

UPTAKE OF RADIOACTIVE SODIUM (^{24}Na) BY *NEREIS DIVERSICOLOR* MUELLER AND *PERINEREIS CULTRIFERA* (GRUBE)

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(Text-figs. 1-4)

INTRODUCTION

Nereis diversicolor is typically euryhaline: it is in equilibrium with normal sea water (Schlieper, 1929; Beadle, 1937), and develops hypertonicity in more dilute media which it is able to maintain indefinitely. The maintenance of this steady state is preceded by a transition period. During this there is a rapid uptake of water accompanied by a loss of salts from the worm, resulting in a fall in the osmotic pressure of the body fluids, and then a subsequent water loss which, according to Ellis (1937), is not accompanied by an uptake of salts. Worms accommodated to 25% sea water weigh about 140% of their original weight (Beadle, 1931). Beadle (1937) found that when the steady state is attained the body fluid concentration in 50% sea water approaches a value which is about 5% higher than that of the external medium, whereas in 25% sea water the concentration of the body fluid is equivalent to about 44% sea water. His worms were collected from the Northumberland coast, and concentration measurements of the body fluid were determined by Baldes's modification of the Hill vapour-pressure method. The results of Schlieper (1929) on worms from Heligoland, where they may live in a salinity as low as 4‰, are based on freezing-point determinations and give a higher internal concentration in the dilute media: after 3 days in 50% sea water the concentration of the body fluid is equivalent to 68% sea water, and after 2 days in 25% sea water equivalent to 49% sea water. These varying results suggest the occurrence of local physiological races.

The method by which this hypertonicity is maintained is open to conjecture. Since the concentration of the body fluids is increased when an animal previously treated with dilute sea water is transferred to an isotonic solution, Beadle (1937) concludes that the mechanism must entail an active process which is not merely controlling the inflow of water. He cites three possibilities: the excretion of a hypotonic fluid by the nephridia; the addition of salts to the body fluids from the tissues, or, as in *Carcinus* (Webb, 1940), an active uptake of salts from the external medium. It is, of course, probable that more than one of these mechanisms is involved. Beadle favours

the first and states that the most reasonable conclusion is that the animal behaves as a normal osmotic system as regards uptake of water and change of internal concentration, but that the volume of the body fluids is continually being reduced by the removal in the nephridia of a fluid hypotonic to the body fluid. In a previous paper Beadle (1931) showed that the respiratory and the weight curves of an animal in dilute sea water are of the same form: they each have an approximately simultaneous maximum and a subsequent fall. He suggests that the extra oxygen consumption is not the result of osmotic work, but rather that of work done by the body-wall muscles in resisting swelling. That hypertonicity is maintained, at least in part, by the active uptake of salts from the environment is, however, suggested by the results of both Schlieper (1929) and Beadle (1937).

The uptake and loss of chloride by an organism is easy to demonstrate. It has been studied for a number of invertebrates as well as anamniotes. Ellis (1939) measured the chloride lost by *Nereis diversicolor* and *N. cultrifera* when placed in 20% sea water, and also its uptake when the worms are transferred back to 100% sea water. Isotopic indicators which are now in use give further scope for studying the passage of ions between an aquatic organism and its environment. By this means Jørgensen, Levi & Ussing (1947) have recorded the uptake of $^{24}\text{Na}^+$ and $^{38}\text{Cl}^-$ by the axolotl, and Abelson & Duryee (1949) the exchange of radioactive sodium by the frog's egg. Jørgensen & Dales (1954), by using the tracer $^{36}\text{Cl}^-$, have shown that at certain dilutions of the external medium there is an active uptake of chloride ions by *N. diversicolor* and *N. virens* which have been adapted to the diluted medium, and so are in a steady state with respect to their chloride content.

