The Secretion of Oxygen into the Swim-Bladder of Fish

III. *The role of carbon dioxide*

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ABSTRACT The secretion of carbon dioxide accompanying the secretion of oxygen into the swim-bladder of the bluefish is examined in order to distinguish among several theories which have been proposed to describe the operation of the rete mirabile, a vascular countercurrent exchange organ. Carbon dioxide may comprise 27 per cent of the gas secreted, corresponding to a partial pressure of 275 mm Hg. This is greater than the partial pressure that would be generated by acidifying arterial blood (about 55 mm Hg). The rate of secretion is very much greater than the probable rate of metabolic formation of carbon dioxide in the gas-secreting complex. It is approximately equivalent to the probable rate of glycolytic generation of lactic acid in the gas gland. It is concluded that carbon dioxide brought into the swim-bladder is liberated from blood by the addition of lactic acid. The rete mirabile must act to multiply the primary partial pressure of carbon dioxide produced by acidification of the blood. The function of the rete mirabile as a *countercurrent multiplier* has been proposed by Kuhn, W., Ramel, A., Kuhn, H. J., and Marti, E., *Experientia*, 1963, **19**, 497. Our findings provide strong support for their theory. The unique structure of the gas-secreting complex of the swim-bladder of the bluefish, *Pomatomus saltatrix* L., is described.

Gases are brought into the swim-bladder of teleost fishes against a pressure equal to the ambient hydrostatic pressure. Abyssal fishes living at depths of 4000 meters or more successfully inflate their swim-bladders at pressures which may exceed 400 atmospheres (Marshall, 1960). This remarkable secretion is accomplished by both surface living and deep sea fish through the action of a gas-secreting complex which includes the gas gland in contact with the gases in the swim-bladder lumen and the rete mirabile supplying blood to the gas

gland. The rete mirabile is a large distinct organ (which may be single or separated into multiple retia) formed of several thousand closely packed parallel capillaries in which the afferent and efferent streams flow countercurrent one to another.

The structure of the rete is ideally suited to both the creation and the maintenance of pressure differences along its length. There is general agreement that the large pressures of gases found in the swim-bladder are generated in the rete mirabile (Marshall, 1960; Denton, 1961; Kuhn *et al.*, 1963), the gas gland serving some other function. The mechanism by which the rete operates to generate gas pressures is much less certain. Three modes have been proposed, each involving different critical assumptions about the permeability of the capillary walls to the several substances under consideration (Denton, 1961). These assumptions are not now open to direct test and the experimentalist must be content to make such deductions as he can from the composition of the secreted gas and the state of the blood in samples drawn from the several vessels. In this communication the secretion of carbon dioxide into the swim-bladder is examined in order to distinguish among the several modes in which the rete mirabile has been considered to operate.

It has long been known that carbon dioxide accompanies other gases entering the experimentally emptied swim-bladder (Jacobs, 1932; Akita, 1936; Copeland, 1952; Fänge, 1953). The transient carbon dioxide partial pressure is variable and may be large: up to 0.1 atmosphere in cod (Fänge, 1953; Scholander *et al.*, 1956), up to 0.25 atmosphere in scup (Wittenberg, 1961), and as high as 0.27 atmosphere in European strains of perch (Jacobs, 1930). For the most part, however, it is low. Large partial pressures of carbon dioxide may exist somewhere in the secretory apparatus without necessarily or always being reflected in the composition of the secreted gas mixture because individuals of species such as toadfish, whose secretory product is characteristically low in carbon dioxide (0.04 atmosphere, average) occasionally will produce a gas mixture of which 18 per cent is carbon dioxide (Wittenberg, 1961; Wittenberg and Wittenberg, 1961).

The gas gland, which in most species forms a strongly developed continuous layer of cells interposed between the gas gland capillaries and the gases in the swim-bladder lumen, may impede the passage of carbon dioxide from those capillaries to the lumen. Indeed, we have previously considered evidence that this cell layer in reality may act as a barrier to gas diffusion (Wittenberg, 1958; Wittenberg and Wittenberg, 1961). In order to study the functioning of the rete mirabile we sought and found in the bluefish, *Pomatomus saltatrix* L., an animal in which the gas gland capillaries lie virtually exposed to the gases in the swim-bladder lumen. (A similar situation probably also is found in jacks, Carangidae, and to a much lesser extent in scup, *Stenotomus versicolor* M., and the John Dory, *Zeus faber*.)

In the following discussion, we assume that the gases brought into the bluefish swim-bladder are in equilibrium with the gases dissolved in the blood of the gas gland capillaries. Analysis of the gas then approximates the inaccessible quantity, the partial pressure of carbon dioxide and other gases in the blood.

MATERIALS AND METHODS

Bluefish were captured in a fixed net at Woods Hole, Massachusetts, and transferred to laboratory tanks with minimal handling. They were allowed at least 24 hours at 18–20°C to recover from the effects of capture before being used for experiments. It was necessary to anesthetize the very active fish before taking gas samples. For this purpose they were immersed briefly in a strong solution of tricaine methane sulfonate (MS 222, Sandoz Pharmaceuticals Co., Hanover, New Jersey) in sea water until almost quiescent; the samples were obtained rapidly; and the fish immediately returned to sea water where they were pushed through the water until swimming and ventilation of the gills began.

Experimentally Induced Gas Secretion

Gas secretion was induced by emptying the swim-bladder. Samples for analysis of the composition of the secreted gas were obtained 2 to 6 hours later, at which time 20 to 130 per cent of the original volume had been replaced. Only a single sample of secreted gas could be taken from each fish since the animals tolerated handling poorly.

