THE OSMOTIC RELATIONS OF THE EARTHWORM

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(With Two Text-figures)

I. INTRODUCTION

Interest in the water-relations of the earthworm appears to date from the work of Overton in 1904. In his paper 'Neununddreissig Thesen über die Wasserökonomie der Amphibien und die osmotischen Eigenschaften der Amphibienhaut' one 'thesis', the last, is devoted to the earthworm. He observed that an earthworm suspended in air lost water by evaporation and that when the partially dehydrated worm was placed in a U-tube of water with the mouth and anus above the surface, water was absorbed. He concluded that the surface of the animal was readily permeable to water in both directions and from this he argued that the water entering under osmotic forces must be got rid of by the excretory organs; such activity on the part of the nephridia he also claimed to have observed.

In 1925 Adolph & Adolph included the earthworm in an investigation of fresh-water animals which had as its object a study of the regulation of body volume. Their experiments consisted mainly in weighing worms at intervals after immersion in various solutions. No direct measurements were made of the osmotic pressure of the body fluids, but from the weight changes and from changes in the chloride content of the medium they calculated corresponding changes in internal osmotic pressure. They demonstrated that the initial weight changes, following transference from one medium to another, could be accounted for in general by the movement of water under osmotic forces, and that these initial changes were followed by slower changes under which the animal tended to return to its original weight at the beginning of the experiment. This work was continued by Adolph (1927), who gave the following picture of the earthworm's water-relations. Water enters by the skin and leaves by the intestine, there being no evidence that the nephridia are concerned: the exchanges of water which take place between the body fluids and external solutions are mediated entirely by the skin, the gut output being adjusted according to supply: water does not 'leak' into the worm under the influence of higher internal osmotic pressure because the skin is the seat of 'osmotic forces' which oppose this tendency.

Both Overton and Adolph observed that, on handling incidental to weighing, weight was lost by the worm. Wolf (1940) made a special study of this point and concluded that by far the greater part of this loss was due to the expulsion of fluid from the nephridiopores as originally suggested by Overton. Wolf did not commit himself to any comprehensive interpretation of water exchanges in the earthworm. Adolph (1943) accepted Wolf's conclusion that the loss of water was mainly via the nephridiopores without any further comment upon his own conclusions of 1927.

Prior to the appearance of Wolf's paper, Maluf (1939) described an investigation of the water-relations of the earthworm. Most of his experiments consisted in following changes in the weight of the worm, kept in water or in solutions, after various ligatures had been applied. He made the further important observation that the worm could take up chloride from very dilute solutions, even with both ends ligated, which implied that this active uptake was via the skin and not via the gut. Maluf took the view that water entered the worm by diffusion through the skin under osmotic forces, that it was stored in the gut and that it was got rid of through the nephridia and through the gut; he assumed that the fluids leaving these organs were hypotonic to the body fluids, but he did not verify this assumption by any measurements of osmotic pressure; he believed the gut to be of paramount importance in osmotic and volume regulation, as it were coming to the aid of the nephridia when the task was beyond their powers. In a later paper (1940), having measured the osmotic pressure of the gut fluids and found it to be only slightly less than that of the body fluids, Maluf retracted his earlier statement that the gut played an active part in osmotic regulation and returned to the view that the nephridia alone were responsible for getting rid of the excess of water and that they presumably must excrete an hypotonic fluid, as originally proposed by Overton.

The osmotic relations of the earthworm were also investigated by Stephenson (1945), who measured chloride content and conductivity of the body fluid and also changes in weight when the worms were kept in various solutions. He drew attention to the fact that volume changes on the one hand and chloride (and conductivity) changes on the other were not intimately correlated as Adolph had supposed. He further showed that, in addition to maintaining a higher internal chloride concentration in dilute solutions, the earthworm was able to maintain a lower internal chloride concentration in concentrated solutions, and this led him to the view that 'the earthworm is certainly no longer a fresh-water animal in so far as its osmotic relationships with the environment are concerned'—a view not shared by other workers in this field.

