THE ADAPTATION OF GUNDA ULVAE TO SALINITY

III. THE ELECTROLYTE EXCHANGE

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(With Five Text-figures.)

In previous papers (Pantin, 1931; Weil and Pantin, 1931) the environment and water exchange of the estuarine flatworm, Gunda ulvae, have been described. It is found on the sea shore at the mouths of small streams, where it is subject to violent salinity changes.

The worm can live normally in sea water for an indefinite period, but it can only survive continuous immersion in fresh water for a few days. Previous experiments showed that this is related to the fact that the worm is not impermeable to water, but reacts to the difference of osmotic pressure between the tissues and the external fresh water by swelling: that is, it behaves as though it were covered with a semi-permeable membrane. The worms cannot indefinitely withstand this swelling without permanent injury. While it is in progress slight mechanical injury causes cytolysis of the worm—no doubt partly caused by sheer mechanical distension.

The presence of calcium in the stream water in which the organism lives reduces both the rate of swelling and the general deleterious effect of fresh water.

Now in an estuarine organism subjected to fresh water there must be a tendency not only to gain water but also to lose salts. Previous work suggested that Gunda itself tends to lose salts in a fresh-water medium: its semi-permeability with respect to water and salts is incomplete. No organism can afford to lose the whole of its salt content and, since Gunda normally survives fairly long periods of immersion in fresh water, control of the rate of loss of salts would be of vital importance.

The worms used were obtained by the Marine Biological Laboratory, Plymouth, from the estuary of a small stream at Wembury, South Devon. They were kept in an aquarium at Cambridge during the course of the experiments.

The term "permeability" in this paper is used simply to describe the ingress and egress of salts and water into and out of the worm under the given conditions, irrespective of the mode of transport or of the nature of the final equilibrium.
METHODS.

In the following experiments worms were transferred from sea water to distilled water or other dilute solutions, and the rate of accumulation of electrolytes in the external medium determined.

The small size of the worms and the necessity for rapid and frequent determinations of the amount of salts lost rendered chemical estimation unsuitable. The most convenient, rapid and delicate method was found to be the determination of the electrolyte content of the external medium by measuring the electric conductivity after Gray's (1920) method, using a Kohlbransch bridge.

A small cell was used, which contained the worms together with 1.5 c.c. of fluid. The latter was kept stirred by bubbles of air passing through the cell from a small orifice at the base. Five or ten worms were usually put in the cell at the same time in order to magnify the effect. The worms were selected to be of the same size, 4 mm. × 1 mm., when normally extended in locomotion.

In order to reduce as far as possible initial errors in conductivity due to adherent sea water, the worms were thoroughly washed with the same solution as that in the cell to which they were to be transferred. This process, however, took about 2 minutes, during which time salts might truly be lost by the animal, and consequently the first conductivity determination may give slightly too low a value for the initial rate of salt loss.

All conductivity measurements were corrected for temperature. The cell was also calibrated for all the solutions used. Equivalent additions of electrolyte were found to produce the same changes of conductivity. The difference between the initial conductivity of any solution and the conductivity after worms had been immersed in it for a given time was, therefore, proportional to the amount of electrolyte which had diffused out of the worm.

It was at first feared that CO₂ produced by the worms during activity might influence the apparent loss of electrolytes as measured by conductivity. But this did not appear to be the case. The stirring of the solution by bubbling prevented accumulation of CO₂, and differences of activity in any batch of worms were not apparently accompanied by changes in the rate of increase in the conductivity of the solution.

Results obtained with these methods were easily reproducible with batches of worms in the same condition.

For comparison with conductivities mentioned in this paper the following were the conductivities in reciprocal ohms found for solutions at 15°C. in the cell used:

\[
\begin{align*}
\text{NaCl } 0.004 M & \quad 94.8 \times 10^{-6} \frac{\text{I}}{\Omega}; \\
\text{CaCl}_2 0.002 M & \quad 98.0 \times 10^{-6} \frac{\text{I}}{\Omega}; \\
\text{Cambridge tap water} & \quad 95.8 \times 10^{-6} \frac{\text{I}}{\Omega}.
\end{align*}
\]
The duration of survival of the worms in dilute solutions was greatly affected by mechanical treatment during experiments, particularly in calcium-free solutions. Whereas the majority of worms were able to survive for about 20 hours in distilled water, the continual agitation by air bubbles in the conductivity experiments hastened the onset of cytolysis.

