

WATER COMPARTMENTS AND OSMOREGULATION IN THE PARASITIC NEMATODE *PSEUDOTERRANOVA DECIPIENS*

M. FUSÉ*, K. G. DAVEY†

Department of Biology, York University, North York, Ontario, Canada M3J 1P3

and R. I. SOMMERVILLE

Department of Zoology, University of Adelaide, Adelaide, South Australia

Accepted 21 September 1992

Summary

A study of the time course of penetration of $^3\text{H}_2\text{O}$ into whole worms suggests that worms immersed in a hypo-osmotic environment (15% artificial sea water) reach full exchange equilibrium more slowly than worms in an iso-osmotic environment (40% artificial sea water). The apparent water content, determined by dry mass, matches that determined by $^3\text{H}_2\text{O}$ exchange when worms are immersed for 24h in 40% artificial sea water (ASW), but the water content measured by $^3\text{H}_2\text{O}$ exchange is lower when worms are kept in a hypo-osmotic environment for 24h. These differences disappear after 48h. No such differences are apparent when sacs, consisting of cylinders of body wall lacking their intestines and pseudocoelomic fluid and closed at both ends by ligatures, are immersed in either 40% or 15% ASW for 24h. The placing of ligatures at the head, but not at the tail, results in a failure of worms immersed in 40% ASW or 15% ASW containing $^3\text{H}_2\text{O}$ to achieve full exchange equilibrium within 24h. These results suggest that although worms immersed in an iso-osmotic environment drink, those immersed in a hypo-osmotic environment do not, a conclusion supported by studies involving the addition of [^{14}C]inulin to the medium. The application of ligatures to the head and tail of worms immersed in 40% ASW results in a slower penetration of $^3\text{H}_2\text{O}$ into the pseudocoelomic fluid, whereas similar ligatures do not further retard the penetration in worms exposed to 15% ASW. The results are consistent with a model which sees the pseudocoelomic fluid as consisting of two compartments containing water, one of which exchanges more slowly than the other.

Introduction

The codworm, *Pseudoterranova* (*Phocanema*, *Porrocaecum*, *Terranova*) *decipiens* (Myers, 1959; Likely and Burt, 1989) is an anisakid nematode found as the infective third-stage larva in the muscle of cod. When ingested by seals, the worm moults twice to

*Present address: Department of Zoology, University of British Columbia, Vancouver, BC, Canada.

†To whom reprint requests should be addressed.

Key words: nematode permeability, volume regulation, water relations, *Pseudoterranova decipiens*.

become an adult in the gastrointestinal tract (McClelland, 1980). The third-stage larva, removed from the cod, can be induced to moult *in vitro* when provided with appropriate stimuli (Townsend *et al.* 1963), and the worm has been used in a number of studies related to development (Davey, 1988). These studies, and similar studies on the trichostrongylid *Haemonchus contortus*, have demonstrated that the act of ecdysis associated with the infective process is marked by alterations in the distribution of water in the tissues of these nematodes (Davey, 1979; Davey and Rogers, 1982). These observations have prompted a more detailed study of osmoregulation in the dormant third-stage larva of *P. decipiens*, and the preceding paper (Fusé *et al.* 1993) has provided unequivocal evidence that this worm is capable of strict osmoregulation when exposed to a wide range of salinities over a 24h period. The principal site of the osmoregulatory mechanism is in the body wall of the nematode.

Earlier studies on the water relations of *P. decipiens* during ecdysis to the fourth stage *in vitro* led to the conclusion that there were two compartments in the dormant third-stage larva. In the larger of these compartments, water was freely available for exchange, while water in the second compartment, constituting as much as 20% of the total water content, is not so readily available and exchanges much more slowly. Part of the slowly exchanging compartment was identified with the 'excretory' cell (Davey, 1979), but that cell, with a total water content of only 0.5mg, is too small to account for the entire slowly exchanging compartment.

The present paper examines the possible existence of physiological compartments in *P. decipiens* in the context of osmotic regulation by the dormant third-stage larva at 5°C, in the absence of the complications associated with development.

