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SECRETION OF NITROGEN INTO THE SWIMBLADDER OF FISH. I. SECRETION BY FISHES NEARLY LACKING CIRCULATING HEMOGLOBIN. ROLE OF THE RETE MIRABILE[1]

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Abstract

Toadfish (*Opsanus tau*) essentially lacking circulating erythrocytes were prepared by repeated exchange transfusion with serum. The rate of nitrogen secretion is not changed by removal of the erythrocytes. Oxygen secretion is slowed drastically. This shows that nitrogen secretion does not require erythrocytes and is not driven by oxygen secretion. In the absence of circulating erythrocytes, oxygen and nitrogen are brought into the swimbladder in proportion to their concentrations in blood plasma. Carbon dioxide partial pressure in the secreted gas mixture is three to fourfold greater than the pressure generated by acidifying arterial blood. This implies counter-current multiplication of the small increment of carbon dioxide pressure brought about by acidification of the blood. In the presence of blood buffers, increased carbon dioxide pressure will increase blood bicarbonate. Three independent estimates indicate that, during gas secretion, gas gland blood is near pH 6.5. Total carbon dioxide (CO_2, HCO_3^-, $CO_3^=$) is increased from the arterial value near 2 m*M* to about 14 m*M*, divided nearly equally between carbon dioxide and bicarbonate anion. The increment in total blood carbon dioxide concentration together with the well-known increment in lactate anion may serve to salt out inert gases from solution in blood plasma.

Introduction

The swimbladder of marine and fresh water teleost fishes is a gas filled sac which serves primarily to make the fish neutrally buoyant. The largest part of the gas brought into the swimbladder of most fish is oxygen, at a pressure very close to the external hydrostatic pressure. The difference in gas partial pressure in the contents of the swimbladder and in the lake or sea water is large in fishes living at any considerable depth, for the hydrostatic pressure increases about 1 atm with each 10 m depth; while the partial pressures of gases dissolved in lake or sea water are relatively independent of depth (Biot, 1807) and remain near 0.2 atm oxygen and 0.8 atm nitrogen. The partial pressure of oxygen in the swimbladders of marine fish living at 3000–6000 m depth is commonly 300 to 500 atm (Wittenberg *et al.*, 1980). A fish with a functional swimbladder has been captured from a depth of 7160 m (Nielsen and Munk, 1964); if the contained gas were 90% oxygen the partial pressure of oxygen would have been 644 atm.

Nitrogen and other chemically inert gases are brought into the swimbladder at partial pressures that greatly exceed their partial pressure in the surrounding

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water. J. S. Haldane (1898), considering the analyses of swimbladder gas made by Biot (1807) 90 years earlier, realized that although nitrogen and argon make up only about 10% of the swimbladder gases, their partial pressure may be very great at the enormous pressures at which fish live in the deep sea. Haldane's estimate of the partial pressure of nitrogen in the swimbladders of deep sea fish has been extended from 7–8 atm to as high as 50 atm (Delaroche, 1809; Richard, 1895; Schloesing and Richard, 1896; Scholander *et al.*, 1951; Scholander and van Dam, 1953; Scholander, 1954; Kanwisher and Ebeling, 1957; Nielsen and Munk, 1964; Douglas, 1967; Wittenberg *et al.*, 1980).

Hüfner (1892) reported that the swimbladder gases of whitefish (*Coregonus acronius*) captured from the bottom of the Bodensee at a depth of 60 to 80 m contained 99% nitrogen. At the depth at which the fish were captured, the partial pressure of nitrogen in the swimbladder would have been 6–8 atm. Subsequently, nearly pure nitrogen at 10 atm pressure has been found in the swimbladders of many deep-living fresh and saltwater salmonids, coregonids, osmerids, and catostomids (Saunders, 1953; Scholander *et al.*, 1956a; Tait, 1956; Sundnes *et al.*, 1958; Sundnes, 1963; Sundnes *et al.*, 1969; Fahlén, 1970).

In Hüfner's (1892) classic study, the fraction of nitrogen in the swimbladder gas of most fish was 99%. However, substantial concentrations of oxygen (10–50% of the total gases) were found in some individual fish examined by Hüfner and by most later workers (Saunders, 1953; Tait, 1956; Fänge, 1958; Sundnes *et al.*, 1958; Fahlén, 1968; Fahlén, 1970). Sundnes (1963) finds that *Salmo* and *Coregonus*, which have 99% nitrogen in the swimbladder while residing for some weeks at one depth, may have as much as 70% oxygen while moving to deeper water during their annual spawning migration. Fahlén (1967b) emptied the swimbladder of *Coregonus* experimentally and found that the gases were replaced with a gas mixture containing up to 61% oxygen, and 6.5% carbon dioxide.

