

OSMOREGULATION IN THE PARASITIC NEMATODE *PSEUDOTERRANOVA DECIPIENS*

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Summary

When subjected to hyper- or hypo-osmotic stress at 5°C for 24h, third-stage larvae of the parasitic nematode *Pseudoterranova decipiens* do not exhibit changes in mass or in the osmotic pressure of the pseudocoelomic fluid. Immersion in solutions containing ³H₂O demonstrates that exchange with the water in the pseudocoelomic fluid is substantially complete within 24h. Sacs composed of cylinders of body wall without the intestine and pseudocoelomic fluid do not gain weight when immersed for 24h in hypotonic medium. Metabolic poisons abolish the ability of whole worms and sacs to maintain their weight when immersed in hypotonic media. These observations support the conclusion that the nematode is capable of at least short-term osmoregulation and that the site of osmoregulation is the body wall. The observations that more fluid is passed from the anus in some hypo-osmotically stressed worms and that worms ligatured at the tail exhibit a small increase in mass when exposed to hypo-osmotic conditions may indicate that the intestine plays a minor and subsidiary role in osmoregulation.

Introduction

The nematodes occupy an important position in phylogeny. They represent the first major group of animals with a body cavity, the pseudocoelom. At the same time, they are among the most numerous multicellular animals on earth, occupying habitats as diverse as the soil, fresh water and the sea, and exist not only as free-living forms but as parasites in both plants and animals. Because the free-living nematodes are small, and because the larger parasitic forms are difficult to maintain in a healthy state outside the host, our knowledge of the physiology of these interesting animals is limited.

In particular, little is known of the capacity of nematodes to regulate their internal osmotic pressure. Experiments aimed at studying osmoregulation in small nematodes have been complicated by difficulties in obtaining adequate samples of body fluids for

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precise measurements of ionic and osmotic concentrations. The only reliable studies on the osmotic pressure of the body fluids of a nematode have been conducted on *Ascaris lumbricoides* (Schopfer, 1925, 1932; Hobson *et al.* 1952; Harpur and Popkin, 1965). However, when *Ascaris* is removed from its normal environment in the intestines of pigs, it enters a physiological decline from which it never recovers, and measurements taken during this period are thus of limited applicability (Harpur, 1963, 1964; Davey, 1964; Harpur and Popkin, 1973). A variety of indirect methods has been used to study osmoregulation in nematodes, but definitive experiments have not been possible (Wright and Newall, 1976, 1980).

The codworm, *Pseudoterranova* (*Phocanema*, *Porrocaecum*, *Terranova*) *decipiens* (Myers, 1959; Likely and Burt, 1989) is an anisakid nematode which occurs as the infective third-stage larva in the muscle of the Atlantic cod, *Gadus morhua*. If the fish host is eaten by a seal, the nematode develops through the fourth stage to the adult in the intestines (McClelland, 1980*a,b*). Because the third-stage larva is in a state of developmental quiescence in the cod, worms removed to a holding solution can be stored at 5°C and will retain for many weeks their capacity to develop when subjected to appropriate conditions (Davey, 1976). The worms are large enough to permit dissection and the sampling of body fluids. While there is considerable variation in size, third-stage larvae are approximately 6cm in length and weigh approximately 25mg.

Earlier studies on the control of ecdysis have revealed that the distribution of water among the compartments of both *P. decipiens* and *Haemonchus contortus* changes in a controlled way during development (Davey, 1979; Davey and Sommerville, 1974; Davey and Rogers, 1982). These observations suggest that nematodes may have the capacity to osmoregulate. This paper explores that possibility using the quiescent third-stage larva of *P. decipiens* at 5°C, thereby avoiding the complicating effects of the changes in the distribution of water that accompany development.

Materials and methods

Third-stage infective larvae of *P. decipiens* were shipped on ice from Sydney, Nova Scotia, after being removed from codfish muscle at various fish processing plants. Arrangements were made to maintain worms in 0.9% NaCl on ice until they were received and subsequently stored at 5°C in 40% artificial sea water (ASW). All experiments were conducted at 5°C and were carried out over a period of 18 weeks from the time the worms were received.

100% ASW solution consisted of salts in the following concentrations (in mmol l⁻¹): NaCl 401.78, KCl 8.85, CaCl₂·2H₂O 10.00, MgCl₂·6H₂O 53.94, NaHCO₃ 2.26, Na₂SO₄ 27.60, MgSO₄ 30.41, H₃BO₃ 0.42 (Pantin, 1964). This solution has an osmotic pressure of 1000mosmolkg⁻¹. Solutions at various osmotic pressures were made by diluting 100% ASW solution with appropriate quantities of double-distilled water.

