THE WATER AND ELECTROLYTE EXCHANGE OF *NEREIS DIVERSICOLOR* (MÜLLER)

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(With Nine Text-figures)

It has been shown by McCutcheon & Lucke (1928) that in glucose solutions isotonic with 40 per cent sea water, the eggs of the sea-urchin *Arbacia* absorb twice as much water as they do from 40 per cent sea water, and that this increased imbibition is prevented by a small concentration of calcium ion in the glucose solution, even in the absence of the other cations of sea water. Pantin (1931 *a*) showed that the Platyhelminth, *Procerodes* (*Gunda*) *ulvae*, which in its estuarine habitat withstands tidal

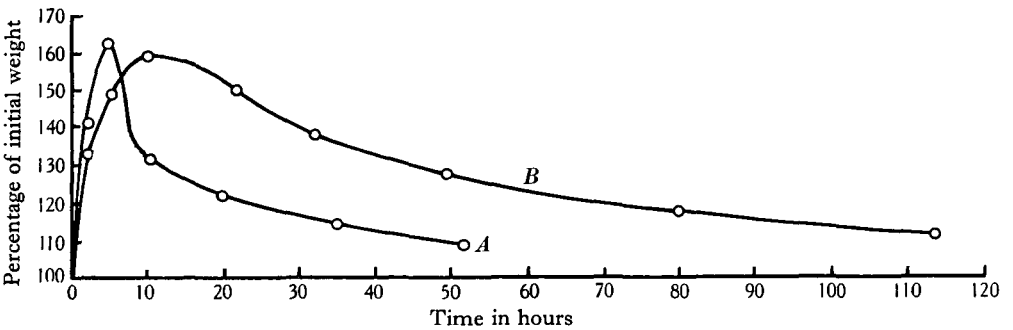

Fig. 1. Weight regulation of: *A*, Roscoff worms and *B*, Bangor worms, in 20 per cent sea water.

changes in osmotic pressure, is only able to survive in very dilute salt solutions if these contain calcium.

The present writer, Ellis (1933), working at Roscoff, found that the regulatory powers of the polychaete *Nereis diversicolor* are also dependent on the presence of the calcium ion. Repetition of this work on *N. diversicolor* from Plymouth and from the Menai Straits, Bangor, has given similar, although not identical, results. Fig. 1 shows on the same co-ordinates the time-weight curves of *N. diversicolor* from Roscoff (curve *A*) and from Bangor (curve *B*), the worms in each instance being in 20 per cent sea water. It is clear that there is a considerable difference between the powers of weight regulation of the worms from the two geographical positions. On curve *A* (Roscoff worms) each point represents the average percentage weight of ten worms, weighed separately. As worms from this source show only slight variability in their time-weight curves, ten worms are sufficient to give a "standard" curve.

Worms taken from the Menai Straits, however, or from Plymouth, show a much higher variability, and it is therefore essential, in order to obtain reproducible curves of weight regulation, to use larger numbers of animals. On the curve shown (*B*, Fig. 1) each point represents the average weight of seventy-five worms, weighed separately. Separate weighing of the worms is always necessary, for if several worms are placed in the same vessel they invariably come together in an inextricably tangled mass after about 20 hours and, moreover, fail to regulate in the same manner as isolated worms. The failure of weight regulation under the circumstances described may be due to inadequate oxygen concentration at the centre of the "bolus" of worms.

Fig. 2 shows the time-weight relations of Roscoff (curve *A*) and Bangor (curve *B*) worms in 20 per cent calcium-free sea water. Curve *C* is the time-weight curve of

