

PHYSIOLOGICAL STUDIES ON *MARPHYSA GRAVELYI* SOUTHERN

IV. STRUCTURE OF NEPHRIDIA*

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INTRODUCTION

IN *Marphysa gravelyi* Southern there was circumstantial evidence of formation of hyposmotic urine (Krishnamoorthi & Krishnaswamy, 1966b & c). Since excretion of hyposmotic urine has been associated with the size and structure of organs of excretion in a number of annelids (Grobber, 1881; Jurgens, 1935) and crustaceans (Schwabe, 1933; Peters, 1935; Hynes, 1954), it appeared a study of the morphology and histology of nephridia would help in understanding their role in the physiological adaptation of *M. gravelyi* to a changing brackish water environment.

MATERIAL AND METHODS

From a collection of worms obtained from Adayar, a few equal in size with those that formed the experimental animals in earlier studies of this series (Krishnamoorthi & Krishnaswamy, 1966a, b & c), were selected and narcotised with Chloral Hydrate, Menthol or 30% alcohol to ensure an extended condition. A number of fixatives like Duboseq Bouin, Zenker's Fluid and Susa were tried and discarded in favour of Bouin's fluid which yielded the best results. The worms were starved a day or two before they were fixed, this being necessary to remove from the gut fine particles of mud and sand which impede obtaining good sections. Serial sections at eight micra were cut; stained with Haematoxylin either Heidenhain's or Ehrlich's and counter-stained with Borax Carmine or Orange G. All diagrams were made with the aid of a *Camera lucida*. An ordinary ocular micrometer was used for measurements that are recorded here, under constant magnification, 5×8.

STRUCTURE OF THE NEPHRIDIUM

(a) *Previous work* : Nephridia in Eunicidae were first studied by Ehlers (1864) in *Eunice harasil*, *E. limosa*, *E. rubrocincta*, *E. siciliensis*, *Omuphis tubicola* and *Marphysa sanguinea*. However, it was not until 1900, that Goodrich pointed out the homology of the large trumpet shaped nephrostome with the coelomostome of primitive forms and explained the composite nature of the mixonephridia. Fage (1906) figured the coelomostome of the eunicids, *Hyalinoecia tubicola* and *Lumbri-conereis impatiens*, as simple and smooth. But Goodrich (1945) observed deep grooves on the ciliated inner surface. Aiyar (1933) confirmed the mixed nature of the nephridium in *M. gravelyi*.

