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# SURVIVAL OF ANAEROBIC PERIODS BY TWO INTERTIDAL POLYCHAETES, *ARENICOLA MARINA* (L.) AND *OWENIA FUSIFORMIS* DELLE CHIAJE

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Borden (1931) found that the lugworm *Arenicola marina* L. did not increase its rate of oxygen consumption after short (2 h) periods under anaerobic conditions, and concluded that there was no evidence that these animals could go into debt for oxygen after the supply of oxygen in the blood had been used. She calculated that there was sufficient oxygen in the blood to last the worm about 1 h, calculating that the oxygen capacity per gramme worm was 0·037 ml., and the oxygen consumption per gramme hour was 0·031 ml. As the period during the experiment when oxygen was excluded from the animals was 2 h, anaerobic respiration would have continued for about 1 h, which should have been sufficient to necessitate an easily measurable increase in the oxygen consumption on return to normal conditions if a debt for oxygen had been produced. In vertebrates, and some invertebrates, such a debt would be incurred by glycolysis, the lactic acid produced being reoxydized to glycogen in the presence of free oxygen, and thus producing an initially increased oxygen consumption on return to aerobic conditions.

The absence of an oxygen debt after a period of anaerobic conditions may be due to one or more of several causes. During an anaerobic period, a poikilothermic animal may (1) reduce its metabolic rate to a very low level, (2) metabolize glycogen to an end-product other than lactic acid, (3) excrete the lactic acid produced, or (4) utilize protein or oil rather than glycogen as an energy source. In polychaetes there is also the possibility that lactic acid could be accumulated in the coelomic fluid and only slowly reoxidized. In all these instances no marked increase in oxygen consumption would be expected on return to normal aerobic conditions.

The method of surviving anaerobic periods by polychaetes is interesting in view of the regular or occasional subjection of some species to such conditions, especially in the intertidal zone, and of their ability to survive such conditions in the laboratory. Borden (1931) found *Arenicola* to be unaffected by short periods, and Hecht (1932) found that lugworms would in fact survive for as long as 9 days without oxygen. *Owenia fusiformis* Delle Chiaje is even more resistant; von Brand (1927) found that this species could survive for 21 days under strictly anaerobic conditions. Packard (1905) found *Amphitrite* and

*Nereis* to survive a day; Jacubowa & Malm (1931) investigated the survival of several species of polychaetes, and found them to survive for periods of from 1 to 10 days. Neither Borden (1931) nor Hecht (1932) made glycogen or lactic acid determinations on *Arenicola*, but von Brand (1927) found 0·38% of the wet weight in the lugworm was glycogen, and 5% in *Owenia*; in an earlier paper (Dales, 1957*a*) I found 1–2% in *Arenicola*, and in the present work 2% in *Owenia*.

The long survival time of *Owenia* coupled with the high glycogen content reported by von Brand (1927) suggests that survival of long anaerobic periods may be made possible by metabolism of glycogen. Also the knowledge that *Arenicola* can survive anaerobic periods much longer than could be endured solely by utilization of oxygen in the blood, yet does not show an increased oxygen consumption on return to normal aerated conditions, indicates that if glycogen breakdown does occur, this either does not lead solely or mainly to lactic acid as in vertebrate glycolysis, or the lactic acid is wholly excreted.

Consequently, the glycogen content of various parts of the body of *Arenicola* and of *Owenia* has been measured in worms under normal aerobic, and under varying periods of anaerobic conditions. The oil content, lactate and pyruvate content have also been estimated, and the distribution of glycogen and oil studied histochemically. The results are discussed in relation to the actual conditions with which these worms may contend in nature. The *Arenicola* were mostly collected at Chalkwell, Essex, rather small worms being used for convenience in the experiments, but some duplicate experiments were done on Plymouth worms which were much larger. The *Owenia* studied were collected from Tor Abbey sands, Devon, and from near St Mawes in Cornwall.

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## METHODS

Worms were placed in a 'Thermos' flask with ice immediately on collection, and dealt with as soon as possible on return to the laboratory, always within 3–4 h. The glycogen content of animals described here as 'fresh', are those so treated and weighed immediately on reaching the laboratory.