Pseudocoelomic fluid (PCF) was collected from worms for determinations of osmolality as described by Davey (1979). Two methods were employed for determinations of the osmolality of the PCF. In most cases, the PCF from individual worms was collected in a drawn-out Pasteur pipette and expelled as a drop under 20 µl of

type 'B' immersion oil (R. P. Cargill Laboratories, Inc., New Jersey, USA). A subsample of about 5 nl was taken up in a micropipette, using suction from a micrometer syringe (Gilmont Instruments Inc., Great Neck, New York 11021) filled with immersion oil, and discharged into the oil-filled carrier of the osmometer. Osmolality determinations were made, based upon the depression of freezing point, on a Clifton direct reading nanolitre osmometer (Clifton Technical Physics, Hartford, New York 12838) calibrated against the manufacturer's standards. In a few instances, osmolality of PCF was determined in a vapour pressure osmometer (model 5100C, Wescor, Logan, Utah, USA). This required the pooling of the PCF from 2–5 worms in order to achieve the sample size required.

Changes in body mass were used as an indication of the uptake or loss of water. Worms were lifted from containers on the tines of curved forceps and quickly blotted on bibulous paper (Fisher Scientific, Toronto, Canada) in order to remove surface water. Water that remained trapped in tightly coiled worms was removed by suction with a Pasteur pipette connected to an aspirator at the time that worms were placed on the paper. Worms were transferred rapidly into pre-weighed scintillation vials containing ASW at 5°C. Worms were out of solution during these procedures for no longer than 10s. Masses were determined on an analytical balance with a sensitivity of 0.01mg.

Vials with or without worms were quickly weighed, and the difference in these masses was defined as the worm mass. Because measurements were made on larvae with varying mass from as low as 12mg to as high as 40mg, changes in mass over a 24h interval were expressed as a percentage of the initial body mass.

Worms were maintained in vials of chilled ASW on ice except during blotting and suction-drying. Weighing of each specimen took less than 1min. Experiments demonstrated that any condensation that might occur on the chilled vials as they were removed from the ice for weighing contributed less than 0.01mg to the total mass recorded (data not shown). This value is less than 0.8% of the body mass of the smallest worms.

In some experiments, worms were ligatured in chilled ASW, with ophthalmic surgical silk thread (IR465, Irex Surgical Instruments, Toronto, Ontario), 1–2mm from the anterior tip of the worm, posterior to the nerve ring and excretory pore (head ligatures), or 1–2mm from the posterior tip, anterior to the anal opening (tail ligatures).

In order to study the role of the body wall in osmoregulation, the body wall was isolated as a cylindrical 'sac' preparation. The head and tail of whole worms were cut off 1–2mm from the ends, eliminating the nerve ring and excretory pore at the anterior end and the anal opening at the posterior end. The intestine was removed with a pair of forceps. The excretory cell was left in place along the muscle layer of the body wall, in the pseudocoelomic cavity. The cylinders of body wall were left in chilled 40% ASW for 30min in order to allow it to enter the pseudocoelom. These cylinders were then ligatured 1 mm from each end with ophthalmic surgical thread.

Worms from which excess water had been removed with filter paper prior to sampling the PCF often released fluid from the anus. To measure this fluid more carefully, worms were held for 24h in distilled water (0% ASW) or in 15%, 40% or 60% ASW. Each was then wiped with filter paper, examined under a dissecting microscope until fluid appeared, or for 4min, whichever came first. The volume and osmolality of the anal fluid

were determined and, as soon as fluid had been released, the PCF was collected as described above and osmolality determined.

The metabolic inhibitors *o*-hydroxydiphenol (*o*-HP), 2,4-dinitrophenol (DNP) and potassium cyanide (KCN) were applied either to sacs or to whole worms in control and hypo-osmotic conditions. The inhibitor *o*-HP was dissolved in chilled ASW at a concentration of $10^{-3} \text{ mol l}^{-1}$. KCN was dissolved in warm ASW at a concentration of $10^{-3} \text{ mol l}^{-1}$, and all vials containing KCN solutions were covered with aluminium foil to protect against degradation by light. DNP ($10^{-3} \text{ mol l}^{-1}$) was dissolved in warm ASW and worms were presoaked in 40% ASW with DNP at 5°C for 24h before being transferred to 40% or 15% ASW with DNP for another 24h, to allow for complete permeation of the inhibitor across the body wall.

In order to verify that the worms were permeable to water, groups of worms were exposed to 15% or 40% ASW containing $^3\text{H}_2\text{O}$ (Amersham, Toronto, Canada; 5 Ci ml^{-1}) at 5°C. At various intervals, worms were removed from this solution, rinsed rapidly in 15% or 40% ASW without $^3\text{H}_2\text{O}$, blotted and the PCF sampled as described above. The sample (usually $1 \mu\text{l}$, but occasionally $0.5 \mu\text{l}$) was transferred to a glass scintillation vial containing 10.0ml of ACS scintillation fluid (Amersham, Toronto, Canada). The samples were counted by scintillation spectrometry, as were samples of the incubation medium similarly added to ACS. The counts from the worms were converted to volume equivalents of water by dividing by the specific activity of the incubation medium (typically about $1.25 \times 10^8 \text{ disintegrations min}^{-1} \text{ ml}^{-1}$). Five worms were used for each time period.

