GAS SECRETION IN FISHES LACKING RETE MIRABILE

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(With Plate 11)

In most fishes with a closed swimbladder the gas secretion is confined to a special organ, the so-called red gland, which consists of a secretory epithelium supported by a counter-current capillary system, the rete mirabile. All the fishes investigated which possess this structure are capable of accumulating oxygen, and most of them also nitrogen, in the swimbladder against a pressure gradient. In other fishes the rete mirabile is lacking, and instead the secreting epithelium lines the swimbladder directly. Many fishes in this category apparently secrete only nitrogen.

Two lines of thought have been formulated in an attempt to explain the gas secretion. According to the oldest theory the glandular epithelium secretes acid into the blood, which through the Bohr effect dissociates oxygen from the haemoglobin. The primary oxygen release would accordingly take place in the blood of the gland capillaries, and from these oxygen would diffuse through the epithelium into the swimbladder. Because of seemingly insurmountable difficulties inherent in this theory a second view has been proposed, to the effect that the gas accumulation takes place by a process of cellular secretion whereby the glandular epithelium extracts the gases from the surrounding tissue and actively secretes them into the swimbladder.

The main difficulty with the acid-secretion theory becomes apparent when we consider gas accumulation against high pressures. The ultimate requirement must then be that the dissociation pressure of the oxygen produced by acidifying the haemoglobin would be as high, or higher, than the partial pressure of the oxygen found in the swimbladder. It has been shown by optical methods that this requirement is far from fulfilled in many deep sea fishes (Scholander & van Dam, 1954). Very likely the Bohr or Root effect plays a certain 'booster' role in the secretion process, especially at shallow depths, but it is incapable of explaining one of the most striking aspects of the phenomenon, namely, secretion against high pressures. In most fishes the oxygen accumulation is accompanied by accumulation of nitrogen and argon as well, and both of these inert gases may enter against considerable tension gradients, a situation left entirely unexplained by the acid-secretion theory.

If a closed container is filled with fully oxygenated blood and all of the oxygen is released from the haemoglobin by, for instance, ferricyanide, the tension developed is simply the ratio between the oxygen capacity and the solubility coefficient, and may amount to some 2–3 atmospheres oxygen pressure in fish blood. In the presence of a rete mirabile counter-current system such a primary pressure effect, if caused by a non-diffusible agent, would easily be multiplied by a factor of several hundred, but without a rete no more pressure could be produced in the blood than would correspond to the above ratio. This makes it attractive to consider what gas pressures may develop in a swimbladder where the rete mirabile system is lacking.

In the present investigation we have accordingly studied the blood of some salmonid fishes lacking a rete mirabile and yet capable of secreting oxygen and nitrogen against considerable pressure. It will be seen that our data cannot be explained in terms of the acid-secretion theory, but point rather to an active cellular process.

MATERIAL AND METHODS

The fishes for this investigation, *Coregonus lavaretus* and *Salmo alpinus*, were obtained in the Ransfjord lake in Norway and *Coregonus acronius* in the Bodensee in Germany. They were caught by gill nets at various depths, ranging from 2 to 70 m. Gas samples were taken from live fish immediately the net reached the surface. The gas was analysed in a syringe analyser (Scholander, van Dam, Claff & Kanwisher, 1955).

Gas samples from the whitefish *C. acronius* were taken in syringes sealed with a saturated LiCl solution, and analysed by mass spectrometer for A/N_2 and $^{14}N/^{15}N$ ratios.

Blood samples were drawn from live fish by heart puncture, and were analysed for oxygen and nitrogen capacity in a Scholander–Roughton syringe. The fish were kept breathing in an irrigated rubber mask to avoid lactic acid formation. The procedure for oxygen analysis in fish blood (Scholander & van Dam, 1956) was modified in two respects: (1) the sequence of acid sulphate and potassium ferricyanide was reversed; and (2) the bicarbonate content of the potassium ferricyanide solution was doubled.

The nitrogen was analysed according to a modification of the above procedure. Three times the amount of blood was used and the oxygen was absorbed by the Fieser reagent (Edwards, Scholander & Roughton, 1943).

The depth of buoyancy was determined by the gas volume in the swimbladder divided by the submerged weight of the fish, according to the method of Saunders (1953).

