Some aspects of the carbohydrate metabolism of the cornea

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A review of certain features of the carbohydrate metabolism of cornea is presented. The possible mechanisms oxidizing the reduced form of triphosphopyridine nucleotide (TPNH) generated in the hexose monophosphate shunt pathway are considered. One such mechanism is the oxidation of TPNH through the lactic dehydrogenase reaction. Evidence indicates that glucose oxidation through the shunt mechanism is inhibited by low levels of iodoacetate. Since oxidation through the citric acid cycle is not inhibited, the mechanism reoxidizing TPNH formed in the shunt mechanism appears to be different from that involved with the citric acid cycle. Moreover, the addition of pyruvate overcomes the iodoacetate inhibition of the shunt mechanism. These findings support the contention that oxidation of TPNH may proceed through the lactic dehydrogenase reaction. Certain requirements must be met before this mechanism can function under normal physiologic conditions: (1) diphosphopyridine nucleotide (DPN) must be in the oxidized form, or at least, DPNH must be of sufficiently low concentration so that it would not compete with TPNH for pyruvate; (2) a pH lower than 7.4 greatly enhances this mechanism; and (3) sufficient amounts of pyruvate must be present.

In regard to glucose metabolism, the cornea can be classified along with those tissues which exhibit aerobic glycolysis. Thus, the metabolism of the cornea would fall into the same category as that of the brain, leukocytes, retina, tumor cells, lens, and erythrocytes.

In considering the metabolism of the cornea, it is important to keep in mind the possible interactions which may exist between the different layers. There are many indirect evidences that metabolic interactions occur between the various layers, but, since this subject is discussed elsewhere in the Symposium, they will not be brought up here. The epithelium and endothelium appear metabolically active as evidenced by an oxygen consumption of approximately 6 μL per milligram per hour,[1] high levels of enzymes[1] and of pyridine nucleotides.[3] However, the main bulk of the cornea, the stroma, which consists largely of collagen imbedded in a polysaccharide ground substance, is sparsely populated with cells and seems metabolically inert. As pointed out by Herrmann and Hickman,[4] the apparent metabolic inactivity of the stroma is due to the abundance of the connective tissue component. If the rate of metabolism were expressed on a basis of cellular content, the stroma would probably prove to be as active as the epithelial layers.

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Aerobic glycolysis

The main characteristic of the metabolism of the cornea is the high rate of aerobic glycolysis.[1] By aerobic glycolysis

we mean the accumulation of lactic acid during the metabolism of glucose in the presence of oxygen. Under these conditions lactate accumulates because the Embden-Meyerhof pathway is more efficient than the aerobic mechanisms involved in the complete oxidation of glucose. That is, the breakdown of glucose to pyruvate and then to lactate occurs more rapidly than the combustion of pyruvate to CO_2 through the citric acid cycle and its associated aerobic mechanisms. In those tissues which do not exhibit aerobic glycolysis, the citric acid cycle keeps pace with the Embden-Meyerhof pathway and therefore no lactate accumulates.

Aerobic glycolysis has been observed in excised cattle cornea[5] and in all layers of the rabbit cornea.[1] DeRoetth,[5] upon comparing the accumulation of lactic acid in excised cattle corneas with and without epithelium, concluded that the epithelium had no aerobic glycolysis. Only when the epithelium was removed or damaged was aerobic glycolysis observed. This may be a species difference between the rabbit and cattle corneas. However, Langham[1] has pointed out that the absence of aerobic glycolysis in the epithelium of cattle cornea is inconsistent with the observation that lactate normally accumulates in the epithelial layer. On the other hand, the accumulation of lactate may be the result of an active transfer of this metabolite from the stroma to the epithelium. This was suggested by Herrmann and Hickman[4] who found that in an excised, intact cornea the lactate depot in the stroma was depleted upon incubation. In contrast, the disappearance of lactate from the stroma of a denuded cornea was not observed. The transfer of lactate from the stroma to the epithelium would necessitate an active mechanism since it would have to overcome not only a concentration but also an electropotential gradient. Further experiments are needed to determine the exact fate of lactate which disappears from the stroma in the presence of the epithelium. A detailed study is also needed to establish the absence of aerobic glycolysis in the epithelium of cattle cornea. Preliminary experiments have revealed that the concentration of inorganic phosphate is extremely critical for glycolysis in isolated corneal epithelium.[6] It may be possible that in DeRoetth's experiment, in which a cattle cornea was immersed in a relatively small volume of Krebs-Ringer bicarbonate buffer, an insufficient level of phosphate was the factor limiting the extent of glycolysis.