METHODS

The tracer element ^{24}Na with a half-life of 14.9 h allows only short-term experiments to be carried out. It is clean to work with, however, and the slight amount adsorbed on to the surface of an organism is readily removed by washing in water. Sodium comprises the predominant cation of the environment of a marine animal, and the experiments of Ellis (1937) have shown that in *N. diversicolor* it is essential in the weight regulation associated with osmotic control. The uptake of the element by a living organism can be followed by using the apparatus designed by Arnott & Fossey (1952). It consists of 8 G.M. Tubes (20th Century, G 10 P6) set in a ring around a Perspex tube and connected in parallel. A second Perspex tube surrounds the counters and the whole is shielded by a lead castle, which can be opened at the top to the inner Perspex tube. For the present experiments a disc of sheet cork, with a hole centrally placed, was fitted horizontally into the top of the inner Perspex tube, and through the hole was slipped a boiling tube: the rim of the tube is held by the cork and the rest of it, which may contain the animal to be assayed, is exposed to the counters. A similar use of γ -Müller tubes as a

means of assaying different sources of radioactivity has been described by Freedberg, Ureles & van Dilla (1949) and Veall & Vetter (1952).

^{24}Na obtained from A.E.R.E. Harwell as $^{24}\text{Na}_2\text{CO}_3$ has the high specific activity of 32 mc/g. Tracer amounts never greater than 16 mg were added to each litre of sea water, which raises the sodium content by a maximum of 0.0148%. Each experimental worm was in 100 ml. of activated water in a darkened jar: the jar was shielded at the opening, and the water kept saturated with oxygen at normal atmospheric pressure. Experiments were maintained at a constant temperature, usually 14°C; others were at 5°C. Sea water used for the experiments had a salinity of 35‰. Its sodium was labelled with ^{24}Na , and the activity of 1 ml., indicating the presence of 10.8 mg of inactive sodium, was recorded at the beginning of the experiment. Such a standard was used to calculate the uptake of sodium by a worm under specific experimental conditions. Distilled water was added to the sea water to give the various dilutions, and hypertonic sea water was obtained by slow evaporation of the normal sea water at a temperature not higher than 60°C.

UPTAKE OF SODIUM BY *NEREIS DIVERSICOLOR* AND *PERINEREIS CULTRIFERA* IN WATER OF VARYING SALINITIES

The uptake of sodium by *Nereis diversicolor* Müller was first studied on worms which were accommodated to the various salinities of sea water to be used in the experiments. These animals would be in a steady state with respect to their sodium content. Prior to the experiment the wet weight of each worm was recorded, and those weighing about 1 g were chosen. These worms were then placed in sea water of a salinity of 35, 33, 17.5, or 9‰, or in hypertonic water, which was kept saturated with oxygen at normal atmospheric pressure and was at a constant temperature of 14°C. The weight of all these individuals had reached a relatively steady value at 36 h (Beadle (1937) found that the regulation of the osmotic pressure is completed in advance of volume regulation and well within this time). Each animal was then dried on filter-paper, the weight recorded, and it was placed in the experimental vessel which contained sea water of the same salinity as that from which it had been taken, but with the tracer sodium added. After a period ranging from 1 to 2 h the worm was removed from the vessel, washed rapidly in three changes of sea water and presented to the counting apparatus. For animals in dilute sea water the uptake of sodium per g wet weight was calculated on the original weight of tissue, the osmotic uptake of water being neglected; allowance was made for the slight loss of weight due to starvation.

The results of such an experiment are shown in Fig. 1. In sea water of a salinity of 35‰ the uptake of sodium per hour ranges from 275 to 240 $\mu\text{g/g}$ wet weight; the higher figures appear to be associated with the more active worms. In a salinity of 17.5‰ the uptake per hour reaches an average of 161 $\mu\text{g/g}$ wet weight, which is rather more than might be expected if the worm

behaves as a simple osmometer; in water of about half this salinity (9‰) the uptake may even exceed this figure, reaching an average of 180 $\mu\text{g/g}$ wet weight. In lesser dilutions, as in a salinity of 33.5‰, the sodium influx is proportional to the degree of dilution, indicating a passive exchange of ions. For worms adapted to hypertonic sea water of a salinity of 43‰ the uptake is slightly less than might be expected. The hypertonicity of worms in brackish water may involve the uptake of ions in different proportions from the normal, since Cole (1940) found a differential accumulation in *Homarus* in similar conditions.

