

CILIARY ACTIVITY AND OXYGEN UPTAKE IN *BRANCHIOSTOMA LANCEOLATUM* (PALLAS)

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INTRODUCTION

Although the lancelet has been the subject of extensive studies by many workers, there is no detailed account of oxygen uptake by this animal. Nicol (1960) quotes a figure of 35.8 ml./kg./hr. at 16° C. calculated from a paper by Vernon (1895). However, Zeuthen (1947) has emphasized the importance of taking size into consideration, while, in addition, temperature, external oxygen concentration and variations in activity are also factors of importance in determining the level of oxygen uptake which have not been considered with regard to lancelets.

METHODS

In the course of the investigation it was necessary to measure the concentration of dissolved oxygen in a series of sealed respiration vessels at frequent intervals. For this purpose, it was convenient to use the miniature oxygen electrode used by us in an earlier investigation (Newell & Courtney, 1965) and similar to that described by Naylor & Evans (1963). This consisted of a tubular silver anode of 0.5 mm. external diameter in which was sealed an insulated platinum cathode of 0.2 mm. diameter. The electrode was sealed into a glass capillary to prevent bending and a polystyrene membrane was applied across the surface to prevent undue stirring effects (Fig. 1). A potential difference of 0.7 V. was applied for 0.5 sec. at 15 sec. intervals across the electrode and the resultant current was amplified and recorded on a galvanometer.

As Kanwisher (1959) has pointed out, oxygen electrodes consume 2×10^{-4} ml. O₂/hr. for each microamp. of current flow and thus may produce apparent 'respiratory' effects. However, in the micro-electrode used, the current output was only of the order of 0.5 μ A recording for only 0.5 sec. at 15 sec. intervals (i.e. the electrode was consuming oxygen for a total of only 2 min. in each hour). Moreover, the actual consumption would be only 1×10^{-4} ml. O₂/hr. with continuous recording so that with the pulsed system the utilization rate was 1×10^{-4} ml. O₂/30 hr., a rate which we regarded as negligible.

All the animals used in the experiments were taken from their habitat at Helgoland by means of a dredge. They were then kept in the laboratory under flowing sea water at 16° C. for not more than 1 day before being placed in the respiration vessels described above. In all instances, however, the lancelets were allowed a further 24 hr. to adjust to the particular temperature at which the experiment was to be carried out.

RESULTS

A. *Oxygen uptake by lancelets from Helgoland waters*

Three main aspects of oxygen uptake were studied. These were: (1) the effect of external oxygen concentration on oxygen uptake, (2) the effect of size as indicated by the dried weight of the tissues, (3) the effect of temperature.

