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SECRETION OF NITROGEN INTO THE SWIMBLADDER OF FISH. II. MOLECULAR MECHANISM. SECRETION OF NOBLE GASES[1]

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Abstract

Toadfish (*Opsanus tau*) were maintained at 50 m depth, 6 atm total pressure. The partial pressures of argon and nitrogen in the gases brought into the experimentally emptied swimbladder exceed the ambient pressures. The fraction of nitrogen in the gases brought into the swimbladder is nearly independent of depth. This finding is inconsistent with an earlier hypothesis that active oxygen secretion, by forming minute bubbles, drives nitrogen secretion. Toadfish were maintained in seawater equilibrated with mixtures containing oxygen, nitrogen, helium (in previous experiments), neon, argon, krypton and xenon. The more soluble gases are enriched in the mixture brought into the swimbladder, so that the composition of the inert gases brought into the swimbladder is similar to the composition of the gases dissolved in blood plasma. The enhancements, $([Gas/N_2]_{secreted} \div [Gas/N_2]_{ambient})$, of the gases in the mixture brought into the swimbladder are proportional to the solubility of the gases in water. These facts support the hypothesis that salting out of inert gases elevates the partial pressure of nitrogen and other inert gases in the gas gland blood vessels. High gas pressures may be generated by counter-current multiplication of this initial effect.

Introduction

Nitrogen and other chemically inert gases may be brought into the swimbladder of fishes in the face of very large pressures. Hüfner (1892) found nearly pure nitrogen under 5–7 atmospheres pressure in the swimbladders of whitefish captured from deep in the Bodensee. Schloesing and Richard (1896) found in the swimbladder of a deep sea eel captured from 1385 m, 14.0% nitrogen, 0.278% argon, 79.6% oxygen, and 6.1% carbon dioxide. J. S. Haldane (1898) pointed out that this implied active secretion of both argon and nitrogen. At the depth from which the animal was captured, the partial pressures of nitrogen and argon would have been 19.4 and 0.39 atm, twenty-fivefold greater than the corresponding pressures in sea water. While one might conceive of a chemical mechanism to capture and transport oxygen or even nitrogen, the noble gas, argon, forms no compounds, and the mechanism by which it was brought into the swimbladder could not involve formation of a chemical bond to a carrier molecule.

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The facts have been abundantly verified. The mass ratio, argon/nitrogen, in the gases present at the time of capture in the swimbladders of deep (Schloesing and Richard, 1896; Scholander, 1954; Douglas, 1967) and shallow-living (Wittenberg, 1958; Wittenberg *et al.*, 1964) marine fishes and of deep-living coregonids and salmonids (Scholander, *et al.*, 1956; Tait, 1956; Sundness *et al.*, 1958) is always close to that of air, 1.20×10^{-2}. In sharp contrast, the mass ratio, argon/nitrogen, in the gases as they enter the swimbladder may be far from that of air (Wittenberg, 1958). This fact is the starting point of the present study.

Two divergent theories have been offered to account for inert gas secretion. Koch (1934) suggested that inert gases might be expelled from the blood flowing through the rete mirabile if the gas solubilities were somehow depressed through the action of the gas gland. When it was discovered that lactic acid, generated by aerobic glycolysis, was present at high concentration in this blood, Kuhn and his colleagues (Kuhn and Kuhn, 1961; Kuhn *et al.*, 1963) and Scholander and his colleagues (Scholander, 1954; Enns *et al.*, 1967) calculated that salting out of gases with counter-current multiplication of the resulting "single concentrating effect" in the rete mirabile could account for the highest pressures of inert gases found in the swimbladder.

Powers (1932) suggested that gas bubble evolution might somehow be involved, and Wittenberg (1958) attempted to show that generation of oxygen bubbles could provide the driving force for inert gas secretion. The experiments to be presented here remove the experimental basis for the bubble hypothesis.

Here, we have examined the relative rates at which nitrogen and several of the noble gases are brought into the swimbladder. We found that the inert gases and dissolved oxygen were brought into the swimbladder in proportion to their concentrations in the blood plasma. This implies that they were driven from watery solution by some process that depressed their solubility. Salting out is one such process. Processes strongly influenced by relative rates of diffusion of the gases are ruled out.

In an accompanying paper (Wittenberg *et al.*, 1981) we showed that inert gas secretion persisted unchanged in the absence of circulating erythrocytes, and we estimated the increments of lactate anion, protons and carbon dioxide added to plasma of blood circulating through the gas-secreting complex.

Materials and Methods

Gas analyses

Oxygen, carbon dioxide and residual inert gases were determined as previously (Wittenberg *et al.*, 1981).

The ratios of noble gases to nitrogen were determined using a mass spectrometer (model 21-620A of the Consolidated Electrodynamic Corporation). Oxygen was not removed prior to analysis. Air served as a standard for argon and $^{28}N_2$. Unfortunately, the instrument used could not be adapted to determine the ratio of helium to gases of much greater molecular weight. Filaments of earlier mass spectrometers were destroyed by oxygen, and in early experiments (Wittenberg, 1958) it was necessary to remove oxygen from the samples prior to analysis, with some consequent distortion of the mass ratios. This objection applies to the data for helium quoted here.

