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IONIC AND OSMOTIC REGULATION IN AN ACANTHOCEPHALAN

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Abstract.- Adult specimens of the endoparasite Macracanthorhynchus hirudinaceus were incubated under anaerobic conditions at 39°C in a series of dilutions of artificial sea water. Changes in osmolarity and ionic composition of the pseudocoelomic fluid were studied as functions of changes in these various parameters in the external solution. It was determined that the pseudocoelomic fluid is maintained hyperosmotic to the environment between external osmotic concentrations of 0 - 500 mOsmol. The degree of hyperosmotic regulation decreased linearly with increasing external osmolarity. Sodium concentration of the psuedocoelomic fluid was regulated at 85.0 meq./liter against external sodium concentrations varying from 17.5 through 53.0 meq./liter. Above 53.0 meq./liter internal sodium concentration increased linearly with increases of that ion in the external environment but remained above that ion's external concentration. Potassium and calcium concentrations in the pseudocoelomic fluid were regulated such that potassium remained higher internally than externally while calcium was maintained much lower internally than externally. Chloride appears to be passively distributed. Data gathered in this study indicates the presence of weak forms of ionic and osmotic regulatory mechanisms.

The adult stage of the acanthocephalan, Macracanthorhynchus hirudinaceus, is an obligate endoparasite of swine and several other vertebrates including humans (Petrochenko, 1956 and Schmidt, 1971). Very little is known concerning how this organism is adapted to survive the periodic ionic and osmotic fluctuations which characterize the portion of the vertebrate intestine in which it is found. Gettier (1942) and later Van Cleave and Ross (1944) working with another acanthocephalan from the turtle, Neoechinorhynchus emydis noted volume changes associated with different osmotic concentrations of culture media. Read and Rothman (1958) working with Moniliformis dubius from the rat observed that in vivo swelling and shrinking was correlated with the concentration of carbohydrate solutions which the investigators were force-feeding the host rats. Crompton and Edmonds (1969) investigated the osmotic concentration of the pseudocoelomic fluid of Polymorphus minutus and found its osmotic concentration to be similar to that of a simultaneous sample of host intestinal juice. In addition, Crompton (1970) in a later monograph referred to all acanthocephalans as osmoconformers. Branch (1970a, 1970b) in a series of papers examined several aspects of ion and osmotic regulation in Moniliformis dubius. In

addition, on the basis of histochemistry he described the distribution of sodium, potassium and calcium in the body wall. This study was to investigate the way in which internal concentrations of ions in M. hirudinaceus vary with changes in the external ions.

METHODS AND MATERIALS

Specimens of M. hirudinaceus were collected from the small intestine of swine at Hunter Packing Company, East St. Louis, Illinois. Worms were dissected free from the intestine in such a fashion that the proboscis remained embedded in a small portion of the calceous nodule. These specimens designated for later in vitro experiments were placed immediately into a pre-warmed Dewar flask partially filled with intestinal juice. The contents of the flasks were then sealed from direct atmospheric contact by means of a thick cork stopper. Approximately four hours elapsed between the collection of the specimens and the beginning of the in vitro experiments.

Samples of pseudocoelomic fluid for ionic and osmotic analysis were collected immediately after worms were removed from the intestine. Worms removed from the intestine were wiped clean of adhering intestinal juice with clean tissue paper. The anterior-most portion of the praesoma was sectioned and the pseudocoelomic fluid gently "milked" into small sample vials. Approximately 1.0-1.5 ml of pseudocoelomic fluid was collected per worm. No distinction was made between male and female worms, but females being larger probably contributed more fluid than males. In all cases not more than two worms were taken from the same intestine. The total number of worms collected was about 100. In the majority of experiments a composite sample of pseudocoelomic fluid from three to five worms was used. In a few cases individual samples were used in order to determine the range of individual variations. No significant differences were noted between osmolarities or sodium concentrations of pooled samples and individual samples.

