THE WATER RELATIONS OF EARTHWORMS

I. THE ACTIVITY OF THE NEPHRIDIOSTOME CILIA OF *LUMBRICUS TERRESTRIS* L. AND *ALLOLOBOPHORA CHLOROTICA* SAVIGNY, IN RELATION TO THE CONCENTRATION OF THE BATHING MEDIUM

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I. INTRODUCTION

The distribution of earthworms is incompletely known, particularly in relation to soil type, but it is now clear that the different species of earthworms show different soil preferences. This leads one to inquire whether there are physiological differences correlated with the distributional differences between species. This problem, with special reference to water content, forms the subject of this paper, particular attention being paid to the action of hypo- and hypertonic media on the cilia of the nephridiostome.

Both *Lumbricus terrestris* and *Allolobophora chlorotica* are relatively easy to obtain and were chosen for these experiments because of their contrasting ecological distribution. *L. terrestris* is purely terrestrial, but *A. chlorotica* occurs in soil but also submerged in streams and even in Lake Windermere up to 20 m. from the shore (Černosvítov, 1945).

II. MATERIAL AND METHODS

Material

A regular supply of *L. terrestris* was obtained from Newdigate, Surrey. Garden specimens of *A. chlorotica* were collected as required from south-east London. Both species were kept in pots of moist soil at temperatures ranging between 8 and 18°C, and were used within a week of collection. Lake Windermere specimens of *A. chlorotica* were kept submerged in a large tank at temperatures ranging between 8 and 15°C, and were used within a month of collection.

Preparation of nephridia

Living nephridia were dissected out and mounted on slides in the way shown in Fig. 1. Preparations mounted in this way were inverted over a pot of saline and observed through a microscope with a 4 in. objective. By means of a vulcanite chamber with inlet and outlet tubes it was possible to observe the cilia while the bathing fluid was changed.

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**Preparation of the bathing medium**

Adolph (1927) found that the coelomic fluid of *L. terrestris* had a Δ of 0·31° C. This was confirmed by Ramsay (1949a), who also estimated the chloride content and found that only half the osmotic pressure could be accounted for as chloride and suggested (1949b) that the other half is probably due to organic substances. Bahl (1945, 1946) analysed fully the coelomic fluid of *Pheretima posthuma* (Megascolidae), but as the chloride content is half that found for *Lumbricus* it is clear that the composition of the fluid is different in the two species. Thus there is no standard earthworm Ringer solution. Therefore many solutions, based on the above information, were tested.

![Diagram](image)

**Fig. 1.** Diagram to show method of mounting nephridia.

The two which gave the best results were frog Ringer* diluted with half its volume of M/400-NaHCO₃, and the same diluted Ringer mixed with an equal volume of 30 g./l. dextrose. Both fluids had a pH of 7·8–8·0 and a calculated Δ of 0·30° C. In one case the osmotic pressure was entirely due to salts; in the other, half to salts and half to dextrose. Full ciliary activity was maintained for about 22 hr. in the solution with dextrose (henceforth referred to as earthworm saline, or E.S.), whereas in the diluted frog Ringer alone (½ F.R.) full activity was maintained for only 16–20 hr.

As survival was curtailed at temperatures above 18° C. the experiments were made at temperatures ranging from 8 to 18° C., the majority being at 15–17° C., and the variation during any one experiment never exceeded 1° C.

*The frog Ringer had the following composition: NaCl 0·107 M, KCl 0·00165 M, CaCl₂ 0·00113 M, NaHCO₃ 0·0025 M.
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III. RESULTS

1. Ciliary activity in hypotonic fluids

After about an hour in the isotonic medium the nephridia were transferred to a hypotonic medium, dilutions being made with m/400-NaHCO₃. The activity of the cilia was noted periodically and compared with that of control preparations kept in undiluted media. As with other ciliated tissues the transfer of a nephridium to a new salinity often produces responses which gradually disappear as the tissue accommodates itself to the new medium. We must therefore distinguish between the temporary effects of the change and the long-term effects of a new tonicity.