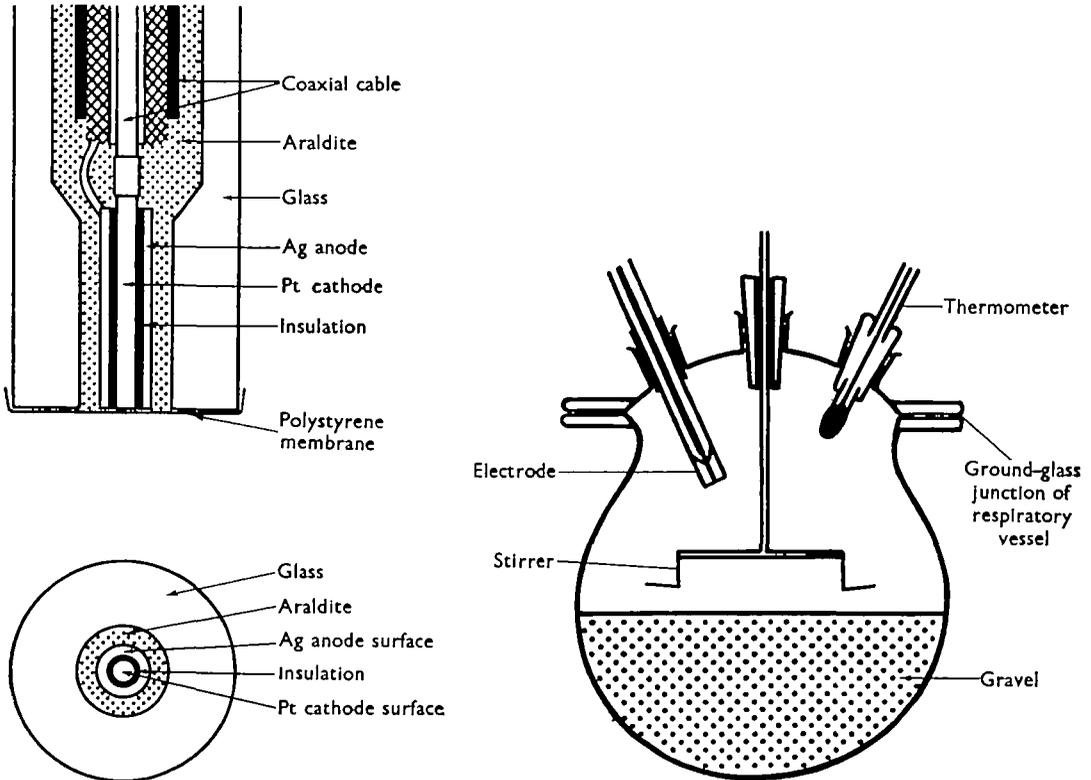


Fig. 1

Fig. 2

Fig. 1. Diagram showing the oxygen electrode used for measuring the concentration of dissolved oxygen in sea water.

Fig. 2. Diagram showing the system used for measuring the respiratory rate of *Branchiostoma lanceolatum* under gravel.

(1) *The effect of external oxygen concentration*

In order to measure oxygen uptake under a series of oxygen concentrations animals were placed individually in a number of sealed respiration vessels of 150 ml. capacity. The animals were allowed to burrow in heat-sterilized 'amphioxus gravel' which covered the bottom of the vessel to a depth of approximately 3 cm. and were thus regarded as being under conditions approaching those of the natural habitat. The overlying water, which had been boiled and aerated, was stirred by a slowly revolving swept-plate vortex generator as described by Bryer (1963). The oxygen concentration of the water above the gravel was measured by means of an oxygen electrode inserted

through the lid of the vessel (Fig. 2). All experiments were made in a constant-temperature room and electrode readings were checked before and after the experiment by means of the micro-Winkler method of Fox & Wingfield (1938). In this way the rate of oxygen consumption of an animal under conditions of steadily decreasing oxygen concentration could be studied.

Such experiments yield variable results. For example, none of the animals depleted the oxygen in the water to below 70% saturation at temperatures 4–10° C., but at higher temperatures an animal might or might not continue to use oxygen at lower saturation values. At 15° C., for example, some individuals of a variety of sizes

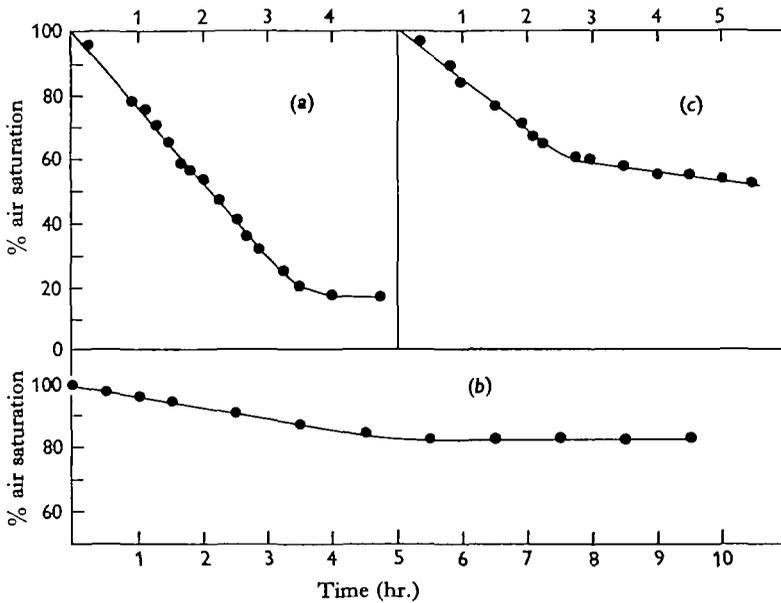


Fig. 3. Graphs showing the percentage of air saturation in water in three vessels of 150 ml. capacity plotted against time in hours. Temperature 15° C. (a) Showing a linear rate of utilization despite a continuous decrease in the concentration of oxygen in the water. The animal weighed 0.0238 g. dry wt. (b) Showing the cessation of oxygen uptake by an animal at 80% of air saturation. The animal weighed 0.0205 g. (c) Showing two successive rates of oxygen uptake by an animal of 0.018 g.

depleted the oxygen in the water linearly down to less than 20% of air saturation. Others, however, used oxygen linearly to approximately 80% saturation and then ceased to consume oxygen even for a period of 4–5 hr. Yet others utilized oxygen at a particular rate and later continued to use oxygen linearly but at a reduced rate and finally ceased to use oxygen as in the case of the individuals mentioned above. Examples of these results, which were obtained from a study of more than twenty-five individuals at this temperature, are shown in Fig. 3.