Results

The stability of worms held in 40% ASW

The experiments reported in this paper were performed on worms held at 5°C in 40% ASW for varying lengths of time up to 126 days. It is important to establish that the capacity of the worms to osmoregulate does not vary with time in storage. Two lines of evidence are presented. First, the osmotic pressure of the PCF does not vary in any marked way in worms stored for longer or shorter times (Table 1). The differences between means in Table 1 are not significant ($0.2 < P < 0.5$). The mean osmolality of these samples was $475 \pm 7 \text{ mosmol kg}^{-1}$ ($N=51$). Second, worms exposed for a 24h period to 15% ASW or distilled water (0% ASW) at 5°C do not gain weight. While this fact in isolation demonstrates that the worms have the capacity to regulate their volume, it also suggests that they may have the capacity to osmoregulate. There are no significant changes in mass for worms maintained for up to 126 days *in vitro* ($P > 0.05$) when these worms are exposed to hypotonic media for 24h. For worms held for 132 days, however, the capacity to withstand exposure to hypotonic media is lost, and such worms exhibit a significant ($P < 0.001$) gain in mass (Table 2).

The effect of immersion in media of different tonicities

Worms stored in 40% ASW at 5°C were transferred to distilled water (0% ASW) or to different concentrations of freshly prepared ASW, including 40%, for either 24h or 240 h

before the osmolality of the PCF was determined. The results are displayed in Fig. 1 as the regression of the osmolality of the medium upon that of the PCF. It is clear that the osmolality of the PCF changes only slightly in worms subjected to severe osmotic stress for 24h, confirming that the worms are capable of osmoregulation over this period. However, worms subjected to the same stress for 240h behave more nearly as osmoconformers, with the osmolality of the PCF changing with the osmolality of the medium in which the worms were immersed, but remaining at an osmotic concentration which is approximately 90mosmolkg^{-1} above that of the medium. In worms exposed to osmotic stress for longer than 10 days, there was no further change in the osmotic pressure of the PCF; levels approximately 90mosmolkg^{-1} above that of the medium were maintained for as long as 30 days (data not shown).

Table 1. *The osmolality of the pseudocoelomic fluid (PCF) of worms immersed in 40% artificial sea water (ASW) for 24h after various times of storage in the same medium*

Days in storage	PCF osmolality (mosmolkg^{-1})
1	471.0 \pm 10.0 (7)
2	435.8 \pm 3.20 (9)*
3	456.0 \pm 11.0 (9)
28	509.0 \pm 19.0 (10)
37	463.0 \pm 14.0 (10)
54	469.0 \pm 20.0 (9)
80	440.2 \pm 6.80 (9)*
112	476.0 \pm 14.0 (6)
126	427.9 \pm 8.20 (8)*

*Values determined with a vapour pressure osmometer. All other values were determined with a Clifton direct reading osmometer.

The numbers in parentheses indicate the number of samples used.

Differences between means were not significant ($0.2 < P < 0.5$). Values are mean \pm S.E.M.

Table 2. *The change in mass of whole worms after immersion in artificial seawater solutions (ASW) of different osmotic concentrations for 24h following various times in storage in 40% ASW*

Days in storage	Mass change %		
	40% ASW	15% ASW	0% ASW
80	-0.67 \pm 0.6	-0.71 \pm 0.7	1.76 \pm 1.3
112	-0.22 \pm 1.3	-0.51 \pm 2.1	ND
120	-0.05 \pm 1.6	0.06 \pm 1.4	ND
126	0.19 \pm 0.6	3.65 \pm 1.4	0.35 \pm 1.6
132	-0.48 \pm 0.4	23.05 \pm 0.3 ^a	43.76 \pm 0.5 ^b

ND, values not determined.

^{a,b}Values are significantly different from controls and from each other, as determined by ANOVA and Duncan's multiple range test.

Values are mean \pm S.E.M.

Changes in mass of whole worms and sacs exposed to different concentrations of ASW for 24h are displayed in Table 3. These data demonstrate that both intact worms and sacs consisting of the body wall are able to maintain their mass in the face of either hyper- or hypo-osmotic stress over a 24h exposure: the percentage change in mass in hyper- or hypo-osmotic media did not exceed that in 40% ASW.

