RESPIRATORY MOVEMENTS IN *HOLOTHURIA FORSKALI* DELLE CHIAJE

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INTRODUCTION

Although the respiratory rate of holothurians has attracted a considerable amount of interest, the results of many workers appear to be contradictory. Nomoura (1926), for example, found that the oxygen consumption of *Paracaudina* is dependent upon the oxygen content of the surrounding water, while Hiestand (1940) found no such relation in *Thyone*. Again, although a considerable amount of data has accumulated on the nature and control of cloacal pumping in holothurians, relatively little work has been done on the relation between the pumping movements of intact animals and their rate of oxygen consumption. Perhaps one reason for this has been the difficulty of making continuous recordings, not only of the oxygen concentration of the pumped water, but also of changes in the oxygen concentration of the coelomic fluid.

These difficulties have been largely overcome by the use of miniature oxygen electrodes which can be inserted into the coelom, whereby continuous recordings of oxygen concentration can be compared with changes in the external oxygen concentration. It is possible at the same time to record volume changes due to water being pumped in and out of the respiratory trees and in this way to correlate pumping movements with oxygen consumption under a wide variety of conditions.

MATERIAL AND METHODS

A. *Apparatus for recording volume changes*

Pantin & Sawaya (1953) recorded a pumping rhythm in *Holothuria grisea* by measuring the volume changes of the body when water was pumped in and out of the respiratory trees. Such volume changes caused movements of a float and were then recorded by means of a lever system on a slowly revolving smoked drum. Essentially the same principle was used in our experiments to record pumping movements in *H. forskali*. The apparatus consisted of two Perspex tubes of 3 in. diameter joined together end to end but with their cavities separated by a rubber membrane into two compartments (Fig. 1). Attached to each compartment was a manometer so that water pumped from one compartment into the other caused a fall in the fluid of one manometer and a corresponding rise in the fluid of the other. The posterior end of the holothurian was inserted through a hole in the centre of the membrane which was supported by a Perspex disk to prevent undue distortion. Movements of the fluid in the manometers due to pumping by the animal were recorded by means of a float which was attached to a lever system whose movements marked a smoked drum.

The diffusion of oxygen into the chambers was prevented by filling the manometers with liquid paraffin. The oxygen concentration in each chamber was measured by means of electrodes inserted through rubber bungs (Fig. 1). The whole apparatus was contained in a water bath to minimize temperature fluctuations.
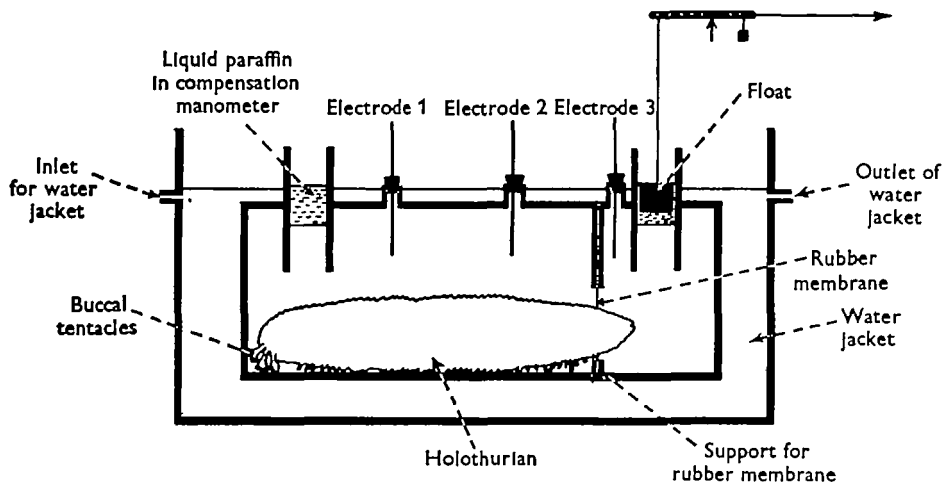

Fig. 1. Diagram showing the apparatus used to record pumping activity in *H. forskali*.

B. *Measurement of dissolved oxygen*

The recording electrodes were similar to those described by Naylor & Evans (1963). They consisted of a silver tube 0·5 mm. external and 0·3 mm. internal diameter which formed the anode and an insulated platinum wire of 0·2 mm. diameter forming the cathode. The platinum wire was inserted into the tube and sealed in with Araldite. The end of the anode and cathode was then ground first with carborundum and then with fine alumina powder and finally coated with a thin film of polystyrene which prevented the electrode being affected by stirring. Such electrodes are not only useful for making continuous measurements of the dissolved oxygen in sea water outside the animal but can also be inserted through a serum needle into the coelom of the holothurian.

