A NOTE ON THE MECHANISM OF SALT AND WATER BALANCE IN THE HETEROTRICHOUS CILIATE, *SPIROSTOMUM AMBIGUUM*

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(With Two Text-Figures)

ALTHOUGH considerable attention has been paid to the kinetics of water balance in the Protozoa, *e.g.* Adolph (1926), and more recently Kitching (1934) and Kamada (1935), comparatively little is known of the instantaneous distribution of salt, water and colloidal substances in the body of a protozoon, and nothing is known of the magnitude of the hydrostatic pressure within the animal and its possible importance in the process of excretion. The experiments described here were made in order to obtain direct information on the total osmotic concentration of the body fluid, the maximum value for the colloid osmotic pressure of the cell contents, and the hydrostatic pressure within the organism.

Spirostomum ambiguum was reared in large numbers from a wild strain, and fed on bacteria from an infusion of wheat. Before an experiment the liquid containing the organisms was decanted from the detritus at the bottom of the vessel, and was centrifuged in such quantity that a deposit of *Spirostomum* about 0·5 cm. deep was formed at the bottom of the centrifuge tube. The clear liquid was withdrawn and the deposit was transferred to the surface of a sintered glass microfilter, and as much as possible of the liquid between the organisms was removed. There remained on the filter a little slime composed for the most part of *Spirostomum* but containing also smaller ciliates such as *Paramoecium* and *Loxodes* and bacteria.

For measuring the vapour pressure of the animal, the Hill thermal method was used, employing medium-sized thermopiles, and strips of cigarette paper as described in Picken (1936, Appendix I). The thermopiles were calibrated with a solution of 0·20 per cent. sodium chloride against distilled water. A thermopile was prepared with one face already covered by a piece of paper soaked in distilled water, and a second piece of paper was rubbed in the deposit of *Spirostomum* and rapidly transferred to the other face of the thermopile. The results of five such determinations in duplicate on two thermopiles are shown in Table I. The consistently higher values given by the second thermopile are due to the fact that there is less interstitial fluid remaining in the mass after the first paper has been coated with the deposit. The mean values are certainly somewhat lower than the actual concentration of the

body fluid, since it is impossible to remove the interstitial fluid completely. Nevertheless they are of the same order as the values found by conductivity measurements on *Paramoecium* (Gelfan, 1927), namely, 0·06 *M* potassium chloride, which is approximately equivalent to 0·35 per cent. sodium chloride. They are, however, nearer to Kamada's estimate of *M*/20 potassium chloride, which is roughly equivalent to 0·23 per cent. sodium chloride.

Some of the deposit obtained by the centrifuge was transferred to the surface of the auxiliary prism of a Zeiss dipping refractometer, and the observation of the field was made through a stop attachment. Table I contains the non-mineral refractive increments measured in five experiments and obtained from the refractive index measurements by subtracting the increment due to a sodium chloride solution of the same vapour pressure as the body fluid. These values are converted into colloid

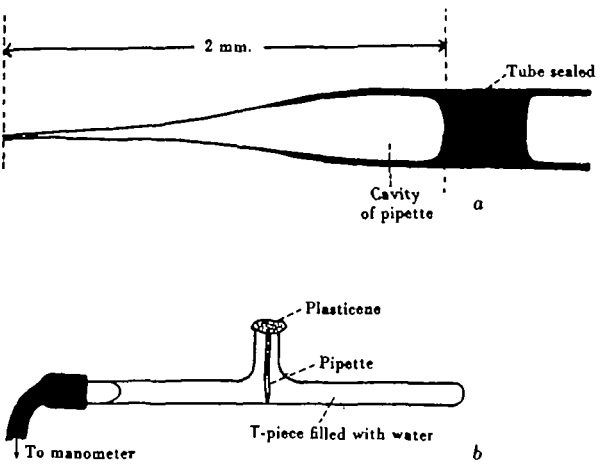

Fig. 1.

osmotic pressures in centimetres of water (column four) by determining the percentage of protein present (considered as vertebrate serum protein) from the factor n_D 1 per cent. protein = 0·00172, and using the graph from Krogh & Nakazawa (1927) to convert this percentage into the equivalent osmotic pressure. Since it has been found that such calculated values are too high for invertebrate body fluids (Picken, 1935 *a*), it is probable that the true colloid osmotic pressure is rather less than the calculated value.

