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Water, Water Compartments and Water Regulation in Some Nematodes Parasitic in Vertebrates[1]

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Abstract: While nematodes are sometimes regarded as osmoconformers, at least one species is capable of short-term osmoregulation over a wide range of osmotic environments, and the principal site of osmoregulation is the body wall. This general osmoregulation is important to the life of the nematode not only in confronting variations in the environment, but also in maintaining its hydrostatic skeleton. There is also evidence suggesting that compartments exist in some nematodes and that water exchange between the compartments is limited and slow. The ability to regulate the internal movements of water is important in molting and in the infective process. Hormones may be the mediators of osmotic control.

Key words: animal parasitic nematode, *Haemonchus contortus*, nematode, osmotic control, osmoregulation, *Pseudoterranova decipiens*, regulation, water compartment.

The ability to regulate the osmotic concentration of body fluids is important to the survival and success of many metazoan groups. Nematodes encounter a wide range of environmental conditions, and might be expected to exhibit some degree of osmoregulation. The definitive review of the subject (29) concludes, on the basis of changes in linear dimensions when worms are immersed in hypo- or hyperosmotic media, that many nematodes are capable of volume regulation, but points out that direct measurements of osmotic changes are lacking. More recently, however, it has become clear that some nematodes parasitic in animals have the capacity to regulate the osmotic concentration of their body fluids, at least in the short term, and that the capacity to regulate the flow of water in and out of various compartments in the nematode body is important to their development and survival. This paper will review the evidence for these phenomena, using two model systems. *Psuedoterranova decipiens* is an anisakid that lives as a third-stage juvenile in the muscles of cod and some other fish, and that lives as an adult in the intestines of seals (23,24). The large third-stage juvenile is about 4.5 cm in length and frequently weighs in excess of 20 mg. It can be kept for many weeks in 40% artificial sea water (ASW) at 5 C; when placed at 37 C, it initiates development and, provided with an appropriate medium, will undergo the molts to the adult (22,28). *Haemonchus contortus* is a trichostrongylid that exists as a free-living third-stage juvenile ensheathed in the second-stage cuticle. When ingested by the definitive host, the sheep, it quickly exsheaths and enters a parasitic existence in the abomasum. A much smaller nematode (the third-stage juvenile is less than 1 mm in length), it has been the subject of the classical studies on exsheathment by Rogers and Sommerville (27) in Australia, who have shown that the principal stimulus leading to exsheathment is elevated CO_2 combined with a temperature of 37 C to 39 C; CO_2 triggers the release of exsheathing fluid into the space between the two cuticles.

Osmoregulation in P. decipiens: When *P. decipiens* are immersed for 24 hours in solutions with osmotic pressures as low as 0 (distilled water) and as high as 800 mOs/kg, the osmotic pressure of the pseudocoelomic fluid remains approximately constant (Fig. 1). Moreover, nematodes immersed in strongly hypo-osmotic media of 150 mOs/kg for 24 hours do not gain weight. These experiments were conducted at 5 C to avoid complications of development, but even at this temperature, experiments with ^3H_2O show that ex-

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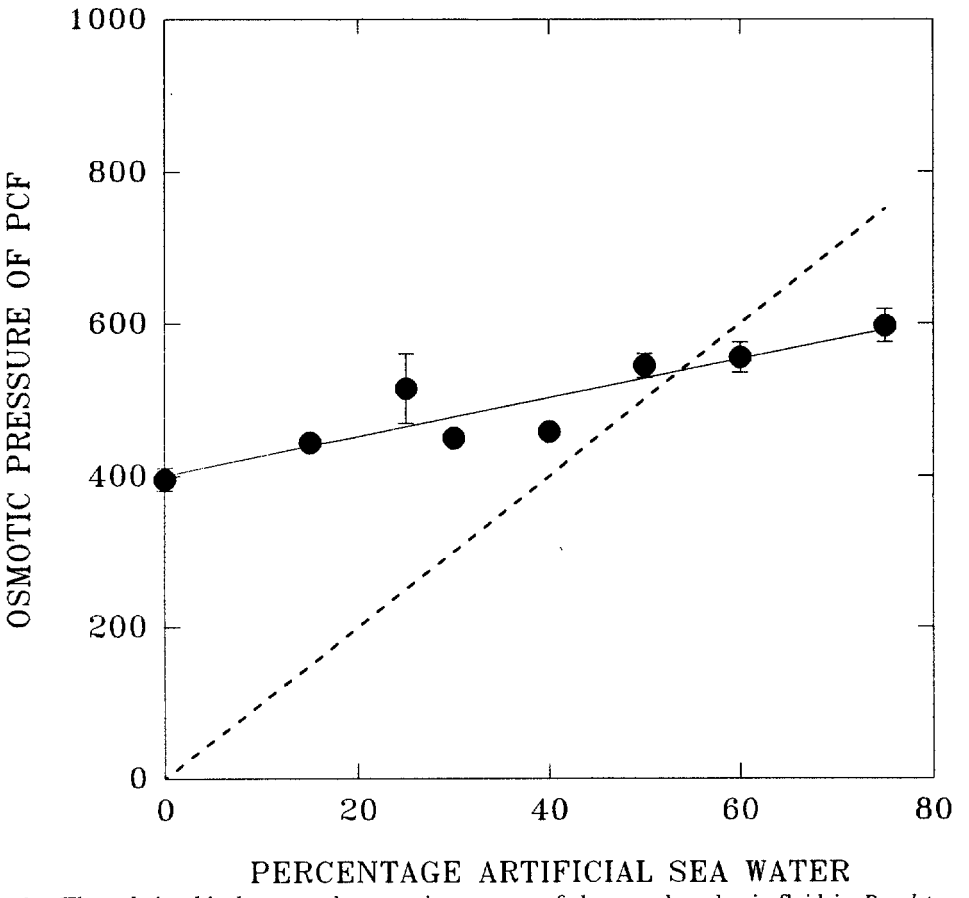

