Reports and Letters

Nitrogen Secretion in the Swimbladder of Whitefish

Hüfner reported in 1892 (*1*) that the swimbladder of whitefish (*Coregonus acronius*) that had been netted on the bottom of the Bodensee at a depth of 60 to 80 m contained 99 percent or more "nitrogen"—that is, unabsorbable gases. The fish were alive and distended when they reached the surface and from the amount of gas they contained one may calculate that they were in neutral buoyancy at the bottom. They could not, therefore, have filled their swimbladders with air at the surface, but the "nitrogen" must have been deposited in the swimbladder at the bottom—that is, against a pressure gradient of 5 to 7 atm (*2*). Hüfner's startling discovery was verified by Saunders (*3*) in a large series of determinations on several species of deepwater physostome fishes in Lake Huron and adjoining waters.

A similar situation exists in deep-sea physoclist fishes, in which the nitrogen tension frequently reaches 10 atm or more, although in this case the main pressure is due to oxygen. Organic gases, if any, are present only as traces (*4*), and the argon-to-nitrogen ratio is near to that in air (*5*). For physostome fishes there is no knowledge so far concerning the identity of the gas they secrete, except that it is unabsorbable. We have therefore analyzed the swimbladder gas of a deepwater coregonid (*Leucichthys johannae*) from Lake Michigan, obtaining information concerning its amount and its content of carbon dioxide, oxygen, nitrogen, organic gases, and argon (*6*).

We were able to secure samples from live fishes as they were hauled aboard the boat. The fish were caught in gill nets set at the bottom at a depth of 100 m. Only lively and externally intact fishes were used. Gas was drawn into 20-ml syringes, lubricated with concentrated lithium chloride, and analyzed in the ½-ml analyzer (*7*), accurate to ±0.015 percent, for CO_2, O_2, and "N_2." Organic gases were analyzed by fitting a combustion chamber onto the oxygen side of the Henderson-Haldane analyzer. Checks on air containing known amounts of acetylene gave satisfactory results. The argon-to-nitrogen ratio was determined by mass spectrometer at the Johns Hopkins University School of Medicine (*8*).

Buoyancy check. The gas from four fishes was pooled; it measured 188 ml. These four fishes, emptied of gas and suspended under water, weighed 15 g. Hence, neutral buoyancy would occur at a total pressure of nearly 12 atm. This is close enough to the total pressure of 11 atm at which the fishes were caught to indicate that the gas must have been deposited in the swimbladder at that depth.

Absorption analysis. The results of analyses of swimbladder gas from ten fishes taken at 100-m depth are given in Table 1. It will be seen that, in agreement with Saunders' data from similar or greater depths, the swimbladder gas in our species consisted of more than 99 percent nonabsorbable gases.

Combustion analysis. Gas from ten fishes was pooled over concentrated calcium chloride and 10-percent tank oxygen was added. Combustion resulted in zero shrinkage and zero CO_2 production. In a second lot of four pooled samples, the gas shrank in triplicate analyses by 0.10, 0.06, and 0.04 percent, with increments in CO_2 of 0.04, 0.00 and 0.01 percent. There is therefore less than 0.1 percent of combustible gas, if any, in the swimbladder, and the nonabsorbable gas consists of nitrogen and argon, with, very likely, traces of other noble gases.

Argon-nitrogen analysis. The argon-to-nitrogen ratio is of particular interest inasmuch as it may give some clue concerning how the nitrogen gets into the swimbladder. If the nitrogen were released from some chemical compound so that it attains a pressure of, say, 10 atm above the nitrogen tension in the water (0.8 atm), the argon would be left behind, so that the argon-to-nitrogen ratio in a swimbladder from a depth of 100 m would be only one-tenth of that in air—that is, 0.1 percent (*9*). Actually, we find that the argon-to-nitrogen ratio averages 0.92 percent, which is very close to the 1.17 percent in air and water (Table 1). In our whitefishes, the argon tension in the swimbladder was accordingly 8 to 10 times higher than it was in the water. These findings, which agree with the data from deep-sea physoclists, lend little support to the idea of a nitrogen secretion by chemical means, whether produced by the fish itself or by bacteria. We observed no free gas in the intestines of the fishes.