The swimbladder of fresh water cyprinids (goldfish, carp, roach, tench) is normally filled with pure nitrogen (Wittenberg, 1958). In fact, Priestley (quoted in de Fourcroy, 1789) used nitrogen from the swimbladder of carp purchased in the market for his chemical experiments. Nevertheless, if the swimbladders of these fish (Moreau, 1876, 1877; Krohn and Piiper, 1962; Evans and Damant, 1928; Wittenberg, 1958) are emptied experimentally, the swimbladder is refilled with a gas mixture containing up to 60% oxygen, and 2–10% carbon dioxide. Oxygen is removed from the swimbladder rapidly (Wittenberg, 1958; Piiper *et al.*, 1962), nitrogen slowly, thus nitrogen is accumulated in the swimbladders of those fishes that secrete gas slowly.

The fraction of nitrogen in the swimbladder gas of deep living marine fish tends to be constant for each species and independent of depth over a wide range (Scholander and van Dam, 1953; Scholander, 1954; Douglas, 1967). This implies that the rates of oxygen secretion and of nitrogen secretion remain in constant proportion for each species.

Oxygen secreted into the swimbladder comes from oxygen dissolved in the water in which the fish lives (Scholander *et al.*, 1956b; Wittenberg, 1961) and is brought to the swimbladder in the blood. Oxygen is released from the blood in the capillaries of the gas gland, a specialized area of the epithelium lining the swimbladder, and in the rete mirabile, a vascular counter-current exchange organ supplying blood to the gas gland (Denton, 1961; Steen, 1970). These structures are massive in fish that secrete oxygen rapidly and against high pressures (Marshall, 1960, 1972; Wittenberg, *et al.*, 1980). They are present, albeit inconspicuous and dispersed as small bundles of as few as two capillaries, in the salmonids, coregonids, and other

fish that secrete gas slowly (Fänge, 1958; Marshall, 1960; Fahlén, 1967b, 1968, 1970, 1971). (Certain of these fishes, described as lacking retia mirabilia (Sundness, *et al.* 1958), in fact have retia (Fahlén, 1967b).) In these fish the rate of oxygen secretion may parallel the degree to which the microretia are developed (Fahlén, 1967b). Herring differ; they lack retia (Fahlén, 1967a; Blaxter *et al.*, 1979) and bring swallowed air into the swimbladder by way of the pneumatic duct.

We conclude that the gas brought into the swimbladder through the action of the gas gland and rete mirabile is always a mixture of nitrogen and oxygen.

In this study we have inquired whether, and how these two secretory processes are linked. We concluded that oxygen secretion and nitrogen secretion are independent processes driven by a common force. To prove this we have required fish lacking or nearly lacking circulating erythrocytes. In addition we have shown that the driving force for nitrogen secretion is generated by two separate processes—generation of lactate anion by aerobic glycolysis and counter-current multiplication of carbon dioxide pressure in the rete. An accompanying paper (Wittenberg *et al.*, 1981) discusses the molecular mechanism by which inert gases are driven from the blood plasma.

Materials and Methods

Toadfish

Toadfish (*Opsanus tau,* L.), weighing 250–600 g, were maintained, with feeding, in running sea water at 21°C. The morphology of the toadfish swimbladder has been described (Fänge and Wittenberg, 1958). Gases in the experimentally emptied swimbladder of fish maintained near atmospheric pressure are replaced in about 24 hours (Fänge and Wittenberg, 1958). All members of the population used in this study belong to the Type I hemoglobin phenotype described by Fyhn and Sullivan (1974) (86 individual fish were examined; all were type I; Sullivan, Bonaventura and Bonaventura, personal communication). This phenotype has six or more electrophoretically distinguishable hemoglobins.

Secretion of gas by toadfish breathing pure oxygen

A 100 liter tank was fabricated from plexiglass. The lid was made gas-tight with an O-ring seal. Temperature was maintained at 21°C by means of an external heat exchanger through which the water was circulated by a pump (TEEL model IP681, Dayton Electric, Chicago). A second pump circulated the water continuously through a bed of dolomitic limestone (Marine Filter Mix, Aquarium Systems Inc., Eastlake, Ohio), a small bed of activated charcoal and a layer of glass wool. A population of bacteria which developed in the filter bed after 5–6 days removed waste products, particularly the very toxic ammonia.

