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DIFFUSION OF OXYGEN THROUGH THE MESOGLOEA OF THE SEA ANEMONE *CALLIACTIS PARASITICA*

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SUMMARY

The diffusion of oxygen through preparations of the mesogloea of *Calliactis parasitica* (Couch) has been measured with a custom-built diffusion cell and an oxygen microelectrode. The mean value for Fick's diffusion coefficient was $7{\cdot}29 \times 10^{-6}\,cm^2\,s^{-1}$ and that for Krogh's diffusion constant $10{\cdot}00 \times 10^{-6}\,cm^3$ oxygen per min per cm^2 area and cm thickness at a pressure difference of 1 atmosphere, at 25 °C. Comparison with the rather few values in the literature for oxygen diffusion through biological materials indicates that mesogloea is similar to connective tissue. The mesogloea of *Calliactis* seems to present a fairly significant barrier to the diffusion of oxygen between the two cell layers, and this is discussed in relation to the route whereby the endoderm obtains its oxygen.

INTRODUCTION

The oxygen requirements of the endoderm of coelenterates may be met either by direct uptake of oxygen from the water in the enteron or by uptake through the ectoderm and subsequent diffusion through the mesogloea. Direct uptake through the endoderm has been found to be substantial in several anthozoans (see Brafield, 1980), often aided by rhythmic movements which periodically expel deoxygenated water from the enteron and replace it with fresh. Such behaviour suggests that the ectoderm and endoderm may each be responsible for taking up most of the oxygen they need, which raises the question of whether the mesogloea which separates the two layers presents a significant barrier to diffusion of oxygen between them. In an attempt to answer this we have measured the rate at which oxygen diffuses through preparations of mesogloea from the sea anemone *Calliactis*. As it turns out that there is rather little quantitative information in the literature about the diffusion of oxygen through biological materials in general, the results are of wider interest than might have been expected.

METHODS

Pieces of body wall about 1·5 by 1·5 cm were cut from living *Calliactis* and the

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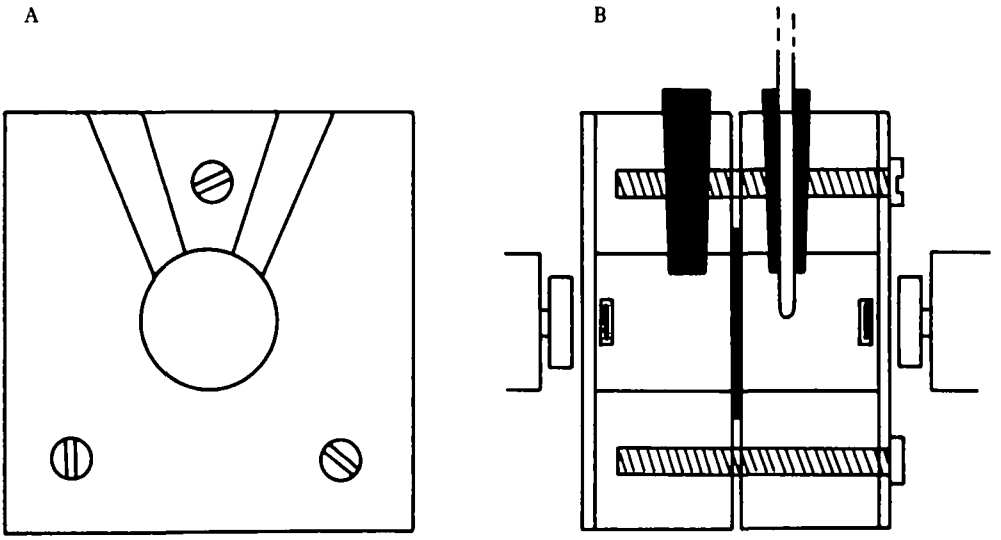

Fig. 1. The diffusion cell seen from the right side (A) and from in front (B). In (B) the preparation of mesogloea, the oxygen electrode, Teflon plugs, magnetic followers and magnet-carrying motors are indicated.