Gas samples were withdrawn from the swim-bladder into syringes fitted with a fine needle and lubricated with a strong sloution of lithium chloride. Samples for mass spectrometer analysis were freed from oxygen by equilibration in the collecting syringe with 0.05 volume of alkaline pyrogallol (pyrogallol 10 gm, in 100 ml, 20 per cent potassium hydroxide which had been boiled to expel dissolved gases. The solution was subjected to vacuum to remove dissolved gas introduced with the pyrogallol).

Oxygen and carbon dioxide were determined in the Fry, 1 ml, gas analyzer (Fry *et al.*, 1949), using the reagents of Scholander and Irving (1947). This procedure gave accurate results with known gas mixtures containing from 2 to 100 per cent carbon dioxide.

Mass spectrometer analyses to determine the ratio argon to nitrogen were performed on a mass spectrometer in the Department of Biochemistry, Columbia University.

Carbon Dioxide Dissociation Curve of Bluefish Blood

Blood was withdrawn from the gill vessel into siliconized syringes containing a minimal volume of a solution of sodium chloride, 0.25 molar, heparin, and potassium oxalate. The blood sample was equilibrated with air for more than 30 minutes to allow the rapid endogenous oxygen consumption to subside. (Presumably oxygen consumption subsides because of the exhaustion of substrates which may include lactic acid released from the muscles during the animal's struggles.) Portions of the sample were equili-

brated at 23°C with large volumes of known mixtures of carbon dioxide in air. It was inadvisable to equilibrate the entire sample because the blood deteriorates with mechanical handling. The equilibrated blood samples were analyzed for the total carbon dioxide content released by acid in the procedure of Holaday and Verosky (1956), in the Natelson (1951) micromodification of the manometric Van Slyke apparatus.

Histological sections were prepared from gas glands fixed in Bouin's fluid during the period of gas secretion and were stained with azan or with hemotoxylin and eosin. Capillary injections were prepared by injecting a large volume of a solution of gelatin, 7 per cent, in India ink into the aorta. The injected gas gland was dissected free, fixed in acid formalin and, following dehydration in alcohol, cleared in benzyl benzoate. Fig. 2 is based on histological sections and on sectioned and dissected India ink–injected preparations.

RESULTS

The Structure of the Gas-Secreting Complex

The gas-secreting complex is large and covers much of the anterior-ventral, interior surface of the swim-bladder. The retia mirabilia, which number about 200, receive blood from short lateral branches of the four or more major radiating branches of the swim-bladder artery (Fig. 1) and supply blood to the gas gland which forms a continuous layer overlying the retia mirabilia.

The structure of the rete mirabile is similar in most species of fish, and the bluefish follows the general pattern. The rete is comprised almost entirely of capillaries. Several thousand afferent capillaries, arising directly from a branch of the swim-bladder artery, are interdigitated in the rete with several thousand efferent capillaries which join to form a branch of the swim-bladder vein. The afferent capillaries supply blood, either directly or through an intermediate blood vessel, to the capillaries of the gas gland. After passing through the gas gland the blood returns to the rete and flows in the efferent capillaries to the swim-bladder vein. The swim-bladder circulation thus forms a loop with the gas gland at its vertex. The blood flow in the efferent retial capillaries is countercurrent to the flow in the afferent retial capillaries. A very large area is provided in the rete for the diffusive exchange of material between afferent and efferent capillaries.

The structure of the rete mirabile of the bluefish was reconstructed from preparations partially injected with India ink (Fig. 2). The formation of the retial capillaries by abrupt arborization of branches of the swim-bladder artery and vein, without intervention of arterioles or venules, is accurately but semidiagrammatically presented. The fusion of the numerous retial capillaries to form a lesser number of gas gland capillaries is also presented accurately. In the interest of clarity only a small number of capillaries are shown in the rete, and these as though they were widely separated. They are actually closely packed. The tortuosity of the retial capillaries, which is a result of

