophys. acta* 74: 340, 1963.

Table III. Substrates of aldose reductase

| Compound | Relative velocity* | Relative maximal velocity | K _m (M) |
|----------------------|--------------------|---------------------------|----------------------|
| DL-Glyceraldehyde | 100 | 100 | 3 × 10 ⁻⁵ |
| D-Erythrose | 96 | 170 | 4 × 10 ⁻⁴ |
| D-Xylose | 60 | 91 | 5 × 10 ⁻³ |
| D-Lyxose | 25 | | |
| D-Ribose | 57 | 94 | 7 × 10 ⁻³ |
| D-Arabinose | 33 | 70 | 2 × 10 ⁻² |
| L-Arabinose | 58 | 80 | 4 × 10 ⁻³ |
| D-Glucose | 10 | 90 | 7 × 10 ⁻² |
| D-Galactose | 24 | 52 | 2 × 10 ⁻² |
| D-Mannose | 1 | | |
| L-Glucose | 1 | | |
| L-Xylose | 2 | | |
| 2-Deoxy-D-glucose | 4 | | |
| 2-Deoxy-D-galactose | 10 | | |
| D-Glucuronolactone | 83 | 92 | 7 × 10 ⁻⁴ |
| Sodium D-glucuronate | 43 | 60 | 4 × 10 ⁻³ |
| Acetaldehyde | 17 | | |
| Propionaldehyde | 89 | | |
| Butyraldehyde | 80 | | |
| Isobutyraldehyde | 90 | 90 | 2 × 10 ⁻⁵ |
| Benzaldehyde | 11 | | |
| Glycoaldehyde | 85 | | |
| Isovaleraldehyde | 10 | | |

Data from Hayman, S., and Kinoshita, J. H.: *J. Biol. Chem.* 240: 877, 1965.

*The relative velocity was determined at substrate levels of 0.01M.

two enzymatic reactions were involved. In the first reaction, catalyzed by the enzyme, aldose reductase, glucose reacts with TPNH, and is reduced to its alcohol form, sorbitol. Sorbitol can be further metabolized to fructose in the presence of DPN and a second enzyme, polyol dehydrogenase. The sorbitol pathway was previously thought to function only in the accessory sexual tissues such as the seminal vesicles and placenta but apparently is active in other tissues as well.¹⁵

That the conversion of glucose to fructose actually takes place in the calf lens was demonstrated by incubating it in the presence of ¹⁴C-glucose.¹⁴ After incubation, glucose and its derivatives which accumulated during incubation were isolated on an ion exchange column and their specific activities determined. As shown in Table II the specific activities of glucose, sorbitol, and fructose were essentially identical. It appears that related sugars and sugar alcohol equilibrated during the course of incubation, and the results are consistent with the view that this occurs via the sorbitol pathway. Another hexitol present in the lens is inositol, but this cyclic sugar alcohol is apparently not synthesized directly from glucose as is sorbitol but by a series of more complicated reactions, for its specific activity was considerably lower.

It has been shown that purified lens aldose reductase reduces a large variety of aldehyde-containing substances.¹³ It is rather surprising that hexoses serve as very poor substrates for this enzyme. As shown in Table III, a comparison of the relative velocities and Michaelis constants (Km) shows that hexoses are less reactive than other related aldehydes. The 3 carbon sugar, glyceraldehyde, appears to be the best substrate. There is some indication that reactivity of the substrate decreases with increasing chain length of the sugars. Glucuronic acid and glucuronolactone are much more reactive than either glucose or galactose, suggesting that the presence of a free aldehydic group, one

not bound in a pyranose structure, facilitates the reaction. The substrate apparently need not be a sugar, for even aliphatic aldehydes can be attacked by aldose reductase.

The very high Michaelis constant (Km) of aldose reductase with glucose indicates that under normal circumstances the glucose concentration in the lens is not sufficiently high to produce appreciable quantities of sorbitol. Hexokinase and aldose reductase actively compete for glucose as it enters the lens. Because the Km of lens hexokinase is 10^{-4} M,¹⁰ and that of aldose reductase is about 10^{-2} M,¹³ most of the glucose would be phosphorylated and channeled into the Embden-Meyerhof pathway. However, when a situation arises in which there is an increase in the uptake of glucose by the lens, the hexokinase becomes readily saturated because of its low concentration. Consequently the glucose level is elevated and this in turn stimulates sorbitol production. If the lens were endowed with high hexokinase activity, an increase in the availability of glucose may not result in high sorbitol production because the active phosphorylating mechanism would tend to maintain a low glucose level. Substantial quantities of sorbitol are formed only when the lens glucose concentration is elevated.