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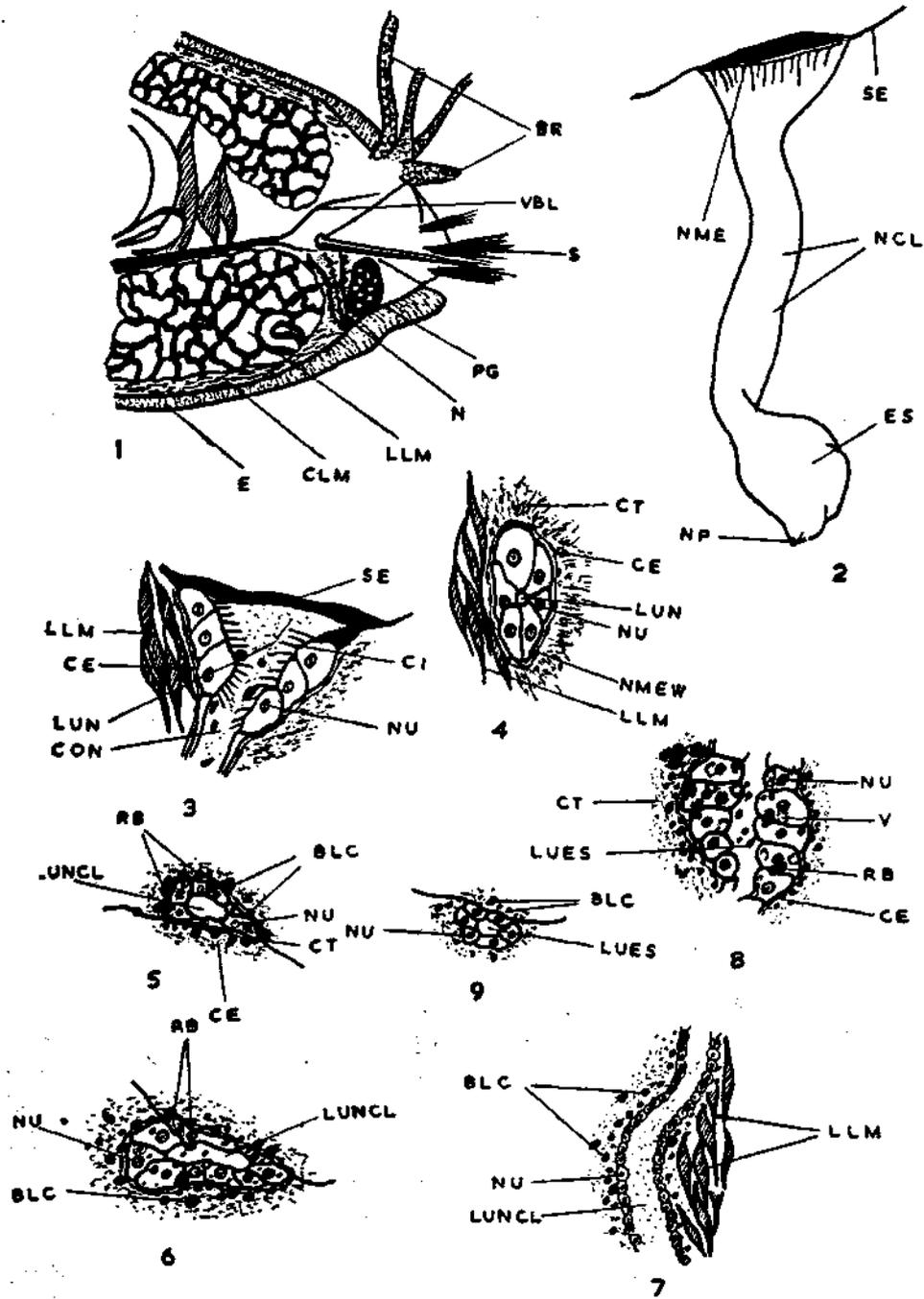
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(b) *Morphology and histology*: In *Marphysa gravelyi*, as in all eunicids, a pair of nephridia are found in each segment except in a few of the anterior and posterior segments. As can be made out from sections (Fig. 1), the nephridium (Fig. 2) is located between the lateral border of the ventral longitudinal muscle and the inner border of the pigment gland. It consists of a wide trumpet-shaped nephrostome opening into the coelome along the outer edge of the ventral longitudinal muscle and a long narrow almost straight nephridial canal which runs outwards piercing the circular layer of muscles and the epidermis to open by the nephridiopore situated near the ventral edge of the neuropodium. While the funnel has been observed in *Marphysa sanguinea* (Cosmovice, 1880), there has been no mention of the external opening. Just before opening to the exterior, the nephridial canal dilates into an end-sac.

The wide, oval mouth of the trumpet shaped nephrostome measuring 80μ , gradually narrows down to 18μ before it imperceptibly fuses with the nephridial canal. The wall of the nephrostome (Figs. 3 & 4) is made up of a single layer of well defined cubical cells 10μ in length. The deeply stained nucleus is invariably at the proximal end of the cells. Only the inner walls of the cells bear numerous cilia about 20μ in length directed towards the nephridial canal. The lumen of the nephrostome is full of concretions, probably, excretory in nature. The nephrostome externally is lined by the coelomic epithelium and excepting at the opening of the nephrostome into the coelomic cavity, is the loose much vacuolated connective tissue. The cytoplasm of the cells is free of any inclusions. The waste matter present in the coelomic cavity appears to be collected by the nephrostome and pumped out by means of cilia through the nephridial canal as observed in a number of polychaetes (Cunningham, 1887).

Immediately following the nephrostome is the nephridial canal which measures about 120μ in length and is uniformly 18μ in width. The lumen of the canal contains concretions throughout its length. The wall of the canal is formed of a single layer of cubical cells about 6μ in length. The cell boundaries are well demarcated. The cytoplasm is densely granular. Among the granules, large bright refringent bodies could be distinguished by their taking a deep eosin stain and by their reflecting surfaces. Such bodies are found outside the cells and also in the coelomic cavity (Figs. 5, 6 & 7). The presence of such bodies within the cells and outside the cells and the uniform granulation of the cytoplasm suggest that this part of the nephridial canal is, perhaps, concerned with filtration. Racovitza (1895) saw, after injecting sepia-black ink into the coelomic cavity of *Leiocephalus leiphygas*, masses of black granules deposited in the cells of the nephridial canal and concluded that the particles may have been ingested by the cells of the walls of the nephridial canal. Schneider (1899) confirmed this observation in *Arenicola marina*, *Travisia forbesi*, *Pectinaria hyperboraea*, *Terebellides stremi*, *Polymnia nebulosa* and *P. nidensis* as well as in a number of oligochaetes just as Willem & Minne (1900) did in many more polychaetes. Spherical and unstained vacant spaces similar to those found in the cells of the nephridial canal in *Pectinaria belgica* and *Terebella conchilega* (Willem & Minne, 1900; Maziariski, 1905) and regarded as water vacuoles concerned in the elimination of water from the coelomic cavity, are also apparent in all the cells forming the nephridial canal in *M. gravelyi* and perhaps have the same function.