The aerobic phase of glucose metabolism

The most important aspect of glucose metabolism is that it provides biologic energy which is necessary to carry out the energy-expending processes. Although the cornea has an active Embden-Meyerof pathway, glycolysis alone appears insufficient to maintain the normally dehydrated state of the cornea. This is well documented by physiologic experiments which indicate that in the absence of oxygen swelling of the cornea results.[7-11]

Since the aerobic mechanisms provide most of the biologic energy in the cornea, it is important to understand the nature of this phase of glucose metabolism. Most of the oxygen-requiring reactions are confined to the epithelial layers; very little is found in the stroma.[1, 4] Since the endothelium is technically difficult to study, information on aerobic metabolism has been obtained from studies of the corneal epithelium.

The Krebs citric acid cycle appears to be functioning in the corneal epithelium. However, the activity of this cycle is insufficient to keep pace with lactic acid production as evidenced by its accumulation. Kuhlman and Resnik[12] have shown that the rabbit cornea was capable of oxidizing the variously labeled lactate to CO_2. In contrast to the oxidation of glucose, an appreciable amount, 28 per cent, of lactate was oxidized by the stroma and endothelium. Glucose oxidation to CO_2 was confined mainly to the epithelium. The results from a study of the relative rates of oxidation of the three carbon atoms of pyruvate appear to be consistent with the

presence of the citric acid cycle in the bovine corneal epithelium.[13]

However, the most unusual aspect of the aerobic phase of glucose metabolism in the cornea is the high activity of the hexose monophosphate shunt mechanism. It was estimated that in bovine corneal epithelium about 65 per cent of the glucose is metabolized by the conventional glycolytic scheme and 35 per cent by way of the shunt mechanism.[14] When this is compared with estimates of shunt activity in other tissues, calculated by the same procedure, the cornea ranks as one of the tissues with the highest activity.[13] More recently, Kuhlman and Resnik[12] have estimated that in rabbit cornea up to 70 per cent of the glucose oxidized to carbon dioxide is by this alternate pathway.

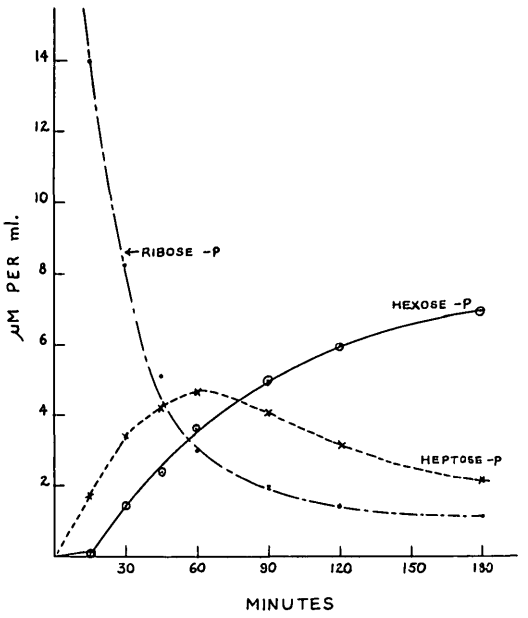

Fig. 1. Utilization of ribose-5-phosphate by corneal epithelium. Reaction mixture containing 0.020M ribose-5-phosphate, dialyzed extract of corneal epithelium (2.3 mg. dry weight) and 0.1M phosphate buffer pH 7.4 making a total volume of 1.5 ml. was incubated at 37.5° C. for varying periods.

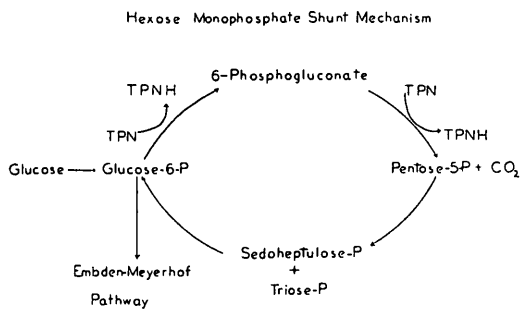

Fig. 2. Hexose monophosphate shunt mechanism.