EVIDENCE FOR LACK OF COUNTER-CURRENT SYSTEM

In whitefish and char the epithelia lining the swimbladder are very similar, consisting of cylindrical cells in the anterior part and cubical cells in the posterior part (Pl. 11). Gas secretion is presumably associated with the cylindrical epithelium. Beneath this layer the tissue is only sparsely supplied with capillaries which do

not form any discernible rete structure. The lack of a functioning rete was independently indicated by the analyses of the nitrogen isotope ratio $^{14}N/^{15}N$ in the gas obtained from the Bodensee whitefish caught at 65 m. depth. The ratio was the same as for atmospheric nitrogen to within $\pm 0{\cdot}3\%$. This lack of separation of isotopes may be taken to indicate that a counter-current diffusion process is absent.

OXYGEN SECRETION WITHOUT A RETE MIRABILE

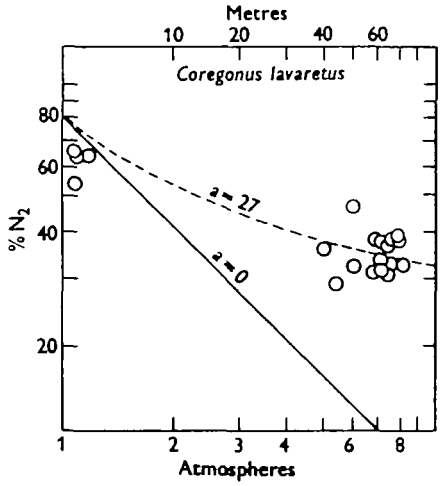
One of the salmonids investigated, *C. lavaretus*, unlike other whitefish, was found to accumulate considerable amounts of oxygen in the swimbladder. The oxygen fraction increased with depth (Text-fig. 1), as it does in deep-sea physoclist fishes (Scholander & van Dam, 1953).

It is a fair assumption that the secretion pressure must equal the buoyancy depth, i.e. the hydrostatic pressure at which the fish is in neutral buoyancy. Unfortunately *C. lavaretus* suffered a conspicuous loss of gas during its ascent to the surface, with the result that estimates of its buoyancy depth were too low (Text-fig. 2). One may safely say, however, that the secretion pressure must lie somewhere between the depth of buoyancy and the depth of catch, i.e. between 3·5 and 5 atmospheres (Table 1).

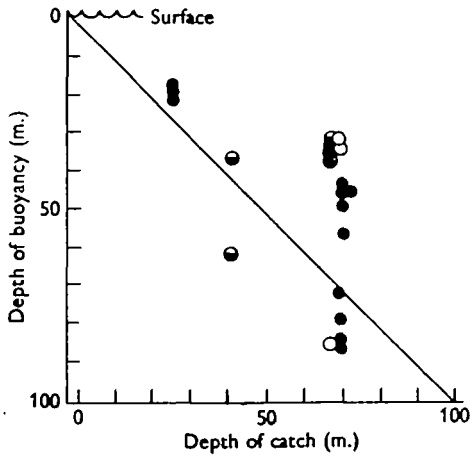
The oxygen capacity of the fish blood at lake temperature (4° C.) was found to be near 8 vol. % (Table 2). It has never been observed that more than 60% of any fish blood could be inactivated by acidification (Scholander & van Dam, 1954), and we can therefore expect no more than 4·8 vol. % oxygen to be released in the whitefish blood. The solubility coefficient for oxygen in water at 4° C. is near 4·4 vol. % and somewhat less in blood, and hence the maximum oxygen pressure which could be developed in the blood would be $4{\cdot}8/4{\cdot}4 = 1{\cdot}1$ atmospheres, which is one-quarter or at best one-third of the observed oxygen tension in the swimbladder. We may therefore conclude that a primary one-step oxygen release in the blood capillaries supplying the swimbladder epithelium cannot produce an oxygen tension as high as exists in the swimbladder of the fish.

SECRETION OF NITROGEN AND ARGON

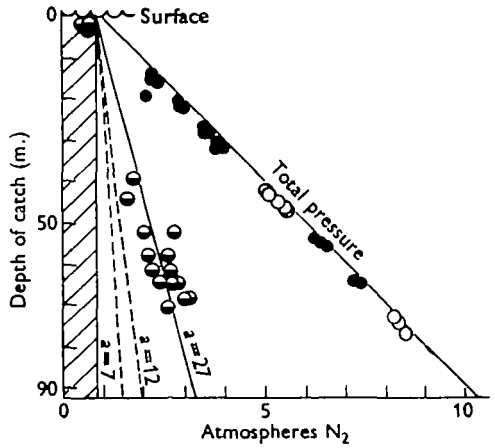
The partial pressure and fractional concentration of nitrogen in the swimbladder gas of the Bodensee whitefish and the char is shown in Text-fig. 3. It is clear that the nitrogen tension in both these fishes could rise to some 7–8 atmospheres pressure as compared to 0·8 atmosphere in the lake water, and the question arises: how does this accumulation of nitrogen against high pressure come about? One possibility would be a liberation of nitrogen from some compound in the blood effected by a secretory product from the epithelial cells. The released nitrogen would then diffuse into the swimbladder, i.e. a process in full analogy with the acid-secretion theory of oxygen. Such a chemical release of nitrogen from the blood is unlikely, because the A/N_2 ratio found in the swimbladder, although low (54–70% of that of air), was not nearly as low as would be expected if the argon were left behind by a chemical secretion of nitrogen (Koch, 1934; Scholander, van Dam & Enns, 1956).