A potential difference of 0·7 V. was applied across the electrode and the resultant current was amplified, the reading being indicated on a galvanometer. A description of the amplifier and electrode characteristics will form the basis of a further communication.

C. *Equilibration of sea water with different gas mixtures*

In order to study the respiratory behaviour of *H. forskali* at different oxygen concentrations, and to check the calibration of individual electrodes, it was necessary to equilibrate sea water with a variety of oxygen and nitrogen mixtures. These were produced by passing the component gases through rotameters so that the flow rates of oxygen and of nitrogen into a 20 l. mixing vessel were known. The mixture was then allowed to flow over water which was stirred by means of a vortex-generator as described by Bryer (1962). The amount of oxygen present could then be checked by means of the micro-Winkler method as described by Fox & Wingfield (1938).

A. *The role of the respiratory trees in gas exchange*

The rate of oxygen consumption of animals kept in sealed vessels of approximately 600 ml. capacity for periods of up to 1 hr. was found to vary between individuals, and with the oxygen concentration of the surrounding water, but was normally between 0·0162 and 0·0465 ml. O_2/g. dry wt./hr. and at 17° C. This result is difficult to compare with that of Hiestand (1940) who found that *Thyone* used 0·013–0·014 ml./ O_2/g. wet wt./hr., and with that of Tao (1930) who found that *Paracaudina* used 0·01– 0·037 ml. O_2/g. wet wt./hr. since our results are expressed in terms of the dried weight of the tissues. Clearly, without data on the wet wt./dry wt. ratio, any closer comparisons are impossible at this stage, particularly as Hiestand (1940) did not remove the perivisceral fluid prior to weighing. This is an important omission since, in *H. forskali*, the perivisceral fluid may vary in any one animal from 5 to 50 ml. within an hour. However, assuming a wet wt./dry wt. ratio of approximately 10:1 (as in many groups) our results for *H. forskali* are approximately one-tenth of that found for *Thyone* and *Paracaudina*. This might be accounted for by the weight of fluid contained in the coelom.

It is well known that the respiratory trees have, at least in part, a respiratory function. Winterstein (1909) found that the oxygen consumption in holothurians is reduced by 50–60% when the anus is covered by a rubber membrane and so prevented from taking in water. Bertiolini (1933) found that reduced methylene blue, when injected into the coelom, coloured the coelomic surface of the rete and of the respiratory trees deep blue. This indicated that oxygen had passed through the walls of the respiratory trees into the perivisceral fluid. It was therefore of interest to investigate the role of the respiratory trees in the respiration of *H. forskali*.

The apparatus described made possible the separation of water pumped by the cloaca from that in contact with the rest of the body by means of the rubber membrane. The oxygen depletion of water pumped in and out of the respiratory trees could thus be compared with the oxygen depletion in the water surrounding the general body surface of the animal. The results of such an experiment are expressed in Fig. 2 from which it is seen that the oxygen in the anterior chamber is steadily depleted while that of the posterior chamber is depleted at a decreasing rate until the oxygen concentration is approximately 60–70% of its normal air-saturation value. After this the rate of depletion is very slow indeed and results in a decline in the total respiratory rate of the animal (Fig. 3). This result agrees with that of Nomoura (1926) who found that the overall respiratory rate of *Paracaudina* also declined as the oxygen concentration of the surrounding water became lower. The reasons for this are discussed on p. 56.

