Metabolism of the Retina

I. RESPIRATION OF CATTLE RETINA*

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In view of the many interesting metabolic properties of the retina it is surprising to find that relatively few investigations on this subject have been reported. The retina is reputed to have high rates of respiration and glycolysis as well as a strong Pasteur effect (1). In this paper observations on the metabolism of the retina dealing with various aspects of respiration are reported. An attempt was made to identify the endogenous substrate capable of sustaining the respiration of retinal tissue. This led to a study of the cofactors and substrates capable of stimulating the respiration of retinal homogenates. In the course of these studies it was possible to obtain evidence indicating the presence of the tricarboxylic acid cycle and the phosphogluconate oxidative pathway in this ocular tissue.

EXPERIMENTAL

Lactic acid was determined by the method of Barker and Summerson (2), hexose by the anthrone method (3), and α-keto-acids by the Friedemann and Haugen method (4). Oxygen consumption was determined in the Warburg apparatus with air as the gas phase and NaOH in the center well. To determine CO_2 production additional flasks were employed, which lacked alkali in the center wells, but contained H_2SO_4 in the side arms. At the end of the incubation period the acid was tipped in, and the CO_2 produced was calculated from the gas evolved, after correction for that present at the beginning of the incubation period. For the calculation it was assumed that oxygen consumption was not altered in flasks which lacked alkali in the center wells to trap the CO_2 formed. The methods used for the incubation of C^{14}-glucose, and recovery and analysis of $C^{14}O_2$ and C^{14}-lactate have been described (5). Cattle eyes were obtained from a local slaughter house.

RESULTS

As shown in Table I, the Q_{O_2} of the intact retina was about 10 and decreased gradually with time. The addition of glucose to the incubation medium did not elevate the O_2 consumption nor did it sustain the rate. It appeared that the respiration was maintained by the presence of endogenous substrate in the retina. Even in homogenates the Q_{O_2} was maintained nearly as high as that of the intact tissue for about 1 hour. With added glucose, the Q_{O_2} of retinal homogenates was stimulated somewhat and did not drop off as markedly as it did without the added substrate. To obtain information on the optimal conditions for the respiration of retinal homogenates, a study was made of the cofactors capable of stimulating respiration and aerobic glycolysis in retinal homogenates supplemented with glucose. It was found that even without any added cofactors retinal homogenates consumed oxygen at nearly half the rate of fortified preparations and, in addition, were able to accumulate some lactic acid (Table II). With fortified homogenates a Q_{O_2} of 14 was frequently obtained during the 1st hour, whereas the intact tissue generally gave a Q_{O_2} of about 10. The presence of all of the cofactors appeared to be necessary for a maximal rate of respiration. Lactic acid formation was depressed by the omission of either $MgSO_4$ or ATP, but not by the omission of DPN.

Although the high endogenous respiration in other tissues (6) has sometimes been attributed to the utilization of glycogen, or of fatty acids (7), R.Q.'s approaching 1 have been reported in studies of the retina (8), and the glycogen content of cattle retina has been reported to be only 92 mg./100 gm. of tissue (9). This level amounts to 2.8 μmoles of glycogen per retina (50 mg. of dry weight) and thus could account for only about 50 per cent of the oxygen consumed in 3 hours by the intact tissue. To demonstrate experimentally that glucose or glycogen was not the principal endogenous substrate supporting respiration, a study of the effect of iodoacetate was undertaken (Table III). It was not possible to find a concentration of iodoacetate which would block glycolysis completely without showing some inhibition of lactate utilization. However, it was possible to show for the intact tissue and retinal homogenates that during the 1st hour of incubation endogenous respiration was inhibited only about 15 per cent by iodoacetate at a concentration that suppressed glycolysis almost completely. Thus during the 1st hour of incubation respiration could be supported by endogenous substrate even in the absence of an active glycolysis. Noteworthy in these experiments (Table III) is the high capacity of the retina for aerobic glycolysis. This is illustrated by the accumulation of large amounts of lactic acid when glucose is added to the medium and demonstrates the fact that the glycolytic rate of this tissue far exceeds the rate at which its tricarboxylic acid cycle operates.

The possibility that the phosphogluconate oxidation pathway might be contributing to the endogenous respiration of the retina was then considered. In view of the report by Eichel (10)

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TABLE I

Respiration of cattle retina

Flasks containing 2 μmoles of DPN, 0.02 μmole of cytochrome *c*, 110 μmoles of phosphate buffer of pH 7.4, glucose as indicated, and either a whole retina or homogenate equivalent to one retina (50 mg. of dry weight) in a final volume of 3.0 ml. were incubated at 37°. Each value reported is the mean of four observations.

