THE PERMEABILITY OF THE SURFACE OF MARINE ANIMALS

By ALBRECHT BETHE

(From the Institute of Animal Physiology, Theodor-Stern House, Frankfort-on-the-Main, Germany)

The opinion that the surfaces of aquatic animals are semi-permeable was chiefly based on the researches of Frédéricq and of Bottazzi, which were carried out on marine forms, and on the experiments of Overton on amphibians. According to these experimenters water alone penetrates the skin, while dissolved substances will not pass through it unless they are soluble in lipoids. Only in a few cases, however, was proof furnished by chemical means that substances dissolved in water do not penetrate into the body of the experimental animals. In the main, in the past reliance was placed on the results of experiments which measured osmotic changes alone. I may be permitted to discuss briefly such experiments.

In accordance with the statements of Frédéricq and of Bottazzi (1) the osmotic pressure of most marine animals save fishes is equal to that of the surrounding sea water. If such marine animals are put into more diluted or more concentrated sea water, the content of salt in the blood and hence its osmotic pressure decreases or increases correspondingly in a very short time.

At the same time as the osmotic pressure changes in diluted sea water, according to Frédéricq and others, the volume of the animals increases and in more concentrated sea water decreases. Consequently the deduction readily was drawn that the equalization of the internal with the external osmotic pressure was produced by the diffusion of water alone. A proof of this presumption could only be given, however, if the increase in volume takes place in all animals and corresponds to the difference in osmotic pressure. But this is not the case, as I will subsequently show.

To examine the permeability of the surface of animals for substances

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dissolved in water, it is best to choose substances which exist both within the animal and in its environment. These are the ions of sea water. But one has to avoid the danger of complicating the process of equalization by causing changes in the osmotic pressure. This is only possible by using artificial sea water, in which one ion alone is either increased or diminished.

I chose for my experiments the same species of animals employed by Frédéricq and by Bottazzi, the common crab (*Carcinus maenas*) representing the crustaceans, the sea-slug *Aplysia* representing the molluscs.

When these animals were kept in artificial sea water containing no Ca, the Ca-content of their blood decreased constantly, as I have shown in a previous paper (2). In the crab it diminished to one-third in about 10 days, in *Aplysia* in 5 hours. If I used artificial sea water free from Mg or K, the content of these ions in the blood decreased in the same way. On the other hand the Ca (Mg or K) in the blood can be augmented, when the animals are kept in sea water with a high content of these ions.

All these facts *show that the surfaces of marine invertebrates are not impermeable for ions* (or their salts) as suggested by Frédéricq, Bottazzi, and Macallum.

Macallum (3) based his hypothesis on his discovery that the body fluids of marine animals living in sea water of varying concentration, always show the same relative relationship of the ions one to another, which differs in a specific manner from the relationship of the same ions in the sea water. I convinced myself of the correctness of this statement in regard to crabs, *Aplysia*, and other marine forms by transferring them from ordinary sea water into sea water either diluted or concentrated. As the relative concentrations of the ions in the outside medium did not change in such experiments, it does not change within the body either. Only when the relation in sea water is changed will it change in the blood also.

On the other hand there were some experiments on the changes in osmotic pressure from which Frédéricq and Bottazzi drew the conclusion that the surface of all marine invertebrates has the quality of a semi-permeable membrane. I was obliged to try to bring their results into harmony with my own, which showed that the surfaces are permeable also for dissolved substances as, for example, for salts.

If, when one brings an animal from ordinary sea water into a more diluted or a more concentrated one, the compensation for the change in osmotic pressure took place merely by the transport of water inwards or outwards, the volume of the animal would have to change. The amount of such change could be calculated beforehand, and the new volume assumed would have to remain constant as long as the animal remained in the altered sea water. Neither the one nor the other condition is fulfilled.

Let us see what experiments with *Aplysia* will show. If we place it in half-diluted sea water the weight should double. But at best it increases only about 60 per cent (Fig. 1). Very soon the weight decreases again and the original weight is nearly reached after 6 to 10 hours. When less diluted sea water (relation of 3 to 1) is used the situation is still clearer. After increasing in weight at first, it decreases again, in many cases after 2 hours, and returns to the original weight in another 2 or 3 hours. The weight may even decrease still further. On transferring the animal into normal sea water, a new and rapid diminution of weight occurs. But a short time later the weight becomes constant (Fig. 1).

These changes of weight are only explainable if in addition to a transfer of water a transfer of salt from within to without also occurs. To ascertain this more precisely, samples of blood were taken at various times after transfer into diluted sea water. As we had found, as stated above, that the relative relations of the blood constituents did not change, it was not necessary to make a complete analysis in each case but an estimate of the Cl content would be sufficient; and this was calculated from the blood sample taken as if the animal consisted of blood alone. On the basis of these experiments it was shown that the increase in weight at the beginning was almost entirely due to the transfer of water alone. But later on considerable amounts of salt, even up to 37 per cent, are lost. In this manner the animal comes into equilibrium with the external osmotic pressure. The lost salt can be recovered almost completely from the outside water. When put back into normal sea water, first a loss of weight owing to transfer of water occurs, which is followed by a pretty rapid transfer of salt to the inside of the animal. This taking up of salt can also be easily shown by blood analyses.

