OXYGEN UPTAKE AND TRANSPORT DURING HYPOXIC EXPOSURE IN THE STURGEON *ACIPENSER TRANSMONTANUS*[1]

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Abstract. Gill ventilation, stroke volume and frequency, % O_2 utilization and oxygen uptake, and dorsal aortic blood oxygen tension, content, pH and oxygen affinity have been determined during normoxia and during a range of hypoxic exposures in the sturgeon, *Acipenser transmontanus*.

In air-equilibrated water gill ventilation was 350 ml/kg/min, % utilization was 35–40%, and oxygen uptake at 15 °C was 55–60 ml O_2/kg/h. Dorsal aortic blood P_{O_2} was 90 mm Hg and blood O_2 content at a normal pHa of 7.84 was 7.0 vol %.

$\dot{V}g$ fell considerably through a reduction in branchial stroke volume when $P_{I_{O_2}}$ was reduced from 150 to 100 mm Hg. Although % utilization remained unchanged, $\dot{V}_{O_2}$ was halved, clearly identifying *Acipenser* as an O_2 conformer with a critical O_2 tension just below air saturation. At a $P_{I_{O_2}}$ of 60 mm Hg $\dot{V}_{O_2}$ was only 15% of that at normoxic levels falling to only 5% at a $P_{I_{O_2}}$ of 30 mm Hg. There was no hypoxic bradycardia.

There was no repayment of an oxygen debt even after severe hypoxic exposure in *Acipenser*, and pHa remained unchanged under all experimental conditions, a response incompatible with lactate or succinate production. It is concluded that the sturgeon reduces total energy expenditure during hypoxic exposure, rather than switching from aerobic to anaerobic metabolism.

Arterial blood	Gill ventilation
Breathing pattern	Oxygen conformer
Extraction coefficient	Oxygen debt

Respiratory processes in some of the extant ancient orders of the bony fishes have come under close scrutiny in recent decades. Much interest has centred on the Dipnoi, both for their air breathing abilities and for their close phylogenetic relationships with the first terrestrial vertebrates (see Johansen, 1970). Respiration in the Holostei

and Chondrostei, the direct ancestors of the Teleost fishes, has also been examined to some extent. The surviving Holosteians *Amia* and *Lepisosteus* are representative of this ancient order, and their respiration, particularly their air breathing abilities, have been investigated (Johansen *et al.*, 1970; Rahn *et al.*, 1971). Aerial gas exchange in the Chondrosteian *Polypterus* has also been studied (Budgett, 1901; Magid, 1966). Perhaps attesting to the selective advantage in archaic fishes of bimodal gas exchange, only two closely related actinopterygian ancestors of the Teleosts – the sturgeon and the paddlefish, *Polyodon* – have survived over 200 million years to present times with a total dependency on aquatic gas exchange. Yet nothing is known of the respiratory processes in either of these primitive fish. In a companion study to this paper the mechanics of gill ventilation have been examined in the sturgeon *Acipenser transmontanus* and unusual ventilatory adaptations for bottom dwelling have been described (Burggren, 1978). In the present paper we report on processes of oxygen uptake and transport during normoxic and hypoxic exposure in the sturgeon *Acipenser transmontanus.*

Methods

Nine sturgeons (*Acipenser transmontanus*) weighing between 0.82 and 1.06 kg (mean weight 0.95 kg) were used in this investigation (see Burggren, 1978, for details of their capture and maintenance). All experiments were performed at 15 C $\pm \frac{1}{2}$ C.

