

# The Secretion of Oxygen into the Swim-bladder of Fish

## *I. The transport of molecular oxygen*

JONATHAN B. WITTENBERG

From the Department of Physiology, Albert Einstein College of Medicine, Yeshiva University, New York, and the Marine Biological Laboratory, Woods Hole, Massachusetts

**ABSTRACT** Fish were maintained in sea water equilibrated with a gas mixture containing a non-equilibrium mixture of the three molecular species of oxygen,  $O^{18}-O^{18}$  (mass 36),  $O^{18}-O^{16}$  (mass 34), and  $O^{16}-O^{16}$  (mass 32). Analyses in the mass spectrometer, of the gases secreted into the swim-bladder showed that no change in the relative abundance of these three molecular species had occurred during the secretory process and that therefore no exchange of atoms between oxygen molecules had occurred. Scission of the oxygen-oxygen bond probably does not occur during the transport process.

It is concluded that the active transport of oxygen into the swim-bladder by the gas gland is a transport of molecular oxygen.

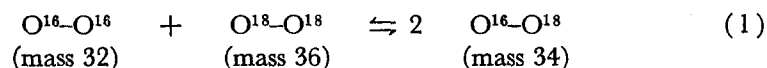
Gases are brought into the swim-bladder of fishes through the combined action of a glandular epithelium, the gas gland, and a vascular structure, the rete mirabile, supplying blood to the gland. Oxygen constitutes the major portion, up to 95 per cent, of the gas mixture elaborated by the gland and is the substance transported. The partial pressure of oxygen in the swim-bladders of fish living at great pressures in the depths of the sea may exceed 150 atmospheres (Biot, 1807; Richard, 1895; Schloesing and Richard, 1896; Scholander and van Dam, 1953). The partial pressure of oxygen in the swim-bladder of these fish is commonly 500-fold or in one case, 10,000-fold (Kanwisher and Ebeling, 1957) greater than the partial pressure of oxygen in the ambient sea water. Biot (1807), the discoverer of the phenomenon, stated that these pressure differences were achieved by active secretion of oxygen.

Nitrogen and argon, although constituting only a small proportion of the total gases may also be present at partial pressures far greater than the ambient. They are brought into the swim-bladder by a physical mechanism (Wittenberg, 1958) which is a corollary of oxygen secretion.

Although Haldane (1922) had early suggested that blood oxyhemoglobin is the source of oxygen brought into the swim-bladder, it is now clear that a substantial proportion of the oxygen enters through a truly cellular active transport process (discussed in Wittenberg and Wittenberg, 1961). The experiments presented here and in the accompanying paper (Wittenberg and Wittenberg, 1961) are designed to elucidate the chemical mechanism by which cells transport oxygen against large pressure gradients.

The source of the oxygen brought into the swim-bladder is the oxygen dissolved in the sea water and, by that token, the blood of the fish, since fishes maintained in sea water equilibrated with oxygen-18 labeled air brought oxygen of unchanged isotopic abundance into the swim-bladder (Scholander *et al.*, 1956).

The present experiments utilize isotopic oxygen ( $O^{18}$ ) to establish whether oxygen molecules are transported intact, or whether during the transport process they are cleaved to their component atoms. Cleavage of the oxygen-oxygen bond could result in the exchange reaction:



It will be shown by mass spectrographic analysis of secreted oxygen that such exchange between oxygen molecules does not take place during the transport process, and that oxygen molecules therefore are transported intact.

## EXPERIMENTAL

### *Choice of Experimental Animals*

Woodland (1911) in his classic account of the structure of the gas gland recognizes two general types of gland: (I) those in which the glandular epithelium is composed of a single layer of cells and, (II) the massive type in which the glandular epithelium is many layered. In addition, in some species a much folded single cell layer secondarily assumes a massive character. These morphological differences apparently underlie differences in function (Wittenberg, 1958). The toadfish exhibits a powerful development of the single layered type of glandular epithelium (Fänge and Wittenberg, 1958). This animal and the eel, another example of Woodland's type I, were found to differ greatly from the scup, a representative of Woodland's type II, in the mode of evolution of gas. Sea robins, with a secondarily massive epithelium, were intermediate. For the present study, the toadfish, *Opsanus tau* (L.), Woodland's type I, and the scup, *Stenotomus versicolor* (Mitchill), Woodland's type II, were selected because they represent the extremes of the two patterns of gas evolution encountered.