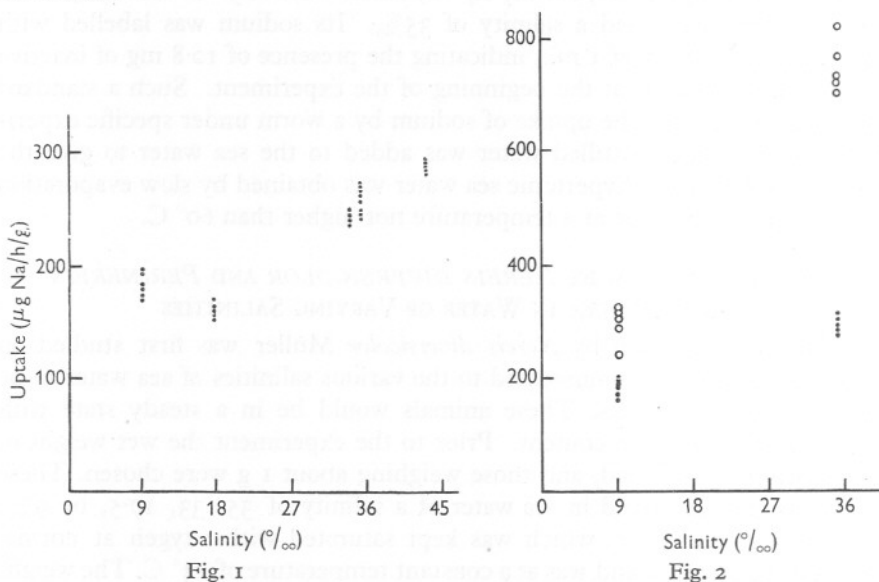


Fig. 1. *Nereis diversicolor*. Rate of uptake of sodium in sea water of different salinities. (Rate expressed as micrograms of sodium per hour per gram wet weight.)

Fig. 2. *Perinereis cultrifera* and *Nereis diversicolor*. Rate of uptake of sodium in sea water of 9‰ and 35‰ salinity. Rings indicate results for *Perinereis* and dots for *Nereis*.

Jürgens (1935) has shown that respiration takes place through the epithelium of the gut of *Nereis diversicolor* as well as through the integument. It may be that the gut wall can serve as an area of active absorption of ions for a worm in a hypotonic medium. To test this the previous experiment was repeated with a series of animals which had the body tied anteriorly and posteriorly with nylon thread to close the passage to the gut, and another series acted as a control. Both sets of worms were transferred from normal to diluted sea water, some in 50‰ and others in 25‰, and they were weighed at intervals for a comparison of the weight regulation. When accommodation was completed the uptake of sodium was measured. The results (Table I) showed that the weight regulation of worms which had the gut obstructed, and were in

50% or 25% sea water, followed the normal course; and the uptake of sodium by these individuals showed only slight individual variations from the results of the control specimens.

These results suggest that only the integument is concerned with the uptake of salts, or that, when necessary, the integument can compensate for the loss of the gut surface as an area of transport to and from the body tissues.

TABLE I. UPTAKE: $\mu\text{g Na/h/g}$ WET WEIGHT

| | Salinity | |
|----------------|----------|-------|
| | 9‰ | 17.5‰ |
| Gut obstructed | 180 | 175 |
| | 150 | 160 |
| | 170 | 140 |
| Controls | 183 | 164 |
| | 200 | 150 |
| | 170 | 170 |