From longitudinal and transverse sections (Figs. 8 & 9), the walls of the end-sac could be seen to be made up of a single layer of cubical cells 8μ in length. As the cells are thick the lumen of this enlarged part of the nephridial canal is as narrow as elsewhere. The cytoplasm is granular, especially at the proximal end of the cells close to the lumen. Refringent bodies and water vacuoles such as those seen in the



FIGS. 1-9. 1. Diagrammatic cross-section of the worm, *Marphysa graveyi* Southern, indicating the relation of nephridium to other structures in the body cavity. X 56. 2. Diagrammatic representation of a single whole nephridium of *Marphysa graveyi*. X 400. 3. Longitudinal section of the nephrostome. X 450. 4. Cross-section of the nephrostome. X 450. 5. Cross-section through the nephridial canal. X 450. 6. Cross-section through the middle part of the nephridial canal. X 450. 7. Longitudinal section of the nephridial canal. X 450. 8. Longitudinal section of the end-sac. X 450. 9. Cross-section of the end-sac. X 450.

cells of the nephridial canal are very scarce suggesting that filtration may not be a functional feature of this sac. Coelomic epithelium and connective tissue are, however, present surrounding the end-sac, as in the case of the nephridial canal and the nephrostome. In its morphological features and in the absence of cellular concretions, it resembles the end-sac of the nephridium of earth-worms like *Pontoscolex corethrurus*, *Thamnodrilus crassus* (Bahl, 1942a & b) and *Pheretima posthuma* (Bahl, 1919) and of crustaceans like *Carcinus maenas* and *Potamobius fluviatilis* (Picken, 1936).

(c) *Blood supply* : The nephridium in *M. gravelyi*, is supplied with blood by a branch of the ventral blood vessel, resembling in many respects the situation obtained in *Eunice* sp. (Goodrich, 1900). The main branch of the ventral vessel supplies blood to the parapodia as well as the branchiae. But before it proceeds to the parapodia and the branchiae proper it gives off a subsidiary branch to the nephridium which breaks into capillaries on the nephridial body and is brought back to the general circulation of the blood from the epidermis (Fig. 1). Several of the capillaries end blind in the form of dilations within the nephridia as in *Marphysa sanguinea* (Fuchs, 1907). Such blind ending capillaries, known as ampullae, are of common occurrence in oligochaetes (Stephensen, 1930) and have also been observed in *Arenicola* sp. (Benham, 1891) and *Lanice conchilega* (Meyer, 1888). Although Ewer (1941) noted them in *Travesia forbesii*, he concluded that their possible function must remain a matter of conjecture. However, considering their intimate 'juxtaposition' (Krishnan, 1952) with the nephridial body, the number of such ampullae could be a convenient measure of the metabolic activity of the nephridium. With this object in view, serial sections obtained from a number of polychaetes were examined and the number of blind ending capillaries noted (Table I) as in previous studies (Krishnamoorthi, 1963a). The number of blind ending capillaries in *M. gravelyi* ranged from 66 to 75 with a mean of 71.23 ± 0.37 .

TABLE I
Number of blind-ending capillaries on the nephridium of *M. gravelyi*

S. No.	1	2	3	4	5	6	Mean
1	70	72	72	70	72	72	71.33
2	66	74	70	68	72	76	71.00
3	73	70	68	68	74	72	70.83
4	70	70	71	75	69	72	71.17
5	72	72	70	71	70	72	71.17
6	68	74	75	72	72	70	71.87
							Mean : 71.23
							s.d. 2.23
							s.e. ± 0.37

(Key to lettering)

BR : Branchiae ; BLC : Blind ending capillaries ; CE : Coelomic epithelium ; CI : Cilia ; CT : Connective tissue ; CLM : Circular layer of muscles ; CON : Concretions ; E : Epidermis ; ES : End-sac ; LLM : Longitudinal layer of muscles ; LUN : Lumen of nephrostome ; LUES : Lumen of end-sac ; LUNCL : Lumen of nephridial canal ; N : Nephridium ; NP : Nephridiopore ; NU : Nucleus ; NCL : Nephridial canal ; NME : Nephrostome ; NMEW : Nephrostomial wall ; PG : Pigment gland ; RB : Refracting bodies ; S : Setae ; SE : Septum ; V : Vacuoles ; VBL : Ventral blood vessel.

