IONIC REGULATION IN THE CILIATE *SPIROSTOMUM AMBIGUUM*

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It is generally accepted that the cytoplasm of fresh-water Protozoa is hypertonic to the medium. This is based on circumstantial evidence and indirect measurements of the osmotic pressure of the body fluids. Inorganic ions are likely to account for a large part of the osmotic pressure, and in certain Protozoa radioactive isotopes may be used to determine the concentrations of ions. The isotope technique has been used to demonstrate that cells in Metazoa, namely nerve, muscle and erythrocytes, accumulate ions and carry out ionic regulation. Evidence is presented in this paper concerning the tonicity of the body fluids of the heterotrichous ciliate *Spirostomum ambiguum* and concerning its powers of ionic regulation.

The concentration of sodium, potassium and bromide have been measured in animals grown in medium containing these ions. The rates of entry and loss of these ions have been measured and the results are discussed in the light of current theories of the mechanisms of ionic regulation in other cells.

MATERIALS AND METHODS

Spirostomum ambiguum was chosen for the experiments as it is easily cultured, it is relatively large and it survives the experimental conditions. It was cultured in the following medium:

	mM/l.
Potassium chloride	0·5
Sodium chloride	2·0
Magnesium chloride	0·2
Calcium chloride	0·5
Potassium dihydrogen phosphate	0·1 (approx)
Potassium hydroxide	0·01 ,,

The medium was made up with glass distilled water and the pH was adjusted to 6·3 by addition of the KH_2PO_4–KOH buffer. Boiled wheat grains were added as a source of food for the bacteria on which *Spirostomum* feeds.

^{42}K was obtained as the 'Specpure' carbonate from A.E.R.E., Harwell, and converted to the chloride by addition of hydrochloric acid; the excess acid being removed by evaporation. When the ^{42}K had decayed the concentration of potassium in the experimental solution was confirmed by measurement with a flame photometer. ^{22}Na was supplied by the Radiochemical Centre, Amersham, as a sodium chloride solution. ^{82}Br was obtained from A.E.R.E., Harwell, as ammonium bromide. The compound was converted to sodium bromide by dissolving it in sodium hydroxide and evaporating the water and ammonia.

In each experiment approximately 50,000 animals were removed from the cultures and concentrated in a small volume of medium by centrifuging at approximately 15 g. for 1 min. The animals were then kept for 3 days in unlabelled medium under experimental conditions to come into a steady state before being transferred to a similar but labelled medium. Several samples of the radioactive medium were taken with an 'Alga' pipette for determination of the radioactivity per unit concentration of medium. At suitable times samples of several hundred animals were taken from the labelled solution. These animals were washed free of labelled solution by centrifuging with six changes of unlabelled medium. The time taken for washing was recorded so that correction could be made for the washing of labelled ions out of the animals. From the last wash the animals were transferred to a planchette with a 0·1 ml. pipette operated by a hypodermic syringe. A sample of the last washing medium was also taken with the same pipette to enable correction to be made for any residual radioactivity of the medium on the animals The count of this sample was never more than one or two counts above the background count; it was used to correct the sample for background count and that of contaminating medium. The animals on the planchette were fixed and stained with a few drops of saturated aqueous picric acid and photographed. Photographing the animals made it possible to carry out the slow process of counting them after all the samples had been assayed for radioactivity. This permitted many more samples to be taken during the useful life of the isotope. The planchettes were dried in an oven at 100° C. before being assayed for radioactivity.

The radioactivity was assayed with a Geiger–Muller end-window counter. The time in half seconds for 1000 counts was recorded for all samples, so that the standard deviation of any count is approximately 3% of the total count. In experiments with ^{42}K and ^{82}Br the counts were corrected for decay to a zero time by calculation using the measured half-lives of the isotopes.