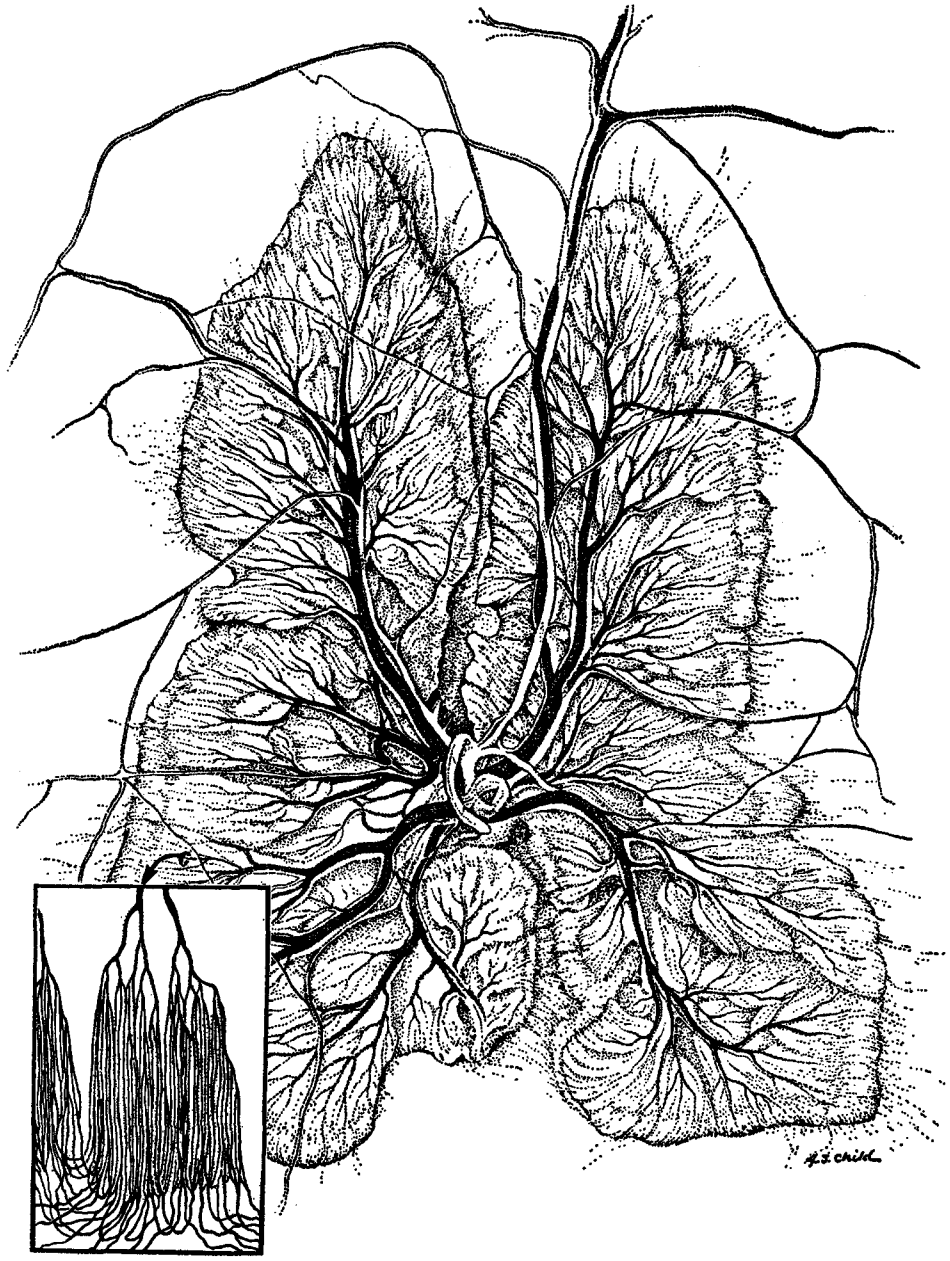

FIGURE 1. Surface view of the gas-secreting complex of the bluefish, showing the major blood vessels. Posterior direction is upward. The retia are multiple and are not resolved at this magnification. The branching of the arterial tree to supply three retia is diagrammed in the insert.

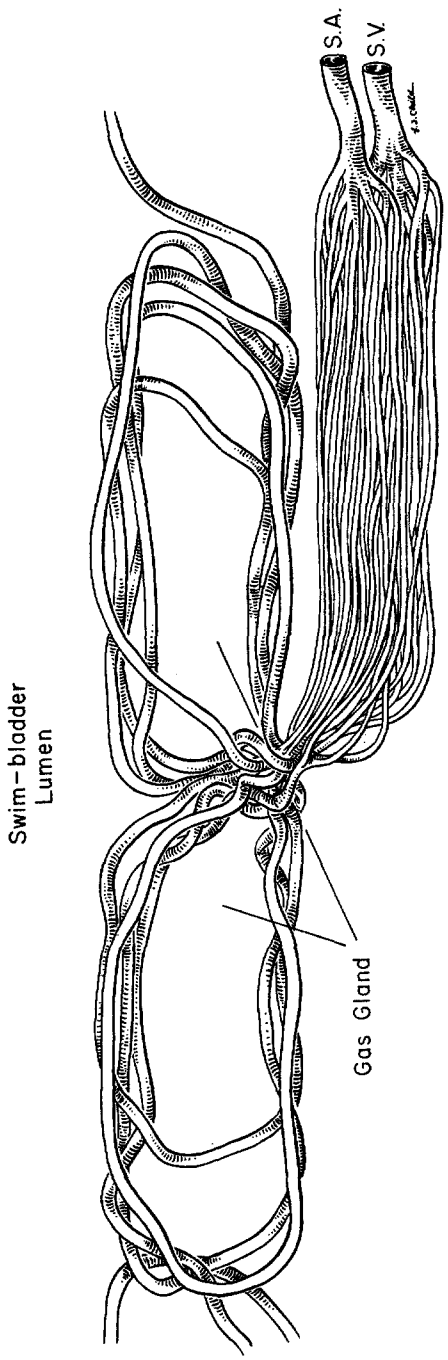

FIGURE 2. Diagram of a section through a small area of the gas-secreting complex showing a rete mirabile and gas gland capillary loops. The upper horizontal limb of capillary loops is in contact with the gas-filled swim-bladder lumen, as indicated diagrammatically in Fig. 4. S. A., swim-bladder artery. S.V., swim-bladder vein.

their formation by branching of the large vessels, is exaggerated. In longitudinal section these capillaries are seen to be very straight and, as shown, run the full length of the rete. The average length of the retial capillaries in the bluefish is 1.1 mm (range 0.6 to 1.5 mm). In cross-section the capillaries of the rete are seen to be in intimate contact and in different species in orderly