(i) Long-term effects of hypotonicity

The duration of activity in the various hypotonic solutions is shown in Table 1. In any one column, the solutions are seen to fall into three groups:

(a) Those in which the dilution is slight; in these the cilia remain active for as long as in isotonic media, although they show a slight falling off in vigour in the last few hours.

(b) Those in which the dilution is intermediate; in these some of the individual preparations behave as in group (a) and the others as in group (c).

(c) Those in which the dilution is extreme; in these the preparations are rapidly arrested, the duration of activity varying inversely with dilution.

When the different columns are compared, the following conclusions clearly emerge:

(a) The resistance of the preparation to dilution depends on the basic saline, § F.R., giving greater resistance than E.S. There is an approximate correspondence between the results for a given dilution of § F.R. and those for double that concentration of E.S. As the salt concentration in E.S. is half that in § F.R., these results suggest that salt concentration is more important than osmotic pressure in determining the long-term effects.

(b) In either medium, the nephridia of A. chlorotica are more resistant to dilution than those of L. terrestris.

(c) The nephridia of garden and Windermere specimens of A. chlorotica were compared in E.S. only, but no significant difference was observed in their long-term salinity relations.

(d) The cilia of L. terrestris and A. chlorotica are able to withstand a dilution of the medium much greater than they are likely to experience under natural conditions. Ramsay (1949a) has shown that L. terrestris regulates the osmotic concentration of its coelomic fluid, and variations are therefore unlikely to be very large.

(ii) Temporary effects of change

In both species the temporary effects were excitatory—acceleration of the beat or increased amplitude, or both together.
Generally there was a time lag of about 1 min. before these effects appeared, but in some cases they occurred within 4 sec. of transference. Four seconds was the time required to commence observations after transferring preparations.

The duration of the effects was variable and apparently bore no relation to the degree of dilution in either *L. terrestris* or *A. chlorotica* (Table 2). The results hardly allow a comparison between E.S. and § F.R. The only generalization that can be made is that in the higher concentrations there were no temporary effects and that in the lower concentrations they invariably occurred. In intermediate concentrations of § F.R. temporary effects occurred in some only of the preparations.

The results are essentially the same for the two species, and the results for garden and Lake Windermere specimens of *A. chlorotica* agree well.

The nephridiostome cilia of these earthworm species differ from the cilia of molluscan gills and the nephridial cilia of the polychaete *Arenicola marina*, in that there is no inhibition of the cilia after a sudden drop in osmotic pressure, the temporary effect being acceleration and increased amplitude of the beat. Wells, Ledingham & Gregory (1940) found that after a change from 100 to 20 % or less a phase of excitation preceded the inhibition of *Mytilus* short abfrontal cilia. Pilgrim (1953) found that the frontal and food-groove cilia behaved in a similar way when subjected to a change from 100 to 50 % or lower. Pilgrim also found that with smaller reductions in concentration the frontal cilia sometimes showed hyperactivity without inhibition.

(iii) *Return to isotonic media*

The effect of return to an undiluted medium was investigated in *L. terrestris* for 25 and 30 % § F.R. and for 40 % E.S. After 15 min. in the hypotonic medium preparations were transferred back to 100 %.

In all cases about 15 sec. after transfer the activity of the cilia was reduced for a time, after which the activity returned to the normal level. Inhibition lasted for 3–8 min. when the change was from 25 % § F.R.; for 2–12 min. when the change was from 30 % § F.R.; and for 55 sec. to 8 min. when the change was from 40 % E.S.

(2) *Ciliary activity in hypertonic fluids*

The experimental procedure was the same in these experiments as in those with hypotonic fluids but only E.S. was used. As before it is necessary to distinguish between the temporary and the long-term effects of a change.

(i) *Long-term effects of hypertonicity*

The duration of ciliary activity at various degrees of hypertonicity is shown in Table 3. As in the case of hypotonic fluids, one can distinguish between a slight change, which does not significantly shorten the duration of ciliary activity, an extreme change which rapidly suppresses movement altogether, and an intermediate change which sorts out the individual preparations into two groups. Even in those
Table 1. Long-term effects of hypotonic media
The first figure in each case is the time until activity ceased altogether. The second (in brackets) is the time until activity was noticeably less than that of a preparation in the undiluted medium. The third figure gives the number of preparations used, and the fourth the number of worms from which these were made.