Abrupt exposure to high oxygen pressure injures toadfish. Accordingly, three to five 250-300 g fish were placed in the tank filled with air-equilibrated sea water, and the air replaced slowly by oxygen gas delivered from a bubbler at the bottom of the tank. The flow was more than 1 l per min. After 24 hours, the fish were restrained without anesthetic, and the swimbladder was emptied, using a syringe fitted with a fine needle. This initial gas sample was discarded. Thereafter gas samples were withdrawn for analysis at 24–48 h intervals. Three successive samples of secreted gas were often obtained from the same animal.

Gas analyses

Oxygen, carbon dioxide and residual inert gas were determined by the method of Scholander *et al.* (1955). Gas samples containing a large fraction of carbon dioxide were analyzed in the apparatus described by Fry *et al.* (1949) using the reagents of Scholander and Irving (1947).

Determination of blood hemoglobin

Washed red blood cells from a measured volume of blood were hemolyzed in distilled water; the solution was adjusted to pH 8.6 by the addition of sodium borate buffer, and the stroma removed by low speed centrifugation. The concentration of oxyhemoglobin was determined using a Beckman model DU spectrophotometer taking $\epsilon_{578nm} = 14.1 \cdot 10^3\ M^{-1}\ cm^{-1}$. Values are expressed as millimoles heme per liter blood (m*M* heme).

Saline and anticoagulant

A balanced salt solution for *Opsanus* was devised following the suggestions of Wolf and Quimby (1969) using the ionic composition of toadfish blood given Lahlou *et al.* (1969). It contained NaCl (170 m*M*), KCl (5.5 m*M*), $CaCl_2$ (1.5 m*M*), $MgCl_2$ (1.0 m*M*), $NaHCO_3$ (10 m*M*), KH_2PO_4 (0.6 m*M*). After aeration, the pH was 7.4. Heparin (500 IU/ml) was added to saline used to lubricate syringes or fill cannulae.

Serum

Blood was drawn from the heart or gill vessels. Red cells were removed by centrifugation and the hemoglobin-free plasma pooled and frozen. The clot discovered on thawing was removed by centrifugation at about 10,000 *g*.

Experimentally produced anemia

Procedures for prolonged cannulation of the dorsal aorta of free-swimming fish (Smith and Bell, 1964, 1967; Holeton and Randall, 1967) were adapted to the toadfish. Large toadfish (400–600 g) were anesthetized with Tricaine methanesulfonate (MS 22, Sandoz), and fine polyethylene tube (PE-10, Clay Adams, Inc.) filled with heparinized saline was implanted in the dorsal aorta at a point about 4 cm posterior to the vent. The PE-10 polyethylene tubing was connected to a one meter length of polyethylene tubing of larger bore (PE-50, Clay Adams, Inc.) filled with heparinized saline. Every 12 h blood equivalent to about 5% of the animal's weight was withdrawn from the unrestrained free-swimming fish using a syringe connected to the free end of the indwelling cannula. Since plasma proteins are replaced slowly in toadfish (Haschemeyer, 1973), this blood was replaced by an equal volume of toadfish serum.

Anemic toadfish showed no signs of distress while swimming freely, but were little able to withstand struggling or the stress of capture in a net. Accordingly, to obtain gas samples, Tricaine (400 mg per liter) was added to the sea water and the gas sample taken as soon as anesthesia was produced. Gas samples were taken every 48 hours.

Oxygen Equilibrium of Toadfish Blood

Blood from toadfish immobilized by intramuscular injection of d-tubocurarine (15 mg per kg) was drawn into syringes containing dry heparin, and was stirred

for 20 minutes under oxygen to permit the consumption of any excess metabolites. Portions of blood were adjusted to known pH by the addition of small volumes of 0.2 *M* lactic acid in 0.2 *M* NaCl. The pH was verified at the end of each experiment and found unchanged. A drop of such blood was placed in a layer of 625-mesh woven stainless steel cloth, 0.1 mm thick, sandwiched between layers of teflon film (0.5 mil thick, Dilectrics Corporation, New York). Capillary flow brings the film of blood to a uniform thickness. This assembly was held in a pressure cuvette (Beckman RIIC, Ltd.) maintained at 20°C. Spectra were recorded using a Cary model 17 recording spectrophotometer equipped with a scattered transmission accessory. Calculations of fractional saturation of the hemoglobin with oxygen were made from the sum of optical density changes at 560 and 576 nm.

pH of toadfish blood equilibrated with oxygen and varying pressures of carbon dioxide

Portions of blood were equilibrated with 3 successive portions of each of the gas mixtures listed in Table I at 20°. The pH was determined with a Radiometer pH meter using a combined glass and reference electrode (Ingold Electrodes Incorporated, Lexington, Massachusetts).