It is clear that there is considerable variation in the effect of external oxygen concentration on oxygen consumption by lancelets (Fig. 3) and it was further observed that any individual may display several rates of oxygen consumption. Such experiments were repeated with animals in sea water alone without gravel present and were also shown over a wide variety of temperatures from 4 to 20° C.

(2) *The effect of size*

The effect of size on oxygen uptake was studied in the same way as the effect of oxygen concentration. Animals were allowed to burrow in sterilized gravel and the depletion of oxygen in the overlying sterile sea water gave a measure of the oxygen

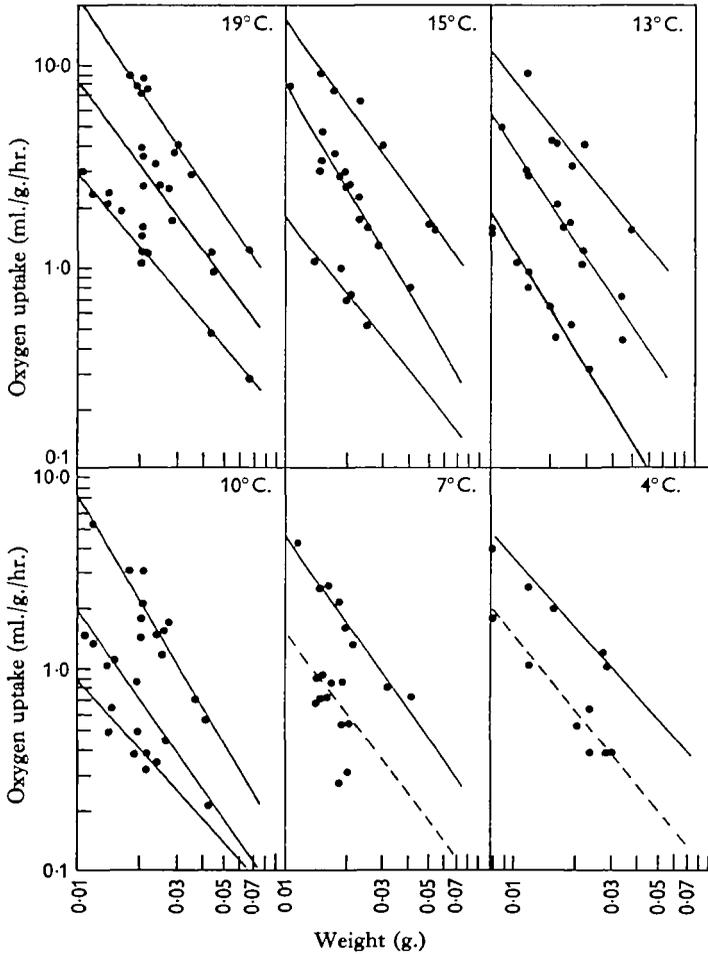


Fig. 4. Graph plotting the log. of the respiratory rate in ml./g. dry wt./hr. against the log. of the dry wt. for a series of temperatures. All animals were allowed to adjust to the temperature for 12 hr. before measurements were made and were buried in sterilized gravel throughout the experiment. It will be noted from the Table 1 (p. 5) that there is no significant difference between the slopes of the lines *a*, *b* and *c* ($F(1)$ in Table 1) but the F test between levels ($F(2)$) confirms the presence of three distinct regression lines. It was not possible to distinguish clearly between the regression lines *b* and *c* at temperatures of 7° C. and below. Their probable positions are indicated by the broken line. (See appendix for calculation of regression lines.)

uptake by the animal. As has been shown above, any one animal may exhibit several rates of oxygen utilization so that a graph plotting oxygen uptake in ml./g./hr. against the dry weight of the animal will necessarily be complicated particularly as some animals exhibit only one or other of their possible rates.

