Aerobic Glycolysis and Its Role in Maintenance of High O_2 Tensions in the Teleost Retina[1] (35657)

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Both oxygen tension and glucose availability at the cellular level are known to affect the metabolism of carbohydrate by tissues and they are referred to as the Pasteur and Crabtree effects, respectively. During the past 15 years, little work has been reported on the study of these two effects (1).

The teleost retina is characterized by a high rate of glycolysis, and the energy derived therefrom provides the bulk of that needed for photochemical visual processes. The additional energy needed is derived from the complete oxidation of glucose by the tricarboxylic acid cycle (TCA).

Oxygen tensions have been measured at the margin of the pigmented epithelium of the trout retina and were found to be approximately 400 mm Hg, some 20 times greater than arterial P_{O_2} (2). The authors hypothesized that the high oxygen tensions were due in part to a shift of the oxygen dissociation curve to the right, caused by a significant lowering of the pH which resulted from a rapid rate of acid metabolite production by cells in this locale. The release of oxygen from hemoglobin coupled with a countercurrent diffusion multiplying system results in the generation of high O_2 tensions. It was further speculated that lactic acid derived from a high rate of aerobic glycolysis and CO_2 from TCA cycle activity were the major acid metabolites causing the Bohr shift.

To our knowledge, only one attempt has been made to measure aerobic glycolysis in the teleost retina. In this *in vitro* study de Vincentiis (3) found that lactic acid production by retinas of a marine teleost subjected to 100% oxygen was slightly inhibited by high oxygen but unfortunately the absolute rates of glycolysis were not given. Davson (4) interprets de Vincentiis' report as indicating an absence of aerobic glycolysis in the teleost retina.

The experiments reported here represent an attempt to determine quantitatively the level of aerobic and anaerobic carbohydrate metabolism of rainbow trout retinas *in vitro*. In addition, the effects of oxygen concentration (P_{O_2}) and glucose concentration on carbohydrate metabolism were investigated.

Methods. Rainbow trout (*Salmo gairdneri*) were obtained from the Michigan Department of Natural Resources at Grayling, Michigan. The trout were 2–2½ years old, between 23 and 28 cm in length, and weighted from 380 to 480 g.

The trout were killed by severing the spinal cord at the cervical level and both eyes were enucleated. Eye dressing forceps were carefully inserted into a small circular incision in the posterior surface of the sclera and the retina and choroid were freed from the sclera by gentle scraping. The retina, with its adhering choroid and vitreous body, was removed from the globe and placed in sterile Ringer solution. The retina was separated from the choroid and vitreous body, blotted dry, weighed and placed in the appropriate incubation media. The retinal weights ranged between 290 and 450 mg with never more than a 50-mg variation between retinas from the same fish.

The media and tissue were contained in a sterilized center well constructed of Pyrex glass and having a volume of approximately 2.0 ml. The center well was suspended from a rubber stopper which was fitted into a 165-ml capacity graduated milk dilution bottle (No. 1370, Corning Glassware Co., Corn-

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ing, N.Y.). An 18-gauge needle with attached 3-way plastic stopcock (No. K-75, Pharmaseal Laboratories, Glendale, Calif.) was permanently inserted into the stopper. The needle stopcock assembly provided for the entrance of gas into the incubation flask.

Gas mixtures were made by means of a water displacement spirometer. The spirometer was attached to a mercury manometer which allowed an accurate determination of the pressure within the incubation flasks. The flasks were connected to a multioutlet exhaust manifold. A vacuum was utilized to evacuate over 95% of the air before gas, at atmospheric pressure, was admitted into the flasks. After gassing three times, the pressure in the bottles was again allowed to reach atmospheric pressure before the tissues were incubated.

The retinas were incubated for varying time periods at 13°. Shaking was accomplished by placing the media bottles on a sliding platform, cam driven by an electric motor. The speed at which the platform was driven was adjusted to provide maximum agitation of the media without splashing it from the center well. After the completion of incubation 0.3 ml of the samples were withdrawn, protein was precipitated using $Ba(OH)_2$ and $ZnSO_4$, and glucose was determined by the Glucostat Enzymatic Micromethod (Worthington Biological Corp., Freehold, N.J.). The method of Barker and Summerson (5) was employed for the determination of lactic acid.