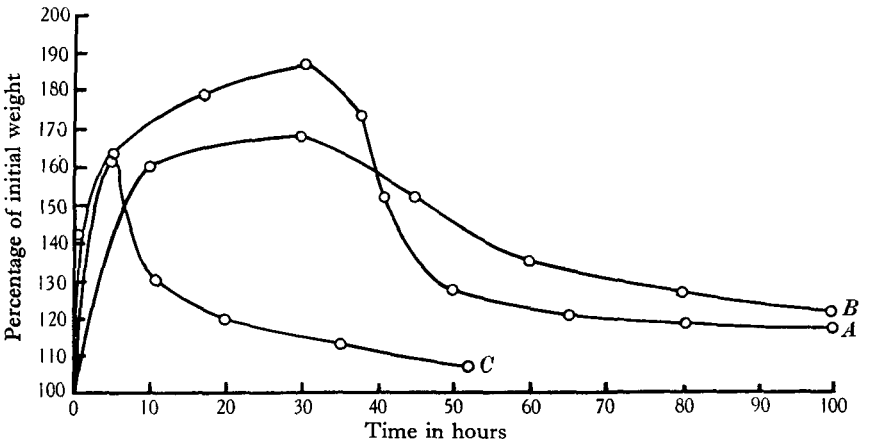

Fig. 2. Weight regulation of: *A*, Roscoff worms and *B*, Bangor worms in 20 per cent calcium-free sea water. At 30 hours calcium is replaced without alteration of osmotic pressure. Curve *C* shows, for comparison, the weight regulation of Roscoff worms in natural 20 per cent sea water.

Roscoff worms in "complete" 20 per cent sea water. It will be seen that, even after 30 hours, the weight of the animals in calcium-free water is still increasing. At 30 hours calcium is replaced, with the result that the normal weight regulation now begins and the weight falls to the level which would have been reached, had calcium been present from the beginning. It will be seen from the curves that the Roscoff worms, in spite of their superior powers of regulation in natural dilute sea water, are markedly less able to control their water intake in calcium-free sea water.

For the following reasons it is probable that the above differences between the worms from Brittany and those from Wales are not due merely to differences in experimental conditions. The experiments at Roscoff and Bangor were carried out at the same temperature, 17° C. In both instances the worms were used soon after collection. In any case, whether the worms are kept in the laboratory in 100 per cent or in diluted sea water, their history has no effect on the *rate* of weight regulation (although, as will be shown below, prolonged exposure to dilute sea water has other important effects on the regulation). In both sets of experiments ordinary

aerated water was used (i.e. with the dissolved air in equilibrium with the atmosphere), so that the tension of dissolved oxygen cannot have been a significant variable. Moreover, the same differences are observed whether natural or artificial 20 per cent sea water is used. Therefore there is no possibility that the observed differences are due to chemical differences between the brackish water from the two sources. It seems reasonable to conclude, therefore, that the physiological difference between the worms from the two geographical positions is racial rather than environmental.

The following observations apply to worms collected from Bangor or Plymouth; *N. diversicolor* from these sources being physiologically similar.

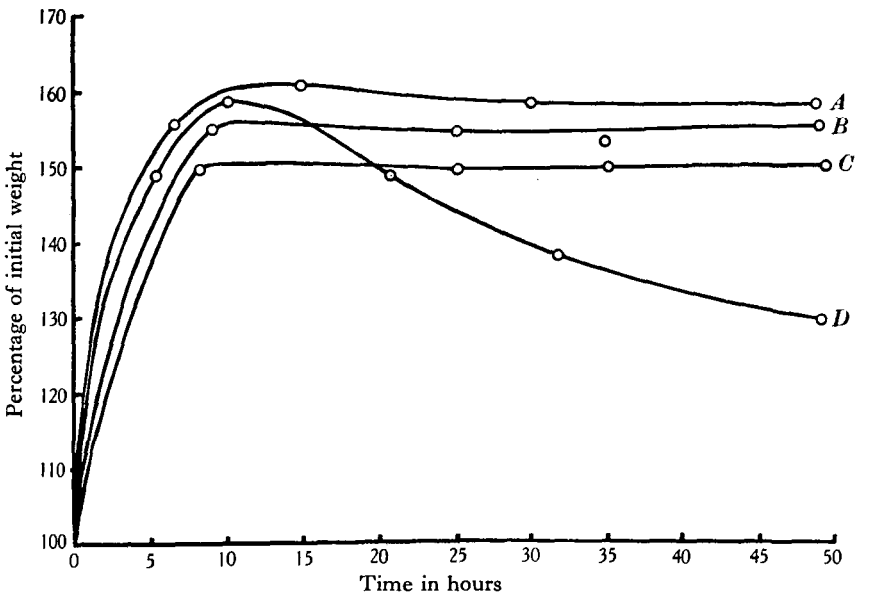

Fig. 3. Weight regulation in: *A*, sodium-free 20 per cent sea water; *B*, potassium-free 20 per cent sea water; *C*, magnesium-free 20 per cent sea water. Curve *D* shows the weight regulation of a control in natural 20 per cent sea water.
(This and all subsequent curves refer to Bangor worms.)