RELATION OF LOSS OF SALTS TO SWELLING.

The conductivity of fresh waters and other dilute solutions rose continuously when worms were placed in them. At the same time, the worms swelled. The worms not only lost salts when greatly swollen but continuously from the moment of immersion: the rate of loss actually fell while swelling proceeded. The semi-permeability is, therefore, decidedly incomplete.

In Fig. 1 the rate of loss of salts of a group of five worms (curve 1) in 1.5 c.c. of distilled water is shown. After this experiment the worms were allowed to recover in sea water for 24 hours. Three of the worms were then placed in distilled water, and the rate of swelling of each measured by the method previously described (Weil and Pantin, 1931). The results are shown in curves 2, 3 and 4.

Both the rate of salt loss and the rate of imbibition of water fell during the immersion. This does not necessarily argue a progressive reduction in the relative permeability of the worm because, as swelling proceeds, both these changes cause
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The internal salt concentration of the worm as a whole, to fall. Consequently the driving force is less. Thus in Fig. 1, curve 1, the rate of loss of salts at 10 minutes is to the rate at 75 minutes roughly as 1 is to 0.4. But the volume of the worm at 75 minutes is about double that at 10 minutes and, as will be seen later, the worm contains after 75 minutes only about 80 per cent. of its original salt content. The general concentration of salts in the worm has, therefore, fallen during the interval to roughly 0.4 times the original concentration: the rate of salt loss is thus falling at roughly the same rate as the force driving the salts out. It must, of course, be fully realised that the worms cannot be treated as simple osmotic machines, but the calculation shows that there is no reason to suppose, on the present evidence, that the fall in rate of salt loss is due to a progressive change in the permeability of the worm.

Certain conditions, however, do affect the rate of loss of salts. It will be seen from Fig. 1 that after some 60 minutes, when the worm has swelled to about double its volume, it becomes very sensitive to mechanical injury and bursts easily. This bursting is preceded by a partial disruption of the ectoderm, from which the worm can recover if it is not allowed to progress too far. This disruption is accompanied by a sudden rapid loss of salts. Thus in Fig. 1, curve 1, point A, one worm accidentally touched the electrodes while a conductivity measurement was in progress. As will be seen, an immediate and rapid loss of salts took place. This was allowed to continue till the worm cytolysed. After this the conductivity increased more slowly again, owing to the slow normal loss of salts by the remaining four worms.

The "normal" loss of salts has here been contrasted with that related to the cytolysis of tissues. It might be suggested that this normal loss itself were due to progressive disruption of cells. This does not seem to be the case. Worms allowed to recover in sea water even after a considerable period in fresh water regain their original volume, so that no appreciable amount of tissue can have been destroyed even when large amounts of salts have been lost to the external medium. It must also be remembered that the worms are subjected to completely fresh water for several hours with each tide in their natural environment.

There is frequently some individual variation in the actual rate of leakage of salts in distilled water. This was even found to occur in the same group of worms at different times. Such differences in rate did not usually exceed 20 per cent. Fig. 4, curves 1 and 2, show cases in which the loss of salts in distilled water was measured on two occasions, using the same batch of ten worms. The great difference in rate shown in this case was by far the most exaggerated of those observed in any one group of worms.

These individual variations were found to be almost entirely due to the previous history of the worm. The worms in their normal environment are exposed twice in the 24 hours to fresh water for some hours. The stock worms kept in the laboratory on the other hand were maintained in sea water for many weeks. It was found that such worms on exposure to distilled water or dilute solutions comparable to fresh water lost salts at a greater rate than those which had been subjected to
a few changes of medium. The worms in Fig. 4, curve 1, had been in sea water continuously for some weeks. The same batch was then used in experiments in which they were exposed to fresh water for about 1½ hours twice a day. After a few such exposures Fig. 4, curve 2, was obtained. It is clear that the rate of salt loss is greatly reduced.