Relationship of the sorbitol pathway to the pentose phosphate shunt

A necessary component of the aldose reductase reaction is TPNH. Since the main source of TPNH in the lens is the pentose phosphate shunt mechanism, there is an interaction between the reaction which generates TPNH and that which utilizes it (Fig. 3). Because of this relationship a curious effect is observed in the lens when it is exposed to increasing levels of glucose.¹⁴ As the concentration of glucose in the medium is increased, there is a stimulation in the oxidation of glucose-6-phosphate via the pentose shunt, as evidenced by an increased rate of CO₂ production from carbon 1 but not from

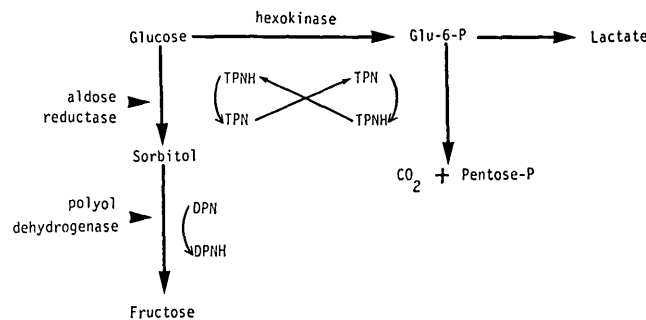


Fig. 3. Interaction of aldose reductase with the dehydrogenases of the shunt mechanism.

carbon 6 of glucose. As shown in Fig. 4 the stimulation in the rate of oxidation of C-1 of glucose is observed as the glucose level in the medium is increased from 5 to 30 mM. In this range of concentrations about 1 to 4 μ moles of C-1 of glucose are oxidatively decarboxylated, whereas only a small and constant amount of CO_2 resulted from the C-6 atom of glucose. With increasing levels of glucose, there is also no stimulation in lactate production.¹⁴ Apparently the hexokinase reaction in the rabbit lens is saturated when the glucose concentration is 5 mM. in the medium so that a further increase in sugar is not accompanied by an increase in the rate of lactate or CO_2 production through the glycolytic and citric acid path-

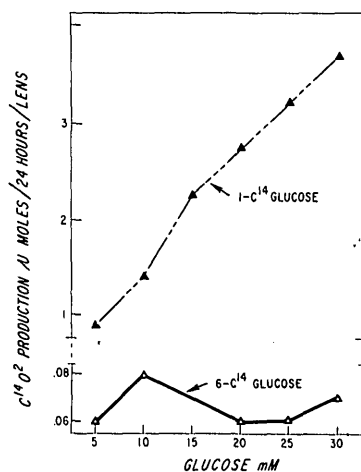


Fig. 4. Stimulation of glucose-6-phosphate oxidation through the pentose phosphate shunt by increasing levels of glucose. (Data from Kinoshita, J. H., et al.: *Biochim. et biophys. acta* 74: 340, 1963.)

ways. Perhaps only in the lens where there is a low concentration of hexokinase is it possible to demonstrate an increase in the rate of oxidation of glucose-6-phosphate through the shunt mechanism by increasing the availability of glucose. The elevated concentration of glucose in the lens stimulates the aldose reductase reaction to increase the rate of reoxidation of TPNH, which in turn stimulates the pentose phosphate shunt mechanism.