Permeability of the nematode to water

While a more detailed consideration of water movements will form part of a future paper, it is essential to explore in a preliminary way the permeability of the nematode to water in order to establish the mechanism by which the apparent osmoregulation occurs. Fig. 2 sets out the time course of the uptake of $^3\text{H}_2\text{O}$ into the PCF of worms exposed for various lengths of time to $^3\text{H}_2\text{O}$ in either 40% or 15% ASW. While there is some evidence that worms exposed to 15% ASW reach an exchange level slightly lower than that reached by worms in 40% ASW, it is clear that exchange between the medium and

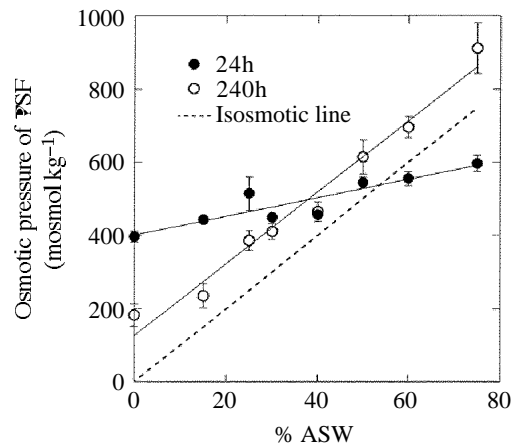


Fig. 1. The regression of the osmolality of the external medium upon that of the pseudocoelomic fluid (PCF) for worms exposed to different concentrations of ASW at 5°C for either 24h or 240h. The coefficient of correlation, r^2 , is greater than 0.9, and the slopes of the two lines are significantly different ($P < 0.0001$). The broken line indicates the line of isosmocity.

Table 3. *The change in mass of whole worms and body wall preparations (sacs) immersed in artificial sea water (ASW) of various osmotic concentrations for 24 h*

ASW	Mass change (%)	
	Whole worms	Sacs
0%	0.23±1.14 (27)	0.53±1.34 (31)
15%	-0.46±1.03 (34)	-0.34±1.70 (17)
40%	-0.45±0.63 (34)	1.17±2.34 (9)
60%	-0.10±0.88 (38)	-0.12±1.42 (13)

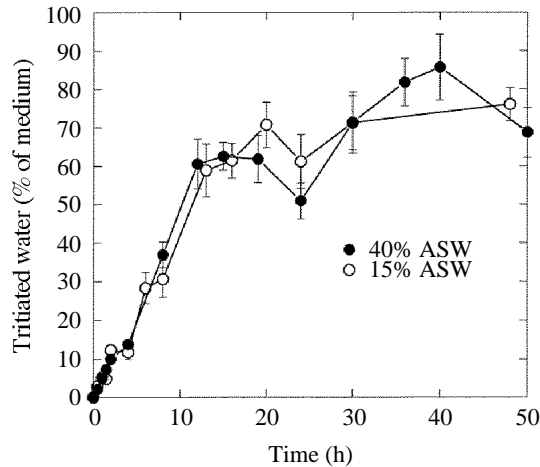


Fig. 2. The uptake of $^3\text{H}_2\text{O}$ into the PCF of worms exposed for varying times to either 40% or 15% ASW containing $^3\text{H}_2\text{O}$. Each point is the mean of samples from five worms, and the data are expressed as a percentage of the specific activity of the medium \pm S.E.M.

the water in the PCF of the worm is substantially complete in both 40% ASW and 15% ASW within 24h.

Metabolic requirement for osmoregulation

In order to explore whether the ability to osmoregulate over a 24h period is dependent on an intact metabolism, three metabolic inhibitors were employed. Immersing *P. decipiens* in o-HP ($10^{-3} \text{ mol l}^{-1}$) in 15% ASW for 24h resulted in 75% mortality (data not shown). Most of the dead worms had suffered ruptures of the body wall, with the gut protruding through the rupture: those that had not ruptured were rigid and bloated in appearance. Those worms immersed in the inhibitor in 40% ASW did not die, and did not appear to be bloated.

Worms were exposed to the general electron transport inhibitor DNP by presoaking in $10^{-3} \text{ mol l}^{-1}$ DNP in 40% ASW for 24h before transfer for a further 24h to either 40% or 15% ASW containing inhibitor. The changes in mass are displayed in Fig. 3. Worms in 15% ASW containing inhibitor experienced a significant ($P < 0.001$) increase in mass, while those in 40% ASW containing inhibitor did not.

It proved not to be necessary to pre-soak worms when using KCN as the metabolic inhibitor. Worms transferred to either 0% or 15% ASW containing $10^{-3} \text{ mol l}^{-1}$ KCN for 24h gained significantly ($P < 0.001$) more weight than worms not exposed to KCN or worms exposed to inhibitor in 40% ASW (Fig. 4).