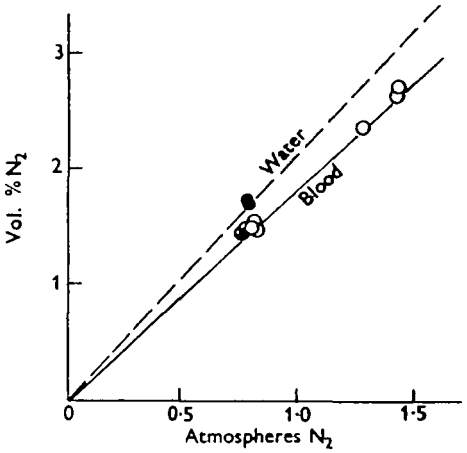
Text-fig. 1



Text-fig. 2



Text-fig. 3



Text-fig. 4

Text-fig. 1. Percentage of nitrogen found in the swimbladder of *Coregonus lavaretus* at various depths. The diagonal line represents the percentage of nitrogen which at any given depth would give a partial pressure of nitrogen of 0·8 atmosphere. *a*, the nitrogen as percentage of the total gas secreted.

Text-fig. 2. Depth of buoyancy of *Coregonus acronius*, *C. lavaretus*, and *Salmo alpinus* in relation to depth of catch. The diagonal is the line of perfect buoyancy, i.e. where depth of buoyancy equals depth of catch. ●, *C. acronius*; ◒, *C. lavaretus*; ○, *S. alpinus*.

Text-fig. 3. Nitrogen tension in the swimbladder of *Coregonus acronius*, *C. lavaretus* and *Salmo alpinus* in relation to depth. *a*, the nitrogen as percentage of the total gas secreted, 7 for *Macrourus*, 12 for *Sebastes*, and 27 *for Coregonus lavaretus*. Cross-hatched area represents nitrogen tension in the lake water. ●, *C. acronius*; ◒, *C. lavaretus*; ○, *S. alpinus*; – – –, physoclist fish.

Text-fig. 4. Nitrogen content in the blood of *Salmo alpinus* and *Coregonus lavaretus* equilibrated at various pressures of nitrogen at 3·8° C. ●, Six water analyses performed by the same method, which agree with the drawn line for the solubility of nitrogen in water; ◒, *C. lavaretus*; ○ *S. alpinus*.

Table 1. *Partial pressure of oxygen and nitrogen in the swimbladder of whitefish* (Coregonus lavaretus) *calculated from depth of catch and depth of buoyancy*

No. of specimens	Depth of catch (mean values) (m.) A	Depth of buoyancy (mean values) (m.) B	pO_2 atm. (mean values)		pN_2 atm. (mean values)	
			A	B	A	B
10	1–2	0	0·4	0·4	0·7	0·7
8	40	38	3·5	3·1	1·7	1·6
12	70	45	5·0	3·5	2·9	1·9

Table 2. *Oxygen capacity of haemoglobin in blood of salmonids* (*blood adjusted to pH 7·8 and saturated with air. Physically dissolved oxygen subtracted*)

Species	No. of specimens	Temp. (° C.)	Vol. %	Average
Coregonus lavaretus	9	3·8	6·6–9·4	8·0
Coregonus acronius	4	15·0	4·6–5·0	4·8
Salmo alpinus	2	3·7	6·4–9·0	7·7
Salmo alpinus	3	2·8	8·7–9·9	9·3

If, nevertheless, such a process took place it would have to be capable of releasing up to 7–8 times the normally dissolved nitrogen in order to account for the observed nitrogen tension in the swimbladder. It would seem that such a large amount of dissociable nitrogen would be easy to detect. Blood from char and whitefish was therefore equilibrated with nitrogen at various pressures at 4° C., and the total amount of molecular nitrogen given off by acid ferricyanide and vacuum was determined gasometrically. From this it was established that the amount of nitrogen present in the blood was no greater than that physically dissolved, and that the nitrogen content followed Henry's law exactly (Text-fig. 4).

We may conclude that there is no positive evidence for chemical release of nitrogen into the blood, but indeed very strong evidence against it.