Secretion of gas by toadfish at six atmospheres pressure

Toadfish were maintained in live cars at the bottom of the deepest water available to us, a 50 m deep depression in the sea floor (Lat. 41° 27′ 2″ N; Long. 70° 57′ 9″ W) near Cuttyhunk Island, Massachusetts. The depth and temperature were determined with a bathythermograph, and the depth was corroborated with a calibrated depth sounder. The pressure at 50 m depth corresponds to 5 atm hydrostatic pressure from the overlying water column plus 1 atm atmospheric pressure, a total of 6 atm. The water at this location is stirred by strong tidal currents. The temperature at depth was 19.6°C, the surface temperature 21–22°C.

The swimbladder was emptied by means of a syringe fitted with a fine needle. The fish were lowered to depth. After 48 h the fish were brought to the surface; the swimbladder gases withdrawn immediately, and the fish returned to depth for a second 48 h period.

Secretion of gas by toadfish breathing a mixture of oxygen, nitrogen and noble gases

The apparatus was the same as that described (Wittenberg *et al.*, 1981) except that gases were recirculated continuously from a 100 l external reservoir. A bed of "Baralyme" (Thomas A. Edison Co., Stuyvesant Falls, New York) interposed between the tank and reservoir served to remove carbon dioxide. The recirculating gases contained argon-free oxygen and a mixture of inert gases. In one experiment this mixture contained: neon, 2.1%; argon 2.0%; krypton, 2.0%; nitrogen, 93.9%. In a separate experiment, the mixture contained argon, 1.9%; krypton, 2.0%, xenon, 2.0%, and nitrogen, 94.1%. At the beginning of each experiment seawater in the tank was equilibrated with three successive portions of the gas mixture; these portions were discarded and the reservoir filled. Oxygen in the gas mixture was replenished daily or more frequently with argon-free oxygen so as to maintain the fraction of oxygen in the gas mixture at 21%. Samples of the recirculating gas mixture were taken frequently for comparison with the gases brought into the swimbladder. Fish were introduced into the tank at least 24 h before the swimbladder was emptied initially. The initial gas sample was discarded and subsequently samples were taken for analysis every 12 or 24 hours. Frequently 3 or 4 successive samples were obtained from each fish.

Presentation of mass spectrographic data

The mass spectrometer reports the ratios of the number of ions of each apparent mass. We take nitrogen as a common point of reference and consider the ratios of the mass abundance of each inert gas to the abundance of mass 28, diatomic ^{14}N. To compare gases brought into the swimbladder with ambient levels we define a quantity, "enhancement" (relative to nitrogen), which is a ratio of the experimentally determined mass ratios:

$$\left(\frac{\text{noble gas}}{\text{mass 28}}\right)_{\text{secreted}} \div \left(\frac{\text{noble gas}}{\text{mass 28}}\right)_{\text{ambient}}$$

Here, ambient is taken as the gas phase with which the sea water is in equilibrium.

The mass ratio, noble gas/nitrogen, will be different for gases dissolved in water and for the gas phase with which the water phase is in equilibrium. For instance, the mole fractions of argon and nitrogen in air are 0.00934 and 0.7808, respectively.

Their mass ratio is $^{40}Ar/^{28}N_2 = 0.0120$. Argon, which is 2.19-fold more soluble in water than nitrogen (at 20°C), is enriched 2.19-fold in the gases dissolved in air-equilibrated water. Consequently the mass ratio in the dissolved gases is $^{40}Ar/^{28}N_2 = 2.19 \times 0.012 = 0.026$.

Results

Secretion of gas at six atmospheres pressure

Refilling of the swimbladder at depth is slow; a small fraction of the original volume was replaced in 24 h and about half in 48 h. In shallow water the original volume would have been restored in 18–24 h (Fänge and Wittenberg, 1958; Wittenberg, 1958).

The partial pressure of argon in the secreted gas exceeded ambient in about one half of the fishes (Fig. 1A); and the partial pressure of nitrogen exceeded ambient in about one third of the individuals (Fig. 1B). The proportion of oxygen in all samples was 75% or greater.

Secretion of argon by fishes in air-equilibrated water

The enhancement of argon ranged from a small number to approximately 2. The enhancement tends to be small in the initial secreted sample and greater in subsequent samples (Fig. 2). The enhancement of argon is not different in fish maintained at 1 atm or 6 atm (Fig. 2).

