THE SITE OF FORMATION OF HYPOTONIC URINE IN THE NEPHRIDIUM OF *LUMBRICUS*

By J. A. RAMSAY, *From the Zoological Laboratory, University of Cambridge*

(*Received* 16 *October* 1948)

(With Three Text-figures)

I. INTRODUCTION

The structure of the nephridium of *Lumbricus* is known in some detail. The first adequate description is that of Gegenbaur (1853) and is correct in essentials. Goehlich's (1890) description is less accurate, as is pointed out by Benham (1891) who contributed further details of the structure of the nephridiostome and of the histology of the canal. Maziarski (1901, 1905) made a further study of histology; I have not had access to his second paper but it is summarized in the review by Meisenheimer (1910). Rosen (1911) re-described the nephridiostome but not altogether satisfactorily; the most recent and most accurate description is by Goodrich (1932).

Although these accounts are substantially in agreement there is some confusion in the names given to different parts of the nephridium and it is therefore necessary to give a brief description in order to define the terms to be used in this paper (see also Fig. 1).

The nephridium opens internally by the *nephridiostome* (Goodrich prefers this word to the more usual 'nephrostome'). Details of the structure of the nephridiostome are not relevant to the work described in this paper. The nephridiostome is followed by the *narrow tube* which is about 30μ in diameter, thin-walled and ciliated over certain regions. It traverses the first loop, then the second loop, then traverses the first loop a second time in the reverse direction. At the base of the first loop it is succeeded by the *middle tube*. The lumen suddenly enlarges to about 60μ or more; the walls become much thicker, bear cilia and have brown granules in the cytoplasm. The middle tube runs from the base of the second loop almost to its tip, gradually decreasing in diameter to about 20μ, and then opens into the *wide tube*. In the wide tube four regions can be distinguished. At its commencement it shows a wide dilatation, up to 100μ or more in diameter, and bends around the tip of the second loop; this is known as the *ampulla*. It is not histologically distinguishable from the succeeding region, known as the *proximal segment*, which is characterized by having thick walls with radial striations in the basal portion of the cytoplasm and what appears to be a brush border adjacent to the lumen. The wide tube traverses the second loop from tip to base and during this part of its course it decreases in diameter, its walls become thinner, and in histological features it shows a gradual reversion towards the narrow tube. This narrower portion of the

wide tube may be spoken of as the *middle segment*, but it is not sharply demarcated from the proximal segment; it leaves the second loop and traverses the first loop. At the base of the first loop there is a sudden transition to the *distal segment*, about 70μ in diameter, with thicker walls showing the same basal striations as the proximal segment but lacking the brush border. The distal segment describes a wide sweep around the ventral part of the nephridium and enters upon the third loop. About half-way towards the tip of the third loop it opens into the *bladder*, but not at the point where the diameter of the canal appears to enlarge; the distal segment continues