Aplysia, on being transferred from normal sea water to a more concentrated one, very quickly decreases in weight, but this loss of weight remains behind that arrived at by calculation. After a short time the weight increases again and becomes constant. But in none of the experiments hitherto performed has the original weight been
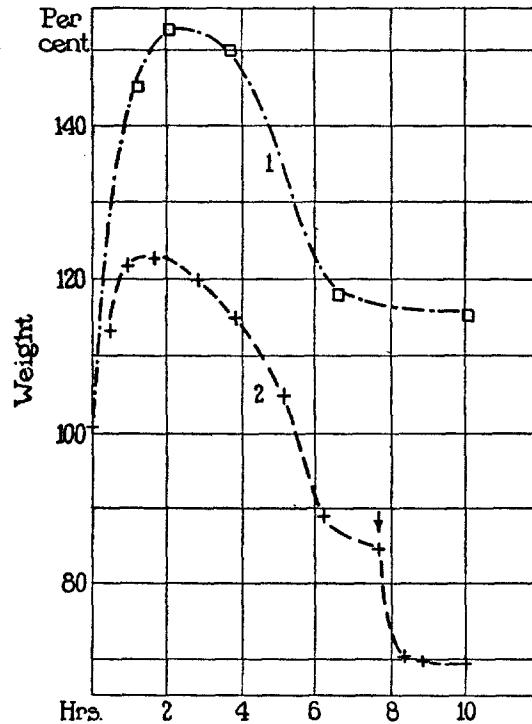

FIG. 1. Change of weight of *Aplysia* in diluted sea water: (1) half concentrated, (2) three-fourths concentrated. ↓ normal sea water.

regained. In this case, too, calculation shows that not only does water leave the body but salt also enters it.

In fact, the *compensation to osmotic-pressure changes* in *Aplysia* takes place *in large part by transport of water*. But this is not the only cause. In *addition a transport of salt takes place*, which is only revealed by exact analysis.

In the hard-shelled crabs the reactions are quite different. Here a

transfer of water is difficult to prove. Within the limits of the method there is *no change of weight*. Hence the *compensation for the osmotic-pressure changes* in hypotonic or hypertonic solutions *can only take place by transfer of salt*. It hardly could be expected that the result would be otherwise. If the compensation were to be effected by water transfer, the animals in diluted sea water would be likely to burst as there is no room within the shell for the entering water. Or in concentrated solutions a negative pressure would be established within the carapace, which can not shrink.

The experiments performed up to the present make it evident that osmotic pressure can not be the only effective force producing the compensation which occurs on transferring the animals into more or less concentrated sea water. But in addition it seems that the compensation is in part produced by the salts or their ions, in obedience to the laws of diffusion, passing through the skin from places of higher to that of lower concentration. This question can be decided by very simple experiments.

Animals transferred to sea water, diluted not with distilled water but *with a solution of a non-electrolyte isotonic with sea water, ought not to change their weight* as the osmotic pressure of the outside solution should remain equal to that of the blood. But in fact all species of *soft-skinned animals* experimented with *shrunk in such solutions permanently*, till at last their blood became so viscous that death occurred. On the contrary, hard-skinned animals which are unable to shrink die much faster because they lose nearly all their blood. The death of some marine animals in isotonic solutions of cane sugar, often referred to in the literature, does not depend, as has been generally accepted, upon the toxicity of pure sugar solutions but on the concentration (thickening) of the body fluids.

I am now going to report on experiments of this kind. I will speak first about the soft-skinned animals covered with a mucous membrane and again will take *Aplysia* as my example. Figure 2 shows the loss of weight of *Aplysia* on being transferred into sea water diluted either to one-quarter or one-half with an isotonic solution of cane sugar. Both curves are from the averages of a large number of experiments. You see how in the one case the weight has decreased to one-half after as little as 6 hours, and in the other case after 18 hours, and this

in spite of the fact that the osmotic pressure was the same outside and within the animal. After losses of weight of such an extent the blood
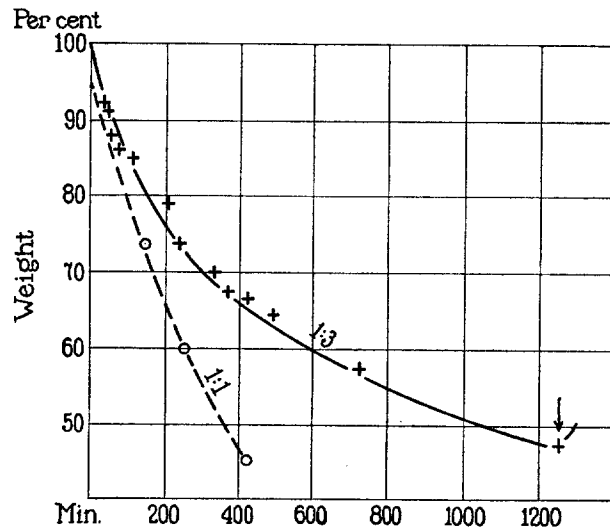