The uptake of sodium from 100% and 25% sea water was studied for *Perinereis cultrifera* in the same way as for *Nereis*. *Perinereis* is a more typically marine form, and although it can withstand a lowering of the salinity of sea water, its weight curve in brackish water increases to a higher value than that of *Nereis diversicolor*, and there is no subsequent fall. In 25% sea water the animal becomes sluggish. The results of an experiment in which both polychaetes were used for comparison are shown in Fig. 2. From these it can be seen that *Perinereis* is the more permeable to sodium ions: in normal sea water the uptake is nearly 3 times greater than in *Nereis*, and in water of about a quarter this salinity the uptake is at least 1.5 times as great. In the dilute medium even this species takes from the environment a relatively greater amount of sodium than worms in normal sea water: Wells & Ledingham (1940) state that in 25% sea water *Perinereis cultrifera* maintains a slight degree of hypertonicity. The lower permeability of the integument of *Nereis* must be of importance in diminishing the amount of work necessary to maintain osmotic independence. These results, however, are contrary to those of Ellis (1939). In measuring the chloride output of *N. diversicolor* and *Perinereis cultrifera* in 20% sea water he records a higher loss of chloride from the former than from the latter, and suggests that *Nereis diversicolor* swells less in dilute sea water because of the more rapid loss of salts relative to water intake.

The influx of ions to the tissues of worms which are transferred from dilute to normal sea water is high, and is approximately inversely proportional to the dilution of the medium from which they are taken. To estimate this the following experiment was carried out. The exchange of sodium per hour by twelve specimens of *N. diversicolor* which had been living in sea water of a salinity of 35‰ was calculated, and these individuals were then transferred

to a more dilute medium, four worms being placed in water of each of the following salinities, 27, 17.5 and 9‰. After 40 h they were returned to normal sea water containing ^{24}Na and their uptake of sodium per hour was again measured—when returned to normal sea water the weight of the worm is reduced with the loss of water. The results are shown in Fig. 3, and the results of a similar experiment for a comparison between *N. diversicolor* and *Perinereis cultrifera* are given in Table II.

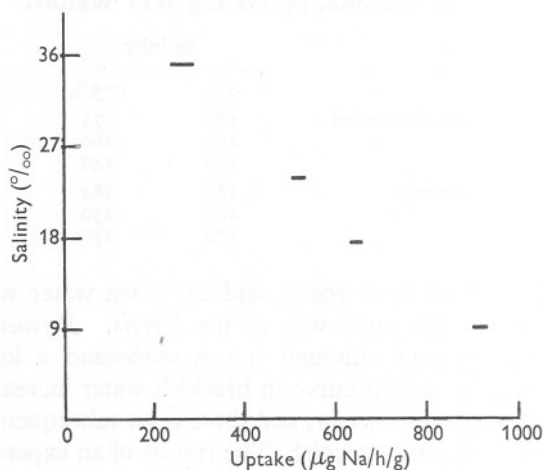


Fig. 3. *Nereis diversicolor*. The thickened bar at 35‰ salinity is the range of the rate of uptake of sodium per hour (per gram wet weight) for twelve worms. At the other three salinities is similarly shown the range of uptake by four worms when returned to sea water of 35‰ salinity from brackish water.

TABLE II. $\mu\text{g Na/h/g}$ WET WEIGHT OF WORM

| | Uptake per hour in normal sea water (salinity 35‰). Average of four worms | Uptake per hour on return to normal sea water after 26 h in dilute sea water (salinity 9‰). Average of four worms |
|------------------------|---|---|
| <i>N. diversicolor</i> | 260 | 969 |
| <i>P. cultrifera</i> | 720 | 2960 |

The increased uptake on return to normal sea water presumably compensates for the loss of salts in the lower salinities. The increase is about 3 times greater for *P. cultrifera* than for *Nereis diversicolor*.

The apparatus which has been used for these experiments makes it possible to follow the rate of uptake of radioactive sodium to the level at which equilibrium is reached, and the amount of the tracer element in the worm ceases to increase. From this level can be determined the total amount of exchangeable sodium. For *N. diversicolor* accommodated to water of a

salinity of 9‰ this is approximately 3.2 mg/g wet weight. For worms living in a salinity of 17.5‰ there may be only a slight increase over this amount, though it is doubled when the animals are in sea water of full salinity (35‰) and for an average of twenty individuals was 6.3 mg/g wet weight. There would thus appear to be a correlation between the osmotic pressure of the body fluid and the amount of exchangeable sodium. In hypertonic sea water the amount increases: for the average of six worms in water of a salinity of 51‰ it reached a value of 7.2 mg/g wet weight. *Perinereis* has a higher permeability than *Nereis*, yet the total amount of exchangeable sodium per g wet weight in 100 and 25‰ sea water is slightly less than for *Nereis*.