It was calculated that cloacal gas exchange initially accounts for approximately 60% of the total oxygen uptake by the animal, a figure which agrees well with that given by Winterstein (1909).
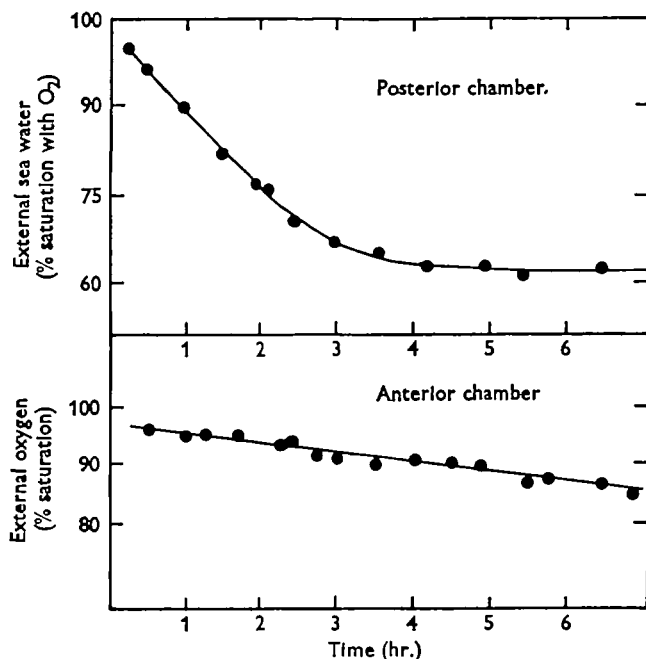
Fig. 2. The oxygen uptake through the cloaca (above) and the general body surface (below),
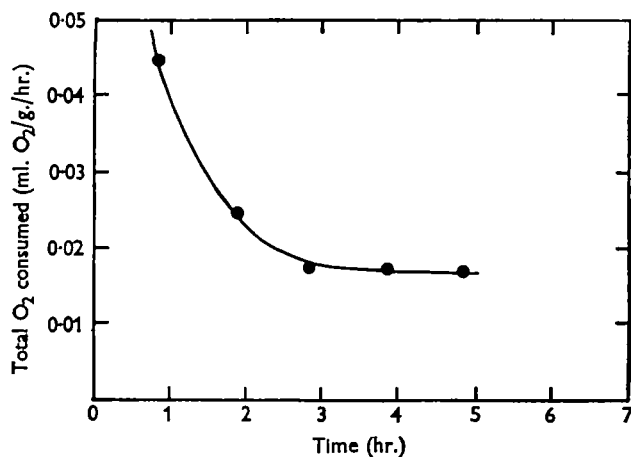plotted against the time in hours. The temperature was 17° C.

Fig. 3. The total oxygen consumed by an animal, plotted against the time in hours. The
temperature was 17° C.

B. *Pumping rhythms in intact* Holothuria forskali

Pantin & Sawaya (1953) have described a regular pumping rhythm in *H. grisea*.
A series of approximately ten cloacal contractions, each driving approximately 1 ml.
of sea water into the respiratory trees, is followed by a contraction of the body wall
which ejects the accumulated water. Lutz (1930), working on isolated strips of
circular muscle from the cloacal wall of *Stichopus*, has shown that the amount of
oxygen available to the preparation modified the amplitude of the contractions. A fall

in available oxygen initially increased the amplitude of the contractions and induced a periodicity in the pumping rhythm with periods of inhibition of 2–3 min. But further depletion of the oxygen in the surrounding fluid, or treatment of the preparation with cyanide, caused contraction to stop. There appears to be little information, however, on the effect of oxygen concentration on the nature and rate of cloacal pumping in intact holothurians.
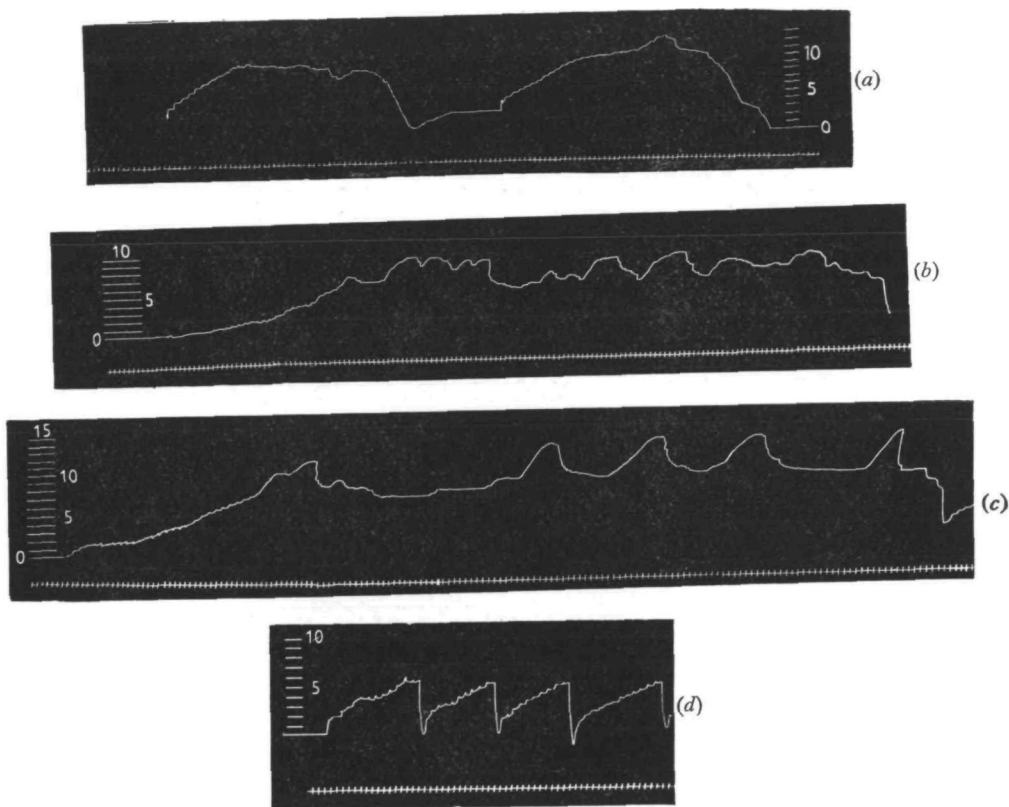