Carbon dioxide pressure of acidified toadfish blood

Blood was withdrawn from the heart of toadfish immobilized by intramuscular injection of d-tubocurarine into syringes whose dead space was filled with a strong solution of heparin in 0.2 *M* NaCl. A portion of the blood sample was retained in the syringe and not exposed to air. A second portion ws equilibrated with air for 30 min at 20°C. Each portion was made first weakly, then strongly acidic by the addition of small volumes of lactic acid, 0.2 or 0.5 *M* in 0.2 *M* NaCl, and the pH and P_{CO_2} determined using a Radiometer E 5036-0 P_{CO_2} module and a PHM 72 Acid Base Analyser.

Results

Toadfish nearly lacking blood hemoglobin

The blood hemoglobin concentration in normal toadfish of the population studied here was 2.35 ± 0.46 m*M* heme (mean and S.D. of 24 individuals, range 1.8–3.03).

Table I

pH of toadfish blood equilibrated with gas mixtures containing oxygen and carbon dioxide at 20°C.

	pH				
	CO_2, atmosphere				
Animal	0.0	0.040	0.088	0.135	0.189
520	7.67	7.03	6.74	6.60	6.50
521	8.34	7.25	7.00	6.84	6.71
522	7.89	7.00	6.66	6.54	6.40
523	8.04	6.95	6.64	6.47	6.36
524	7.93	6.99	6.68	6.49	6.38
525	7.80	6.96	6.66	6.47	6.36
mean ± SD	7.95 ± .23	7.03 ± .11	6.73 ± .14	6.57 ± .14	6.45 ± .14

The course of a series of exchange transfusions in which blood is withdrawn and replaced by serum is presented in Figure 1. A working lower limit of blood hemoglobin concentration is reached at 0.05 m*M*, about 2% of the normal value. Below this limit, removal of red blood cells appears to be countered by the mustering of new cells by the animal. The initial slope of the relation of Figure 1 suggests that about half of the blood volume is replaced at each exchange transfusion.

Composition of swimbladder gas in severely anemic fish

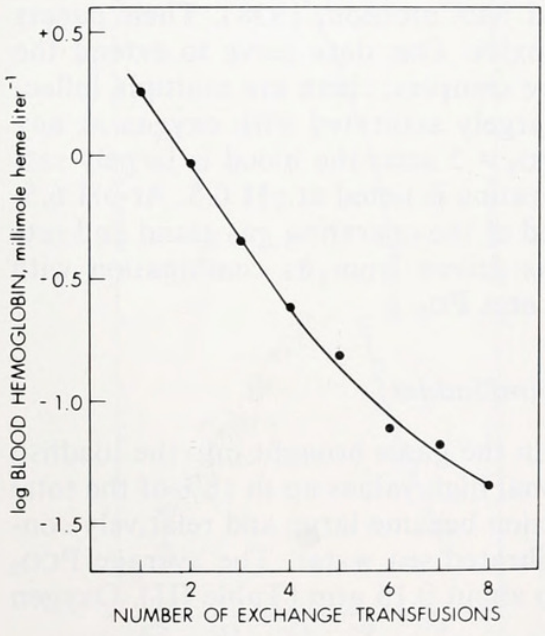
The fraction of oxygen in the gases brought into the swimbladder is much diminished as the level of blood hemoglobin is made less (Fig. 2). It does not, however, fall to zero but approaches about 35% in the most anemic fish examined. The ratio, $Ar/^{28}N_2$ (mean of the samples from the six most anemic fish in Fig. 2), is $1.40 \pm 0.10 \times 10^{-2}$.

It may be relevant to recall that the fraction of oxygen in gases secreted by toadfish whose blood hemoglobin is entirely combined with carbon monoxide also does not fall to zero (Wittenberg and Wittenberg, 1961).

Rate of gas secretion in severely anemic fish

The rate of replacement of oxygen in the experimentally emptied swimbladder falls precipitously to a low and more or less constant value as the level of blood hemoglobin is lowered.