It was found that this abnormally high rate of loss of salts only takes place during the first time of immersion in fresh water after a prolonged rest in sea water, and that after this reproducible results are easily obtained.

The effect was found to occur not only with distilled water but also with Cambridge tap water and with NaCl 0·004 M in distilled water.

**PROPORTION OF SALTS LOST.**

Fig. 2 shows the progressive loss of salts in distilled water by two batches of worms from different stocks. Batch A consisted of ten worms and Batch B of five.

For the ordinates, the observed conductivity is divided by the number of worms so as to show the increase in conductivity per worm. The experiments were continued till cytolysis was complete, and the tissues of the worm formed an emulsion in the distilled water. Most of the increase of conductivity after 60 minutes is not due to normal leakage of salts but to the progressive cytolysis of successive worms.
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It will be seen that the different batches show a considerable difference in the rate at which cytolysis occurs. On the other hand, the initial normal leakage represented by the first part of the curve is approximately the same in the two cases.

In Fig. 2 it will be noticed that in both cases, A and B, the conductivity per worm of the solution at complete cytolysis corresponds to about the same figure, \(5 \times 10^{-5} \frac{I}{\Omega}\). In any experiment in which one worm of standard size happened to cytolyse, an increase in conductivity of approximately this same magnitude was observed. This figure may be taken as a measure of the total electrolyte content of the worm. On the basis of Hill and Kupalov’s work (1930) we may reasonably assume that such electrolytes are entirely osmotically active in the living tissues. It must also be assumed that large quantities of electrolytes of high specific conductivity are not produced by metabolism at the time of cytolysis. This assumption is probably valid; first, because the absolute concentration of salts in Gunda is high and relatively very large amounts of electrolyte would have to be produced at cytolysis to affect the total conductivity; secondly, the absolute osmotic pressure of frog’s muscle, as determined by Hill and Kupalov, is of the same magnitude as that found from the freezing point of expressed muscle juice: if the production of large quantities of electrolytes took place on cytolysis of tissues this would not be the case.

Accordingly it is possible to construct an ordinate scale in Fig. 2 giving the per cent. of salts lost by the worm to the external medium at any given time.

Since the worms were chosen of standard size in all experiments and since the cytolysis increment in conductivity was always of the same order, similar scales can be applied to the other experiments. This has been done in Fig. 1. The maximum swelling of the worms occurs at a time when about 75 per cent. of the total salts are still present in the worm. Any effects, therefore, which occur during the first hour or so of immersion in fresh water are probably related to the imbibition of water rather than loss of salts by the worm.

From the conductivity increment for the cytolysis of a single worm, it seems that the latter contains a fairly high proportion of free electrolytes. Thus the conductivity of \(4 \times 10^{-8}\) molar NaCl was found to be \(95 \times 10^{-8} \frac{I}{\Omega}\). An increment of \(5 \times 10^{-5} \frac{I}{\Omega}\) is, therefore, equivalent to a NaCl concentration of \(\frac{5}{95} \times 4 \times 10^{-3}\) molar: i.e. \(\frac{20}{95}\) millimols of NaCl per litre. This is dispersed in 1.5 c.c. of distilled water in the conductivity vessel, and the amount of NaCl thus corresponds to \(1.5 \times 10^{-3} \times \frac{20}{95}\) millimols.

Now sea water is isotonic with \(0.56\) molar NaCl, and \(1.5 \times 10^{-3} \times \frac{20}{95}\) millimols of NaCl correspond to \(1.5 \times 10^{-3} \times \frac{20}{95} \times \frac{1}{0.56}\) c.c. = 0.6 c.mm. of such a solution.