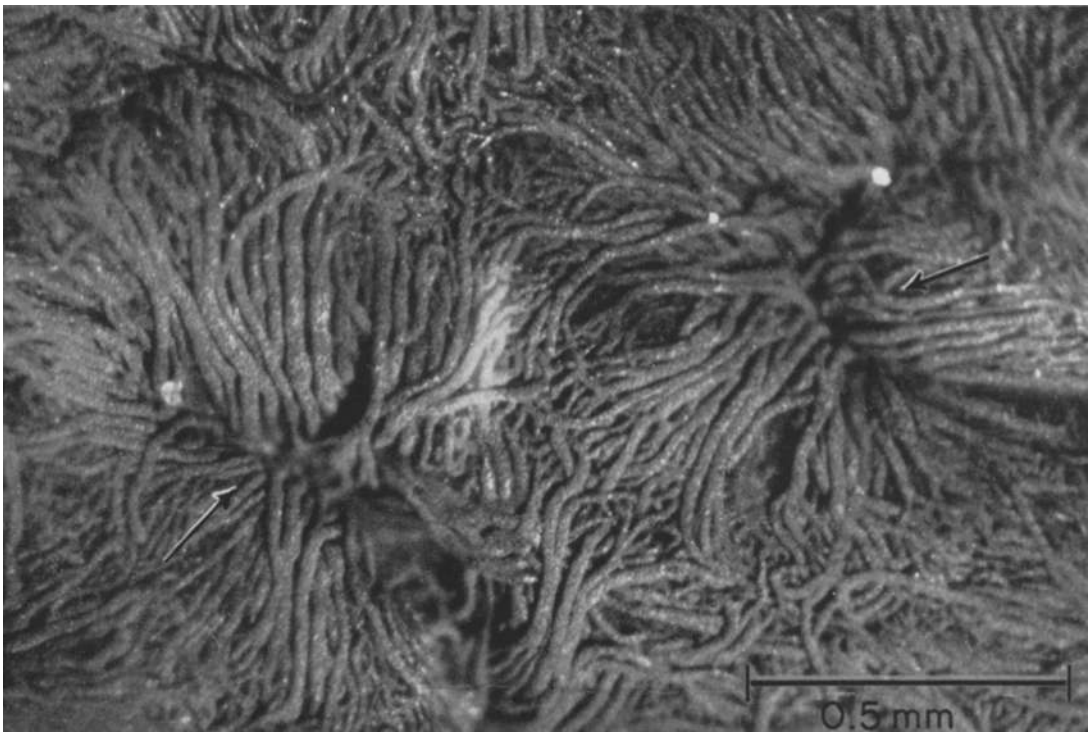

FIGURE 3. Photograph of the luminal surface of an India ink–injected bluefish gas-secreting complex showing the capillaries. Two areas in which the capillaries descend to their junctions with the underlying retia are indicated by arrows.

hexagonal or cubic array (Woodland, 1911; Krogh, 1922; Scholander, 1954). The contact between arterial and venous capillaries is continuous and very intimate, a fact best appreciated from electron micrographs (Dorn, 1961; Fawcett, D., and Wittenberg, J. B., in preparation).

The capillaries of the rete are continuous with those of the gas gland, although some fusion and consequent reduction in number of capillaries occur at the junction. The gas gland capillaries (Fig. 2) radiate from the rete deep in the gas gland, then bend abruptly and follow a tortuous course to the luminal surface of the gas gland where they coalesce to form larger capillaries which course extensively on the luminal surface (Fig. 3). The fusion of the

rising limbs of the capillaries at the luminal surface is not portrayed in Fig. 2, because it was not conspicuous in the histological sections and sectioned injection preparations from which this figure was mainly constructed. The junctions are, however, very clearly seen in Fig. 3, a photograph of the luminal surface of the gland. After they have passed across the gland surface, the capillaries descend abruptly to reenter the rete from which they originated, or a neighboring rete. The direction of blood flow in these capillary loops has not been established.

The capillaries form a densely packed, almost continuous layer on the luminal surface of the gas-secreting complex. Fig. 3, a photograph of an India ink–injected gland, illustrates the density with which the capillaries are displayed and the great length of the individual, largely unbranched, capillaries exposed to the lumen. The average length of a single capillary exposed to the lumen is 480 microns (range 300 to 800 microns). This unique development may be regarded as an adaptation to provide a very large area for the exchange of substances between the capillary blood and the gases in the swim-bladder

The gas gland consists of a mass of irregularly arranged, large (average diameter 20 to 30 microns) eosinophilic cells. There is no suggestion of differentiation into layers although the cells near the luminal surface are small. The rising limbs of the gas gland capillaries wind among the cells and provide an abundant area for exchange of materials. The layer of tissue overlying the capillaries on the luminal surface is exceedingly thin and not easily resolved in the light microscope; in electron micrographs it may be seen to be cellular (D. E. Copeland, personal communication). The location of the gas gland capillaries in bluefish may be contrasted with that in many other fishes (Fig. 4).