The calculation of the mean volume of a population of animals was based on measurements of photographs of a representative sample of contracted animals. A sample of animals was concentrated into a small volume of medium and transferred to a microscope slide which was held in position with a circle of paraffin wax. When a condenser was discharged through the drop via two electrodes embedded in the wax, the animals contracted. Photomicrographs of the contracted animals were taken. The animal appears as an ellipse on the photograph, and assuming that it is a prolate spheroid the volume may be calculated as $\frac{4}{3}\pi ab^2$, where a and b are major and minor semi-axes. A micrometer scale was photographed with each sample.

THEORETICAL CONSIDERATIONS

In the experiments to be described the animals were transferred from one medium in which they had reached a steady state to another of the same composition but containing labelled ions. The tracer ion then exchanges with its counterpart inside the animal and eventually the specific activity of the tracer inside the animal becomes the same as that of the medium. There is no evidence to show that tracer ions

behave differently from the normal species. With a knowledge of the specific activity of the tracer in the medium, the radioactivity when it has reached a steady state in the animal and the amount of body water, the internal concentration may be calculated. This value does not necessarily correspond to the total concentration of the ion inside the animal; only material which will exchange with the labelled ion is measured. Morphological or chemical isolation of the ion inside the animal would give a measured value below the true value.

The equation from which transfer constants may be calculated for the entry of labelled ions into a single cell is given by Davson (1951) as

$$\frac{d\,C_{\text{in}}}{dt} = K_{\text{in}}\,C_{\text{out}} - K_{\text{out}}\,C_{\text{in}}. \tag{1}$$

K_{in} is the transfer constant from out to in and has dimensions of hours^{-1}. K_{out} is the transfer constant from in to out and also has the dimensions of hours^{-1}. t is the time in hours during which the animals are in the labelled solution. C_{in} is the concentration of the labelled ions in the cell. C_{out} is the concentration of labelled ions in the medium and may be regarded as constant when the volume of medium is very large compared with that of the animals.

As there is no net change of concentration of labelled ions when the steady state is reached it follows that

$$\frac{C_{\text{in}}}{C_{\text{out}}} = \frac{K_{\text{in}}}{K_{\text{out}}}. \tag{2}$$

The solution of equation (1) for K_{out}, in the case of animals in labelled medium of constant concentration, is

$$-K_{\text{out}}\,t = \ln\left(1 - \frac{C_{\text{in}(t)}}{C_{\text{in}(t=\infty)}}\right), \tag{3}$$

where t is the time the animals are in the radioactive solution. For practical purposes $C_{\text{in}\,(t=\infty)}$ may be regarded as a steady value obtained at finite time.

In the case of animals which have taken up labelled ions and are then transferred to a similar but unlabelled medium, the solution of equation (1) is

$$-K_{\text{out}}\,t = \ln\left(\frac{C_{\text{in}(t)}}{C_{\text{in}(t=0)}}\right), \tag{4}$$

where t is the time in the unlabelled solution. As the ratios of the values of C_{in} are used in equations (3) and (4), the units in which they are measured are not important and for convenience counts per animal per minute are used in the calculation of K_{out}.

The experimental data when plotted according to equations (3) or (4) should result in straight lines if the animal behaves as a single-compartment system. The value of K_{out}, as measured, is not an absolute transfer constant as it is dependent on the size of the animal. K_{out} is related to the absolute rate constant K'_{out} by the equation

$$K_{\text{out}} = K'_{\text{out}}\,\frac{A}{V}, \tag{5}$$

where K'_{out} is the absolute rate constant as cm. hours^{-1}, V is the volume of the animal in cm.^{3} and A is the surface area in cm.^{2}.

EXPERIMENTAL RESULTS

Uptake — *Experiments with* ^{42}K

In the first experiment the level of potassium was measured over a period of 55 hr. in animals kept at room temperature. Results of this experiment are shown in fig. 1, where the count per animal per minute is plotted against time in the radioactive solution. In all experiments each point is the mean activity determined for 50–500 animals, and all samples at a given time were washed together. The scale on the right of the graph indicates the calculated concentration of potassium in the animal on the assumption (to be discussed later) that the animal is 100% water.