Branchial water flow in *Acipenser* was quantified by modification of the technique of van Dam. The ventrally located tubular mouth recessed into the relatively inflexible cartilagenous undersurface of the head of the sturgeon afforded a highly convenient anatomical arrangement for partitioning buccal water intake from opercular water expulsion without mechanical restriction of the branchial pumps (Fig. 1). The open end of a condom, which had a slightly greater diameter than that of the mouth, was fastened with interrupted stitches through the coarse, scaleless skin around the mouth. The closed half of the condom was then cut off and discarded, and the resulting very flexible rubber tube encompassing the sturgeon's mouth was stretched
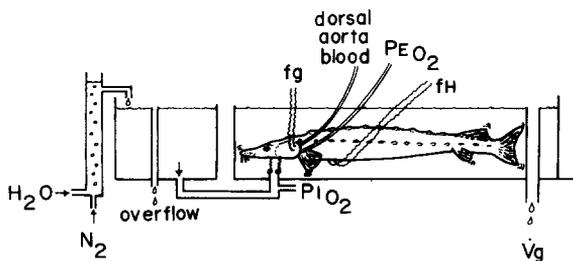
Fig. 1. Diagrammatic representation of the experimental apparatus used to quantify gill ventilation in the sturgeon.

over a plastic tube rising through the floor of a Plexiglass holding chamber (fig. 1). This plastic tube served to convey inhalent water drawn by the action of the fish's respiratory pumps from a water supply chamber. Because the tube diameter was very large, pressure gradients responsible for flow through it were very small. Hence, although the tube was flexible, transmural pressures were never great enough to cause any collapse or significant narrowing of it. Both chambers were fitted with fire-polished overflow standpipes adjusted to equalize water levels between chambers. Water continually overflowed from the water supply chamber to maintain a constant water pressure head. All water drawn in the mouth and over the gills passed into the chamber in which the fish was located and overflowed down the standpipe. Timed collection of this overflow yielded branchial water flow. Sturgeon could be rendered immobile by simply turning them on their backs in the water. Thus the brief operation for attachment of the rubber sleeve over the mouth was easily performed without chemical anaesthetic and with no apparent disturbance to the fish. Once in the experimental apparatus, the sturgeon remained relatively still, though they were free to make swimming movements as they were in no way restrained except for a pivotal point at the mouth (fig. 1). One source of error in this method of ventilation measurement lay in the ability of the sturgeon to ventilate its gills with water drawn in the top of the opercular slit rather than in through the mouth (Burggren, 1978), which could lead to an underestimation of $\dot{V}g$ using the present technique. Estimates of $\dot{V}_{O_2}$ made by the Fick principle, a method dependent upon accurate measurement of ventilation volumes, were compared with direct respirometry measurement of $\dot{V}_{O_2}$ in 4 sturgeon. $\dot{V}_{O_2}$ by the Fick principle was underestimated by less than 5% in all of the fish, and it was assumed that the proportion of gill ventilation arising from opercular water intake was thus negligible. Also, on the basis of dye injection experiments (Burggren, 1978) there were no apparent changes in the proportion of buccal and opercular water intake during hypoxic exposure.

The oxygen partial pressure of water passing into the mouth was adjusted by introducing a variable flow of nitrogen through a gas exchange column containing water destined for the water supply chamber (fig. 1). Inhalent water was sampled from the tube immediately below the sturgeon's mouth. Exhalent water from the opercular cavity was sampled via a cannula attached to a 1-ml glass syringe. Davis and Waters (1969) and Piiper et al. (1977) have commented on the difficulty of obtaining representative mixed exhaled water samples when sampling from a single cannula. A previous X-ray analysis of opercular cavity water flow patterns in Acipenser (Burggren, 1978) has revealed that almost all branchial water is exhaled in a discrete stream along the lower one third of each opercular slit. Moreover, a careful series of experiments involving repeated sampling of exhalent water indicated that a P_{O_2} variation of less than 1–2 mm Hg occurred in different regions of the exhalent stream. During sampling periods in the present experiments the tip of the cannula was held 1 mm inside the ventral region of the right opercular slit. Each exhalent water sample was collected during the course of at least 5 consecutive

ventilatory cycles. Oxygen consumption was then calculated indirectly from O_2 utilization and gill ventilation data.

