

SWIMBLADDER PERMEABILITY TO OXYGEN

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SUMMARY

The permeability of the swimbladder to gas was measured as the conductance to oxygen in seven species of fish. The low over-all conductance resides in the low diffusion constant, K , of the middle layer of the wall (submucosa) while the outer and inner layers (tunica externa and mucosa) have diffusion constants similar to other vertebrate tissues.

The low diffusion constant is due to a low diffusion coefficient, D (not to a low-solubility coefficient), apparently caused by multiple layers of very thin (about $0.02 \mu\text{m}$) and broad (up to $100 \mu\text{m}$) crystals, clearly identifiable in electron micrographs, and presumably consisting of guanine. Crystals occupy only a small fraction of tissue volume. They are flexible as a consequence of their extreme thinness, and subject to being elaborately folded when the mucosa and submucosa of the secretory part of the swimbladder is contracted during reabsorption of gas. The low conductance of the swimbladder wall reduces the metabolic energy needed to maintain swimbladder volume.

INTRODUCTION

The swimbladder of teleost fish is a gas-filled sac which confers near-neutral buoyancy. Some fishes can fill their swimbladders by swallowing air, but most species secrete gases into the swimbladder from the blood by means of a gas gland and the adjoining rete mirabile. The total pressure of the gas inside the swimbladder is nearly identical to the hydrostatic pressure in the water in which a fish finds itself, and the gases in the swimbladder will, in general, have higher partial pressures than in the blood. In the case of oxygen, for example, the partial pressure in the blood will typically not exceed 0.2 atm , while the partial pressure in the swimbladder may approach the hydrostatic pressure at which the fish is found, which may be several hundred atmospheres (Scholander & Van Dam, 1953). Some recent reviews of swimbladder function are those of Fänge (1966), Alexander (1966) and Steen (1970).

Since the gas partial pressures within the swimbladder exceed those in the surrounding tissues, there will be diffusion of gases out of the swimbladder, through its wall. If the fish is to maintain its swimbladder volume, it must secrete new gas to replace that lost by diffusion. It would therefore appear advantageous to limit gas diffusion through the swimbladder wall.

The idea that the swimbladder wall might be relatively impermeable to gases is not new. Bohr (1892, 1894) observed that the excised swimbladder of cod maintains

a high concentration of oxygen for days. He concluded that the wall of the swimbladder must be 'almost impermeable to oxygen' (Bohr, 1894). Fänge (1953) made similar observations on *Ctenolabrus rupestris*.

Direct measurements of swimbladder permeability have been reported by Denton, Liddicoat & Taylor (1970, 1972) on the conger (*Conger conger*) and Kutchai & Steen (1971) on the European freshwater eel (*Anguilla anguilla*). The measured permeabilities were considerably lower than those of other vertebrate tissues.

The mechanism proposed to explain the low permeability of the swimbladder is that solid materials present in its wall act as a barrier to diffusion (Scholander, 1954). Two solids have been mentioned: collagen, of which a 'special kind' is found in the swimbladder (Fänge, 1966), and crystalline guanine (Scholander, 1954; Fänge, 1966, 1958). There is no evidence that swimbladder collagen is any more a diffusion barrier than the collagen of other vertebrate tissues. The role of guanine is supported by the fact that in both *Conger* and *Anguilla*, the layer of the wall which contains guanine crystals has low permeability compared to the layers without guanine.

The purpose of our research was (1) to determine swimbladder permeabilities in various teleosts, and (2) to investigate the nature of the permeability barrier.

DIFFUSION AND DEFINITIONS

The following presents some theoretical relationships as necessary background.

Diffusion

Steady-state diffusion of a substance through a flat lamina of homogeneous material is described by Fick's equation

$$\frac{dn}{dt} = -DA\frac{dc}{dx}, \quad (1)$$

where dn/dt is the amount of substance crossing the lamina per unit time, i.e. the diffusion flux; dc/dx is the concentration gradient of the substance within the lamina in the direction of diffusion; A is the area through which diffusion occurs; and D is the *diffusion coefficient*. The minus sign makes the flux positive in the direction in which the concentration gradient is negative.

A difficulty arises in the case of the diffusion of gases. We apply different partial pressures of the gas to the two sides of the lamina and measure the flux, but we cannot compute D from this information alone, because the concentration gradient within the lamina is unknown. The concentration gradient depends on the solubility coefficient of the gas in the lamina, which is generally unknown. Krogh (1919) handled this problem by using the fact that if the concentration of the gas in the lamina is proportional to the partial pressure, p , the concentration gradient will be

$$\frac{dc}{dx} = \alpha\frac{dp}{dx}, \quad (2)$$

where α is the *solubility coefficient* for the gas in the lamina. He then used Fick's equation in the form

$$\frac{dn}{dt} = -DA\alpha\frac{dp}{dx} = -KA\frac{dp}{dx}, \quad (3)$$

where K is the *diffusion constant*, which replaces $D\alpha$. For a homogeneous lamina of thickness l ,

$$\frac{d\mathcal{p}}{dx} = \frac{\Delta\mathcal{p}}{l}, \quad (4)$$

where $\Delta\mathcal{p}$ is the partial pressure difference across the lamina. The proportionality between diffusion flux and $\Delta\mathcal{p}/l$ breaks down if the solubility coefficient, α , is not constant at all partial pressures. An example in which equation (4) does not apply is the case of facilitated diffusion of oxygen through haemoglobin solutions (Scholander, 1960).

It is sometimes convenient to use a simplification of Fick's equation such as

$$\frac{dn}{dt} = -GA\Delta\mathcal{p}, \quad (5)$$

where K/l is replaced by G , which is the *conductance* of the lamina to the gas. The conductance is the flux per unit area for each unit partial pressure difference across the lamina.

If several laminae are combined, the steady-state diffusion through the laminate is related to the diffusion constants and thicknesses of the individual laminae by

$$\frac{dn}{dt} = -\frac{1}{\sum \frac{l_i}{K_i}} A \Delta\mathcal{p}. \quad (6)$$

Here the term $1/\sum (l_i/K_i)$ is the conductance of the laminate. This can be multiplied by the total thickness of the laminate, $\sum l_i$, to give a kind of overall diffusion constant, \bar{K} , for the laminate (by analogy to a single lamina, for which $G = K/l$, or $G l = K$). Having done this, we can describe the flux through the laminate by

$$\frac{dn}{dt} = -\bar{K} A \frac{\Delta\mathcal{p}}{\sum l_i}. \quad (7)$$

It should be noted that $\Delta\mathcal{p}/\sum l_i$ is *not* the partial pressure gradient within the laminate, for $d\mathcal{p}/dx$ will generally have a different value in each lamina. In the Results section the symbol K will be used to designate \bar{K} for the composite layers as well as for single layers of the swimbladder wall.

As explained, the diffusion constant, K , of a material depends on both the diffusion coefficient, D , and the solubility coefficient, α . The steady-state flux of a gas gives no information about D and α separately, but only their product, K . However, D and α can be evaluated by measuring the diffusion flux as a function of time following a step change in the partial pressure on one side of the lamina. This technique was described by Barrer (1968) and by Berkenbosch (1967). Briefly, one determines a parameter of the diffusion response to a step change in $\Delta\mathcal{p}$ called the time lag, L . The diffusion coefficient in the lamina is given by

$$D = \frac{l^2}{6L} \quad (8)$$

and the apparent solubility coefficient, α_{app} by

$$\alpha_{app} = \frac{K}{D}. \quad (9)$$

There is presently no general theory to predict the effect of impermeable inclusions in a material on its overall permeability. Present theory can predict the effect for some specific arrangements of impermeable inclusions, such as regular and random arrays of impermeable spheres, or regular lattices of impermeable blocks (Barrer, 1968). However, these methods cannot be applied to irregular arrangements such as those of the crystals in the swimbladder.

Clark oxygen electrode

We used a membrane covered oxygen electrode, introduced by Clark (1956). Extensive treatments are given by Carritt & Kanwisher (1959), Carey & Teal (1965), Berkenbosch (1967) and Berkenbosch & Riedstra (1963*a, b*), and only certain basic features will be described here.

The basic principle is that oxygen is consumed by reactions at the cathode. As the oxygen adjacent to the cathode is depleted, oxygen begins to diffuse to the cathode through the plastic membrane covering it. A steady state is eventually established where the $p(O_2)$ adjacent to the cathode is reduced to essentially zero, and oxygen diffuses through the membrane at a rate proportional to the $p(O_2)$ external to the membrane, the cathode area, and the conductance of the membrane. The electrolyte layer will not significantly influence the flux since it is very thin and has a higher diffusion constant than the membrane. Four electrons flow through the circuit for each O_2 molecule which reacts at the cathode, thus the oxygen flux results in a proportional electrode current. Given the external $p(O_2)$, cathode area, and the resulting electrode current, the conductance of the membrane can be calculated (Berkenbosch, 1967). In order for such measurements to be accurate, the cathode diameter must be large relative to membrane thickness, so that diffusion in from the edges of the cathode is small relative to that coming straight through the membrane. Berkenbosch (1967) made measurements on teflon membranes using this method and found that it was accurate so long as the diameter of the cathode was at least 20 times the membrane thickness.