In a preliminary notice (Ramsay, 1946) it was reported that urine had been collected directly from the nephridiopores of the earthworm and had been found to be strongly hypotonic to the coelomic fluid. These preliminary observations have now been confirmed and extended and are described in more detail in the present paper. Although this work has had as its ultimate object an investigation of the physiology of nephridia, it was felt that this investigation could be undertaken with more confidence if some of the controversial issues regarding the earthworm's water balance could first be settled. Accordingly, some of the lines of study pursued by Stephenson were re-opened, with results now to be reported.

II. MATERIAL AND METHODS

For this work only worms of the species *Lumbricus terrestris* L. were used. I am indebted to Dr A. C. Evans of Rothamsted for instruction in the correct recognition of *L. terrestris* from among the other species with which it is liable to be confused.

For collection of the body fluids it was usual to have the worm lightly anaesthetized by short exposure to 15% alcohol and to pin it out on a board when it had become motionless and flaccid. The various fluids were allowed to rise into pipettes of pyrex glass, approximately 1 mm. in diameter, and coated with paraffin-wax on the outside. Collection of the coelomic fluid presented no difficulties. For collection of blood the pipette was inserted into the dorsal vessel and when this was successfully accomplished the blood would surge up into the pipette at each contraction of the dorsal vessel.

The collection of urine involved a more elaborate procedure. It is a simple matter to insert a pipette into the nephridiopore of an earthworm which is at rest, but an active worm loses little time in getting rid of it. The collection of urine from a single

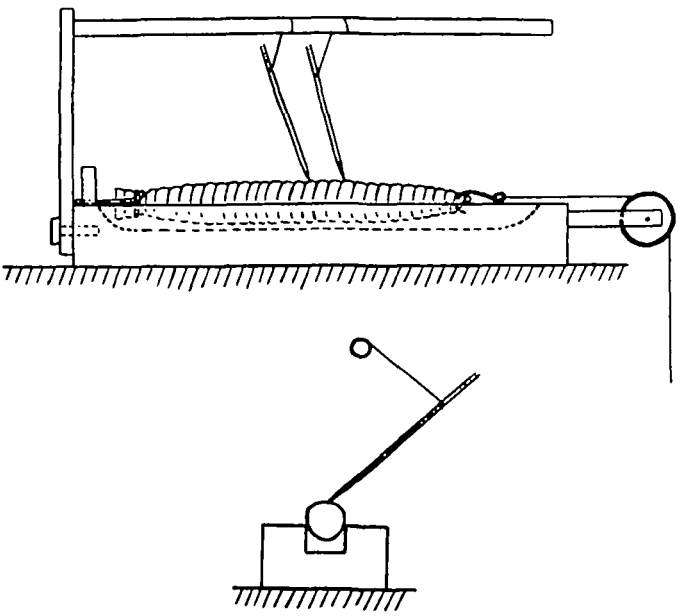

Fig. 1.

nephridiopore in sufficient quantity for analysis takes 1 hr. or more and there is reason to believe that changes occur in the urine of worms which suffer prolonged exposure to anaesthetics. It is not altogether easy, without extensive injury to the worm, to impose restraints which will keep it still. The method finally adopted was as follows. A ligature was applied around the clitellum and the posterior part of the worm was cut away. The anterior part was laid out on its side in a narrow shallow trough (Fig. 1). It was held at the posterior end by a pin passing through the clitellum and anteriorly a hook was passed through the peristomium and connected to a weight over a pulley. The effect of this was to keep the worm normally fully extended, but to allow it to shorten in response to a powerful contraction which, if resisted, would result in tearing out the attachments. With the worm thus lying on its side and extended, the dorsolateral openings of the nephridiopores were in a favourable position.