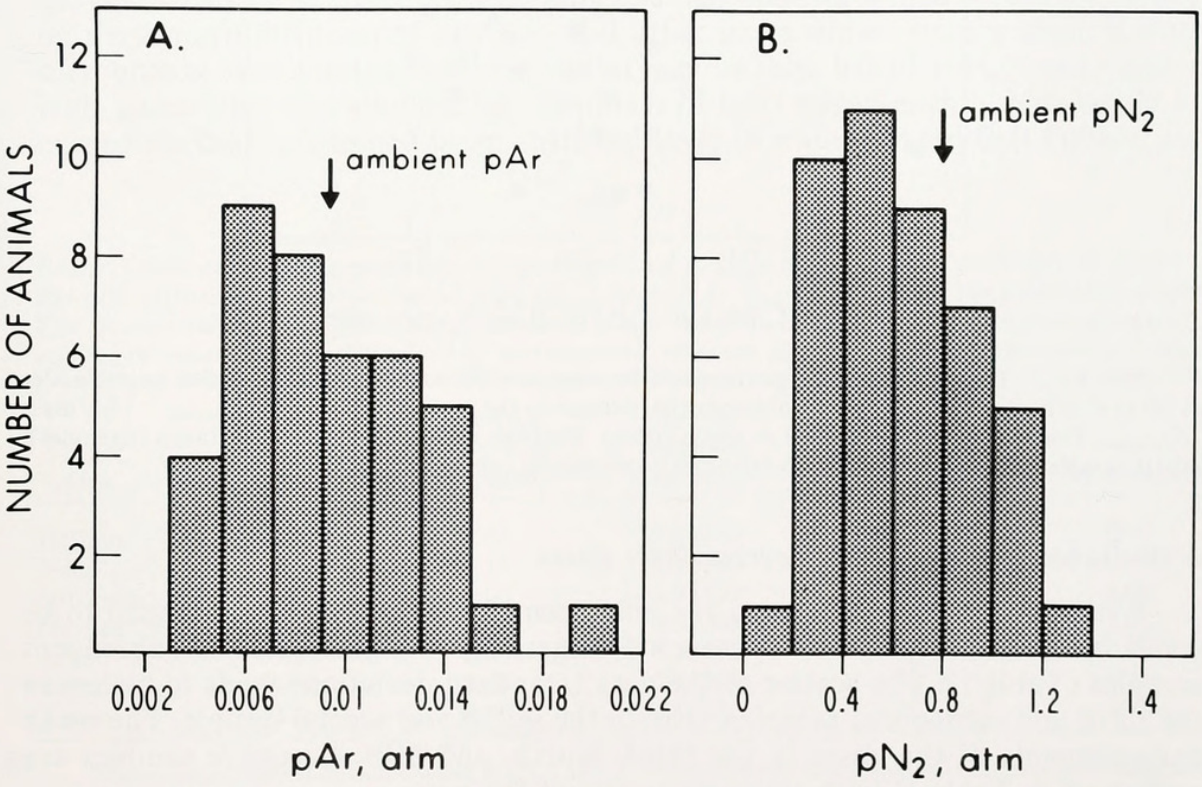

FIGURE 1. Partial pressure of argon, (pAr) A, and partial pressure of nitrogen, (pN_2) B, in gases brought into the experimentally emptied swimbladder of toadfish maintained at 50 m depth, equivalent to 6 atm total pressure. The arrows indicate the partial pressures of argon and nitrogen in air-equilibrated sea water.