Materials and methods

Third-stage infective larvae of *P. decipiens* were shipped on ice from Sydney, Nova Scotia, and stored at 5°C in 40% artificial sea water (ASW) as described in a previous paper (Fusé *et al.* 1993). All of the experiments described in this paper were performed on worms that had been in storage for fewer than 124 days, and care was taken throughout the experiments to maintain the worms at 5°C. The composition of 100% ASW, which has an osmotic pressure of 1000mosmolkg⁻¹, has been described in the earlier paper. Solutions at various lower osmotic pressures consisted of dilutions of 100% ASW.

The preparation of worms ligatured at the head (HL), at the tail (TL) or at both head and tail (HTL), excluding the mouth and excretory pore at the anterior end and the anus at the posterior end, has been described previously. Similarly, the preparation of 'sacs', consisting of cylinders of body wall lacking the head and tail, and with the intestine removed, and ligatured at either end, has also been described (Fusé *et al.* 1993). The cylinders of body wall were left in 40% ASW for 30min before ligatures were applied in order to allow the medium to replace much of the pseudocoelomic fluid (PCF).

Worms were weighed, and total water content was determined by dry mass, as described in the previous paper (Fusé *et al.* 1993). The time course of penetration of ³H₂O into whole worms was determined by immersing worms in 40% or 15% ASW containing ³H₂O at approximately 10000disintegrations min⁻¹ ml⁻¹. At various time intervals, worms were

removed, rinsed rapidly in two changes of chilled 15% or 40% ASW, blotted gently on bibulous paper and placed in a glass scintillation vial in 1.0ml of NCS tissue solubiliser (Amersham, Toronto, Canada) at room temperature overnight. The solubiliser was neutralised with 40 μ l of glacial acetic acid, followed by 10.0ml of scintillant (ACS, Amersham, Toronto, Canada). The vials were counted by liquid scintillation spectrometry, with quench correction by external standards. Samples (10 μ l) of the medium were also counted, and the uptake of $^3\text{H}_2\text{O}$ by the worm was expressed as volume equivalents in microlitres.

In some cases, worms and sacs were exposed to ASW containing $^3\text{H}_2\text{O}$ for fixed time periods and were weighed before counting. This permitted the apparent content of water determined by exchange levels of $^3\text{H}_2\text{O}$ to be expressed as a percentage of the wet mass. Samples of PCF from worms exposed to ASW containing $^3\text{H}_2\text{O}$ for fixed periods were collected and counted as described earlier (Fusé *et al.* 1993). As before, the results are expressed as a percentage of the specific activity of the medium.

To determine whether fluid enters *P. decipiens* via the intestine, worms were exposed to 15% or 40% ASW containing [^{14}C]inulin (Amersham-Searle, Toronto, Canada, 1850kBqmmol $^{-1}$). It was assumed that inulin would not pass across the body wall or the intestinal epithelium. Each worm was placed in a separate vial in 2.0ml of ASW containing [^{14}C]inulin to a specific activity of approximately 1000disintsmin $^{-1}$ μ l $^{-1}$. After exposure to [^{14}C]inulin, worms were rinsed in two changes of chilled isotope-free medium to eliminate as much of the excess surface inulin as possible. Worms were then solubilized in 1.0ml of NCS in glass scintillation vials at room temperature and counted as described above for experiments with $^3\text{H}_2\text{O}$. Samples of the medium were similarly treated and counted, and the results were expressed as microlitre equivalents of medium per worm.

All percentage values were transformed to arcsine values prior to statistical analysis. Student's *t*-test and analysis of variance (ANOVA) were used to test for differences between the means of two groups or a number of groups, respectively. Duncan's multiple-range test was employed after ANOVA when a significant difference was found between groups in order to determine within which groups the significant differences resided. The percentage data have been presented for display as means retransformed from the arcsine values, together with the coefficient of variation, referred to here as the standard error of the mean (S.E.M.).

Results

Water contents and exchange volumes

The time course of uptake of $^3\text{H}_2\text{O}$ into whole worms immersed in 40% or 15% ASW appears in Fig. 1. Differences in the size of the nematodes produce standard errors of the means that are relatively large. Nevertheless, it is clear that during the period 24–36h, those worms exposed to hypo-osmotic conditions have reached a level of exchange of $^3\text{H}_2\text{O}$ significantly lower than that achieved by worms exposed to iso-osmotic conditions.