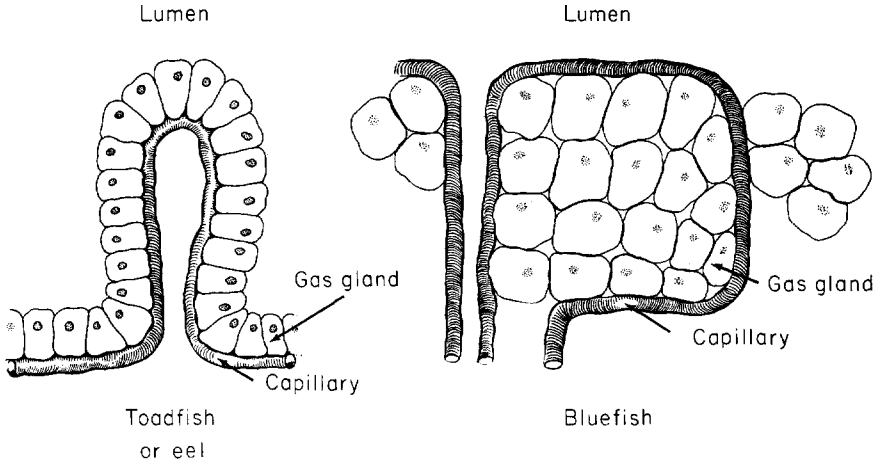

FIGURE 4. Diagram comparing the relative positions of capillaries and gland cells in the gas glands of eels and toadfish and bluefish. The diagram of the toadfish gas gland is redrawn from Fänge and Wittenberg (1958).

The wet weight of the rete mirabile-gas gland complex was in three individuals: 0.43, 0.76, and 0.52 gm. The weights of the fish were 818, 1210, and 926 gm respectively. The average weight of the gas gland per kilo fish, 0.57 gm per kilo, is similar to that of scup, 0.53 gm per kilo (Ball *et al.*, 1955), with which it will be compared.

The Rate of Gas Secretion

Gas secretion in bluefish is conspicuously fast. Less than 4 hours frequently suffices for complete restoration of the original gas volume of the experimentally emptied swim-bladder.

All the other marine fish studied require significantly longer times to replace the swim-bladder gas volume: eels 10 to 18 hours, toadfish, 18 to 24 hours, sea robins 48 hours, tautog 24 hours (Wittenberg, 1958), *Fundulus heteroclitus* about 48 hours (Copeland, 1952), and cod 10 hours (Scholander *et al.*, 1956). Scup frequently require only 6 hours, although most individuals require 10 to 12 hours. The pinfish is stated to require only 4 to 9 hours (McCutcheon, 1962).

Composition of the Secreted Gas

The composition of several samples of gas newly secreted into the bluefish swim-bladder is presented in Table I. The greatest part of the gas is oxygen, comprising 65 to 85 per cent of the total.

The fraction of nitrogen (plus argon) is small; the average of 40 samples is 3.0 per cent (range 1.6 to 5.1 per cent). For comparison the fraction of nitrogen

TABLE I

COMPOSITION OF GAS SECRETED INTO THE SWIM-BLADDER OF BLUEFISH

Animal No.	Composition			
	CO_2	O_2	N_2	$\frac{100\ Ar}{N_2}$
	per cent	*per cent*	*per cent*	
173	23.1	74.8	2.1	1.90
180	33.4	64.6	2.0	1.89
181	37.0	61.2	1.8	2.03
182	35.8	62.6	1.6	1.92
252	28.1	69.0	2.9	1.91
254	20.8	75.3	3.9	1.68
255	25.4	71.5	3.1	1.92
275	22.1	73.8	4.1	1.79
276	24.6	72.4	3.0	1.69
278	30.9	67.5	1.6	1.54
279	18.7	79.2	2.1	1.89
280	34.4	63.2	2.4	1.90
Average				1.84

in the gases secreted into the toadfish swim-bladder (in which an inert gas-secreting mechanism is thought to be highly developed) usually ranges from 4 to 7 per cent, with many higher values (Wittenberg, 1961).

The ratio of argon to nitrogen, average 1.84×10^{-2} (Table I), is greater than in air, 1.12×10^{-2}, and less than that of the gases dissolved in air-equilibrated water, 2.6×10^{-2}.

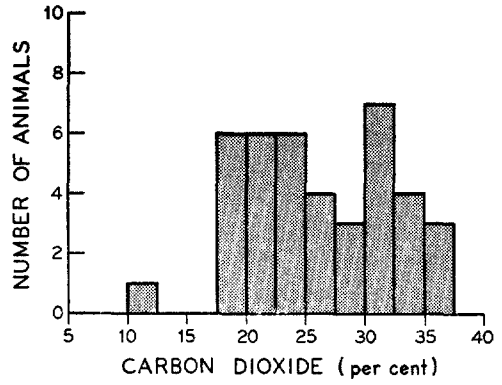

FIGURE 5. Carbon dioxide content of gases secreted into the bluefish swim-bladder.

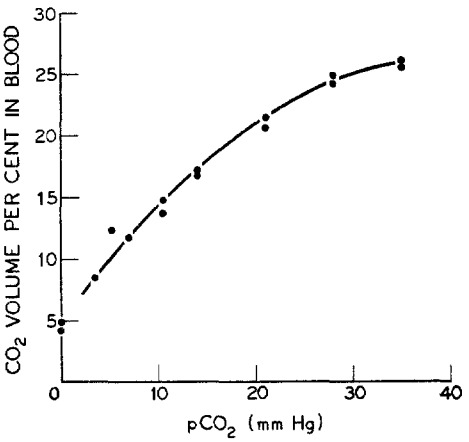

FIGURE 6. Carbon dioxide dissociation curve of bluefish blood.

Carbon dioxide comprises a very large fraction of the secreted gas; representative data are given in Table I, and all the data in Fig. 5. Only a single sample contained less than 17 per cent. Thirty-nine samples contained from 17 to 37 per cent carbon dioxide, and since all values within this range occur with about the same frequency, it is legitimate for purposes of this discussion to consider only the highest value, 37 per cent. At 20°C this corresponds to a partial pressure of 275 mm Hg.