Since both worms and sacs appeared to osmoregulate, the energy requirements of the body wall were examined by immersing sacs in 40% or 0% ASW in the presence of $10^{-3} \text{ mol l}^{-1}$ KCN (Fig. 5). Sacs immersed in 40% ASW with KCN did not show an appreciable change in mass after 24h. In contrast, sacs immersed in 0% ASW with KCN

showed a significant increase in mass compared to controls and compared to sacs immersed in cyanide-free 0% ASW ($P < 0.001$).

Release of fluid from the anus

Worms were held in 0%, 15%, 40% or 60% ASW for 24h and then examined for release of fluid from the anus as described in Materials and methods. For those that released more than a trace of fluid, the volume was determined. Under the microscope,

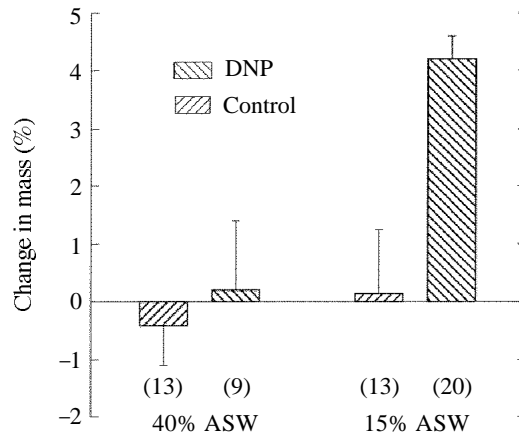


Fig. 3. The effect of DNP on mass changes, as a percentage of the initial mass, of worms exposed for 24h to 40% or 15% ASW. Worms were pre-soaked for 24h in 40% ASW containing $10^{-3} \text{ mol l}^{-1}$ DNP before exposure to the inhibitor in the experimental solutions. Control worms were not exposed to inhibitor. The numbers in parentheses represent the numbers of worms in each experimental group, and the vertical bars indicate the S.E.M.

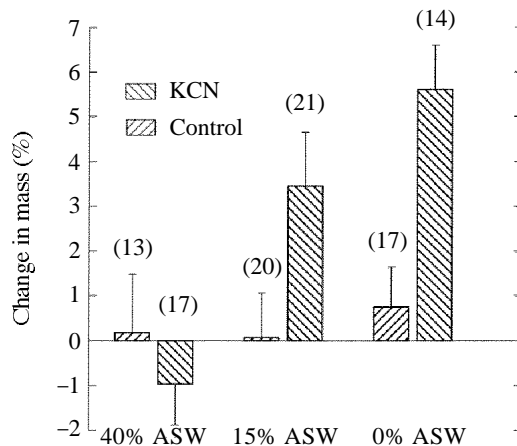


Fig. 4. The effect of KCN ($10^{-3} \text{ mol l}^{-1}$) on mass changes, as a percentage of the initial mass, of worms exposed for 24h to 40%, 15% or 0% ASW. Control worms were not exposed to inhibitor. The numbers in parentheses represent the numbers of worms in each experimental group, and the vertical bars indicate the S.E.M.

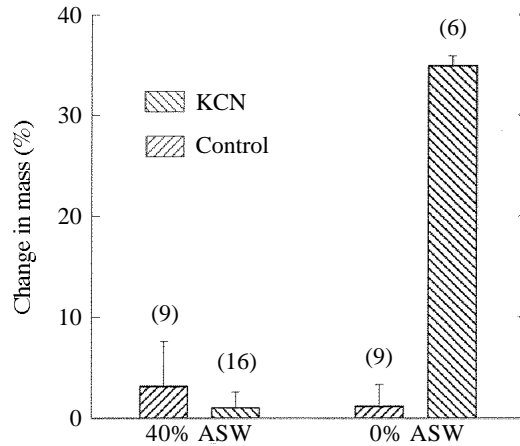


Fig. 5. The effect of KCN ($10^{-3} \text{ mol l}^{-1}$) on mass changes, as a percentage of the initial mass, of sacs exposed for 24h to 40% or 0% ASW. Control sacs were not exposed to inhibitor. The numbers in parentheses represent the numbers of sacs in each experimental group, and the vertical bars indicate the S.E.M.

the fluid released from the anus was seen to contain a very fine flocculent precipitate. The data obtained are displayed in Table 4. There appears to be a greater tendency for worms exposed to 0% ASW to release fluid, while worms exposed to more concentrated media released smaller amounts of fluid with less frequency. For example, 14 of the 20 worms exposed to 60% ASW failed to produce fluid during the 4min for which they were examined.

The osmotic pressure of the fluid released from the worms was determined, along with the osmotic pressure of the PCF from the same worm. There was no correlation between the osmotic pressure of the fluid released from the anus and that of the PCF (data not shown).