In order to reduce the variation caused by differences in the size of the worms, the apparent percentage water content, determined by exchange of $^3\text{H}_2\text{O}$ after 24h or 48h,

was compared with the percentage water content determined by dry mass. These data (Fig. 2) show that worms immersed in 40% ASW achieve an exchange level with $^3\text{H}_2\text{O}$ which is identical to the water content determined by dry mass in less than 24h. By contrast, those worms exposed to hypo-osmotic conditions exhibit a water content determined by exchange of $^3\text{H}_2\text{O}$ lower than that determined by dry mass by a small but significant amount ($P < 0.001$). By 48h, the difference has disappeared, although the total

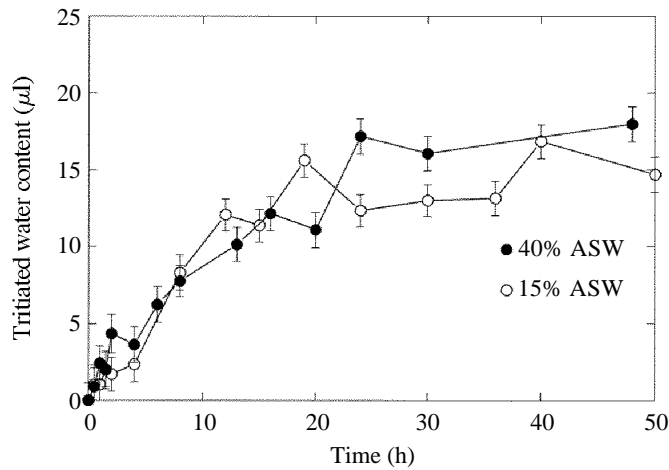


Fig. 1. The uptake of $^3\text{H}_2\text{O}$ into whole worms exposed for various times to either 40% or 15% ASW containing $^3\text{H}_2\text{O}$. Each point is the mean value of five worms, and the data are expressed as volume equivalents in μl of $^3\text{H}_2\text{O} \pm \text{S.E.M.}$

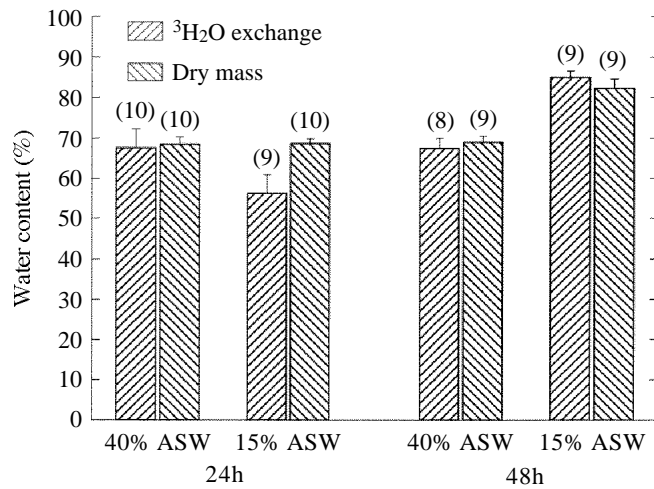


Fig. 2. Apparent percentage water content determined by $^3\text{H}_2\text{O}$ exchange or dry mass of worms exposed to 40% or 15% ASW for 24h or 48h. The numbers in parentheses indicate the numbers of worms in each experimental group, and the vertical bars indicate the S.E.M.

water content of these osmotically stressed worms is now higher than those maintained in 40% ASW.

A similar experiment was performed on sacs, consisting of cylinders of body wall ligatured at both ends. As shown in Fig. 3, within 24h the water content determined by exchange with $^3\text{H}_2\text{O}$ was identical to that determined by dry mass both in sacs exposed to iso-osmotic conditions and in those exposed to hypo-osmotic conditions. These results confirm (i) that the body wall of *P. decipiens* is sufficiently permeable to water for exchange conditions to be achieved within 24h; and (ii) that sacs are capable of osmoregulation, since there is no increase in water content under hypo-osmotic conditions.