Ventilation frequency was recorded either usually or with copper electrodes implanted in the operculae and attached to a Biocom 2991 Impedance Convertor writing out on an E and M Physiograph 6-chart recorder. Heart rate was recorded using copper electrodes implanted in the skin on the ventral surface over the heart. The electrodes were connected to a Physiograph AC preamplifier and Cardiotach, the recorded signal being printed out on the chart recorder.

Dorsal aorta blood was sampled via a PE90 cannula. Attached to the tip of the cannula was the shaft of a 21 ga Huber point hypodermic needle which was chronically implanted into the dorsal aorta through the roof of the mouth during MS 222 anaesthesia. The cannula was led out the top of the opercular slit and stitched lightly to the back. Blood and water oxygen tensions and blood pH were measured with water-jacketed Radiometer electrodes and a Radiometer 27 or pHM 71 acid-base analyzer. Blood O_2 content was determined according to Tucker's method.

Oxygen dissociation curves for sturgeon blood were determined using the technique of mixing known proportions of deoxygenated and fully oxygen saturated (air equilibrated) blood (Edwards and Martin, 1966). Blood was tonometered at a fixed P_{CO_2} level for 20 min before construction of curves was begun, and the entire curve consisting of 5 points was completed within 45–50 min of a change in blood P_{CO_2}. The O_2 content of air equilibrated blood at each P_{CO_2} level was measured and the proportions of saturated/unsaturated blood used for each point on the O_2 dissociation curve were subsequently corrected. Hematocrit was determined after centrifugation at 12,000 rpm for 5 min.

Results

Two sets of experiments were undertaken. Steady state levels of gill ventilation frequency (fg) and volume ($\dot{V}g$), oxygen utilization (% util) and uptake ($\dot{V}_{O_2}$), and heart rate (fh) in response to different levels of hypoxia were monitored in 6 *Acipenser transmontanus* without any previous anaesthetic exposure or chronic cannulation. 2 of these sturgeon and an additional 3 fish were then later anaesthetized and a cannula chronically implanted in the dorsal aorta to permit arterial blood sampling.

Figure 2 illustrates a representative experiment in a sturgeon before, during, and after exposure to an inhalent oxygen tension (PI_{O_2}) of approximately 100 mm Hg. When first placed in the apparatus this sturgeon and the others exhibited an elevated $\dot{V}g$ and ventilatory stroke volume, % utilization was comparatively low, and $\dot{V}_{O_2}$ was high. After 1–2 h in normoxic water, however, gill ventilation and $\dot{V}_{O_2}$ began to decrease as the fish became accustomed to the apparatus, and then usually stabilized 2–3 h after the beginning of the experiment. Breathing and heart rate were nearly constant in each fish during this acclimation period. Upon a stepwise reduction of PI_{O_2} from 155 mm Hg to 100 mm Hg, $\dot{V}g$ decreased within 1–2 min.

Time (min)
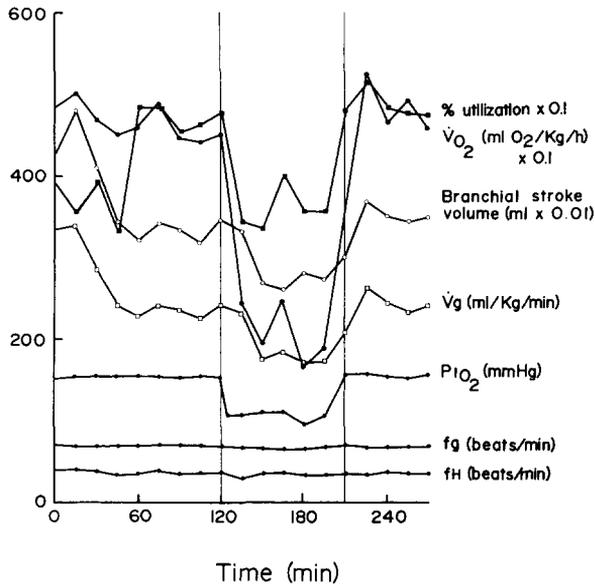
Fig. 2. Representative ventilatory responses to a 90-min period of hypoxic exposure in a 0.88 kg *Acipenser transmontanus*. The area between the vertical lines represents the period of hypoxic exposure.