The rate of inert gas secretion into the swimbladder of severely anemic fish is the same as the rate at which inert gas is brought into the swimbladders of normal



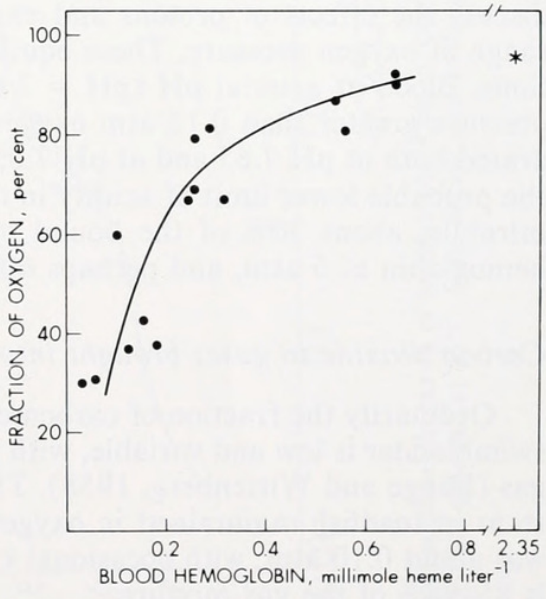


FIGURE 1. (Left) Blood hemoglobin concentration after successive exchange transfusions of an individual toadfish. Exchange perfusion was done every 12 hours.

FIGURE 2. (Right) Composition of the gas brought into the swimbladder as a function of blood hemoglobin concentration. The fraction of oxygen is expressed as a fraction of the sum of oxygen plus nitrogen, the fraction of carbon dioxide being variable. Dots represent individual gas samples; two successive gas samples were occasionally obtained from the same fish. The asterisk represents the mean of 50 samples of secreted gas and 24 blood samples from normal toadfish.

TABLE II

Rate of nitrogen secretion by toadfish, expressed as the fraction of the initial gas volume of the swimbladder replaced by inert gas (nitrogen plus argon) per 24 h.

Condition	Number of animals	Number of gas samples[b]	Rate of nitrogen secretion mean ± SD
Normal Fish; 1 atm	31	79	0.042 ± 0.035
Normal Fish; 6 atm	43	53	0.040 ± 0.035
Normal fish which had secreted gas at 6 atm and were subsequently maintained at 1 atm	20	20	0.052 ± 0.034
Severely anemic fish[a]	11	12	0.041 ± 0.034

a) Blood hemoglobin less than 0.29 mM. The normal hemoglobin level is 2.3 mM.

b) Often, three successive gas samples were obtained from each normal fish at 1 atm and two successive gas samples were obtained from normal fish at 6 atm.

fish at 1 atm or at 6 atm total pressure, (Table II). Details of the experiment in which fish were maintained at 6 atm pressure are presented elsewhere (Wittenberg *et al.*, 1981).

Oxygen equilibrium of toadfish blood

Oxygen equilibria were determined to a maximal P_{O_2} of 10 atm at 20°C using whole blood. In the relations presented in Figure 3, the solid symbols represent our data; the open symbols present data of the pioneers of this field, (Root, 1931; Green and Root, 1933; Root, *et al.*, 1939; Hall and McCutcheon, 1938). Their papers discuss the effects of protons and carbon dioxide. Our data serve to extend the range of oxygen pressure. These equilibria are complex; there are multiple inflections. Blood at arterial pH (pH = 7.65) is largely saturated with oxygen at any pressure greater than 0.12 atm oxygen. At P_{O_2} = 5 atm, the blood is largely saturated both at pH 7.65 and at pH 7.0; desaturation is noted at pH 6.5. At pH 6.5, the probable lower limit of acidity in the blood of the operating gas gland and rete mirabile, about 20% of the bound oxygen is driven from its combination with hemoglobin at 5 atm, and perhaps 60% at 1 atm P_{O_2}.

Carbon dioxide in gases brought into the swimbladder

Ordinarily the fraction of carbon dioxide in the gases brought into the toadfish swimbladder is low and variable, with occasional high values up to 18% of the total gas (Fänge and Wittenberg, 1958). This fraction became large and relatively constant in toadfish maintained in oxygen-equilibrated sea water. The average P_{CO_2} was about 0.10 atm, with occasional values to about 0.15 atm (Table III). Oxygen is 85–95% of the gas mixture.

pH and carbon dioxide pressure generated by acidifying toadfish blood

We wish to know whether the carbon dioxide pressure found in the swimbladder could have been generated by acidifying arterial toadfish blood. We assumed arterial P_{CO_2} is less than 2 torr, and equilibrated the blood with air. Steen (1963b)