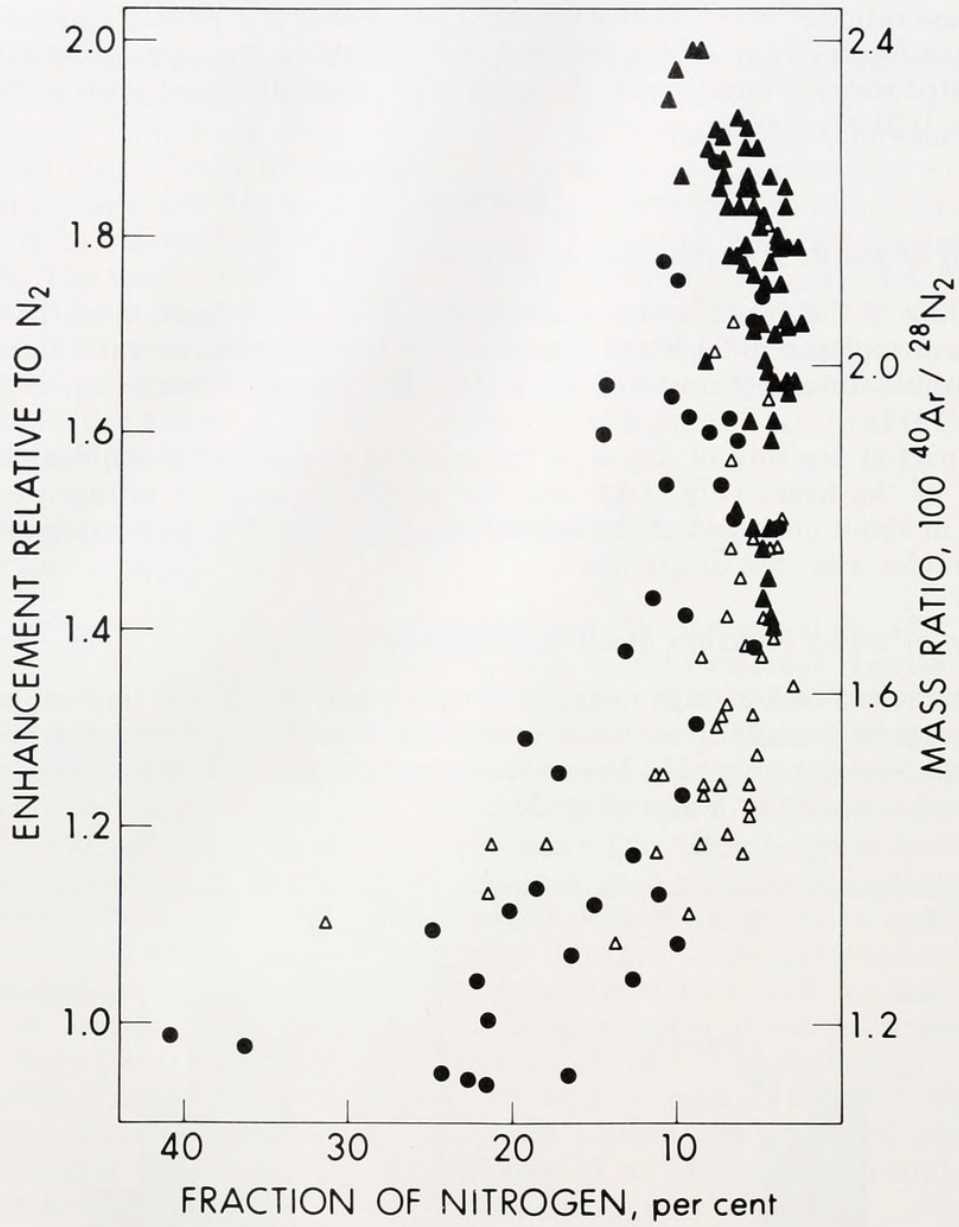

FIGURE 2. Enhancement of argon in gases brought into the experimentally emptied swimbladder at 50 m depth and at the surface. Enhancement, defined in the text, is: $(Ar/mass\ 28)_{secreted} \div (Ar/mass\ 28)_{ambient}$. Toadfish maintained at 50 m depth (dots). Toadfish maintained in shallow tanks (triangles); initial samples of replaced gas (open triangles); subsequent samples (closed triangles).

Simultaneous transport of several inert gases

With the exception of xenon, the enhancement of each inert gas tended to be small in initial samples and approach a larger, reproducible value in subsequent samples (Table I). The scatter of the data (standard deviation) tends to be less in the third and subsequent samples, than in the initial and second sample. The mean enhancements of the gases in the third, fourth, and fifth successive samples are compared in Table II with some properties of the gases.

In Figure 3 the enhancements of xenon, krypton, neon, and helium are plotted against the enhancement of argon in the same gas sample. These relations are linear with slopes characteristic of each gas.

TABLE I

Enhancement of noble gases brought into the swimbladder in successive replenishments of the swimbladder gas. Enhancement is defined in the text. Numbers are given as the mean and standard deviation. The number of samples is given in parentheses.

	Enhancement			
Sample	Neon	Argon	Krypton	Xenon
1	0.463 ± 0.08 (6)	1.36 ± 0.17 (15)	2.30 ± 0.45 (14)	3.95 ± 0.34 (5)
2	0.530 ± 0.12 (6)	1.72 ± 0.14 (15)	3.03 ± 0.38 (14)	4.45 ± 0.24 (7)
3	0.595 ± 0.05 (2)	1.87 ± 0.04 (9)	3.22 ± 0.11 (8)	4.27 ± 0.31 (6)
4	0.677 ± 0.07 (2)	1.92 ± 0.06 (8)	3.23 ± 0.15 (7)	4.15 ± 0.21 (4)
5		1.97 (1)	3.26 (1)	4.23 (1)
		mean of samples, 3, 4, and 5		
	0.636 ± 0.07 (4)	1.90 ± 0.05 (18)	3.23 ± 0.12 (16)	4.22 ± 0.26 (11)

DISCUSSION

In this study we use the shallow-living toadfish to explore a phenomenon of deep waters, the secretion of nitrogen and other inert gases against high pressure. We first establish that toadfish can bring inert gases into the swimbladder to greater than ambient partial pressure (Fig. 1A, 1B).

Wittenberg (1958) considered that the undoubted active accumulation of oxygen could provide the driving force for inert gas accumulation. He argued that oxygen might be secreted as a stream of small bubbles which would be invaded by the inward diffusion of nitrogen and other gases drawn from a shell of water surrounding each bubble, and that the oxygen bubbles would sweep their cargo of inert gases into the swimbladder. The mass of inert gas in each bubble would not exceed the mass contained in an unstirred layer of water around that bubble, and,

TABLE II

Enhancement of the noble gases and oxygen compared to their diffusivity and solubility in water. Relative diffusivity is approximated from the reciprocal of the square root of the molecular weight. The Bunsen solubility coefficient is the volume of gas (at 0°C, 1 atm pressure) absorbed per unit volume of water at the temperature of measurement when the partial pressure of that gas is 1 atm.

Gas	Molecular Weight	Diffusivity Relative to Nitrogen	Bunsen Solubility Coefficient	Solubility Relative to Nitrogen	Enhancement
Helium	4.00	2.65	0.00873	0.560	0.51
Neon	20.18	1.18	0.01045	0.670	0.636
Nitrogen	28.02	1.00	0.01559	1.00	1.00
Oxygen	16.00	0.94	0.03103	1.99	2.0
Argon	39.94	0.84	0.03412	2.19	1.90
Krypton	83.80	0.58	0.06264	4.02	3.23
Xenon	131.3	0.46	0.1122	7.20	4.22

Mean enhancement of noble gases from Table I. Enhancement of oxygen is from toadfish essentially lacking circulating erythrocytes (Wittenberg *et al.*, 1981). Enhancement of helium is from the second and third successive samples secreted by an eel (Wittenberg, 1958). Solubilities from Weiss (1970, 1971a, and 1971b), Benson and Krause (1976), Wilhelm *et al.* (1977) and Weiss and Kyser (1978), at 20°C.