However, when such data are plotted for a large number of animals of widely differing weights ranging from 0.01 to 0.055 g., interesting results are obtained. Fig. 4 shows the log. of the respiratory rate in ml./g./hr. plotted against the log. of the dry weight in grams for a large number of animals at a variety of temperatures from 4 to 20° C. It will be seen that three possible respiratory rates may be shown by an animal of any given weight at temperatures of 10° C. and higher. Further, there appear to be three possible rates only, since no gradation of one into the other occurred. The results of the *F* test confirm the presence of these three distinct regression lines (Table 1.). This suggests that the rates are not an expression of variations in body movement, since this would be expected to give a continuous series of possible rates of oxygen consumption, from zero when the animal was immobile to a maximum during full swimming activity. In any event the animals spent their time buried in gravel and extensive movement would be unlikely and rapid swimming impossible.

Table 1. *Analysis of covariance*

	Equation of regression line	$S_{(b)}$	$S_{z,y}$	D.F. ($n-2$)	r	<i>F</i> test
19°						
A	$Y = -1.56-1.43X$	0.21	0.12	7	0.93**	(1) 0
B	$Y = -1.82-1.39X$	0.14	0.07	8	0.95**	(2) 31.616**
C	$Y = -1.96-1.21X$	0.08	0.06	10	0.97**	—
15°						
A	$Y = -1.71-1.49X$	0.15	0.08	4	0.99**	(1) 1.318
B	$Y = -2.46-1.67X$	0.28	0.11	12	0.93**	(2) 249.84**
C	$Y = -2.52-1.42X$	0.36	0.06	3	0.86	—
13°						
A	$Y = -1.40-1.23X$	0.25	0.10	4	0.91*	(1) 2.7
B	$Y = -2.52-1.67X$	0.06	0.04	8	1.0**	(2) 231.7**
C	$Y = -2.53-1.36X$	0.15	0.07	7	0.95**	—
10°						
A	$Y = -2.93-1.94X$	0.28	0.12	10	0.90**	(1) 2.258
B	$Y = -2.51-1.45X$	0.22	0.12	5	0.98**	(2) 119.71**
C	$Y = -2.03-0.97X$	0.36	0.07	5	0.74	—
7°						
A	$Y = -2.23-1.45X$	0.16	0.07	6	0.97**	—
4°						
A	$Y = -1.33-0.89X$	0.17	0.08	3	0.92	—

Where $F(1) = \frac{\text{mean squares for regression coefficient}}{\text{mean squares of within samples}}$

and $F(2) = \frac{\text{mean squares for adjusted means}}{\text{mean squares for common}}$.

(Two asterisks indicate a probability of < 0.001)

Each rate agrees with Zeuthen's (1947) correlation between oxygen uptake and body size and seems to be an expression of some activity which is either present or absent in an 'all or nothing' fashion. As is shown in Table 1 (*F*(1)) there is no significant difference between the slopes of the lines. A possible explanation of these data lies in the ability of the animal to change its rate of ciliary activity and this is discussed on p. 9.

(3) *The effect of temperature*

As would be expected, and is apparent from Fig. 5, oxygen uptake is affected by temperature, being faster at high than at low temperatures. Fig. 5 shows the effect of temperature on oxygen uptake by an animal weighing 0.02 g. It will be seen that between 10 and 20° C. the Q_{10} of the fastest rate *a* is 3.5; of the medium rate *b* is 5.0; and of the slowest rate *c* is 3.5. That is, the medium rate *b* is more affected by a change in temperature than either rates *a* or *c*. Animals in gravel probably exhibit only rates *a* or *b* at temperatures below 10° C. (see Fig. 4 and Table 1).