The weight curves of worms in 20 per cent potassium or magnesium-free sea water show that these ions also are essential for weight regulation. In Fig. 3, curve *B* shows the time-weight relations in potassium-free 20 per cent sea water, curve *C* in magnesium-free water of the same concentration. Curve *D* is the "normal" curve when all ions are present. The sodium ion is also necessary for weight regulation, for worms fail to regulate in sea water in which sodium chloride is replaced by an isotonic concentration of glucose. Curve *A* shows the behaviour in such a sodium-free artificial sea water.

The action of these ions on the weight regulation must, however, be quite different from the action of calcium, as is shown by the following experiment. If a worm be allowed to regulate for 100 hours in "complete" diluted sea water and is then placed in sea water of the same concentration but lacking calcium, its weight

rises up to, or above, the original maximum. Curve *A*, Fig. 4, is the time-weight curve of a worm which, having risen to 160 per cent of its initial weight and subsequently fallen to 120 per cent in 20 per cent sea water, was then placed in 20 per cent calcium-free sea water. Worms which have regulated and are then placed in magnesium or potassium-free sea water, undergo little or no rise in weight. Curve *B*, Fig. 4, is that of a worm which was placed in 20 per cent magnesium-free sea water after regulation in complete 20 per cent sea water. The curves for sodium and potassium-free sea water are similar to that for magnesium-free water. It should be added that the behaviour of worms in calcium-free sea water is quite different from that in sea water lacking one of the other three cations. In potassium,

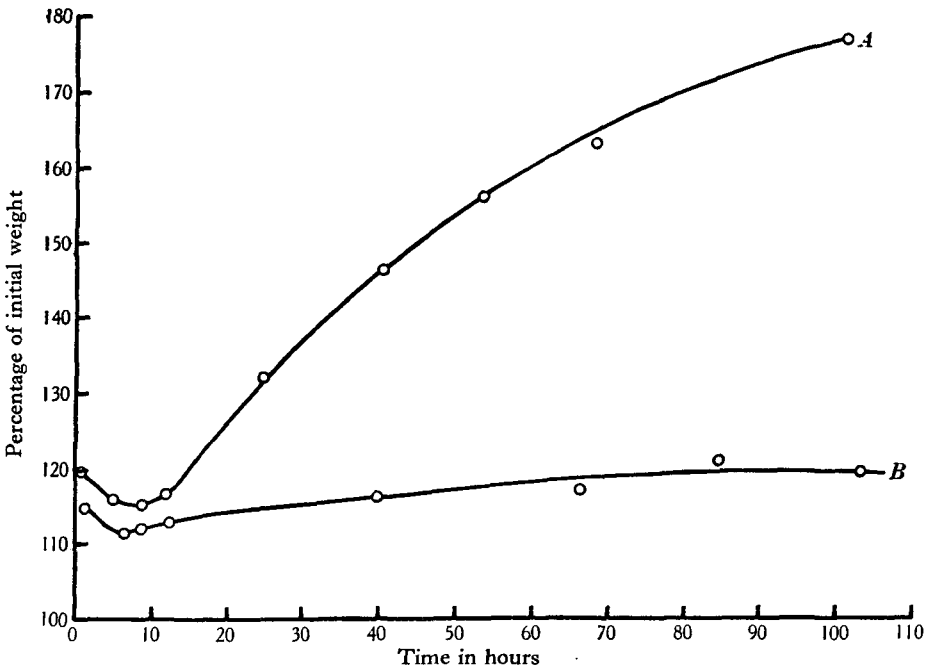

Fig. 4. Reabsorption of water on transference from 20 per cent sea water (in which there was complete weight regulation) to: *A*, calcium-free 20 per cent sea water; *B*, magnesium-free 20 per cent sea water.

sodium or magnesium-free water the worms preserve their normal appearance and movements for about 100 hours, after which they begin to lose pigment and blood. But after a very short time in calcium-free water, 100 per cent or dilute, all movement ceases. Roscoff worms show this effect after 1 hour, Bangor worms not until 7 hours. The worms coil themselves into close helices, and the tails become red with extravasated blood. The effect of restoring calcium is remarkable. Within a minute or even less, the extreme tip of the tail twitches slightly, and during the next hour, after which recovery is complete, the twitches grow larger and spread to the anterior end.