From these studies it appears that the factors which favor sorbitol formation would include an environment in which there is low hexokinase, high aldose reductase, and high pentose shunt activities. The distribution and activities of these enzymes were studied by Hayman¹⁵ in the epithelium, cortex, and nucleus of calf lens (Table IV). As might be expected, the activities of these enzymes were highest in the capsule-epithelium segment of the lens. Although glucose-6-phosphate dehydrogenase and hexokinase activities were higher in the cortex than the nucleus, aldose reductase was equally as active in the old as in young lens fibers. A high ratio of activity of aldose reductase to that of hexokinase, AR/HK, would favor sorbitol production. This ratio is highest in the nucleus, but the TPNH generating system in the nucleus is extremely low so that high sorbitol production is not likely. The epithelium has a high AR/HK ratio and also an extremely active shunt mechanism, so that the highest sorbitol production would most likely be in this area of the lens. This possibility may

Table IV. Enzyme activities in various parts of calf lens

| | <i>Capsule plus epithelium</i> | <i>Cortex</i> | <i>Nucleus</i> |
|-----------------------------------|--------------------------------|---------------|----------------|
| Hexokinase (HK) | 0.9 | 0.2 | 0.02 |
| Aldose reductase (AR) | 7.9 | 0.9 | 1.2 |
| Glucose-6-phosphate dehydrogenase | 6.6 | 3.2 | |
| AR/HK ratio | 8.8 | 4.5 | 60 |

Hexokinase activity was measured with 2-deoxyglucose and aldose reductase activity with glyceraldehyde. Activities are given as micromoles per minute per gram wet weight of tissue. Data were obtained by Hayman.¹⁵

be important since the epithelium is the main site of the active transport activities and because of the possible implication of sugar alcohols affecting these transport mechanisms.^{16, 17}

The second enzyme of the sorbitol pathway, polyol dehydrogenase, oxidizes sorbitol to fructose with DPN as the cofactor. This enzyme is found in a number of tissues.¹⁸ The specificity of the enzyme restricts its action to those sugar alcohols which possess stereochemical configurations I and II of the first four carbon atoms (Fig. 5). Sorbitol and xylitol are of configuration I and thus are suitable substrates for polyol dehydrogenase. On the other hand, dulcitol has an entirely different configuration (Fig. 5), and conse-

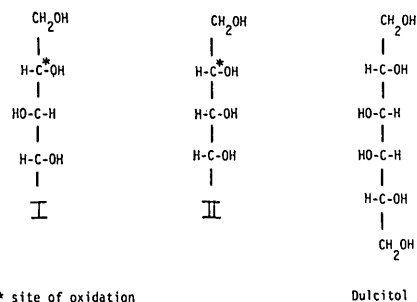


Fig. 5. Specificity of polyol dehydrogenase. Configurations I and II are the substrate requirements for polyol dehydrogenase.¹⁸

quently is a poor substrate for this enzyme. Polyol dehydrogenase purified from sheep liver was shown to have a much lower K_m for xylitol than sorbitol.¹⁸ From some of the properties studied the lens polyol dehydrogenase must be identical with or at least very similar to the liver enzyme.¹⁴

Of the cataractogenic sugars, galactose, glucose, and xylose, the properties of the enzymes indicate that xylose would be metabolized more rapidly through the sorbitol pathway than would be the hexoses. The conversion of galactose to its sugar alcohol form would not be as favorable as that of xylose but much more so than glucose. However, dulcitol is not readily converted to its keto sugar while xylitol and sorbitol are readily attacked by polyol dehydrogenase.

The role of the sorbitol pathway in normal lens metabolism is not clearly defined. From the substrate specificity studies of aldose reductase it appears that glucose is not the primary substrate for this enzyme. Since compounds such as glyceraldehyde or glucuronic acid are more appropriate substrates for aldose reductase, the major role of this enzyme may be in the metabolism of substrates other than hexoses. Hayman¹⁵ has explored the possibility that a reduction of glucuronic acid or glucuronolactone to L-gulonate may lead to the synthesis of ascorbic acid. However, incubation of ¹⁴C-glucuronic acid or ¹⁴C-glucuronolactone in rabbit lens did not lead to a significant incorporation into ascorbic acid. Apparently L-gulonate is formed but instead of proceeding to ascorbic acid, it is oxidatively decarboxylated. That the lens is able to metabolize glucuronic acid suggests the presence of the uronic acid pathway.

Another possible function of the sorbitol pathway was suggested by Kuck.¹⁹ The conversion of glucose to fructose through the sorbitol pathway may serve as a transhydrogenation system in that TPNH is utilized and DPNH is formed (Fig. 3). It is generally accepted that

for TPNH to lead to energy production a transhydrogenation to DPNH is required. The DPNH formed can be reoxidized in the mitochondria producing high-energy phosphate. The sorbitol pathway may thus serve as a means of harnessing the oxidative steps of the pentose phosphate shunt mechanism to derive biological energy.

