The Dependence of the Oxygen-Concentrating Mechanism of the Teleost Eye (*Salmo gairdneri*) on the Enzyme Carbonic Anhydrase

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ABSTRACT Microoxygen polarographic electrodes were constructed and used to measure oxygen tension (P_{O_2}) in the eyes of rainbow trout (*Salmo gairdneri*). The values obtained are compared with arterial blood and environmental water P_{O_2} and indicate that there is an oxygen-concentrating mechanism in the eye supplying oxygen to the avascular retina. Anatomically similar retes suggest that the mechanism is similar to the one which exists in the swim bladder. Elimination of the arterial blood supply to the choroidal gland rete mirabile of the eye (through pseudobranchectomy) and the consequent lowering of ocular oxygen tensions implicate the choroidal gland as one of the major components of the oxygen-concentrating mechanism. After pseudobranchectomy the presence of ocular P_{O_2} above that of arterial blood is indicative of a secondary structure in the eye capable of concentrating oxygen. Inhibition of carbonic anhydrase, using acetazolamide, is shown to result in complete suppression of the oxygen-concentrating mechanism. A hypothesis is advanced for the participation of retinal-choroidal and erythrocyte carbonic anhydrase in the oxygen-concentrating mechanism.

INTRODUCTION

Only a few vertebrates other than mammals possess well-vascularized retinas. Since the retina has a high oxygen demand it would seem that this lack of retinal vascularization might lead to difficulties in oxygen supply to this tissue. Wittenberg and Wittenberg (1) have shown that many marine teleosts can concentrate oxygen in the eye to levels above environmental water oxygen tensions, and that the magnitude of this ability is directly correlated with the anatomical development of the choroidal rete mirabile (choroidal gland).

The choroid rete is a horseshoe-shaped structure which surrounds the optic nerve and lies within the choroid proper (Barnett [2]). It consists of a parallel

arrangement of arterial and venous capillaries and is similar to the rete mirabile of the swim bladder; an anatomical arrangement which is thought to act as a countercurrent diffusion multiplier for the concentration of oxygen (Berg and Steen [3], Kuhn et al. [4]) and CO_2 (Wittenberg, Schwend, and Wittenberg [5]) in the gas bladder.

Choroidal glands are found only in those fish which possess pseudobranchs, remnants of the first gill arches, situated anteriodorsally in the opercular cavity (Walls [6]). The pseudobranch contains a higher concentration of the enzyme carbonic anhydrase than any other teleost tissue (Maetz [7], Hoffert [8]) but the physiological role of this structure and the carbonic anhydrase produced by it is still unknown (Parry and Holliday [9]). A pseudobranch receives its arterial blood supply from the first efferent gill artery (Allis [10], Vervoort [11]) which after entering the gland subdivides into a capillary system and then reunites to form the ophthalmic artery, the only vessel supplying arterial blood to the choroidal rete.

Copeland (12) has shown that after pseudobranchectomy fish are no longer capable of refilling their swim bladders with gases, and Fange (13) has obtained similar results using fish which had been treated with a carbonic anhydrase inhibitor. These results strongly indicate participation of carbonic anhydrase in the oxygen-concentrating mechanism of the swim bladder and the possibility that the pseudobranch may be a source of the carbonic anhydrase.

The purpose of this study was to determine the extent to which the freshwater teleost, *Salmo gairdneri*, which has a well-developed choroidal rete, is able to maintain a superatmospheric ocular oxygen tension. The role of carbonic anhydrase and the glandular pseudobranch in the oxygen-concentrating mechanism of the choroidal rete was studied.

MATERIALS AND METHODS

Rainbow trout were obtained from the Grayling State Fish Hatchery operated by the Michigan Department of Conservation. Fish weighing between 100 and 150 g were kept in the laboratory at 13°C with periods of 16 hr light and 8 hr darkness.

Microoxygen polarographic electrodes with tip diameters between 10 and 20 μ were constructed in a manner similar to that described by Silver (14) and used to measure oxygen tension (P_{O_2}) in the eye. These electrodes were found to be insensitive to changes in pH from 3.9 to 8.4 and in salinity from 0.0 to 2.0% NaCl. They were also insensitive to movement of fluid at the sensing tip and the response time of the electrodes was about 99% full response within 2 min. All measurements were made at 13°C $\pm$ 0.5°C to eliminate the effect of temperature on the sensitivity (2–3%/°C) of the polarographic electrodes. The polarographic circuitry was similar to that used by Montgomery and Horowitz (15), except that a Grass Model 5P1 low level DC preamplifier and DC driver amplifier (Grass Instruments Co., Quincy, Mass.) connected to a Beckman 10 inch potentiometric recorder, Model 1005 (Beckman

Instruments, Inc., Fullerton, Calif.) were used for amplification and recording of variations in current output from the electrodes. The response of the electrodes was linear over a range of P_{O_2} from 6 to 700 mm Hg.