tissue of both sides scraped away with a scalpel to expose the mesogloea. The rate of diffusion of oxygen across such a preparation was measured by means of a specially made Perspex cell (Fig. 1) somewhat similar to that used by Chapman & Pardy (1972) for following the movements of glucose and glycine across the mesogloea of *Corymorpha*. A suitable piece of mesogloea was clamped between the two half-cells by three screws. The cavity in each half-cell was 1 cm in diameter and 1 cm deep, giving a volume of 0·785 cm^3 and a 0·785 cm^2 area of mesogloea in contact with the sea water in the half-cells. Two holes in the top of each half-cell allowed them to be filled with sea water. Fully aerated sea water was pipetted into the right half-cell and partially deoxygenated sea water into the left. The latter concentration was obtained by bubbling a mixture of 96 % nitrogen and 4 % oxygen through stock sea water. For each experiment, the oxygen concentration of air-saturated sea water (in mg dm^{-3}) at the appropriate temperature and salinity was taken from tables. The temperature throughout was 25 ± 1 °C and the salinity of the sea water between 33 and 34 ‰.

An oxygen electrode in a split plug of Teflon was placed in one of the holes of the right half-cell and a solid Teflon plug in the other. Two similar solid plugs were placed in the holes of the left half-cell. The miniature oxygen electrode (1·7 mm in diameter), produced by Searle Medical Products (Soutter, Conway & Parker, 1975; Brafield, 1980), was connected by way of the appropriate P_{O_2} meter to a strip-chart recorder. Thus the fall in oxygen concentration in the right half-cell, due to oxygen diffusing through the mesogloea to the lower concentration in the left one, was monitored continuously. By placing fully aerated water in both sides, the fall in concentration in the right half-cell due to consumption by the electrode (and possibly by the mesogloea) could be measured and subtracted from the fall found in an experiment with deoxygenated water in the left half-cell, to arrive at a true indication of the rate of oxygen diffusion through the mesogloea.

The water in each half-cell was stirred continuously by a glass-covered magnetic follower spinning in response to a small electric motor carrying a magnet (Fig. 1). Each motor was clamped in a horizontal position with its magnet as close as possible to the outer face of each half-cell. The volume of water in the right half-cell, after making allowance for space occupied by the magnetic follower and the oxygen electrode, was 0·766 cm^3. Before and after each experiment the piece of mesogloea was placed between two cover slips and its average thickness measured with high-quality calipers to the nearest 0·025 mm. This method provided an accuracy of about 3 %, as the thickness of most preparations was about 0·8 mm. Transverse sections of whole animals of about 25 mm diameter, fixed in an expanded condition, show the mesogloea to be about 1 mm thick, suggesting that our experimental pieces of mesogloea were in a fairly natural state and not altered too greatly by the process of preparation.

Two aspects of the method as described above deserve some amplification. First, while filling the left half-cell with partially deoxygenated water there was a danger that the oxygen concentration could rise through invasion of oxygen from the air. This was avoided by liberally over-filling the cavity before inserting the Teflon plugs. To check this, the oxygen electrode was placed in the left half-cell on occasion, and always indicated that the oxygen concentration there was, initially, a fifth of the air-saturation level, as intended. Secondly, as oxygen diffused from the right half-cell to the left one, the diffusion gradient became less steep, of course, and so the rate of diffusion also fell. This resulted in a curved trace on the strip-chart recorder. For calculation purposes, however, we only used the first part of a trace (usually the initial 20 min), where no curve was discernible, so avoiding having to take account of the change in diffusion rate with time (see Kawashiro, Scheid & Piiper, 1976). In addition, by using this method we could safely ignore back-diffusion from left to right.

RESULTS

Fick's diffusion coefficient (see, for example, Chapman & Pardy, 1972, p. 644) can be expressed for this purpose as the rate of transfer of oxygen (from right to left across the mesogloea, in mol s^{-1}) divided by the area of mesogloea (in cm^2) and by the concentration gradient. The latter is the difference in oxygen concentration between the right and left sides (in mol cm^{-3}) divided by the thickness of the intervening mesogloea (in cm). To take an example, the molar difference in one case (calculated from the oxygen concentrations of the right and left half-cells) was $0{\cdot}174 \times 10^{-6}$ mol cm^{-3}. The thickness of the mesogloea was 0·085 cm, giving a concentration gradient of $2{\cdot}047 \times 10^{-6}$ mol cm^{-4}. The oxygen transfer rate (calculated from the fall in oxygen concentration in the right half-cell) was $12{\cdot}36 \times 10^{-12}$ mol s^{-1}. The area of mesogloea exposed to the sea water was 0·785 cm^2, giving a value for Fick's coefficient of $(12{\cdot}36 \times 10^{-12})/(2{\cdot}047 \times 10^{-6} \times 0{\cdot}785)$ or $7{\cdot}69 \times 10^{-6}$ cm^2 s^{-1}. Similarly calculated values for twelve experiments with five preparations of mesogloea (ranging in thickness from 0·07 to 0·0925 cm) are shown in Table 1.