In the experiments the weights of all the *Arenicola* refers to whole animals minus tails, coelomic fluid and gut. The weights of the *Owenia* refers to complete animals

including gut and coelomic fluid. In both cases wet weights were obtained after drying on filter-paper; although the *Owenia* were small, the body is smooth and cylindrical and the animal is easily and quickly dried.

The experimental animals were placed in 500 ml. conical flasks half filled with sea water, with three small *Arenicola* or twenty *Owenia* in each. The *Owenia* were not removed from their tubes but these were seen to be clean before being used; the *Arenicola* were washed before use in an experiment. Experiments were performed in a constant temperature room at 18° C. To obtain anaerobic conditions a stream of nitrogen from a cylinder was passed through alkaline pyrogallol wash-bottles into a trap from which a number of closable jets protruded, each of which could be connected to an experimental flask. A stream of nitrogen was maintained through the water until the oxygen content was negligible before the animals were introduced. Then a very slow but continuous stream was maintained for the duration of the experiment, the gas escaping through a bunsen valve in the stopper closing the flask. In a few instances the oxygen contents of the water during and at the end of an experiment was checked with Winkler's reagents, and found to be no more than would be expected in the reagents. The gas was therefore not passed over heated copper as well as through alkaline pyrogallol as recommended by von Brand (1946) as the cylinders of nitrogen used were sufficiently pure. In all the experiments the animals were fasting.

Glycogen was estimated by hydrolysis to glucose which was then determined by Hagedorn & Jensen's method (1923) as described in a previous paper (Dales, 1957*a*). Lactate was estimated by the method of Barker & Summerson (1941), the tissue being fixed by dropping the rapidly weighed tissue into ice cold 10% trichloroacetic acid. Pyruvate was estimated by Lu's method (1939) using 2:4-dinitrophenlyhydrazine and extracting with ethyl acetate. The final colour in both lactate and pyruvate determinations was measured with a 'Unicam' S.P. 500 spectrophotometer, the lactate at 570 m$\mu$, pyruvate at 550 m$\mu$, against standards of known purity.

Total oil was measured gravimetrically by the method of Sperry (Glick, 1955). The tissue was disintegrated in a Griffeth pattern tissue disintegrator and dried with acetone which was then blown off in a current of nitrogen, the sample being left to dry in a nitrogen-filled desiccator. The dried tissue was weighed and then extracted with 2:1, $CHCl_3$:MeOH in the cold, the extract poured off and the tissue re-extracted with three successive portions of solvent. The extract was poured through fat-free filter-paper into a flat-bottomed tube, and the paper rinsed through. The combined extract was then stood in a large volume of distilled water overnight, and the aqueous layer then removed (without flocculent interface) by suction. The solution was then transferred to a small flask and evaporated to dryness *in vacuo*, the oil redissolved in $CHCl_3$ and transferred to a small weighing bottle weighing rather less than 1 g. The $CHCl_3$ was evaporated and the sample left in a nitrogen-filled desiccator until ready for weighing. The weight of the oil was determined by difference after redissolving away the oil with $CHCl_3$. An aperiodic microchemical balance with a sensitivity of 0·1 mg was used.

Distribution of glycogen and oil was studied by the methods previously described (Dales 1957*a*). Glycogen was stained by Best's method after embedding in ester wax (Smyth & Hopkins, 1948), and oil with acetylated sudan black B (Casselman, 1954) in propylene glycol (Chiffelle & Putt, 1951), after formal-calcium fixation (Baker, 1946), frozen sections being cut from gelatin embedded tissue.

## RESULTS

### Arenicola marina

The results of estimations of glycogen content of the body wall of animals under various conditions are summarized in Table 1. It will be seen that the glycogen content falls under conditions of fasting when aerated, but that much more glycogen is used under anaerobic conditions. Although the glycogen content of the gut was found to be much lower than that of the body wall, the concentration in 'fresh' animals and those under anaerobic conditions was also measured. The fall is not statistically significant. To test whether the glycogen content would rise in fasting worms after an anaerobic period (as in vertebrate glycolysis) the glycogen content was measured at the end of a 40 h period under anaerobic conditions and after a further 6 h under fresh aerated sea water. The concentration did not, however, approach that of the fresh control worms.