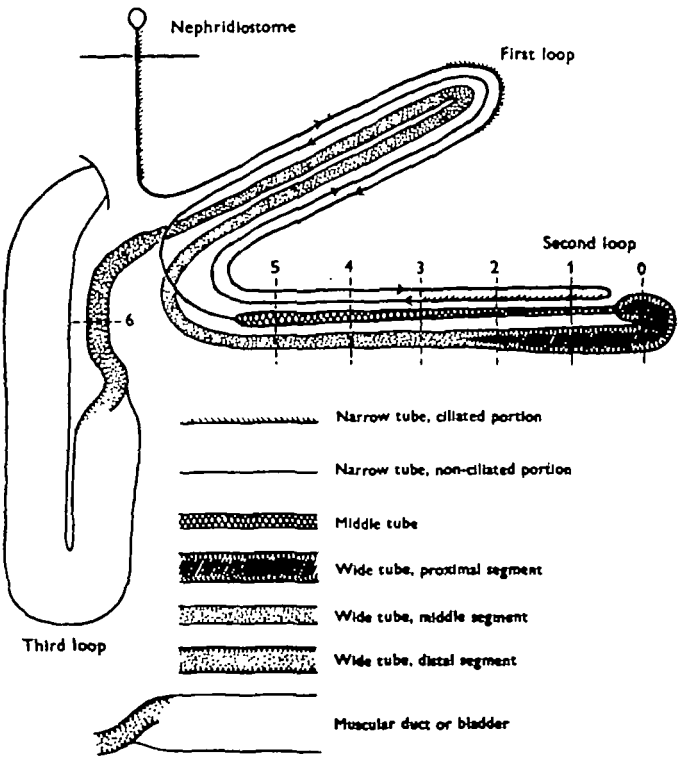

Fig. 1. Diagram of the nephridium of *Lumbricus* (from the account by Meisenheimer). For reasons of clarity the loops are shown displaced from their normal positions. The numbers indicate certain positions at which samples were collected, and are referred to in the text.

for a short distance in the wall of the bladder, opens out into a gutter and ends. The wall of the bladder is provided with a very thin epithelial lining surrounded by muscle fibres. The bladder completes the third loop and enters the body-wall under the ventral part of the nephridium to open eventually at the *nephridiopore* which may be either dorsolateral or ventrolateral in position. The whole organ is covered with a layer of peritoneal epithelium which forms a membrane uniting the three loops. The whole of the canal with the exception of the bladder is intracellular.

About the function of the nephridium less is known. By collecting directly from the nephridiopores it has been shown that the nephridium eliminates considerable

quantities of urine which is strongly hypotonic to the body fluids; the nephridium therefore plays an active part in water-balance (Ramsay, 1946, 1949*a*). In the latter paper the work of others bearing upon the problem of water-balance is referred to and discussed, and there is no need to summarize it here.

Apart from water-balance, physiological work is almost entirely restricted to the study of 'athrophagocytosis' which occurs in the middle tube. Contributions were made by Kowalevsky (1889), Schneider (1896), Cuenot (1898), Willem and Minné (1900) and Maziarski (1905); it was established that dyes and other substances, injected into the coelom, accumulated in the middle tube, but whether they reached that position directly from the coelomic fluid (elimination) or by being removed from the urine in its passage down the canal (resorption) could not be decided. Cordier (1933, 1934) carried out a more extensive series of observations, using colloidal particles of known size, and by analogy with similar work on the amphibian kidney he took the view that the process was one of resorption. Cordier's work will be considered in more detail in § IV of this paper.

The information available for other Oligochaeta is admirably reviewed by Bahl (1947). The only other genus for which comparable information is available is *Pheretima*, which Bahl himself has studied. *Pheretima* possesses three kinds of nephridia: (1) open septal nephridia, discharging into the intestine, (2) closed integumentary nephridia, discharging to the exterior, (3) closed tufted nephridia, discharging into the pharynx. These organs have been fully described and biochemical analyses have been carried out upon the blood, coelomic fluid and urine. From a comparison of these analyses Bahl concludes that the coelomic fluid enters the nephridial canal, that during its passage to the exterior potentially valuable constituents are resorbed and that an hypotonic urine is eliminated.

It is clear that Bahl's views about the nephridia of *Pheretima* have much in common with the Ludwig-Cushny filtration-resorption theory of the vertebrate kidney; and when one reflects upon the outstanding contribution made to this field by Richards and his associates one cannot but wonder whether similar methods of micro-sampling and micro-analysis might be applicable to the nephridium. In work of this kind the importance of choosing suitable material can hardly be overestimated. From the reviews of Bahl (1947) and of Goodrich (1945) it clearly emerges that *Lumbricus* offers many advantages as compared with other Oligochaeta such as *Pheretima*; in addition to being readily procurable, *Lumbricus* has nephridia all of the same kind, opening separately to the exterior, of relatively large size, accessible in dissection and adequately described. Accordingly, it was decided to proceed with the investigation of *Lumbricus* in an attempt to discover the site of formation of hypotonic urine.