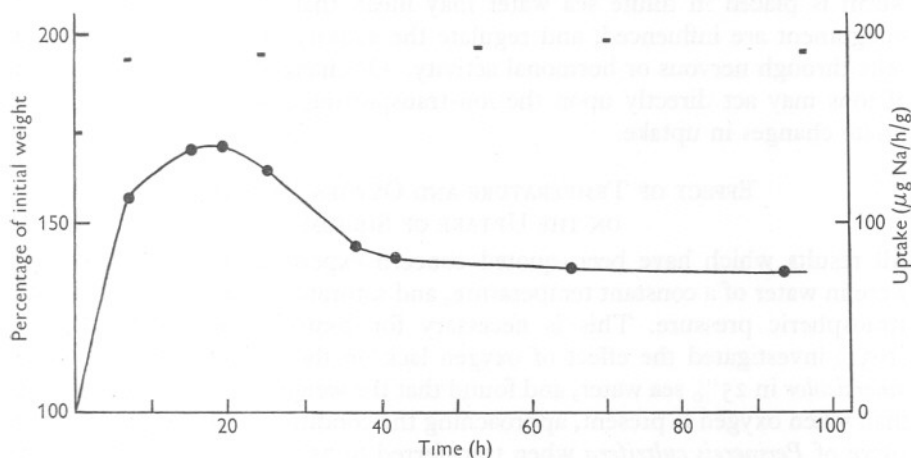


Fig. 4. *Nereis diversicolor*. Uptake of sodium per hour (per gram wet weight) during the period of weight regulation when transferred from sea water of 35‰ salinity to that of 9‰ salinity. Continuous line indicates weight regulation.

UPTAKE OF SODIUM BY *NEREIS DIVERSICOLOR* DURING THE PERIOD OF ACCOMMODATION TO DILUTE SEA WATER

No study has yet been concerned with the uptake of ions by *N. diversicolor* during the period of osmotic swelling and subsequent weight adjustment in water of low salinity. For this purpose freshly collected specimens were kept for a period of 36 h in normal sea water (35‰). Twenty worms were then weighed individually and each isolated in a vessel containing water of a salinity of 9‰. At a temperature of 14°C the typical weight curve followed the course shown in Fig. 4. At varying intervals of time along this curve certain worms were placed in activated sea water for an hour or two, so that an estimate of the sodium uptake at that point in the weight regulation could be made. Afterwards they were returned to the inactive water. The handling of worms in this way did not appear to have any adverse consequences. The results show that throughout the period of osmotic adjustment, which involves

varying volume changes, there is an active uptake of sodium from the brackish water at an approximately constant level. The only variation is at the beginning of the experiment when it is lower, though not sufficiently low to suggest that there is any serious time lag in the establishment of the active uptake. An experiment for worms transferred from water of a salinity of 35‰ to that of 17.5‰ gave similar results.

Thus it seems that, as in *Eriocheir* (Krogh, 1939), the passage of ions through the integument is unrelated to the passage of water, though the two events take place simultaneously. This suggests two independent transport processes. The rapidity with which the active uptake of ions is established when the worm is placed in dilute sea water may mean that chemoreceptors in the integument are influenced, and regulate the activity of the ion-transporting cells through nervous or hormonal activity. Or changes in the concentration of ions may act directly upon the ion-transporting cells and induce appropriate changes in uptake.