Fresh samples of pseudocoelomic fluid sealed in glass vials were immediately tested for pH with Hydrion paper. Individual and composite samples were then centrifuged at 3000 x g for 15 minutes to separate the reproductive cells from the pseudocoelomic fluid. The supernatants of the samples were then placed in clean sample vials and frozen until the time for analysis. The osmolar concentrations of various samples of pseudocoelomic fluid were determined on an Osmette Precision Freezing-point Osmometer. This instrument was calibrated with 100 and 500 mOsmol standards purchased from the Precision Instrument Corporation. The osmolarity of each sample was determined three times and the mean reported. The concentration of sodium and calcium in the samples of pseudocoelomic fluid were determined by atomic absorption spectrophotometry on a Beckman Model 979 Atomic Absorption Spectrophometer. The methods of analysis followed those of Willis (1960a and 1960b). Potassium was determined by flame emission spectrophotometry on an ILS flame spectrophotometer. The methods of analysis conformed to standard clinical procedures for the determination of potassium in human serum. The concentration of chloride in pseudocoelomic fluid was determined by Whitehorn's modification of the Vollhard method (Furman, 1963).

To test the effect of changes in the external ionic and osmolar concentration on the composition of the pseudocoelomic fluid, twenty-five large

female worms were divided into five groups of five worms each and incubated for three hours in a series of dilutions of artificial sea water. The solutions employed were: distilled water (0 mOsmols); 20% sea water (212 mOsmols); 30% sea water (300 mOsmols). The various solutions of sea water were prepared by volume dilution of 100% sea water with doubly distilled and deionized water. Artificial sea water was obtained from Aquarium Systems Inc., 1450 E. 29th Street, Wickliffe, Ohio, 44092. During the three hour incubation all solutions were maintained at 39°C in a water bath and periodically gassed with nitrogen and carbon dioxide to reduce pO_2 to a level more nearly resembling that of the host intestine. Following the period of incubation, the pseudocoelomic fluid of each group of worms was collected and analyzed with respect to its ionic and osmolar concentrations.

In order to determine the ability of M. hirudinaceus to regulate its volume, groups of three large female worms were incubated at 39°C in each of the following dilutions: 10%, 30%, 50%, and 10% sea water fortified with sucrose to 542 mOsmols. At one hour intervals each group of worms was removed and weighed on a Mettler Model P162 balance and then replaced in the solution. After each weighing the solutions were gassed with nitrogen and carbon dioxide and resealed with Parafilm. After three hours of incubation sodium fluoride was added to each group to a final concentration of 0.05M. After one hour one final weighing was taken. Simultaneously, the above experiment was repeated exactly with the exception that all solutions were maintained at 10°C.

RESULTS

The pH range of all samples was in the 6.4 - 6.8 range and the osmolar concentration of the pseudocoelomic fluid was found to be 348.0 mOsmols ± 3.2 (S.E.M.). Sodium and potassium concentrations in these composite samples were 89.5 and 10.0 meq/liter respectively, and the calcium concentration was 2.5 meq/liter. The chloride concentration was found to be 101.0 meq/liter ± 6.0 (S.E.M.).

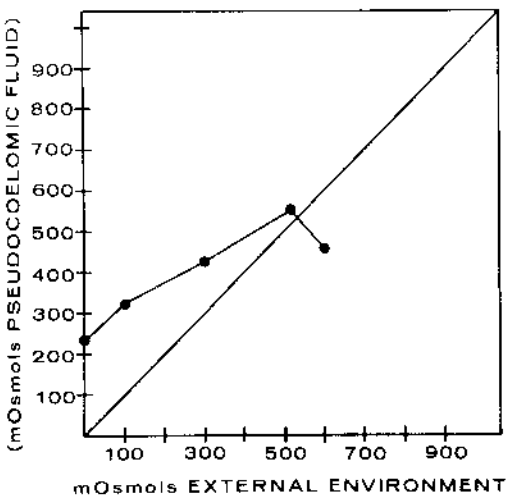

Figure 1. Osmolar concentration of the pseudocoelomic fluid of specimens of M. hirudinaceus incubated in increasing concentrations of artificial sea water.

Figure 1 shows the effect of incubation in a series of artificial sea water dilutions on the osmolarity of the pseudocoelomic fluid. After four hours in distilled water, the osmolarity of the pseudocoelomic fluid of the group was 232 mOsmols. As the external osmolar concentration increased between 0 and 50% sea water (300 mOsmols), the osmolar concentration of the pseudocoelomic fluid of each group was above that of the external environment. At 500

mOsmols the pseudocoelomic fluid was isosmotic with the external medium. Beyond this point the osmolar concentration of the pseudocoelomic fluid was observed to become more dilute than the external environment.