The pipettes were drawn to fine (15 μ) points from 0·5 mm. diameter pyrex tubing; they were varnished on the outside with bakelite so as to give a surface upon which a layer of wax could adhere firmly. The tips were dipped in molten paraffin-wax and a short length of very fine cotton was attached to the other end with sealing-wax. Above the trough was a brass rod, covered with plasticine. When a pipette was taken for use the cotton thread was laid over the plasticine and pressed into it. The pipette was then taken in forceps and (under a low power binocular) was inserted into a nephridiopore, where it remained suspended obliquely by the thread and pressing into the nephridiopore under its own weight. Since it conformed freely to the movements of the worm it was not readily dislodged. Several pipettes could be mounted in this way upon the same worm.

During the process of insertion the pipette was liable to become choked with mucus and when this happened it had to be discarded. After a successful insertion a small quantity of urine rose into the pipette, but the accumulation of urine was not continuous. Light scratching of the skin with a needle usually resulted in a local relaxation of both longitudinal and circular muscles and at the same time the urine could be observed to rise in the pipette. The preparation was covered with a perspex box (to prevent desiccation) and at intervals of about 20 min. the response to scratching was observed. The collection was continued for as long as urine continued to rise in the pipette in response to the stimulus, but after it had failed to do so on three successive occasions the pipette was withdrawn. A yield of 1 cu.mm. from a single nephridiopore was above the average; this was enough for two determinations of vapour pressure or for two determinations of chloride, or for one of each, but where smaller quantities were obtained they were usually pooled.

It was not generally feasible to carry out the analysis of fluids immediately after collection. For preservation over periods of a few hours or up to 2 days the two ends of the pipette were sealed off with paraffin-wax and the pipette was kept in a refrigerator. For preservation over longer periods the two ends were sealed off by fusion and the contents were sterilized by immersing the pipette in boiling glycerine (120° C.). This naturally had the effect of coagulating the proteins and the coagulum had to be separated by centrifuging. In the case of urine and coelomic fluid the coagulum is either non-existent or very slight. In the case of blood it is always very extensive and only a small proportion of clear fluid can be separated by centrifuging. The effect of this treatment upon the vapour pressure of coelomic fluid and blood and upon the chloride content of coelomic fluid was tested and was not found to be significant; in the case of blood no determination of chloride was possible on untreated samples.

Osmotic pressure was measured as vapour pressure by the Hill-Baldes thermoelectric method (Baldes & Johnson, 1939) and chloride by the micro-method of Wigglesworth (1937). In a few cases, where stated, use was made of a freezing-point method for osmotic pressure (see Table 2). The accuracy of these determinations varied according to the amount of fluid available for repetition of the measurements; where possible four measurements were made of vapour pressure and two of chloride on each sample and such results are believed to be accurate to $\pm 0{\cdot}02\%$ NaCl.

Both osmotic pressure and chloride content are expressed as that concentration of NaCl%, which exerts the same osmotic pressure or has the same chloride content; and in this connection a 1% solution of NaCl is understood to be 1 g. NaCl dissolved in water and made up to 100 c.c. In converting osmotic pressures to freezing-point depression for comparison with the results of others the relation 1% NaCl ≡ 0·585° C. △ has been used.

The media used in the experiments to be described were various concentrations and dilutions of frog ringer, of osmotic pressure varying between 0·0065 and 1·44% NaCl; tap water, equivalent to 0·02% NaCl, was also used. The vessels containing the worms were placed in a constant temperature room at 17° C. and aerated continuously. Under these conditions, and provided that the osmotic pressure of the medium lay between 0·01 and 1·0% NaCl, the worms survived well, less than 10% of deaths occurring in 3 weeks; as these limits were departed from the time of survival became progressively shorter.