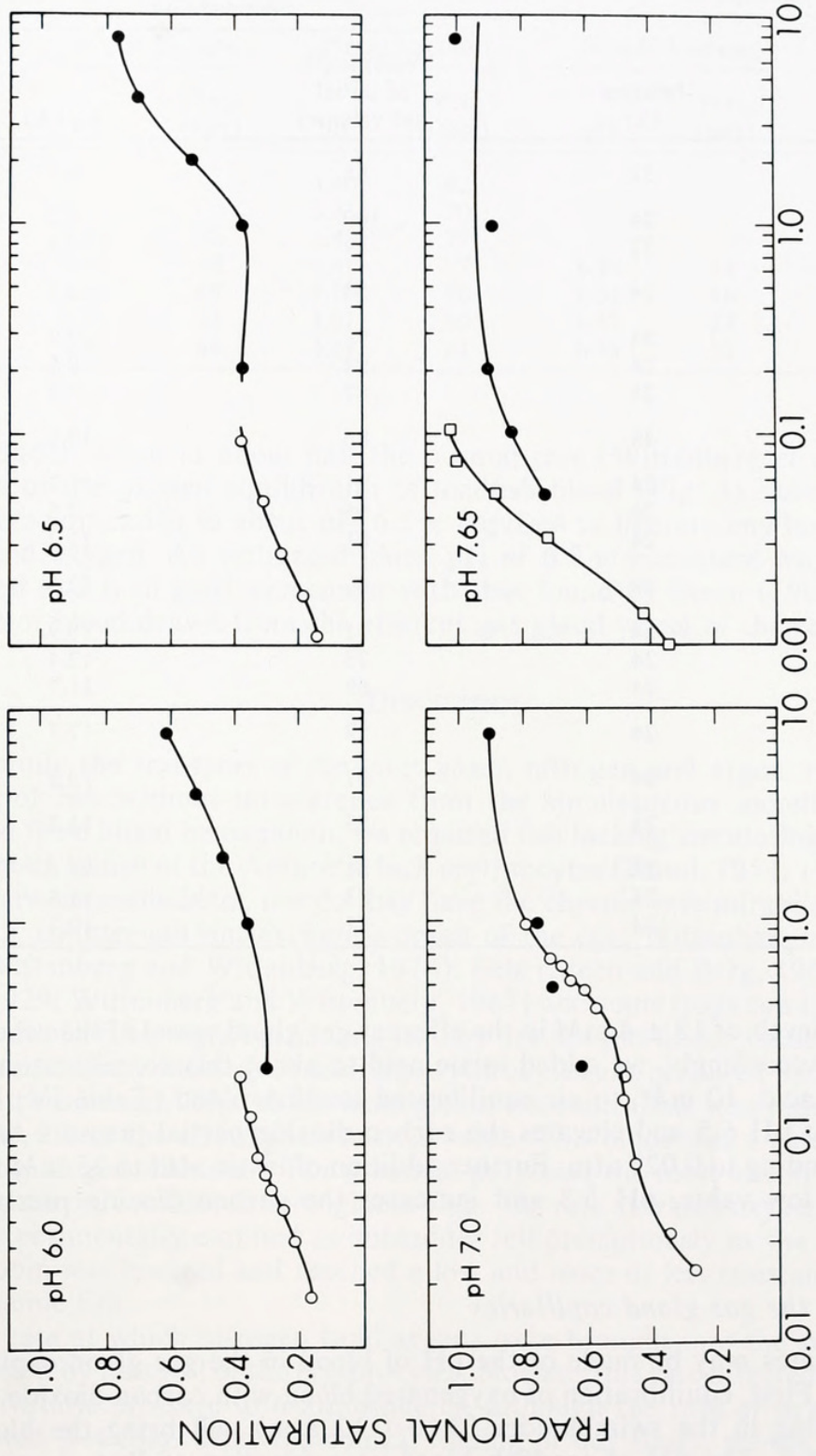


FIGURE 3. Oxygen equilibrium curves of toadfish whole blood. Open squares: data of Root (1931) obtained at 20°C and pH 7.60–7.99. Open circles: data of Green and Root (1933) obtained at 25°C. Solid circles: present data obtained at 20°C.

TABLE III

Composition of swimbladder gas of toadfish maintained in oxygen-equilibrated sea water. Samples marked A, B, C are successive samples from the same animal.

Animal	Interval (h)	Secreted Gas	
		Volume (% of initial gas volume)	CO_2 (%)
241	32	88	6.5
240A	24	100	6.5
B	32	79	7.1
503	24	50	8.5
504A	24	98	9.9
B	24	97	8.5
C	24	87	7.2
242	48	85	10.0
502A	24	81	11.2
B	24	85	7.5
C	24	81	11.2
239	48	63	12.0
509A	24	91	10.9
B	24	73	12.4
C	24	69	11.7
507	24	59	12.7
510	24	97	13.2
506	24	55	13.3
501A	24	81	14.5
B	24	83	11.6
C	24	71	10.7

records lactate levels of 13 ± 4 m*M* in the efferent gas gland vessel of the eel during gas secretion. Accordingly, we added lactic acid to about this concentration. Addition of lactic acid, 10 m*M*, to air equilibrated toadfish blood (Table IV) lowers the pH to about pH 6.5 and elevates the carbon dioxide partial pressure to 18 ± 5 torr, corresponding to 0.023 atm. Further addition of lactic acid to 25 m*M* brings the pH to the low value, pH 5.3 and increases the carbon dioxide pressure to 0.054 atm.