Nevertheless, potassium, sodium and magnesium are essential for weight regulation. This result should be contrasted with that of Pantin (1931 *a*) who found

that the presence or absence of these ions was without influence on the ability of *Gunda* to survive in dilute solutions. An explanation of this apparent contradiction will be suggested later.

The large decrease in weight, almost down to the initial weight, after the initial rise in dilute sea water would suggest that the body wall is not completely, or even approximately semi-permeable, but that the initial water gain is accompanied by a salt loss. This was investigated in the following manner.

Four worms, weighing in all about 1·5 g., are washed rapidly in distilled water to remove adhering salt water, then dried, weighed, and placed in a measured volume (20 c.c.) of 20 per cent sea water in a beaker.[1] The diameter of the beaker is such that the 20 c.c. of water forms a shallow layer, sufficient but not much more than sufficient, to cover the worms. The beaker and its contents are now kept in a state of gentle agitation by a mechanical arrangement. Under these conditions the worms do not congregate into a bolus, as described above, and the weight regulation is the same as that for isolated worms. After 2 hours the worms are removed from the beaker. As each worm is lifted out (with a seeker) the salt water adhering to it is washed back into the beaker with distilled water from a wash bottle. The worms are dried, reweighed, and replaced in a fresh 20 c.c. of sea water, the second beaker and its contents being treated in the same way as the first. The chloride content of the first sample is then titrated with $N/10$ $AgNO_3$, potassium chromate being used as an indicator. Comparison of this titre with that of a control 20 c.c. of 20 per cent sea water gives the chloride output from the worm during the first 2 hours. The output in subsequent 2-hour periods is found in the same way. A further control showed that the salt lost during the rapid washing with distilled water is too little to be measurable by the method used.

Such experiments show that, after a worm is placed in dilute sea water, there is a rapid initial salt output, which falls almost to zero in about 12 hours, that is, by the time the weight curve has reached its maximum. The four curves of Fig. 5 show the salt loss in four concentrations of sea water.

Although titrations were continued up to 150 hours, it was found that there is no salt intake associated with the water loss which begins at 12 hours, continuing until about 100 hours. The salt loss being irreversible, the fall in osmotic pressure of the body fluid, which must result from the salt loss and water intake, can only be reversible if the body wall acquires, during the 12 hours of water intake, an impermeability, or greatly reduced permeability, to electrolytes. The weight regulation would then be accompanied by an active excretion of water against the osmotic gradient. If, on the other hand, the body wall does not become semi-permeable, the body fluid must become and remain, approximately isotonic with the external medium. Weight regulation would then consist merely in the expulsion, by some mechanical means, of the excess volume of body fluid, without alteration in the osmotic pressure of the body fluid.

[1] These quantities, which were found empirically, are such that, while the change in concentration of the sea water, due to salt loss from the worm, is sufficiently large to be readily measurable, it is not so large as seriously to change the osmotic gradient between body fluid and external medium.

To discover which of these theoretically possible views is correct, all that is necessary is to take a worm which has come to equilibrium in 20 per cent sea water (i.e. after 100 hours in this medium), and replace it in 100 per cent sea water. On