The assumption that the $p(O_2)$ adjacent to the cathode is essentially zero is supported by the fact that the conductance of membranes as determined by the response of O_2 electrodes agrees well with conductance determined by other methods (Berkenbosch, 1967). This could not occur if there were any significant $p(O_2)$ at the cathode. The validity of our method for measuring tissue permeability does not depend on the assumption that $p(O_2)$ at the cathode is zero. It does, however, require that the electrode current increase linearly with increasing external $p(O_2)$.

MATERIALS AND METHODS

Fish collection and holding. The fishes used in this study were collected in the vicinity of Beaufort, North Carolina. The following species were studied:

Atlantic croaker	<i>Micropogon undulatus</i> (Linnaeus)
Spot	<i>Leiostomus xanthurus</i> Lacépède

Weakfish	<i>Cynoscion regalis</i> (Bloch and Schneider)
Silver perch	<i>Bairdella chrysura</i> (Lacépède)
Black sea bass	<i>Centropristus striata</i> (Linnaeus)
Pigfish	<i>Orthopristis crysoptera</i> (Linnaeus)
Conger	<i>Conger oceanicus</i> (Mitchell)

Conger were caught in wire-mesh crab traps, and other species were caught either with hook and line or by trawling. Fish were held in running sea water for up to 2 weeks until used for experiments.

Anatomy. According to the terminology proposed by Fänge (1953), the swimbladder wall comprises three layers: mucosa, submucosa and tunica externa. The tunica externa consists of dense connective tissue and provides structural integrity. The middle layer, the submucosa, is very incohesive and delicate. Its loose structure permits extensive movements of the mucosa relative to the outer tunic.

All the fishes used in this study, with the exception of the conger, have swimbladders in which the mucosa and submucosa are differentiated into two regions, denoted 'secretory' and 'resorbent'. During secretion of gases the secretory region expands to cover most of the inner surface of the swimbladder, while the resorbent region contracts (Fig. 1A). During reabsorption of gases, the secretory region contracts and the resorbent region expands to cover much of the inner surface (Fig. 1B) (Fänge, 1953).

The secretory region usually occupies the ventral or anterior part of the swimbladder, while the resorbent region is dorsal or posterior. In all of the fish of this study, the secretory part is ventral and the resorbent part dorsal.

In eels, the secretory mucosa lines the main chamber of the swimbladder and the resorbent mucosa lines the pneumatic duct. Contraction of the secretory mucosa expels gas from the main chamber into the pneumatic duct and vice versa (Fänge, 1953).

The submucosa underlying the secretory mucosa spreads and contracts with it, and similarly for the submucosa underlying the resorbent mucosa. We therefore shall speak of secretory and resorbent submucosae, without implying any direct role in secretion and reabsorption processes.

Preparation of specimens. Permeability measurements were made on portions of excised swimbladder wall mounted on plexiglass rings, as shown in Fig. 2A. The fish was killed by severing the spinal cord behind the head and eviscerated through a ventral incision, leaving the swimbladder in place. Then the swimbladder was carefully dissected away from the body wall, while still inflated (in the weakfish the anterior end of the swimbladder is firmly attached to the body wall, and when cut through behind this point of attachment, it was caused to deflate). The swimbladder was then slit open along the mid-dorsal line and laid out flat. The secretory and resorbent regions could now be distinguished because (1) the resorbent region has more prominent vascularization, (2) the secretory region has a somewhat pearly or silvery appearance, and (3) the boundary between secretory and resorbent regions is often thickened or folded into a ridge. If the secretory mucosa was strongly contracted toward the ventral region, it was expanded by pulling on the resorbent mucosa until the secretory region covered most of the swimbladder surface. The

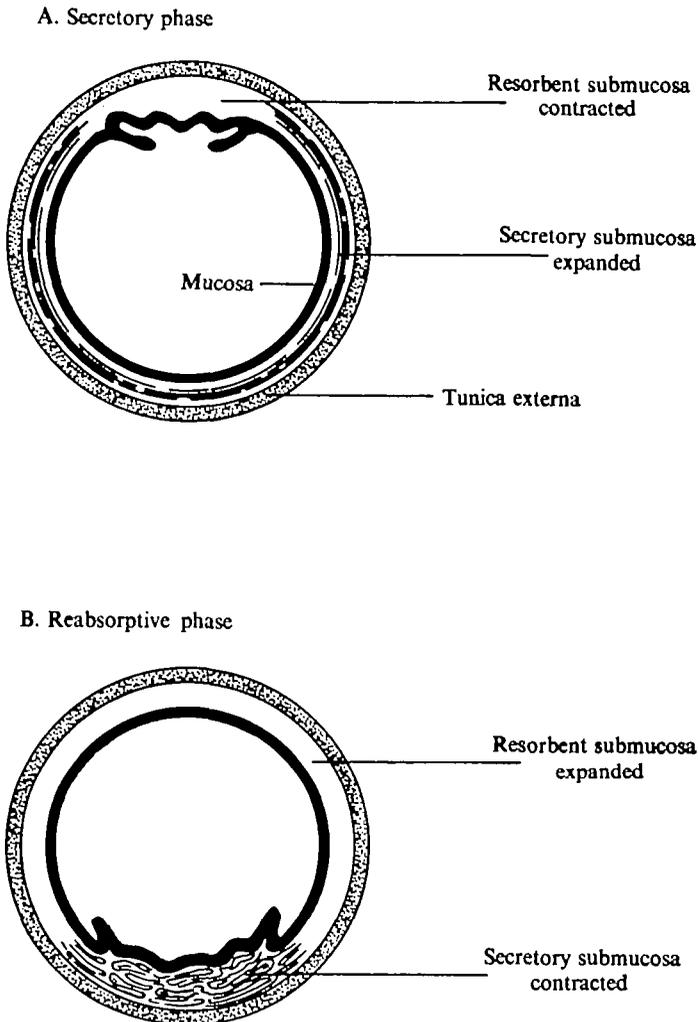


Fig. 1. Swimbladder anatomy. Transverse sections through the swimbladder during (A) secretion and (B) reabsorption of gas. During secretion of gas the secretory region is expanded; during reabsorption of gas the secretory region is contracted.

swimbladder was then laid on the plexiglass ring with the mid-ventral part, just behind the gas gland, centred on the ring. An 'O'-ring was slipped on to hold the specimen in place, and excess tissue was trimmed away. During the whole process the tissue was kept moist with physiological saline solution (Young, 1933).

In conger the tunica externa is only evident on the ventral half of the swimbladder. It is firmly attached to the body wall along its lateral margins, and there are fat deposits around the margin. The fat deposits were trimmed away and the tunica externa was cut loose from the body wall. The tunica externa could then be pulled away from the still inflated inner part of the swimbladder, from which it separates very easily. Specimens of the separated tunica externa were mounted on rings as described above. The inner part of the swimbladder (comprising mucosa and sub-

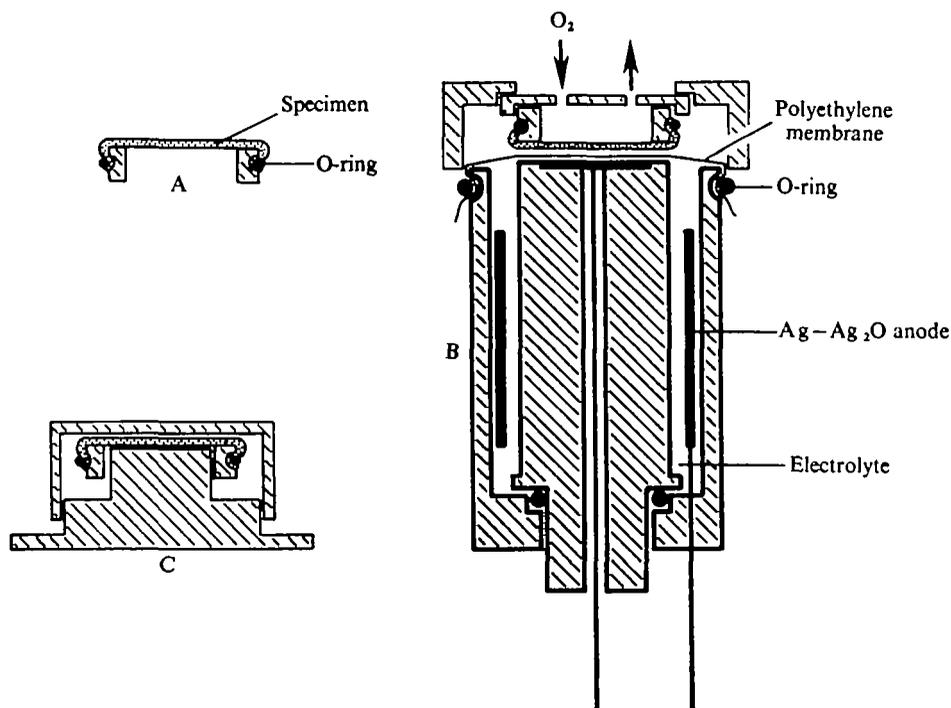


Fig. 2. Apparatus for oxygen conductance and thickness measurement. (A) Specimen mounted on plexiglass ring. (B) Specimen placed on oxygen electrode for conductance measurement. (C) Device used to determine specimen thickness.

mucosa) was then removed, slit open, laid out flat, and made into several preparations on plexiglass rings.