Fig. 4. Kymograph recordings showing the three types of pumping exhibited by *H. forskali*. (*a*) Pumping cycle characteristic of an animal in aerated sea water. (*b*) An intermediate stage between the normal (trace *a*) pumping and that characteristic of oxygen depletion in the cloacal region (trace *c*) (*c*) Rhythm shown by an animal whose anterior end and general body surface was aerated but the water entering the respiratory trees was approaching 70 % saturation and pumping was maximal. (*d*) Rhythm shown by an animal whose anterior end and general body surface was deoxygenated whilst the water pumped by the cloaca was oxygenated. In all instances the temperature was 17° C. The volume of water exchanged is shown in ml. on the scale.

(*a*) Pumping rhythm exhibited in oxygenated sea water

In order to study the effect of oxygen concentration on respiratory pumping in *H. forskali* an animal was placed in the apparatus described and sea water 95 % saturated with air was available to both the general body surface and the respiratory trees. Under these conditions the animal behaves in a different way from that described by Pantin & Sawaya (1953) for *H. grisea*. Instead of a series of cloacal contractions driving water into the trees and being followed by a sudden expulsion

of fluid, approximately 20 ml. is taken up by cloacal pumping and then gradually expelled (Fig. 4a). That is, the water is expelled less violently from the trees and in general the rhythm is much less regular than that described for *H. grisea*.

(b) Pumping rhythms under conditions of reduced oxygen concentration

Soon the oxygen concentration in the water available to the trees becomes lower than that available to the general body surface due to a higher utilization rate through the trees than through the general body surface. Under these conditions of slightly reduced oxygen concentration, a different type of water exchange takes place. Instead of a series of small pumping movements followed by a gradual expulsion, water is taken in by cloacal pumping (the tip of the lever moved upwards) and then follows a series of rapid influxes each followed by expulsion (Fig. 4b, c). Each of these movements is independent of cloacal pumping and may be caused by a set of muscles different from those concerned with the normal pumping rhythm. The net result of such pumping is that the total volume of water pumped in and out of the trees per minute is greatly increased. A further point of difference from the normal rhythm is that water taken in by cloacal pumping is not expelled so that the last series of rapid pumping movements occurs when the volume of the animal is greater by as much as 50 ml. The significance of this will be discussed on p. 54.

It might be expected that the volume of water pumped per unit time would increase steadily as the oxygen concentration of the pumped water decreases. However, when the oxygen concentration of the water in the posterior chamber reaches approximately 61 % of its air-saturation value, the rate of pumping rapidly declines and soon pumping ceases irrespective of the oxygen concentration of the sea water bathing the anterior end and general body surface. At an oxygen concentration corresponding to 60–70% air-saturation at 17° C., as has been noted earlier (p. 47), the uptake of oxygen via the respiratory trees is also minimal. Thus the rate of cloacal pumping in *H. forskali*, as estimated by the movement of the calibrated lever, varies with the concentration of oxygen in the water being pumped (Fig. 5) and ceases when the oxygen concentration is below 60% of its air saturation value. These results agree with, and to some extent amplify, those of Lutz (1930) on the isolated cloacal wall of *Stichopus* (p. 48).

Under the conditions of the experiment the anterior end of the animal was in relatively highly oxygenated water because the rate of oxygen uptake through the general body surface was slow. If, however, the oxygen concentration of the anterior chamber is low while the water pumped into the trees is highly oxygenated, a third entirely different pumping rhythm occurs. Instead of a rather irregular series of cloacal contractions, a regular pumping rhythm, identical with that described by Pantin & Sawaya (1953), is exhibited (Fig. 4d). As before, the rate of cloacal pumping increased as the oxygen concentration of the pumped water became less and also, as in the second rhythm, was inhibited when the oxygen concentration of the pumped water fell below 60% of its air-saturation value.