With this purpose in mind a search was made for a method of measuring osmotic pressure on quantities of fluid considerably smaller than those required by existing methods. This led to the development of a method of freezing-point determination applicable to quantities of the order of 10^{-6} ml. or less. This method is described in another paper (Ramsay, 1946*b*). The present paper describes its application to the nephridium of *Lumbricus*.

II. MATERIALS AND METHODS

Throughout this work full-grown specimens of *Lumbricus terrestris* L. have been used. The worm is opened and pinned out in a dissecting dish under frog ringer in various concentrations and dilutions. The membrane connecting the loops of the nephridium is cut through as necessary and the whole organ excluding the nephridiostome is cut out and transferred to a microscope slide. Excess ringer is quickly drained and blotted and the preparation is at once covered with a layer of liquid paraffin which prevents it from drying up.

The collecting pipettes are drawn from thin-walled 'Vitreosil' silica tubing of about 1 mm. diameter with a steep taper and with openings varying from 5μ for the narrow tube to 20μ for the bladder. The pipette is fixed with sealing-wax to a wider glass tube connected through pressure tubing to a mercury reservoir and screw-plunger. The mercury is driven to the tip and then withdrawn under liquid paraffin which is allowed to fill the tapering portion. The glass tube carrying the pipette is mounted on a micro-manipulator.

The most serious difficulty is that of holding the organ steady while the pipette is being inserted. The nephridial canal is very tough and very extensible and penetration is only possible if it is held firmly and close to the point of insertion. In the case of collections made from the various parts of the canal represented in the second loop it is possible to press down upon the loop with a glass needle, held in a micro-manipulator, and to insert the pipette as shown in Fig. 2*a*, *b*. In the case of the distal segment of the wide tube this method of holding is inadequate; the force which has to be applied to make the pipette penetrate is great enough to drag the tissues under the needle. In order to hold the tube steady a wool-fibre is tied around it, the knot serving to prevent the tissues from being drawn under the needle. In addition it is necessary to use a slide with a cover-glass cemented to it so that the edge of the cover-glass can be used to support the needle (Fig. 2*c*, *d*). In the case of the bladder it is also necessary to apply a ligature in order to retain the fluid when the organ is excised; the ligature is applied around the middle of the third loop which is then cut between the ligature and the base of the loop.

The process of penetration is observed under a $\frac{2}{3}$ in. objective. When it has been successfully accomplished the tip of the pipette is very carefully adjusted so as to lie in the middle of the lumen. On releasing the pressure on the mercury the fluid rises very rapidly into the pipette and the walls of the canal collapse together. After the collapse of the walls the fluid ceases to rise in the pipette, showing that the seal is effective and that the outside medium does not enter the canal through the puncture. The pressure of the mercury is then raised slowly and carefully until the fluid in the pipette shows a tendency to return into the canal. The pipette is then withdrawn, raised into the paraffin layer, and paraffin is drawn in so that the sample is enclosed in a column of paraffin.

Satisfactory penetration cannot be made when there is insufficient pressure in the canal to keep the lumen open and this is more often than not the case with the middle tube and the distal segment of the wide tube. Even when penetration has

been successful it is frequently found that the opening of the pipette has become blocked. Of all parts of the canal the ampulla is the easiest to deal with since the lumen is wide and the walls are transparent; about 80% of attempts on the ampulla are successful. The bladder by reason of its large size is easy to penetrate, but the muscle fibres in its wall often make it difficult to see the tip of the pipette and it is possible to imagine that the pipette has penetrated the bladder when in fact it has merely passed under a fold of the membrane which unites the loops. The narrow tube is more difficult, partly because of its smaller diameter and partly because its course, even in the second loop, is somewhat contorted; about 50% of attempts are