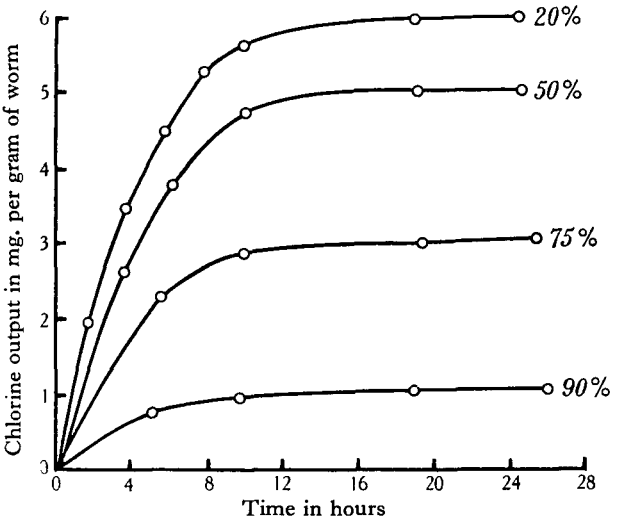

Fig. 5. Salt loss accompanying the initial water intake in dilute sea water. Each curve shows the salt loss in a given dilution of sea water.

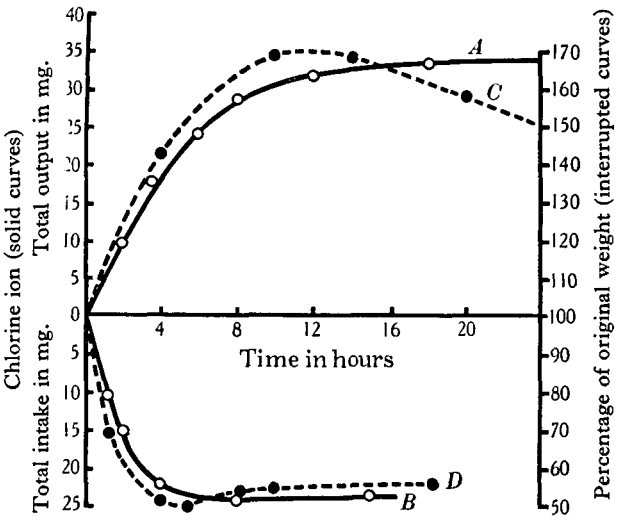

Fig. 6. Curve *A*, Initial salt loss in 20 per cent sea water; Curve *B*, Salt intake on transference back to 100 per cent sea water. See left-hand vertical axis. Solid curves. Curve *C*, Water intake accompanying *A*; Curve *D*, Water loss accompanying *B*. See right-hand vertical axis. Interrupted curves.

the first of the above views it should not take up salt from the 100 per cent sea water, on the second view, it should.

The experiment shows that the worm does abstract salts from the environment under the conditions described. Curve *A*, Fig. 6, shows the salt output of five

worms during the 12-hour period of water intake in 20 per cent sea water. Curve *B*, on the same figure, shows the salt intake when the worms were replaced in 100 per cent sea water, after having been for 100 hours in the more dilute medium. It will be seen from these curves that the salt intake in 100 per cent sea water is about two-thirds of the salt output in 20 per cent sea water. Relative to water loss in 100 per cent sea water the salt intake is, however, the same as the salt loss in 20 per cent sea water, relative to the water intake. This can be seen from Fig. 6 on which water loss and intake are plotted on the same time axis as the salt intake and loss, see dotted curves *C* and *D*. The vertical axis (on the right of the figure) represents the percentage of the weight at the beginning of the salt loss (above the *X* axis) while the part below the *X* axis represents the percentage of the weight at the beginning of the salt intake. The scale of this vertical axis is so chosen that the maximum of the water intake curve coincides approximately with that of the salt output curve. On the same scale the minimum of the water-loss curve (i.e. in 100 per cent sea water) corresponds with the minimum of the salt intake curve, showing that the salt intake per gram of water loss is the same as the salt loss per gram of water intake.

Hence, it would appear probable that during weight regulation the body fluid becomes and remains isotonic with the diluted external medium and that the body wall does not acquire semi-permeability.

Other experiments point to the same conclusion.