The difficulty in this procedure is that as soon as a swimbladder is punctured it collapses and contracts. All specimens were gently stretched before mounting, but there was no means to ensure that they were expanded to the same extent as in the intact animal.

All specimens, except those from conger, were mounted in such a manner that diffusion would be from tunica externa to mucosa. Conger specimens of mucosa plus submucosa were mounted so that diffusion was from mucosa to submucosa. These differences in diffusion direction should not have affected the results. An effect would be possible if the tissue is consuming oxygen, but specimens were treated with cyanide to eliminate oxygen consumption, as described below.

Measurement of oxygen conductance and permeability

Apparatus. The body of the oxygen electrode was machined out of plexiglass. The cathode was a platinum disc 0.953 cm in diameter. The anode was silver, with an exposed surface area of about 16 cm², to fulfil the recommendation of Kanwisher (1959) that the anode area should be at least ten times the cathode area to produce proper temporal response. The electrolyte was 1.0 N-KOH. The response of this electrode to O₂ has a plateau at a polarizing voltage between 0.9 and 1.2 V; 1.1 V

was used throughout this work. The electrode was prepared for use by filling it with electrolyte and applying 3 V for about 30 s to build up Ag_2O on the anode and to clean the cathode (Berkenbosch & Riedstra, 1963*a*). Then the membrane, polyethylene 0.002 cm thick, was applied. The sensitivity of this electrode was about 1.5×10^{-4} A/atm $p(\text{O}_2)$.

Electrode current was measured by passing through a precision resistor ($\pm 1\%$) and recording the potential across this resistor. Resistors between 100 and 10000 Ω were selected as required, to limit recorded potential to less than 0.01 V. This resistor was in series with the electrode, and the effective polarizing voltage thus varied within a 0.01 V range. The plateau of the electrode was sufficiently flat that no significant change in electrode current would be produced by this variation.

To define the area through which diffusion occurred, the inside diameter of the plexiglass rings was equal to that of the cathode. However, between the two surfaces of the specimen, the area available for diffusion is larger than that of the cathode, but the error is negligible so long as cathode diameter exceeds 20 times the membrane thickness (Berkenbosch, 1967). This condition was met for all specimens of this study.

The gases which flowed over the specimen were saturated with water vapour (see Fig. 2B). Temperature was between 21 and 24 °C. At these temperatures and a barometric pressure of 1 atm the $p(\text{O}_2)$ would be from 0.2028 to 0.2042 atm for saturated atmospheric air and 0.9687 to 0.9754 atm for saturated oxygen. The mid-points of these ranges, 0.203 and 0.972 atm, were used for all computation without correction for variations in the barometric pressure. The gas flow rate was high enough to prevent diffusion of room air into the space over the specimen; the positive pressure in this space was less than 0.5 mmH₂O.

Specimen thickness was determined by means of the device shown in Fig. 2C, consisting of a pedestal upon which the specimen, on its mounting ring, could be placed, and a cap resting on the specimen. The device was placed on the stage of a microscope (at $\times 440$) which was focused on an ink dot on the upper surface of the cap. The difference between settings of the graduated fine-focus adjustment with and without the specimen in place indicated the thickness. The pedestal was 0.9 cm in diameter, and the cap exerted a pressure of 0.15 N cm⁻².

Procedure. To determine sensitivity and temporal response, the electrode current was first recorded with nitrogen passing over the electrode membrane (no specimen in place); the nitrogen was then replaced with air and the response recorded. This must be done before each measurement since the sensitivity of an oxygen electrode depends on temperature, increasing about 4%/°C for a polyethylene membrane (Carey & Teal, 1965). Next the specimen, drained of excess saline, was placed on the electrode membrane in such a manner that no air bubbles were trapped between specimen and electrode membrane. The first $p(\text{O}_2)$ was applied until diffusion reached steady state. Then the applied $p(\text{O}_2)$ was switched to a higher value, and the electrode current recorded until diffusion again stabilized. Finally, the specimen was removed and its thickness measured.

In many cases a layer of the specimen was stripped off at this point by pulling it away with fine forceps and with the fingers. The thickness of the remaining tissue and its conductance was then measured. In all species, except the conger, the

stripped-off layer comprised mucosa and submucosa and the tissue remaining on the mounting ring was only the tunica externa.

With conger specimens of mucosa plus submucosa, the silvery submucosa could be removed with forceps and by gently wiping with moist tissue until all the silvery flecks adhering to the mucosa were gone. Thus only the mucosa remained.

Calculations. Specimen conductance is calculated as follows, using these symbols:

G specimen conductance in $\text{cm}^3 \text{O}_{2,\text{STP}} \text{cm}^{-2} \text{atm}^{-1} \text{min}^{-1}$,

S sensitivity of electrode alone ($dI/dp(\text{O}_2)$) in A atm^{-1} ,

$p(\text{O}_2)_1$, $p(\text{O}_2)_2$ two partial pressures of oxygen applied to the electrode with specimen in place,

I_0 , I_1 , I_2 electrode currents produced by $p(\text{O}_2) = 0$, $p(\text{O}_2)_1$, and $p(\text{O}_2)_2$, respectively,

A Cathode area in cm^2 .

The partial pressure difference across the specimen when $p(\text{O}_2)_1$ is applied is $[p(\text{O}_2)_1 - (1/S)(I_1 - I_0)]$, and $[p(\text{O}_2)_2 - (1/S)(I_2 - I_0)]$ when $p(\text{O}_2)_2$ is applied. (10)

The oxygen fluxes, in $\text{cm}^3 \text{O}_{2,\text{STP}} \text{min}^{-1}$, produced by the two applied partial pressures are $3.48(I_1 - I_0)$ and $3.48(I_2 - I_0)$. (One ampere of current corresponds to $3.48 \text{ cm}^3_{\text{STP}}$ of oxygen reacting per minute, according to the stoichiometry of the electrode reactions.)

$$G = \frac{\text{change in flux}}{\text{change in partial pressure difference across specimen} \times \text{area}}$$

$$= \frac{3.48(I_2 - I_1)}{[p(\text{O}_2)_2 - p(\text{O}_2)_1 - (1/S)(I_2 - I_1)] A} \quad (11)$$

An advantage of this form of the equation, involving only changes in current, is that I_0 need not be known since it does not appear in the final equation.

In cases where the conductance of a specimen is determined, a layer removed, and the new, higher conductance determined, the conductance of the layer which was removed can be calculated as follows:

Let $G_{(1+2)}$ be the conductance of a two-layered specimen, and G_1 be the conductance after layer 2 with conductance G_2 is removed, then

$$\frac{1}{G_2} = \frac{1}{G_{(1+2)}} - \frac{1}{G_1} \quad (12)$$

The time-lag attributable to the specimen, L_s , was calculated from the lags of electrode membrane alone, L_e , and electrode plus specimen, L_{e+s} , using the relationship

$$L_{e+s} = L_e \left(\frac{3G_e + G_s}{G_e + G_s} \right) + L_s \left(\frac{G_e + 3G_s}{G_e + G_s} \right), \quad (13)$$

where G_e and G_s are the oxygen conductances of the electrode membrane and specimen. This is a rearrangement of equation (10) in Berkenbosch (1967), and was originally derived by J. C. Jaeger.

The diffusion and apparent solubility coefficients were next calculated using equations (8) and (9).

Oxygen consumption by specimens. Oxygen consumption by the tissue interferes

with diffusion measurements, and specimens were therefore treated with cyanide by placing them for 10 min in saline containing 10 mM-KCN, and rinsing briefly in cyanide-free saline before oxygen conductance was measured.

Untreated specimens did not show proportionality between applied $p(O_2)$ and oxygen flux. For example, the flux produced by air might be only one-twentieth that produced by oxygen, rather than one-fifth as expected. This effect was eliminated by the cyanide treatment. Apparently, at low $p(O_2)$ much of the oxygen which entered the tissue was consumed. When a series of increasing $p(O_2)$'s were applied, before and after cyanide treatment, the conductance calculated from the increase in flux between two high $p(O_2)$'s before cyanide treatment was only slightly less than the conductance after cyanide. This indicates that cyanide treatment does not affect conductance in some way not attributable to its effect on oxygen consumption.