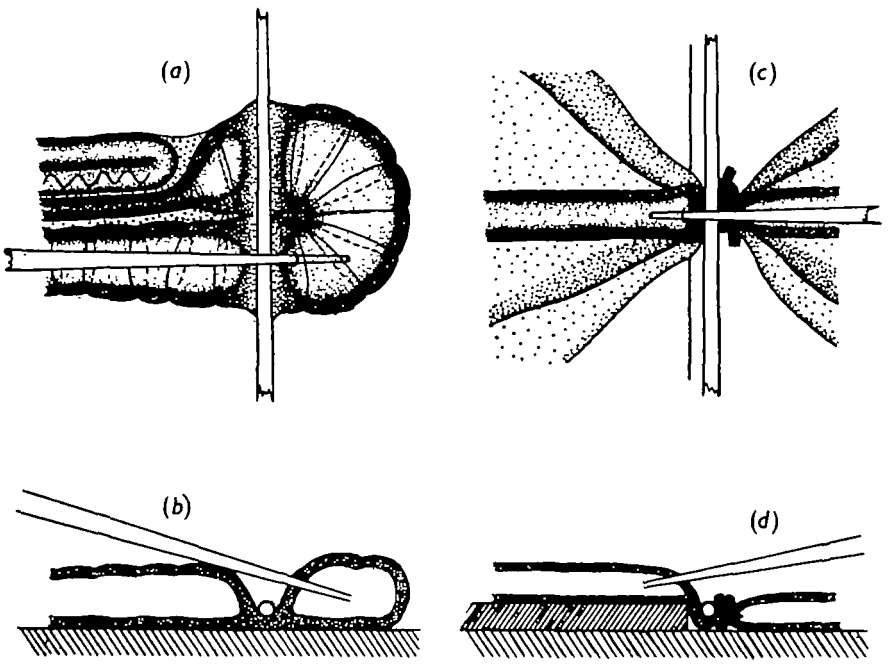

Fig. 2. Methods of collecting urine. *a*, *b*, from the ampulla; *c*, *d*, from the distal segment of the wide tube.

successful. The middle tube and the distal segment of the wide tube are the most difficult, mainly because their walls show a readiness to collapse; only about 20% of attempts are successful, and in the case of the distal segment the time taken to apply the ligature means that fewer attempts are made. The difficulties of collecting from other parts of the canal are too formidable to be overcome by present technique.

The volumes collected from various parts of the canal (excluding the bladder where much more could be obtained) varied from about $0{\cdot}1-5{\cdot}0 \times 10^{-6}$ ml. One determination of freezing-point was made on each sample, and the results are expressed in terms of the osmotically equivalent concentration of NaCl%. The probable error of each determination is 0·01% NaCl.

III. RESULTS

The results of the first series of collections are given in Table 1. As might be expected, the urine in the bladder is strongly hypotonic to the ringer; exceptions such as Serial 8 are probably due to faulty penetration as described under § II. The urine in the distal segment of the wide tube is also strongly hypotonic. The samples taken from the ampulla on 10 August 1948 are very clearly hypotonic and those

Table 1

Serial no.	Date	Worm	Dissection ringer (% NaCl)	Nephridium	Part of canal	Position of puncture	Sample (% NaCl)	Adherent ringer
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1	30. vi. 48	*A*	0·75	(*a*)	Bladder	—	0·05	Not recorded
2				(*b*)	,,	—	0·01	—
3				(*c*)	,,	—	0·11	—
4	5. vii. 48	*A*	0·75	(*a*)	Distal	6, base	0·17	—
5		*B*		(*a*)	,,	6 ,,	0·20	—
6		*C*		(*a*)	,,	6 ,,	0·16	—
7		*D*		(*a*)	,,	6 ,,	0·07	—
8	7. vii. 48	*A*	0·75	(*a*)	Bladder	—	0·71	—
9				(*b*)	,,	—	0·05	—
10				(*c*)	,,	—	0·02	—
11				(*d*)	,,	—	0·16	—
12				(*e*)	,,	—	0·16	—
13				(*f*)	,,	—	0·46	—
14				(*g*)	,,	—	0·27	—
15		*B*	0·75	(*a*)	Ampulla	0, tip	0·72	—
16				(*b*)	,,	0 ,,	0·52	—
17				(*c*)	,,	0 ,,	0·69	—
18				(*d*)	,,	0 ,,	0·56	—
19				(*e*)	,,	0 ,,	0·97	—
20				(*f*)	,,	0 ,,	0·61	—
21	8. vii. 48	*A*	0·75	(*a*)	Narrow, asc.	3, tip	0·87	—
22				(*b*)	,, ,,	3 ,,	0·79	—
23				(*c*)	,, ,,	3, base	0·79	—
24				(*d*)	,, ,,	3 ,,	0·77	—
25	9. vii. 48	*A*	0·75	(*a*)	Narrow, asc.	3, tip	0·71	—
26				(*b*)	,, ,,	4, tip	0·76	—
27				(*c*)	,, ,,	4 ,,	0·69	—
28				(*d*)	Narrow, des.	3, tip	0·76	—
29				(*e*)	Narrow, asc.	1, tip	0·80	—
30				(*f*)	,, ,,	5, tip	0·74	—
31	10. vii. 48	*A*	0·75	(*a*)	Ampulla	0, tip	0·36	—
32				(*b*)	,,	0 ,,	0·32	—
33				(*c*)	,,	0 ,,	0·34	—
34				(*d*)	,,	0 ,,	0·33	—
35				(*e*)	,,	0 ,,	0·36	—
36				(*f*)	,,	0 ,,	0·32	—