<table>
<thead>
<tr>
<th>Percentage of isotonic solution</th>
<th>L. terrestris</th>
<th>A. chlorotica (garden)</th>
<th>Earthworm saline</th>
<th>A. chlorotica (lake)</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>Duration</td>
<td>Preparations</td>
<td>Worms</td>
<td>Duration</td>
</tr>
<tr>
<td>80</td>
<td>20 hr. (16-19 hr.)</td>
<td>15</td>
<td>3</td>
<td>&gt; 21 hr. (19-20 hr.)</td>
</tr>
<tr>
<td>60</td>
<td>19 hr.</td>
<td>18</td>
<td>5</td>
<td>90 min. (20-30 min.)</td>
</tr>
<tr>
<td>50</td>
<td>&gt; 20 hr. (16-19 hr.)</td>
<td>15</td>
<td>3</td>
<td>&gt; 21 hr. (19-20 hr.)</td>
</tr>
<tr>
<td>40</td>
<td>20 hr. (16-19 hr.)</td>
<td>20</td>
<td>4</td>
<td>&gt; 21 hr. (19-20 hr.)</td>
</tr>
<tr>
<td>30</td>
<td>1-2 hr. (11-15 min.)</td>
<td>30</td>
<td>6</td>
<td>&gt; 21 hr. (19-20 hr.)</td>
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<tr>
<td>20</td>
<td>1 hr. (5-10 min.)</td>
<td>40</td>
<td>8</td>
<td>&gt; 21 hr. (19-20 hr.)</td>
</tr>
<tr>
<td>10</td>
<td>20-40 min. (1 min.)</td>
<td>15</td>
<td>3</td>
<td>&gt; 21 hr. (19-20 hr.)</td>
</tr>
<tr>
<td>5</td>
<td>3-15 min. (30-60 sec.)</td>
<td>15</td>
<td>3</td>
<td>&gt; 21 hr. (19-20 hr.)</td>
</tr>
</tbody>
</table>

Table 2. Duration of temporary effects of change
The effects begin after a time lag of about 1 min. except in those marked*, in which the effects begin within 4 sec. of transfer to the diluted medium.

<table>
<thead>
<tr>
<th>Percentage of isotonic solution</th>
<th>L. terrestris</th>
<th>A. chlorotica (garden)</th>
<th>Earthworm saline</th>
<th>A. chlorotica (lake)</th>
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<td></td>
</tr>
<tr>
<td></td>
<td>Duration</td>
<td>Preparations</td>
<td>Worms</td>
<td>Duration</td>
</tr>
<tr>
<td>80</td>
<td>No effect</td>
<td>15</td>
<td>3</td>
<td>No effect</td>
</tr>
<tr>
<td>60</td>
<td>No effect</td>
<td>15</td>
<td>3</td>
<td>No effect</td>
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<td>No effect</td>
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<td>3</td>
<td>No effect</td>
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<td>30</td>
<td>No effect</td>
<td>15</td>
<td>3</td>
<td>No effect</td>
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<tr>
<td>25</td>
<td>No effect</td>
<td>15</td>
<td>3</td>
<td>No effect</td>
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<tr>
<td>20</td>
<td>No effect</td>
<td>15</td>
<td>3</td>
<td>No effect</td>
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<td>15</td>
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<tr>
<td>5</td>
<td>No effect</td>
<td>15</td>
<td>3</td>
<td>No effect</td>
</tr>
</tbody>
</table>