EFFECT OF TEMPERATURE AND OXYGEN DEFICIENCY ON THE UPTAKE OF SODIUM

All results which have been quoted concern experiments in which worms were in water of a constant temperature, and saturated with oxygen at normal atmospheric pressure. This is necessary for comparative results. Beadle (1931) investigated the effect of oxygen lack on the weight curve of *Nereis diversicolor* in 25% sea water, and found that the weight rises to a higher value than when oxygen is present, approaching the condition of the normal weight curve of *Perinereis cultrifera* when transferred to 25% sea water. Moreover, there is no subsequent loss of water from the tissues, which suggests that this is an active process carried out only in the presence of oxygen. Oxygen deficiency also has an effect on the uptake of sodium ions. When *Nereis* is taken from normal sea water and placed in brackish water with a low oxygen content—water through which nitrogen has been passed, or even water which is not kept saturated with oxygen—the rate of active uptake of sodium is above the normal, and the total amount of exchangeable sodium increases; these differences become greater with decreasing salinity. For a worm taken from 100% and placed in 25% sea water the influx in 20 h is twice as great as when the water is saturated with oxygen: for a worm already accommodated to 25% sea water, and therefore with a lower oxygen consumption, it is less.

A low temperature also influences the weight adjustment of worms which are transferred from normal to dilute sea water. Beadle (1937) working with *N. diversicolor* collected from the estuary of the River Blyth, Northumberland, found that 'during the winter months the increase in weight in 25% sea water was greater and more prolonged than during the summer', though his graph shows the reverse of this. He does not, however, give the winter temperatures. Results of my experiments carried out at 5° C agree with his

statement. At 5° C the weight curve for animals placed in 25‰ sea water rose slowly during 24 h to over 1.8 times the weight in normal sea water, which exceeds the weight increase at 14° C, and it showed no reduction for the following 24 h. At all salinities the penetration of sodium was slower at this lower temperature. Particularly noticeable is the fact that worms in 25‰ sea water, where more osmotic work is needed, take up less per h per g wet weight than those in 50‰ sea water. It is known that high temperature reduces the degree of hypertonicity which must be maintained by an animal for survival in dilute media. Pannikar (1940) suggests that it is for this reason that the most active colonization of fresh and brackish water by marine animals takes place in the tropical areas. May this not also be due to the fact that the processes concerned with osmoregulation are carried out more speedily at higher temperatures and so are less impeding?

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SUMMARY

In sea water of a salinity of 35‰ saturated with oxygen at normal atmospheric pressure and at a temperature of 14° C, the uptake of sodium by *Nereis diversicolor*, which is in equilibrium with its environment, ranges from 275 to 240 µg/h/g wet weight. The higher uptake appears to be associated with the most active worms. When the salinity is reduced by half the uptake, for worms accommodated to this dilution, averages 160 µg/h/g wet weight, and at 9‰ it averages 180 µg, which shows that there is an active uptake of salts against the concentration gradient. For a salinity slightly higher or lower than that of normal sea water the influx of the salt is approximately proportional to the degree of dilution or concentration. The closing off of the intestine has no effect on the rate of uptake.

The integument of *Perinereis cultrifera* is more freely permeable to ions than that of *Nereis diversicolor*, the influx of sodium being about 3 times greater in normal sea water, and at least 1.5 times as great in 25‰ sea water. When worms are transferred from dilute to normal sea water the uptake of sodium is high compensating for the loss of salts; it is about 3 times greater in *Perinereis* than in *Nereis*.

For *Nereis* in sea water of salinity 9‰ the total amount of exchangeable sodium approaches 3.2 mg/g wet weight: in normal sea water it is about double this amount.

During the period of accommodation to dilute sea water by *N. diversicolor* there is an active uptake of sodium, which remains at an approximately constant level throughout the period of weight fluctuation, and is of

approximately the same value as for worms already in equilibrium with their environment.

Oxygen deficiency increases the rate of uptake from dilute sea water and also the total amount of exchangeable sodium. A low temperature (5° C) causes a greater imbibition of water during the period of accommodation to dilute sea water, it slows down the weight adjustment and reduces the rate of uptake of sodium.

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