To study the effect of varying oxygen concentrations, the retinas were weighed and placed in 0.5 ml of modified Medium 199 (Grand Island Biological Co., Grand Island, N.Y.) consisting of an Earles base and modified to contain 1.25 g/liter $NaHCO_3$, 0.5 g/liter glucose and no phenol red. The medium in equilibrium with 5% CO_2 at 13° had a pH of 7.0 and an osmolarity of 289 mOsm/kg. The rubber stoppers with suspended center wells were securely inserted into the milk dilution bottles and the bottles gassed with either 95% N_2–5% CO_2 or 95% O_2–5% CO_2. Incubation times were ½, 1, and 3 hr.

The effects of varying glucose concentrations were investigated by incubating the retinas in Mammalian Krebs Saline Medium having an osmolarity of 289 mOsm/kg and a pH of 7.6. Glucose was added to give concentrations ranging from 100 mg % to a maximum of 200 mg %. The retinas were prepared as above and incubated for 1 hr in 99.5% O_2–0.5% CO_2.

TABLE I. Effect of Sonification on Anaerobic Retinal Metabolism in Phosphate Buffered Saline with 100 mg% Glucose for 5 hr at 13°.

Treatment	Glucose utilized (μM/hr/g-wet)	Lactate produced (μM/hr/g-wet)
Whole	4.79 ± 0.36 (2)[a]	5.79 ± 0.03 (2)[a]
Sonified	0.61 ± 0.44 (2)	0.12 ± 0.08 (2)

Note: Mean ± SE (number of observations).
[a] Significant difference between sonified and whole $p < 0.001$.

Following cellular disruption, glucose utilization and lactic acid production were measured. One intact retina was removed and placed in a center well containing 0.5 ml of Phosphate Buffered Saline (PBS) (Grand Island Biological Co., Grand Island, N.Y.) containing 100 mg % glucose. The contralateral retina was homogenized in PBS by means of a Sonifier Cell Disrupter fitted with a micro-tip (Heat Systems Co., Melville, L.I., N.Y.). During the sonification procedure, excessive heating was prevented by immersion of the test tube containing the homogenate in ice water. The tissue preparations were incubated in 95% N_2–5% CO_2 for 5 hr.

Results. Data presented in Table I clearly indicate that under anaerobic conditions utilization of glucose and lactic acid production by trout retinas are profoundly affected by cellular disruption. Retinal homogenates had a level of glucose utilization some 87% below that of intact retinas, whereas, lactic acid production was virtually absent (98% inhibition) in homogenized retinas.

The effects of increasing glucose concentrations on the *in vitro* carbohydrate metabolism of intact retinas (nonsonified) are illustrated in Table II. As glucose concentration increased both glucose utilization and lactic acid production increased, the latter showed the more proportional increase. Glucose con-

TABLE II. Effect of Varying Glucose Concentrations on Aerobic Retinal Metabolism in Modified Mammalian Krebs Saline Medium with Bicarbonate Buffer for 1 hr at 13°.

Media	Glucose utilized (μM/hr/g-wet)	Lactate produced (μM/hr/g-wet)	EMP[a] activity (%)	TCA[b] activity (%)
100 mg %	7.55 ± 0.20 (3)	7.54 ± 0.11 (3)	49.9	50.1
150 mg %	8.04 ± 0.31 (3)	11.14 ± 0.30 (3)	69.2	30.8
200 mg %	10.07 ± 0.16 (3)	14.99 ± 0.35 (3)	74.4	25.6

Note: Mean ± SE (number of observations).
[a] Embden–Meyerhof pathway.
[b] Tricarboxylic acid cycle.

centrations in excess of 100 mg % resulted in significant inhibition of TCA cycle activity. For example, at a glucose concentration of 100 mg % aeorbic glycolysis (lactic acid production) accounted for some 50% of the glucose utilized, and this value increased to about 75% at 200 mg % glucose. Results for glucose utilized and lactic acid produced at different glucose levels are significantly different at the $p<0.05$ level (Student t test) with the exception of glucose utilization between 100 and 150 mg % glucose in the media.