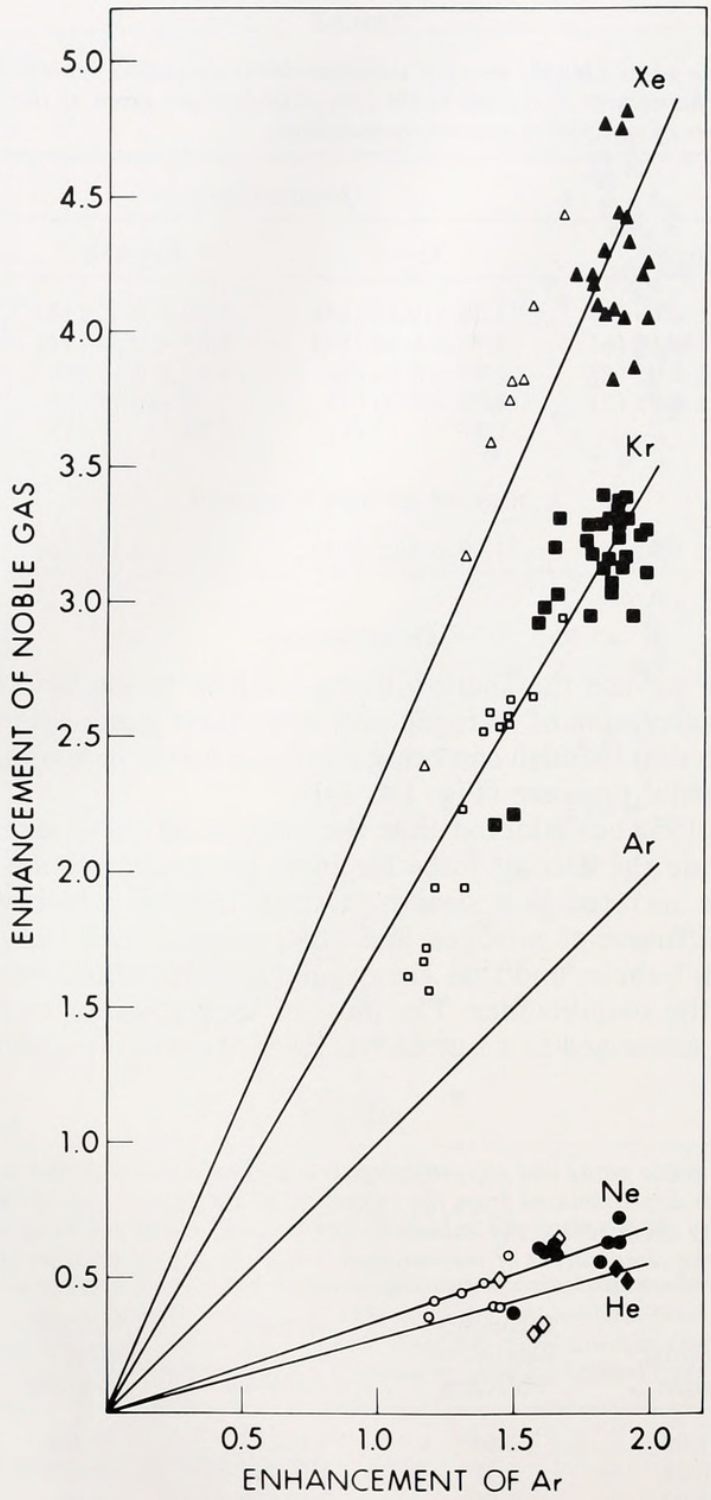

FIGURE 3. Enhancement of noble gases relative to the enhancement of argon. Enhancement is defined in the text. Points represent individual gas samples. A line of unit slope is drawn for argon. The other lines are least squares fits constrained to pass through the origin. Data for helium are from Wittenberg (1958). Open symbols, initial samples of secreted gas. Closed symbols, subsequent samples. Four successive gas samples were usually obtained from each fish.

since unstirred layers tend to be always of about the same thickness, would be a function of the size of the bubble independent of pressure. The mass of oxygen in each bubble would depend on the size of the bubble and the pressure. We reason that on this hypothesis, unless bubble size is dependent on depth, the fraction of

nitrogen in the secreted gas should be much less at depth. The data presented in Figure 2 show no difference in gas composition with depth. We reject the hypothesis.

These findings remove all experimental support for the concept that oxygen secretion provides the driving force for nitrogen transport. They have no bearing, however, on the manner in which gases are released from the surface of the gas gland. It may well be that cytoplasmic crystaloid bodies (Copeland, 1969; Brooks, 1970; Morris and Albright, 1975, 1977) and surface specializations (Copeland *et al.*, 1980) serve as nucleation sites for gas release.

We turn now to the simultaneous transport of the several inert gases at one atmosphere total pressure. The measured enhancements in individual gas samples, for instance of argon in Figure 2, are scattered over an enormous range. The net accumulation of gas in the toadfish swimbladder represents an ongoing balance of secretion by the gas-secreting complex of the anterior swimbladder chamber and resorption by the specialized resorbing epithelium of the posterior chamber (Fänge and Wittenberg, 1958). Although the experimental protocol was designed to emphasize gas secretion and minimize resorption, individual differences in the extent of resorption will remain. Individual gas samples differed in the intensity or vigor of enhancement of all of the gases in that sample at the time the sample was elaborated. The changes which we see in intensity or vigor do not stem from variations in the rate of secretion, which remained always about the same, and vary only weakly with the fraction of nitrogen in the sample (Fig. 1).