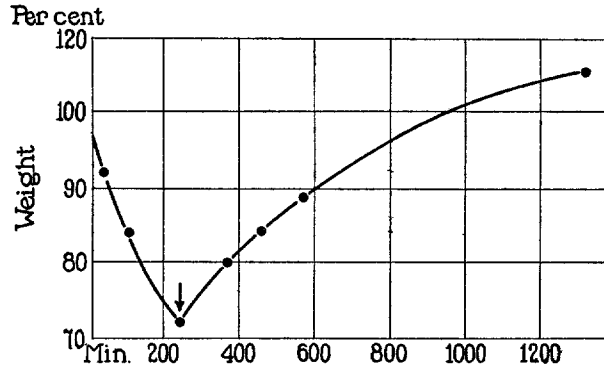FIG. 2. Loss of weight of *Aplysia* in a mixture of isotonic cane sugar solution and sea water. ↓ normal sea water.

FIG. 3. Loss of weight in 1 part of cane sugar solution and 3 parts sea water, and recovery in sea water ↓ .

has become so thick that it can hardly be squeezed out after cutting the skin. In normal animals it is quite liquid and flows out by itself quite easily when they are wounded. The Cl-content does not de-

crease much relatively, but calculated on the weight of the animal it is quite considerable up to 50 per cent.

These occurrences may be accounted for as follows: The osmotic pressure is equal outside and inside, but the salt concentration is lower outside than within. As the surface is permeable for salt, salt begins to diffuse out. As the result of this, the osmotic pressure becomes lower inside than outside and water passes to the outside. And this procedure is repeated again and again and an equilibrium
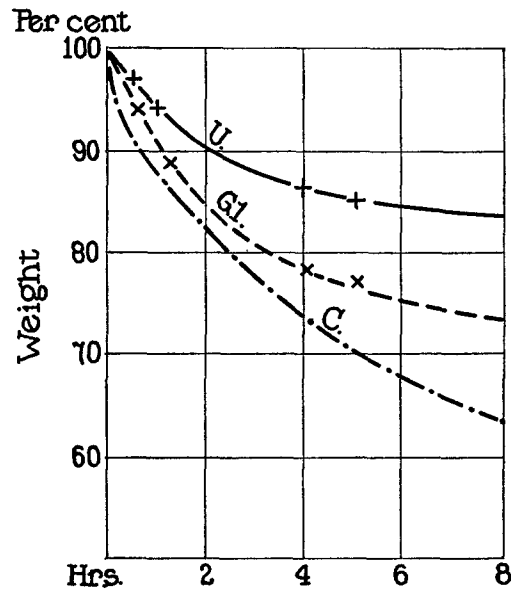

FIG. 4. Loss of weight in 1 part isomolecular solution of urea (U) or glycerine (Gl) or cane suger (C) and 3 parts of sea water.

can not be achieved. Theoretically the animals would lose all their water if the volume of the external solution were sufficiently great. The process ceases because the circulation of the blood stops.

If the animals are transferred at the right time into normal sea water, they swell quickly again, regaining from the sea water salt as well as water (Fig. 3). In some cases it may be noted that the original weight is even exceeded. This permits the assumption that the skin is not quite impermeable for cane sugar. Indeed, it can be shown by blood

analysis that this non-electrolyte does enter to a certain extent. *Other non-electrolytes penetrate more quickly*, dextrose more so than cane sugar, glycerine still more rapidly, and urea more rapidly still. In accord with this, in equal time periods, the losses of weight are smaller with urea than with glycerine and still smaller than with cane sugar (Fig. 4).

Experiments were made with this method of transfer into sea water diluted with isotonic non-electrolyte solutions with other species of soft-skinned animals. If a permanent loss of weight takes place, which can be reversed by retransfer into normal sea water, there is clear proof that the surfaces are permeable for salts, and much more permeable for the salts than for the non-electrolyte used. All the species of animals experimented upon as yet, *e.g.*, the worm *Sipunculus*, the Echinoderm *Holothuria*, and various medusa and pelagic molluscs, showed such reversible losses of weight.

Entirely different, as already mentioned, are the facts ascertained with the hard-shelled animals. As yet I have employed for these experiments only the crab *Carcinus maenas*. Losses of weight could hardly be observed with this form by transfer into sea water diluted with cane sugar solution. It has already been mentioned that even in highly hypertonic solutions these animals showed hardly any loss of weight. But in this form there was evident a marked *thickening of the blood* which parallels the great reduction in salt content.

CONCLUSIONS

The surfaces of all marine invertebrates which have been experimented upon are permeable for water and also for both the salts or their ions which are in solution in their blood and in sea water. The forces which tend to bring the salt content of the blood into equilibrium with the salt content of the surrounding sea water are just as great as the forces which strive to prevent osmotic differences. The skin of these animals, save in the cases where special modifications have arisen, serves only as a protecting barrier preventing the loss of the body colloids.

REFERENCES

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2. Bethe, A., *Pflüger's Archiv.*, 1928, 221, 344-362.
3. Macallum, A. B., *Journ. Physiol.*, 1903, 29, 213-241.