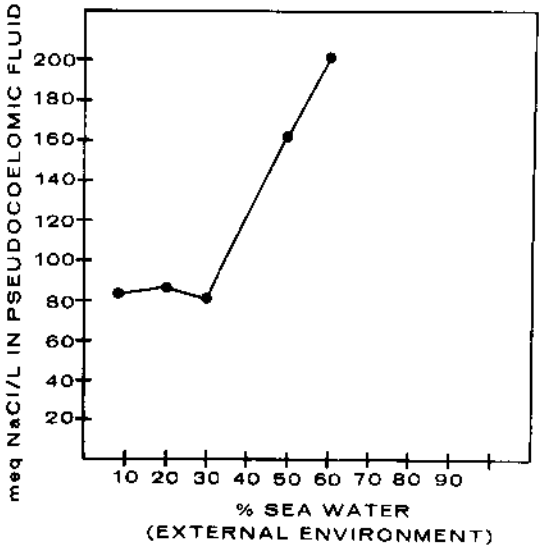

Figure 2. Sodium ion concentration of the pseudocoelomic fluid of specimens of M. hirudinaceus incubated in increasing concentrations of artificial sea water.

Figure 2 illustrates the effects of changes in the external sea water concentration on the sodium concentration of the pseudocoelomic fluid. The pseudocoelomic sodium concentration of groups incubating in 10, 20 and 30% sea water remained relatively stable at 86.0 meq/liter between external concentrations of that ion in 10-40% (44.0-176.0 meq/liter) sea water. In concentrations of sea water above 40% the internal sodium concentration was proportional (x4) to the external concentration.

In 30% sea water there was a sodium concentration of 86.2 meq/liter in the pseudocoelom as opposed to an external concentration of 132 meq sodium/liter (Table 1). The concentration of chloride in the pseudocoelom was lower than the concentration of that ion in 30% sea water. Potassium concentration in the pseudocoelomic fluid was found to be 10 meq/liter, somewhat higher than that ion's concentration in the external environment. The calcium concentration maintained internally in 30% sea water was 2.5 meq/liter as opposed to an external concentration of 1.35 meq/liter.

TABLE 1. Ion concentration of pseudocoelomic fluid of acanthocephalan in 10% and 30% sea water solution.

	10% sw		30% sw	
	Inside	Outside	Inside	Outside
Na^+	84.2 meq/l	44.0 meq/l	86.0 meq/l	132.0 meq/l
K^+	15.0	0.92	10.0	2.76
Ca^{++}	1.30	0.46	2.5	1.35
Cl^-	15.0	53.0	101.0	159.0

When acanthocephalans were incubated in 10% sea water, differences in ion

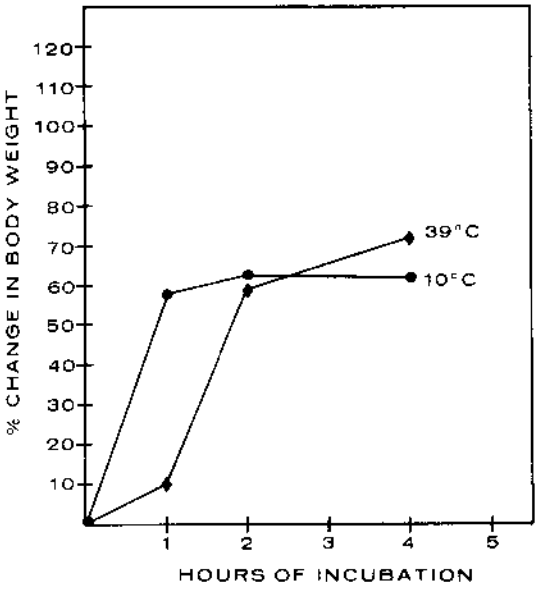

Figure 3. Percent change in body weight with time of groups of acanthocephalans incubated in 10% sea water at 10° and 39°C. Fluoride was added after three hours of incubation.

distribution from those maintained in 30% sea water were noted. Sodium ion was maintained at approximately the same level as in 30% sea water, although the external concentration of this ion was almost one-third the concentration in 30% sea water. The internal chloride concentration maintained in 10% sea water of 15 meq/liter was still lower than that of the external environment (53 meq/liter). The potassium ion gradient maintained was 15.0 meq/liter internal vs. 0.92 meq/liter external. Calcium ion concentration of the pseudocoelom was 1.3 meq/liter vs. 0.46 meq/liter external concentration.