The enhancement of each gas tends toward a high constant value if the first samples of gas secreted by each fish are put aside. The mean of the enhancements of the third, fourth, and fifth successive samples are ranked in Table II together with some properties of the gases. In the absence of blood hemoglobin to which it may bind, oxygen will behave as any other gas. The enhancement of oxygen in fish essentially lacking circulating hemoglobin (Wittenberg *et al.*, 1981) is ranked with the enhancements of the noble gases in Table II. The enhancements of the gases bear no simple relation to molecular weight or to its correlate, diffusivity.

The enhancement of each gas may be compared to that of others within any particular sample. This is done in Figure 3 where the enhancements of xenon, krypton, neon, and helium are plotted against the enhancement of argon in the self-same sample. Linear relations emerge with slopes characteristic of each gas. These slopes express the relations:

$$\left(\frac{\text{noble gas}}{\text{argon}}\right)_{\text{secreted}} \div \left(\frac{\text{noble gas}}{\text{argon}}\right)_{\text{ambient}}$$

These are the smoothed enhancements, normalized relative to argon. These, together with values for nitrogen and oxygen taken from Tables I and II, are used to construct Figure 4.

Many solution properties of the gases may correlate with enhancement; since these properties are intimately interrelated they will change together in a homologous series of gases. We focus attention on solubility solely because, in salting out, we perceive a mechanism by which gas solubility may be depressed and partial pressure elevated. A plot, Figure 4, of the enhancements relative to argon versus the solubilities of the gases in water shows a linear function which falls off at higher values. Helium, neon, nitrogen, argon, and oxygen follow the linear relation fairly closely; krypton deviates somewhat, and xenon deviates substantially. We conclude that enhancement is closely related to solubility.

Salting out of gases from aqueous solution obeys the empirical Setschenow

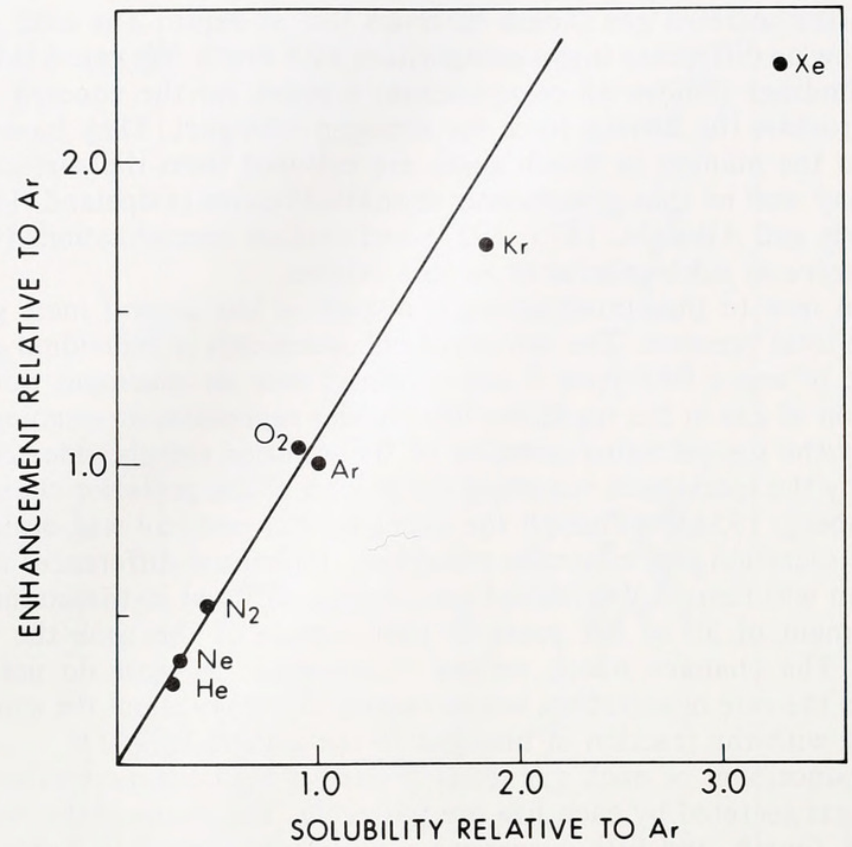

FIGURE 4. Enhancement relative to argon of noble gases, nitrogen and oxygen as a function of their solubility in water. Enhancement is defined in the text. Enhancements of the noble gases are the slopes of the lines in Figure 3. Enhancements of nitrogen and oxygen estimated from the data of Table I taking the enhancement of argon relative to nitrogen as 1.90.

relation which states that at constant temperature the logarithm of the solubility is a linear function of the salt concentration:

$$\log (S_O/S) = kC \qquad (1)$$

here S_O and S are bunsen solubility coefficients in the absence and presence of salt; k is a constant different for each gas and each solute, and C is the concentration of the solute. All of the gases considered here follow this relation closely without systematic deviation (Morrison and Billett, 1952; Morrison and Johnstone, 1955; Weiss, 1970, 1971a, 1971b; Weiss and Kyser, 1978). Values of the salting out coefficient are collected in Table III. We note that the salting out coefficients of the several gases are of similar magnitude. From this and from equation 1, we note that the ratio S_O/S, for a given increment of solute concentration, is similar for all gases studied. Thus, a given increment of added solute drives off the same fraction of each dissolved gas. It follows that the composition of the gases salted out is similar to the composition of the gases dissolved in the plasma. The amount of each gas driven off by any increment of solute is proportional to its solubility and the partial pressure (of that gas) with which the plasma was equilibrated at the gill. The more soluble gases will be enriched in the gases brought into the swimbladder.