The mean volume of one worm is 1.2 c.mm. and there are thus present in this volume electrolytes equivalent to 0.6 c.mm. of a NaCl solution isotonic with the
external medium, sea water. In view of the facts that some non-electrolytes are bound to be present, that the specific conductivity of the electrolytes themselves in the worm is probably considerably less than that of NaCl, and that the volume of "free" water is probably of the order of 75 per cent. of the volume of the worm, this value is reasonable for an organism normally in osmotic equilibrium with sea water (cf. Hill and Kupalov, 1930).

LIMITING INTERNAL SALT CONCENTRATION.

The lowest electrolyte concentration which can be reached within a surviving worm cannot easily be determined when distilled water is the medium, owing to the ease with which cytolysis occurs. It is not possible to determine how far values such as those corresponding to the points d in Fig. 2 are due to leakage of salts from normal worms.

On the other hand, the worms can survive and are recoverable after a very long sojourn in solutions such as Cambridge tap water, containing calcium. In one experiment, ten worms left for 18½ hours in Cambridge tap water increased the conductivity by $44 \times 10^{-5} \Omega$ units, at which value the conductivity was almost steady. All the worms appeared to be healthy and eight of the ten recovered completely on transference to sea water: the two others partly recovered, but ultimately cytolysed in the sea water. Now allowing $5 \times 10^{-5} \Omega$ for the cytolysis increment for an individual worm, it follows that the ten worms had lost $\frac{44}{50}$ of the total salts, i.e. 88 per cent.

The worms are, therefore, able to survive in such solutions with 10–15 per cent. of their original internal salt content. Since, in such solutions, the volume of the worm is increased to about 160 per cent. of the original volume, the actual internal concentration of salts is probably of the order of 6–10 per cent. of the original concentration in sea water. It is of interest that this value is of the same order as the concentration of salts in the fluids of fresh-water Invertebrates.

Even this low concentration of salts is far higher than that of the external medium (about = 0·7 per cent. sea water) and, since the organism is certainly permeable to water and salts, such a concentration difference must involve the performance of work. Schlieper (1929) found that the rate of respiration of many estuarine organisms is enhanced in dilute media. Beadle (1931) finds that in Gunda itself the rate of respiration is roughly doubled under similar conditions. This increase is apparently directly connected with the maintenance of the internal salt concentration, for worms in dilute sea water increase the degree of swelling when the respiration is depressed by cyanide. Beadle further finds that, whereas in Nereis diversicolora the high rate of respiration falls after a time in dilute sea water, the high rate is maintained in the case of Gunda ulvae. This may well be correlated with the far greater degree of adaptation to water of low salinity in the case of Gunda, the organism living temporarily in a medium which is actually fresh water (Pantin, 1931).
FACTORS AFFECTING SALT LOSS.

It has been shown that survival of the worms in dilute solutions and their imbibition of water depends greatly upon the salts present in those solutions. The worms survive far better in their own stream water than in distilled water. Within the range of pH normally met by the worms this factor is without significance. The salts present, however, exert a considerable effect. Dilute solutions of non-electrolytes, such as glycerol, of roughly the same osmotic pressure as the stream water, are no more effective in maintaining the worms in normal condition than distilled water. Dilute NaCl (0.004 M) is also unable to maintain the normal condition: mere osmotic pressure is thus not the factor concerned. But the river water itself and Cambridge tap water, both of which contain significant quantities of CaCO$_3$ in solution (0.002 M), and also CaCl$_2$ in distilled water (0.002 M), are able to maintain the worms in normal condition for a very long period and greatly to reduce the rate of imbibition of water. This indicates that the presence of calcium salts in the river water is the chief factor. It is, therefore, necessary to investigate the effects of these factors on the rate of loss of salts.
The effects of distilled water and Cambridge tap water.

Like the stream water, Cambridge tap water is a hard water, and the survival and behaviour of the worms in both these was found to be the same. For convenience Cambridge tap water was, therefore, usually employed as a “normal fresh water” control.