The further reactions of the shunt mechanism also point out the importance of this pathway in the cornea. The utilization of the pentose phosphate formed in the decarboxylation of glucose phosphate is remarkably rapid in the cornea.[15] The rate at which ribose-5-phosphate is utilized in extracts of corneal epithelium is shown in Fig. 1. With only 2.3 mg. of the corneal preparation over 10 μmoles of ribose phosphate are utilized in 30 minutes. With the disappearance of ribose phosphate, the first product to appear is sedoheptulose phosphate followed by the appearance of hexose phosphate (Fig. 1). Thus, all the steps of this cycle (Fig. 2), beginning with the decarboxylation of glucose-6-phosphate, the recombination of pentose phosphate to sedoheptulose phosphate and triose phosphate, and, finally, the resynthesis of hexose phosphate have been demonstrated.

Because of the effectiveness of the pentose phosphate cycle it can be shown that the carbon 2 atom of glucose is also oxidized by the shunt mechanism. On the second turn of the cycle the carbon 2 atom of glucose becomes situated in the number 1 position of glucose. Then, as the newly synthesized glucose undergoes the reactions of the shunt mechanism, the carbon 2 atom of the original glucose molecule is decarboxylated. In rabbit cornea[12] and in bovine corneal epithelium[13] it was shown by different techniques that considerable quantities of the carbon 2 atom of glucose are oxidized through the shunt mechanism. This has not been clearly demonstrated in other tissues.

Mechanisms for the reoxidation of TPNH

In the hexose monophosphate oxidation pathway, there are two reactions

which require triphosphopyridine nucleotide (TPN) in the oxidized form (Fig. 2). In these reactions the pyridine nucleotide is reduced (TPNH) by accepting electrons from the substrates oxidized. Since TPN is available only in limited quantities, once reduced it must be reoxidized; otherwise the shunt mechanism would cease without appreciable amounts of CO_2 being produced. In a tissue such as the cornea in which the shunt mechanism is so active, it is important to consider the mechanisms involved in the reoxidation of TPNH. For TPNH to be a source of biologic energy, its reoxidation must proceed through the electron transmitting system and its associated phosphorylation mechanism. The TPN-cytochrome C reductase, which may channel TPNH into the electron transmitting system, is one possible means by which this is accomplished. However, there has been no conclusive evidence that TPNH generated outside of the mitochondria has been actually geared to oxidative phosphorylation. In fact, Kaplan and associates[16] observed that the oxidation of TPN in isolated mitochondria of liver did not yield significant amounts of high energy phosphate bonds.

Transhydrogenase is another enzyme which may participate in the oxidation of TPN by the following reaction: TPNH + DPN = TPN + DPNH. The reduced diphosphopyridine nucleotide (DPNH) formed can then be oxidized through the electron transmitting system with the production of energy. There is no evidence that this enzyme functions in the cornea.

The third possible scheme by which reoxidation may take place is by the TPNH-linked dehydrogenases. Through these dehydrogenases it is now generally accepted that in most tissues TPNH formed in the shunt mechanism serves as a hydrogen donor in the synthesis of fatty acid, steroids, and in reductive amination reactions.[17] In the cornea there are two other dehydrogenases which participate in similar reactions. A survey indicated that those tissues which have high activity of the shunt dehydrogenases also have a high activity of glutathione reductase.[18] From this, the possibility that a coupling of these dehydrogenases may function to oxidize TPNH and reduce glutathione was proposed. Recently, Carson[19] has pointed out that in erythrocytes the interaction of the dehydrogenases of the shunt mechanism and glutathione reductase controls the level of glutathione. A defect in the glucose-6-phosphate dehydrogenase, as observed in the primaquine-sensitive individuals, produces a lowering of the glutathione content in red cells. This interaction of dehydrogenases is also demonstrable in the corneal epithelium[15] and in lens,[18] where glutathione is in abundance, but the importance of this mechanism in these tissues has not been ascertained.