Thus the type of pumping rhythm exhibited by *H. forskali* is dependent upon the oxygen concentration of the water surrounding the anterior end and general body surface of the animal. When the oxygen concentration around the anterior end is high, a rather variable rhythm occurs and water is expelled only gradually; when

the anterior end is in water which is poor in oxygen, a regular pumping rhythm consisting of 10–15 cloacal pumps followed by a sudden expulsion of water occurs. The fact that Pantin & Sawaya (1953) have recorded only the latter type of rhythm in *H. grisea* is probably because their apparatus depended upon enclosing the general body surface of the animal in a fixed volume of water. In this case, after a time, the concentration of oxygen around the body wall would fall to a sufficiently low value to initiate a rhythm similar to that illustrated for *H. forskali* in Fig. 4*d*; at this oxygen concentration the less regular cycle characteristic of high oxygen concentrations would be inhibited.
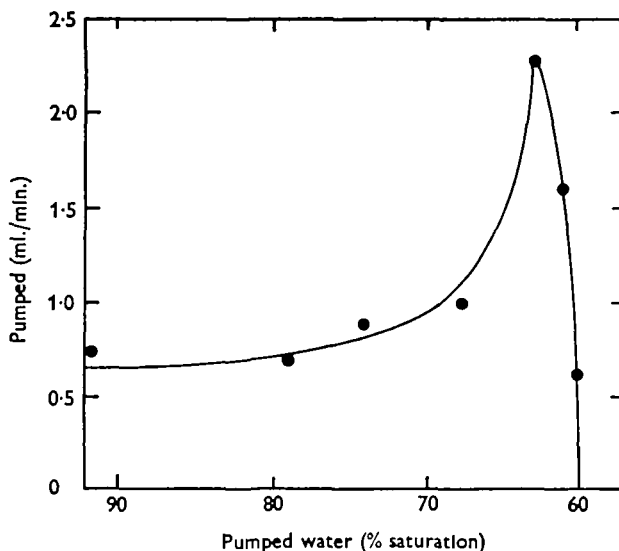

Fig. 5. The pumping rate of an animal, plotted against the percentage air-saturation of the pumped water. The temperature was 17° C.

(c) Inhibition of pumping

It is significant that both types of pumping were inhibited when the oxygen concentration in the pumped water fell below 60% of its air saturation value. That is, although the type of pumping appears to depend upon the oxygen concentration around the anterior end and general body surface of the holothurian, cessation of pumping seems to be controlled by the oxygen concentration of water entering the cloaca. The reason for this inhibition of pumping becomes apparent only when the efficiency of the pumping mechanism as a means of abstracting oxygen from sea water is compared over a series of gradually decreasing oxygen concentrations. Fig. 6 shows the relation between the rate of cloacal pumping and the absorption efficiency

$$\frac{\text{ml. } O_2 \text{ absorbed}}{\text{ml. } O_2 \text{ available}} \text{ per ml. water pumped.}$$

For example, from Fig. 6 it is seen that when the ratio of oxygen absorbed/oxygen available per ml. water pumped is 0·5, the pumping rate is 0·8 ml./min. Therefore, if the ratio of oxygen absorbed/oxygen available drops to 0·25, the pumping rate must double, i.e. must be 1·6 ml./min. The broken line in Fig. 6 indicates this theoretical

value. It is seen that despite increased water flux, cloacal pumping becomes progressively less effective as an oxygen absorbing mechanism as the oxygen concentration of the pumped water diminishes.

This, in itself, would set a limit to the advantages of increased cloacal pumping under conditions of reduced oxygen concentration. The final stimulus for the cessation of pumping, however, probably resides in the effect of low oxygen concentrations on the cloacal muscles responsible for pumping (p. 55).
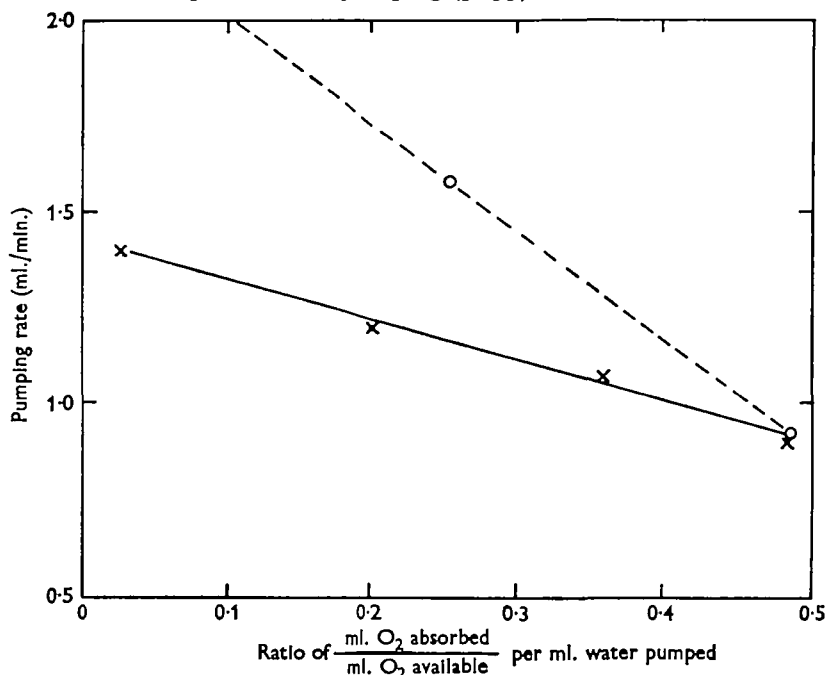

Fig. 6. The pumping rate of an animal, plotted against the ratio of vol. O_2 absorbed/vol. O_2 available per unit volume of pumped water. The temperature was 17° C.