(Facing p. 768)
Table 3. Duration of ciliary activity in hypertonic media

<table>
<thead>
<tr>
<th>Percentage of isotonic solution (E.S.)</th>
<th>Lumbricus terrestris</th>
<th>Aliolobophora chlorotica (garden)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration</td>
<td>Preparations</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>&gt;20 hr.</td>
<td>40</td>
</tr>
<tr>
<td>250</td>
<td>&gt;20 hr.</td>
<td>25</td>
</tr>
<tr>
<td>300</td>
<td>5 min.</td>
<td>23</td>
</tr>
<tr>
<td>400</td>
<td>40 sec.</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 4. Appearance and subsidence of vesicles in hypotonic media

<table>
<thead>
<tr>
<th>Percentage of isotonic solution</th>
<th>Time of appearance</th>
<th>L. terrestris</th>
<th>A. chlorotica (garden)</th>
<th>Earthworm saline</th>
<th>A. chlorotica (lake)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>No vesicles</td>
<td>-</td>
<td>15</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>60</td>
<td>No vesicles</td>
<td>-</td>
<td>15</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>50</td>
<td>No vesicles</td>
<td>-</td>
<td>15</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>40</td>
<td>30-60 sec.</td>
<td>+</td>
<td>20</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>50</td>
<td>30 sec.-2 min.</td>
<td>+</td>
<td>74</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>25</td>
<td>60 sec.-4 min.</td>
<td>-</td>
<td>31</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>45</td>
<td>60 sec.-4 min.</td>
<td>-</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>60 sec.-4 min.</td>
<td>-</td>
<td>15</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>30-35 sec.</td>
<td>-</td>
<td>15</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>45 sec.</td>
<td>-</td>
<td>15</td>
<td>3</td>
<td>-</td>
</tr>
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</table>
preparations which remained active for many hours, the degree of activity was nearly always less than in the controls at 100%. The cells of *L. terrestris* are rather more resistant to the long-term effects of hypertonicity than are those of *A. chlorotica*.

(ii) **Temporary effects of change**

The typical temporary effect of suddenly transferring preparations from the isotonic to a hypertonic medium was a slowing or complete cessation of ciliary movement. The duration of reduced activity or of inhibition, the time taken for inhibition to become complete, and the time for the first flickering activity after inhibition to reach the final degree of activity, were all extremely variable. In some cases inhibition was not seen at all; in others the period of inhibition was broken by a few minutes of flickering activity. When inhibition occurred, the cilia slowed and stopped within a few minutes.

The variability of the duration of inhibition may be illustrated for the figures for 200% E.S.; reduced activity or inhibition times at this concentration ranged from 4 min. to 2½ hr. in *L. terrestris* and from 40 min. to 3 hr. in *A. chlorotica*. In these experiments, as in the return to normal from a hypotonic fluid, slowing and inhibition occur when the medium is suddenly changed for a more concentrated one and in this respect the earthworm nephridiostome cilia resemble those of the *Mytilus* gill (Pilgrim, 1953).

(iii) **Return to isotonic solution**

Under natural conditions very little dilution of the coelomic fluid takes place because of the powers of osmoregulation of the worm, but there is no defence against desiccation and the consequent concentration of the coelomic fluid. It is therefore of interest to know whether a period in hypertonic media impairs the ability of the cilia to function when returned to an isotonic medium.

After at least two hours in E.S. after mounting, preparations from *L. terrestris* were transferred to 200 and 300% E.S. and left for 21 hr. in the case of 200% E.S. and for between 19½ and 24½ hr. in the case of 300% E.S. They were then returned to 100% E.S. and observed.

In all cases ciliary activity began after 8-30 sec. The lip cilia were usually, but not invariably, the first to show slight movement which gradually increased, nearly or quite to the normal degree of activity. The central cell and pre-septal canal cilia burst abruptly into nearly full activity and reached the normal activity level after 1 min. After recovery, full activity was maintained for at least 6 hr.

(3) **The swelling of cells in hypotonic media, and its relation to the temporary acceleration of the beat**

(i) **The formation of vesicles**

During the experiments with moderately hypotonic media (e.g. 40%) it was observed, in both species, that bubble-like vesicles appeared around the margin of the upper lip of the nephridiostome a minute or so after the change of medium and
then gradually subsided. The vesicles began to subside 10–20 min. after their appearance, and took 1–3 hr. to disappear completely. They remained permanently in the greatest dilutions, and were absent in the least (Table 4).

