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THE FAILURE OF OXYGEN TRANSPORT IN A FISH AT LOW LEVELS OF AMBIENT OXYGEN

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Abstract-1. Complete deoxygenation of arterial blood results in the failure of oxygen consumption in brown bullheads *(Ictalurus nebulosus)* at low levels of ambient oxygen.

2. The arterial deoxygenation apparently results from inability of the blood to load oxygen across the diffusion barrier of the gills, since gill ventilation and perfusion continue after oxygen uptake ceases.

3. The ambient oxygen tension at which this occurs differs appropriately in two groups of bullheads known to have different blood oxygen affinities as a result of thermal acclimation. The oxygen affinity of the blood is therefore strongly implicated as a factor limiting oxygen uptake at low levels of ambient oxygen.

INTRODUCTION

Oxygen consumption in relation to ambient oxygen supply has been determined in many animals, especially fishes. It is, however, the supply of oxygen to the tissues which influences the rate of aerobic metabolism and Johansen & Vadas (1967) have underlined the importance of considering internal as well as ambient oxygen tensions. The best known level of metabolic sensitivity to oxygen shortage is the "critical oxygen tension" (Prosser & Brown, 1961) which is equivalent to Fry's "incipient lethal level" (Shepard, 1955) and is defined as the level below which oxygen consumption is unable to continue at the standard rate. Below the critical oxygen tension the oxygen transport system is presumably unable to satisfy the demand of the tissues for oxygen and oxygen consumption decreases, eventually to a level below which further oxygen uptake ceases. Various names have been applied to this level, of which "residual level" is perhaps most useful (named because of the method in which it is usually determined). It is the aim of this paper to describe and evaluate physiological events which account for the ambient oxygen tension at which aerobic metabolism ceases in the brown bullhead, *Ictalurus nebulosus*.

Firstly, it is proposed to describe the relationship between internal oxygen tension and the cessation of oxygen consumption. Secondly, it is proposed to investigate the importance of the oxygen affinity of the blood of bullheads in fixing the residual level of ambient oxygen. In this regard some work done by Hall (1966) is of considerable interest. Hall showed a strong correlation between the oxygen affinity of the blood and the ability of eighteen rodent species to extract oxygen from the environment. The brown bullhead is an ideal animal for further investigation because it adjusts the oxygen affinity of its blood seasonally (Grigg, 1969) as follows. The oxygen affinity of the blood of warm-acclimated fish is higher than that of cold-acclimated fish measured at the same temperature. Therefore, if a relationship exists between oxygen affinity of the blood and residual oxygen tension in the water, cold- and warm-acclimated fish should show different results in determinations of residual oxygen tension.

MATERIAL AND METHODS

Specimens of *Ictalurus nebulosus* (43-127g) were taken by angling in a small pond at Eugene, Oregon, U.S.A. In the laboratory, fish were acclimated for at least three weeks to cold (9-10°C) and warm (24-25°C) well-aerated water and fed on earthworms. Residual oxygen tension was determined by placing a fish in 2800-m1 Fernbach flasks filled with aerated water, sealed with rubber stoppers and placed in a water-bath at the required experimental temperature. A sampling system allowed withdrawal of samples for determination of the oxygen tension of the water (PiO_2 mm Hg) using a temperature-stabilized Beckman Macroelectrode and a Spinco Model 160 gas analyser. As the fish consumed oxygen it steadily reduced the oxygen tension of the water at a rate proportional to oxygen consumption until a steady level was reached (as in Fig. 1). This level could be accurately determined and represents the "residual oxygen tension". Brown bullheads are so tolerant of hypoxia that they survived this procedure. No attempt was made to achieve a measurement of "standard metabolism" and the reported values can be regarded as measurements of "routine oxygen consumption" as defined by Fry (1957). Black *et al.* (1954) have described the effects of carbon dioxide in modifying residual oxygen tension in a number of fresh-water fishes. In their experiments with *Ictalurus nebulosus* they found that carbon dioxide levels lower than 180 mm Hg had no effect on the residual oxygen tension. Assuming the respiratory quotient was little different from 1, and considering the solubility of carbon dioxide in water, the accumulation of carbon dioxide in the present experiments could not exert any influence on the results.

In five experiments this procedure was carried out on fish with the dorsal aorta cannulated via lateral entry to the caudal artery through an incision in the tail. The cannula was passed through the rubber stopper of the respirometer flask and blood samples were withdrawn as hypoxia developed. The oxygen tension of these samples was determined and the blood was then returned to the fish. Results of such an experiment are seen in Fig. 1. In addition, the cannula was connected to a Statham pressure transducer actuating a Grass Polygraph and in this way heart rate could be monitored.

RESULTS

Arterial oxygen tensions were sampled during the course of five experiments, the results from which are shown in Figs. 1 and 2 and more extensively in Table 1. It is clear that oxygen consumption ceased only when the arterial blood became completely deoxygenated. The blood pressure trace recorded from the dorsal aorta showed that the heart continued to beat slowly and gill ventilation continued after oxygen consumption ceased.