In this table, and in Table 2, the capital letters (column 3) are used to indicate different worms, the small letters (column 5) indicate different nephridia of the same worm. Columns 6 and 7 together describe the conditions under which the sample was collected, e.g. Serial 21, collection was made from the ascending limb of the narrow tube in the second loop, with the clamping needle at position 3 (see Fig. 1) and with the point of the pipette on the side of the needle nearer to the tip of the second loop. Column 4 gives the concentration (in terms of osmotically equivalent NaCl) of the ringer under which the worm was opened, and column 9 (Table 2 only) gives the concentration of the ringer adherent to the preparation.

taken on 7 August 1948 are also hypotonic, but less so. The samples taken from the narrow tube appear on the average to have a slightly higher concentration than the ringer.

This last result was somewhat unexpected and it seemed possible that the ringer surrounding the preparation on the slide might have become concentrated by evaporation. (It is also conceivable that the ringer might become diluted by urine leaking from the bladder.) To test this possibility six preparations were made in succession, following the normal routine exactly, and samples were taken of the ringer adhering to the preparation. These samples were found to vary from 0·75–0·8% NaCl with an average value of 0·79% NaCl as compared with 0·75% NaCl for the stock.

The preparation of the nephridium in these experiments involved the use of a binocular microscope first on a long arm over the dissecting dish, then on an ordinary stage mounting while the preparation was arranged on the slide. The slide had then to be transferred to a special stage screwed to the bench where it was viewed through a compound microscope. The micro-manipulator carrying the pipette was mounted on a large and heavy adjustable stand. It was not considered practicable to enclose all this apparatus in a moist chamber. Variations in the concentration of the ringer on the slide were therefore accepted, but in the second series of collections, after a successful collection had been made, a sample of the ringer adherent to the preparation was taken as well. Use was also made of a less concentrated ringer in the dissecting dish. The results of the first series had shown that the urine in the bladder and in the distal segment was strongly hypotonic to the ringer and it was not considered necessary to repeat these observations. In the second series attention was restricted to the narrow tube, the middle tube and the ampulla.

The results of the second series are given in Table 2. In two cases they appear to be anomalous: Serial 2, in which the adherent ringer is abnormally concentrated, and Serial 9, in which the sample collected from the middle tube shows a value nearly double that of the other samples. Neglecting these two cases the average values work out as follows:

	Urine (% NaCl)	Adherent ringer (% NaCl)
Narrow tube	0·51	0·54
Middle tube	0·51	0·56
Ampulla	0·33	0·52

In order to put all these results on a comparable basis the value for adherent ringer in each case has been equated to 100 and the value for the urine has been scaled up in proportion; a value of 0·79% NaCl has been assumed for the adherent ringer in the first series. Treated in this way all the data are presented graphically in Fig. 3.