III. RESULTS

(*a*) *Osmotic pressure and chloride content of the coelomic fluid in relation to the medium*

After a change of medium the osmotic pressure and chloride content of the coelomic fluid become practically constant at their new value in 3 or 4 days. Table 1 gives the results of an experiment in which worms were transferred from tap water to

Table 1. *Worms were placed in tap water in constant temperature room on* 6 *January* 1948: *transferred to frog ringer* ≡ 0·75% NaCl *on* 20 *January* 1948: *returned to tap water on* 3 *February* 1948

Date	(1)		(2)		(3)		(4)		Average	
	O.P.	Cl	O.P.	Cl	O.P.	Cl	O.P.	Cl	O.P.	Cl
21. i. 48	0·75	0·45	0·74	0·43	0·77	—	0·78	0·44	0·76	0·44
23. i. 48	0·88	0·56	0·90	0·56	0·83	0·47	0·91	–	0·88	0·53
29. i. 48	0·90	0·49	0·94	0·54	0·97	0·53	0·97	0·46	0·94	0·50
26. i. 48	0·95	0·53	0·99	0·48	0·96	0·50	0·97	0 49	0·97	0·50
2. ii. 48	0·90	0·46	0·95	0·44	0·90	0·45	1·02	0·49	0·94	0·46
4. ii. 48	0·60	0·29	0·61	0·34	0·57	0·29	0·57	0·31	0·59	0·31
6. ii. 48	0·54	0·30	0·52	0·22	0·63	0·27	0·59	0·26	0·57	0·26
9. ii. 48	0·57	0·36	0·51	0·29	0·66	0·32	0·52	0·29	0·56	0·31
Controls in tap-water										
20. i. 48	0·50	0·27	0·54	0·24	0·56	0·26	0·55	0·26	0·54	0·26
2. ii. 48	0·66	0·31	0·59	0·27	0·56	0·29	0·59	0·28	0·60	0·29
9. ii. 48	0·52	0·25	0·52	0·23	0·54	0·25	0·58	0·25	0·54	0·24

frog ringer ≡ 0·75% NaCl and kept in this medium for 14 days before being returned to tap water. Four worms were taken at a time. Four determinations of vapour pressure and two of chloride were made on the coelomic fluid of each worm and the average values (accurate to ±0·02% NaCl) of these measurements are listed in the table. These values for individual worms are reproduced to show the range of variation from one worm to another. The average values for each set of four worms indicate the rate at which adjustment to the new medium proceeds.

Fig. 2 summarizes the results of various experiments with different media in which the worms were allowed to remain for at least 7 days before the coelomic fluid was collected for analysis. From this figure the osmotic pressure of worms kept in tap-water is seen to be 0·53% NaCl. This corresponds to a freezing-point depression of $\Delta = 0{\cdot}31°$C. which is exactly the value given by Adolph (1927) for worms kept in tap-water; it is not in good agreement with the results of Maluf who gives $\Delta = 0{\cdot}45°$C. (1939) and $\Delta = 0{\cdot}59°$C. (1940).