Fig. 3 shows the results of a series of experiments performed on a single batch of ten worms. The abscissa gives the time after transference from sea water to the solution. By comparing curves 1 and 3 it will be seen that, in the presence of the hard water, the rate of loss of salts by the worms is greatly reduced in comparison with distilled water. The worms were allowed to recover in sea water for 24 hours after each experiment.

**Osmotic pressure.**

Fig. 4, curves 2 and 3, show the effect of distilled water and of 0.008 M glycerol (approximately isotonic with the river water) on the rate of loss of electrolytes of the same batch of ten worms. It will be seen that the loss in the two cases is almost identical. Simple osmotic pressure does not, therefore, seem to be effective.
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Hydrogen-ion concentration.

In Fig. 5 are shown the effects on the same batch of ten worms, of NaCl 0·004 M in distilled water pH 6·6, and of a NaHCO₃ solution of similar concentration containing sufficient HCl to bring the pH to 8·0. Here again it will be seen that there is no appreciable difference in the rate of loss of salts.

By considering the slope of curves 3 and 4 in Fig. 3 it will be seen that pH is without sensible effect on the rate of loss of salts in solutions containing similar concentrations of calcium salts.

The action of salts.

Fig. 3 shows the effects on the same batch of worms of distilled water, NaCl 0·004 M, Cambridge tap water (0·002 M Ca) and CaCl₂ 0·002 M. NaCl solutions usually depress the rate of loss of salts slightly, though in many experiments no difference could be seen between its effect and that of distilled water. The mere presence of electrolytes at approximately the same concentration as in the control is not of itself an important factor.

However, it will be seen from the slopes of curves 3 and 4, that solutions containing calcium, either as carbonate as in the natural stream water or Cambridge tap water, or simply as CaCl₂ in distilled water, depress considerably the rate of loss of salts.
DISCUSSION.

It is evident that, in regard to the permeability to water, to the normal activity of the animal and its power of survival, and also to the rate at which the animal loses salts to a fresh-water medium, the presence of calcium is the chief controlling factor.

In what way calcium acts is not certain. It is difficult to suppose that all its effects are simply due to the action of this ion in maintaining the mucus secreted by the animal in a normal condition (Weil and Pantin, 1931)—though this factor undoubtedly controls the normal locomotion of the animal. It is possible that the calcium acts to some extent directly by reducing the permeability of the cell to water and perhaps to salts. Such an action has been demonstrated in Arbacia eggs by McCutcheon and Lucke (1928). In this case we are certainly not concerned with cells normally adapted to a change in composition of the medium (Weil and Pantin, 1931).

It is, however, very significant that, as has already been shown, worms left for long periods in hard water (containing CaCO₃) are able to survive, and at the same time retain electrolytes in their tissues at a far higher concentration than in the external medium. Work must be done to enable the organism to do this, and the experiments of Beadle (1931) indicate that this is related to an observed increase in oxygen consumption.

Such a system as this is of great interest. Although the worm in sea water may perhaps be considered as approximately in osmotic equilibrium with the external medium, yet when the medium changes to fresh water the condition passes into that of a steady state which is far removed from equilibrium. In this state the osmotic difference across the surface of the animal is counterbalanced by processes deriving their energy from metabolic activity.

The steady state does not involve water transport alone, but also the movement of electrolytes, since Gunda is permeable to these and yet can maintain a relatively high internal salt content when in fresh water. Molecules of both solvent and of the various solutes must therefore be transported in opposition to osmotic tendencies, through metabolic activity. But even cells apparently in osmotic equilibrium with external medium approach this condition. The relative salt concentrations inside a marine organism differ from those in sea water, yet as Bethe (1929) has shown in Aplysia and Carcinus, the organisms are permeable to these same salts. Here also there is a steady state in which work must be done to maintain the difference in individual ion concentrations. This differs from the case of Gunda (and presumably from fresh-water organisms) only to the degree to which the steady state depends on the transport of molecules of solvent as well as those of solute. The mechanism of salt and water regulation in Gunda and in fresh-water organisms may, therefore, well be only an elaboration of mechanisms controlling the “steady state” of salt distribution in marine organisms. Macallum (1926) has pointed out the importance of the excretory function in the transition of marine organisms into fresh water.
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What parts of the Platyhelminthes are actually concerned with this function there is at present no experimental evidence to show, but sections cut of Gunda ulvae by Miss E. Weil and Mr Beadle indicate that the swelling of the worms in dilute solutions is due almost entirely to swelling of the endoderm and parenchyma with the production of large vacuoles in the gut cells. The ectoderm scarcely swells at all: perhaps it functions merely as a passive semi-permeable membrane allowing water imbibed into the parenchyma and endoderm to be excreted into the gut by way of the vacuoles.