While it would be unwise to base any definitive conclusions on these preliminary observations, they could be taken to imply that substantial amounts of fluid might be

Table 4. Release of fluid from the anus of *Pseudoterranova decipiens* after exposure to distilled water or to 15%, 40% or 60% ASW

Volume released	Water	15% ASW	40% ASW	60% ASW
No fluid	10	11	10	14
Trace*	3	8	5	3
>10nl	7	1	4	3
Mean volume (nl±S.E.M.)	88±42	(20)	62±16	36±22

*<10nl: actual volume not determined.

Worms were dried with filter paper and then examined under low magnification until fluid was produced, or for 4min, whichever came first.

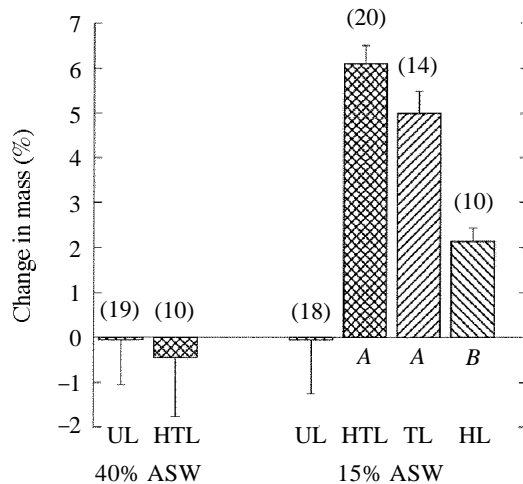


Fig. 6. The effects of ligatures on changes in mass, as a percentage of initial worm mass, in worms immersed in either 40% or 15% ASW for 24h. UL, no ligatures; HTL, head and tail ligatures; TL, tail ligatures; HL, head ligatures; The numbers in parentheses represent the numbers of worms in each sample. Bars with the same letter are not significantly different from each other, but are significantly different from the other bars ($P < 0.001$). Bars without letters are not significantly different from one another.

discharged from the anus of worms in a hypotonic medium. Subsequent experiments were designed to test this possibility.

Ligatures

To explore the role of the alimentary tract or the excretory system in osmoregulation, ligatures were placed on whole worms to eliminate sites of entry or exit of water. Worms were ligatured at both the head and tail (HTL) or at the head (HL) or tail (TL) alone. Ligatures were placed at the head posterior to the excretory pore, and thus eliminated both the mouth and the excretory pore as sites for the exchange of water. Tail ligatures were applied anterior to the anus. The effects of ligaturing on mass changes in worms exposed to 40% and 15% ASW are shown in Fig. 6.

There was no significant change in mass in whole or HTL worms in 40% ASW or in whole worms in 15% ASW. Worms ligatured at the tail, however, did show significant increases in mass when immersed in 15% ASW for 24h ($P < 0.001$). There was no significant difference between worms ligatured at both the head and tail and those ligatured at the tail alone. HL worms in 15% ASW also exhibited a significant increase in mass. However, it is important to note that this increase was very small, and was significantly less than the increases seen in HTL and TL worms.

Discussion

This paper provides the first unequivocal evidence for osmoregulation by a nematode over a wide range of osmotic concentrations. It attempts to localize sites of

osmoregulation in the parasitic codworm *P. decipiens*, and it describes some osmotic responses of this nematode in hypo-osmotic conditions.

P. decipiens is a parasitic nematode living as a third-stage larva in the muscles of cod. It can be maintained in the laboratory at 5 °C in 40% ASW for some months, and this arrangement raises questions about the validity of results obtained under these artificial conditions as a model for physiological regulation *in vitro*. Three lines of evidence support the view that nematodes maintained under these conditions respond to their environment in a manner which reflects *in vivo* responses.

First, *P. decipiens* stored in this way will, when provided with appropriate stimuli, develop to the fourth-stage larva in an apparently normal fashion (Davey, 1971). Second, data provided in the current paper (Table 1) demonstrate that the osmotic pressure of the PCF does not change over an extended period of storage in 40% ASW at 5 °C. Third, the apparent capacity to withstand osmotic stress over a 24h period remains intact for about 3.5 months of storage in 40% ASW at 5 °C. After a prolonged period in storage, however, this capacity deteriorates rapidly. Thus, *P. decipiens* stored in 40% ASW at 5 °C is in a stable condition, and is not subject to the slow, irreversible physiological and biochemical decline characteristic of *Ascaris lumbricoides* when it is removed from its host (Harpur and Popkin, 1965). Eventually, the worm does deteriorate after at least 126 days in storage, but this deterioration is rapid and unmistakable and has been avoided by using worms stored for less than 126 days.