It appears reasonable to conclude that an active process producing hypotonic urine is certainly at work in the ampulla, is possibly at work in the middle tube and

Table 2

Serial no.	Date	Worm	Dissection ringer (% NaCl)	Nephridium	Part of canal	Position of puncture	Sample (% NaCl)	Adherent ringer (% NaCl)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1	11. vii. 48	*A*	0·75	(*a*)	Middle	2, base	0·52	0·71
2				(*b*)	,,	3 ,,	0·50	1·14
3				(*c*)	,,	3 ,,	0·82	0·74
4	12. vii. 48	*A*	0·75	(*a*)	Middle	1, base	0·68	0·70
5*				(*b*)	,,	1 ,,	0·42	0·41
6	13. vii. 48	*A*	0·45	(*a*)	Middle	2, base	0·49	0·49
7				(*b*)	,,	3 ,,	0·36	0·49
8		*B*		(*a*)	,,	3 ,,	0·41	0·49
9		*C*		(*a*)	,,	3 ,,	0·72	0·47
10		*D*		(*a*)	,,	0, base	0·49	0·51
11		*E*		(*a*)	,,	3 ,,	0·42	0·52
12	14. vii. 48	*A*	0·45	(*a*)	Ampulla	0, tip	0·29	0·51
13				(*b*)	,,	0 ,,	0·31	0·52
14				(*c*)	,,	0 ,,	0·29	0·49
15		*B*		(*a*)	,,	0 ,,	0·33	0·51
16				(*b*)	,,	0 ,,	0·32	0·52
17				(*c*)	,,	0 ,,	0·47	0·55
18	15. vii. 48	*A*	0·45	(*a*)	Narrow, asc.	2, tip	0·51	0·56
19				(*b*)	,, ,,	2 ,,	0·51	0·50
20		*B*		(*a*)	Narrow, des.	2 ,,	0·52	0·54
21		*C*		(*a*)	Narrow, asc.	1, tip	0·52	0·56

See legend to Table 1.
* In this experiment the ringer was diluted on the slide.

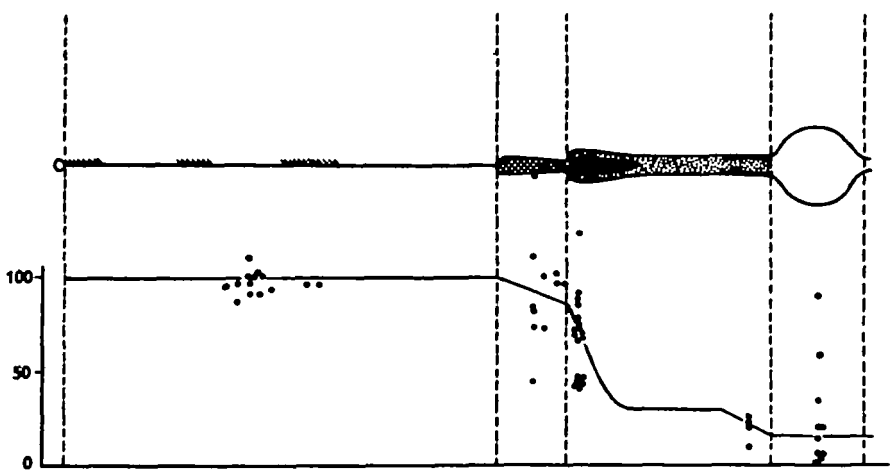

Fig. 3. To show the osmotic pressure of the urine at different levels in the nephridium. Conventions as in Fig. 1. The osmotic pressure of the ringer surrounding the nephridium has been equated to 100. Individual observations are shown as points; the line drawn through the points represents the interpretation placed upon them.

is probably not at work in the narrow tube. The results do not indicate whether the formation of hypotonic urine is due to the addition of water or to the resorption of osmotically active substances from the urine.