TABLE 1. *ARENICOLA:* GLYCOGEN IN THE BODY WALL AND GUT

| | No. of estimations | Mean (wet) weight (g) | Extreme weights | Mean total glycogen (mg) | Standard deviation total glycogen | Mean concentration glycogen (mg/g) | Standard deviation glycogen concentration |
|---|---|---|---|---|---|---|---|
| Body wall | | | | | | | |
| 'Fresh' | 40 | 0·398 | 0·116–0·975 | 6·80 | 3·85 | 16·82 | 4·57 |
| Fasting, 40 h aerated | 30 | 0·263 | 0·088–0·819 | 3·42 | 2·51 | 11·95 | 3·51 |
| Fasting, 40 h under anaerobic conditions | 19 | 0·310 | 0·093–0·655 | 2·55 | 2·01 | 7·03 | 3·90 |
| Fasting, 40 h under anaerobic conditions; aerated 6 h | 12 | 0·219 | 0·097–0·305 | 2·11 | 1·17 | 9·85 | 4·60 |
| Gut | | | | | | | |
| 'Fresh' | 15 | 0·151 | 0·069–0·296 | 1·27 | 0·63 | 8·32 | 2·86 |
| Fasting, 40 h under anaerobic conditions | 15 | 0·078 | 0·028–0·138 | 0·52 | 0·81 | 7·11 | 2·54 |

Oil content of the body wall in 'fresh' worms and those subjected to anaerobic conditions for 48 h was also measured (Table 2), and there was found to be no change.

Concentration of lactate in the body wall was not significantly different in 'fresh' worms, and those subjected to anaerobic conditions for 42 h, aerated controls or those returned for 6 h to fresh aerated sea water (Table 3). As any lactate secreted might be broken down by bacteria in the medium, in order to test whether any secretion of lactate occurs under anaerobic conditions, one series of experiments was done under the same conditions as before, but using specially cleaned glassware and boiled and autoclaved sea water to which 100 $\mu$g/ml. of streptomycin sulphate had been added. A detectable, but negligible amount of lactate was found in the medium, after 40 h under

anaerobic conditions with three worms (total mean weight 10 g) in 250 ml. sea water (Table 3).

Pyruvate was not detectable in the tissues of worms after 48 h under anaerobic conditions.

TABLE 2. *ARENICOLA*: OIL CONTENT OF BODY WALL

| | No. of estimations | Mean (dry) weight (g) | Extreme weights | Mean total oil (mg) | Standard deviation total oil | Mean oil concentration (mg/g) | Standard deviation oil concentration |
|---|---|---|---|---|---|---|---|
| Group A | | | | | | | |
| 'Fresh' | 15 | 0·3380 | 0·2152–0·7115 | 17·1 | 7·0 | 50·20 | 5·59 |
| After 48 h under anaerobic conditions | 14 | 0·3877 | 0·2174–0·7694 | 18·8 | 6·7 | 49·20 | 6·52 |
| Group B | | | | | | | |
| 'Fresh' | 13 | 0·1775 | 0·1132–0·2968 | 7·6 | 1·8 | 43·90 | 5·55 |
| After 48 h under anaerobic conditions | 11 | 0·0951 | 0·0658–0·1420 | 4·2 | 1·3 | 44·46 | 6·52 |

Group A = Plymouth worms (August, large); B = Chalkwell worms (October, small).

TABLE 3. *ARENICOLA*: LACTIC ACID IN THE BODY WALL

| | 'Fresh' | Fasting, aerated 42 h | Fasting, under anaerobic conditions 42 h | Fasting, under anaerobic conditions 42 h; aerated 6 h | Concentration in the external medium after 40 h under anaerobic conditions |
|---|---|---|---|---|---|
| No. of estimates | 9 | 12 | 15 | 10 | 10 |
| Mean concentration (mg/g) | 0·297 | 0·338 | 0·365 | 0·362 | 0·007 |
| Standard deviation of mean | 0·164 | 0·180 | 0·165 | 0·215 | 0·0036 |

## Owenia fusiformis

The glycogen content of whole 'fresh' *Owenia* and those that had been subjected for 5 days and 9 days to anaerobic conditions are shown in Table 4. There appears to be no fall in glycogen content. Consequently, no measurements of lactate were made. Estimations of oil content (Table 5) of 'fresh' worms and those subjected to 5 days of anaerobic conditions showed no significant change in concentration. There was no significant fall in weight after 9 days under anaerobic conditions, nor did the green pigment in the intestine (Dales, 1957*b*) appear to decrease in quantity.