The swimbladder gas in our deepwater coregonid consists of some 99-percent pure nitrogen gas. This has been brought into the swimbladder against a pressure gradient of 10 atm by some process that is capable of concentrating argon as well. A similar nitrogen-argon transport is realized in deep-sea physoclist fishes, in which, however, the main action is oxygen secretion. Vascular *rete* structures have not been described for coregonid fishes, and the question therefore arises whether the epithelial cells lining the swimbladder might be the site for the secretion.

The deposition of nitrogen and argon against considerable concentration gradients in the swimbladder of fishes sug-

Table 1. Composition of the swimbladder gas in whitefish taken at a depth of 100 m (combustible gases 0.00 to less than 0.10 percent).

Fish	CO_2 (%)	O_2 (%)	$N_2 + A$ (%)	Pressure of $N_2 + A$ (atm)	Ratio ($100A/N_2$)
1	0.24	0.01	99.75	11.0	
2	2.13	0.20	97.67	11.0	
3	0.47	0.00	99.53	10.9	
4	0.52	0.28	99.20	10.9	1.16
5	0.64	0.21	99.15	10.9	0.69
6	1.13	0.07	98.80	10.9	0.67
7	0.27	0.02	99.71	11.0	1.08
8	0.31	0.02	99.67	11.0	0.96
9	0.73	0.01	99.26	10.9	0.71
10	0.61	0.01	99.38	10.9	1.17
Air	0.03	20.94	79.03	0.79	1.17

gests, in the absence of other explanations, the possibility of a cellular mechanism for the secretion of inert material.

P. F. SCHOLANDER
L. VAN DAM
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

THEODORE ENNS
Department of Medicine, Johns Hopkins University, Baltimore, Maryland

References and Notes

1. G. Hüfner, *Arch. Anat. u. Physiol. Physiol. Ab.* 54 (1892).
2. P. F. Scholander *et al.*, *Biol. Bull.* 101, 178 (1951).
3. R. L. Saunders, *Can. J. Zool.* 31, 547 (1953).
4. P. F. Scholander and I. van Dam, *Biol. Bull.* 104, 75 (1953).
5. T. Schloesing and J. Richard, *Compt. Rend.* 122, 615 (1896); P. F. Scholander, *Biol. Bull.* 107, 260 (1954).
6. Contribution No. 816 from the Woods Hole Oceanographic Institution. This investigation was supported by a grant from the National Science Foundation. We are indebted to Peter I. Tack of Michigan State University for help in arrangements to get the whitefish and for determining the species. We were able to secure samples from live fishes as they were hauled aboard the boat through the courtesy of E. L. Hill of Grand Haven, Mich.
7. P. F. Scholander, *J. Biol. Chem.* 167, 235 (1947).
8. F. P. Chinard and T. Enns, *Am. J. Physiol.* 178, 203 (1954).
9. H. Koch, *Rev. questions sci. (Belg. France)* 26, 385 (1934).

* Present address: Institute of Zoophysiology, University of Oslo, Oslo, Norway.

15 July 1955

New Scheme for Performance of Osmotic Work by Membranes

There is now a wealth of literature dealing with the fact that a variety of cell membranes are able to remove inorganic salts and other neutral organic molecules from dilute solutions and transport them through the membrane into more concentrated solutions (*1*). This "active transport" of molecules against a concentration gradient requires energy that is thought to be provided by the metabolic activity in the neighborhood of the membrane. Several proposals have been made regarding the mechanism of active transport (*2*), all of which are possible, but perhaps none of which is the simplest mechanism that could be described.

It is the purpose of this communication to suggest that in the simplest case, active transport can be performed by a single enzyme. This enzyme, which acts as a "carrier" of the transported species, is confined between two closely spaced semipermeable membranes and is engaged in the conversion of a substrate S into products P. Let us examine the state of affairs when a substrate diffuses into the enzyme "sandwich" from the left-hand side. Inside the membrane, the enzyme-substrate complex ES is formed and diffuses to the right, driven by its own concentration gradient. If the ES complex binds another ion or molecule, this species will be transported to the right as a "passenger." On the way over to the right side of the membrane, the ES complex is broken down, forming the products and the free enzyme E which we suppose for the moment can no longer bind the "passenger species" in question. Thus, the passenger species is continually being removed from the left and deposited on the right in the membrane. In the steady state, the back diffusion of the free passenger species is just balanced by the flux of ES (with bound passenger molecules) to the right.