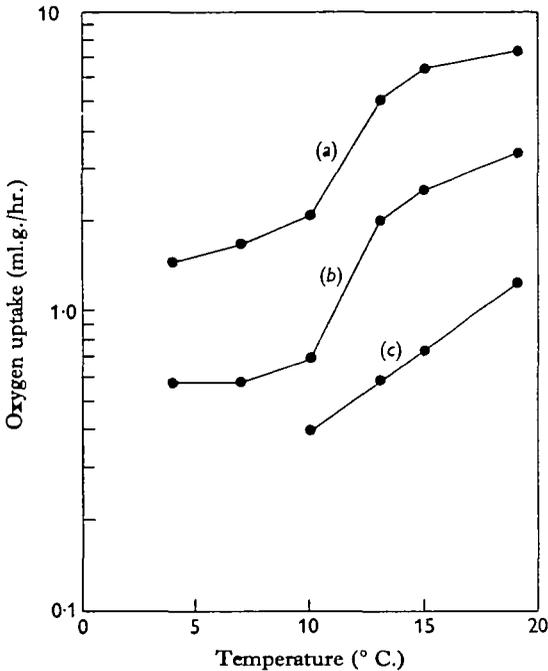


Fig. 5

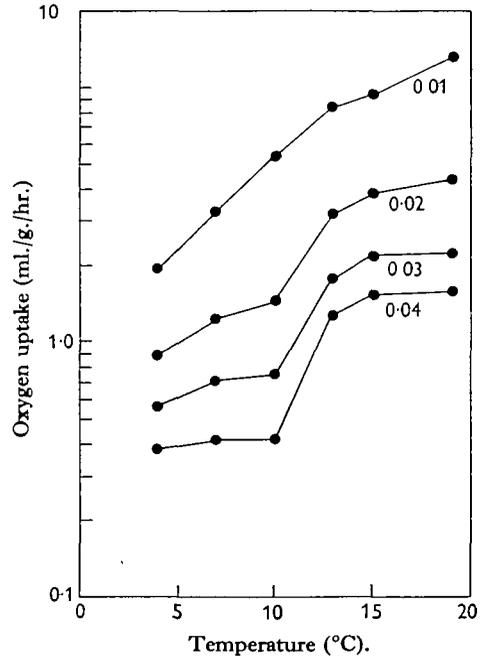


Fig. 6

Fig. 5. Graph showing the relationship between oxygen uptake and temperature for an animal of 0.02 g. Compiled from data illustrated in Fig. 4.

Fig. 6. Graph showing the effect of temperature on the respiratory level *a-b* in animals of 0.01 g., 0.02 g., 0.03 g. and 0.04 g. dry wt., respectively. Compiled from data in Fig. 4.

B. *Variations in the respiratory levels*

It has been shown that individual animals can exhibit three different rates of oxygen uptake. It is reasonable to suppose that when the intermediate rate is exhibited (rate *b* in Fig. 4) the 'basal metabolic rate' *c* is also occurring. Similarly during the fast rate of oxygen consumption (rate *a* in Fig. 4) rate *b* accounts for a proportion of the total uptake. It therefore becomes possible to study quantitatively the effect of size and temperature on the mechanisms responsible for the different respiratory levels *a-b*, *b-c* and *c*; this would be difficult to carry out by any other method in the intact animal.

Fig. 6 shows the effect of temperature on the respiratory level *a-b* in four animals of

different sizes. It will be seen that in the small animal (0.01 g. dry wt.) a reduction of temperature from 10 to 4° C. resulted in this level being reduced by a factor of 0.6. The larger animals are less affected and tend to respond to a fall in temperature in such a way as to maintain this level of respiration. It is suggested (p. 9) that the oxygen uptake *a-b* may be associated with increased ciliary activity during feeding.

Clearly, temperature change has an important effect on the respiratory level *b-c* (Fig. 7) but, at temperatures below 10° C., animals of all sizes tend to maintain a constant respiratory uptake, despite changes in temperature.