Prior to and after each in vivo or in vitro P_{O_2} determination the electrodes were calibrated by measurement of the current output in solutions of known P_{O_2}. The P_{O_2} of these solutions was calculated from measurements of dissolved oxygen using the azide modification of the Winkler method (Orland [16]). Technical grade nitrogen, room air, and oxygen were bubbled through carboys of distilled water to obtain solutions with a wide range of oxygen tensions. Standard curves of current vs. P_{O_2} were constructed each time the electrodes were used.

Arterial blood samples were obtained from the dorsal aorta using a heparinized syringe. The blood was placed under mineral oil, avoiding at all times any exposure to atmospheric oxygen. The nucleated erythrocytes utilized a small but significant amount of oxygen during the sampling period so electrode current was recorded over a 15 min interval and a current vs. elapsed time graph was prepared. The curve was extrapolated to zero time which corresponded to the time the blood sample was drawn. The current at this time was used for determining arterial P_{O_2} through reference to the calibration graph. That the P_{O_2} decay during the recording interval was not due to O_2 utilization by the microelectrode was shown by the fact that there was only a 0.15 mm Hg/hr P_{O_2} decline over a 6 hr interval when the electrode was placed in a 1.0 ml sample of distilled water which was covered with a 6 cm column of mineral oil.

In a typical experiment a fish was placed on its side in a water-filled chamber and a 13 gauge needle placed through both the upper and lower jaws. A rubber catheter was placed in the fish's mouth and aerated water was pumped over the gills at the rate of 100 ml/min. The cornea was punctured using a 22 gauge needle and the polarographic electrode lowered by hand into the eye until it came in contact with the retina. The electrode was held in this position for the duration of the measurement.

The effect of carbonic anhydrase inhibition on ocular P_{O_2} was determined 24 hr after fish had received a 5 mg/100 g dose of a 5 mg% solution of acetazolamide (Diamox, Lederle Laboratories, American Cyanamide Co., Pearl River, N. Y.). Unilateral pseudobranchectomies were performed by scraping away the pseudobranch using an electric cautery iron. 24 hr after pseudobranchectomy ocular P_{O_2} was determined in the same manner as described above.

RESULTS

In vivo ocular P_{O_2} measurements indicate that the rainbow trout is capable of creating oxygen tensions in the eye which are 20 times those of arterial blood and 3.5 times those of the environmental water (Table I). The low P_{O_2} values found for arterial blood agree with published data from this laboratory on the per cent saturation of arterial blood (Hoffert and Fromm [17]). 24 hr after Diamox treatment the mean ocular P_{O_2} was reduced to a value not significantly different from that for arterial blood P_{O_2} (Table I), indicating a direct dependence of the oxygen-concentrating mechanisms on the enzyme carbonic anhydrase.

Values for vitreous body P_{O_2} were obtained by placing electrodes in excised eyes (in vitro studies). It was noted during these in vitro studies that utilization of oxygen by ocular structures led to an exponential decline of oxygen. A semilogarithmic plot of the results was made and the curve extrapolated

TABLE I

NORMAL P_{O_2} VALUES FOUND AT THE RETINA, IN ARTERIAL BLOOD, AND IN ENVIRONMENTAL WATER

Comparison of these values with those found after inhibition of carbonic anhydrase is shown.

Treatment	P_{O_2}, mm Hg			Hematocrit	Arterial pH
	Retina	Arterial blood	Water		
Control	445±68.5(16)	21±2.2(12)	133±3.7(16)	29±0.5(10)	7.61±0.03(22)
Diamox	25±5.1(6)	22±2.8(6)	130±3.4(6)	42±4.0(6)	7.38±0.03(17)

Mean ± SE (number of observations).

Normal P_{O_2} at the retina is greater than blood or water at the $p = 0.01$ level. Control P_{O_2} at the retina is greater ($p = 0.01$) than after Diamox treatment.

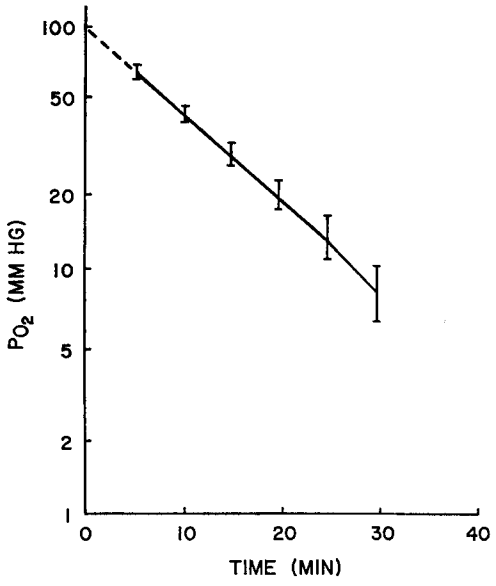

FIGURE 1. Semilogarithmic graph of mean ±SE in vitro P_{O_2} values recorded from the vitreous chamber. The dotted line represents extrapolation of graph to zero time, which corresponds to the P_{O_2} in the vitreous at the time the spinal cord was cut. Measurements were at 13°C with 11 observations per point.