Electron microscopy

Specimens, still on the rings, were fixed for 4 h in the refrigerator in 4% glutaraldehyde in Millonig's phosphate buffer (Pease, 1964). Specimens were then rinsed, removed from the rings, and stored in the same buffer for 4-6 weeks. Then they were postfixed for 2 h in 2% osmium tetroxide in 0.1 M sodium cacodylate, pH 7.4, with 4% sucrose and 0.01 M $CaCl_2$. They were dehydrated in an ethanol series, followed by propylene oxide and embedded in Spurr embedding medium (Polysciences, Inc., Warrington, Pa., U.S.A.). Sections approximately 70-100 nm thick (as indicated by their pale gold to silver colour) were cut using glass knives. They were expanded with chloroform vapour and picked up on carbon-stabilized Formvar-coated grids. They were observed and photographed with a Hitachi HS-7 electron microscope.

Cutting of cross-sections was easiest if the direction of knife motion was parallel to the plane of the specimen. Coated grids were necessary because otherwise the section pulled apart in the electron beam. This is a problem that has arisen in other studies on tissues containing guanine crystals (e.g. Best & Nicol, 1967). Apparently the embedding medium does not adhere strongly to the crystals of guanine, and separates from them in the electron beam if the section is not adequately supported. No such problems occurred when coated grids were used.

Accuracy. The accuracy of the results is specified in terms of systematic error and imprecision as described by Eisenhart (1969) and Ku (1969*a, b*). Briefly, *systematic error*, sometimes called bias, is the amount by which the long run mean of determinations on a single specimen could differ from the true value. *Imprecision* refers to the variability among replicate determinations and its contribution to the standard error of a reported mean value based on different specimens. Imprecisions given here are based on the standard deviation of replicate measurements on single specimens divided by \sqrt{n} , where n different specimens contributed to a reported mean value.

The estimated systematic errors and imprecisions of the quantities reported in this study (where % refers to the reported mean values) are:

Quantity ...	G	l	K	D	α_{app}
Systematic error	± 5 %	± 5 %	± 10 %	± 10 %	± 10 %
Imprecision	2.5 %	3 μ m	2.5 %	5 %	5 %

Table 1. Oxygen conductance and diffusion constant (K) for intact swimbladder wall (mean \pm S.E. (n))

	Oxygen conductance, G (10^{-3} cm ³ O ₂ cm ⁻² atm ⁻¹ min ⁻¹)	Thickness (10^{-3} cm)	Diffusion constant, K (10^{-3} cm ³ O ₂ cm ⁻² atm ⁻¹ cm min ⁻¹)
Croaker	9.03 \pm 1.09 (35)	3.42 \pm 0.88 (15)	0.204 \pm 0.053 (15)
Spot	8.56 \pm 1.11 (37)	2.97 \pm 0.72 (17)	0.245 \pm 0.060 (17)
Weakfish	7.86 \pm 1.49 (3)	—	—
Silver perch	7.16 \pm 1.45 (7)	6.04 \pm 2.7 (5)	0.438 \pm 0.196 (5)
Sea bass	6.74 \pm 0.66 (36)	2.63 \pm 0.57 (20)	0.176 \pm 0.039 (20)
Pigfish	5.17 \pm 0.63 (29)	3.57 \pm 0.88 (16)	0.134 \pm 0.034 (16)
Conger*	0.90 \pm 0.23 (7)	3.90 (4)	0.035

* The values for conger are calculated from separate determinations on tunica externa and mucosa plus submucosa, using equation (12).

The systematic errors in G and K are larger for conger mucosa ($\pm 10\%$ and $\pm 15\%$, respectively). The systematic error in l and K of the mucosa plus submucosa of all species except conger is larger (possibly as great as 50%) as a result of tissue deformation during thickness measurement.

The systematic error in G was obtained as follows. For each elemental quantity entering into the computation of G (e.g. cathode area, electrode current, applied $p(\text{O}_2)$) the maximum possible deviation from its assumed value was estimated. The extreme possible values were then used to calculate values for G which would result from the most unfavourable combination of errors in the elemental quantities. The value for systematic error in G given above is based on half the difference between the maximum and minimum values so obtained. The imprecision in G was determined by replicate measurements on plastic membranes with conductances spanning the range found in swimbladder specimens (polyethylene, $G = 3 \times 10^{-4}$ cm³ O₂ cm⁻² atm⁻¹ min⁻¹; polyvinylidene chloride (Saran), $G = 2 \times 10^{-6}$).

The systematic error in l was estimated by measuring the thickness of tissue (frog abdominal wall muscle) using the method already described, punching out a known area, weighing it, and then calculating its thickness using the density of the tissue.

The systematic errors and imprecisions of the other quantities were derived from those of G and l using the propagation of error formulae given by Ku (1969b).

The magnification of electron micrographs was checked with a carbon-grating replica (Ernest & Fullam, Inc., Schenectady, N.Y.). The systematic error was less than $\pm 5\%$.

RESULTS

Oxygen conductance and diffusion constant

Intact swimbladder wall. The oxygen conductances, G , of the intact ventral wall of the swimbladder are shown in Table 1 and Fig. 3. These conductances range from 5 to 9×10^{-5} cm³ O_{2,STP} cm⁻² atm⁻¹ min⁻¹, with the exception of the conger which is an order of magnitude lower. Table 1 includes the mean thickness of the specimens and since the wall thickness of the conger swimbladder is not appreciably thicker than the others, its low conductance must be related to a low diffusion constant, K .

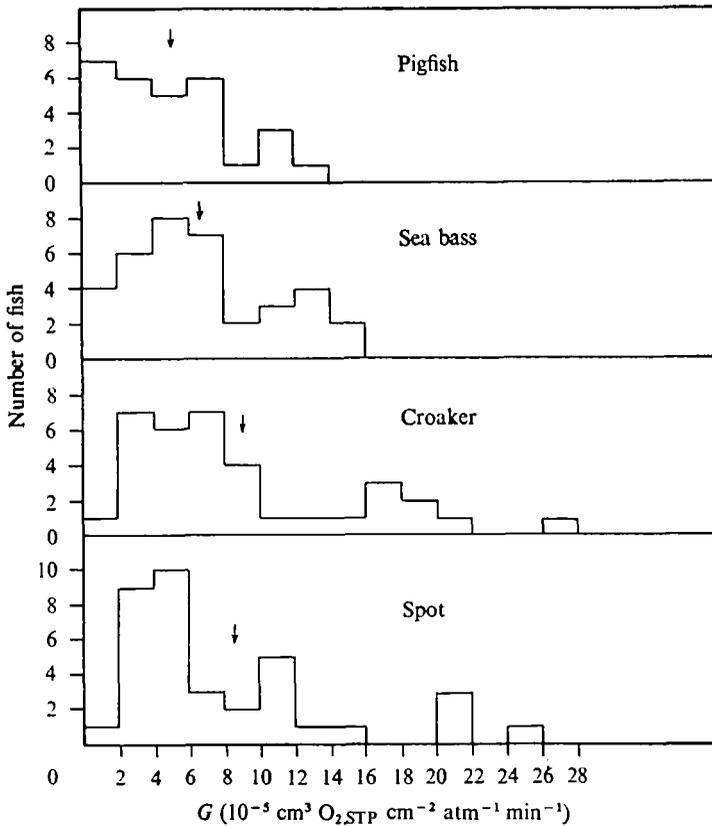


Fig. 3. Frequency distribution of oxygen conductance (G) of the intact swimbladder wall of four species of fish. Arrows indicate means.

Fig. 3 shows that there is a great deal of variability within each species. This variability did not appear to be correlated with the size of the fish from which the specimen came, nor do we believe it to be due to gross mechanical damage to the specimens (each specimen was examined under a dissecting microscope and only those with no evident damage were used). Some variability was undoubtedly caused by the manner in which the specimens were handled and mounted, and furthermore, the degree of expansion of the secretory part was not controlled.

Tunica externa. The conductances, G , and diffusion constants, K , of the tunica externa are given in Table 2. In every case except that of the silver perch, the conductance of the tunica externa is several times that of the intact wall. Thus the tunica externa is usually not the main barrier to diffusion. In the silver perch, however, the conductance of the tunica externa is only about 50% greater than that of the intact wall, so it is more important than the inner layers in reducing diffusion.

The diffusion constants, K , of the tunica externa fall within a narrow range, except for that of the conger which is somewhat higher, and silver perch which is substantially lower than the other values. The relatively low conductance for the silver perch is due to both its low diffusion constant and its greater than average thickness.