Three different incubation periods using gas concentrations consisting of 95% N_2–5% CO_2 or 95% O_2–5% CO_2 were employed to study the effects of varying O_2 concentrations on retinal metabolism. After one-half- and 1-hr incubations in modified Medium 199, there was no apparent change in the physical appearance of the retinas. After 3 hrs, samples of media which contained the retinas were turbid, but gross observations of the retinas indicated that they had remained intact. Under anaerobic conditions glucose utilization was higher, and there was a greater production of lactic acid than under aerobic conditions for the first two time periods (Table III), but there was no significant difference when a 3-hr incubation period was employed. The rate of glucose metabolism significantly decreases with increasing incubation time.

Discussion. Kornblueth, Yardine-Yaron, and Wertheimer (6) observed that glucose utilization by the mammalian retina is dependent on the integrity of the cells; no information was given on lactic acid production. When rainbow trout retinas were sonified, resulting in the rupture of cellular membranes, glucose utilization and lactic acid production showed an 87 and 98% reduction, respectively. Thus the glycolytic pathway exhibited a somewhat greater disruption than the TCA cycle. In some cases retinas were torn into two or three pieces, but it was found that the metabolism did not differ from that of intact retinas. These results show that glucose utilization and lactic acid production (glycolysis) both depend on cellular integrity and the data lend support to the hypothesis proposed by Graymore (7) that cellular disruption causes dilution of essential co-factors resulting in decreased metabolism.

The Crabtree effect is observed in the teleost retina exposed to high levels of glucose.

TABLE III. Glucose Utilization and Lactic Acid Production by Retinal Tissue for ½-, 1-, and 3-hr Incubations at 13° in Modified Medium 199.

Incubation time	Anaerobic (95% N_2–5% CO_2)		Aerobic (95% O_2–5% CO_2)	
	Glucose utilized (μM/hr/g-wet)	Lactate produced (μM/hr/g-wet)	Glucose utilized (μM/hr/g-wet)	Lactate produced (μM/hr/g-wet)
½ hr	7.30 ± 0.37 (10)[a]	16.04 ± 0.66 (10)[a]	4.13 ± 0.32 (10)	10.19 ± 0.93 (10)
1 hr	3.93 ± 0.21 (6)[a]	9.52 ± 0.50 (6)[a]	2.40 ± 0.30 (6)	6.77 ± 0.13 (6)
3 hr	1.63 ± 0.32 (10)	3.67 ± 0.56 (10)	1.36 ± 0.15 (10)	3.44 ± 0.18 (10)

Note: Mean ± SE (number of observations).
[a] Significant difference at $p<0.01$ level between anaerobic and aerobic treatment.

When media glucose was increased from 100 to 150 mg %, a significant fall in the oxidation of glucose by the TCA cycle occurred. Gatt, Krimsky, and Racker (8) have offered a possible explanation of the Crabtree effect, but the physiological significance of the effect remains dubious. The Crabtree effect has previously been described only in mammalian retinal tissue and leucocytes *in vitro*, but now has been substantiated in the teleost retina. Over the normal physiological range of blood glucose (50–100 mg %) one would not encounter a Crabtree effect *in vivo*. It is known that the retina has a fairly constant metabolic rate and changes in blood glucose could be buffered by endogenous glycogen assuring an optimum glucose concentration for the retinal cells irrespective of changes in blood glucose.

Lactate is produced in amounts in excess of that which can be accounted for on the basis of glucose utilization (Table III). A plausible explanation for this observation is the presence of endogenous lactate and glycogen. Substantial deposits of highly mobile glycogen in the teleost retina have been demonstrated recently in our laboratory with histochemical techniques, and the retina and attached remnants of the vitreous body probably contain some endogenous glucose. The total complement of endogenous glucose when added to the incubation media might result in an underestimation of the total glucose utilization calculated using media glucose concentrations alone. Similarly lactic acid production may have been overestimated because of variations in the concentration of lactate in the retina and attached vitreous. Both of these errors would be additive in overestimating the contribution of the Embden–Meyerhof pathway (EMP) to glucose catabolism.