In this particular fish, % util. also fell significantly, though in most other fish % util. did not change with this level of hypoxia (fig. 3). $\dot{V}_{O_2}$ fell precipitously within 5–10 min of hypoxic exposure. There was little change in fg at this level of hypoxia and the marked fall in $\dot{V}g$ was due therefore to a reduction in branchial stroke volume. Heart rate in this fish also showed no change with the reduction in $P_{I_{O_2}}$.

Ventilation at these new levels usually stabilized after 30–45 min of hypoxic exposure. With a step-wise increase in $P_{I_{O_2}}$ back to air saturated levels, ventilatory performance rapidly returned to the previous normoxic levels. Occasionally there was a slight 'overshoot' in $\dot{V}g$ and $\dot{V}_{O_2}$ (fig. 2), though in most instances there was not.

Figure 3 shows mean values ± 1 standard deviation of ventilatory performance and heart rate in 6 *Acipenser* exposed to levels of $P_{I_{O_2}}$ of 155, 105, 60 and 30 mm Hg. In each experiment a steady-state ventilatory performance was allowed to develop and was recorded. $P_{I_{O_2}}$ was then reduced in a stepwise fashion from air-saturated levels. The responses were then noted when a new steady state was achieved after 1/2–1 h, and it is only these data which are included in fig. 3. Each fish was then returned to normoxic water for a further 1 h before another period of exposure to hypoxia was given. In this fashion any cumulative effects of hypoxic exposure on gill ventilation were minimized.

Gill ventilation decreased linearly as $P_{I_{O_2}}$ was decreased from 155 mm Hg to approximately 60 mm Hg. There was little further change in $\dot{V}g$ at more severe levels of hypoxia, but $\dot{V}g$ at 60 mm Hg was already just 35% of that at air saturation. This reduction in $\dot{V}g$ with decreasing O_2 was mostly the result of a similar
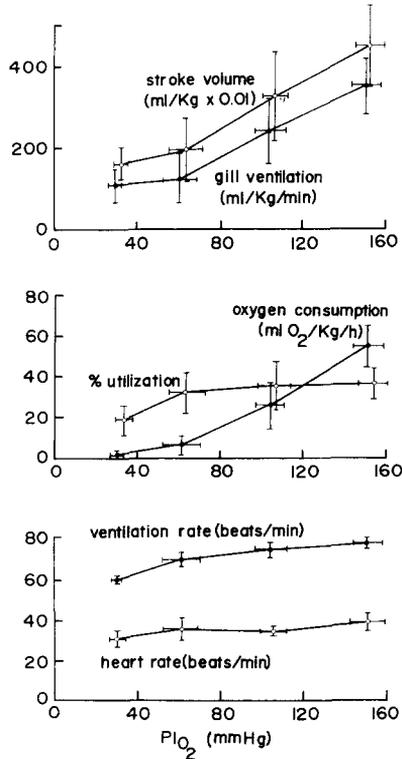
Fig. 3. Ventilatory parameters and heart rate in *Acipenser transmontanus* during exposure to 4 levels of hypoxia. Mean values ± 1 standard deviation are given. Data from 6 fish are presented.

decline in ventilatory stroke volume: fg decreased by only 7–10 beats/min from a PI_{O_2} of 155 to 60 mm Hg (fig. 3). Only at very severe levels of hypoxia below 60 mm Hg did fg begin to show greater decreases. Similarly, fg showed almost no change from normoxic levels during all but the lowest values of PI_{O_2}. Although $\dot{V}g$ progressively decreased with hypoxia, % utilization decreased little from normoxic levels above 60 mm Hg. Below this level % util. decreased sharply. Oxygen uptake of *Acipenser*, approximately 55 ml O_2/kg/h at 15 C.in normoxic water, showed a marked depression with even a small reduction in PI_{O_2}. At 100 mm Hg, $\dot{V}_{O_2}$ had fallen by approximately 50% and at a PI_{O_2} of 60 mm Hg, $\dot{V}_{O_2}$ was only 10% of that at normoxic levels. Sturgeon usually showed no overt signs of distress at low O_2 tensions, though at 30 mm Hg some of the fish exhibited brief bursts of struggling. All fish tested survived 30 min of exposure to an inhalent PI_{O_2} of 25–35 mm Hg.