When the percentages of body weight gains were calculated for specimens incubated for four hours in several dilutions of artificial sea water, a certain degree of volume regulation was noted. In 10% sea water (Figure 3) at 10°C an increase of more than 50% body weight within the first hour of incubation was noted while those incubating in 10% sea water at 39°C increased only 15% during the same period. By the second hour both groups had increased in weight approximately the same amount.

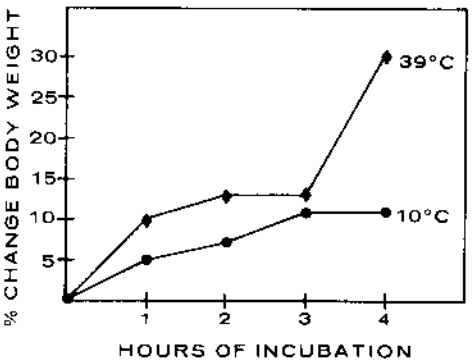

Figure 4. Percent change in body weight of groups of M. hirudinaceus incubated in 30% sea water at 10° and 39°C. Fluoride added after three hours incubation.

Acanthocephalans incubated in 30% sea water at 39°C (Figure 4) reached equilibrium in swelling after two hours at +15% body weight. These specimens incubated in 30% sea water at 10°C reached the same final equilibrium point but one hour later than the group at 39°C. When sodium fluoride was added to the medium after three hours of incubation, no effect was noted on the group at 10°C, but those incubating at 39°C began to swell rapidly doubling their body weight within the next hour.

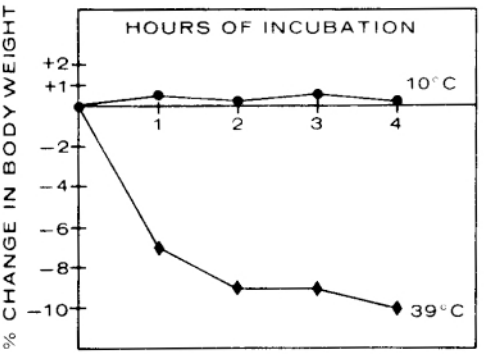

Figure 5. Percent change in body weight of groups of worms incubated in 50% sea water at 10°C and 39°C. Fluoride added after three hours incubation.

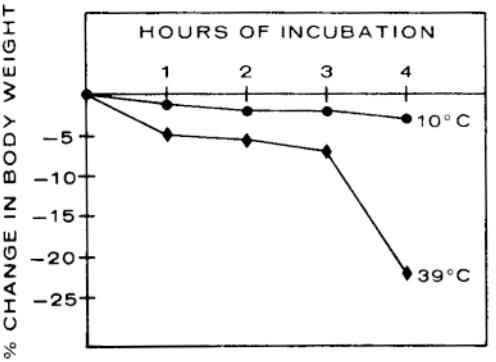

Figure 6. Percent in body weight of groups of acanthocephalans incubated in 10% artificial sea water fortified with sucrose to 542 mOsmols at both 10° and 39°C. Fluoride added after three hours of incubation.

When specimens were incubated in 50% sea water (Figure 5) at 10°C a weight gain of less than 1% was noted. Addition of sodium fluoride had no effect on weight gain or loss. Those specimens in 50% sea water at 39°C lost weight down to about -9% weight. After the addition of sodium fluoride, a further, but probably insignificant weight loss was noted. Incubation of specimens in 10% sea water, (542 mOsmols), (Figure 6) at 10°C was accompanied by a small but steady weight decrease which was also relatively insensitive to addition of fluoride. Other specimens in the same medium but at 39°C lost weight down to about -9% just as the group in 50% sea water at 39°C but this equilibrium was much more sensitive to fluoride addition.