The appearance and subsidence of the vesicles reminds one of the temporary excitation of the cilia produced by a downward salinity change, and the possibility of a relation between the two phenomena was investigated.

Fig. 2. Diagram of longitudinal section of nephridiostome of *L. terrestris* to show structure. (After Goodrich.)

Fig. 3. (a) Camera-lucida drawing of longitudinal section (at right angles to that shown in Fig. 2) of *L. terrestris* nephridiostome. (b) Camera-lucida drawing of a living nephridiostome of *L. terrestris* in 30% F.R.

Very similar blister-like appearances may be seen in cells in the absence of external salinity change—e.g. the bubbling seen in tissue culture cells, and Amoebae when dividing and when exposed to anaesthetics, which seem to be due to syneresis (Goldacre & Lorch, 1950; Goldacre, 1952).

The vesicles in the present experiments appeared to be formed regularly on the marginal cells of the upper lip, usually one to each cell. (The structure of a nephridiostome is shown in Figs. 2 and 3.) Some cells had no vesicles, while others appeared to have several (Fig. 3). Since no cilia were carried out with the membrane of the vesicle, the non-ciliated part of the marginal cells appears to bear the vesicles.

To eliminate the possibility that the vesicles might have been either swollen cells of the coelomic epithelium or connective tissue cells, the site of the vesicles was
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investigated using *L. terrestris* because of its greater size. Preparations of whole nephridiostomes and sections contributed much evidence but were not conclusive, so Medawar’s trypic digestion method was used (Medawar, 1941).

Isolated nephridiostomes were transferred from § F.R. to 0·5 % trypsin in § F.R. (pH adjusted to 7·8 with sodium carbonate using phenol red as indicator) and incubated at 30° C. for 1 hr. Then they were transferred to a slide in a drop of the fluid and a cover-slip was placed over them. On tapping the cover-slip with a needle the cells separated from one another. These acted as controls. Others were transferred from § F.R. to 25 % F.R. for 5 min., then to 0·5 % trypsin in 25 % § F.R. (pH 7·8), and treated in the same way as the controls. These preparations showed beyond doubt that the vesicles are on the marginal cells. One part in 10,000 methylene blue was added to the dissociating fluid and the results recorded by camera-lucida drawings (Fig. 4).

It is possible to vary the salt concentration of a solution without altering the total osmotic pressure, by using dextrose solution as the diluent. If, indeed, the vesicles are due to variations in the salt concentration rather than in the osmotic pressure, the same results should be obtained when the salt concentration is brought down, e.g. to 20 %, while the osmotic pressure is held constant. *L. terrestris* preparations were transferred to § F.R. diluted to 25, 20 and 10 % with isotonic dextrose solution. No vesicles appeared in any of these solutions, and there was neither temporary acceleration nor increase in amplitude of the ciliary beat. In 25 % all preparations lasted as long as the controls with the same degree of activity. In 20 % more than half were as good as the controls, the rest failing to survive the night. In 10 % some preparations showed reduced activity after one hour and maintained this for more than 20 hr., in others the cilia stopped beating after about 1 hr. Preparations on being transferred from § F.R. to a dextrose solution of the same osmotic pressure lasted for only 40 min. Thus it seems possible that a change in the osmotic concentration is responsible for the temporary effects on the cilia, and the vesicles, whereas the salt concentration is responsible for the long-term effects.

(ii) Relation of swelling of cells to temporary effects of change

Experiments were made to study more exactly the time relations between cell swelling and temporary effects on the ciliary beat following a change of medium. It is possible to measure the width of the marginal cells at their widest part with a vernier eyepiece, but it is difficult to be sure of measuring the same cell again, even when the preparation is under constant observation, except in those cases where there are accidental topographical marks. The length of the marginal cells was measured in some preparations, and in others the depth could be seen in optical section and measured.