pH of blood in the gas gland capillaries

Three estimates may be made of the pH of blood in the gas gland capillaries of the toadfish. First, equilibration of oxygenated blood with carbon dioxide at the pressure obtaining in the swimbladder (0.10–0.12 atm) will bring the blood to about pH 6.5–6.7. The pH of toadfish blood as a function of P_{CO_2} is given in Table IV.

Second, addition of lactic acid to the concentration, 10 m*M* which probably obtains in the gas gland capillaries, will bring the blood to pH 6.5 (Table IV).

Third, toadfish maintained at 6 atm pressure continue to bring oxygen into the

TABLE IV

Carbon dioxide pressure generated by adding lactic acid to toadfish blood.

	Heart blood				Air equilibrated blood			
	10 m*M* Lactate		25m*M* Lactate		10 m*M* Lactate		25 m*M* Lactate	
Animal	pH	P_{CO_2} (torr)	pH	P_{CO_2} (torr)	pH	P_{CO_2} (torr)	pH	P_{CO_2} (torr)
748			5.40	62				
748			5.33	70				
749	6.44	32	5.45	77			5.50	28
750	6.46	30	5.41	77	6.65	11	5.40	33
751	6.30	39	5.11	90	6.51	16	5.14	43
752	6.32	35	5.07	89	6.48	22	5.05	59
753	6.45	44	4.81	87	6.58	22		

swimbladder, albeit at about half the normal rate (Wittenberg *et al.*, 1981). Inspection of the oxygen equilibrium of toadfish blood (Fig. 3) shows that at this pressure acidification to about pH 6.5 is required to liberate any large fraction of the bound oxygen. An estimated blood pH of 6.5 is consistent with all the data presented and is in good agreement with that found by Steen (1963b) by actual analysis of blood drawn from the efferent gas gland vessel of the eel.

DISCUSSION

To study the transport of the inert gases, nitrogen and argon, into the swimbladder of fish without interference from the simultaneous secretion of oxygen liberated from blood hemoglobin, we required fish lacking circulating erythrocytes. The famous icefish of the Antarctic lack erythrocytes (Ruud, 1954, 1965), but they do not have swimbladders, nor do they have the choroid rete mirabile (the oxygen-secreting, counter-current exchange organ of the eye; Wittenberg and Haedrich, 1974; Wittenberg and Wittenberg, 1974). Eels (Steen and Berg, 1966), toadfishes (Hall, 1929; Wittenberg and Wittenberg, 1961) and some frogs can survive without functional blood hemoglobin as long as they are not put under stress. In this study toadfish essentially lacking circulating erythrocytes are prepared by successive exchange transfusions. The blood hemoglobin concentration was reduced to about 0.05 m*M*, 2% of the already low normal value. This blood was a pale straw yellow detectably tinged with pink. For practical purposes, the most anemic fish we have prepared can be considered hemoglobin-free. The rate at which oxygen was replaced in the experimentally emptied swimbladder fell precipitously as the level of blood hemoglobin was lowered and reached a low and more or less constant value in the most anemic fish.

The rate at which nitrogen (and argon) were brought into the swimbladder is not affected by removal of the erythrocytes. Nor was this rate affected by increasing the hydrostatic pressure to 6 atmospheres, although the rate of oxygen secretion was slowed 2–4 fold. This, the central finding of our study, proves that nitrogen secretion into the swimbladder is not driven by oxygen secretion. Erythrocytes are not required for nitrogen secretion.

In the absence of erythrocyte hemoglobin, to which it may bind reversibly, oxygen behaves as any other gas. The gas mixture brought into the swimbladder of essentially erythrocyte-free fish contained oxygen and nitrogen approximately

in the proportion 35:65. This is different from their proportion in air, 21:79, but is the same as their proportion, 35:65, in the gases dissolved in water which has been equilibrated with air. It follows that oxygen (in the absence of chemical binding) and nitrogen are brought into the swimbladder in proportion to their *concentrations* in the blood plasma. This implies salting out of nitrogen, oxygen and other gases from their solution in the plasma of the gas gland and rete capillaries (Wittenberg *et al.* 1981).