CONCLUSIONS

We have shown that the maximum oxygen pressure which may be obtained by acidifying fish blood is much less than the observed oxygen tension in the swimbladder. We have been unable to show that our fishes possess means of adding in any way to the molecular nitrogen which is physically dissolved in the blood. Inasmuch as they lack a counter-current multiplying system in the form of a rete mirabile we must conclude that the principal pressure build-up of oxygen and nitrogen does not take place in the blood but is a function of the epithelium lining the swimbladder. This agrees with the findings that the dissociation pressure of oxygen obtained by acidifying blood from various deep-sea fishes frequently falls short of the observed oxygen tension in the swimbladder (Scholander & van Dam, 1954).

SUMMARY

1. Gas secretion has been studied in three species of salmonid fishes, one of which accumulates both nitrogen and oxygen against considerable gradients, while the other two accumulate only nitrogen. None of these three species possesses a counter-current system (rete mirabile) which could step up a slight primary pressure effect.

2. Neither the nitrogen nor the oxygen capacity of the blood is large enough to account for the tensions found in the swimbladder by a simple one-step diffusion process.

3. One is forced therefore to consider a mechanism for active transport of these gases by the cells lining the swimbladder.

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REFERENCES

EDWARDS, G. A., SCHOLANDER, P. F. & ROUGHTON, F. J. W. (1943). Micro gasometric estimation of the blood gases. III. Nitrogen. *J. Biol. Chem.* **148**, 565–71.

KOCH, H. (1934). L'émission de gaz dans le vésicule gazeuse des poissons. *Rev. Quest. sci.* (ser. 4), **26**, 385–410.

SAUNDERS, R. L. (1953). The swimbladder gas content of some freshwater fish with particular reference to the physostomes. *Canad. J. Zool.* **31**, 547–60.

SCHOLANDER, P. F. & VAN DAM, L. (1953). Composition of the swimbladder gas in deep sea fishes. *Biol. Bull., Woods Hole*, **104**, 75–86.

SCHOLANDER, P. F. & VAN DAM, L. (1954). Secretion of gases against high pressures in the swimbladder of deep sea fishes. I. Oxygen dissociation in blood. *Biol. Bull., Woods Hole*, **107**, 247–59.

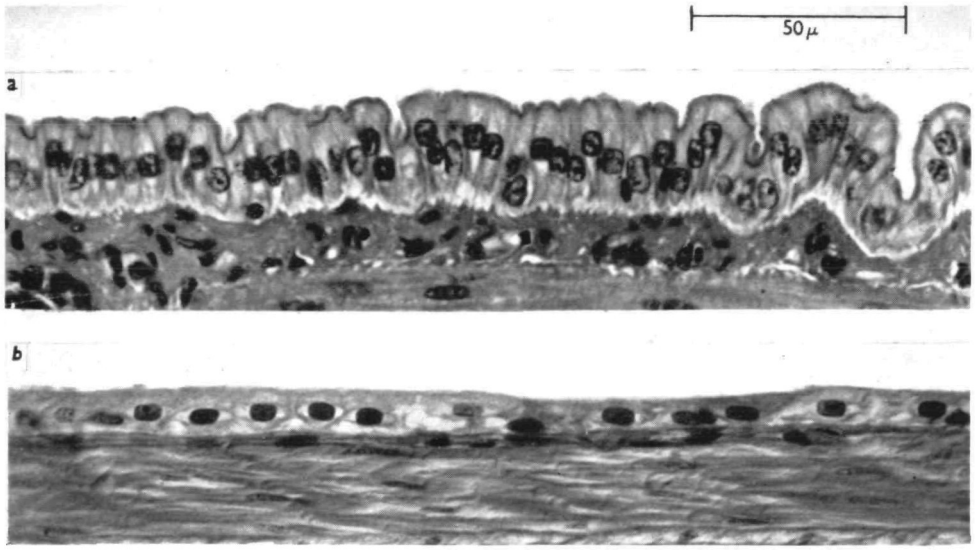
SCHOLANDER, P. F. & VAN DAM, L. (1956). Micro gasometric determination of oxygen in fish blood. *J. Cell. Comp. Physiol.* **48**, 529–32.

SCHOLANDER, P. F., VAN DAM, L., CLAFF, C. L. & KANWISHER, J. W. (1955). Micro gasometric determination of dissolved oxygen and nitrogen. *Biol. Bull., Woods Hole*, **109**, 328–34.

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

EXPLANATION OF PLATE

The epithelium lining the swimbladder of *Coregonus lavaretus*; *a*, cylindrical cells of the anterior part; *b*, cubical cells of the posterior part.



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