FIG. 1. The relationship between the osmotic pressure of the pseudocoelomic fluid in *Pseudoterranova decipiens* and that of various concentrations of artificial sea water in which worms had been immersed for 24 hours at 5 C. There are at least 10 worms for each concentration, and the vertical bars indicate the standard error of the mean. The solid line is a regression line with $r^2 > 0.9$, and the broken line indicates the line of iso-osmocity. Based on a figure in an earlier paper (16).

change is complete before 24 hours. Thus, the cuticle is permeable to water, and the worms are capable of controlling internal osmotic pressure (16,17).

This ability to control the osmotic pressure in these dormant worms is, however, short-lived; exposure of the worms to osmotic stress for 48 hours leads to an increase or decrease in the osmotic pressure of the pseudocoelomic fluid, and by 10 days of exposure, the worms are effectively osmoconformers (17). The reasons for this failure have not as yet been explored, but it is possible that such worms, faced with the profound energetic demands of osmoregulation and with no access to nutrition, simply deplete their reserves.

The head and tail of *P. decipiens* can be removed, the intestine withdrawn from the cylinder of body wall tissue, and the cylinder fashioned into a sac by ligating the two ends. When such preparations are transferred from 40% ASW (400 mOs/kg; isosmotic) to 15% ASW (150 mOs/kg) for 24 hours at 5 C, they do not gain weight. If such preparations are exposed to KCN, they gain weight, but the effect of KCN is reversible upon return to KCN-free medium. Thus, the capacity to osmoregulate resides in the body wall of the nematode and is dependent upon an intact energy metabolism (17).

Volume regulation in H. contortus: The evidence for osmoregulation in *H. contortus* is less direct, but measurements of volume

and water content at the time of exsheathment are suggestive. The volume of juveniles can be calculated from morphometric data. Measurements of length and diameter made just before and 30 minutes after the worms are exposed to CO_2 as well as elevated temperature reveal that a juvenile loses a total of 32 pl in total volume (9).

Measuring exchange volumes with ^3H_2O in *H. contortus* is complicated by the presence of the sheath and the fluid-filled space between the sheath and the third-stage juvenile. The sheath can be removed from the juvenile by treatment with NaOCl, but this procedure in itself results in the loss of 17 pl from the esophagus (12). Nevertheless, when such "desheathed" juveniles are now subjected to the normal exsheathing stimulus of CO_2 at 38.5 C, they lose a further 15–19 pl of water as measured by exchange with ^3H_2O (9,12). If the loss of volume of 17 pl (largely or entirely from the esophagus) due to the NaOCl treatment is included, the total loss of water is about 35 pl, a value that agrees well with the estimate of volume loss obtained by morphometrics.

Thus, the exsheathing stimulus results in a reduction of the volume of the worm by about 17%. Most of this loss appears to be in the form of water, representing a net loss of 27% of the water content of the juvenile (Table 1). These changes argue for a capacity to regulate the water content of the worm, although the evidence is indirect.