The "single concentrating effect" expresses the equilibrium increase in the partial pressure of each gas brought about by salting out. Counter-current multiplication of the single concentrating effect may be looked on as a kinetic process in which transport of the more diffusible gases is favored since they pass more

TABLE III

Coefficients for salting out of some gases by sodium chloride.

Gas	[1]Data of Weiss (1971a, 1971b) and of Weiss and Kyser (1978) 20°C	[2]Data of Morrison and Johnstone (1955) and Morrison and Billet (1952) 25°C	[2]Data of Eucken and Herzberg (1950) 20°C
Nitrogen	0.136	0.121	
Oxygen	0.127	—	
Helium	0.096	0.081	
Neon	0.106	0.097	
Argon	0.128	0.133	0.139
Krypton	0.134	0.146	
Xenon	—	0.149	0.150

[1] Data for salinity of sea water has been recalculated for equivalent ionic strength of sodium chloride, taking S = 10 0/00 equivalent to I = 0.20239.

[2] Expressed as moles per 1000g water.

rapidly from outflowing to inflowing capillaries of the rete mirabile (Kuhn *et al.*, 1963). This kinetic effect would tend to decrease the enhancements of the relatively less diffusible krypton and xenon and cause them to depart from the linear relation shown in Figure 4. In fact, the measured resistance of the walls of the retial capillaries to gas diffusion is large (Rasio and Goreski, 1979).

The "single concentrating effect" (Kuhn *et al.*, 1963) that can be generated by salting out is small; the addition of even 0.02 *M* salt would depress solubility less than 1%; and the rate of inert gas secretion estimated on this basis is much less than the measured rate (Scholander, 1954; Kuhn *et al.*, 1963; Enns *et al.*, 1967). For this reason we consider the potentially greater effect that could be generated by perturbing the binding of gases to blood proteins. Plasma proteins do not bind nitrogen (Van Slyke *et al.*, 1934; Steen 1963). Human hemoglobin binds significant amounts of nitrogen (Van Slyke *et al.*, 1934), oxygen at sites other than the heme (Sendroy *et al.*, 1934) and, at known sites, xenon (Schoenborn, 1965). Binding of nitrogen, oxygen and argon in the erythrocytes of some fishes is depressed about 5 per cent by acidification, with apparent pK = 7.5 (Steen, 1963; Abernethy, 1972). However we show that erythrocytes play no necessary part in nitrogen secretion (Wittenberg *et al.*, 1981). We conclude that non-specific gas binding to proteins plays at most a minor role in inert gas secretion.

All facts presented here support the hypothesis that salting out of inert gases from their solution in the blood plasma generates the small "single concentrating effect." High pressures of these gases may be generated by counter-current multiplication of the single concentrating effect in the rete mirabile. Substances which may be responsible for salting out the inert gases are lactate anion, carbon dioxide and bicarbonate anion (Wittenberg *et al.*, 1981).

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LITERATURE CITED

ABERNETHY, J. D. 1972. The mechanism of secretion of inert gases into the fish swimbladder. *Aust. J. Exp. Biol. Med. Sci.* **50:** 365–374.

BENSON, B. B., AND D. KRAUSE. 1976. Empirical laws for dilute aqueous solutions of non-polar gases. *J. Chem. Phys.* **64:** 689–709.

BROOKS, R. E. 1970. Ultrastructure of the physostomatous swimbladder of rainbow trout (*Salmo gairdneri*). *Z. Zellforsch.* **106:** 473–483.

COPELAND, D. E. 1969. Fine structural study of gas secretion in the physoclistous swimbladder of *Fundulus heteroclitus* and *Gadus callarius* and in the euphysoclistous swimbladder of *Opsanus tau*. *Z. Zellforsch.* **93:** 305–331.

COPELAND, D. E., R. L. HAEDRICH, AND J. B. WITTENBERG. 1980. Gas secretion in the swimbladder of shallow water fishes compared to a deep ocean fish (3000 meters). *Biol. Bull.* **159:** 449.

DOUGLAS, E. L. 1967. Studies of gas secretion in the swimbladder of fishes. Ph.D. Dissertation, University of California, San Diego. Pp. 117 University Microfilms Inc., Ann Arbor, Michigan.

ENNS, T., E. L. DOUGLAS, AND P. F. SCHOLANDER. 1967. Role of the swimbladder rete of fish in secretion of inert gas and oxygen. In: *Advances Biol. Med. Phys.* (J. H. Lawrence, and J. W. Cofman, Eds.), Vol. **2,** Pp. 231–244, Academic Press, New York.

EUCKEN, A., AND G. HERZBERG. 1950. The Salting-out effect and ion hydration. *Z. Physikal. Chem.* **195:** 1–23.

FÄNGE, R., AND J. B. WITTENBERG. 1958. The swimbladder of the toadfish (*Opsanus tau*). *Biol. Bull.* **115:** 172–179.

HALDANE, J. S. 1898. Secretion and absorption of gas in the swimming-bladder and lungs. Part I—Swimming-bladder. *Sc. Progr.* **7:** 120–130.