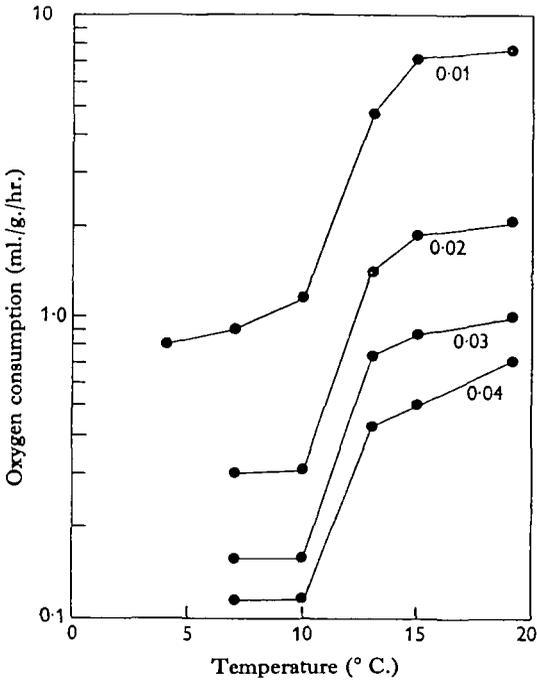


Fig. 7

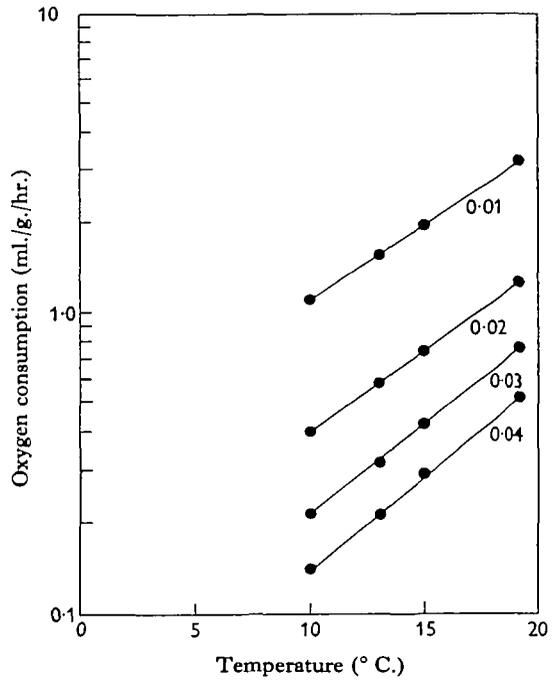


Fig. 8

Fig. 7. Graph showing the effect of temperature on the respiratory level *b-c*. Compiled from the data presented in Fig. 4 for animals of weight 0.01, 0.02, 0.03 and 0.04 g. dry wt., respectively.

Fig. 8. Graph showing the effect of temperature on the uptake of oxygen by inactive animals of 0.01, 0.02, 0.03 and 0.04 g. dry wt., respectively. Compiled from data presented in Fig. 4.

The effect of temperature on the 'basal metabolic rate' *c* is different from that on the respiratory levels *a-b* and *b-c*. Instead of a rapid increase in oxygen uptake between 10 and 15° C., followed by a flattening of the slope between 15 and 19° C., there is a direct relationship between temperature and the log. respiratory rate (Fig. 8). This rate was never exhibited by animals in gravel, at temperatures below 10° C., but was sometimes recorded from animals in sea water alone.

Therefore whatever the mechanisms underlying the respiratory levels *a-b* and *b-c* there is a variable effect of size and temperature upon them. The marked effect of low temperatures upon the oxygen uptake *a-b* of small animals could explain the high mortality of young animals of the Helgoland population during the cold winter

1962/63 (Courtney & Webb, 1964). Although, as they point out, inhibition of the 'coughing' reflex at very low temperatures may also have been important, it is evident that the size of the population in temperate waters is determined by the mortality of the young animals which occurs each winter. (This problem forms the basis of further work by one of us, W.A.M.C.)

The slope of the curves for *a-b* and *b-c* in Figs. 7 and 8 suggests that the metabolism of the animal is suited to temperatures above 10° C. This result agrees with that of Courtney & Webb (1964), who showed that overt activity in lancelets from both Helgoland and Naples increased rapidly between 9 and 20° C. From this and other experiments it is concluded that *B. lanceolatum* is basically a Mediterranean animal which can, however, tolerate temperatures as high as 27° C. and as low as 3° C.

C. Activity patterns in lancelets

It has been shown (Fig. 4) that an animal of a particular weight and at a particular temperature may exhibit three different levels of oxygen uptake. There are no intermediate rates of consumption and it was suggested that this indicated some oxygen consumption mechanism which operated in an 'all or nothing' fashion. During the experiments movement of the animals was restricted by the gravel in which they were buried and therefore it seems probable that it was variations in pharyngeal ciliary activity which accounted for the different levels of oxygen uptake.

(1) Variations in ciliary activity in intact animals

In order to determine whether different pharyngeal ciliary patterns could account for this variation fifty animals were placed in aerated sea water in sealed Petri dishes at 15° C. and observed with a binocular microscope. All animals were found to be capable of either passing water rapidly through the pharynx or else of maintaining a much slower stream. In addition, some of the animals were observed to stop the ciliary current so that no water entered the pharynx. So far as could be observed, there was no intermediate rate of flux between the fast, medium and zero rates observed.