Values of dorsal aorta blood P_{O_2} and hydrogen ion concentration ([H^+]) measured in 5 sturgeon over the same range of inspired P_{O_2} over which ventilatory responses were determined are presented in fig. 4. Dorsal aorta P_{O_2} ranged from approximately 70–100 mm Hg in air-saturated water, falling to less than 20 mm Hg at levels of hypoxia below 60 mm Hg. [H^+] in 4 of 5 sturgeon showed no change
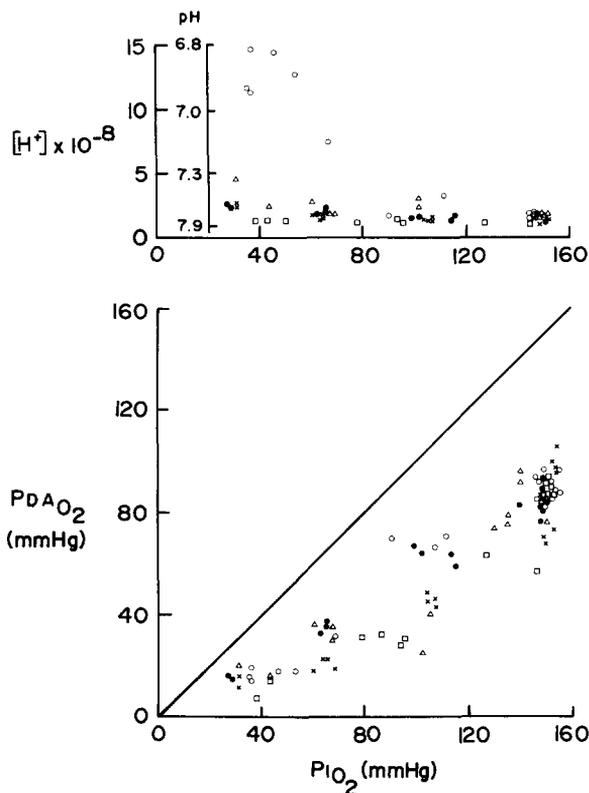
Fig. 4. Plasma hydrogen ion concentration (and pH) and dorsal aorta blood P_{O_2} at different ambient oxygen levels in 5 *Acipenser transmontanus*. Measurements from each fish are represented by a different symbol.

with a progressive reduction in PI_{O_2}, though in 1 fish extreme levels of hypoxia were accompanied by a great increase in plasma $[H^+]$.

Figure 5 presents the relationship between dorsal aorta P_{O_2} and expired water oxygen tensions (PE_{O_2}). Under normoxic conditions PE_{O_2} ranged from 90–120 mm Hg (mean value 105 ± 12 mm Hg). In 3 of 5 fish examined dorsal aorta blood P_{O_2} was occasionally up to 15 mm Hg greater than expired P_{O_2} during normoxia, though in most instances dorsal aorta P_{O_2} was 10–40 mm Hg below PE_{O_2}. A greater dorsal aortic blood P_{O_2} than expired water P_{O_2} was never observed at values of PE_{O_2} below 80 mm Hg occurring during hypoxic exposure. At low values of ambient oxygen, dorsal aortic blood P_{O_2} showed less variability from measurement to measurement and from fish to fish, and tended to values 15–25 mm Hg below PE_{O_2}.