HÜFNER, G. 1892. Zur physikalischen Chemie der Schwimmblasengase, *Arch. Anat. u. Physiol., Physiol. Abt.* 54–80.

KOCH, H. 1934. L'emission de gas dans la vesicule gazeuze des poissons. *Rev. Questions Scientifique*, series 4, **26:** 385–409.

KUHN, W., AND H. J. KUHN. 1961. Multiplikation von Aussalz-und anderen Einzeleffekten fur die Bereitung hoher Gasdrucke in der Schwimmblase. *Z. Elektrochem.* **65:** 426–439.

KUHN, W., A. RAMEL, H. J. KUHN, AND E. MARTI. 1963. The filling mechanism of the swimbladder. Generation of high gas pressures through hairpin countercurrent multiplication. *Experientia.* **19:** 497–511.

MORRIS, S. M., AND J. T. ALBRIGHT. 1975. The ultrastructure of the swimbladder of the toadfish, *Opsanus tau* L. *Cell Tissue Res.* **164:** 85–104.

MORRIS, S. M., AND J. T. ALBRIGHT. 1977. Cytochemical study of the lamellar bodies in the swimbladder of the toadfish *Opsanus tau* L. *Cell and Tissue Res.* **185:** 77–87.

MORRISON, T. J., AND F. BILLETT. 1952. The salting out of non-electrolytes, Part II. The effect of variation of non-electrolyte. *J. Chem. Soc.* Pp. 3819–3822.

MORRISON, T. J., AND N. B. B. JOHNSTONE. 1955. The salting out of non-electrolytes. Part III. The inert gases and sulfur hexafluoride. *J. Chem. Soc.* Pp. 3655–3659.

POWERS, E. B. 1932. The relation of the respiration of fishes to environment. *Ecol. Monographs* **2:** 385–473.

RASIO, E. A., AND C. A. GORESKI. 1979. Capillary limitation to oxygen distribution in the isolated rete mirabile of the eel (*Anguilla anguilla*). *Circ. Res.* **44:** 498–503.

SCHLOESING, T., AND J. RICHARD. 1896. Recherche de l'argon dans les gaz de la vessie natatoire des poissons et des Physalies. *Compt. Rend. Acad. Sci.* (Paris) **122:** 615–617.

SCHOENBORN, B. P. 1965. Binding of xenon to horse haemoglobin. *Nature* **208:** 760–762.

SCHOLANDER, P. F. 1954. Secretion of gases against high pressures in the swimbladder of deep sea fishes. II. The rete mirabile. *Biol. Bull.* **107:** 260–277.

SCHOLANDER, P. F., L. VAN DAM, AND T. ENNS. 1956. Nitrogen secretion in the swimbladder of whitefish. *Science* **123:** 59–60.

SENDROY, J., R. T. DILLON, AND D. D. VAN SLYKE. 1934. Studies of gas and electrolyte equilibria in blood. XIX. The solubility and physical state of uncombined oxygen in blood. *J. Biol. Chem.* **105:** 597–632.

STEEN, J. B. 1963. The physiology of the swimbladder of the eel *Anguilla vulgaris*. I. The solubility of gases and the buffer capacity of the blood. *Acta Physiol. Scand.* **58:** 124–137.

SUNDNES, G., T. ENNS, AND P. F. SCHOLANDER. 1958. Gas secretion in fishes lacking rete mirabile. *J. Exp. Biol.* **35:** 671–676.

TAIT, J. S. 1956. Nitrogen and argon in salmonoid swimbladders. *Canad. J. Zool.* **34:** 58–62.

VAN SLYKE, D. D., R. T. DILLON, AND R. MARGARIA. 1934. Studies of gas and electrolyte equilibria in blood. XVIII. Solubility and physical state of atmospheric nitrogen in blood cells and plasma. *J. Biol. Chem.* **105:** 571–596.

WEISS, R. F. 1970. The solubility of nitrogen, oxygen and argon in water and sea water. *Deep-Sea Res.* **17:** 721–735.

WEISS, R. F. 1971a. The effect of salinity on the solubility of argon in sea water. *Deep Sea Res.* **18:** 225–230.

WEISS, R. F. 1971b. Solubility of helium and neon in water and seawater. *J. Chem. Eng. Data.* **16:** 235–241.

WEISS, R. F., AND T. K. KYSER. 1978. Solubility of krypton in water and seawater. *J. Chem. Eng. Data.* **23:** 69–72.

WILHELM, E., R. BATTINO, AND R. J. WILCOCK. 1977. Low pressure solubility of gases in liquid water. *Chem. Rev.* **77:** 219–262.

WITTENBERG, J. B. 1958. The secretion of inert gas into the swimbladder of fish. *J. Gen. Physiol.* **41:** 783–804.

WITTENBERG, J. B., M. J. SCHWEND, AND B. A. WITTENBERG. 1964. The secretion of oxygen into the swimbladder of fish. III. The role of carbon dioxide. *J. Gen. Physiol.* **48:** 337–355.

WITTENBERG, W., D. K. WITTENBERG, AND J. B. WITTENBERG. 1981. Secretion of nitrogen into the swimbladder of fish. I. Secretion by fishes nearly lacking circulating hemoglobin. Role of the rete mirabile. *Biol. Bull.* **161:** 426–439.