The Krebs cycle

The presence of most of the enzymes of the Krebs cycle has been demonstrated by Ely²⁰ and by Wortman and Becker.²¹ However, the low O₂ consumption and the fact that most of the glucose metabolized in the lens accumulates as lactic acid preclude the possibility of the existence of a very active citric acid cycle. Moreover the site of Krebs cycle activity is known to be the mitochondria. The paucity of these organelles in the lens also points to a low aerobic phase of glucose metabolism. The mitochondria are probably confined to the epithelium and the superficial lens fibers.

A study of the metabolism of labeled pyruvate has shown some interesting features.²² The oxidation of the carboxyl carbon of pyruvate to CO₂ in the lens is much more active than the oxidation of the other two carbons of pyruvate. We had previously shown that, regardless of the different activity of the Krebs cycle in various tissues, there appears a consistent pattern in the relative rates of oxidation of the three carbon atoms of pyruvate or of lactate; that is, independent of the rate of oxidation of pyruvate to carbon dioxide, a predictable ratio of the oxidation rates of the pyruvate carbons could be observed.²² The lens is the only tissue that did not fit the pattern. A summary of the oxidation rates of various carbon atoms of pyruvate is given in Table V. The ratios were established by comparing the oxidation rates of C₂ and C₁ of pyruvate relative to C₃. In most tissues the carbonyl carbon (C-2) of pyruvate is oxidized 1.4 to 1.7 times as rapidly as the

Table V. Relative rates of oxidation of the carbon atoms of pyruvate or lactate

| Tissue | CH ₃ (C ₃) | -CO or -CHOH (C ₂) | -COOH (C ₁) |
|--------------------------------|--------------------------------------|---|----------------------------|
| Diaphragm (rat) | 1 | 1.4 | 1.7 |
| Kidney (rat) | 1 | 1.4 | 2.3 |
| Liver (rat) | 1 | 1.6 | 4.3 |
| Mammary gland (rat) | 1 | 1.7 | 3.4 |
| Corneal epithelium (cattle) | 1 | 1.6 | 4.4 |
| Lens (calf) | 1 | 1.7 | 10.6 |

Data were obtained from Kinoshita, J. H., and Merola, L. O.: *Exper. Eye Res.* 1: 53, 1961.

methyl carbon (C-3), while the carboxyl carbon (C-1) is oxidized at a rate two to four times that of the methyl carbon. These ratios are observed in tissues with a very active Krebs cycle as in kidney or in tissues with a very sluggish Krebs cycle as in the cornea. The unique feature in the lens is that, although the ratio of the oxidation rate of carbonyl carbon to methyl carbon is like that of other tissues, the rate of oxidation of carboxyl carbon is much more rapid than was expected. Apparently the oxidation of carboxyl carbon of pyruvate in lens is geared to a dismutation reaction, in which one molecule is decarboxylated and another molecule is reduced to lactate.²² The two carbon atoms remaining after decarboxylation are in some way incorporated into glutamic acid.²² Thus we have a mechanism by which glucose is converted to glutamate and undoubtedly contributes to the high level of glutamic acid found in the lens.

Conclusion

In the lens the biological energy necessary for the maintenance of transparency, synthesis, and repair is supplied primarily by the reactions that break down glucose to lactic acid. The low levels of the enzymes associated with aerobic oxidation of glucose restrict the lens metabolism mainly to anaerobic glycolysis. One factor which regulates lens glycolysis is the hexokinase enzyme. It is the unusual set of

circumstances of low hexokinase, relatively high aldose reductase, and high pentose phosphate shunt activities which render the lens a favorable site for sorbitol production.

The qualitative aspects of lens metabolism have been delineated by the studies of a number of investigators. Some of these have been covered in this review. What is needed now is more information about the quantitative aspects of lens glucose metabolism. Some information is available about the major pathways involved, but questions such as how significant are the α -glycerophosphate cycle, the uronic acid pathway, transhydrogenation reaction through the sorbitol pathway, and the conversion of glucose to glycogen are some of the questions still unanswered. Also in all the studies so far we have been regarding the lens as a whole; more information is needed concerning the qualitative and quantitative aspects of glucose metabolism of the various areas of the lens.

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