IV. DISCUSSION

With one possible exception, Serial 19 of Table 1, all the samples of urine collected from the ampulla or beyond it have proved to be hypotonic to the medium surrounding the nephridium, and the average value of osmotic pressure is unmistakably lower in the bladder than in the ampulla. The conclusion appears to be well-founded that a large part of the osmotic work producing hypotonic urine is carried out in the wide tube. It cannot be claimed with certainty that hypotonic urine is not formed proximal to the ampulla. Although a difference in concentration having an average value equivalent to 0·03% NaCl has been shown to exist between the ringer and the urine in the narrow tube, this figure is based on only four observations in which a direct comparison was made. Other evidence speaks in favour of the view that there is, in fact, no significant difference in concentration. When the ringer bathing a preparation is diluted, the narrow tube immediately—in a matter of 10 sec.—increases in diameter; the middle tube usually increases in diameter also, but this takes some minutes to become apparent, and a change in the diameter of the ampulla is not noticeable until much later, if it occurs at all. This observation suggests that the wall of the narrow tube is relatively freely permeable to water and therefore implies that the urine contained in it has the same osmotic pressure as the surrounding medium. The position in regard to the middle tube is less satisfactory. Even if Serials 2 and 9 (Table 2) are neglected the scatter of the remaining observations is still considerable. It has been shown that this part of the canal is capable of taking up colloidal substances (Cordier, 1933, 1934) and it would be rash to deny the possibility of its taking up osmotically active substances as well.

It is a fair criticism that these points might have been settled if more observations had been made; but there are good reasons for believing that an extension of the work along the present lines would be unprofitable at the moment.

(*a*) Measurements of the osmotic pressure and chloride content of the coelomic fluid (Ramsay, 1949*a*) indicate that NaCl accounts for about 50% of the total osmotic pressure, assuming that sodium and chloride ions form a large proportion of the inorganic substances present. Other osmotically active substances, probably organic, must be responsible for the other 50%. A similar state of affairs is found in the haemolymph of mosquito larvae (Wigglesworth, 1938; Beadle, 1939). Measurements of the osmotic pressure and chloride content of the urine indicate that the non-chloride fraction of the urine is considerably reduced as compared with that of the coelomic fluid. This reduction may conceivably be brought about by resorption of organic substances in parts of the canal proximal to the ampulla, and if this is the case it will not show itself in experiments in which organic-free frog ringer is used. This possibility cannot be tested until an analysis of the coelomic fluid has been made and an artificial medium can be prepared which is a better substitute for the coelomic fluid than is frog ringer.

(*b*) The method used for the collection of samples necessitated the interruption of the normal flow on the nephridial canal. The urine may have remained longer in the various sections of the canal and the process of forming hypotonic urine may have proceeded further than it would do under normal conditions. It is proper to point out that we know extraordinarily little of the factors which control the rate of flow. That the osmotic pressure of the medium is one of these factors is indicated by the following observation. A number of worms were allowed to remain in frog ringer equivalent to 1·4% NaCl for one week and were then opened under this same medium. The nephridial bladders, almost without exception, were found to be empty, and when the nephridia were excised and examined it was seen that the whole canal was somewhat shrunken in appearance and that ciliary activity was practically at a standstill. When the medium was replaced with frog ringer equivalent to 0·45% NaCl the narrow tube and later the middle tube opened up more fully and the cilia throughout the organ began beating. When portions of the opened worm were transferred to a dish containing the more dilute ringer the filling of the bladders became apparent in about half an hour, and after one hour some of the bladders appeared to be fully distended. The bladders of the control remained contracted.

(*c*) The method of collection also involved the cutting of the blood-vessels to the nephridium. It is obviously desirable, if technically practicable, to observe the flow of urine and to collect samples with the nephridium *in situ* and with its blood supply intact.

For these and other reasons the results reported in this paper are claimed to show no more than that ability to form hypotonic urine is associated with the wide tube of the nephridium and perhaps with the middle tube as well. The possibilities of improving technique have to be explored and a considerable amount of preliminary work has to be done before the present line of attack can be renewed with reasonable hope of advance.