Estimated Values of Some Parameters of Gas Secretion

THE CARBON DIOXIDE CONTENT OF BLUEFISH BLOOD The carbon dioxide content of bluefish arterial blood cannot be determined directly because the

animal struggles violently during handling thus altering the composition of the blood, and because freshly drawn blood consumes oxygen vigorously and presumably forms carbon dioxide. This quantity may be estimated from the carbon dioxide dissociation curve of bluefish blood (Fig. 6) which relates the carbon dioxide content to the partial pressure of carbon dioxide with which the blood is in equilibrium. The arterial blood is probably nearly equilibrated with sea water at the gills, and the arterial pCO_2 of marine fish has been estimated to be less than 2 mm Hg (Fry, 1957). Even venous pCO_2 is estimated not to exceed 10 mm Hg (Fry, 1957). For purposes of discussion an arterial pCO_2 of 2 mm will be assumed, and the corresponding value (Fig. 6) of the carbon dioxide content of arterial blood will be taken to be 6 volumes per cent.

CARBON DIOXIDE PARTIAL PRESSURE GENERATED BY ACIDIFYING THE BLOOD Addition of a strong acid such as lactic acid (pK 3.8) to the blood will convert the carbonate and bicarbonate ions to dissolved carbon dioxide (pK 6.1). The carbon dioxide partial pressure so generated is defined by the volume of carbon dioxide liberated (6 volume per cent) and the absorption coefficient of carbon dioxide in water.

It is equal to 55 mm Hg, which is very much less than the maximum carbon dioxide tension found in the swim-bladder, 275 mm Hg. In order to generate pCO_2 equal to 275 mm Hg, the arterial blood would have to contain 30 volumes per cent CO_2 which is more than its capacity at pCO_2 = 40 mm Hg (Fig. 6). Since at partial pressures of carbon dioxide greater than 40 mm, the carbon dioxide dissociation curve becomes a straight line whose slope is governed by the solubility of carbon dioxide in plasma, the arterial pCO_2 required to yield pCO_2 = 275 mm Hg on acidification may be approximated as 275 − 40 = 235 mm Hg.

RATE OF CARBON DIOXIDE SECRETION A 1 kilo fish (assuming 35 per cent CO_2 in the secreted gas; 46 ml of swim-bladder volume per kilo fish; and a secretion rate of 46 ml per 4 hours) brings into the swim-bladder 4 ml of carbon dioxide per hour. This is a large but not necessarily maximum value; the determined values (Table II) are lower because gas is reabsorbed continuously, as has been shown in separate experiments, and carbon dioxide is resorbed preferentially.

The rate of metabolic carbon dioxide generation may be estimated by comparison with scup, *Stenotomus versicolor*, since the gas-secreting complex of this species is reasonably similar in structure to that of the bluefish, and the pattern of gas secretion exhibited by scup in extreme instances approaches that of bluefish. Ball *et al.* (1955) have found the oxygen consumption of the scup gas-secreting complex to be 0.16 ml per kilo fish per hour (at 30°C) which is equivalent (assuming the respiratory quotient is 1.0) to 0.16 ml carbon dioxide produced per kilo fish per hour.

Ball *et al.* (1955), to whom we owe the discovery of aerobic glycolysis in the gas-secreting complex, find that the gas-secreting complex may produce lactic acid at a rate sufficient to liberate from bicarbonate more than 2 ml carbon dioxide per kilo fish per hour. The rate of aerobic glycolysis is much larger than the rate of oxygen consumption.

These and related values are collected in Table II.

TABLE II

NUMERICAL VALUES OF SOME PARAMETERS OF GAS SECRETION IN BLUEFISH AND SCUP

Parameter	Units	Bluefish	Scup
Weight of gas-secreting complex	Grams	0.57	0.53
Swim-bladder volume	Milliliters	46	46
Rate of gas secretion*			
Total gas	Milliliters per hour, median and range	6.4 (3.0–14.7)	4.5 (2.4–7.2)
Carbon dioxide		1.8 (0.6–4.0)	0.4 (0.1–1.1)
Nitrogen (plus argon)		0.16 (0.09–0.50)	1.0 (0.2–2.9)
Oxygen consumption of complex‡	Milliliters per hour		0.16
Metabolic generation of carbon dioxide§	Milliliters per hour		0.16
Glycolysis	Milliliters per hour‖		0.7–2.7
Estimated minimum blood flow	Milliliters per hour	67	

All data are expressed per kilogram wet weight of fish.

* Based on secretion rates of 40 bluefish and 36 scup (Wittenberg and Wittenberg, unpublished data). Representative data are presented in Table I and in Wittenberg (1961).

‡ Ball *et al.* (1955).

§ Assuming respiratory quotient is 1.0.

‖ Carbon dioxide liberated from bicarbonate by lactic acid.

BLOOD FLOW IN THE GAS-SECRETING COMPLEX The rate of fluid flow is a parameter of great interest in understanding the operation of any countercurrent system. If, as will be shown, carbon dioxide evolved into the swim-bladder originates in the blood, the minimum gas gland blood flow is set by the requirement to evolve carbon dioxide. Assuming that the carbon dioxide content of arterial blood is 6 volumes per cent, the blood flow must exceed 67 ml per kilo fish per hour, or 118 ml per gm gas-secreting complex per hour.