The oxygen content of dorsal aortic blood (C_{O_2}) at a normal dorsal aorta P_{O_2} of 90–100 mm Hg during normoxia ranged from 5.0 to 9.5 vol % with a mean value in 5 fish of 7.0 ± 1.7 vol %. These variations in C_{O_2} arose in part from differences in hematocrit, which ranged from 23% to 28% (mean value 25.4 ± 2.1%). The reduction
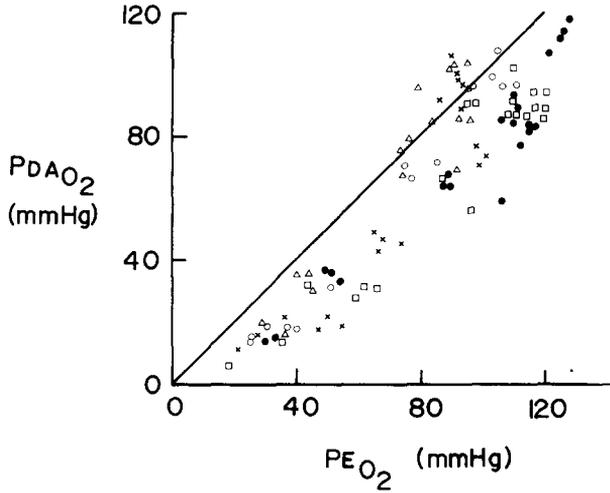
Fig. 5. Relationship between dorsal aorta P_{O_2} and expired water P_{O_2} in 5 *Acipenser transmontanus*.
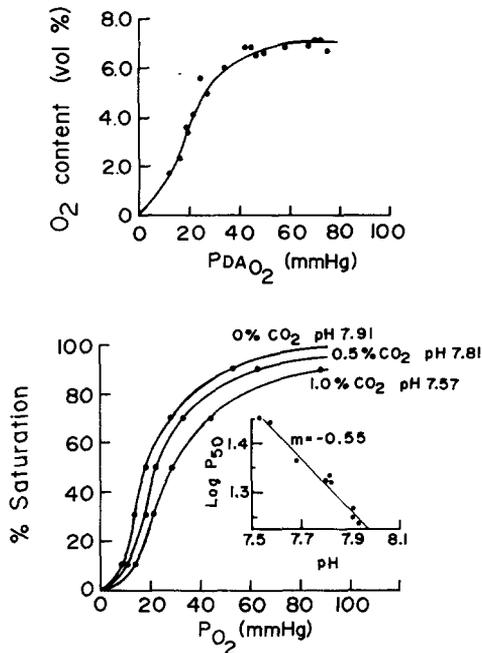
Fig. 6. (A) Relationship between dorsal aorta blood oxygen content and dorsal aorta P_{O_2} in a 0.80 kg sturgeon. (B) Oxygen dissociation curves at three different levels of CO_2 for *Acipenser transmontanus* blood at 15 °C. Also indicated is the blood pH measured at 50% saturation. The inset indicates the magnitude of the Bohr shift for this blood.

in C_{O_2} with decreasing P_{O_2} during hypoxia closely reflected the S shape of the O_2–Hb dissociation curve of sturgeon blood (fig. 6A). Oxygen dissociation curves for *Acipenser* from curves determined separately on blood from 3 sturgeons are presented in fig. 6B. P_{50} of blood equilibrated with 0.5% C_{O_2} (3.5 mm Hg) at 15 C was 21.5 mm Hg, and the Bohr shift between pH 7.57 and 7.91 was −0.55.

Discussion

Acipenser left the major stream of bony fish evolution over 200 million years ago, yet aspects of ventilatory performance in undisturbed sturgeon, at least under normoxic conditions, differ little from those determined in distantly related, recently evolved teleost fish. A branchial water flow of 350 ml/kg/min and oxygen utilizations of 30 to 40% fall well within the range reported for teleost and elasmobranch fish (see Shelton, 1970) and a $\dot{V}_{O_2}$ of approximately 55 ml O_2/kg/h at 15 C is comparable with that of other fairly sedentary, non pelagic fish. Nearly full O_2 saturation occurs in dorsal aortic blood, though the O_2 capacity of the blood of the sturgeon also reflects the oxygen transport needs of a comparatively inactive fish.