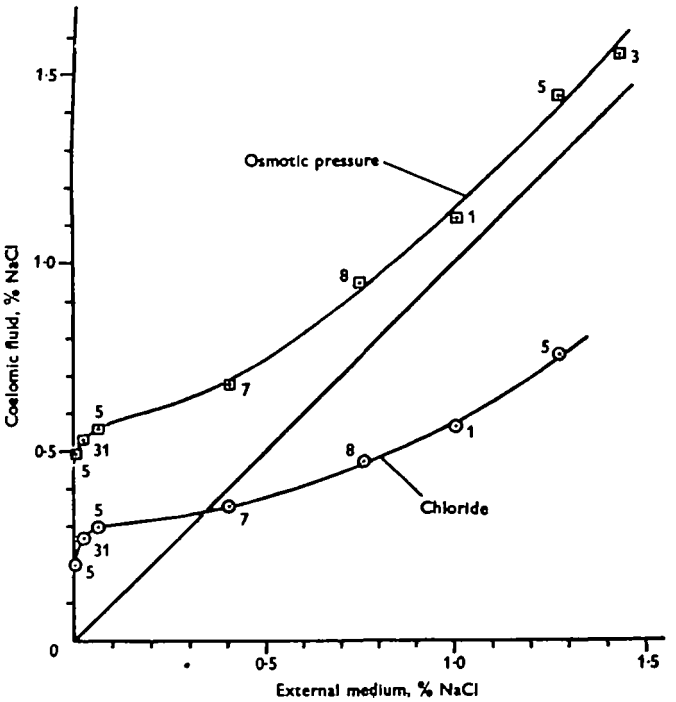

Fig. 2.

As the osmotic pressure of the medium is increased, the osmotic pressure of the coelomic fluid also increases and is always in excess of that of the medium. On the other hand, in media equivalent to 0·35% NaCl or over, the chloride content of the coelomic fluid is less than that of the medium, as Stephenson found; the chloride values given in Fig. 2 are in very close agreement with the values given by Stephenson. It is clear that the earthworm has no powers of osmotic regulation in concentrated media, such as the chloride relations, unsupported by measurements of osmotic pressure, might seem to suggest.

(*b*) *Osmotic pressure and chloride content of the urine in relation to the medium*

The observations under this heading are assembled in Table 2. The values for osmotic pressure and for chloride show considerable variation from one collection to another, even from the same worm—more, in fact, than can be accounted for as

Table 2

			Urine		Coelomic fluid (from Fig. 2)	
Medium (% NaCl)	Date	Worm	O.P.	Cl	O.P.	Cl
Tap water ≅ 0·025	18. vi. 46	*A*	0·10	—		
			0·12	—		
	20. vi. 46	*A*	0·06	—		
			0·05	—		
	28. vi. 46	*A*	0·19	—		
			0·14	—		
	30. vi. 46	*A*	0·08	—		
	6. iv. 47	*A*	0·06	0·01		
		B	0·07	0·04		
		C	0·07	0·03		
	25. ii. 48	*A*	0·15	0·01		
		B	0·14	0·02		
			0·05	0·01		
		C	0·09	0·05		
	26. ii. 48	*A*	—	0·03		
		B	—	0·02		
	Average		0·10	0·02	0·53	0·27
Ringer ≅ 0·65	26. v. 47	*A*	0·24	—		
			0·28	—		
			—	0·12		
		B	0·14	0·04		
			—	0·05		
	27. v. 47	*A*	0·16	0·10		
			0·20	—		
			0·22	—		
			0·38	—		
		B	0·19	—		
			—	0·19		
	Average		0·23	0·10	0·85	0·43
Ringer ≅ 0·75	19. xii. 46	*A*	0·30	—		
		B	0·40	—		
	20. xii. 46	*A*	0·35	—		
	12. vi. 47	*A*	—	0·06		
	13. vi. 47	*A*	—	0·21		
	3. ii. 48*	*A*	0·41	0·22		
	4. ii. 48*	*A*	0·27	0·15		
	5. ii. 48*	*A*	0·17	0·13		
	7. ii. 48*	*A*	0·22	0·09		
		B	0·27	—		
	Average		0·30	0·14	0·95	0·48
Ringer ≅ 1·27	28. ii. 48*	*A*	1·38	0·50		
	3. iii. 48*	*A*	1·36	—		
	Average		1·37	0·50	1·40	(0·75)

* Freezing-point method used.