Accompanying the carbon dioxide 4.3 to 11.0 ml of oxygen per kilo fish per hour are evolved into the swim-bladder (Table II). This corresponds to the removal of 6.3 to 16.5 ml oxygen for each 100 ml of blood at the calculated minimum flow. The oxygen capacity of bluefish blood is about 14 volumes per cent. The actual difference in oxygen content between the swim-bladder arterial and venous blood is known to be small (Scholander, 1956; Steen,

1963 *c*); accordingly the true blood flow must be much larger than the estimated minimum, which in itself is large.

DISCUSSION

Ball, Strittmatter, and Cooper (1955) discovered that the gas-secreting complex of the swim-bladder is outstanding in its capacity to convert glucose into lactic acid, and that it can do so even in the presence of one atmosphere of oxygen. These authors rightly realized that the glycolytic generation of the strong acid, lactic acid, must play a crucial role in the liberation of blood carbon dioxide and oxygen into the swim-bladder.

Although the generation of acid by active gas glands had been discovered by Hall (1924), the identification of the acid as lactic acid was new. Kuhn, Moser, and Kuhn (1962) seeking evidence to support their important theory of rete function, measured the lactic acid concentration of the efferent and afferent blood streams of the rete mirabile of the eel. During gas secretion the efferent blood contained up to 50 mg per cent (0.0056 mole per liter) more lactic acid than the afferent. Steen (1963 *c*), also working with eels, was able to sample not only the afferent and efferent vessels but also the vessels carrying blood from the rete to the gas gland and returning from the gas gland to the rete. The largest part of the lactic acid added to the blood (54 mg per cent; 0.006 mole per liter) originates in the gas gland. The blood leaving the gas gland is acid (pH 6.5 to 6.9) as expected. Some lactic acid (26 mg per cent; 0.003 mole per liter) is added to the afferent stream in its passage through the rete. This acid either may have been generated by local glycolysis or may have diffused across the retial capillary wall from the efferent stream.

In some fish which secrete gas only slowly and intermittently, the lactic acid arises from tissue glycogen (Copeland, 1952; Fänge, 1953). In the species considered here the substrate of glycolysis must be blood glucose and the reaction at pH 7.5 or 6.5 may be expressed:

$$1 \text{ glucose} \rightarrow 2 \text{ lactate}^{(-)} + 2H^{(+)} \qquad (1)$$

It is evident that this reaction not only generates acid but also increases the total solute concentration.

With this background we may proceed to evaluate the source of the carbon dioxide evolved into the bluefish swim-bladder. The carbon dioxide may be a product of metabolism in the gas-secreting complex or it may be brought into the swim-bladder by the blood. Carbon dioxide enters the swim-bladder at a maximum rate of 4 ml per kilo fish per hour (Table II). The probable rate of generation of carbon dioxide by oxidative metabolism (Table II), 0.16 ml per kilo fish per hour, accounts for only 5 per cent of the total. By exclusion swim-bladder carbon dioxide must be provided by the blood. The rate of glycolytic generation of lactic acid (Table II), making allowance for dif-

ferences in species and the difficulties of measurement, appears to be sufficient to liberate carbon dioxide at the required rate.

It may be remarked that the gas gland effluent blood of the bluefish must be very acid. Bluefish blood incubated in air to decrease endogenous respiration and equilibrated with 30 per cent and 40 per cent carbon dioxide in oxygen, was acid, pH 6.3 and 6.2 respectively.

We may now examine the theories of rete mirabile function. Haldane (1922) introduced the concept of countercurrent flow into biology. He considered the rete mirabile to act as a *countercurrent exchanger* in a manner analogous to an industrial heat exchanger. In this view the rete would allow high concentrations of carbon dioxide and acidity to be built up by preventing carbon dioxide from escaping from the site of its production in the gas gland. This might be done by simple diffusion of carbon dioxide (and possibly acid) from the efferent to the afferent capillaries of the rete. It will be appreciated that a *countercurrent exchanger* will *maintain* an already established concentration difference, but cannot create anew or replenish a declining difference in concentration between its ends.

A countercurrent exchanger is a barrier to diffusional loss. If the rete mirable acts only as a countercurrent exchanger, the carbon dioxide partial pressure at the swim-bladder end may not exceed the partial pressure generated by acidifying a volume of blood. As we have seen, the best estimate of this partial pressure is 55 mm Hg, which is only one-fifth of the partial pressure of carbon dioxide, 275 mm Hg, frequently found in the bluefish swim-bladder. The argument is reinforced by examining the condition of the arterial blood required to generate a carbon dioxide partial pressure equal to 275 mm. The required systemic arterial carbon dioxide partial pressure would have to exceed 40 mm Hg and would probably have to be more than 235 mm Hg. We conclude that Haldane's theory must be modified to explain carbon dioxide secretion in the bluefish.

Haldane (1922) and Wittenberg and Wittenberg (1961) have considered that oxygen might be actively transported by the retial cells from efferent to afferent capillaries of the rete. Making reasonable assumptions about the permeability of the cell membranes (Leaf, 1959), it is easy to construct an analogous model for a process in which the generation of lactic acid in the retial cells drives a transcellular transport of carbon dioxide from efferent to afferent capillaries. Since, however, carbon dioxide secretion can be accounted for completely by the addition of lactic acid to the blood, and since Steen (1963 *c*) has shown that this lactic acid originates largely in the gas gland, the hypothesis of an active transcellular transport need not be entertained for carbon dioxide.