The analogy between the various sections of the nephridial canal and the various sections of the vertebrate nephron, pointed out by Cordier (1934), is worthy of further consideration. In collaboration with Gérard, Cordier studied the uptake of colloidal particles of known size in amphibian nephrons with open nephrostomes (Gérard & Cordier, 1934). When injected into the body these substances accumulate in the wall of the proximal convoluted tubule, the finer particles being taken up at the proximal end and the larger particles at the distal end. Cordier repeated these experiments on *Lumbricus* and found that the finer particles were taken up at the proximal end of the middle tube and the larger particles about the middle of the middle tube. In comparing the amphibian nephron with the oligochaet nephridium he very naturally laid stress upon the similarity of function between the proximal convoluted tubule of the nephron and the middle tube of the nephridium, and he regarded the whole of the wide tube of the nephridium as representing the distal convoluted tubule of the nephron. On the basis of this analogy Cordier ventured to predict that a process of water-resorption would be found to occur in the wide tube, such as had been shown to occur in the distal convoluted tubule. This predic-

tion was made at a time when the functions of the different parts of the amphibian nephron were still uncertain; but with the completion of the work of Richards and his associates (Richards, 1938) it became clear that the distal convoluted tubule was the site not only of water-resorption but also of salt-resorption—in fact, that it was the site of formation of hypotonic urine. The present work, although it does not confirm or refute Cordier's prediction about water-resorption, has lent support to his conception of the wide tube as the analogue of the distal convoluted tubule.

V. SUMMARY

1. Methods for the collection of samples of urine from different parts of the nephridium of *Lumbricus* are described.

2. The osmotic pressures of these samples have been measured by determination of freezing-point depression and have been compared with the osmotic pressure of the medium surrounding the nephridium.

3. The results of this comparison indicate that the ability to form hypotonic urine is certainly present in the wide tube, is possibly present in the middle tube and is probably not present in the narrow tube.

4. The analogy between the nephridium and the vertebrate nephron is discussed.

REFERENCES

BAHL, K. N. (1947). *Biol. Rev.* **22**, 109.
BEADLE, L. C. (1939). *J. Exp. Biol*, **16**, 346.
BENHAM, W. B. (1891). *Quart. J. Micr. Soc.* **32**, 293.
CORDIER, R. (1933). *C.R. Soc. Biol., Paris*, **113**, 906.
CORDIER, R. (1934). *Arch. Biol., Paris*, **45**, 431.
CUENOT, L. (1898). *Arch. Biol., Paris*, **15**, 79.
GEGENBAUR, C. (1853). *Z. wiss. Zool.* **4**, 221.
GÉRARD, P. & CORDIER, R. (1934). *Biol. Rev.* **9**, 110.
GOEHLICH, G. (1890). *Zool. Beitr.* **2**, 133.
GOODRICH, E. S. (1932). *Quart. J. Micr. Soc.* **75**, 165.
GOODRICH, E. S. (1945). *Quart. J. Micr. Soc.* **86**, 113.
KOWALEVSKY, A. (1889). *Biol. Zbl.* **9**, 65.
MAZIARSKI, J. (1901). *C.R. Soc. Biol., Paris*, **53**, 259.
MAZIARSKI, J. (1905). *Poln. Arch. biol. med. Wiss.* **2**, 1.
MEISENHEIMER, J. (1910). *Ergebn. Zool.* **2**, 275.
RAMSAY, J. A. (1946). *Nature, Lond.*, **158**, 665.
RAMSAY, J. A. (1949*a*). *J. Exp. Biol.* **26**, 46.
RAMSAY, J. A. (1949*b*). *J. Exp. Biol.* **26**, 57.
RICHARDS, A. N. (1938). *Proc. Roy. Soc.* B, **126**, 398.
ROSEN, F. (1911). *Z. wiss. Zool.* **98**, 135.
SCHNEIDER, G. (1896). *Z. wiss. Zool.* **61**, 363.
WIGGLESWORTH, V. B. (1938). *J. Exp. Biol.* **15**, 235.
WILLEM, V. & MINNÉ, A. (1900). *Mem. Acad. roy. Belg.* **58**, 1.