Table 2. Oxygen conductance and diffusion constant (K) for the tunica externa of the swimbladder wall (mean \pm S.E. (n))

	Oxygen conductance, G (10^{-3} cm ³ O ₂ cm ⁻² atm ⁻¹ min ⁻¹)	Thickness (10^{-3} cm)	Diffusion constant, K (10^{-3} cm ³ O ₂ cm ⁻² atm ⁻¹ cm min ⁻¹)
Croaker	35.7 \pm 1.9 (20)	3.16 \pm 0.16 (18)	1.10 \pm 0.05 (18)
Spot	56.6 \pm 4.3 (16)	2.57 \pm 0.14 (13)	1.35 \pm 0.06 (13)
Weakfish	29.3 \pm 1.7 (5)	4.80 \pm 0.32 (5)	1.11 \pm 0.05 (5)
Silver perch	11.5 \pm 1.2 (7)	4.71 \pm 0.52 (6)	0.56 \pm 0.03 (6)
Sea bass	64.1 \pm 3.6 (21)	2.11 \pm 0.15 (21)	1.28 \pm 0.04 (21)
Pigfish	42.1 \pm 2.9 (20)	3.27 \pm 0.25 (19)	1.28 \pm 0.04 (19)
Conger	73.2 \pm 5.3 (4)	2.47 \pm 0.11 (4)	1.79 \pm 0.13 (4)

Table 3. Oxygen conductance and diffusion constant (K) for mucosa plus submucosa of swimbladder wall (mean \pm S.E. (n))

	Oxygen conductance, G (10^{-5} cm ³ O ₂ cm ⁻² atm ⁻¹ min ⁻¹)	Thickness (10^{-3} cm)	Diffusion constant, K (10^{-3} cm ³ O ₂ cm ⁻² atm ⁻¹ cm min ⁻¹)
Croaker	14.1 \pm 3.1 (20)	0.38 \pm 0.04 (15)	0.0282 \pm 0.0046 (15)
Spot	12.9 \pm 3.5 (15)	0.57 \pm 0.05 (11)	0.0851 \pm 0.0297 (13)
Weakfish	10.8 (3)	—	—
Silver perch	18.8 \pm 4.8 (5)	0.67 \pm 0.04 (4)	0.129 \pm 0.021 (2)
Sea bass	6.51 \pm 1.12 (19)	0.51 \pm 0.03 (21)	0.0431 \pm 0.0081 (19)
Pigfish	5.07 \pm 0.95 (17)	0.47 \pm 0.12 (16)	0.0214 \pm 0.0058 (16)
Conger	0.91 \pm 0.22 (7)	1.43 \pm 0.22 (4)	0.0166 \pm 0.0071 (4)

Mucosa plus submucosa. The conductances and diffusion constants of mucosa plus submucosa are given in Table 3. The mucosa plus submucosa contributes a certain fraction of the total diffusion resistance of the intact swimbladder wall. In most species the mucosa plus submucosa account for some 80% of the total resistance, while in the silver perch it contributes only 37%. In the conger, however, 99% of the total resistance to diffusion resides in the mucosa plus submucosa.

In the conger it was possible to determine separately the conductances and diffusion constants of the mucosa and the submucosa. The observed values were, for mucosa $G = 301$ and for submucosa 9.91×10^{-5} cm³ O₂ cm⁻² atm⁻¹ min⁻¹. Thus the conductance of the mucosa is so high relative to that of the submucosa that it is an insignificant factor in the conductance of the intact swimbladder.

In the spot the peritoneum on the ventral surface of the swimbladder is black, the pigment presumably being melanin. The possibility that this layer might be a significant barrier to diffusion was examined by determining the conductances of two specimens of tunica externa of spot before and after stripping off the black peritoneum. In both cases the conductance increased by about 20% when the black peritoneum was removed, and this represents only a small effect on conductance of the intact swimbladder.

Diffusion coefficient, D , and apparent solubility coefficient, α

The time course of the diffusion response following a step change in applied $p(\text{O}_2)$ was used to calculate D and α , as described before. The time to reach steady state

Table 4. *Oxygen diffusion coefficients (D) and apparent solubility coefficients (α_{app}) for swimbladder tissues (mean \pm S.E. (n))*

	Diffusion constant, K (10^{-3} cm ³ O ₂ cm ⁻² atm ⁻¹ cm min ⁻¹)	Diffusion coefficient, D (10^{-8} cm ² s ⁻¹)	Apparent solubility coeff., α_{app} (cm ³ O ₂ cm ⁻³ atm ⁻¹)
Tunica externa			
Croaker	1.04	0.67 \pm 0.09 (5)	0.027 \pm 0.002 (5)
Sea bass	1.21	0.52 \pm 0.03 (5)	0.039 \pm 0.001 (5)
Pigfish	1.22	0.56 \pm 0.06 (3)	0.037 \pm 0.003 (3)
Conger	1.73	0.53 \pm 0.03 (3)	0.055 \pm 0.003 (3)
Submucosa			
Conger	0.00082	0.00067	0.021
	0.00529	0.0030	0.029
	0.00847	0.0049	0.029
	0.00972	0.0069	0.024

diffusion ranged from about 2 min for thin tunica externa up to twenty minutes for the least permeable specimens of conger mucosa plus submucosa. The diffusion coefficients and apparent solubility coefficients calculated from these response curves are given in Table 4.

The diffusion coefficients, D , for the tunica externa were similar in the four species for which we obtained data. Unfortunately, it was not possible to obtain data for the mucosa plus submucosa of any fish, except the conger (these layers from the other fish could not be manipulated separately). The diffusion coefficients for the conger submucosa were strikingly low, in the order of 1/100 of those of other tissues (see Discussion). It is particularly important to note that the individual values for conger submucosa differ by a factor of ten, and yet the apparent solubility coefficient is similar for all the samples. This means that the differences in K cannot be ascribed to differences in gas solubility, i.e. the cause must reside in other characteristics, presumably inclusions of crystalline material (guanine).

Morphology

The secretory and resorbent parts of the swimbladder in all of the species studied are visually distinguishable by the greater vascularization of the resorbent part and by a somewhat pearly or silvery shine or reflectiveness of the secretory part. The source of the reflectiveness is evidently in the submucosa; it persists when the mucosa is stripped away, and the isolated mucosa is not reflective.

The reflectiveness of the submucosa is due to the presence of crystals, presumably of guanine. Crystals can be obtained by teasing the tissue apart; such crystals may not be intact for they appear irregular in outline as if their edges have been torn. This is in contrast to the hexagonal guanine crystals which can be obtained from the retinas of fish (Best & Nicol, 1967).

When a suspension of isolated crystals is viewed in a microscope between crossed polarizers, they are bright when viewed approximately edge-on and disappear when they settle flat on to the slide. When a specimen of secretory mucosa plus submucosa is laid flat on a slide and viewed between crossed polarizers, it appears predominantly

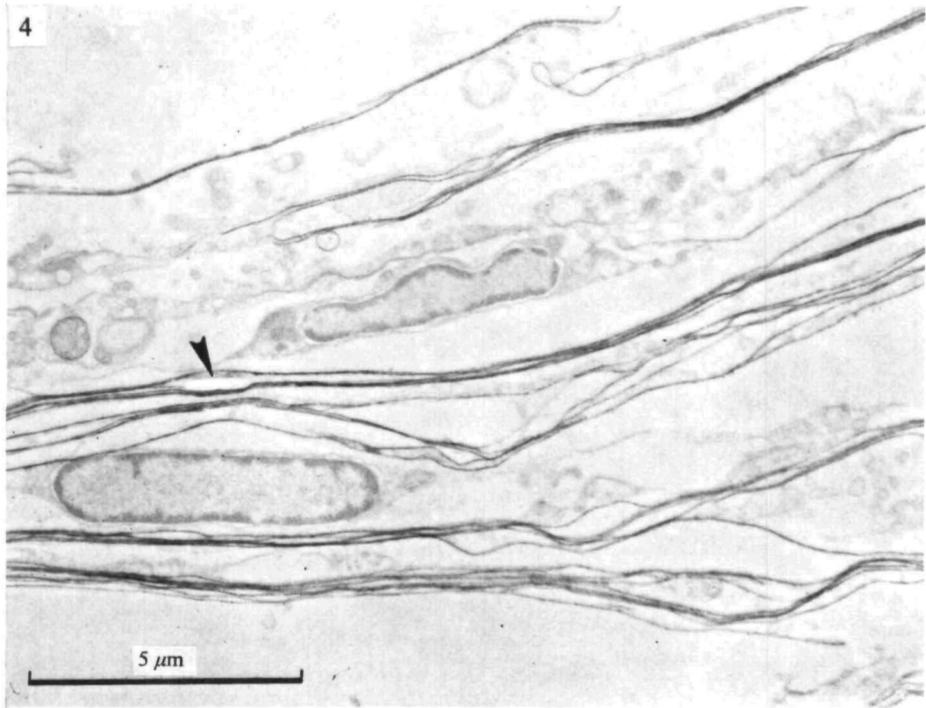


Fig. 4. Cross-section of secretory submucosa of pigfish. Layers of crystals are seen as thin, long, dark lines stretching from one side of the photograph to the other. Arrow points to a hole where the embedding medium has separated from a crystal.