If the kinetics can be adequately described by the Michaelis-Menton expression, we have (*3*):

$$S + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} (ES) \xrightarrow{k_3} E + P$$

where S, E, and ES denote the molar concentration of the species. When a steady state has been attained, we have the following equations for the conservation of mass:

$$D_S(d^2S/dx^2) - k_1SE + k_2(ES) = 0 \quad (1)$$

$$D_{ES}[d^2(ES)/dx^2] + k_1SE - k_2(ES) - k_3(ES) = 0 \quad (2)$$

$$D_E(d^2E/dx^2) - k_1SE + k_2(ES) + k_3(ES) = 0 \quad (3)$$

where D_S, D_E and D_{ES} represent the diffusion constants of the species.

Although the following assumptions are probably not necessary for the operation of the transporting membrane, we make them in order to solve this set of equations easily: (i) k_2 is small and can be neglected; (ii) the concentration of free enzyme inside the membrane is not appreciably affected by reaction with the substrate. This is comparable to the assumption of negligible atmosphere depletion in flame kinetics (*3*), a problem that has recently been solved by Smith (*4*). The solution to Eq. 1 becomes

$$S = S_0\exp(-cx)\,;\; c^2 = k_1E/D_S \quad (4)$$

Assuming that all the substrate that enters the membrane is converted to P, we have a solution to Eq. 2:

$$(ES) = D_Sc^2S_0/D_{ES}(c^2 - a^2) \cdot [c/a \cdot \exp(-ax) - \exp(-cx)]$$
$$(ES)_0 = D_Sc^2S_0/D_{ES}a(a + c)\,;\quad a^2 = k_3/D_{ES} \quad (5)$$

Referring to Fig. 1, we see that for all values of x there will be a flux of ES to the right. Likewise, there will be a continual return flux (from right to left) of the free enzyme E, although this is not evident under the assumption that E is constant.

In order that active transport occur, the passenger species, or those molecules that are transported against their gradient, must be bound more (or less) strongly to ES than they are to E. In the case where 1 mole of ES binds only 1 mole of a neutral passenger species R, we have:

$$D_R(d^2R/dx^2) + (KR/1 + KR) \cdot D_{ES}(d^2(ES)/dx^2) = 0 \quad (6)$$

where K is the equilibrium constant for the binding reaction. Solving Eq. 6 for the case of complete binding, $KR/(1 + KR) \cong 1$, we have

$$R - R_0 = D_{ES}/D_R \cdot [(ES)_0 - (ES)] \quad (7)$$

The maximal concentration achieved by the membrane would be

$$R_\infty - R_0 = D_{ES}/D_R \cdot (ES)_0$$

One can imagine many ways in which the binding characteristics of the enzyme-substrate complex might be different from that of the free enzyme. For instance, a slight change in the $p\mathrm{K_a}$ of titratable groups that are in the neutral pH region will cause a change in the gross charge of the enzyme. Since electroneutrality must prevail in the immediate neighborhood of the protein, this means that a different number of counter ions will accompany the enzyme-substrate complex than accompany the free enzyme. Such pK shifts have been observed (*5*).

The interpretation of active transport along these lines is attractive for the following reasons. (i) It is in terms of enzyme reactions that are better understood and are encountered elsewhere in biological systems. (ii) It avoids postulating extreme differences in oxidation-reduction potentials, differences in voltage, differences in catalytic surfaces, and so forth.

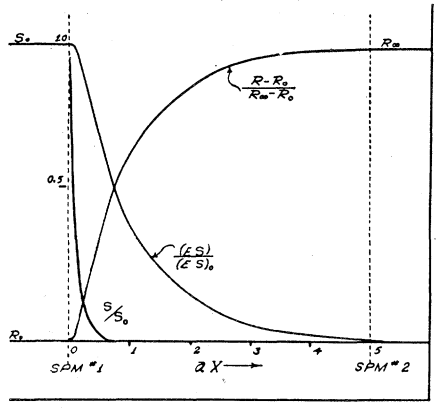

Fig. 1. Relative concentrations of the substrate S, the enzyme-substrate complex ES, and the transported passenger species R between two semipermeable membranes, $SPM\#1$ and $SPM\#2$. These have been calculated from equations 4, 5, and 7, respectively, assuming $c/a = 10$.