Tissue	Glucose, 100 μmoles	Q_{O_2}		
		1st hour	2nd hour	3rd hour
		μl./hr./mg. dry weight		
Whole retina		10.6 ± 0.4	8.4 ± 0.8	6.0 ± 0.9
Whole retina	+	10.5 ± 0.7	9.7 ± 1.0	8.1 ± 1.6
Homogenate		9.1 ± 0.9	4.8 ± 0.8	2.0 ± 0.3
Homogenate	+	10.3 ± 1.0	8.0 ± 0.8	6.8 ± 1.0

TABLE II

Effect of supplements on respiration of cattle retina homogenates

Each flask contained homogenate equivalent to 11.7 mg. of dry weight, 30 μmoles of glucose, 110 μmoles of phosphate buffer of pH 7.4, and supplements as indicated in a final volume of 3.0 ml. Flasks were incubated at 37°. The supplement mixture contained 9 μmoles of $MgSO_4$, 6 μmoles of DPN, 6 μmoles of ATP, and 0.12 μmole of cytochrome *c*.

Additions	Q_{O_2}				Lactic acid formed in 4 hours
	1st hour	2nd hour	3rd hour	4th hour	
					μmoles
Supplement mixture	14.3	13.8	9.4	5.0	27.5
Without $MgSO_4$	12.4	8.8	5.3	5.2	8.8
Without DPN	9.2	6.2	4.8	3.7	29.7
Without ATP	9.6	8.2	6.8	6.2	10.2
Without cytochrome *c*	8.7	7.5	3.8	2.4	28.6
With no supplements	6.4	5.3	4.2	3.0	8.3

TABLE III

Effect of inhibitors on respiration of cattle retina

Flasks containing either one retina (50 mg. of dry weight), 110 μmoles of phosphate buffer of pH 7.4, and additions as indicated in a final volume of 3.0 ml., or retinal homogenate equivalent to 17 mg. dry weight, 9 μmoles of $MgSO_4$, 6 μmoles of DPN, 6 μmoles of ATP, 0.12 μmole of cytochrome *c*, 110 μmoles of phosphate buffer of pH 7.4, and additions as indicated in a final volume of 3.0 ml. were incubated at 37°.

Tissue	Additions			Q_{O_2}			Lactic acid found after 3 hours
	Glucose	Iodoacetate	Lactate	1st hour	2nd hour	3rd hour	
	μmoles	*μmoles*	*μmoles*				*μmoles*
Whole retina	0	0	0	10.3	9.1	5.9	0.67
	0	15	0	8.6	2.1	0.8	0.98
	100	0	0	9.8	8.3	6.7	98.3
	100	15	0	8.2	2.0	0.6	1.2
Homogenate	0	0	0	10.5	5.2	3.2	0.52
	0	0.7	0	9.3	3.0	1.4	0.34
	0	0	30	11.8	11.6	11.0	23.7
	0	0.7	30	11.0	9.6	8.7	24.1
	30	0	0	13.7	11.4	8.1	33.4
	30	0.7	0	10.2	5.9	2.9	1.2

TABLE IV

C^{14}-glucose metabolism of cattle retina

Cattle retina (50 mg., dry weight) was incubated in 8.0 ml. of modified Krebs-Ringer buffer of pH 7.4 for 1 hour at 38°. The values are corrected to an initial specific activity of the 1-C^{14}-glucose which was 350 c.p.m. per μmole. The values are reported as the mean of four observations.

Substrate	Gas phase	Glucose utilized	Lactate produced		Pyruvate added	$C^{14}O_2$
		μmoles	*μmoles*	*c.p.m.*	*μmoles*	*c.p.m.*
1-C^{14}-glucose	Air	48	84	14,400	0	406
6-C^{14}-glucose	Air	46	87	16,125	0	347
1-C^{14}-glucose	N_2				0	34
1-C^{14}-glucose	N_2				160	520
6-C^{14}-glucose	N_2				0	25
6-C^{14}-glucose					160	35