C. *Evidence for an oxygen store*

It has been shown (p. 50) that when the oxygen concentration of the water bathing the cloacal region falls to approximately 60% of its air-saturation value pumping ceases. Under such circumstances the rate of oxygen consumption by the tissues may be expected either to decrease or to remain constant. If the rate of oxygen consumption remains constant, then three types of response might be expected to occur. Either (a) the respiratory exchange through the general body surface may increase, or (b) the tissues may develop an oxygen debt, or (c) the tissues may utilize an oxygen store.

Oxygen uptake through the general body surface remains approximately constant irrespective of the concentration of oxygen in the water being pumped into the respiratory trees (p. 3, Fig. 2). Any slight increase in oxygen intake through the general body surface following cessation of pumping by the cloaca is never sufficient to bring the total oxygen absorption by the animal up to its normal level. Thus the animal must either cease to use oxygen or use some internal oxygen store or develop an oxygen debt.

That the animal does, in fact, continue to utilize oxygen after pumping has ceased is indicated by the following experiment. An animal was placed in the apparatus (Fig. 1) and oxygen measurements were made at 10 min. intervals. Oxygen was absorbed steadily through the general body surface at a rate of 0·016 ml./g./hr. whilst uptake through the cloaca was 0·035 ml./g./hr. initially but soon fell to approximately 0·0121 ml./g./hr. and was reduced to zero when the oxygen concentration of the pumped water aproached 65 % of air saturation (Fig. 7).
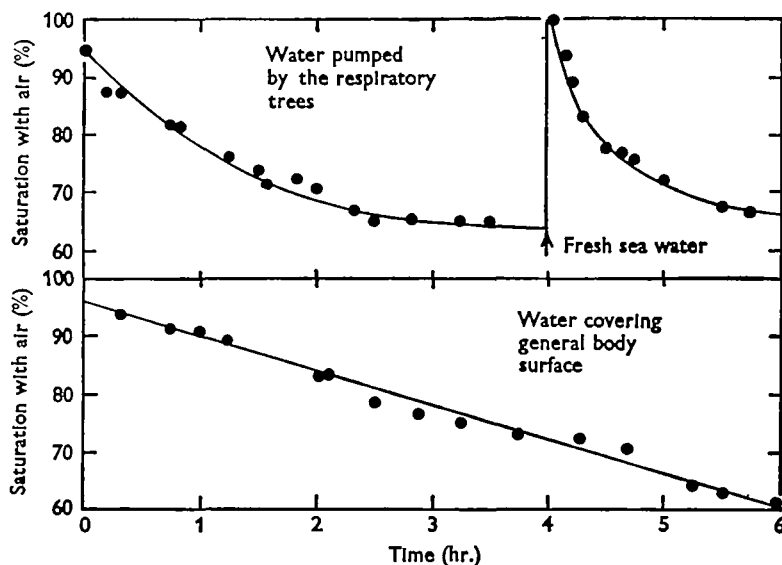

Fig. 7. The effect of prolonged oxygen deprivation on the oxygen absorption rate when fresh sea water was supplied to the cloacal region. The temperature was 17° C.

After 1 hr. during which respiration continued through the general body surface at a rate of 0·016 ml./g./hr., but no oxygen absorption took place through the cloaca, the oxygen concentration of the water available to the cloaca was returned to 100% of air saturation. Readings were taken at 5 min. intervals and it will be seen (Fig. 7) that oxygen was apparently absorbed very rapidly via the cloaca at a rate of 0·057 ml./ g./hr. After approximately 0·5 hr. the absorption rate was 0·0134 ml./g./hr., that is, approximately the normal rate of oxygen uptake as noted earlier in the experiment.

Thus a period of oxygen depletion and cessation of pumping was followed by a period of enhanced oxygen uptake when the oxygen was returned to its initial high concentration. This suggests that after cloacal pumping had ceased, the respiratory rate of the animal was not reduced appreciably. Instead, either an oxygen store was used, or an oxygen debt was developed by the tissues being later repaid when the oxygen concentration of the pumped water was restored to normal.