Compartments in P. decipiens: Two lines of evidence point to the existence of water compartments in *P. decipiens.* In order to understand the first of these lines of evidence, it is necessary to understand something about the control of development. When worms are transferred to 37 C in 0.9% NaCl, the formation of a new cuticle is initiated, but the old third-stage cuticle is not removed; the worms remain ensheathed. If they are incubated in a complete medium, ecdysis of the third-stage cuticle occurs about 3 days later. If worms cultured in 0.9% saline are exposed to insect juvenile hormone or certain of its mimics at an appropriate time, ecdysis occurs, primarily as a result of the activation by juvenile hormone of the pathway leading to the release of molting fluid into the space between the two cuticles (4,5). If worms are cultured for 12 hours at 37 C in 0.9% NaCl containing ^3H_2O, an exchange level equivalent to 13.6 mg of water is achieved. When farnesyl methyl ether (FME), a mimic of juvenile hormone, is present in the medium, the exchange level is near 20 mg. By contrast, when determinations of water content are made by dry mass, these are identical for unactivated worms and activated worms at about 18.5 mg (3). These data are best explained by assuming that in unactivated worms there is a compartment containing water that is less freely exchangeable than water in other compartments and that activation removes whatever barrier to exchange exists.

The second line of evidence is more direct and involves third-stage juveniles kept at 5 C. In worms maintained in 40% ASW (isosmotic) and exposed to ^3H_2O, exchange is complete within 24 hours and indicates a water content close to 70%; in

TABLE 1. Summary of volume changes associated with exsheathment in *Haemonchus contortus*, based on data in earlier papers (9,12).[a]

	Whole worm	Oesophagus
Morphometrics (pl)		
Before ecdysis	190	37
After ecdysis	158	20
Volume loss	32	17
Percentage loss	17	46
^3H_2O exchange (pl)		
Before ecdysis	130	
After ecdysis	95	
Water loss	35	
Percentage loss	17	
OPD (°)		
Before ecdysis		57
After ecdysis		102
Estimated loss (%)		44

[a] The figures under morphometrics are derived from linear measurements of worms and represent the volume of the worms in picoliters. Those under ^3H_2O exchange represent the water volume as determined by ^3H_2O exchange. Those for the optical path difference (OPD) on the oesophagus represent the degrees of rotation of the polarizing analyzer, and an increase in OPD signifies a decrease in volume.

worms maintained in 15% ASW, exchange levels indicating a water content of less than 60% are observed. In both cases, the water content by dry mass is about 70% (17) (Fig. 2). These apparently mysterious effects are explained by the observation that worms in 15% ASW do not drink, and thus the only route for the penetration of water into the body is via the cuticle. Penetration here is sufficiently slow so that some slowly exchanging compartment does not reach equilibrium within 24 hours. On the other hand, in those worms maintained in 40% ASW that do drink, water can exchange more rapidly across the intestinal epithelium. Several lines of evidence indicate that an important component of the slowly exchanging compartment is represented by the pseudocoelomic fluid. For example, sac preparations, which contain no pseudocoelomic fluid, exhibit no trace of two compartments: the water content by exchange or by dry mass is identical (Fig. 2). Thus, the slowly exchanging compartment is not surrounded by membranes. Possibly one or more of the molecules that make up the solution of proteins in the pseudocoelomic fluid have the capacity to bind water (the concentration of proteins exceeds 40 mg/ml). While there are no systematic observations to support this view, various fibrous elements have been reported in the pseudocoel of many nematodes (30), and the fibrous proteins, elastin and collagen, are known for their ability to bind water.

Compartments in H. contortus: Haemonchos contortus is too small to permit direct measurements of the sort made on *P. decipiens,* but results obtained by quantitative interference microscopy suggest that compartments may exist. Interference microscopy measures the optical path difference (OPD) between light passing through a tis-