indicating the presence of a high concentration of glucose 6-phosphate dehydrogenase in the retina, it seemed worthwhile to establish whether the operation of this pathway might account for the respiration occurring in the presence of iodoacetate. To determine the significance of the phosphogluconate oxidation pathway in the aerobic metabolism of glucose, intact retinas were incubated with either glucose-1-C^{14} or glucose-6-C^{14}. As shown in Table IV, no evidence of any significant preferential oxidation of the carbon 1 (C-1) atom of glucose was observed in the retina, unlike other ocular tissues (11, 12). The rate at which the C-1 and C-6 of glucose was oxidized to CO_2 was about the same, as indicated by the C-1 to C-6 ratio of 1.2. Furthermore, the amount of C^{14} incorporation into lactic acid was about the same from either 1-C^{14}- or 6-C^{14}-glucose. These results strongly suggest that the phosphogluconate oxidation pathway does not play a significant role in the utilization of glucose by the retina. Although the phosphogluconate oxidation pathway does not appear to play an active role, it is possible to obtain evidence suggesting the presence of this pathway in the retina. In those tissues which possess a lactic dehydrogenase capable of reacting to some extent with TPN it is possible to couple the oxidation of 1-C^{14}-glucose to $C^{14}O_2$ by the dehydrogenases of the phosphogluconate pathway to the reduction of pyruvate (13). In an atmosphere of nitrogen the retina was capable of stimulating the oxidation of 1-C^{14}-glucose with pyruvate. As shown in Table IV, in the absence of pyruvate there occurs only a small amount of 1-C^{14}-glucose oxidation, but the addition of pyruvate in the medium markedly stimulates the C-1 oxidation. No stimulation of C-6 oxidation of glucose was observed under the same conditions. It is interesting to note that the corneal epithelium and retina, known for their high content of lactic dehydrogenase, are the tissues in which the coupling of the dehydrogenases of the glycolytic mechanism and the phosphogluconate pathway is most easily demonstrated.

The observations made thus far seemed to rule out glucose or glycogen as the principal endogenous substrate supporting respiration in the presence of iodoacetate. It seemed likely that lactic acid might be contributing significantly to endogenous respiration. An attempt was made to relate changes in the initial quantities of lactic acid and hexose to the amounts of pyruvate and CO_2 produced in retinal homogenates incubated for 1 hour. Initially cattle retinas contain from 1 to 3 μmoles

TABLE V

Utilization of endogenous substrate by cattle retina

Flasks containing 2 μmoles of DPN, 0.02 μmole of cytochrome *c*, 110 μmoles of phosphate buffer of pH 7.4, retinal homogenate equivalent to 46 mg. of dry weight, and inhibitors as indicated in a final volume of 3.0 ml. were incubated at 37° for 1 hour. Initial concentrations of lactate, hexose, and pyruvate, expressed as μ atoms of carbon, were, respectively, 17.3, 16.1, and 0.3. The results are the mean of four observations.

Inhibitors	μ atoms of carbon utilized			μ atoms of carbon formed			μ atoms of oxygen consumed		Per cent accounted for by products formed
	Lactate	Glucose	Total	Pyruvate*	CO_2	Total	Observed	Expected†	
None	−9.6	−3.6	−13.2	+6.0	+9.8	+15.8	14.6	10.9	75
Iodoacetate (15 μmoles)	−12.0		−12.0	+6.1	+6.7	+12.8	9.7	7.7	79
Arsenite (12 μmoles)	−9.4	−4.9	−14.3	+12.8	+5.9	+18.7	10.0	8.0	80

* The α-keto acid accumulating in the presence of arsenite was converted to the 2,4-dinitrophenylhydrazone. The absorption spectrum was identical with that of the derivative prepared from pyruvic acid. The 2,4-dinitrophenylhydrazone of the reaction product and of pyruvic acid each migrated at the same rate in 1 per cent Na_2CO_3, *n*-butanol:ethanol:H_2O (50:10:40) by volume, phenol saturated with water, and 3 per cent NH_3.

† The expected oxygen consumption was calculated from the quantities of pyruvate and CO_2 formed, on the assumption that these compounds arose from lactate or hexose.

TABLE VI

Effect of various substrates on retinal respiration

Cattle retina homogenate equivalent to 11.5 mg. of dry weight was incubated for 1 hour at 37° in flasks containing 9 μmoles of $MgSO_4$, 6 μmoles of DPN, 6 μmoles of ATP, 0.12 μmole of cytochrome *c*, 110 μmoles of phosphate buffer of pH 7.4, and 30 μmoles of substrate as indicated.