Acipenser is clearly an oxygen conformer, similar to a number of other fish (Hall, 1929; Hughes and Umezawa, 1968; Marvin and Heath, 1968), but there are several unusual facets to the ventilatory, cardiac and metabolic responses to low ambient oxygen in the sturgeon. Decreases in $\dot{V}g$ during hypoxia are almost totally mediated by changes in stroke volume rather than breathing rate (fig. 3). While large adjustments in stroke volume are usually evident in other fishes during hypoxic exposure, there is also a change in ventilatory frequency either to greater rates in O_2 regulators (van Dam, 1938; Randall and Shelton, 1963; Holeton and Randall, 1967) or to reduced rates in conformers (Hughes and Umezawa, 1968). Heart rate in *Acipenser* also shows virtually no changes over a wide range of ambient O_2 levels. A vagally-mediated bradycardia in response to hypoxia has been documented in a large number of widely divergent fishes (see Randall, 1968, 1970; Butler and Taylor, 1971; Marvin and Burton, 1973), so the absence of a bradycardia during hypoxia in this Chondrosteian is distinctive.

The relationship between ventilation volume and % utilization at varying levels of hypoxia in *Acipenser* is also dissimilar to that of many other fishes. Generally, in many species over a range of gill ventilation, the lowest % oxygen utilizations are associated with the highest values of $\dot{V}g$ (see Shelton, 1970), arising in part from the fact that at a higher $\dot{V}g$ transit times through the gills of water are decreased. In *Acipenser*, however, oxygen utilization decreased only very slightly while $\dot{V}g$ fell from over 50 ml/kg/min to less than 200 ml/kg/min (fig. 3). At the lowest values of $\dot{V}g$ during hypoxia, oxygen utilization was reduced from 30–40% to less than 20%, presumably related to the reduction in water/blood O_2 gradients driving this gas across the gills.

Perhaps the most interesting finding lies in the metabolic responses of *Acipenser*

to an hypoxic environment. Unlike other oxygen conformers, the lack of any compensatory increases in ventilation and oxygen consumption after a return from hypoxic to normoxic conditions indicates that there is no repayment of an oxygen debt which would result from a switch to anaerobiosis. Moreover, the lack of any consistent blood pH change during hypoxic exposure while oxygen uptake falls precipitously, further indicates an absence of any shift to anaerobic processes involving lactate or succinate production. Hence, the sturgeon appears to reduce total energy expenditure during periods of reduced oxygen availability. The fall in gill ventilation will contribute to the reduction in energy expenditure. but as the cost of ventilation in fish is often less than 10% of the total oxygen uptake (Jones and Randall, 1978) it cannot account for the whole of the fall in oxygen uptake. Clearly other tissues must greatly reduce energy expenditure, but whether this applies to all tissues equally or whether there is variable blood distribution to the organs is unknown. It is important to remember that the sturgeon survived extreme hypoxic exposure with $\dot{V}_{O_2}$ reduced to 5–10% of normoxic levels for 25–35 min. What happens to brain and cardiac function during this period requires investigation, especially as there appeared to be no oxygen debt accumulated.