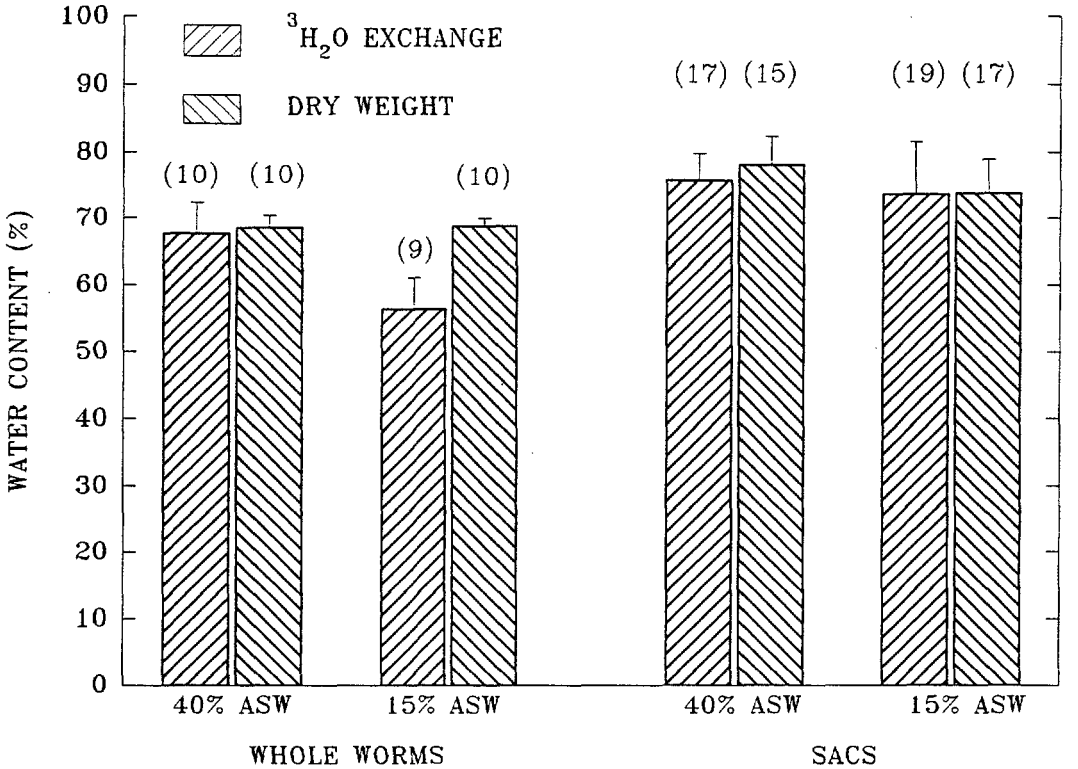

FIG. 2. Apparent percentage water content of *Pseudoterranova decipiens* as intact worms and as ligatured sacs exposed to 40% or 15% artificial sea water for 24 hours at 5 C. The numbers in parentheses indicate the numbers of worms or sacs in each experimental group, and the vertical lines indicate the standard error of the mean. Based on data taken from an earlier paper (17).

sue and that not passing through the tissue. It provides a measure of the dry mass of the tissue, and changes in OPD over a short time reflect differences in water content or volume; the OPD is inversely proportional to the water content of the tissue (1,14).

In *H. contortus,* the OPD both of the esophagus and of the excretory cell exhibit sharp increases at the time of exsheathment, with the ratio of the OPD before exsheathment to that after exsheathment in both cases amounting to 0.44 (12). Since changes in the OPD are inversely proportional to changes in water content, and hence volume, this suggests that these tissues lose about 44% of their volume over a very short time. Because the esophagus is likely the source of the exsheathing fluid, this loss may be a reflection of that function. As can be seen in Table 1, there is good agreement between the loss as determined by morphometrics and that determined by interference microscopy. Because the excretory cell is very small, it has not been possible to provide morphometric data. In both cases, of course, the dry mass has increased, indicating a loss in water and a decrease, as a result, in volume. Given that the water loss for the entire worm at this stage is only 27%, this may constitute evidence of a differential loss in volume from some tissues.

Compartments and the ecdysial process in P. decipiens: In *P. decipiens,* ecdysis from the third to the fourth stage can be made to occur in vitro either by culturing the worms in a complex medium (28) or by adding FME to a saline solution (2). In either case, ecdysis results from the secretion into the space between the two cuticles of a complex mixture of enzymes that erode the old cuticle in a circumferential ring near the anterior of the worm, allowing a cap to come off and the fourth stage to emerge. The source of the enzymes is the so-called excretory cell (8).

The so-called "excretory cell" in *P. decipiens,* the name notwithstanding, has never been implicated in the process of excretion or osmoregulation. Its histology, cytochemistry, and ultrastructure characterize a gland specialized for the secretion of proteins (11). Others have noted similar properties of the excretory cell in other species (21,25). It would, perhaps, be better termed as an "exodigestive gland" (21), possibly derived from an esophageal gland.

In *P. decipiens,* the excretory cell is large and may weigh up to 1 mg or more. In the unactivated third stage, the gland is full of paracrystalline secretory granules that swell and lose their paracrystalline organization when the cell is preparing to secrete the ecdysial fluid (11). This suggests an influx of water, a hypothesis that is borne out by experiment. When the worm is activated by incubation with a mimic of insect juvenile hormone, the water content of the excretory cell increases from 76.7% to 82.1% as determined by dry weight (3), or by 147 nl as determined by exchange with ^3H_2O (6). This influx of water into the excretory cell is, of course, only part of a broader redistribution of water already alluded to within the worm; at the time of activation of the worm, the total water content of the worm does not change, but water in some compartments of the worm becomes more freely available for exchange (3).