Maximum pumping occurred, as would be expected from Fig. 5, at a time when the oxygen concentration of the water had fallen to approximately 65% of its air saturation value. Replacement of highly oxygenated water around the cloaca was followed by a high pumping rate which would be expected to facilitate the replacement of deoxygenated water in the coelom by oxygenated water. This mixing of deoxy-

genated water from the coelom with the freshly oxygenated water outside accounts for the rapid fall in oxygen concentration in the cloacal chamber as shown in Fig. 7.

Direct evidence of the presence of stored oxygen is best obtained by a study of changes in the oxygen concentration of the coelomic fluid both before and after the cessation of pumping. Such measurements can be made relatively easily by means of the oxygen micro-electrode described on p. 46. An intact holothurian was placed in the apparatus with the cloacal region protruding through the rubber membrane and the oxygen concentration of the pumped water was measured. At the same time the general body surface was kept in oxygenated sea water and an electrode was inserted through the serum needle into the coelom.
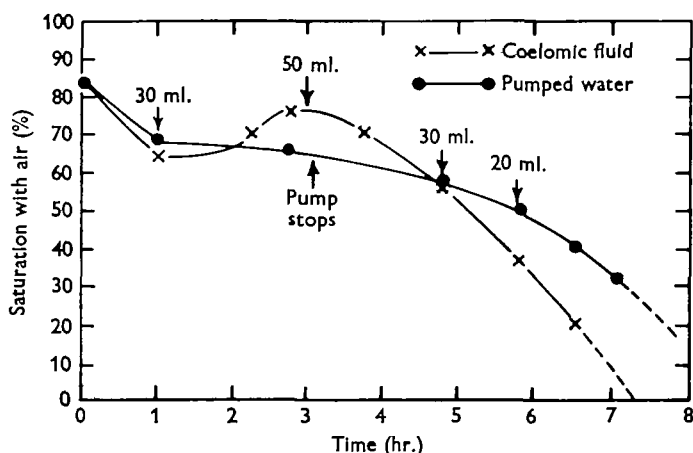

Fig. 8. The changes in the oxygen concentration in the water surrounding an animal and also in the coelomic fluid. Volume changes in the coelomic fluid are also indicated. The temperature was 17° C.

The results of this experiment are shown in Fig. 8. It is seen that the oxygen concentration of the coelomic fluid is initially similar to that of the water being pumped in and out of the cloaca. But when the oxygen concentration of the pumped water falls to approximately 62 % of its air-saturation value, the concentration of dissolved oxygen in the coelom rises and continues to rise until it reaches as much as 76 % of its saturation value. This event is associated with an increase in the volume of the animal of up to 100 ml. and was shortly followed by cessation of pumping. The oxygen concentration of the coelomic fluid then falls steadily.

As is shown in Fig. 8, oxygen is made available to the coelomic fluid in excess of respiratory uptake. The explanation of this increase in the oxygen concentration of the coelomic fluid resides in the ability of the animal to absorb water. Throughout the later phases of the pumping behaviour described on p. 50, the total volume of the coelomic fluid increases considerably (Fig. 4 b, c). The immediate effect of this increase in volume is to cause a rise in the oxygen concentration of the coelomic fluid. This becomes especially evident since the water is taken into the trees at, for example, 80 % saturation and is not liberated into the coelom immediately. Thus when the oxygenated water absorbed is finally liberated into the coelom the oxygen concentration is higher than that of the coelomic fluid whose oxygen concentration is thus observed to rise as the imbibed water mixes with it.

D. *The function of the oxygen store*

We have shown that the nature of the pumping rhythm in *H. forskali* is dependent upon the oxygen concentration of the water bathing the general body surface of the animal, and that pumping stops when the oxygen concentration of the water falls to approximately 60–70% of air saturation. Following this, the oxygen concentration of the coelomic fluid rises and this is brought about by the release of oxygenated water into the coelomic fluid from the respiratory trees. Three main problems arise in this context; first, to determine the factor which causes cloacal pumping to stop at an oxygen concentration of 60–70% air saturation. Secondly, whether the amount of oxygen held by the coelomic fluid is sufficient to last the animal until pumping is resumed. Finally the extent to which deoxygenation occurs under natural conditions.

Lutz (1930) has shown that oxygen lack affects the activity of the pumping muscles of *Stichopus*. Certainly pumping in *H. forskali* ceases at a quite definite oxygen concentration (p. 50) and if an animal is placed in nitrogenated sea water, the body loses all turgidity as if the longitudinal body-wall muscles had lost the ability to contract. If this is so, the stimulus for the cessation of pumping is probably simply the effect of low oxygen on the pumping muscles themselves. Such a mechanism would have the advantage that the coelomic oxygen concentration would remain high enough for the body-wall muscles to maintain their activity for some time and for the animal to move to a higher oxygen concentration. It is interesting to note that when depleted of oxygen, *H. forskali* seems to be able to move actively towards a source of oxygen and animals will cluster around an aerator when the cloacal muscles have been inhibited by oxygen lack.