### *Preparation of the Gas Mixture*

Oxygen gas containing a substantial proportion of the molecular species  $O^{18}-O^{18}$  (mass 36) was prepared by the electrolysis of water containing 60.5 atom per cent

$O^{18}$  and 1.31 atom per cent  $O^{17}$  (footnote <sup>1</sup>). The labeled oxygen, 0.7 liter, was passed over soda lime to decompose ozone formed in the electrolysis (Jahn, 1908) and was added to approximately 45 liters of an air-oxygen mixture containing 35 per cent of normal oxygen. The resulting mixture was used in all the experiments reported here. The composition of this mixture appears in Table I.

### *Apparatus*

The fish were maintained in a chamber made from an 8 liter desiccator fitted with a ground glass connection through which passed inlet and outlet tubes for the circulating gas mixture (Fig. 1). A tube through which sea water could be added or

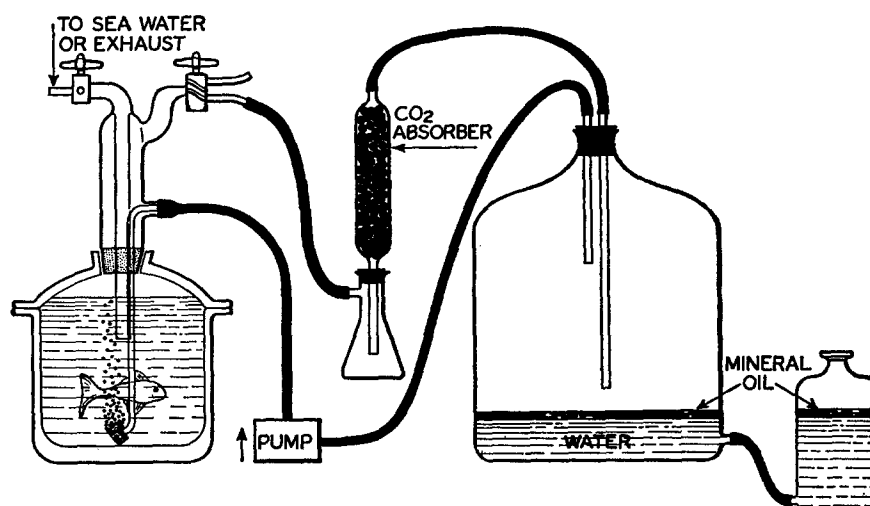


FIGURE 1. Fish chamber and gas recirculating system.

removed and a stopcock opening to the atmosphere were provided to permit the introduction and removal of fish without contamination of the isotopic gas mixture by air. After the fish were introduced, the chamber was completely filled with sea water, the displaced air escaping through the open stopcock. The stopcock was reversed and a part of the sea water withdrawn by suction to permit the isotopic gas mixture to occupy the top of the chamber and allow room for foaming. A pump circulated the gas in the system at the rate of 0.5 liter per minute. A large bottle served as a reservoir for the gas mixture and an attached leveling bottle allowed for volume changes in the system. Carbon dioxide was removed from the circulating gases by a commercial absorbant.<sup>2</sup> Gas analysis established that oxygen or nitrogen introduced into the circulating system was equilibrated with the water phase in 20 minutes.

### *Procedure*

The gas initially present in the swim-bladder was removed by puncture, and the animals were maintained in the experimental chamber for a time judged sufficient

<sup>1</sup> Purchased from the Weizmann Institute of Science, Rehovoth, Israel; lot P9X24.

<sup>2</sup> Baralyme; Thomas A. Edison Inc., Stuyvesant Falls, New York.

for the replacement of the original volume of the gas by secretion. The scup were kept in the chamber for 8, or in one instance, 10 hours. Toadfish were allowed to remain in the chamber for 24 hours. The secreted gas was withdrawn from the swim-bladder into a syringe and after removal of  $\text{CO}_2$  with KOH was transferred over mercury to sealed tubes for storage. The toadfish were returned promptly to the experimental set-up and as many as six successive samples of secreted gas were obtained from one animal. Scup were used only once.

The gas samples were analyzed for oxygen and carbon dioxide by the method of Scholander *et al.* (1955).

Isotopic analyses were performed on a mass spectrometer at the Department of Biochemistry of Columbia University. I am indebted to Dr. D. Rittenberg and Mr. I. Sucher for these analyses.

#### RESULTS AND DISCUSSION

The composition of the gas mixture administered to the fish and the compositions of the gas secreted by the two species of fish are presented in Table I. The proportion of oxygen is much greater in the secreted gas than in the gas mixture administered. The relative abundance of the three molecular species of oxygen,  $\text{O}^{18}\text{-O}^{18}$  (mass 36),  $\text{O}^{18}\text{-O}^{16}$  (mass 34), and  $\text{O}^{16}\text{-O}^{16}$  (mass 32), is precisely the same in the secreted gas and in the gas administered. Certain samples, however, (notably 152*a* and 158*a*), in each case the first sample drawn from the fish, show evidence of mixture with air probably arising from residual gases in the swim-bladder or from solution in the tissues of the fish. The value of  $K$ , the apparent equilibrium constant for the reaction given in equation (1), provides a sensitive measure of the degree of equilibration of the three molecular species. At equilibrium, the value for this constant is 4. The values of 0.033 seen for this constant in Table I emphasize that both the oxygen administered and the secreted oxygen are non-equilibrium mixtures and are equally far from equilibrium. It is therefore clear that no exchange between oxygen molecules, equation (1), has taken place during the transport process.