errors of analysis (which are ±0·04% NaCl for osmotic pressure and ±0·03% NaCl for chloride where only one measurement of each can be made). The average values, however, show quite clearly that in tap water the urine has an osmotic pressure of 0·10% NaCl, being strongly hypotonic to the coelomic fluid (as previously shown) and that the fraction attributable to chloride is so small as scarcely to be measurable. It is conceivable that the non-chloride matter in the urine represents the animal's normal nitrogenous excretion. From Wolf's results the urine production may be taken as 60 c.c./100 g. worm/24 hr. If nitrogen is present as two atoms of nitrogen per molecule of excretory matter, the total nitrogen excreted works out as 5·4 mg. N/100 g. worm/24 hr. Lesser (1908) gives a figure of 6 mg. N/100 g. worm/24 hr. But in experiments in which nitrogenous excretion is determined by analysis of the water in which the animal has been living one cannot be certain that all the nitrogen does, in fact, pass out by way of the nephridia. The view that the non-chloride matter in the urine may be accounted for as normal nitrogenous excretion is therefore put forward with reserve.

In media equivalent to 0·65 and 0·75% NaCl the urine, although more concentrated than in tap water, is still strongly hypotonic to the coelomic fluid, and of a total osmotic pressure of 0·3% NaCl about half is due to chloride which now makes its appearance in significant concentration. In media equivalent to 1·27% NaCl the urine seems to be almost isotonic with the coelomic fluid, but in view of the small number of observations this result must be accepted with caution.

It was very noticeable that the amount of urine obtainable from worms kept in frog ringer, especially in the higher concentrations, was much less than for worms kept in tap water. Collections from several nephridiopores had to be pooled and even then it was not always possible to determine both osmotic pressure and chloride on the same sample. For 3 or 4 days after the worms had been transferred to a concentrated medium, e.g. equivalent to 0·75% NaCl, no urine whatever could be collected, but after a fortnight enough was available to make the attempt worth while. In still higher concentrations, e.g. equivalent to 1·27% NaCl, worms seldom survived as long as a fortnight and in spite of much effort in this direction only two collections were obtained from worms in such concentrated media.

(*c*) *Osmotic pressure and chloride content of the blood in relation to the coelomic fluid*

The osmotic pressures of the blood and of the coelomic fluid were compared in thirty-one cases in which the worms had been freshly taken from soil, or had been kept in artificial media, or had been subjected to special treatment. In four cases the osmotic pressures were identical; in all other cases the osmotic pressure of the blood was slightly lower, the average difference being 0·053% NaCl. As already mentioned, the chloride of the blood can only be determined when the haemoglobin has been coagulated and removed; and it is difficult to separate clear fluid from the clot in quantities sufficient for analysis. In two cases only was the blood chloride determined; as compared with the coelomic fluid the blood chloride was found to be lower by 0·01% NaCl in one case and by 0·03% NaCl in the other.

The difference in osmotic pressure is significant in relation to the accuracy of analysis; the difference in chloride is barely so. It is conceivable that when water enters through the skin it first enters the blood-stream and then diffuses into the coelomic fluid, and if this were so the lower osmotic pressure of the blood might be accounted for. Attempts were made to abolish or exaggerate the difference by collecting these fluids during exposure to desiccation and shortly after the return of desiccated worms to water; but no significant changes in the osmotic difference were observed as a result of such treatment.

Particular attention was paid to this point since Bahl (1945) has shown that in *Pheretima* the osmotic pressure of the blood ($\Delta = 0{\cdot}40$–$0{\cdot}50°$C.) is considerably higher than the osmotic pressure of the coelomic fluid ($\Delta = 0{\cdot}285$–$0{\cdot}31°$C.). A difference of this order is astonishing and appears to be unique in the animal kingdom.