We have seen that, in the presence of calcium salts, Gunda can control its permeability to water and salts far more effectively than in distilled water. In the hard water of its own stream it approaches the "homoiosmotic" condition of a fresh-water organism: in distilled water it is "poikilosmotic" and swells to cytolysis just as a purely marine organism would do (cf. Schlieper, 1929).

This again raises the question of the rôle of the calcium content of fresh waters in the migration of marine organisms into fresh water. We have seen that the effect of calcium on the permeability of Gunda to water seems to be related to the more general effect of this ion on purely marine (and other) cells. If migration from the sea into fresh water depends upon the elaboration of such a mechanism, the calcium content of the fresh water is of great importance. Now, although sea water is far richer in almost all salts than any fresh water, yet a hard water such as that of the "Gunda" stream at Wembury contains an amount of calcium which is by no means negligible in comparison with the amount in sea water.

Table I shows the concentrations of the chief ions of sea water, together with the proportions of the ions in hard and soft fresh waters expressed as percentage of the amounts found in sea water.

<table>
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<th>Ion</th>
<th>Gm. per litre in sea water*</th>
<th>Percentage of ion concentration in sea water</th>
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<tr>
<td></td>
<td></td>
<td>Gunda stream†</td>
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<tr>
<td></td>
<td></td>
<td>Plymouth tap water†</td>
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<tr>
<td></td>
<td></td>
<td>Cambridge tap water‡</td>
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</tbody>
</table>

Table I shows the concentrations of the chief ions of sea water, together with the proportions of the ions in hard and soft fresh waters expressed as percentage of the amounts found in sea water.

Table I.

* Analysed by Dittmar (1884).
† Analysed by Pantin (1931).
‡ Analyised by Cambridge University Department of Chemistry.

It will be seen at once that, in the transition from the sea into fresh water, the individual ion concentrations change enormously, except in the case of transition into hard waters. In the latter the carbonate content is actually increased and the calcium content is by no means reduced to negligible limits, especially when the absence of the antagonistic ion Na⁺ is considered.
Provided, therefore, that an organism can withstand the large change in osmotic pressure at all, there is present in a hard water a sufficient quantity of one of the very elements whose presence is required for the maintenance of the normal condition of the tissues.

SUMMARY.

1. The rate of loss of salts by the estuarine worm, *Gunda ulvae*, on transference from sea water to various dilute solutions has been studied by measurement of the electric conductivity of the solutions.

2. Salts are lost by the worms from the moment of immersion in dilute solutions. Conditions affecting the rate of loss of salts are discussed.

3. The relation between the amount of salts lost and the total electrolyte content of the worm was determined. It is shown that the worms only lose 25 per cent of their salts during the time that they imbibe a volume of water from the dilute solution equal to their initial volume.

4. The limiting internal salt concentration of worms surviving in waters containing calcium is about 6–10 per cent of the normal concentration in sea water. No such limiting value can be found for distilled water, since salts are lost continuously till cytolysis occurs. The significance of the limiting concentration is discussed.

5. The effect of osmotic pressure, pH, dilute solutions of NaCl, NaHCO₃, glycerol, CaCl₂ and CaCO₃ are studied. The presence of calcium reduces the rate of loss of salts. Other factors do not seem to influence this rate.

6. The relation of calcium to the maintenance of normal permeability to water and salts in the worm, and the significance of this to the problem of migration into fresh water are discussed.

REFERENCES.