DISCUSSION

M. hirudinaceus is a weak hyperosmotic regulator up to external concentrations of 500 mOsmols. Ascaris, a nematode of similar habitat has also been noted to practice limited hyperosmotic regulation. Based on freezing point depression data of swine intestinal juice (Read, 1950) showing it to vary in concentration between (-0.073 and -0.940) °C (30 400 mOsmols) it is probable that such regulation does occur in vivo and its extent exactly parallels the extent of most osmotic fluctuations in the swine intestine. Such an adaptation may function in the bulk absorption of nutrients from the intestine. Based on the measurements of in vivo ion concentrations in the pseudocoel it can be estimated that if these ions occur in an osmotically active form that they would account for approximately 60% of the apparent osmotically active fraction. While the chemical nature of the remaining osmotically active fraction is not yet known, the following data indicate it to be a relatively large non-diffusable anion i.e. protein. In both 10 and 30% sea water (Figures 3 and 4 and Table 1) internal chloride concentrations are much lower than external concentrations. This distribution is in accordance with a Donnan equilibrium which might be present if the pseudocoel contained large

amounts of some large non-diffusable anion. In acanthocephalans other than M. hirudinaceus (McAlister and Fisher, 1972) the concentration of the dissacharide trehalose has been reported high enough in the pseudocoelom to contribute to the osmolarity of the pseudocoelomic fluid. The presence of this solute alone, however, would not account for the apparent Donnan equilibrium.

It is also possible that chloride distribution is governed by a different manner from that discussed above. The epicuticle of M. hirudinaceus was described by Crompton (1963) and later by Wright and Lumsden (1969) to consist of a cross linked polyelectrolyte (mucopolysaccharide) gel. If such an epicuticular gel contained many negatively charged sialic acid residues it might be capable of producing a chloride distribution indistinguishable from a Donnan equilibrium. Weinstein (1968) and Katchalsky (1964) have shown that synthetic polyelectrolyte gels can create and maintain asymetric ion distributions. Therefore, it might be possible for trehalose to play a major role in osmo-regulation but an asymetric chloride distribution still to be detected.

Data gathered in these studies (Fig. 2, Table 1) suggest the presence of weak regulatory mechanisms for both sodium and potassium. From Figure 3 and Table one can deduce that the potassium concentration of the pseudocoelom is maintained at levels much higher than external concentrations. It would therefore appear that potassium is accumulated from the environment. Sodium ion (Figure 2) is regulated within a very narrow range at about 83.0 meq/liter against both inward and outward diffusion gradients. Since the electrical potential gradient of the body wall was not determined and the energy dependence of this phenomenon not explored it would be presumptious to refer to an active transport of either sodium or potassium. Branch (1970a) studying the acanthocephalan from the rat (Moniliformis dubius) found similar evidence for an inward transport of both sodium and potassium against a concentration gradient. The range of sodium regulation noted matched well with reported average values of sodium concentration in the intestinal juice of the jejunum. For example, Read (1950) lists a value for sodium concentration in intestinal juice of the dog jejunum to be 126-152 meq/liter and Hobson, Stephenson and Beadle (1952) list a value of 126 meq/liter for the swine. Furthermore, it is significant that the average sodium concentration of pseudocoelomic fluid from specimens taken directly from the host was 89.5 meq/liter, a value within the regulated range.

Experiments designed to reveal volume regulatory ability (Figures 3, 4 and 5) indicate M. hirudinaceus to be a weak volume regulator. Two types of mechanisms may be postulated to account for the data recorded. Contraction of the circular body wall muscles may be involved in resisting sudden volume changes such as those associated with an extremely hypotonic environment (Figure 3). This would explain why the group in 10% sea water at 39°C resisted initial volume change. That this resistance to volume change may depend on metabolic energy may be inferred from the lack of resistance to swelling by the group at 10°C. The reason that the NaF had no effect on either group may be that swelling to the maximum extent may have occurred and the elastic limit of the body wall reached. Also, one hour may not represent the true extent of the resistence to swelling but only an exhaustion of energy reserves.