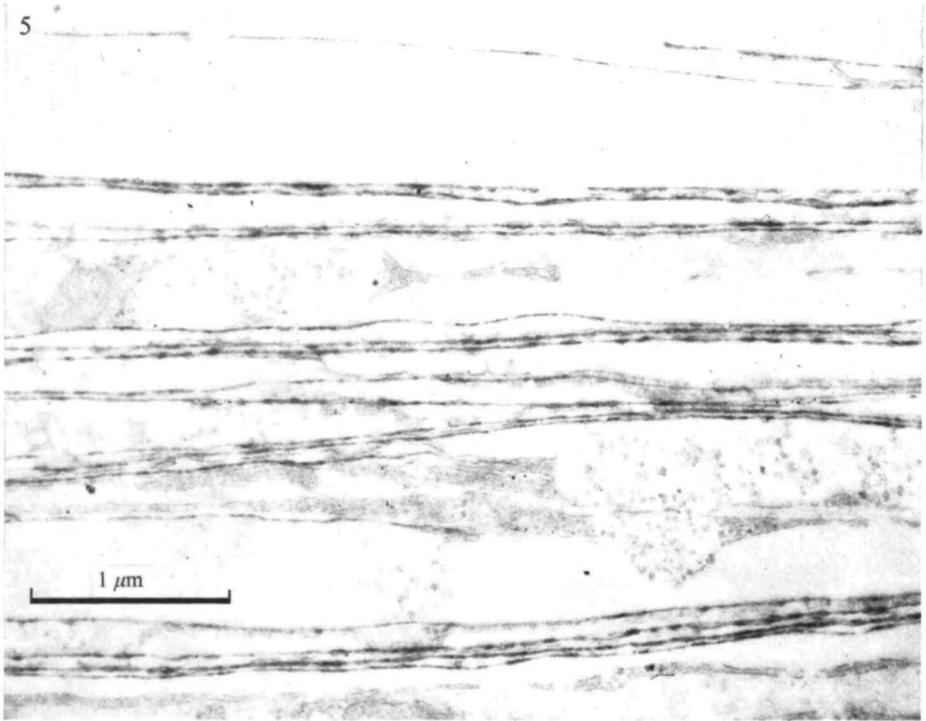


Fig. 5. Cross-section of secretory submucosa of conger.

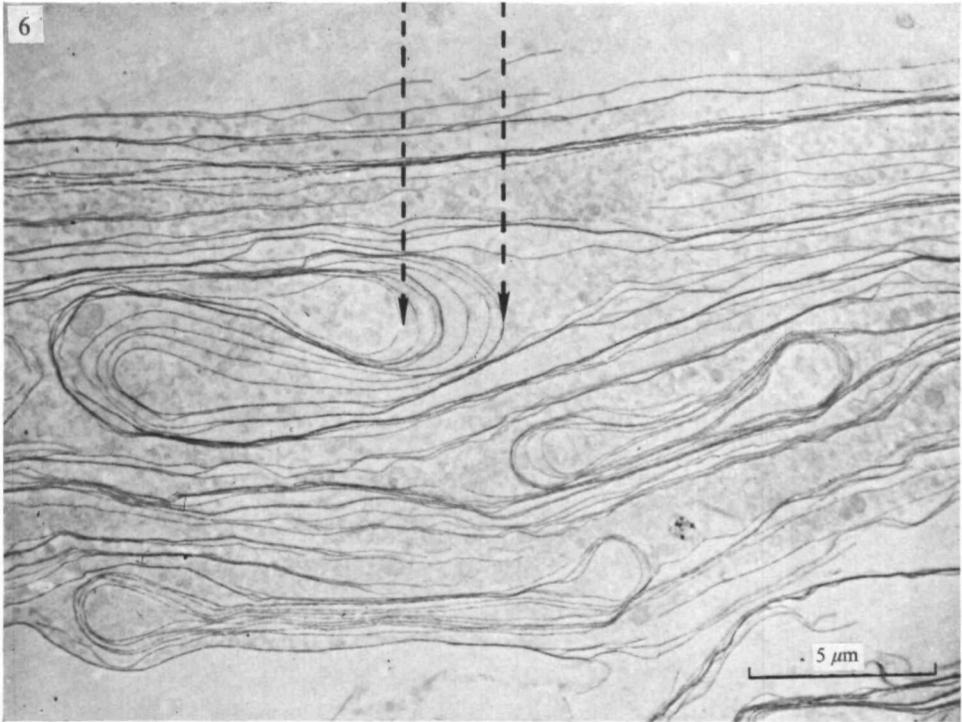


Fig. 6. Cross-section of secretory submucosa of spot showing extensive foldings of the layers of crystals. Dashed lines indicate our interpretation of Fig. 7 as explained in text.

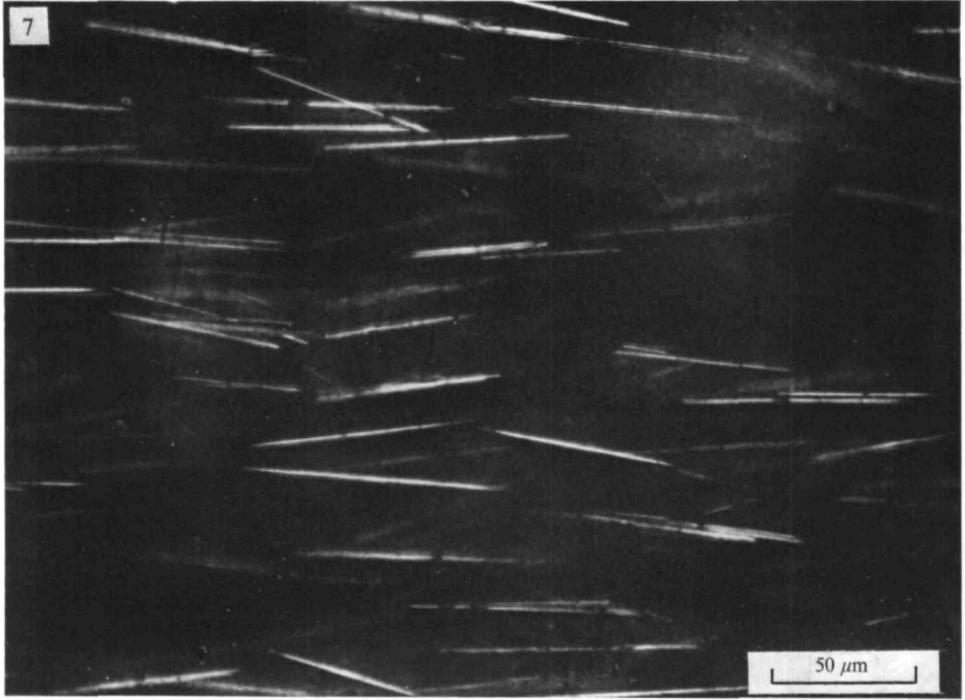


Fig. 7. Surface view of mucosa plus submucosa of conger photographed through polarizing microscope.

dark, with scattered bright areas often in the form of straight-line segments (see Fig. 7).

The submucosae of three species (conger, pigfish and spot) were examined by electron microscopy. The crystals in the secretory submucosa appear as narrow, dense lines (Figs. 4, 5). The correspondence between these dense lines and the guanine crystals is supported by the birefringence of these elements. A thick section (about $0.5\text{--}1\ \mu\text{m}$) examined in the polarizing light microscope shows a pattern of bright lines, and an adjacent thin section (about $0.07\ \mu\text{m}$) used for electron microscopy shows thin dense lines. The patterns of birefringent and dense lines in adjacent sections are in detailed correspondence, indicating identity of the structures, presumably guanine crystals. This conclusion is also supported by the manner in which the embedding medium sometimes pulls away from the dark lines during sectioning or upon exposure to the electron beam. One such separation is indicated by an arrow in Fig. 4. Similar separation is commonly seen in sections of other guanine-containing tissues, such as elasmobranch tapetum lucidum (Best & Nicol, 1967).

The general arrangement of the crystals is in layers roughly parallel to the plane of the swimbladder wall. The number of crystals is rather large. Along transects across the submucosa of conger the number ranged from 100 to 150. In transects of the submucosa of spot, the number of crystals ranged from 40 to 80, averaging about 50.

The single crystals are thin sheets of considerable width and breadth. Their thickness can be estimated from the electron micrographs. For those of the conger the range was 10–25 nm, for pigfish 15–20 nm, and for spot 15–35 nm. These thicknesses are similar to the value of 20 nm given for *Conger conger* swimbladder crystals by Denton *et al.* (1972). The exact thickness is difficult to estimate accurately because the images of the crystals vary in width and density along their length (Fig. 5). This may well be an artifact of sectioning, the somewhat uneven thickness of the sections being caused by flaws in the knife.

The width and breadth of the crystals are substantial, for in most photographs of sufficient magnification to resolve the crystals clearly, one or both ends are outside the field of view. By examining a series of contiguous photographs of a specimen of spot submucosa, it was possible to locate some complete crystal sections which were $90\ \mu\text{m}$ in length. This length is more than a thousand-fold greater than the estimated thickness of the crystals, and judging from the difficulty in finding the ends of the crystals in photographs, a majority of the crystals may be appreciably longer.