The other possibility is that the reoxidation of TPNH may involve lactic dehydrogenase. The evidence for this mechanism is examined in detail here since the initial exploration into this area was stimulated by a study with the corneal epithelium.[20] It was shown that an interaction of the dehydrogenases of the shunt mechanism with lactic dehydrogenase (LDH) was possible. This reaction is not only confined to the cornea, since it was found to occur in retina,[21, 22] ascites tumor cells,[23] lens,[6] and in leukocytes.[24] The common property of all these tissues is that they exhibit active aerobic glycolysis. The stimulation of the oxidation of 1-C-14 glucose to C-14-O_2 by pyruvate was the manner by which this coupling was shown to occur in the corneal epithelium.[19] Even under nitrogen, in the presence of added pyruvate, the carbon 1 atom (C-1) of glucose was rapidly converted to CO_2. The reason for this was that the LDH of the corneal epithelium is capable of functioning with TPNH. Thus, glucose was oxidatively decarboxylated through the shunt mechanism with a release of CO_2 and TPNH. The TPNH was reoxidized with the reduction of pyruvate to lactate mediated by LDH. Previous to this study, DPN was thought to be the only coenzyme with which this enzyme would

function. Although DPN is the preferred coenzyme, LDH is capable of reacting with TPNH, and, since this enzyme is present in such high quantities in the cornea,[2] the activity with TPNH may be of considerable significance. Studies of LDH in the corneal epithelium have revealed some interesting features.[21] Separation of LDH activities by electrophoresis on starch paste has revealed the presence of three peaks of activity (Fig. 3). Multiple forms of LDH from beef cornea were also demonstrated by another technique.[25] As shown in Fig. 2, each of these fractions was active with TPNH although the activity with DPNH was always higher. The pH optimum studies indicated that the LDH activity with TPNH was low at pH 7.4 but steadily increased acid to this pH until a peak was reached near 5.5.[21] It may be argued that, since the TPNH activity of LDH is low at pH 7.4, it is of little physiologic significance. However, the participation of LDH should not be ruled out on this basis alone since the intracellular pH of the corneal epithelium is not known and pyruvate does stimulate the decarboxylation of 1-C-14 glucose.

Preliminary studies on the effect of iodoacetate on CO_2 production from pyruvate and glucose support the view that the mechanism of oxidation of TPNH formed in the shunt mechanism is not by the direct intervention of the electron transmitting system (Table I). Iodoacetate did not seem to affect the oxidation of 2-C-14 and 3-C-14 pyruvate in bovine corneal epithelium. Apparently, inhibition of the reoxidation of TPNH generated in the isocitric dehydrogenase reaction did not occur; otherwise the oxidation of pyruvate through the Krebs cycle would have been severely curtailed. Since the site of this reaction is in the mitochondria, the oxidation of TPNH was probably through the electron transmitting system. In contrast to this, the oxidation of 1-C-14 glucose was inhibited by iodoacetate. The oxidation through the shunt mechanism, obtained from the C-14-O_2 data of C-1 minus C-6, was inhibited by 85 per cent at 10^{-3} M and

Table I. Effect of iodoacetate on the oxidation of glucose and pyruvate in bovine corneal epithelium

Labeled substrate	*Additions*		*C-14-O_2 produced (μmoles)*	*Lactate formed (μmoles)*
	IAA	*Pyruvate*		
1-C-14 glucose				
	0	0	5.4	10.5
	0	0.02M	6.2	14.5
	10^{-3}M	0	0.9	1.2
	10^{-3}M	0.02M	3.9	
	5×10^{-4}M	0	2.1	2.0
	5×10^{-4}M	0.02M	5.5	
6-C-14 glucose				
	0	0	0.7	
	0	0.02M	0.5	
	10^{-3}M	0	0.2	
	5×10^{-4}M	0	0.3	
	5×10^{-4}M	0.02M	Negligible	
2-C-14 pyruvate				
	0	0	4.2	
	10^{-3}M	0	4.4	
3-C-14 pyruvate				
	0	0	2.3	
	10^{-3}M	0	2.6	