(d) *Excretory surface relative to the size of the worm* : A study of the structure of the nephridium in *M. gravelyi*, has revealed that excepting the nephrostome, the entire nephridial canal takes part in the process of excretion. The length of the canal—implying the number of cells—may, therefore, be taken as an index of the 'excretory capacity' of the nephridium of an animal. Although the length, the breadth and the height of the nephridial body may give a picture to compare with (Krishnan, 1952), it is not accurate to take into account those sections of the nephridium which do not participate in filtration and reabsorption. In order to arrive at some value likely to be constant and comparable for different genera/species of polychaetes, the ratio between the length of the worm to the length of the nephridial canal was determined in *M. gravelyi* and it is seen that (Table II) *M. gravelyi* has a mean ratio of $1 : 0.478 \pm 0.061$ and ranged from $1 : 0.401$ to $1 : 0.536$ in the six worms studied.

REMARKS

In *Marphysa gravelyi* Southern, the nephridium is simple in form and structure, similar to that obtained either in *Onuphis eremita* (Krishnamoorthi, 1963a) or *Diopatra variabilis* (Krishnamoorthi, 1951) both belonging to the family Eunicidae. In all the three species the nephridium belonging to the mixonephridial type (Goodrich, 1945), consists of a wide funnel, the nephrostome, and a long uncoiled narrow canal, the nephridial canal, which opens out by the nephridiopore at the base of the neuropodium. Its topography in relation to other organ systems, is much the same in all the three forms. While the nephrostome situated closely along the septum dividing two consecutive segments, opens into that segment previous to the one that contains the nephridial body, the nephridiopore gains its exit in the same segment as that holds the nephridial body. The disposition of the connective tissue and the coelomic epithelium around the nephridia in *M. gravelyi* is much similar to that observed in *O. eremita* and *D. variabilis*. However, the nephridia in *M. gravelyi* differ from those of the others in the possession of an end-sac and resemble in this respect the nephridia of Oligochaetes (Bahl, 1942, 1945) and many Crustaceans (Picken, 1936; Panikkar, 1941a). Although the presence of refringent bodies and vacuoles in the cells of the nephridial canal point to its function as the site of filtration, in the absence of any such concretions and the cytoplasm remaining uniformly granular in the cells forming the end-sac, it is difficult to attribute a precise function to the end-sac in the renal processes of excretion. Bahl (1945b, 1947) as also Picken (1936) assign a function of reabsorption to the end-sac. Whether a similar function could be attributed to the end-sac in *M. gravelyi*, a study of the nephridial physiology alone could reveal. But taking into consideration the structural similarities, it may be possible to assign a similar function i.e., reabsorption, to the end-sac, in *M. gravelyi* Southern. Since *M. gravelyi* is a hyporegulator (Krishnamoorthi & Krishnaswamy, 1966c), the importance of reabsorption of salts and water that may otherwise be lost, needs no further emphasis and, perhaps, the possession of an end-sac is an adaptational response.

Based on circumstantial evidence, Beadle (1937) presumed that hyposmotic urine was being formed in *N. diversicolor*. Examining the nephridia of *N. diversicolor* and *Perinereis cultrifera*, Jurgens* (1935) associated the formation of hyposmotic urine in *N. diversicolor*, with the long coiled nephridial canal which provided ample epithelium for reabsorption, whereas it was a simple sac in *P.*

* More recently this has been questioned by Jones (1957) and Oglesby (1965).

TABLE II
Ratio of the excretory surface to the length of the worm in M. gravelyi

S. No.	a	b	c	d	e	f	g	h	i	j	k	l
1	195	411	6	8	13	5	78	40	64116	32880	96996	0.497
2	179	253	6	8	18	5	108	40	54648	20240	74888	0.418
3	250	397	6	8	13	6	78	48	61932	38112	100044	0.401
4	229	475	6	8	13	5	78	40	75100	38000	113100	0.494
5	249	470	6	8	15	6	90	48	84600	45120	129720	0.524
6	209	431	6	8	15	5	90	40	77580	34480	112060	0.536
	219										104301	0.478-Mean 0.156-s.d. ±0.061 s.e.

S. No.

Length of the worm in/mm.

No. of Segments.

Length of each cell of the middle part of the nephridial canal in μ .Length of each cell of the end-sac in μ .

No of cells in the middle part.

No. of cells in the end-sac.

Length of the middle part in μ ($c \times e$).Length of the end-sac in μ ($d \times f$).Total length of the middle part in μ ($g \times b \times 2$).Total length of the end-sac in μ ($h \times b \times 2$).Sum total of the excretory surface in μ ($i+j$).