The distance between the crystals and their distribution across the thickness of the submucosa are not uniform. Instead, a number of crystals often lie close together to form a layer which is separated from adjacent layers by crystal-free spaces (Fig. 5). The distance between the mid-planes of crystals within a layer is often as little as 50 nm, i.e. of the same magnitude as the crystal thickness. The individual crystals within a layer are not resolved in the light microscope, and the bright bands seen when sections are viewed in the polarizing microscope are layers of several crystals, rather than individual crystals.

Although the crystals for the most part lie in the plane of the swimbladder wall, they may be elaborately folded (Fig. 6). These folds may explain the bright line

Table 5. *Oxygen diffusion constants (K) in swimbladder and other tissues at 20 °C** ($10^{-5} \text{ cm}^3 \text{ cm}^{-2} \text{ atm}^{-1} \text{ cm min}^{-1}$)

Swimbladder tissues		Other tissues	
Intact wall (7 species) (1)	0.034-0.42	Water (4)	4.25
Tunica externa (7 species) (1)	0.55-1.7	Cardiac muscle (rat) (5)	2.1
Mucosa plus submucosa 5 species (1)	0.021-0.126	Cerebral cortex (rat) (6)	2.3
<i>Conger oceanicus</i> (1)	0.016	Lung tissue (rat) (4)	2.0
<i>C. conger</i> (2)	0.015†	Skeletal muscle (rat) (7)	1.9
Eel (3)	0.103‡	Skeletal muscle (frog) (8)	1.4
Mucosa		Connective tissue (frog) (8)	1.1
<i>C. oceanicus</i> (1)	1.6	Urinary bladder (cat) (9)	1.9
<i>C. conger</i> (2)	0.61		
Eel (3)	0.43‡		
Submucosa			
<i>C. oceanicus</i> (1)	0.010		
<i>C. conger</i> (2)	0.009§		
Eel (3)	0.060‡		

(1) This study. (2) Denton *et al.* (1972). (3) Kutchai & Steen (1971). (4) Grote (1967). (5) Grote & Thews (1962). (6) Thews (1960). (7) Kawashiro *et al.* (1975). (8) Krogh (1919). (9) Van Liew & Chen (1975).

* Determinations at other temperatures were adjusted using a $+1\%/^{\circ}\text{C}$ as the temperature dependence of K (Krogh, 1919; Grote, 1967). Values from Kutchai & Steen (1971) and the present study were treated as if determined at 22.5°C , the midpoint of the actual range in both studies.

† Calculated as 2.0 times the reported value for the diffusion constant of nitrogen, which applies to water (Hüfner, 1897, from Denton *et al.* 1972), connective tissue (Krogh, 1919) and conger mucosa (Denton *et al.* 1972).

‡ Kutchai & Steen referred to the mucosa plus submucosa as the 'intact swimbladder' made up of a 'silvery outer layer' (= submucosa) and an 'inner layer' (= mucosa).

§ Calculated from above value for mucosa plus submucosa, assuming that the submucosa makes up 60% of the thickness, as shown by Denton *et al.* (1972).

segments which can be seen when looking through the submucosa (Fig. 7). We suggest that each of these line segments, which have been interpreted as crystals (Kutchai & Steen, 1971), represents a tangential view of a folded layer of crystals, as indicated by dashed lines in Fig. 6. In our view, the electron micrographs give no support for the existence of rod-shaped crystals or long, narrow plates.

DISCUSSION

Oxygen conductance

The property of greatest functional significance is the oxygen conductance of the intact swimbladder wall. This property determines the rate of oxygen loss by diffusion from the swimbladder for specified gas pressures inside the swimbladder and in the surrounding tissues.

The oxygen conductances reported in Table 1 are in good agreement with previous reports. The lowest conductance we observed was $0.9 \times 10^{-5} \text{ cm}^3 \text{ O}_2 \text{ cm}^{-2} \text{ atm}^{-1} \text{ min}^{-1}$ for *Conger oceanicus*, and for the six other species examined between 5 and 9×10^{-5} . Kutchai & Steen (1971) reported a value of 3.5×10^{-5} for the eel, and we estimated from Denton *et al.* (1972) 0.8×10^{-5} for *Conger conger*. The lower observed conductance for *Conger* is in accord with the fact that this species is found at depths

down to 300 m, while the other species that have been studied are from shallower coastal waters.

The diffusion constant, K , for a number of vertebrate tissues and for swimbladders are listed in Table 5. Compared to other vertebrate tissues, the overall diffusion constant of the intact swimbladder wall is low. With the exception of the silver perch, the diffusion constant of the intact swimbladder wall is about one-tenth or less that of the other vertebrate tissues. This means that oxygen will be lost by diffusion through the swimbladder wall more slowly than if the wall were composed of more typical vertebrate tissue.

The low diffusion constant of the intact swimbladder wall is due to only one of its constituent layers, the secretory submucosa. The other layers have diffusion constants of the same magnitude as ordinary vertebrate tissues. The tunica externa (Table 2) has diffusion constants between 1.1 and 1.8×10^{-5} , with the exception of the silver perch for which it is somewhat lower, 0.56×10^{-5} . The diffusion constant for the mucosa alone was determined by use only for the conger, for which the value was 1.6×10^{-5} . For the mucosa of *C. conger*, Denton *et al.* reported 0.63×10^{-5} . Kutchai and Steen also reported a rather low value for the mucosa of the European eel, 0.43×10^{-5} . There are several possible reasons for this difference. First, the figure given by Denton *et al.* is the product of the mean oxygen conductance from two specimens and the mean thickness of six specimens; for the single specimen for which both conductance and thickness were known, the diffusion constant was 1.0×10^{-5} . Secondly, it is possible that Denton *et al.* did not remove the submucosa as completely as we did, and any crystals adhering to the mucosa would reduce the value for the diffusion constant. The crystals are transparent and it is difficult to see whether removal is complete. Kutchai and Steen analysed some of their specimens for guanine and found a trace present; this may indicate incomplete removal of guanine. It is unlikely that the difference is due to the fact that our tissue was not consuming oxygen (due to cyanide treatment) whereas those of Denton *et al.* and Kutchai and Steen were. Denton *et al.* reported permeability of the mucosa to oxygen about twice that to nitrogen, which is the ratio to be expected in the absence of significant oxygen consumption, assuming that the ratio should be close to that in water. Significant oxygen consumption should result in lower oxygen permeabilities relative to nitrogen.

It has been suggested by Denton *et al.* (1972) that heavily pigmented layers could act as a diffusion barrier. We were able to determine the conductance of the black peritoneum on the ventral surface of the swimbladder of spot, and observed conductances so high that it would not significantly affect the overall conductance of the intact wall.

Diffusion and solubility coefficients

Diffusion coefficients, D , and apparent solubility coefficients, α_{app} , of oxygen in various tissues, including results of the present study, are given in Table 7.

The diffusion coefficient of oxygen in the swimbladder tunica externa is at the low end of the range for vertebrate tissues. The only previously studied tissue with equally low diffusion coefficient is the rabbit cornea (Takahashi, Fatt & Goldstick, 1966). The cornea contains a large amount of fibrous collagen, and its dry weight

Table 6. *Oxygen diffusion coefficients (D) and apparent solubility coefficients ($\alpha_{app} = K/D$) of various tissues at 20 °C**

	Diffusion coefficient, D (10^{-6} cm ² s ⁻¹)	Apparent solubility coefficient, α_{app} (cm ³ O ₂ cm ⁻³ atm ⁻¹)
Water (1)	2.3	0.031
Methaemoglobin soln. (33 %) (2)	0.95	0.032
Erythrocytes (2)	0.80	0.030
Heart muscle (rat) (2)	1.35	0.026
Cerebral cortex (rat) (3)	1.4	0.027
Lung tissue (rat) (2)	1.5	0.022
Cornea (rabbit) (4)	0.44	—
Swimbladder:		
Tunica externa (4 species) (5)	0.49–0.63	0.028–0.057
Submucosa (<i>C. ocaenicus</i>) (5)	0.006	0.026

(1) Grote (1967). (2) Grote & Thews (1962). (3) Thews (1960). (4) Takahashi *et al.* (1966). (5) This study.

* Determinations at other temperatures were adjusted using +2.5 %/°C for D and -1.5 %/°C for α_{app} (1).

is about 22 % of the wet weight. The tunica externa likewise contains large amounts of fibrous collagen, and in one sample the dry weight was about 30 % of the wet weight.

The apparent solubility coefficient, α_{app} , in the tunica externa ranged from a little less than to almost twice the value for water. Some of the other tissues in Table 6 also have values similar to that of water. This is somewhat remarkable since one might expect that a tissue with a high protein content would have a reduced solubility coefficient relative to pure water. Takahashi *et al.* (1966) assumed that the oxygen solubility in rabbit cornea was reduced in proportion to the volume fraction of protein.

The true value of α in tissue could indeed be greater than that in water. One reason is the presence of lipids, in which oxygen is some 5 times as soluble as in water. Another reason is that oxygen can apparently bind to proteins. Thus, the solubility coefficient of oxygen in solutions of deactivated hemoglobin is higher than in saline alone (Sendroy, Dillon & Van Slyke, 1934; Christoforides & Hedley-Whyte, 1969).