The effect of ligatures

In order to identify the sites responsible for the differences in permeability that appear in intact worms, the water content determined by exchange with $^3\text{H}_2\text{O}$ and by dry mass was determined for worms ligatured at the head, at the tail and at both the head and tail. These data appear in Fig. 4 and can be compared with the data in Fig. 2 for unligatured worms. In HL or HTL worms, there is a reduction in the apparent water content determined by exchange of $^3\text{H}_2\text{O}$ in worms immersed in 40% ASW, while the TL worms do not exhibit that difference. Thus, the presence of a ligature on the head, but not the tail, slows the rate of exchange even in iso-osmotic conditions, and worms do not achieve exchange levels within 24h. Ligatured worms exposed to 15% ASW exhibit increases in water content determined by dry mass, a finding in accord with the data on weight gains in our earlier paper (Fusé *et al.* 1993). However, the presence of a ligature does not further interfere with the exchange level achieved by 24h compared to that of unligatured worms exposed to 15% ASW.

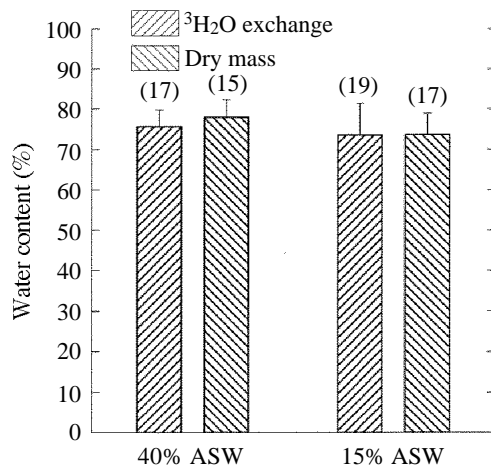


Fig. 3. Apparent percentage water content determined by $^3\text{H}_2\text{O}$ exchange or dry mass of sacs exposed to 40% or 15% ASW for 24h. The numbers in parentheses indicate the numbers of worms in each experimental group, and the vertical bars indicate the S.E.M.

These findings led us to examine the uptake of $^3\text{H}_2\text{O}$ into the PCF in greater detail. While our earlier studies on the time course of the uptake of $^3\text{H}_2\text{O}$ into the PCF revealed no statistical differences between the rate of uptake in worms exposed to 40% ASW and that in worms exposed to 15% ASW, there was some suggestion that the PCF in worms

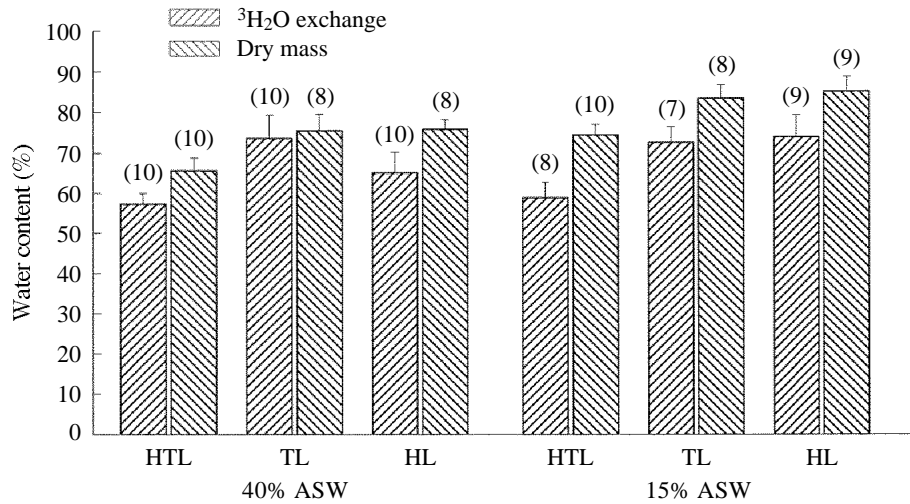


Fig. 4. Apparent percentage water content determined by $^3\text{H}_2\text{O}$ exchange or dry mass of worms ligatured at the head and tail (HTL), at the tail (TL) or at the head (HL) and exposed to 40% or 15% ASW for 24h. The numbers in parentheses indicate the numbers of worms in each experimental group, and the vertical bars indicate the S.E.M.