back to zero time, which corresponded to the time the fish was killed and the eye removed (Fig. 1). The mean vitreous body P_{O_2} was only 23.2 % of the value for retinal P_{O_2}. Following Diamox treatment vitreous P_{O_2} was about 28 % of the value for retinal P_{O_2}. The vitreous body P_{O_2}'s for the Diamox-treated fish were obtained in vivo, by raising the electrode a few millimeters away from the retina. The oxygen tension gradient between the retina and

the vitreous body, as indicated in Table II, may result from the rapid utilization of oxygen by the retina.

After pseudobranchectomy there was a significant decrease in the retinal P_{O_2} on the side of the pseudobranchectomy (Table III) but it was still significantly higher than that of arterial blood.

TABLE II

COMPARISON OF OCULAR P_{O_2} AT RETINA WITH THAT FOUND IN THE VITREOUS BODY OF CONTROLS AND DIAMOX-TREATED FISH

Treatment	P_{O_2}, mm Hg	
	Retina	Vitreous body
Control	445±68.5(16)	103±6.7(11)
Diamox	25±5.1(6)*	7±1.0(6)*

Mean ± SE (number of observations).
* Control larger than Diamox at the $p = 0.001$ level.

TABLE III

EFFECT OF UNILATERAL PSEUDOBRANCHECTOMY ON OCULAR P_{O_2} VALUES AT THE RETINA

Treatment	P_{O_2}, mm Hg	
	Retina	Arterial blood
Control	239±24.7(5)	20±1.2(5)
Pseudobranchectomy	57±9.6(5)	20±1.2(5)

Mean ± SE (number of observations).
Contralateral (control) eye has higher P_{O_2} ($p = 0.03$) than treated eye.
P_{O_2} higher in treated eye than in arterial blood at the $p = 0.01$ level.

DISCUSSION

The mean ocular P_{O_2} value for the controls reported in Table I leaves no doubt that the freshwater teleost, *Salmo gairdneri*, is capable of concentrating oxygen in the eye. This value is more than 400 mm Hg higher than arterial P_{O_2} and 300 mm Hg higher than environmental water P_{O_2}. Data presented in Table II indicate that a large gradient exists between P_{O_2} in the choroidal rete and in the vitreous body. The rather rapid decline in ocular P_{O_2} in excised eyes is believed to be due to a high rate of oxygen utilization by the retina.

In fish treated with Diamox the oxygen-concentrating ability of the eyes was virtually eliminated and since this drug is a specific inhibitor of carbonic anhydrase the importance of this enzyme in the oxygen-concentrating mechanism is indicated. Similar results have been reported by Fange (13) with

respect to the action of a less potent carbonic anhydrase inhibitor on the oxygen-concentrating mechanism of the swim bladder. Diamox did not affect the P_{O_2} of arterial blood and Hoffert and Fromm (17) reported that Diamox had no statistically significant effect upon the per cent saturation of the hemoglobin or the O_2-carrying capacity of trout blood even though it lowered blood pH about 0.23 of a pH unit.

Unilateral pseudobranchectomy results in functional removal of the choroidal rete mirabile of the ipsilateral eye since the only arterial blood supply to the rete comes by way of the pseudobranch. We found an expected decrease in the ocular P_{O_2} following pseudobranchectomy but the mean value after pseudobranchectomy was still significantly higher than arterial blood P_{O_2}. This relatively high P_{O_2} is probably due to the presence of a second but smaller rete in the eye of the rainbow trout. Barnett (2) has described the lentiform body as being anatomically similar to the larger choroidal rete mirabile and it was found that following pseudobranchectomy high ocular P_{O_2} was localized in the area supplied by the lentiform body. This structure may therefore be looked upon as a secondary oxygen concentrator. It should be noted that the lentiform body receives blood from the retinal artery, a branch of the internal carotid, and not from the ophthalmic artery which supplies the choroid rete (Barnett [2]).

Mechanism of Ocular Oxygen Concentration

The vascular similarities between the rete mirabile of the choroid and swim bladder suggest that the oxygen-concentrating mechanism in these two systems may be similar. In both systems blood from the arterial capillaries of the rete supplies a capillary bed which lies adjacent to the structures in which the high P_{O_2} values have been recorded. These capillary beds are the choriocapillaris network of the choroid proper and the capillary network in the gas gland of the swim bladder. Blood leaving these networks returns to the venous capillaries of the rete. Functional similarities of these retia are indicated by the parallel action of Diamox.