Alternatively, the experimental data can be used to calculate Krogh's diffusion constant (Krogh, 1941, p. 19), which in this case is the amount of oxygen (in cm^3) diffusing per min through mesogloea with an area of 1 cm^2 and a thickness of 1 cm, at a pressure difference of 1 atmosphere. In the experimental example used above to

Table 1. *Fick's diffusion coefficient, F ($cm^2 s^{-1}$) and Krogh's diffusion constant K ($cm^3 O_2 min^{-1}$ per cm^2 area and cm length at a pressure difference of 1 atmosphere), at 25 °C, calculated from 12 experiments with five preparations of* Calliactis *mesogloea*

F × 10^6	K × 10^6
7·43	9·84
5·52	7·31
7·39	9·97
6·92	9·32
7·69	10·59
9·18	12·64
6·20	8·54
5·94	8·29
5·94	8·29
9·51	13·26
6·67	9·29
9·06	12·64
Mean 7·29	Mean 10·00

illustrate calculation of Fick's coefficient, the rate of oxygen transfer ($12{\cdot}36 \times 10^{-12}$ mol s^{-1}) is the equivalent of $16{\cdot}62 \times 10^{-6}$ cm^3 min^{-1}. The oxygen concentration difference between the right and left half-cells was equivalent to 0·17 of an atmosphere, and the mesogloeal area and thickness 0·785 cm^2 and 0·085 cm respectively. So in this case Krogh's constant is $(16{\cdot}62 \times 10^{-6} \times 0{\cdot}085)/(0{\cdot}785 \times 0{\cdot}17)$ or $10{\cdot}59 \times 10^{-6}$. Values for Krogh's constant calculated from the results of twelve experiments are shown in Table 1, alongside the corresponding values for Fick's coefficient.

DISCUSSION

We have found no information in the literature on the rate of diffusion of oxygen through mesogloea but it is interesting to compare our average value for Fick's coefficient of $7{\cdot}3 \times 10^{-6}$ $cm^2 s^{-1}$ (Table 1) with the mean values of $0{\cdot}47 \times 10^{-6}$ and $0{\cdot}57 \times 10^{-6}$ for diffusion of glucose and glycine respectively through the mesogloea of *Corymorpha palma* (Chapman & Pardy, 1972). It is not surprising that our value for oxygen diffusion through mesogloea is much higher than those for the diffusion of glucose and glycine as the oxygen molecule is considerably smaller (and consequently more mobile) than those of the monosaccharide and the amino acid. Our mean of $7{\cdot}3 \times 10^{-6}$ $cm^2 s^{-1}$ seems reasonable by comparison with the figure of 0·00104 cm^2 min^{-1}, equivalent to $17{\cdot}3 \times 10^{-6}$ $cm^2 s^{-1}$, quoted by Altman & Dittmer (1974, p. 1580) for diffusion of oxygen through water (fresh water, not sea water, at 15 °C), and that of $16{\cdot}2 \times 10^{-6}$ $cm^2 s^{-1}$ for human plasma (at 25 °C) found by Goldstick, Ciuryla & Zuckerman (1976).

Rhythmic contractions of coelenterates, and the neural mechanisms that control them, continue to attract attention (e.g. Lawn, 1980; Robbins & Shick, 1980; Leonard, 1982). In anthozoans, rhythmic movements seem frequently to be concerned with irrigation of the enteron; the enteric P_{O_2} is often low, and endodermal oxygen