It will be recalled (see above) that if a worm which has come to equilibrium in 20 per cent sea water then be placed in 20 per cent calcium-free sea water, the weight regulation is reversed. This second water intake, equal to or greater than the initial water intake, differs from the initial intake in that it is not accompanied by a comparable salt loss. Curve *A*, Fig. 7, shows the salt loss associated with an initial water intake (in 20 per cent sea water) of 67 per cent of the body weight. Curve *B*, on the same figure, shows the salt loss which accompanied the re-imbibition of 72 per cent of water in 20 per cent calcium-free water, after the worm had been for 100 hours in 20 per cent "complete" sea water. Although the water intake in calcium-free water is slightly larger than the initial intake, the salt loss is negligibly small in relation to the original salt loss. Curve *C* shows the salt loss accompanying the intake of 59 per cent water in another worm in 20 per cent sea water. After 200 hours this worm was transferred to 20 per cent calcium-free sea water, with the result that there was a re-intake of 59 per cent of water and a salt output, shown in curve *D*, which, while relatively larger than that shown on curve *B*, is still negligible, compared with the initial salt loss.

This large water intake, practically unaccompanied by salt loss, suggests that the body fluid, after weight regulation, is no longer hypertonic to the environment. Possibly the water entering the worm from a calcium-free environment, after regulation in "complete" 20 per cent sea water, is largely taken up by the cells of the body wall. It is known that the concentration of anions in protoplasm is markedly less than in sea water or the body fluid of a marine invertebrate. In *Arbacia* eggs, for instance, the chloride concentration is only one-twentieth of that in sea water (Pantin, 1931*b*). Hence we should expect that the intake of a given volume of water

by the cells would be accompanied by a much smaller chloride loss than the uptake of the same amount of water by the body fluid. This would also explain why worms transferred from 100 per cent sea water to 20 per cent calcium-free water take up more water than those transferred to complete 20 per cent sea water (see Fig. 2 above).

On the hypothesis that the regulation of water exchange in *Nereis* is, as we have shown reason to believe, mechanical rather than osmotic, it is possible to offer an explanation of the inhibition of regulation in the absence of potassium, magnesium, and sodium ions, which, as we have seen, are not concerned in the water exchange of *Arbacia* or of *Gunda* (McCutcheon & Lucke, 1928; Pantin, 1931*a*). We may suppose that, while absence of calcium has a purely osmotic effect, similar to that on

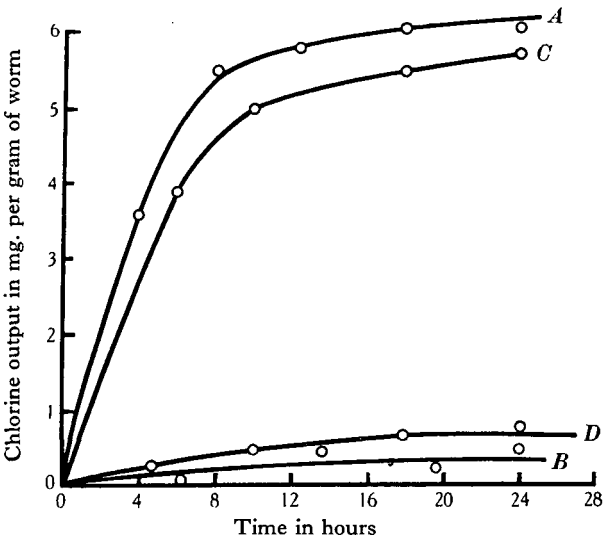

Fig. 7. Curve *A*. Salt loss accompanying initial water intake in 20 per cent sea water. Curve *B*. Salt loss accompanying reabsorption of water on transference to 20 per cent calcium-free sea water after 100 hours in 20 per cent natural sea water. Curve *C*. Initial salt loss of another batch of worms, also in 20 per cent sea water. Curve *D*. Salt loss accompanying reabsorption of water on transference to 20 per cent calcium-free sea water after 200 hours in 20 per cent natural sea water.

the protoplasm of *Arbacia* and *Gunda*, the absence of the other cations affects only the mechanical expulsion of water.

There is a further important difference between the effect of absence of calcium on *Gunda* and on *Nereis*. Pantin (1931*a*) found that in absence of calcium the salt loss for a given external osmotic pressure was greatly increased. Curve *B*, Fig. 8, shows the initial salt loss from *N. diversicolor* in 20 per cent calcium-free water, compared with the salt loss in 20 per cent complete sea water (curve *A*). These two curves, which show the average salt loss from 150 worms in fifteen experiments, clearly show that the absence of calcium is without effect on the salt loss. The slight apparent *decrease* of salt loss in calcium-free water is rather too small to be considered significant, but certainly there is no increase. The explanation of this may be that in *N. diversicolor* the permeability of the body wall is in any event as high as it can be, whether calcium is present or not. This maximal permeability may be an

adaptation to prevent undue swelling in brackish water, for the amount of swelling will be in inverse relation to salt loss.