A second factor is that the α_{app} in tissues (calculated as K/D) may not equal the true solubility coefficient, α . Kawashiro *et al.* (1975) showed that an inequality between α and α_{app} can arise when a tissue is inhomogeneous with respect to diffusion and solubility coefficients. We have no information on the lipid content or other possible inhomogeneities within the tunica externa, and therefore cannot draw any conclusions in regard to the variability in the α_{app} .

The diffusion coefficient in the submucosa of conger is extremely low. The α_{app} in the same tissue is essentially the same as in other tissues. The implication is that the diffusion coefficient, D , is reduced by factors which do not markedly affect the apparent solubility coefficient, α_{app} . Can this striking observation be explained by available information?

The presence of a small volume fraction of thin, impermeable sheets of guanine crystals in the tissue would reduce the diffusion coefficient without greatly affecting

oxygen solubility. The guanine content of conger submucosa was determined by Denton *et al.* (1972) to be about 2.4% of the wet weight. They give values for the densities of guanine (1.73 g cm^{-3}) and tissue (1.07 g cm^{-3}), and the volume fraction of guanine in the submucosa therefore is about 0.015. This would reduce the solubility coefficient by 1.5%, which is insignificant relative to the approximately 99% reduction of the diffusion coefficient relative to other tissues. This interpretation is also in accord with the morphological findings which will be discussed next.

The finding that the main resistance to the diffusion of oxygen through the swimbladder wall resides in the submucosa refers specifically to the so-called 'secretory' part of the submucosa of the swimbladder. As shown in Fig. 1, the secretory submucosa may expand to cover most of the inner surface of the swimbladder, thus presumably reducing the permeability of the entire surface of the swimbladder. Conversely, the secretory submucosa may contract and cover only a small part of the inner surface of the swimbladder, thus facilitating the loss of gas through most of the surface. These movements of the secretory submucosa are of importance in connexion with the secretion and reabsorption of gas (Fänge, 1953), and the morphology of the submucosa and the arrangement of the guanine crystals are therefore important.

The electron micrographs gave clear evidence that the guanine crystals are located mainly in the plane of the swimbladder wall. The birefringence suggests that the sheets are crystalline in nature, thus explaining their low permeability. The layering of multiple sheets of such crystals is an ideal morphological arrangement for reduction of permeability to gases.

The striking picture of folded sheets (Fig. 6) is readily understood in view of the extensive movement of the secretory submucosa. As this layer contracts during the phase of gas reabsorption, folding of the layers is inevitable. As the secretory submucosa expands again, the layers of guanine crystals will unfold and again assume an orientation as flat sheets parallel to the plane of the swimbladder wall. The same structural characteristic of folded layers was reported for the swimbladder of the toadfish by Morris & Albright (1975). Their fig. 11 shows tightly packed layers of dense material, located in extensive foldings similar to those we observed. Although they did not identify the material as crystalline guanine, their description is in accord with this interpretation.

Loss of swimbladder gas and rate of replacement

With information about conductance of swimbladder wall, we can now evaluate the rate of loss by diffusion and relate this to known rates of gas secretion.

The rate (dn/dt) at which oxygen diffuses out through the swimbladder wall is a function of the conductance of the wall (G), the swimbladder surface area (A), and the partial pressures of oxygen in the swimbladder ($p(\text{O}_2)_{\text{sb}}$) and in the tissues at the outer surface of the swimbladder ($P(\text{O}_2)_t$). The relationship is given by the following equation, which is simply equation (5) applied to the swimbladder:

$$dn/dt = GA(p(\text{O}_2)_{\text{sb}} - P(\text{O}_2)_t). \quad (14)$$

The swimbladders of many fish contain a gas mixture consisting of mainly oxygen, some nitrogen (e.g. 90% oxygen, 10% nitrogen), and a small amount of CO_2

(Scholander & van Dam, 1953). However, some fish have a gas in their swimbladder which contains more nitrogen than oxygen. Each of these gases will have its own rate of diffusion through the swimbladder wall, but for the sake of simplicity we will assume that oxygen is the only gas in the swimbladder.

The surface area of the swimbladder of a fish of given body mass can be predicted as follows. The volume of the swimbladder of marine fish is about $0.05 \text{ cm}^3/\text{g}$ body mass (Alexander, 1966). If the swimbladder were spherical, its surface area would be $A = 0.66 \times m_b^{2/3}$ (A in cm^2 , body mass in g). Since the swimbladder is usually somewhat elongated, the coefficient will be greater than 0.66. In the following calculations we will use data appropriate for eels, and we will therefore use the coefficient derived from data given by Denton *et al.* for the conger, 0.82.

Assuming that the swimbladder contains only oxygen, $p(\text{O}_2)_{sb}$ will equal the hydrostatic pressure, P_w , at which the fish finds itself. The $p(\text{O}_2)$ at the outer surface of the swimbladder will be 0.2 atm or less when the fish is near the surface (Alexander, 1972), but could possibly be somewhat higher at greater depth due to the effect of hydrostatic pressure on oxygen solubility (Enns, Scholander & Bradstreet, 1965). This effect, however, is insignificant for our purposes because at a depth of 1000 m it would increase the $p(\text{O}_2)$ in the water by a factor of no more than 1.14 (Fenn, 1972).

Inserting the above values in equation (14) gives the expected rate of oxygen loss as

$$dn/dt = G \times 0.82 m_b^{2/3} (P_w - 0.2), \quad (15)$$

where dn/dt is in $\text{cm}^3 \text{ O}_{2,\text{STP}} \text{ min}^{-1}$, G is conductance in $\text{cm}^3 \text{ O}_{2,\text{STP}} \text{ cm}^{-2} \text{ atm}^{-1} \text{ min}^{-1}$, m_b is body mass in grams, and P_w is water pressure in atm.

Such a loss of oxygen must be evaluated relative to the ability of the fish to secrete gas into the swimbladder to replace that lost by diffusion. The rate of loss becomes a problem if it exceeds the maximum rate of secretion, or if the metabolic cost of secretion to replace the loss becomes excessive.

Maximum sustainable swimbladder pressure. There will be some value of $p(\text{O}_2)_{sb}$ at which a fish must secrete oxygen as fast as it can, simply to keep up with the rate of loss by diffusion. This pressure, which we will call the *maximum sustainable* $p(\text{O}_2)_{sb}$, depends on the oxygen conductance of the swimbladder wall and the maximum rate of secretion of which the fish is capable.

The eel, when held in an aquarium, can refill its swimbladder in 10–18 h following complete emptying (Wittenberg, Schwend & Wittenberg, 1964). A 1 kg eel would have a swimbladder volume of about 50 cm^3 , and the most rapid filling (10 h) would correspond to a secretion rate of $0.083 \text{ cm}^3 \text{ O}_{2,\text{STP}}$ per minute.

The effect of $p(\text{O}_2)$ in the swimbladder on oxygen secretion rate is unknown, but we will follow Alexander (1966), who implied a linear decrease in the rate of nitrogen secretion from its value at the surface to zero at the maximum nitrogen pressure which can be generated. Kuhn *et al.* (1963) predicted that the maximum oxygen $p(\text{O}_2)$ that the eel can theoretically produce is 2000 atm. The rate of oxygen secretion, R , would then be

$$R = R_1 \left(1 - \frac{p(\text{O}_2)_{sb}}{2000} \right), \quad (16)$$

where R_1 is the rate at $p(\text{O}_2)_{sb} = 1 \text{ atm}$.

The conductance of the eel swimbladder can be estimated from Kutchai & Steen (1971), whose values for the diffusion constant and a representative thickness yield a conductance of $3.5 \times 10^{-5} \text{ cm}^3 \text{ O}_2 \text{ cm}^{-2} \text{ atm}^{-1} \text{ min}^{-1}$.

These values lead to a predicted maximum sustainable $p(\text{O}_2)$ in the swimbladder of a 1 kg eel of 29 atm, corresponding to a depth of 280 m, assuming that oxygen is the only gas in the swimbladder. If we use the conductance value for the conger, $0.9 \text{ cm}^3 \text{ O}_2 \text{ cm}^{-2} \text{ atm}^{-1} \text{ min}^{-1}$, the maximum sustainable $p(\text{O}_2)$ in the swimbladder would be about 107 atm, or 1060 m depth (on the assumption that the secretion rate in the conger is the same as in the common eel).

These estimates of a limiting pressure are much smaller than the 2000 atm which an eel presumably can generate (Kuhn *et al.* 1963). Since the estimated maximum sustainable $p(\text{O}_2)$ in the swimbladder is such a small fraction of the pressure capability, the secretion rate will be only slightly reduced at the maximum sustainable $p(\text{O}_2)$ in the swimbladder. This may be an advantage in possessing a pressure capability greatly in excess of what would otherwise seem necessary. In any event, these rough estimates of maximum sustainable pressure seem reasonable since they exceed by a substantial margin the depth at which the eel and the conger normally live.

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