The evidence that *P. decipiens* is capable of osmoregulation is simple and direct. Intact worms do not exhibit significant changes in mass when transferred for 24h to either hypo- or hyper-osmotic media, even when the difference in osmotic pressure between the body fluids of the nematode and the medium is as high as 400mosmolkg⁻¹. Similarly, the osmotic pressure of the PCF remains relatively constant in worms exposed for 24h to media of widely differing tonicities.

This capacity to withstand severe osmotic stress is not a function of altered permeability, since worms exposed to ³H₂O exhibit a pattern of uptake of the labelled water into the PCF in which a plateau is reached in well under 24h, suggesting that exchange of ³H₂O is complete by this time. This may appear to be a relatively low rate of exchange, but it is important to recognise that these measurements are made at 5 °C: in developing worms maintained at 37 °C, exchange is complete in about 3h (Davey, 1979). While the preliminary data presented here contain some suggestion that the exchange level in worms exposed to 15% ASW is marginally less than that in worms exposed to iso-osmotic conditions, and while a second paper will explore permeability more fully, it is clear that the worms are freely permeable to water. A further confirmation that the body wall in particular is permeable to water comes from the studies on sacs, in which preparations poisoned with KCN behave as osmometers and gain weight when exposed to hypo-osmotic conditions.

Since the total water content of an average worm weighing about 30mg is about 21mg, the total flux over 24h would involve at least this mass of water. If the worm were not osmoregulating, fluxes of this order would result in increases in mass or dilutions of the PCF easily detectable by the methods employed here.

The conclusion that *P. decipiens* is permeable to water is in accord with a variety of

studies on other species of nematodes. Non-feeding infective larvae of species like *Nippostrongylus brasiliensis*, which are enclosed within two cuticles, show enhanced pulsations of the excretory ampulla as the osmotic pressure of the external medium is reduced, suggesting that the cuticle is permeable (Weinstein, 1952). Measurements of permeability coefficients calculated using $^3\text{H}_2\text{O}$ and [^{14}C]inulin on whole animals, indirect measurements of water fluxes from changes in mass or length of whole worms and measurements of changes in mass of ligatured animals and sac preparations have been conducted on a wide variety of nematodes (Schopfer, 1932, Stephenson, 1942; Hobson *et al.* 1952; Anya, 1966; Myers, 1966; Viglierchio, 1974) and, in each case, the authors have concluded that the nematode is freely permeable to water.

One of the principal sites of osmoregulation in *P. decipiens* appears to be the body wall, since sacs, in which the intestine is absent, exhibit no alteration in weight when exposed to hyper- or hypo-osmotic conditions for 24h. The tissues that remain are the muscles and the hypodermis. The preparation also includes the large 'excretory cell', which, in spite of its name, is known to be the source of the enzymes involved in the shedding of the cuticle at ecdysis (Davey and Kan, 1968). The opening of its duct to the outside is not included in sacs. The hypodermis is primarily a syncytium in which the nuclei are located in the lateral chords (Kan and Davey, 1965). Embedded in the syncytium of *P. decipiens* and closely applied to the cuticular surface is a series of small cells (M. Fusé, K. G. Davey and R. I. Sommerville, unpublished observations). These have been described in several nematodes (Bird, 1971) and are usually referred to as 'hypodermal glands' or 'bacillary band cells'. On structural grounds, it has been suggested that these cells are sites for osmoregulation (Wright, 1968). The body wall has also been implicated rather indirectly as a site for the regulation of ions in *A. lumbricoides* (Hobson *et al.* 1952), and has been suggested as a possible site for the transport of ions and water in *Xiphinema index* and in some trichuroids (Roggen *et al.* 1967; Wright and Chan, 1973).

While the body wall of the nematode is capable of osmoregulation, the evidence presented in this paper also suggests that the intestine may be an additional site for osmoregulatory control. The evidence is far from conclusive, but the observation that nematodes exposed to hypo-osmotic conditions void fluid from the anus with greater frequency and in greater volumes than those subjected to hyperosmotic stress, together with the observation that tail-ligatured worms exposed to hypo-osmotic conditions increase in weight, lend support to the view that the anus may be a route for the removal of excess fluid. However, it is important to remember that the method of fluid collection requires handling of the nematodes, a procedure that perturbs osmoregulation (M. Fusé, K. G. Davey and R. I. Sommerville, unpublished data). Determinations of the osmotic pressure of the expelled fluid, not presented here, reveal that there is no relationship between the osmotic pressure of the fluid and that of the PCF of the worm from which the fluid is expelled. There are other possible explanations for the behaviour of ligatured worms. For example, the known endocrine centres in *P. decipiens* are in the head and tail (Davey, 1988) and, while there is thus far no evidence for hormonal control of osmoregulation in nematodes, the putative ecdysial hormone in *P. decipiens* exerts its action through a redistribution of water in the nematode (Davey, 1979). Other hormones controlling osmoregulation may exist.