The averages of at least 4 experiments are expressed in micromoles formed every 3 hours per 100 mg. dry weight of bovine corneal epithelium. The reaction vessel contained sheets of bovine corneal epithelium (500 mg. wet weight) in 6.0 ml. of Krebs-Ringer bicarbonate buffer containing 120 μmoles of labeled substrate. Incubation was carried out in a gas phase of 95 per cent O_2 and 5 per cent CO_2 for 3 hours at 38° C. Procedures employed were the same as those given in previous papers.[13, 20]

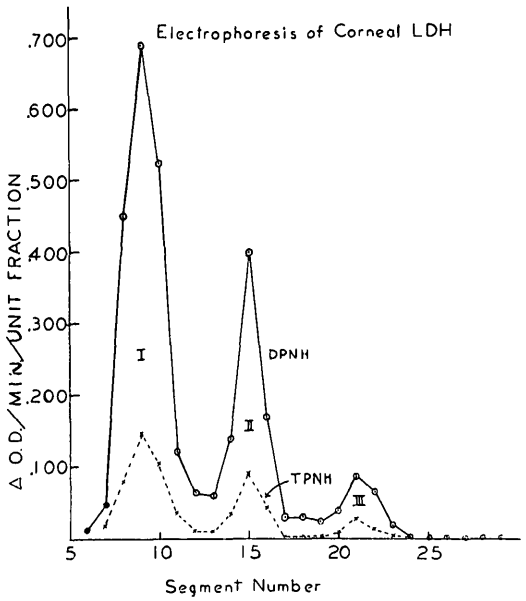

Fig. 3. Separation of the lactic dehydrogenase activity of corneal epithelium.

by 62 per cent at 5×10^{-4} M. At the 10^{-3} M iodoacetate level, the inhibition was overcome only partially by the addition of pyruvate. However, at the lower level of iodoacetate (5×10^{-4} M), the addition of pyruvate completely restored the C-1 oxidation of glucose through the shunt mechanism to normal rates. The results support the hypothesis that the reoxidation of TPNH formed in the shunt mechanism is not by the same mechanism as that which oxidizes the TPNH generated in the isocitric dehydrogenase reaction.

As evidenced by a decrease in lactate production, iodoacetate inhibited glycolysis (Table I). The concomitant inhibition of glycolysis and the shunt mechanism is consistent with the hypothesis that pyruvate serves as the electron acceptor of TPNH. The C-1 oxidation of glucose may have been depressed because pyruvate, normally generated through glycolysis, was not sufficiently available. When pyruvate was added to the inhibited system, the decarboxylation was accelerated. Although these results do not establish conclusively that this system is functioning, they strongly suggest that this is the case.

The use of iodoacetate in this type of experiment was previously employed by Cohen and Noell[22, 26] in the study of the metabolism of the retina. Moreover, Wenner[23] is also of the opinion that in ascites tumor cells, pyruvate is linked to C-1 oxidation of glucose through LDH in the manner proposed for the corneal epithelium and retina. These investigators have expressed the opinion that in ascites tumor cells and in retina the principal electron acceptor for TPNH oxidation is pyruvate since the iodoacetate inhibition of the decarboxylation of C-1 of glucose is overcome by pyruvate. This can be further interpreted to suggest that glycolysis governs the rate of the hexose monophosphate shunt mechanism.

The study of the effects of Pontocaine on glucose metabolism in the corneal epithelium,[27] however, reveals that the hexose monophosphate shunt is not dependent solely on glycolysis. The effect of Pontocaine on corneal glucose metabolism is summarized in Table II. The results indicate that in the presence of 0.003 M Pontocaine a marked drop in C-1 oxidation of glucose was observed. In contrast to the iodoacetate experiment, the oxidation of C-14 pyruvate was also inhibited. On the other hand, the Embden-Meyerhof pathway was not affected. In fact, because the further combustion of glucose to CO_2 was essentially abolished, glycolysis appeared to increase in the presence of Pontocaine. In the absence of the drug about 9 μmoles of 1-C-14 glucose and 13 μmoles of 6-C-14 glucose were converted to C-14 lactate. The difference is due to the considerable quantities of C-14 from 1-C-14 glucose lost as CO_2 in the shunt mechanism. Pontocaine prevented this oxidation and thus the same quantity, 18 μmoles, of C-14 was recovered in lactate from each form of labeled glucose. In the presence of the drug, the tissue loses its capacity to oxidize glucose to CO_2 either through the shunt mechanism or citric acid cycle. This is consistent with the findings of Herrmann and co-workers[28] who were