Substrate	Q_{O_2}			Substrate	Q_{O_2}		
	1st hour	2nd hour	3rd hour		1st hour	2nd hour	3rd hour
None	8.0	4.7	3.7	None	5.7	4.0	3.0
Glucose	12.8	10.1	8.2	Glucose	11.1	8.3	7.9
L-Lactate	14.9	9.0	7.6	α-Ketoglutarate	10.7	7.5	4.6
Pyruvate	10.3	8.0	7.7	Succinate	13.4	7.4	4.8
Citrate	14.4	10.0	7.5	Fumarate	9.5	6.5	5.0
DL-Isocitrate	12.7	6.5	4.8	L-Malate	11.9	7.0	5.9

of hexose per retina (50 mg., dry weight) and from 4 to 5 μmoles of lactic acid. The results, expressed as μ atoms of carbon, Table V, indicated that considerably more lactic acid was utilized than hexose. Together the quantities of lactate and hexose utilized were sufficient to account for the major portion of the pyruvate and CO_2 formed. These products accounted for about 75 per cent of the oxygen consumed. In the presence of iodoacetate lactic acid utilization was unimpaired; no hexose was utilized. In this experiment the amount of CO_2 and pyruvate produced was equivalent to the lactic acid consumed and sufficient to account for nearly 80 per cent of the oxygen utilized. In the presence of arsenite considerably more pyruvate accumulated than in its absence. No α-ketoglutarate accumulated, although retinal tissue is reported to contain 1.83 mg. of glutamic acid per gm. of tissue (14), amounting to 2.9 μmoles per cattle retina, and has been shown to accumulate α-ketoglutarate when incubated with glutamic acid in the presence of arsenite (15). These experiments suggest lactate to be a major endogenous substrate contributing to respiration in the presence of iodoacetate and indicate that at least 25 per cent of the observed endogenous respiration cannot be accounted for by the utilization of hexose or lactate.

The fact that lactic acid appeared to be an important endogenous substrate suggested that an active tricarboxylic acid cycle was functioning in the retina. The ability of the intermediates of the tricarboxylic acid cycle to stimulate oxygen consumption in retinal homogenates, as shown in Table VI, supports the view that the tricarboxylic acid cycle is operating in this tissue. A more detailed study of the tricarboxylic acid cycle in retinal mitochondria is now being undertaken.

DISCUSSION

The very high rate of respiration, (Q_{O_2} = 31) that has been attributed to the retina is based on Warburg's data (16) in which various rat tissue slices including retina, were compared. However, data compiled by deBerardinis (17) indicate considerable variation between species in the rate of oxygen consumption of the retina. In our experiments the Q_{O_2} of cattle retinas was approximately 10 which is within the range previously reported by Greig and Munro (18). It should be noted that in these studies from 1 to 2 hours elapsed between the removal of the cattle eyes at the slaughter house and the addition of retinal tissue to the flasks. However, retinas from eyes stored for 2 hours consumed oxygen at about the same rate as those used after 1 hour. Moreover, experiments in which the Q_{O_2} was determined for rabbit retinas, removed immediately after death and also 1 hour thereafter, indicated that there was no significant decrease in the respiratory capacity of the retina upon brief storage in the intact eye.

The outstanding metabolic property of the retina seems to be

its capacity for aerobic glycolysis. In these studies *in vitro*, the aerobic oxidative mechanisms of the cattle retina cannot keep pace with the glycolysis and consequently lactic acid accumulates. In view of these results, it seems somewhat surprising that the retina *in situ* does not have a higher concentration of lactic acid. Adequate supplies of glucose must be available to this tissue since it is vascularized. Yet one is left with the impression that only a small fraction of the total capacity for glycolysis is ever utilized. Possibly lactate is actually formed at a much higher rate than is apparent, but diffusion into surrounding tissues never allows it to reach an unfavorable level. If this is so, the diffusion of lactate is probably in the direction of the choroid and not into the vitreous humor since only a small amount of lactate is found in the latter tissue. The existence of a regulatory mechanism is a more attractive possibility. There may be some control of the glycolytic mechanism *in vivo* which is not operative under conditions *in vitro*. These questions on glycolysis and the important relationship to the Pasteur effect are problems currently being investigated.

SUMMARY

The utilization of endogenous lactic acid and hexose appears to contribute significantly to the endogenous respiration of cattle retina homogenates. Tricarboxylic acid cycle substrates increase the oxygen consumption of homogenates.

The utilization of glucose by the retina appears to occur almost exclusively through glycolysis and the tricarboxylic acid cycle, although under suitable conditions the hexose monophosphate shunt pathway can be activated.

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