The behaviour of worms on being transferred from dilute sea water, in which they have come to equilibrium, back into 100 per cent sea water, affords a further confirmation of our hypothesis that there is no osmotic regulation of the body fluid. Consider a worm which, having taken in 60 per cent of its weight of water, subsequently expels nearly all this water, so that when it reaches equilibrium its weight is only about 10–20 per cent higher than its initial weight. Its body fluid, according to our hypothesis, is now isotonic with the environment (20 per cent sea water). But the weight of the worm is little higher than the initial weight. Hence if we

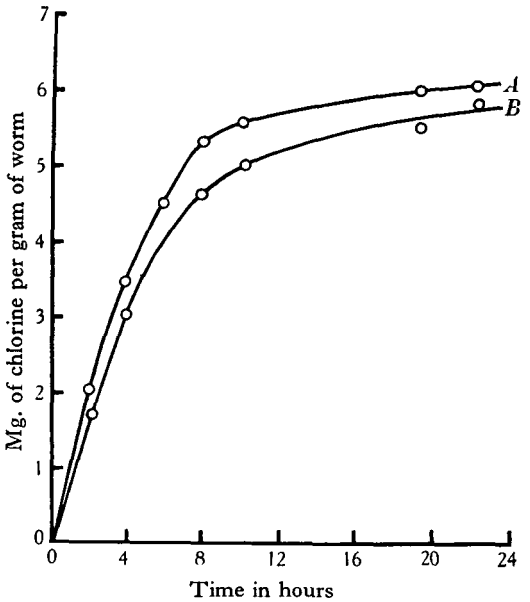

Fig. 8. Curve *A*. Salt loss (per gram of worm) accompanying the water intake in 20 per cent sea water (72 worms). Curve *B*. Salt loss in 20 per cent calcium-free sea water (78 worms).

replace the worm in 100 per cent sea water we should expect the resulting osmotic outflow of water to bring the body weight well *below* the initial weight.

This is in fact so. Curve 1, Fig. 9, is the usual curve of weight regulation in 20 per cent sea water. At 100 hours, the worm is transferred to 100 per cent sea water. Curve 1*a* shows the resulting drop in weight, taking the worm down to 80 per cent of its initial weight in sea water. It will be seen from the curve that the initial fall in weight is succeeded by a slight rise, but that the greater part of the water loss is irreversible. In the experiment shown on Fig. 9 the worm was kept in 100 per cent sea water for 100 hours, but in other experiments worms transferred from 20 per cent sea water to 100 per cent, were kept there for 800 hours without rise in weight. At 150 hours Fig. 9, the worm was transferred back to 20 per cent sea water. The resulting time-weight curve (2) is exactly similar to the first (1), except that the whole curve has shifted towards the negative side of the vertical axis. At 250 hours the worm

is again replaced in sea water, and the water loss now takes the weight down to 70 per cent of the initial weight. The remaining curves (3, 3 *a* etc.) show further repetitions of the process described. Curve 3 *a* on the upper part of the diagram is repeated below. These curves show that after 500 hours alternately in 20 and 100 per cent sea water, the weight has fallen almost to half the initial weight. It follows, from this remarkable progressive reduction in weight, that the mechanical activity which reduces the volume of body fluid does not persist after the animal has come to equilibrium in 20 per cent sea water. That is, although work is done to reduce the body volume, no work is done to maintain the reduction. Suppose that a given animal in 20 per cent sea water, having risen to 160 per cent of its initial weight, then reduces its weight to 115 per cent. If this 45 per cent reduction had to be