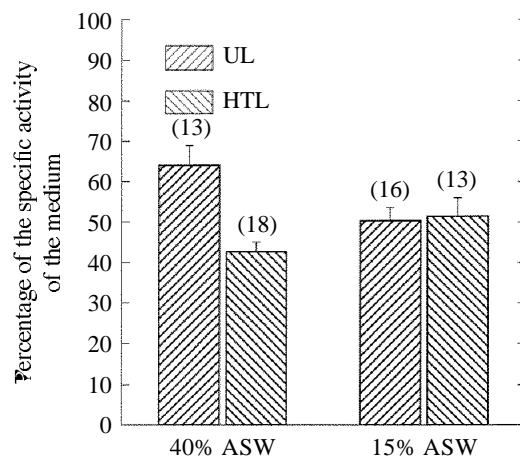


Fig. 5. The penetration of $^3\text{H}_2\text{O}$ into the PCF of intact (UL) worms or worms ligatured at the head and tail (HTL) and immersed for 24h in either 40% or 15% ASW containing $^3\text{H}_2\text{O}$. The numbers in parentheses indicate the numbers of worms in each experimental group, and the vertical bars indicate the S.E.M.

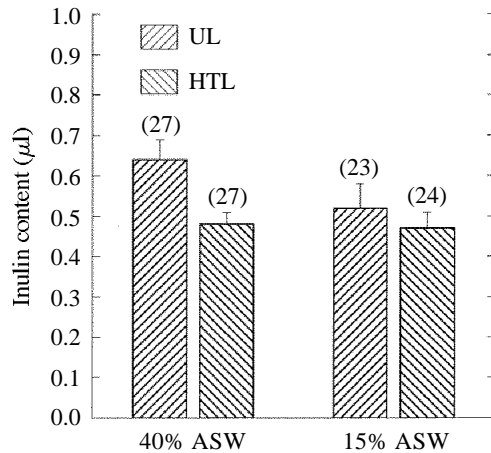


Fig. 6. The uptake of [^{14}C]inulin by intact (UL) worms or worms ligatured at the head and tail (HTL) and immersed for 24h in either 40% ASW or 15% ASW containing [^{14}C]inulin. The numbers in parentheses indicate the numbers of worms in each experimental group, and the vertical bars indicate the S.E.M.

exposed to hypo-osmotic conditions exhibited a lower plateau (Fusé *et al.* 1993). The interpretation of these data, however, was rendered difficult by the large S.E.M. values resulting from the smaller sample sizes imposed by the availability of worms. Accordingly, intact or HTL worms were exposed for 24h to 40% or 15% ASW containing $^3\text{H}_2\text{O}$, and the PCF was sampled for scintillation counting. The results appear in Fig. 5. These reveal that, in intact worms exposed to 15% ASW, the penetration of $^3\text{H}_2\text{O}$ into the PCF is significantly less than for worms in iso-osmotic conditions. The presence of ligatures slows the rate of penetration of $^3\text{H}_2\text{O}$ into the PCF in worms immersed in 40% ASW, but does not affect further the level in worms immersed in 15% ASW.

The role of drinking

These findings focused our attention on the head as a possible site of entry of water in worms exposed to iso-osmotic conditions. Intact or HTL worms were immersed in either 40% or 15% ASW containing [^{14}C]inulin, and counted 24h later as described. The results appear in Fig. 6. There was a significant ($P < 0.001$) increase in the amount of [^{14}C]inulin associated with unligatured worms immersed in 40% ASW compared to ligatured worms. This difference was absent in worms exposed to 15% ASW. The [^{14}C]inulin associated with ligatured animals is clearly material that has adhered to the surface of those animals. In unligatured animals, the [^{14}C]inulin content is the sum of the material on the surface and that imbibed by the worm. In worms immersed in 40% ASW, the amount of inulin detected is greater in unligatured worms than in ligatured worms, and it can be concluded that such worms imbibe the medium. In contrast, there is no difference in inulin content between ligatured and unligatured worms immersed in 15% ASW, the amount of inulin remaining at the level characteristic of ligatured worms in

40% ASW. Thus, whereas worms immersed in 40% ASW drink, worms immersed in a hypotonic medium do not imbibe amounts of medium detectable by these methods.