Bilateral pseudobranchectomy does not interfere with the blood supply to the swim bladder rete mirabile, yet under these conditions there is an inhibition of the fish's ability to refill its swim bladder with gases (Copeland [12]), thus implying a functional relationship between the pseudobranch and swim bladder. It has been suggested that this relationship involves the enzyme carbonic anhydrase which occurs in a high concentration in the pseudobranch. Pseudobranch carbonic anhydrase, if it is released into the blood, would also be available to the oxygen-concentrating mechanism of the eye. In addition there is also erythrocyte carbonic anhydrase and carbonic anhydrase from the retina and choroid (Hoffert [8]). At this time only a hypothetical role in the

oxygen-concentrating mechanisms of the eye (see below) can be assigned to these three carbonic anhydrases.

The most widely accepted hypothesis for the participation of the swim bladder rete mirabile in oxygen concentration is that this structure functions as a countercurrent diffusion multiplier of an "initial single concentrating effect" for oxygen (Kuhn et al. [4], Berg and Steen [3]). The initial single concentrating effect is generally believed to result from acidification of blood (by lactic acid) in the gas gland. This leads to an increase in the P_{O_2} (through the Bohr, Root, or salting out effect) of the blood returning to the venous side of the rete.

In order for the countercurrent diffusion multiplication to be effective, the P_{O_2} increase should occur while the blood is traversing the choriocapillaris

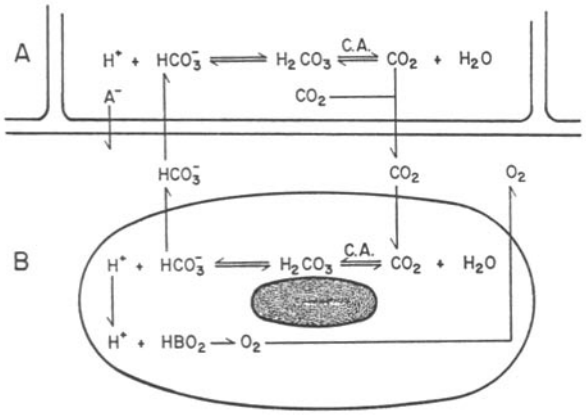

FIGURE 2. Representation of the role played by retinal-choroidal and erythrocyte carbonic anhydrase in increasing the rapidity of the single concentrating effect. A, retinal and/or choroidal cells. B, nucleated erythrocyte.

of the eye. Thus the acidifying agent must enter the erythrocytes and effect the dissociation of oxygen from hemoglobin before these cells enter the venous vessels of the rete. The slow diffusion of H^+ through biological membranes precludes postulation that intracellular pH changes (hence dissociation of O_2 from HbO_2) are due to movement of hydrogen ions into red blood cells from the plasma.

It is therefore proposed that choriocapillaris CO_2 is high due to either a high rate of CO_2 production by the retina or through its liberation from HCO_3^- in the presence of a metabolic acid. Bicarbonate would be presented to the retinal and/or choroidal cells in exchange for the anion of the metabolic acid. Formation of CO_2 in this manner would be enhanced by the presence of C.A. in the retinal and choroidal cells. The CO_2 formed would then rapidly diffuse into the erythrocytes present in the choriocapillaris network (Fig. 2).

Hydration of this CO_2 is effected by RBC carbonic anhydrase with subsequent formation of hydrogen ions and bicarbonate. Bicarbonate which diffuses into the plasma (chloride shift) could diffuse from the venous to the arterial side of the rete and be presented again to the retinal and/or choroidal cells in exchange for organic acid anions. Bicarbonate as a source of swim bladder CO_2 has been proposed by Wittenberg, Schwend, and Wittenberg (5).

An increase in erythrocyte P_{CO_2} will not in itself produce a significant dissociation of HbO_2 (Craw et al. [18]) which implicates the important role played by erythrocyte carbonic anhydrase. Forster and Steen (19) reported that the rate of dissociation of O_2 from HbO_2 is governed by the time required for hydration of CO_2 since the dissociation of carbonic acid into H^+ and HCO_3^- and the subsequent release of O_2 from HbO_2 both occur almost instantaneously.

The mechanism proposed emphasizes the key role played by erythrocyte and choriocapillaris carbonic anhydrase in catalyzing the hydration (in RBC) of CO_2 or dehydration (in choriocapillaris) of H_2CO_3, reactions which in turn effect a rapid dissociation of HbO_2. It also offers an explanation for the entry of lactate (which occurs in the gas gland of fishes (Steen [20]) or other anions into the plasma of the choriocapillaris.

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