The late Professor Werner Kuhn and his collaborators in an important series of papers (Kuhn and Kuhn, 1961; Kuhn, Moser, and Kuhn, 1962;

summarized in Kuhn, Ramel, Kuhn, and Marti, 1963) have considered that the rete mirabile can operate as a *countercurrent multiplier* capable of generating high concentrations of gases toward one end of the rete. The salient features characterizing the countercurrent multiplier system are (*a*) a physicochemical effect (*e.g.* a salting-out effect) which brings about a decreased solubility of the species to be concentrated as it passes through the region of the vertex of the capillary loops (in this case the gas gland), and (*b*) the juxtaposition of the afferent and efferent capillaries in the rete mirabile so that optimal diffusive transfer can take place from efferent to afferent capillaries. For the system to operate the capillary walls must be permeable to the species to be concentrated and impermeable to water and to solutes added at the vertex which bring about the decreased solubility of the species to be concentrated.

The operation of the countercurrent multiplier system may be understood by considering a sequence of single steps. As blood passes the vertex of the system, a physicochemical effect brings about a decreased solubility of the species to be concentrated. Since the concentration of this species is not changed, its activity is enhanced in the efferent relative to the afferent stream. Diffusive transfer of this species from efferent to afferent retial capillaries will take place until the activity in the two streams becomes equal. The result of a single step is to augment both the concentration and the activity of this species in the afferent stream. The ratio of the concentration in the afferent relative to the efferent stream achieved in a single step, referred to by Kuhn *et al.* as the "equilibrium concentrating factor," depends only on the magnitude of the physicochemical effect occurring at the vertex. Kuhn *et al.* refer to the equilibrium concentrating factor for any species as the "*single concentrating effect*" for the species. In a second step in the operation of the countercurrent multiplier system blood from the afferent capillaries will be swept past the vertex into the efferent capillaries, and the events repeated with an enhanced initial concentration in the efferent stream. Repetition of this process may cascade the concentration at the vertex to very high values. Kuhn *et al.* treat rigorously the manner in which the single concentrating effect is multiplied by an enormous factor in a countercurrent flow system.

Koch (1934) had suggested that gases would be accumulated in the swim-bladder if the solubility of gases in the blood were decreased in the region of the gas gland. The difficulty was to find a plausible method of decreasing the solubility. All authors prior to Kuhn *et al.* had tacitly assumed that the afferent and efferent blood streams were similar in composition. Kuhn *et al.* challenged this assumption with the proposal that the gas gland elaborates a substance, shown to be lactic acid (Kuhn *et al.*, 1962; Steen, 1963 *c*), which brings about a decrease in the solubility of gases in the efferent stream. This decrease in solubility produces the single concentrating effect.

Steen in his careful study of swim-bladder function in the eel (1963 *a*, *b*, *c*)

had analyzed blood from the several blood vessels of the gas-secreting complex. The changes found in lactate ion, hydrogen ion, oxygen, and carbon dioxide content all accord with Kuhn's hypothesis and provide elegant evidence for the reality of the hypothesis.

It is necessary to test the central idea of Kuhn's hypothesis, the idea that a single concentrating effect can be multiplied. Carbon dioxide secretion provides a favorable case. The single concentrating effect is very large and may be estimated simply. Addition of lactic acid to the blood passing through the gas gland increases the carbon dioxide partial pressure not only by a small salting-out effect but largely by liberating carbon dioxide from bicarbonate ion. The partial pressure so generated, the single concentrating effect, is estimated to be 55 mm Hg. If countercurrent multiplication occurs, the carbon dioxide partial pressure developed at the swim-bladder end of the rete is expected to be very much larger than the single concentrating effect. This we find to be so. The concentration achieved in the swim-bladder, 275 mm Hg, is very much larger than the single concentrating effect, which is 55 mm Hg. This finding provides the strongest evidence that Kuhn's hypothesis truly describes the events in the rete mirabile leading to carbon dioxide secretion.

It follows that the capillary walls must be permeable to carbon dioxide and relatively impermeable to lactic acid.

The structure of the bluefish gas gland is unique among the more than one hundred species of fish we have examined. All gas glands (except in species in which the gas-secreting complex is diffuse or weakly developed) are intensely vascular. The difference lies in the position of the capillaries. In the majority of species the gas gland capillaries lie below the cells, with which they are intimately associated (Woodland, 1911). In the eel and the toadfish, which typify the most extreme case (Fänge and Wittenberg, 1958), virtually every gas gland cell has a capillary at its base (Fig. 4) and the cells form a continuous layer exposed to the lumen. In contrast, in the bluefish the capillaries are exposed to the lumen (Fig. 4) where they form a dense, ordered, essentially continuous layer (Fig. 3) essentially isolating the gas gland cells from the gases in the lumen. This structure suggests that gas exchange takes place at the capillaries. The chemical events leading to secretion may be presumed to take place in the blood as a consequence of the addition of metabolites by the gas gland. The winding ascending limbs of the capillaries provide a large area of contact for exchange of substances between the gas gland cells and the blood.

For the experimentalist this structure provides a fortunate circumstance. What use is it to the fish? Schools of bluefish feed both on the bottom at some depth and at the surface, and probably make frequent and abrupt migrations between these environments (Bigelow and Schroeder, 1953; Lyman, 1955). We offer the suggestion that the structure of the gas-secreting complex in

bluefish is an adaptation to the very rapid gas secretion demanded by this unusual way of life.

Preceding articles in this series are: Wittenberg, J. B., *J. Gen. Physiol.*, 1961, **44**, 521; Wittenberg, J. B., and Wittenberg, B. A., *J. Gen. Physiol.*, 1961, **44**, 527.

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