IV. DISCUSSION

A few animals normally living in fresh water are able to survive transference to sea water (e.g. the eel) and in such animals it is found that the body fluids are hypertonic to the medium when this is fresh water and hypotonic to the medium when this is sea water. But in most fresh-water animals, as the osmotic pressure of the medium is raised, the osmotic pressure of the body fluids rises also until the limit of toleration is reached; the body fluids are never hypotonic to the medium. In this the earthworm is no exception. Nevertheless, although the osmotic pressure of the coelomic fluid is always greater than that of the medium, the chloride of the coelomic fluid may be less than that of the medium, as is the case in media equivalent to 0·35% NaCl or over. This is not without its parallel in other fresh-water animals; the larva of *Aedes aegyptii*, studied by Wigglesworth (1938), shows precisely this relationship. It is also characteristic of fresh-water animals that they eliminate urine which is hypotonic to the body fluids and are able to make good loss of salts by absorption from very dilute solutions; in the case of the earthworm the production of hypotonic urine, reported previously, has now been confirmed, and the ability to take up chloride from very dilute solutions has been demonstrated by Maluf (1939). By indirect methods Wolf (1940) has estimated the rate of urine production as approximately 60% of the body weight in 24 hr., which may be compared with the corresponding figures of up to 30% for fresh-water fishes and an average of 0·5% for marine fishes (see Krogh, 1938). In all these respects the earthworm behaves like a fresh-water animal and there seems to be little reason for doubt on this matter.

It is perhaps worth while to point out that the ability of the worm to maintain the chloride of its coelomic fluid below that of the medium does not require us to postulate an active process of salt excretion against a concentration gradient. In media equivalent to 0·35% NaCl or over salt will no doubt tend to diffuse in, possibly aided by the process of active uptake which is known to occur in more dilute media. But since the internal osmotic pressure is always greater than that of the medium, water will also tend to enter; and further, since living tissues are in general

very much more permeable to water than to salts, it may well be that the net effect of this entry of salt and water is to dilute the coelomic fluid. Under such conditions the worm will be able to maintain the chloride of the coelomic fluid lower than that of the medium and at the same time will be able to excrete an hypotonic urine.

No important body of facts is in conflict with the view that under all the conditions herein considered (1) water enters passively through the skin with the osmotic gradient, (2) salt enters through the skin, either passively with the concentration gradient (as in concentrated media) or by active absorption against the concentration gradient (as in dilute media) and (3) an hypotonic urine is eliminated, involving an active process of secretion against a concentration gradient. It does not seem to be necessary to postulate any other active processes involving osmotic work. The outlines of the picture are attractively simple but it is unlikely that this simplicity will be maintained when the details come to be filled in. We assume, for example, that the difference in osmotic pressure is the force which causes water to pass through the skin; but Adolph has stated (1927) and re-affirmed (1943) that when a desiccated earthworm is allowed to recover in water 'the rate of intake increases many fold at deficits that do not even double the believed osmotic pressure of the body fluids'. Again, Stephenson has investigated the relation between body volume and the osmotic pressure of the medium. It appears that the body volume is a maximum in a medium equivalent to 0·35% NaCl and declines as the medium becomes more concentrated or more dilute. This observation is difficult to reconcile with a simple conception of the water relations such as has been outlined above.

These and other anomalies must be borne in mind, but until more facts are available it does not seem profitable to discuss them in detail.

V. SUMMARY

1. Osmotic pressure and chloride concentration have been determined for the coelomic fluid, blood and urine of earthworms kept in various saline media.

2. About 50% of the osmotic pressure of the coelomic fluid and of the blood can be accounted for as chloride. The blood is very slightly hypotonic to the coelomic fluid.

3. As the concentration of the medium is increased the osmotic pressure of the body fluids also increases and is always greater than that of the medium; the chloride increases proportionately, but is less than that of the medium when the latter exceeds 0·35% NaCl.

4. The urine is always strongly hypotonic to the body fluids except possibly in the most concentrated media (over 1·0% NaCl).

5. The osmotic relations of the earthworm are such as are characteristic of fresh-water animals generally.

I wish to record my thanks to Mr J. L. Parkinson for supplying me with two vapour pressure thermocouples and for advice on the construction of these instruments. I also wish to thank Dr S. Smith and Dr G. B. B. M. Sutherland, each of whom kindly lent a galvanometer. Nearly all the measurements of vapour pressure in this work were carried out by my assistant Miss J. Gukenbiehl.

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