## DISCUSSION

It is quite clear that in *Arenicola* metabolism of glycogen under anaerobic conditions leads to products other than lactic acid. This is consistent with the findings of von Brand (1946), who concluded that invertebrates generally metabolized glycogen to a mixture of acids; which acids are produced by *Arenicola* has not been determined. Thus, while an oxygen debt may be avoided, the resources may be wasted if normal activity is continued. Vertebrate glycolysis may be more economical of material, but this incurs an oxygen

debt which can only be cleared in a short time by an efficient respiratory mechanism. Even if true glycolysis existed in the lugworm, it might be that the less efficient respiratory mechanism would be unable to clear the debt within a reasonable period.

TABLE 4. *OWENIA*: GLYCOGEN CONTENT OF WHOLE WORMS

| | No. of estimations | Mean (wet) weight (g) | Extreme weights | Mean total glycogen (mg) | Standard deviation mean total glycogen | Mean concentration glycogen (mg/g) | Standard deviation concentration |
|---|---|---|---|---|---|---|---|
| 'Fresh' | 17 | 0·059 | 0·027–0·104 | 1·215 | 0·53 | 21·0 | 8·8 |
| After 10 days in laboratory (aerated) | 10 | 0·055 | 0·033–0·073 | 1·064 | 0·30 | 19·9 | 4·5 |
| After 5 days under anaerobic conditions | 32 | 0·059 | 0·023–0·096 | 0·978 | 0·41 | 20·7 | 7·9 |
| After 9 days under anaerobic conditions | 34 | 0·053 | 0·025–0·089 | 1·048 | 0·45 | 20·2 | 6·4 |

TABLE 5. *OWENIA*: OIL CONTENT OF WHOLE WORMS

| | No. of estimations | Mean (dry) weight* (g) | Extreme weights* | Mean total oil (mg) | Standard deviation total oil | Concentration of oil (mg/g) | Standard deviation of concentration |
|---|---|---|---|---|---|---|---|
| 'Fresh' | 10 | 0·1961 | 0·1647–0·2337 | 11·6 | 1·54 | 59·27 | 8·28 |
| After 5 days under anaerobic conditions | 12 | 0·1887 | 0·1370–0·2245 | 12·2 | 1·84 | 64·30 | 3·45 |

* Twenty worms weighed together.

It also seems likely that in nature anaerobic periods would be short, and the glycogen used could easily be replenished. Intertidally, the burrows are probably rarely uncovered for more than 9 h, and commonly for much less, although Hecht (1932) found some worms in a situation covered only by spring tides. Presumably aerial respiration (the trapping of bubbles of air in the burrow, described by van Dam, 1938, Wells, 1945, 1949) would play an important part in the lives of these worms, and anaerobic periods are more likely to be encountered where a layer of stagnant water remains over the burrow openings. But even if exposed for 9 h to completely anaerobic conditions, the glycogen concentration of the body wall would fall only by 2 mg/g, and the worm would lose 11% of its total glycogen. Probably lugworms are rarely subjected to such long periods, and even when they are, there is every indication that the activity of the animal would be reduced. In the experiments the worms became rather quiescent after some time without oxygen, though still showing spontaneous body-wall contractions and bursts of activity. This agrees with Wells' findings (1949). Dr J. D. Jones found no significant difference in lactate content in worms dug at the beginning and end of a 5 h intertidal period (private communication).