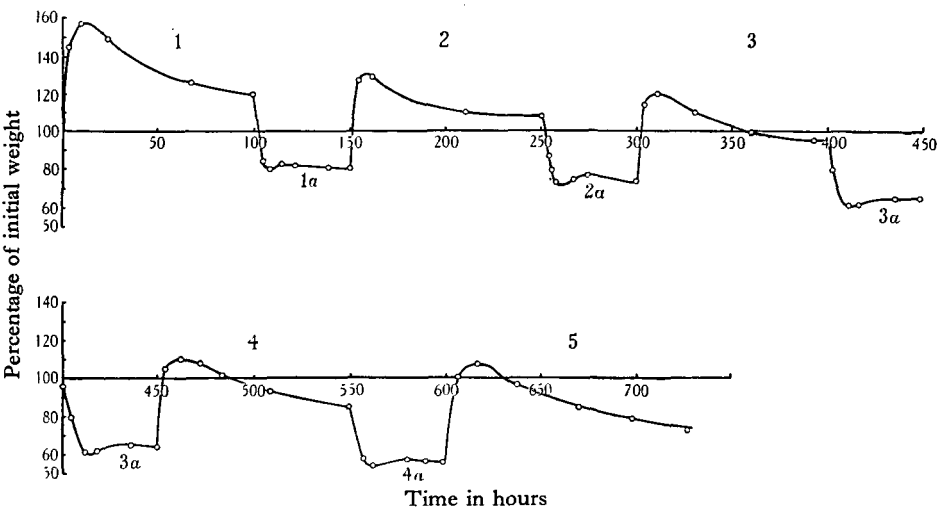

Fig. 9. Curves showing percentage change of weight of worms exposed alternately to 20 and 100 per cent sea water. Those sections of the curve marked 1, 2, 3, 4, 5, show the percentage weight in 20 per cent sea water; those marked 1 *a*, 2 *a*, etc., show the weight in 100 per cent sea water. Curve 3 *a* in the upper section is repeated in the lower section.

maintained by mechanical activity (e.g. by increased ciliary activity in the nephridia), we should expect that, on replacing the animal in 100 per cent sea water, this mechanical activity would fall off, and finally stop. We should therefore expect that the greater part of the weight reduction which occurs on re-transference to 100 per cent sea water, would be reversible. As this is not so, it may be concluded that the water loss, under the conditions described, is a passive osmotic loss, that it is not due to inhibition of a previously existing mechanical activity, and that the weight equilibrium attained in dilute sea water is therefore not maintained by extra mechanical activity.[1] As the curves show, a small proportion of the water loss is reversible, and this may represent the inhibition of a residual activity, persisting in 20 per cent sea water after 100 hours.

[1] Although, of course, like every apparent *status quo* in the organism, it must represent the resultant of dynamic equilibria: there is no reason to believe that *increased* metabolism should be necessary for the maintenance of the weight equilibrium in dilute sea water.

This final experiment, therefore, reinforces the conclusion, reached also from other data, that the weight regulation of *N. diversicolor* is not accompanied by osmotic regulation.

SUMMARY

1. The rate of weight regulation in diluted sea water of *Nereis diversicolor* from Brittany (Roscoff) is much greater than that of the same species from the Menai Straits (Wales) or Plymouth. This difference is believed to be racial and not due to environmental factors.

2. Weight regulation in diluted sea water is inhibited by the absence of any one of the chief cations found in normal sea water.

3. Water is reabsorbed by *N. diversicolor* if, after weight regulation, the worms are transferred to water of the same osmotic pressure but containing no calcium. No reabsorption of water occurs in solutions lacking sodium, potassium, or magnesium.

4. The uptake of water from diluted sea water is accompanied by a loss of salts from the worm: this loss is not affected by the presence or absence of calcium. The subsequent water loss in diluted sea water is not accompanied by an uptake of salts from the water. Only a very small loss of salts occurs when weight-regulated worms absorb water from calcium-free solutions.

5. If a "regulated" worm be transferred from dilute sea water back to 100 per cent sea water, there is a water loss and a salt intake. As a result of the water loss the weight of the worm is now only 80 per cent of the initial weight in 100 per cent sea water. By repeated transferences from 100 to 20 per cent sea water, and back again, the weight in 100 per cent sea water can be reduced to half the initial weight.

6. It is concluded that weight regulation is not accompanied by osmotic regulation.

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