We enquire, what substances present in this plasma at enhanced concentration may be responsible for salting out the gases? In the eel which has a gas-secreting complex remarkably like that of the toadfish (Fänge and Wittenberg, 1958), the blood vessels are accessible and a partial answer is known. The cells of both the rete mirabile (Rasio, 1973) and gas gland (Kutchai, 1971) generate lactic acid by aerobic glycolysis of glucose brought in by the flowing blood. Arterial lactate in the eel ranges from about 2–10 m*M*. About 2.5 mmol lactic acid per l is added to the blood in its transit through the inflowing capillaries of the rete (bringing the blood to about pH 7.2). A further 5–6 mmol lactic acid per l is added in the capillaries of the gas gland, bringing the total lactic acid concentration to 13 ± 4 m*M* and bringing the blood to about pH 6.7 (Kuhn *et al.*, 1962; Steen, 1963b). We assume similar lactate levels in the gas gland capillaries of toadfish.

We learn the concentration of total carbon dioxide (CO_2, HCO_3^-, $CO_3^=$) in these vessels from the composition of the swimbladder gas. We assume only that the partial pressure of carbon dioxide in this blood approaches equilibrium with the gases of the swimbladder lumen, since the capillaries of the gas gland are separated from the gas-filled lumen by a single layer of cells about 30–50 μm thick. During refilling of the experimentally emptied swimbladder, particularly in toadfish maintained in oxygen equilibrated sea water, this carbon dioxide pressure is commonly 0.1 atm with occasional values to 0.15 or even 0.18 atm. This finding is not unusual; values from 0.1–0.37 atm have been reported for other species (Krohn and Piiper, 1962; Fänge, 1953; Scholander, 1956; Scholander *et al.*, 1956b; Wittenberg, 1961; Jacobs, 1930; Wittenberg *et al.*, 1964). The partial pressure of carbon dioxide in the gases brought into the swimbladder exceeds fivefold (bluefish) or three to fourfold (toadfish) the greatest pressure which could be generated by acidifying arterial blood (Wittenberg *et al.*, 1964). The source of this carbon dioxide is arterial blood; oxidative metabolism in the rete or gas gland contributes very little (Wittenberg *et al.*, 1964; Rasio, 1973). It follows that carbon dioxide pressure is assuredly augmented by counter-current multiplication in the rete mirabile (Kuhn *et al.*, 1963; Wittenberg *et al.*, 1964; Kuhn and Marti, 1966; Lesslauer *et al.*, 1966; Sund, 1977). The role of carbonic anhydrase, which is present in the rete at extraordinary concentration (reviewed in Wittenberg and Haedrich, 1974) remains unknown.

The total carbon dioxide concentration (CO_2, HCO_3^-, $CO_3^=$) in blood of the gas gland capillaries of the toadfish (P_{CO_2} = 0.15 atm; pH 6.5; 20°C) may be estimated from the carbon dioxide equilibrium curve of eel blood (Steen, 1963a), since blood buffering in eels (Steen, 1963a) and toadfish (Root, 1931) is about the same. It will be about 14 m*M*, divided about equally between CO_2 and HCO_3^-. This represents an increment of 12 m*M* total carbon dioxide above the arterial blood, which (at an estimated P_{CO_2} = 2 torr), will contain only 2 m*M* total carbon dioxide (Root, 1931).

Conclusion

From the foregoing facts we can deduce the sequence of events leading to the secretion of nitrogen against high pressure in the teleost swimbladder. We focus

attention on blood plasma, since erythrocytes are not required for nitrogen secretion. The primary event must be the glycolytic generation of lactate anion and protons. Protons, reacting with blood bicarbonate, increase the partial pressure of carbon dioxide. This small "single concentrating effect" is cascaded by counter-current multiplication of the pressure of the highly diffusible carbon dioxide in the rete mirabile to give a much elevated partial pressure of carbon dioxide in the gas gland blood plasma. Interaction of carbon dioxide with blood buffers leads to a much increased concentration of total carbon dioxide (CO_2, $H_2CO_3^-$, $CO_3^=$) in the gas gland blood. This increment in the concentration of bicarbonate anion (and other forms of CO_2), in consort with the increment in the concentration of lactate anion, serves to salt out inert gases from their solution in the blood plasma.

Studies of natural populations and analyses of gases brought into the experimentally emptied swimbladder have shown that oxygen secretion and nitrogen secretion are closely linked processes. Protons, arising from dissociation of lactic acid, drive oxygen from its combination with blood hemoglobin, and thus provide the link between oxygen secretion and nitrogen secretion.

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