uptake can represent a significant proportion of the total oxygen consumption (Needler & Ross, 1958; Brafield & Chapman, 1965, 1967; Brafield, 1969; Chapman, 1972; Sassaman & Mangum, 1972, 1974; Jones, Pickthall & Nesbitt, 1977; Brafield, 1980). It therefore seems likely that the oxygen demands of the endoderm are largely met by direct uptake from the enteric water, rather than by ectodermal uptake followed by diffusion through the mesogloea. The relatively low diffusion coefficients for mesogloea that we have found (Tables 1, 2) support this view. On the other hand, Shick, Brown, Dolliver & Kayar (1979) found that the body wall of *Anthopleura elegantissima* was more permeable to oxygen than that of *Metridium senile* and suggested that this is because in *Anthopleura* proportionally more of the body wall thickness is occupied by mesogloea. Their reasoning rests on the assumption that the mesogloea is highly permeable to oxygen as a result of its high water content (91·2 % in *Metridium* mesogloea as against about 84 % for the animal as a whole). In *Calliactis*, however, we have found the water content of the mesogloea to be 70·4 % whereas that of the animal as a whole is 83·5 %, which tends to support our contention that, in *Calliactis* at least, the mesogloea probably presents a significant barrier to oxygen diffusion. Caution is needed when comparing mesogloeal water contents, however. Our value of about 70 % for *Calliactis* may be an underestimate if some water was expressed and not replaced while making the preparation; and the value of about 91 % for *Metridium* mesogloea quoted by Shick *et al.* (1979) refers to a preparation of body wall from which endodermal muscle had been removed but not, apparently, ectoderm (Gosline, 1971, p. 764). In short, if the mesogloea of anthozoan coelenterates does vary in water content it can be expected to have equally variable oxygen diffusion characteristics.

There is little information in the literature on oxygen diffusion rates through biological materials in general, and relatively simple tissues (like mesogloea) in particular. Even today, the values most often quoted are those obtained by Krogh (1919) in his classical experiments and re-presented (1941) in his book (where, incidentally, the value for chitin is misprinted). These and other results are shown in Table 2, where a correction of 1 % per °C has been applied in cases where the original data were

Table 2. *Krogh's diffusion constant (K) for oxygen through various materials, all at 20 °C*

Material	K ($\times 10^6$)	Source
Water	42·5	Grote, 1967
Serum	28·5	Dittmer & Grebe, 1958
Gelatine	28	Krogh, 1919
Cerebral cortex (rat)	23	Thews, 1960
Cardiac muscle (rat)	21	Grote & Thews, 1962
Lung tissue (rat)	20	Grote, 1967
Urinary bladder (cat)	19	Van Liew & Chen, 1975
Skeletal muscle (frog)	14	Krogh, 1919
Connective tissue (frog)	11·5	Krogh, 1919
Mesogloea of *Calliactis*	9·51	Present work
Swimbladder wall (seven teleosts)	0·34–4·2	Lapennas & Schmidt-Nielsen, 1977
Chitin	1·3	Krogh, 1919

Values in cm^3 oxygen diffusing per min through 1 cm^2 area and 1 cm length at a pressure difference of 1 atmosphere.

for temperatures other than 20 °C (see Krogh, 1919, p. 408; Dittmer & Grebe, 1958, p. 10; Lapennas & Schmidt-Nielsen, 1977, p. 190). We have failed to find other comparable values – movement of oxygen from air to blood in the lungs is clearly a special case (see Forster, 1964); and the values for Krogh's constant calculated by Longmuir & Bourke (1960) for three organs of the rat are very high indeed (63×10^{-6} for kidney tissue, 164×10^{-6} for liver and 154×10^{-6} for heart). Longmuir & Bourke pointed out that this was because their material was actively respiring, so that simple diffusion of oxygen was enhanced by mitochondrial oxygen consumption and supplemented by such active processes as protoplasmic streaming. Their experiments were conducted in optimal conditions for respiratory activity; in a system less conducive to high metabolic rates, MacDougall & McCabe (1967) obtained the much lower value for oxygen diffusion through rat liver of 52×10^{-6} (also at 37 °C).

Some of the higher values in Table 2 therefore tend to be for complex cellular systems with a high metabolic rate. The mesogloea of *Calliactis*, on the other hand, contains very few and very small cells, by far the greatest part of the tissue being fibrous (Young, 1974), and so its metabolic rate must be very low. Thus the small size of the diffusion constant for mesogloea is due to the tissue's low respiratory rate and to the dense packing of fibres that oxygen must negotiate while moving through the tissue. It is therefore not surprising that the diffusion constant we have found for *Calliactis* mesogloea is one of the lowest in Table 2, nor that it is so similar to the value for connective tissue. As Krogh took his connective tissue from the abdominal wall of the frog it was presumably areolar tissue, containing both white (collagen) and yellow (elastin) fibres. Our preparations of mesogloea are likely to resemble this fairly closely, as mesogloea frequently contains significant amounts of collagen and may also contain some elastin (Chapman, 1966, 1974). If the mesogloea and connective tissue have a similar composition it is reasonable that they should show similar diffusion constants.

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