There is a linear relationship between Pa_{O_2} and $\dot{V}_{O_2}$ (fig. 7) indicating that oxygen uptake by the tissues is determined by the oxygen gradient between blood and mitochondria. That is, tissue oxygen consumption is simply diffusion limited. Although heart rate in *Acipenser* changed little during hypoxic exposure, it seems probable, however, that there are some changes in cardiac output and tissue perfusion. For instance, if cardiac output and blood distribution remain unchanged during hypoxia and we assume 5% of oxygen uptake under normoxic conditions is utilized by the heart (Jones, 1971) and that because output and distribution are un-
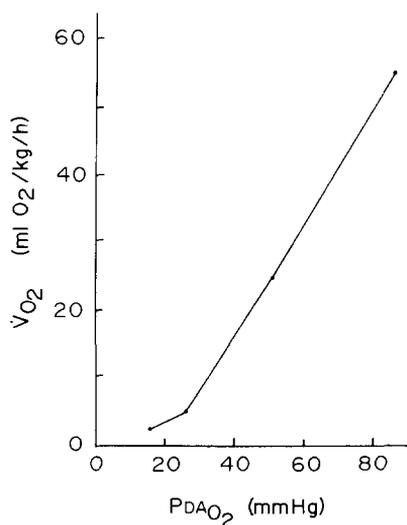
Fig. 7. Relationship between dorsal aorta P_{O_2} and $\dot{V}_{O_2}$ in the sturgeon.

changed energy expended by the heart does not change during hypoxia, then during extreme hypoxia, when $\dot{V}_{O_2}$ is reduced to less than 10% of normoxic values, nearly all the oxygen will be utilized by the heart, clearly an untenable situation. There must be some reduction in cardiac output and blood redistribution to ensure continued cardiac function. Thus, we suggest that there are perfusion, and probably diffusion, limitations determining tissue oxygen uptake during hypoxia in *Acipenser*.

Another unusual characteristic of the sturgeon is that respiratory dependence develops when $P_{I_{O_2}}$ falls just below air saturation. At a $P_{I_{O_2}}$ of 100 mm Hg dorsal aortic blood was still more than 80% saturated with oxygen (fig. 6) but $\dot{V}_{O_2}$ had already fallen by one-half. Why, under these conditions, the sturgeon reduces $\dot{V}g$, and therefore Pa_{O_2} and $\dot{V}_{O_2}$, is not clear. Shelton (1970) has reviewed arguments for and against the widely accepted premise that the appearance of respiratory dependence correlates with transport limitations in the respiratory system. In the case of the sturgeon the selected strategy appears to be to reduce water flow, increasing gill water residence time and so maintain oxygen extraction. This presumably maintains a low cost of ventilation per unit oxygen uptake, the consequence being an overall drop in $\dot{V}_{O_2}$ as $\dot{V}g$ and Pa_{O_2} decrease. Thus the sturgeon has evolved strategies for maintaining costs at a low level rather than maintaining rates of oxygen delivery. As pointed out earlier the metabolic consequences of maintaining the 'cost-benefit' ratio rather than O_2 delivery rate are enormous and require investigation.

Ventilatory and cardiac responses in the sturgeon during the onset and termination of hypoxia began to develop only after 1–2 min. In no instance was a nearly instantaneous response to low ambient O_2, characteristic of other fishes (Bamford, 1974), observed in *Acipenser*. This strongly suggests that externally or superficially located O_2 receptors, should they be present in the sturgeon, do not mediate the ventilatory and cardiac responses to hypoxia. Indeed, the time course of these responses follows changes in the P_{O_2} or dorsal aorta blood, and central sites of oxygen reception may be operant in the sturgeon during hypoxic exposure, as Saunders and Sutterlin (1971) and Bamford (1974) have indicated in some fishes.

Acipenser is unusual amongst vertebrates in that it becomes an oxygen conformer as soon as O_2 levels are reduced below ambient and, during severe hypoxia, oxygen uptake may fall to only 5% of normal. There appears to be no shift to anaerobiosis, the sturgeon simply shuts down metabolism. This fish ventures into very hypoxic water and these adaptations may allow the sturgeon to shelter in deoxygenated benthic regions of lakes, ice covered waters and stagnant regions of estuaries which would be inaccessible to less resistant fishes. While extreme environmental hypoxia resulting in a marked reduction in aerobic metabolism may be only rarely encountered or sought, it nonetheless attests to the tremendous respiratory flexibility of this ancient species.

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