Discussion

The results described in this paper are generally consistent with the principal conclusions of our earlier paper (Fusé *et al.* 1993). These results confirm that worms or sacs immersed in hypo-osmotic media do not gain weight and are capable of osmoregulation over a 24h period. The further experiments with sacs confirm that the principal site of osmoregulation is located in the body wall.

The results presented here also indicate that there is a behavioural component to the ability of *P. decipiens* to withstand hypo-osmotic conditions. It is clear that while worms exposed to 40% ASW imbibe the medium, as shown by the increase in content of [¹⁴C]inulin in unligatured worms compared to HTL worms, worms exposed to 15% ASW do not drink. This conclusion explains the observation that HL or HTL worms immersed in 40% ASW or unligatured worms immersed in 15% ASW do not achieve an equilibrium exchange of ³H₂O within 24h. Clearly, the ability to imbibe the medium is essential to achieve equilibrium exchange within 24h. If the oral route is blocked, either as the result of a ligature or as a result of a failure to drink occasioned by the osmotic pressure of the medium, exchange is limited to the body wall, and the water in the worm does not exchange fully with the water in the medium during the first 24h.

These conclusions, however, lead to a paradox. In sac preparations, the only route available for exchange is *via* the body wall, and the absence of the oral route ought to lead to a failure to achieve equilibrium exchange within 24h. Nevertheless, sacs exposed to ³H₂O for 24h exhibit a water content determined by exchange levels that is undistinguishable from the water content measured by dry mass determination. The following model resolves the apparent paradox.

The difference between sacs and intact animals resides in the absence of the intestine and of the virtual absence of the PCF from sacs. Because the intestine is only one cell thick, it is reasonable to assume that exchange across the intestine would be more rapid than that across the body wall. But even with that reasonable assumption, why is complete exchange within 24h possible in a sac, but is not achieved in an HTL worm or a worm that does not drink as the result of exposure to hypotonic media?

The observations can be explained by assuming that the PCF consists of two compartments. In one compartment, the water is freely available for exchange, while in the other compartment, represented by some component of the PCF, the water is less available. In unligatured worms in 40% ASW, medium is taken into the intestine as the worm drinks, and exchange across the intestine into the PCF compartment is therefore sufficiently rapid to allow the water in the more slowly exchanging compartment to come to equilibrium within 24h. In ligatured worms immersed in 40% ASW, or in worms exposed to 15% ASW, no medium enters the intestine, and the only route for exchange with the PCF is *via* the body wall. This route is too slow to allow the more slowly exchanging compartment in the PCF to come to equilibrium within 24h. In sacs, which contain at best only very small amounts of PCF diluted with 40% ASW, the two

compartments are effectively absent, and exchange is with the 40% ASW contained in the sac.

While this model explains all of the observations presented in this paper, it leaves unanswered the nature and dimensions of the second compartment in the PCF. While no direct evidence bearing on this question is currently available, there are some observations which may be pertinent. It is proposed that the second compartment consists of hydrophilic proteins which bind water or reduce its osmotic activity. We know very little of the origin and composition of the PCF for any nematode. Various fibrous elements are included in the pseudocoelom of many nematodes (Wright *et al.* 1972) and, in *P. decipiens*, these elements appear to be secreted by a large pseudocoelomocyte (Boghen and Davey, 1975). The elements are probably related to elastin or collagen, both well known for their ability to bind water. In addition, the PCF is rich in proteins. While this has not been carefully documented, the viscosity of the PCF and its tendency to form a gel-like material have been noted in the current study. Our own unpublished observations suggest that the protein content of PCF in *P. decipiens* exceeds 40mgml^{-1} . These unsystematic observations suggest that the PCF of *P. decipiens* contains the necessary elements for the existence of the postulated slow-exchanging compartment and that a detailed examination of the composition of the PCF would be of interest. Unfortunately, the collapse of the inshore cod fishery on the East coast of Canada has prevented us from conducting such a study.