Table II. Effect of Pontocaine on the oxidation of glucose and pyruvate in bovine corneal epithelium

Labeled substrate	*Pontocaine*	*C-14 recovered in μmoles*		*% inhibition*
		Lactate	*CO_2*	
1-C-14 glucose				
	0	9.1	5.8	—
	9×10^{-4}M		5.6	0
	1.3×10^{-3}M		4.2	27
	2.0×10^{-3}M		1.6	73
	3.0×10^{-3}M	18.0	1.2	80
6-C-14 glucose				
	0	13.2	0.8	—
	3.0×10^{-3}M	18.5	Negligible	<100
1-C-14 pyruvate				
	0		11.8	—
	1.3×10^{-4}M		9.5	20
	3.0×10^{-3}M		1.2	90
2-C-14 pyruvate				
	0		4.8	—
	3.0×10^{-3}M		Negligible	<100

The averages of at least 4 experiments are expressed in μmoles every 3 hrs. per 100 mg. dry weight of corneal epithelium. Description of the reaction mixture is the same as given in Table I.

the first to show that Pontocaine causes an 80 per cent inhibition of respiration of the cornea. The site of inhibition was not at the dehydrogenase level, nor was it at the stage of the TPNH or DPNH cytochrome C reductases.[27]

Let us consider how the results obtained with Pontocaine bear on the hypothesis of the mechanism of TPNH oxidation involving LDH. Since the Embden-Meyerhof pathway which generates pyruvate is active in the presence of Pontocaine, one might expect no change to occur in the C-1 oxidation of glucose if the mechanism for TPNH oxidation involved pyruvate. However, the results showed a marked lowering of C-14-O_2 production from 1-C-14 glucose. The explanation probably is that a competition of DPNH and TPNH for pyruvate results because Pontocaine also inhibits the reoxidation of DPNH. Since LDH is considerably more active with DPNH as shown in Fig. 3, practically no TPNH is oxidized by this means. It is obvious that for LDH to function with TPNH it is necessary to maintain DPN in the oxidized form.

A summary of facts concerning the reoxidation of TPNH by pyruvate obtained from studies with the corneal epithelium,[20] liver,[29] retina,[22, 26] and ascites tumor cells[23] is as follows: The TPNH formed in the dehydrogenation and decarboxylation of glucose phosphate through the shunt mechanism can be reoxidized by the LDH reaction converting pyruvate to lactate. Pyruvate can serve as an electron acceptor of TPNH provided that DPN is maintained in the oxidized form and sufficient amounts of pyruvate are available. A pH lower than 7.4 greatly facilitates this mechanism. Lactate can be regarded as a reservoir of energy in that it can eventually cause a reduction of DPN which, in turn, can be channeled into the electron transmitting system for the production of ATP. In effect, the TPNH formed in the shunt mechanism is converted to DPNH. Thus, the coupling of the dehydrogenases of the dehydrogenases of the shunt mechanism with LDH provides the tissue with a pyridine nucleotide transhydrogenation mechanism.

The available evidence seems to support the possibility that TPNH formed in reactions localized in the soluble cytoplasm may be oxidized by pyruvate. It is an espe-

cially attractive hypothesis for those tissues which exhibit aerobic glycolysis since it would account for the accumulation of lactate in most cases. The reoxidation of TPNH by pyruvate has been demonstrated not only in the corneal epithelium but also in the retina,[21, 22, 26] ascites tumor cells,[23] and in the liver.[29] A marked increase in the C-1 oxidation of glucose in leukocytes during phagocytosis has been observed, and the evidence obtained strongly suggests that TPNH reoxidation is by the LDH mechanism.[24] However, the main body of evidence supporting this mechanism in the corneal epithelium, retina, and ascites tumor cells comes from the iodoacetate experiments. It is conceivable that iodoacetate is blocking other alternate pathways of oxidizing TPNH and the addition of excess pyruvate circumvents this by forcing the TPNH-linked LDH to come into play although normally it is not functioning. Therefore, other experimental approaches bearing on this problem are needed before a major role can be assigned to this mechanism.

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