In slightly hypotonic situations such as 30% sea water (Figure 4) no

evidence was found to connect ion movements with volume maintenance. The rate of swelling between 10°C and 39°C groups was probably not significantly different and may easily be explained by the passive inward diffusion of sodium down its concentration gradient. However, of the two groups, NaF caused rapid swelling only in the group at 39°C.

Data supporting a role of ion transport in volume regulation was obtained only from those experiments in isosmotic-hypertonic and isosmotic-hypotonic environments (Figure 5). Those groups incubated at 10°C in either 50% sea water on 10% sea water (540 mOsmols) changed little in weight. This result was anticipated since from Figure 1 it was determined that 500 mOsmols was approximately isosmotic with pseudocoelomic fluid. Therefore, in the absence of ion transport (due to low temperature inhibition) and an osmotic gradient no weight change should have occurred. At 39°C, groups incubating in these solutions again lost weight at about the same rate. Since NaF addition to the group in 10% sea water (540 mOsmols) was followed by rapid weight loss, it is tempting to suggest that continued uptake of sodium is involved in maintaining volume. If this is the case then one must infer that very little uptake was occurring in 50% sea water since NaF had no effect on this group.

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LITERATURE CITED

Branch, S. I.,1970a. Moniliformis dubius and Macracanthorhynchus hirudinaceus: Na, K, Ca and Mg content and Na and K active transport. Exp. Parasitol.,25:33-43.

Branch, S. I.,1970b. Moniliformis dubius: Osmotic responses. Exp. Parasitol. 25:95-99.

Crompton, D. W. T.,1963 Morphological and histochemical observations on Polymorphus minutus (Goeze, 1782) with special reference to the body wall. Parasitol.,53:663-685.

Crompton, D. W. T.,1970. An ecological approach to acanthocephalan physiology. Cambridge University Press. London.

Crompton, D. W. T., and S. J. Edmonds.1969. Measurement of the osmotic pressure in the habitat of Polymorphus minutus (Acanthocephala) in the intestine of the duck. J. Exp. Parasitol.,50:69-77.

Furman, N. H.,1963. Standard methods of chemical analysis. D. Van Nostrand Co. Inc. Princeton, N. Y.

Gettier, D. A. 1942. Saline requirements of Neoechinorhynchus emydis. Proc. Helminthological Soc. of Washington.,9:75-78.

Hobson, A. D., W. Stephenson, and L. C. Beadle.1952. Studies on the physiology of Ascaris lumbricoides I. J. Exp. Biol.,29:1-21.

Katchalsky, A. 1964. Polyelectrolytes and their biological interactions. Biophysics J.,4:9-42.

McAlister, R. O., and F. M. Fisher, Jr.1972. The biosynthesis of trehalose in Moniliformis dubius (Acanthocephala). J. Parasitol.,58:51-62.

Petrochenko, V. I.,1956. Acanthocephala of domestic and wild animals. Vol. I. Moscow.

Read, C. P.,1950. The vertebrate intestine as an environment for parasitic helminths. Rice Institute Pamphlet.,37(2):1-94.

Read, C. P., and A. H. Rothman.1958. The carbohydrate requirement of Moniliformis (Acanthocephala). Exp. Parasitol.,7:191-197.

Schmidt, Gerald T.,1971. Acanthocephalan infections of man with two new records. J. Parasitol.,57:582-585.

Van Cleave, H. J., and Elizabeth L. Ross.1944. Physiological response of Neochinorhynchus emydis (Acanthocephala) to various solutions. J. Parasitol. 30:369-372.

Weinstein, John N.,1968. Charge mosaic membranes: Enhanced permeability and negative osmosis with a symetrical salt. Science.,161:70-72.

Willis, J. B., 1960a. The determination of metals in blood serum by atomic absorption spectroscopy-I calcium. Spectorchem. Acta., 16:259-272.

Willis, J. B., 1960b. The determination of metals in blood serum by atomic absorption spectroscopy-II sodium and potassium. Spectrochem. Acta., 16:55-58.

Wright, R. D. and R. D. Lumsden. 1968. Ultrastructural and histochemical properties of the acanthocephalan epicuticle. J. Parasitol., 54:111-123.