Earlier studies on ecdysis in *P. decipiens* also led to the conclusion that there were two compartments in terms of water exchange. One compartment contained water that was freely available for exchange, while the second compartment only became accessible for exchange when the ecdysial hormone was released. The excretory cell was identified as part of the second compartment, based on direct measurements of the water content of individual cells (Davey, 1979). The excretory cell is also present in sacs, but it represents a very small proportion of the total water content of the worm, and hence its potential influence on the results under consideration here would not be detectable by the methods used. Because the worms in the previous study (Davey, 1979) were no longer dormant, and because they were maintained in 0.9% NaCl, it is difficult to make useful comparisons with the present work. Nevertheless, that earlier work identified a slowly exchanging compartment in worms incubated at 37°C , but not activated to ecdyse, of about $5\ \mu\text{l}$. The data from Figs 1 and 2 in the current paper suggest that the dimensions of the slowly exchanging compartment in worms maintained at 5°C is also about $5\ \mu\text{l}$. While we have no data on the volume of PCF contained in a worm, the fact that $1\ \mu\text{l}$ can be collected with ease suggests that the total volume is likely to be in the region of $3\text{--}4\ \mu\text{l}$. Given that the water content of the excretory cell may approach 1mg (Davey, 1979), it is possible that the entire slowly exchanging compartment may be defined by the excretory cell and the PCF.

In summary, a model is proposed which accounts for the distribution of water in normal and hypo-osmotically stressed worms. It is hypothesized that four compartments are available for the exchange of water. The intestine is the site of the most rapid exchange. The body wall is less permeable, but of sufficiently high permeability to allow the establishment of equilibrium exchange of water in sacs in less than 24h. One

component of the PCF is also freely available for the exchange of water, while a second, not necessarily membrane-bound, component of the PCF is not. It is suggested that this component is the rate-limiting step for the equilibrium exchange of $^3\text{H}_2\text{O}$ in intact worms. When worms are in hypo-osmotic conditions, they shut their mouths to eliminate influxes of water into the intestine, and thus slow the rate of entry into the PCF.

References

- BOGHEN, A. D. AND DAVEY, K. G. (1975). A pseudocoelomocyte in the parasitic nematode *Phocanema decipiens*. *Trans. Am. microsc. Soc.* **94**, 203–210.
- DAVEY, K. G. (1979). Molting in a parasitic nematode, *Phocanema decipiens*: the role of water uptake. *Int. J. Parasitol.* **9**, 121–125.
- DAVEY, K. G. (1988). Endocrinology of nematodes. In *Endocrinology of Selected Invertebrate Types* (ed. H. Laufer and R. G. H. Downer), pp. 63–86. New York: Alan R. Liss Inc.
- DAVEY, K. G. AND ROGERS, W. P. (1982). Changes in water content and volume accompanying exsheathment of *Haemonchus contortus*. *Int. J. Parasitol.* **12**, 93–96.
- FUSÉ, M., DAVEY, K. G. AND SOMMERVILLE, R. I. (1993). Osmoregulation in the parasitic nematode *Pseudoterranova decipiens*. *J. exp. Biol.* **175**, 127–142.
- LIKELY, C. G. AND BURT, M. D. G. (1989). Cultivation of *Pseudoterranova decipiens* (sealworm) from third-stage larvae to egg-laying adults *in vitro*. *Can. J. Fish. aquat. Sci.* **46**, 1095–1096.
- MCCLELLAND, G. (1980). *Phocanema decipiens*: molting in seals. *Exp. Parasitol.* **49**, 128–136.
- MYERS, B. J. (1959). *Phocanema*, a new genus for the anisakid nematode of seals. *Can. J. Zool.* **37**, 459–465.
- TOWNSLEY, P. M., WRIGHT, H. G., SCOTT, M. A. AND HUGHES, M. L. (1963). The *in vitro* maturation of the parasitic nematode *Terranova decipiens* from cod muscle. *J. Fish. Res. Bd Can.* **20**, 743–747.
- WRIGHT, K. A., DICK, T. A. AND HAMADA, G. S. (1972). The identity of pseudocoelomic membranes and connective tissue in some nematodes. *Z. Parasitenk.* **39**, 1–16.