(2) The effect of oxygen concentration on ciliary activity

In sealed vessels many animals exhibited a sequence of changes in oxygen uptake (Fig. 3c). First a fast rate of oxygen uptake was shown (rate *a* of Fig. 5) then a slower rate (rate *b*). In some animals an even slower rate (rate *c* of Fig. 5) followed. Finally, in all animals the sequence was concluded by a period of zero oxygen uptake.

The following experiment was performed to investigate whether a similar sequence of activity could be induced in the ciliary currents by lowered oxygen concentrations. An animal was placed between two slides which were separated by a layer of silicone grease so that the animal was in a restricted volume of water. Initially the stream was fast and later became slow before finally stopping. Replacement with oxygenated water resulted in the fast current reappearing. This sequence was observed on five separate animals but it was found difficult to determine the cause of such variations in the inhalant stream in intact animals.

In order to study the cause of such variations in the inhalant current a piece of side

Wall of the pharynx was removed and placed under a coverslip the edges of which were then sealed with silicone grease. The cilia could then be readily observed under a high-power lens. Initially in all preparations examined all the cilia were beating but later, as the oxygen concentration became lower, some of the lateral cilia in the centre of the preparation beat more slowly and finally a few ceased to beat. From this group the inactivity spread along the gill bar and finally even the cilia near the edges of the preparation were affected. There were, however, patches of lateral cilia which continued to beat but even these stopped later. Corresponding with this decrease in the activity of the lateral cilia, the frontal cilia also slowed and stopped and were inactive when most of the lateral cilia had stopped. When the coverslip was removed and oxygenated sea water added, the ciliary activity was restored.

This result suggests that the fast ciliary stream may correspond to the high oxygen-utilization rate (rate *a* in Fig. 5) and is due to the total activity of all the lateral cilia on the gill bars and also of the frontal cilia. These ciliary activities result in the feeding current described by Orton (1913). The slower inhalant stream, which is set up by the lateral cilia and does not involve the frontal cilia, may be the activity underlying the lower oxygen-utilization rate (rate *b* in Fig. 5). Finally, the state in which there is no inhalant stream is probably due to the absence of ciliary activity at low oxygen concentrations and would correspond with the low rate of oxygen uptake by the animal (rate *c* in Fig. 5).*

(3) *Other factors controlling ciliary activity*

As is shown in Fig. 3*a*, the oxygen concentration may be taken to below 30% of air saturation with no variation in the rate of uptake of oxygen, or the rate of uptake of oxygen may cease at high oxygen concentration. This suggests that, even if the activity of the pharyngeal cilia is related to rates of oxygen uptake, some factor other than oxygen concentration also controls ciliary activity in the animal.

Such a supposition receives confirmation when a portion of pharyngeal wall with attached endostyle is removed from the animal and spread on a slide. When a coverslip is placed on the preparation all the lateral cilia immediately stop but the frontal cilia continue to beat. When the coverslip is removed the lateral cilia beat again. This sequence can be repeated by successively removing and replacing the coverslip. The fact that the frontal cilia continue to beat although they are in contact with the glass, while the lateral cilia are not in contact with the glass and yet are inhibited, suggests that the lateral cilia are not being inhibited by mechanical obstruction. Instead, it seems probable that the cilia are responding to pressure changes set up by the weight of the coverglass. However, when the endostylar region is removed, so that the preparation resembles the one which was shown to respond to changes in oxygen concentration, the lateral cilia do not stop beating when a coverslip is placed on the preparation. Instead, the cilia stop in the sequence described earlier, namely, most of the laterals and the frontals, followed later by the remaining laterals.