One may consider the possibility that at some stage in the transport process oxygen may be reduced to hydrogen peroxide which may cross a barrier impermeable to oxygen and which may subsequently be oxidized to the oxygen elaborated by the gland. It would appear that this sequence of reactions is compatible with the present evidence, for the glucose oxidase-catalyzed reduction of oxygen to hydrogen peroxide and subsequent catalase-catalyzed decomposition of the hydrogen peroxide to oxygen proceed without exchange with water (Bentley and Neuberger, 1949). However, the transport of carbon monoxide by the oxygen-transporting systems of the gas gland (Wittenberg and Wittenberg, 1961) rules out this sequence of reactions as the major secretory mechanism.

The combination of transported oxygen with an intracellular hemoglobin

would not be expected to bring about the exchange reaction given in Equation 1. Therefore, the results of the present experiments are compatible with the mechanism of oxygen transport proposed in the accompanying paper (Wittenberg and Wittenberg, 1961).

TABLE I  
COMPOSITION OF SECRETED GAS

Species	Animal	Molecular composition			Oxygen: Relative molecular abundance			K*
		N <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	Mass 36	Mass 34	Mass 32	
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>				
Toadfish	149a†	14.3	2.0	83.7	1.00	1.57	77.9	0.0317
"	" b	6.6	3.1	90.3	"	1.53	71.4	0.0328
"	152a	30.2	2.1	67.7	"	1.73	122.	0.0246
"	" b	18.1	4.6	77.3	"	1.55	75.6	0.0318
"	153a	13.7	6.9	79.4	"	1.56	79.2	0.0307
"	" b	4.6	17.6	77.8	"	1.53	69.1	0.0338
"	" c	3.0	5.0	92.0	"	1.49	68.5	0.0324
"	" d	7.1	4.9	88.0	"	1.50	68.5	0.0328
"	" e	7.4	4.0	88.6	"	1.51	68.1	0.0334
"	" f	9.3	4.6	86.1	"	1.53	69.8	0.0336
"	154	5.7	4.3	90.0	"	1.56	73.5	0.0331
"	156	11.0	1.6	87.4	"	1.54	75.0	0.0316
"	158a	19.1	1.7	79.2	"	1.74	117.	0.0259
"	" b	8.2	1.3	90.5	"	1.58	81.1	0.0307
"	" c	5.1	1.9	93.0	"	1.54	74.8	0.0317
"	159a	6.5	2.4	91.1	"	1.56	79.0	0.0308
"	" b	3.0	5.0	92.0	"	1.54	72.3	0.0328
Scup	136	22.5	22.4	55.1	"	1.54	72.1	0.0328
"	138	19.1	15.1	65.8	"	1.58	76.7	0.0325
"	141	22.3	11.8	65.9	"	1.54	69.0	0.0344
"	142	23.7	7.0	69.3	"	1.54	70.4	0.0337
"	143	21.2	15.8	63.0	"	1.54	71.4	0.0332
"	144	40.3	4.7	55.0	"	1.54	72.4	0.0328
Administered gas mixture								
	Initial	63.2	0.0	36.8	"	1.52	68.6	0.0336
	Final	80.0	0.0	20.0	"	1.52	68.7	0.0336

\* The apparent equilibrium constant of Equation 1.

$$K = \frac{[O_2^{34}]^2}{[O_2^{36}] [O_2^{32}]}$$

† Samples marked a, b, c, are successive samples from the same animal.

The present experiments also obviously confirm the conclusion of Scholander *et al.* (1956) that dissolved oxygen is the sole source of the oxygen secreted into the swim-bladder. They emphasize the conclusion implicit in

the data of these authors that there is no equilibration of dissolved oxygen with the oxygen of water molecules during the transport process.

Apparently the absence of exchange between oxygen molecules (Equation 1) cannot lead to a statement of a mechanism of oxygen transport. It can, however, provide a criterion or restriction which must be fulfilled by any mechanism proposed. Although it is not a strictly necessary conclusion, the results of these experiments are best summarized by saying that the active transport of oxygen by the gas gland is a transport of molecular oxygen.

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