The hydrostatic pressure of the body fluid was measured by means of a micropipette of the size and proportions shown in Fig. 1*a*; the tube was sealed about 2 mm. from the tip. A drop of a very thick culture of *Spirostomum* was placed on a microscope slide and most of the liquid was removed so that the animals were confined to a layer of water not much thicker than themselves. Moving the slide with the left hand and the capillary pipette with the right, an animal was pursued while being kept in the field of the microscope and, in some cases, was successfully impaled on the pipette. On immersing the pipette in the drop of water on the slide a limited

amount of water is taken up and the length of this column of liquid is measured by means of an eyepiece scale. Perhaps one out of ten animals was successfully pierced by the pipette (without putting the pipette right through the animal), and in these cases the length of the water column was momentarily increased, and the increment was measured. The displacement was of the order of one or two divisions of the eyepiece scale. Since the pipette was small, the amount of liquid taken from the animal was not more than a tenth of the total volume of the organism. It was observed that the animal does not collapse completely on tearing the surface, partly because of the rigidity of the envelope and the cortical protoplasm, which do not contract beyond a certain limit, and partly because of the rapid reformation of the surface at the point of injury.

In order to calibrate the pipette, it was sealed by plasticene into the short limb of a T-piece filled with water, and connected to a water manometer (see Fig. 1 *b*). The T-piece was clipped on to a microscope stage and the pipette was observed under the same power as was used in transfixing the animal. The manometer was adjusted until the position of the meniscus in the pipette was the same as the reading of the eyepiece scale when the pipette was dipped into the drop with the *Spirostomum*. The pressure necessary to move the meniscus from this position to the position it had occupied when the *Spirostomum* was pierced was measured by means of the manometer. The pipette was not calibrated, but in every experiment a new pipette was used and a new determination made of the pressure change required to move the meniscus through its observed displacement. The values of hydrostatic pressure measured are shown in Table I. It must be realised that these values are likely to be supernormal, since the animal is always maximally contracted when the measurements are made; on the other hand, the values may be subnormal, since the body fluid probably has a lower surface tension than the culture medium (tap water). This would tend to make the meniscus fall. The relative importance of these two factors cannot as yet be estimated.

Table I

Thermopile	Concentration of body fluid; gm. sodium chloride in 100 c.c. solution	Mean	Refractive increment due to non-mineral solutes	Equivalent colloid osmotic pressure (calc.) cm. of water	Hydrostatic pressure cm. of water
1	0·11	0·13	0·00112	1·6	5
2	0·14				5
1	0·08	0·13	0·00131	1·8	3
2	0·18				3
1	0·11	0·14	0·00322	5·2	3
2	0·16				5
1	0·11	0·15	0·00034	0·4	4
2	0·19				7
1	0·12	0·15	0·00123	1·8	—
2	0·17				—

DISCUSSION

From the data in Table I it is apparent that even in unicellular organisms mechanical factors may be of considerable importance in determining the rate of movement of water through the system; that is to say, the rate of filling of the contractile vacuole may depend (among other factors) on the difference between the tension of the body wall and the osmotic pressure due to colloidal substances in the body fluid. It is possible that the rates at which water and salt move through the organism are regulated to a large extent by the tone of the body wall. Fig. 2 summarises the osmotic conditions inside and outside the organism. It is suggested that the solution entering through the body wall is hypertonic to the external medium (by analogy with other organisms in which osmotic work appears to be done at a surface other than that of the excretory organs. Whether osmotic work is done here or not will not affect the following argument, which is an attempt to show that the rate of filtration into the vacuole is proportional to the hydrostatic pressure and colloid osmotic pressure of the body fluid.

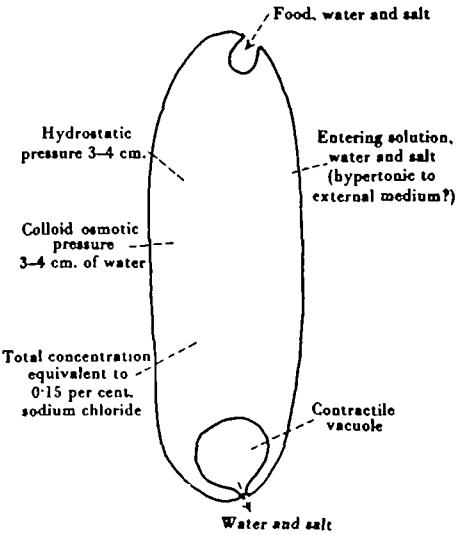

Fig. 2.