It is already well known (Barcroft & Barcroft, 1924; Borden, 1931; Wolvekamp & Vreede, 1941; Wells, 1949; Jones, 1954) that the oxygen dissociation

curve of *Arenicola* haemoglobin shows that oxygen may be taken up at low outside concentrations, lower than usually occurs in an exposed burrow after 5 h exposure which was higher than that of the interstitial water (Jones, 1954) perhaps owing to aerial respiration by the worm. Wolvekamp & Vreede (1941) have criticized Barcroft & Barcroft's original idea (1924) that the oxygen in the blood acted as a store for use by the animal when the burrow was uncovered by the tide, but maintained that it was of use during resting periods in the irrigation of the burrow. More recent writers (Wells, 1949; Jones, 1954) have also inclined to minimize the usefulness of the oxygen in the blood for surviving anaerobic periods. On the other hand, in view of Wells' (1949) observations on aerial respiration and the characteristics of the haemoglobin, do lugworms often have to contend with long anaerobic periods? Linke (1939) found the temperature of the surface water on an *Arenicola* beach to rise as high as 26·2° C, and to 21·3° C at 10 cm depth; Thamdrup (1935) found that the oxygen consumption of *Arenicola* falls above 20° C, and Wells (1949) that under such conditions the normal irrigation of the burrow would cease. Wells found that worms would withstand 7 h in his glass apparatus when this was closed and which, at the time of closure would contain about 0·2 c.c. oxygen. The behaviour was modified completely under such conditions, the worm making only occasional 'testing' movements, though responding at once when a fresh supply of oxygenated water became available. This suggests that, even though glycogen is used under anaerobic conditions, worms conserve their energies by becoming quiescent.

This is certainly so in *Owenia*, in which there was no significant fall in glycogen concentration after 9 days under anaerobic conditions. After a few hours the worm became quiescent, and after some days, coiled up tightly in the middle of the tube and showed no sign of activity. Nothing is known about the characteristics of the haemoglobin in this species. The reason why *Owenia* does not use any of its glycogen, whereas *Arenicola* does, though reducing its activity, may also be related to the site of the glycogen deposits which is mainly in the body wall in the lugworm, but in the coelomic cells in *Owenia*. It is unlikely that these cells constitute reserves to be drawn upon during periods of inanition (Dales, 1957*a*); further work on their function is in progress. Again, while *Arenicola* shows bursts of activity under anaerobic conditions, *Owenia* does not.

Von Brand (1946) found that the quotient of aerobic: anaerobic glycogen consumption was low, although he found a relatively high glycogen content, commenting: 'Whether this indicates an exceptionally great reduction of metabolic rate under anaerobic conditions, or a more pronounced participation of fat or protein in anaerobic degradation processes, or whether it is due to fermentative processes in aerobic conditions has not been established.' The rather large discrepancy between von Brand's figures (1927) for glycogen content of *Owenia*, and those obtained here, may perhaps be accounted for by

the difference in weight, his worms were six times heavier, weighing 300 mg each. Von Brand (1927) found that, under anaerobic conditions, 0·23 g glycogen/100 g worm/24 h was consumed. If consumption continued at this rate, all would have been used in 21 days, the survival time von Brand found. *Owenia* from Tor Abbey sands being much smaller may have relatively less in the body wall, so that the consumption of this could not be detected in the presence of the large quantities in the coelomic cells. This ability to reduce activity may well have adaptive significance as these worms may temporarily be buried by the shifting sand they inhabit.

The ability to reduce the metabolic rate may be general in other polychaetes. There seems to be no correlation between the survival time of different species under anaerobic conditions (von Brand, 1946) and the glycogen content (von Brand, 1927). Indeed those species with the highest values for glycogen are the more specialized tube dwellers, which are often provided with well-developed coelomic cells in which most of the body glycogen is stored, and which seem least likely to encounter anaerobic conditions in nature.

## SUMMARY

Measurements of glycogen in the body wall of *Arenicola* indicate that glycogen is consumed during anaerobic conditions. Estimations of lactate and pyruvate show that neither is accumulated, accounting for the absence of an oxygen debt previously found by other workers, and suggesting that glycogen breakdown leads to other acids. In *Owenia* most of the glycogen is stored in coelomic cells and these deposits are not drawn upon during anaerobic periods, yet this species can survive long periods without oxygen, apparently by becoming quiescent. Oil content in both species has also been measured, and was found not to fall under anaerobic conditions. It is suggested that survival of anaerobic periods may be mainly due to an ability to suspend normal activity.

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