Ratio of the excretory surface to the length of the worm.

cultrifera. With the positive demonstration of the function of nephridia in the osmoregulation of *Sabella pavonina* (Ewer & Ewer, 1943), its importance gained strength. Krishnan (1952) without providing experimental evidence, related the size of the nephridia in *Lycastis indica*, *Nereis chilkaensis* and *Perinereis nuntia* to the habitat. Krishnamoorthi (1962, 63a) provided both experimental and structural evidence to account for the penetration and distribution of a number of polychaetes other than Nereidae in the Adayar estuary.

In *M. gravelyi* also there is circumstantial evidence that hyposmotic urine is being formed (Krishnamoorthi & Krishnaswamy, 1966b & c). If association of bigger nephridia with the formation of hyposmotic urine is accepted and Jørgensen & Dales (1957) suspect that it might well be, it is seen (Table III) that *M. gravelyi* possesses a nephridium which has a ratio between the excretory surface and the length of the worm, considerably higher than ratios obtained either in *O. eremita*,

TABLE III

Comparative statement of ratios of excretory surface to the length of the worm in five species of polychaetes of the Adayar estuary.

S. No.	<i>O. eremita</i> *	<i>L. medusa</i> *	<i>C. insecta</i> *	<i>D. variabilis</i> **	<i>M. gravelyi</i>
1	0.270	0.224	0.355	0.355	0.497
2	0.236	0.213	0.335	0.368	0.418
3	0.254	0.227	0.342	0.330	0.401
4	0.233	0.210	0.348	0.354	0.494
5	0.240	0.224	0.352	0.377	0.521
6	0.246	0.224	0.342	0.359	0.536
Mean :	0.247	0.220	0.346	0.357	0.478
s.d. :	0.011	0.007	0.023	0.050	0.156
s.e. :	±0.004	±0.003	±0.001	±0.020	±0.061

L. medusa and *C. insecta* (Krishnamoorthi, 1963a) or *D. variabilis* (Krishnamoorthi, 1951). While the ratio in *M. gravelyi* was 1 : 0.478, the respective ratios in *O. eremita*, *L. medusa*, *C. insecta* and *D. variabilis* were viz., 1 : 0.247 ; 1 : 0.220 ; 1 : 0.346 and 1 : 0.357. Now, applying the 'student's t' test for the significance of the difference of two sample means, the 't' value between the mean ratios of *M. gravelyi* and *D. variabilis*, was 1.881 and the corresponding probability read from Table III of Fisher & Yates (1948) for 10 d.f. was at the 10% level. Similar values of 't' between *M. gravelyi* and *C. insecta* ; between *M. gravelyi* and *O. eremita* ; and *M. gravelyi* and *L. medusa* respectively were 2.164, 3.787 and 4.230 with the corresponding probabilities for 10 d.f. at 5%, 0.05% and 0.01%. The conclusion is, therefore, irresistible that *M. gravelyi* Southern owes its better powers of osmoconcentration (Krishnamoorthi & Krishnaswamy, 1966c) and volume regulation (Krishnamoorthi & Krishnaswamy, 1966b) to the bigger nephridia that it possesses.

An examination of Table III also reveals that the ratios between *C. insecta* and *D. variabilis* as also between *O. eremita* and *L. medusa* are not significantly different. In the Adayar estuary, *C. insecta* and *D. variabilis* occur together with

*Taken from Krishnamoorthi, 1963a. **Taken from Krishnamoorthi, 1951.

M. gravelyi at Station D (Krishnamoorthi, 1963d), while *O. eremita* and *L. medusa* have hardly crossed the mouth of the estuary, the occurrence of *O. eremita* being restricted to the sandy beach (Station A) and that of *L. medusa* to the marine dominated regions of the estuary (Station B). Viewed in this light, the range and distribution of these species and the advantage gained by possessing a bigger and better vascularised nephridia by *M. gravelyi* Southern and the osmotic independence it has gained thereof, are obvious and well account for its wide distribution in the Adayar estuary.

SUMMARY

M. gravelyi Southern possesses nephridia of the mixonephridial type as obtained in most eunicids. However, a structure comparable to the end-sac of the nephridium of earthworms and crustaceans, is met with in the nephridium of *M. gravelyi*. Histological preparations support the view that it may be taking part in reabsorption, while the nephridial canal takes part in filtration. Not only the ratio of the excretory surface to the length of the worm, but also the magnitude of vascularisation of the nephridium are higher than those obtained in other polychaetes that co-exist with *M. gravelyi* in the Adayar estuary. The probable role of the nephridium in osmoregulation in *M. gravelyi* is discussed.

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