Compartments and exsheathment in H. contortus: In *H. contortus,* the evidence for tissue-specific changes in water content at exsheathment is not so clear, and any changes that do occur represent a decrease in water content rather than an increase as in *P. decipiens.* Exsheathment is accompanied by a large reduction in volume, much of which is a reduction in the water volume (see above and Table 1). We know very little beyond that, except that of the 32 pl total loss, more than half comes from the esophagus, probably in the form of the exsheathing fluid. The precise source and route of the remaining water loss is not clear, but it may reflect a loss from the remaining tissues.

The functions of such a reduction in volume are not clear. Some of the reduction is an inevitable consequence of the rapid se-

cretion of the exsheathing fluid. The remaining water loss may be a means of preparing the worm for its new environment in the abomasum. Alternatively, or additionally, the reduction in volume may assist the worm in escaping from the sheath, once the sheath has been opened by the action of the enzymes in the exsheathing fluid.

Hormonal regulation of water content: While we lack the sort of precise experimental evidence available for some other invertebrates, it is becoming increasingly clear that some of the developmental and physiological events in nematodes are orchestrated by hormones (4,5). In *P. decipiens,* ecdysis is accompanied by histological and ultrastructural changes in both peptidergic and aminergic neurosecretory cells in the ganglia associated with the nerve ring (7,18,19). More direct evidence, however, is provided from studies with tissue extracts.

As has already been pointed out, the activation of *P. decipiens* involves an influx of water into the excretory cell. The influx of water fails to occur in worms in which the head is ligatured. If unstimulated worms receive an injection of an extract of heads from activated worms, the excretory cell takes up water. Because the cell is large, it can be manipulated in vitro and its water volume monitored by ^3H_2O exchange. When cells are exposed directly to extracts of heads from activated worms, the water volume of the cells increases, while extracts from other tissues have no effect (6). Similar experiments involving a number of amines (20) have led to the conclusion that there is an ecdysial hormone in *P. decipiens* that acts on the excretory cell to permit the entry of water. The release of that hormone is caused by the detection of the appropriate stimulus (thus far not identified), which acts via a nor-adrenergic pathway.

As has already been indicated, exsheathment in *H. contortus* involves a reduction in the water content and volume of the worm. Rogers and Sommerville in their classic experiments with a UV pencil have demonstrated that an area in the region of the nerve ring is essential to the process of exsheathment (26). More recently, nor-adrenaline has been implicated in the process (10). Definitive experiments demonstrating that hormones are involved in regulating the events surrounding exsheathment in this species have not yet been done, although all of the results thus far are consistent with the *P. decipiens* model.

Nematodes may possess both diuretic and anti-diuretic hormones. This conjecture rests on the results of experiments in which *P. decipiens* reacts to stress by reducing its capacity to osmoregulate in hypoosmotic conditions. The effect of ligatures placed at the head and (or) tail alters the effect of stress on osmoregulatory capacity. These observations have led to the hypothesis that an anti-diuretic factor is released from the tail region, that a diuretic factor is released from some unknown part of the body, and that the control over the release of both factors resides in the head (13). This hypothesis awaits testing.

The importance of water in the life of nematodes: Osmoregulation is important to any organism, but there may be some special reasons for its importance in nematodes. Obviously, both free-living and parasitic species may encounter very different environments during the life of a single individual, and homeostatic mechanisms such as osmoregulation will be important to the nematodes' capacity to adapt to different environments.

Frequently, nematodes must survive periods of unfavorable conditions by becoming quiescent or entering diapause, and this period is often characterized by reduced water content, while emergence from dormancy is associated with increasing water content (15). Perhaps the capacity to control water content, osmoregulation, is crucial to survival during quiescence and diapause.

Finally, it is important to remember that the movement of nematodes is dependent on a hydrostatic skeleton, in which the contraction of the body wall muscles is op-

posed by the internal hydrostatic pressure of the nematode. The maintenance of an appropriate osmotic pressure in the pseudocoel is important to the hydrostatic pressure. In nematodes in which the capacity to osmoregulate in hypo-osmotic media has been reduced or lost, the animals become excessively turgid and unable to move (16). The maintenance of optimal turgor within the hydrostatic skeleton may explain the aniso-osmotic regulation of body fluids observed in *P. decipiens* (16) and other nematodes (29), by which the nematode maintains the osmotic pressure slightly above that of the medium in which it is immersed.

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