Sixty-three experiments were made, but complete sets of measurements were obtained for only thirty, because of twisting of the preparations. The vulcanite chamber was used for these experiments so that observations could be continued while the medium was being changed. The complete replacement of the fluid in
the chamber was effected in 10 sec. § F.R. was used as the isotonic medium and 30 % § F.R. as the hypotonic medium.

There was a general swelling of the cells before the appearance of the vesicles, which usually continued as the vesicles were formed, but in a few preparations the marginal cells increased in size up to the time of the appearance of the vesicles and then shrank to their original size as the vesicle appeared. In these cases the cell appeared to extrude excess water into the vesicle. After a few minutes the size of the vesicles and of the cells began to decrease again. At maximal swelling the linear dimensions of the cells had increased by 20–30 %. In thirteen experiments acceleration of the cilia occurred before swelling, in five experiments swelling
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occurred first, and in five others acceleration and swelling were simultaneous. Of the remaining seven experiments, in one no acceleration occurred and in the other six the measurements were not made at exactly the right moment to be certain which event occurred first. Ciliary accommodation took place either while the cells were still swelling or while the swelling was at a maximum. From these results it may be concluded that variations in ciliary activity are not directly related to the intracellular water content. Wells et al. (1940) reached the same conclusion for Mytilus gill.

In seventeen experiments the preparations were returned to 100% § F.R. The activity of the cilia was reduced and the cells shrank and became even smaller than they were originally. This suggests that the regulating process involves ion as well as water transference, which would explain the differences in the effects of dilution with § F.R. and E.S. As in the change to a more dilute medium, there was no constant time relationship between the shrinking of the cells and reduction of ciliary activity.

(4) The relative importance of absolute concentration and concentration change

Pilgrim (1953) found that the ratio final/initial concentration, rather than the final concentration or the arithmetic difference between initial and final concentration, was the effective factor producing the dilution effects in Mytilus gill cilia.

An attempt was made to establish whether this is true for the nephridiostome cilia of L. terrestris by comparing the effects of changing to a series of low concentrations from 100% and from 150% E.S. The effects are clearly not due to the arithmetic difference between the concentrations, because a change from 100 to 30% (a difference of 70) gives rise to shock effects, whereas a change from 150 to 80% (also a difference of 70) produces no shock effects. As, however, the duration of the shock effect is very variable in earthworms even when the same salinity change is repeated, no further conclusions can be drawn from these results.

(5) Axial gradients

Kopenhaver (1937) demonstrated than an axial gradient in the water-absorbing properties of the body wall exists in L. terrestris and A. caliginosa. Since there was a possibility that such a difference might exist also in the nephridiostomes in L. terrestris these were taken from the anterior and the posterior regions of the body and compared. No differences were found between them when they were subjected to a sudden change of medium from 100 to 30% § F.R.

IV. SUMMARY

1. Suitable media have been found for maintaining the activity of the nephridiostome cilia of two earthworm species for about 24 hr. at 18° C. or below.

2. The nephridiostome cilia of Lumbricus terrestris are less resistant to hypotonic media, and more resistant to hypertonic media, than those of Alolobophora chlorotica.
3. When the medium is suddenly diluted, the nephridiostome cilia of *L. terrestris* and *A. chlorotica* have a period of hyperactivity and/or increased amplitude of the beat followed by a return to normal activity. The temporary effect after a change to a hypertonic medium takes the form of decreased activity or inhibition.

4. In hypotonic solutions, vesicles are formed on the marginal cells and later disappear provided the dilution has not been too great.

5. A change in the osmotic concentration of the medium is responsible for the vesicles and the temporary effects on the cilia, whereas the inorganic ion concentration is responsible for the long-term effects.

6. There is no axial gradient affecting the activity of the nephridiostome cilia in relation to the osmotic concentration of the medium.

7. No differences were detected between garden and Lake Windermere specimens of *A. chlorotica*.

I wish to thank Prof. G. P. Wells, F.R.S., for his advice, criticism, encouragement and supervision of the investigation; also the Committee of University College, London, for the Margaret Browne Studentship which made the work possible.

REFERENCES