The second problem concerns the total oxygen which can be stored in the coelomic fluid and whether this forms a significant oxygen store. Often approximately 25 ml. of 75% saturated water are taken immediately prior to the cessation of pumping but other animals might absorb up to 75 ml. Taking 50 ml. as a mean volume, this would supply 0·2 ml. O_2. Thus a normal animal would have 0·2 ml. O_2 at its disposal from the time pumping stopped.

The rate of oxygen utilization in the coelom of the animal illustrated in Fig. 8 was as low as 0·023 ml. O_2/hr. Taking into account the uptake through the general body surface this gives a respiratory rate of approximately 0·019 ml./g./hr. Thus assuming that increased absorption of oxygen took place through the body wall, the animal might be expected to survive without additional oxygen supply for approximately 9 hr. This is in approximate agreement with the observed time taken for the oxygen concentration of the coelomic fluid to be reduced to zero in this animal (Fig. 8). Obviously, as Kawamoto (1928) has also pointed out, the volume of the water absorbed is an important factor and may considerably increase the length of time the animal can withstand low oxygen concentrations. An additional factor which tends to prolong the period for which the animal can survive is the rate of absorption through the body surface. As much as 40% of the total oxygen requirement is absorbed more-or-less continuously through the general body surface irrespective of the oxygen concentration of the surrounding fluid (p. 47), so that an animal might be expected to be able to last more than 9 hr. without pumping provided that the surrounding water is oxygenated.

Nomoura (1926) showed that in *Paracaudina* the oxygen consumption was dependent upon the oxygen concentration of the surrounding fluid. It seems probable, therefore, that cessation of pumping followed by the use of an oxygen store accounts for the apparent reduction in overall respiratory rate of *Paracaudina* as in *Holothuria forskali*. Hiestand (1940), however, found no such relationship in *Thyone* so some different mechanism must operate in that animal. The muscles of *Thyone* may also show some differences from those of *Holothuria forskali* and *Paracaudina* for it has been suggested (p. 55) that the muscles of *H. forskali* are unable to contract when the oxygen concentration of the bathing fluid is low. This dependence upon oxygen concentration has also been demonstrated in *Stichopus* by Lutz (1930). If *Thyone* is able to maintain its normal respiration and pumping behaviour in low oxygen concentrations, it seems possible that not only the pumping muscles but also the body-wall musculature may not require such high oxygen concentrations to maintain their activity.

Finally, the problem arises whether *H. forskali* encounters oxygen concentrations of less than 70% saturation in its normal habitat. This is difficult to assess; it is generally assumed that *H. forskali* lives in crevices and feeds on organic debris. It seems possible, therefore, that under these circumstances this animal will encounter deoxygenated conditions when it would be advantageous to cease pumping. As Pantin & Sawaya (1953) point out, volume changes also have important effects on the possible activity of the animal. Thus the volume increase which occurs prior to the cessation of pumping might serve not only to increase the oxygen reservoir (p. 55) but also to increase the rate of movement of the animal. Since *H. forskali* moves towards a source of oxygen when the oxygen concentration of the surrounding medium is low, the additional supply of oxygen within the body might well act as a reserve which is utilized while the animal is seeking new conditions where oxygen is more plentiful.

SUMMARY

1. The respiratory rate of *Holothuria forskali* has been found to be between 0·0162 and 0·0465 ml. O_2/g. dry wt./hr. at 17° C.

2. By separating the water pumped by the cloaca from that in contact with the general body surface it is found that cloacal gas exchange accounts for approximately 60% of the total uptake of oxygen by the animal.

3. Uptake of oxygen through the cloaca occurs only when the pumped water is more than 60–70% saturated with air. At lower concentrations there is no exchange through the respiratory trees and no corresponding increase in absorption through the general body surface.

4. Pumping rhythms in intact *H. forskali* are described. It is shown that there are three main types. In all types the rate of pumping increases as the oxygen in the pumped water becomes depleted. At concentrations corresponding to 60–70% air-saturation, however, pumping ceases and this coincides with the observed reduction in cloacal gas exchange.

5. It is shown that a period of reduced gas exchange through the cloaca is followed by increased uptake of oxygen when pumping is resumed.

6. Measurements of the coelomic oxygen concentration shows that cessation of

pumping is followed by a rise in the oxygen concentration in the coelomic fluid.

7. The function of the coelomic oxygen reservoir is discussed.

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