Aerobic glycolysis (production of lactic acid) has been demonstrated in the teleost retina under *in vitro* conditions at a P_{O_2} of 700 mm Hg. Experiments at different gas concentrations and time periods show that there is 42% less glucose utilized and 33% less lactic acid produced under aerobic conditions than under anaerobic conditions for periods of 1 hr or less (Table III). This inhibitory effect of elevated oxygen on retinal carbohydrate metabolism (Pasteur effect) was described for mammalian retinas (9) several years ago. Craig and Beecher (10) reported that when the oxygen was lowered from 95 to 5%, glycolysis by retinas of white rats was increased to nearly the anaerobic level.

Graymore (11, 12) using mature rat retinas in bicarbonate buffer showed that glycolysis was inhibited some 58% under aerobic conditions, whereas, we found that glycolysis in the trout retina was inhibited only 33% by high oxygen. Cohen and Noell (9) found that a decreased Pasteur effect and increased independence between glycolysis and respiration were associated with increasing age in the rat retina. Mature teleost retinas show less Pasteur effect than the adult mammal thus indicating a greater independence of glycolysis and respiration. The increased degree of compartmentalization of the glycolytic scheme apart from the TCA cycle in the teleost retina favors the production of lactic acid even at elevated oxygen tensions.

Unlike the retina of mammals, the teleost retina is avascular, and in the choroidal area there is an elaborate array of capillaries in juxtaposition allowing for the accumulation of oxygen by a process of countercurrent diffusion multiplication. The high oxygen tensions generated in the choroid region enhance the diffusion of oxygen to the inner layers of the retina and evidence has been presented by Hoffert and Fromm (13) that the teleost retina does consume oxygen through the TCA cycle.

Aerobic glycolysis is assumed to occur in the teleost retina *in vivo* based on the evidence of aerobic lactate production *in vitro*. Graymore (7) attributes a high rate of *in vivo* aerobic glycolysis to a high rate of glucose catabolism which forms the precursors of the TCA cycle faster than they can be oxidized. Because the TCA cycle is saturated, the precursors are converted to lactic acid. Data presented here supports a postualted physiological role for the lactic acid produced by aerobic glycolysis. The fact that the accumulation of acid metabolites (lactic acid and CO_2) reduce the oxygen binding capacity of

hemoglobin was utilized by Fairbanks (14) in a scheme proposed for the generation of superatmospheric oxygen tensions by the teleost choroid. This scheme involves the neutralization of retinal lactic acid by bicarbonate with the ultimate production of CO_2 through the action of retinal carbonic anhydrase.

It is apparent from the above discussion that increased retinal lactic acid production can lead to increased oxygen tensions in the choroid rete mirabile. It is further noted that increased oxygen tensions in turn, through the Pasteur effect, result in decreased aerobic glycolysis, *i.e.*, decreased lactic acid formation. Aerobic glycolysis and the Pasteur effect are believed to be components of a negative feedback loop for control of oxygen tensions in the teleost eye. The existence of a certain degree of aerobic glycolysis insures adequate acidification of hemoglobin and thereby aids in the maintenance of optimum oxygen concentrations. High oxygen concentrations found in the teleost eye appear to be influenced by (1) the magnitude of aerobic glycolysis, (2) inhibition of lactic acid production by oxygen (Pasteur effect), and (3) CO_2 produced by direct oxidation of glucose in the retina.

Summary. Lactic acid production in the presence of high oxygen tensions (aerobic glycolysis) was found to occur in the teleost retina. Forty-two percent more glucose was utilized and 33% more lactic acid was produced under anaerobic than under aerobic conditions. Glycolysis was inhibited 33% by elevated oxygen (Pasteur effect), and it was further demonstrated that glycolysis is dependent upon the integrity of the retinal cells, being almost completely abolished by cellular disruption.

The shift in the oxygen dissociation curve to the right by acid metabolites causes release of oxygen from hemoglobin in the choroid region. This O_2 release explains in part the high P_{O_2} encountered in the vicinity of the retina. The Pasteur effect may function as a possible component of a negative feedback control loop for the generation of high P_{O_2} in trout eyes.

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