Whatever the precise location of the osmoregulatory mechanism, it is dependent on an intact metabolism for its operation. Nothing is known about the energy metabolism of *P. decipiens*, but the major respiratory pathway in the mitochondria of muscle, reproductive tissue and the gut of *A. lumbricoides*, *N. brasiliensis* and *Ascaridia galli* terminates at cytochrome *o*, a pathway which is inhibited by *o*-HP (Barrett, 1976; Paget *et al.* 1988a,b). Exposure of worms to hypo-osmotic conditions in the presence of this inhibitor leads to the death of the worms, with symptoms suggesting that osmoregulation has failed, although it has not been possible to make a definitive determination.

A minor cyanide-sensitive metabolic pathway also exists in nematodes (Barrett, 1976; Paget *et al.* 1988a,b). Both intact animals and sacs exhibit significant increases in mass when exposed to hypo-osmotic conditions in the presence of $10^{-3} \text{ mol l}^{-1}$ KCN. These results, together with those involving the general metabolic poison DNP, lead to the conclusion that the ability to osmoregulate in *P. decipiens* depends on metabolic energy and arises at least partly as the result of metabolic activities located in the body wall of the nematode. Although the data are not shown, worms exposed to $10^{-3} \text{ mol l}^{-1}$ KCN for 24h and then returned to cyanide-free 40% ASW for a few weeks are again able to osmoregulate over a 24h period in cyanide-free 15% ASW, demonstrating that the effect of KCN poisoning on osmoregulation is reversible.

While *P. decipiens* clearly possesses the capacity to control its internal osmotic pressure in the face of severe osmotic stress over a relatively short period, this capacity is insufficient to sustain a constant osmotic pressure over a longer period of exposure. Intact worms exposed to 15% ASW for 24h do not gain weight, but worms exposed to osmotic stress for 10 days gain weight and behave as euryhaline osmoconformers, maintaining the osmotic pressure of the PCF at about $90 \text{ mosmol kg}^{-1}$ above that of their environment. Measurements of the osmotic pressure of the PCF have only been determined for one other nematode, *A. lumbricoides*. Under the conditions of the experiments, however, this nematode was unable to maintain the osmotic pressure of the PCF at a constant level in the absence of osmotic stress (Hobson *et al.* 1952; Harpur and Popkin, 1965). The cause of the eventual failure in osmotic control in *P. decipiens* remains a matter for conjecture. It is possible that it reflects an exhaustion of metabolic reserves, since the medium in which the nematodes are stored contains no nutrients. A similar explanation for the abrupt loss of short-term osmoregulatory capacity after prolonged storage in 40% ASW at 5°C also seems plausible.

The capacity to osmoregulate over such a wide range may appear to be surprising in a parasitic nematode which lives in an apparently constant environment in the tissues of the host. But the demands for osmoregulation are great and varied. Obviously parasitic species must be able to respond to changes in osmotic pressure encountered in the transition between the external environment and the host, and between one host and another. In addition, like other nematodes, regulation of the water balance is critical for both moulting and for movement. *P. decipiens* will encounter, as an egg, the high osmotic pressures of sea water ($1000 \text{ mosmol kg}^{-1}$) as it passes from the intestines of seals in the faeces. In the larval stages, worms are in contact with the blood of copepods and isopods (Oshima, 1987), the intestinal contents, blood and muscles of cod and the stomach and intestinal contents of seals (McClelland, 1980b). In each they must osmoregulate

efficiently. Even within the alimentary tract of the seal, substantial changes in osmotic pressures are likely to be encountered (Mettrick and Podesta, 1974).

The larger nematodes, at least, rely on a 'hydrostatic skeleton' (Harris and Crofton, 1957), in which the internal hydrostatic pressure of the nematode provides an antagonistic force against which the longitudinal muscles contract. The osmotic pressure of the PCF, while considerably greater than the hydrostatic pressure, will nevertheless influence its magnitude. Certainly those worms that gained considerable amounts of weight as a result of exposure to distilled water for 10 days were bloated and rigid and unable to move. The ability to osmoregulate is thus important in the maintenance of the appropriate hydrostatic pressure essential to movement in nematodes; this may explain the maintenance of the osmotic pressure of the PCF at approximately $90 \text{ mosmol kg}^{-1}$ above that of the medium.

The capacity to osmoregulate may also be important during development in nematodes. Ecdysis in *P. decipiens* is accompanied by movements of water among the compartments in the nematode (Davey, 1979). In *Haemonchus contortus*, exsheathment of the third-stage larva is a complex process during which there are rapid alterations in the distribution of water within the nematode (Davey and Rogers, 1982). Understanding the osmoregulatory capacity of the dormant third-stage larva of *P. decipiens* is an important prerequisite to studies on the more complex regulation of water during the rapid changes that follow exposure of the worms to developmental temperatures.

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