Thus apart from the effect of the atrial and velar diameters, which under certain

* It is important to notice that lancelets appear on occasions to exhibit such an extremely slow rate of oxygen uptake that no depletion of oxygen in the surrounding water is detectable (Fig. 3*a* and *b*). Thus although rate *c* (Fig. 5) may be conveniently termed the 'basal metabolic rate', it probably represents the sum of the true basal metabolic rate and a number of other metabolic activities, such as the maintenance of posture and the movement of gut contents.

circumstances undoubtedly modify the velocity of the inhalant stream (Bone, 1961), the speed of current appears to be controlled by two mechanisms both affecting the lateral cilia. The first is oxygen concentration or some other dependent factor, which causes not only most of the lateral, but also the frontal cilia to stop beating. The second mechanism may operate at high oxygen concentrations as well as low ones and causes all the lateral cilia to stop beating. In view of the rapidity of the response, the only mechanism by which this could operate would appear to be by the nervous control of the lateral cilia. The fact that the presence of the endostyle seems essential for the nervous control to operate suggests that this structure plays an important part in the regulation of ciliary activity. It is known that there is a complex nerve network under the endostyle (Bone, 1961), indicating the presence of a local reflex system. Our observations suggest that the subendostylar network exerts an important regulatory effect on the lateral cilia of the gill bars.

This hypothesis finds support in the work of Bone (1958, 1961), who demonstrated that the lateral cilia, but not the frontal cilia, are under nervous control in *Branchiostoma lanceolatum*. Nervous control of lateral cilia has also been demonstrated in *Doliolum* by Fedele (1923) while Knight-Jones (1953) has suggested that the lateral cilia on the bars of *Saccoglossus* are under nervous control. Thus the nervous control of lateral cilia may well be a feature of protochordate gill bars and perhaps the respiratory rate of these organisms is subject to variations similar to those which have been described above for *B. lanceolatum*.

SUMMARY

1. The rate of oxygen uptake by single specimens of *Branchiostoma lanceolatum* has been shown to vary considerably. Some animals in sealed vessels at 15° C. were able to utilize the oxygen linearly down to less than 20% of air saturation. Others ceased to absorb oxygen at high saturations while yet others displayed two different rates in a sequence starting initially with a high rate.
2. There are three possible rates of oxygen utilization, as well as a zero rate, for an animal at any temperature between 10 and 19° C. Animals in gravel exhibit only two rates at lower temperatures.
3. The slowest rate varies exponentially with temperature but the two faster rates show a rapid increase between 10 and 15° C. with little change outside this temperature range.
4. The effect of size and temperature on the increased oxygen uptake during the faster rates of respiration are discussed. It is suggested that ciliary activity of the pharynx could be associated with the extra oxygen utilization.
5. It has been found that there are three inhalant stream velocities, a fast, a slow and a zero rate with no intermediate rates.
6. Study of the isolated portions of the pharyngeal wall confirm that the fast inhalant current is set by the activity of all the cilia to give a feeding stream. The slow stream is set up by the lateral cilia, which continue to beat when the frontal cilia and most of the lateral cilia have been inhibited by lowered oxygen concentrations.
7. The lateral cilia have been shown to be under nervous control and to be inhibited by pressure on the pharyngeal bars. This mechanism depends on the presence of a connexion between the pharyngeal bars and the endostyle.

The work described above was started at the Stazione Zoologica, Napoli, and was completed at the Biologische Anstalt, Helgoland. We are most grateful to the directors and staffs of both these laboratories for providing the facilities and animals necessary for this work, also to the Royal Society of London for use of the Royal Society table at Naples, and to the Central Research Fund of the University of London for a grant to cover the cost of equipment. We should like to express our thanks to Q. Bone and J. E. Webb for their criticism of the manuscript, to P. Gibbs for his help with the statistical treatment of the data, and to T. H. Hesketh for the design and construction of the polarograph. One of us (W. A. M. C.) is financed by the D.S.I.R.

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APPENDIX

Statistical treatment of data shown in Fig. 4

In order to study the data statistically all values were rectified (using logs.) so that the regression line of respiratory rate and size was rectilinear rather than curvilinear. The regression line for each temperature could then be calculated as described below.

Calculation of the regression line

The slope (*b*) of the regression line was calculated from

$$b = -\frac{\Sigma x \cdot y}{\Sigma x^2},$$

where

$$\Sigma x \cdot y = \Sigma(X \cdot Y) - \frac{(\Sigma X)(\Sigma Y)}{n}$$

and

$$\Sigma x^2 = \Sigma(X)^2 - \frac{(\Sigma X)^2}{n} \text{ for } n \text{ observations.}$$

For any value of X ,

$$Y = \frac{\Sigma Y}{n} + b \left(X - \Sigma \frac{X}{n} \right).$$

Two values of Y were calculated for chosen values of X and the regression line was drawn. The statistical data derived from these regression lines by an analysis of covariance is given in Table 1 p. 5.