If the membrane bounding the contractile vacuole is impermeable to protein, the rate of filtration into the vacuole will be proportional to the difference between the hydrostatic pressure and the colloid osmotic pressure of the body fluid. We will assume that the colloid osmotic pressure is directly proportional to the concentration of colloids in the system (which is not strictly true); that the hydrostatic pressure has suddenly fallen so that filtration stops; that the original colloid osmotic pressure of the cell contents is 4 cm. of water; and that the maximum volume of the contractile vacuole represents the volume of liquid taken up by the cell between two contractions of the vacuole. Then in the time between two contractions the colloid osmotic pressure will fall to $4 \cdot (V/V+x)$ cm. of water, if filtration stops (V = volume of *Spirostomum*, x = volume of contractile vacuole). Since the volume of the animal is about $1{\cdot}6 \times 10^{-6}$ c.c. and the volume of the contractile vacuole about $1{\cdot}6 \times 10^{-7}$ c.c., the rate of filtration, R, in the normal animal will be equal to

$$R = K \text{ (hydrostatic pressure} - \text{colloid osmotic pressure)}$$
$$= K\left(4 - 4 \cdot \frac{1{\cdot}6 \times 10^{-6}}{1{\cdot}6 \times 10^{-6} + 1{\cdot}6 \times 10^{-7}}\right)$$
$$= K \cdot 0{\cdot}36.$$

(It is assumed that there is normally equilibrium between hydrostatic pressure and colloid osmotic pressure.)

If the hydrostatic pressure and the colloid osmotic pressure are simultaneously reduced to 1 cm. of water, then the rate of filtration will be equal to

$$R = K\left(1 - 1 \cdot \frac{1{\cdot}6 \times 10^{-6}}{1{\cdot}6 \times 10^{-6} + 1{\cdot}6 \times 10^{-7}}\right)$$
$$= K.0{\cdot}09$$

(assuming that all other factors remain constant).

A fall in the level of hydrostatic pressure and colloid osmotic pressure means then a diminution in the rate of filtration. If this occurs, the rate of salt loss from the animal diminishes because the volume of filtrate formed in a given time is reduced and therefore the amount of non-resorbed solutes excreted is reduced. Accordingly, from the point of view of salt and water balance the whole cycle could be slowed down by diminishing the hydrostatic pressure of the system. If less salt is lost in unit time, less need be taken in through the surface, and the rate of working of both the surface salt absorbing mechanism and the salt-resorbing mechanism of the contractile vacuole could be reduced without lowering the osmotic pressure due to mineral solutes in the organism. Moreover, the rate of food ingestion need not be maintained (assuming that food is required simply as a source of energy and salts). In short, there seems no reason why the organism should not live much less intensely than it does; as far as we can see at present the tone of the body wall is only increasing the total exchange of energy between organism and environment.

For the moment we have overlooked the fact that the slowing down of the salt and water cycle was achieved by reducing the colloid osmotic pressure of the system, and it is perhaps in this fact that the key to the situation lies. The organism lives more intensely than the adjustment of its total osmotic pressure demands, because only by so doing can it reduce the proportion of water to solid in its protein systems. In a subsequent paper (Picken, 1937) it will be shown that there is a tendency for the muscle proteins of animals in the various phyla to become less hydrated as one passes from the "lower" invertebrates to the "higher" invertebrates and from these through the groups of the vertebrates. Even within the same organism various muscles may contain different percentages of water, and the muscles are not in colloid osmotic equilibrium with the body fluid; they are considerably less hydrated than would be the case if they were in simple equilibrium with the surrounding protein solution. The muscle cells show some regulation of the degree of hydration of their substance, and the hydration of the cell proteins in *Spirostomum* may also be regulated, in this case by the hydrostatic pressure within the cell.

Such a control of the solvation of the protein molecules in the cell is to be expected in contractile structures or indeed in any structure in which the rigidity of the system depends in part on the covalent linkage of long chain molecules, a linkage which cannot take place if water molecules are saturating the valences on which cohesion depends. Some control of the state of hydration is conditional for the existence of such a system.

This picture of the cycle of salt and water in *Spirostomum* is intended simply as a possible scheme by which the known facts can be interrelated. Perhaps its chief

value lies in the fact that here, in a unicellular system the importance of the ratio of protein to water is apparent. In the necessity for increasing this ratio lies perhaps the significance of the pace set for the salt and water cycle by the hydrostatic pressure of the body fluid.

SUMMARY

1. Vapour pressure measurements on *Spirostomum ambiguum* suggest that the concentration of the body fluid is at least equivalent to an aqueous solution of 0·15 per cent. sodium chloride.

2. The colloid osmotic pressure (calculated) of the body fluid varies from *c.* 0·5 to 5·0 cm. of water and has an average value of *c.* 2·0 cm. of water.

3. The hydrostatic pressure varies from 3 to 7 cm. of water and has an average value of *c.* 4 cm. of water.

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