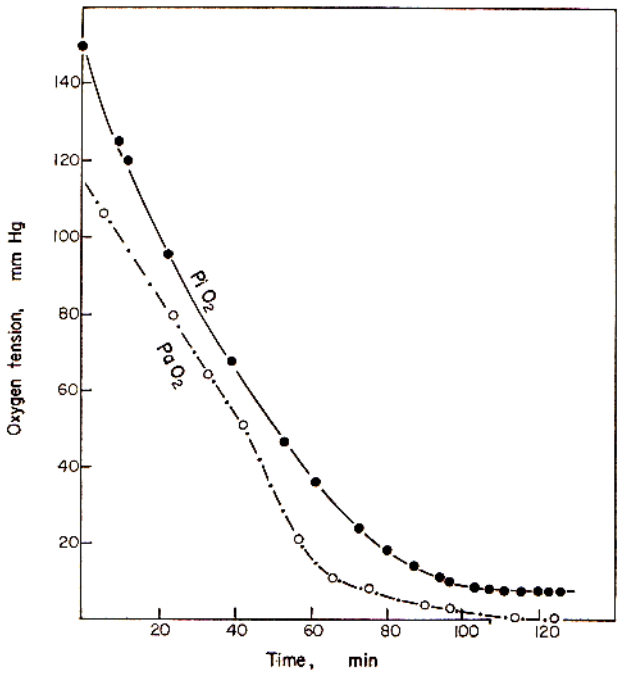

FIG. 1. Results of an experiment in which a brown bullhead with cannulated dorsal aorta depletes the oxygen from a sealed flask down to minimal level, the "residual oxygen tension". PiO_2 is the oxygen tension in the water (closed circles) and PaO_2 is the oxygen tension in the dorsal aorta (open circles).

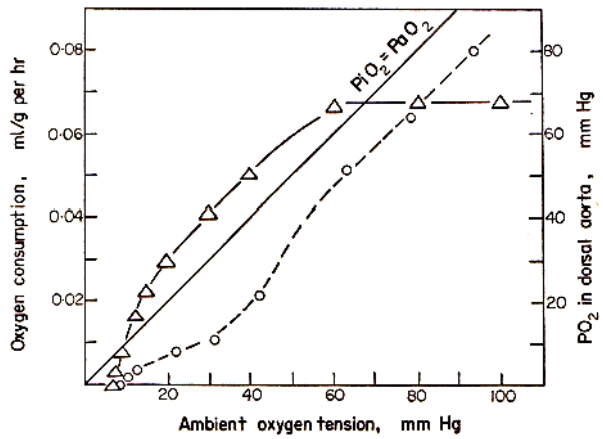

FIG. 2. Oxygen consumption (triangles) and PaO_2 (open circles) in relation to oxygen tension in the water (PiO_2), in an experiment with *Ictalurus nebulosus*.

TABLE 1-COMPARISON OF AMBIENT OXYGEN TENSIONS (PiO2) AT WHICH OXYGEN CONSUMPTION AND ARTERIAL OXYGEN TENSION (PaO_2) EACH BECOME ZERO, IN FIVE SEPARATE EXPERIMENTS WITH *Ictalurus nebulosus*

Acclimation temperature (°C)	Experimental temperature (°C)	$Pi0_2$ (mm Hg) at which O_2 consumption ceased	$Pi0_2$ (mm Hg) at which PaO_2 = O
24	24	7-5	7-5
9	9	5-1	5-8
9	9	5-5	6-0
9	24	9·0	9·0
24	9	4-0	4-0

Blood from bullheads with 24°C thermal history was known to have a higher oxygen affinity (P_{50} = *6* mm Hg at *20°C,* pH *7-8)* than that from bullheads with 9°C thermal history (P_{50} = 10 mm Hg at 20°C, pH 7-8) (Grigg, 1969). If the failure of the blood of bullheads to load at low partial pressures of ambient oxygen is related to the oxygen affinity of the blood, then we would expect residual oxygen tensions determined on warm-acclimated fish to be lower than similar determinations on cold-acclimated fish at the same temperature. Accordingly, determinations of residual oxygen tension were made on both acclimation groups at 9°C and 24°C. The results are seen in Table 2. At both experimental temperatures the residual oxygen tensions recorded from warm-acclimated fish are significantly lower than those from cold-acclimated fish at the same temperature, as predicted.

TABLE 2-"RESIDUAL OXYGEN TENSION" IN COLD- (9°C) AND WARM- (24°C) ACCLIMATED *Ictalurus nebulosus* MEASURED AT BOTH 9°C AND 24°C

Acclimation temperature (°C)	Experimental temperature (°C)	Residual $P0_2$ (mm Hg)	*n*	*t*	P
9	24	9.8 (8.5-12.5)	9	t_{16} = 11.53	0·0001
24	24	6.2 (5.3-7.6)	9	t_{17} = 0.704	N.S.
9	9	5.8 (4.8-7.0)	10	t_{18} = 20.18	0·0001
24	9	3.5 (2.8-4.4)	10		

Mean values are shown, followed by the total range (in parentheses)

DISCUSSION

The continuation of gill ventilation and perfusion when the blood is deoxygenated seems to indicate that the observed arterial deoxygenation results from the inability of the blood to take up oxygen from the water, rather than from a failure of the mechanical processes of gas exchange. This failure to load across the diffusion barrier of the gills is probably related to the oxygen affinity of the blood, an argument which is strengthened by the described agreement between predicted and actual performance of cold- and warm-acclimated bullheads in determinations of residual oxygen tension.

It seems that in the brown bullhead at least, oxygen affinity is strongly implicated as the most significant factor controlling the level of ambient oxygen tension at which oxygen consumption is zero. The observed arterial deoxygenation at this point is of interest, and the tolerance of this deoxygenation shown by bullheads provides a striking contrast with the situation in mammals.

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Key Word Index-Oxygen transport; blood; gills; fish; ambient oxygen tension; *Ictalurus nebulosus*