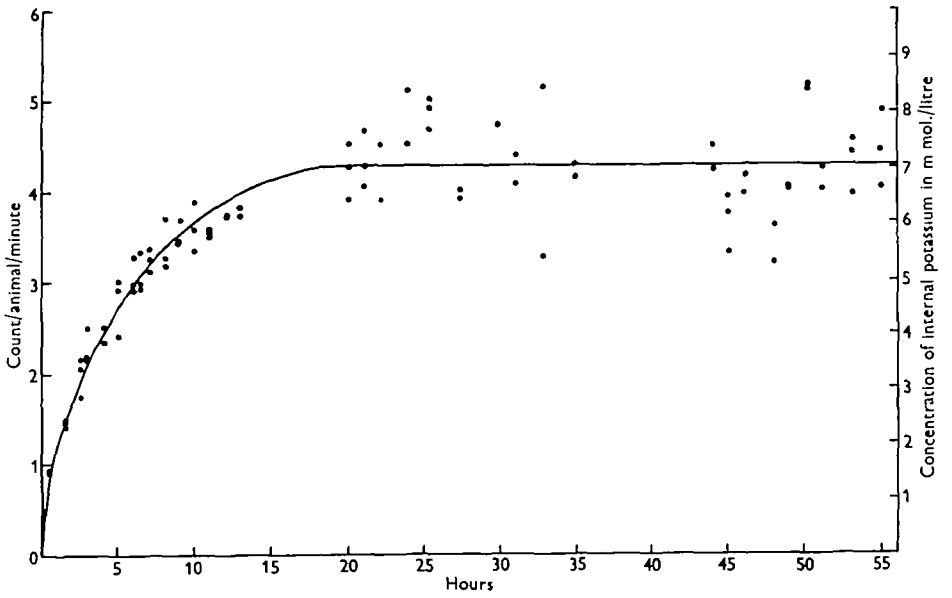

Fig. 1. The uptake of ^{42}K by *Spirostomum* from culture medium.

In Table 1 the results of several similar experiments are summarized. The level of activity in the animal remains fairly constant during an experiment, but from column 3 of Table 1 it may be observed that the mean volume frequently falls off with time. Assuming that the mean volume alters at a constant rate, calculations of mean volumes have been made to obtain the best estimates at the beginning and end of the period during which the level of activity in the animal was measured. The calculated concentrations based on these volumes are shown in column 5 of Table 1. The means of the levels of the radioactivity in the animals during the period stated are listed in column 4 of Table 1 and they are corrected for loss during the washing period from data obtained in Exp. 4.

It is clear from Table 1 that the potassium concentration inside the animal is at least ten times that in the medium.

Table 1

1	2	3 Volume		4 Activity		5
Exp. no.	External potassium concentration (mM/l.)	Time measured (hr.)	Mean volume ± S.E. of mean ($mm.^3 \times 10^{-4}$)	Time measured (hr.)	Mean activity ± *S.E. of mean* (counts/animal/min.)	Internal potassium concentration (mM/l.)
1	0·475	0	59 ± 6·5	20 to 55	4·4 ± 0·05	6·2
		70	37 ± 4·1			7·9
2	0·48	12	74 ± 4·7	20 to 32	17·3 ± 0·09	5·5
		33	62 ± 2·5			6·1
3	0·48	0	40 ± 1·6	18 to 30	10·3	8·5
		72	28 ± 1·3			9·0
4	0·49	0	47 ± 1·4	20 to 72	5·9	6·9
		72	48 ± 1·4			7·1
5	0·1	73	36 ± 1·4	12 to 38	9·1 ± 0·1	8·9
		73	28 ± 1·2	40 to 66	8·2 ± 0·1	10·3
		73	27 ± 1·6	24 to 34	8·4 ± 0·2	10·9
6	0·07	56	32 ± 0·4	10 to 51	8·9 ± 0·1	7·0
13	0·42	0	65 ± 8·6	22 to 54	19·2 ± 0·5	4·5
		44	65 ± 2·6			
22	0·50	5	52 ± 2·5	8 to 12	19·5 ± 0·3	7·6
		20	59 ± 3·1			6·6

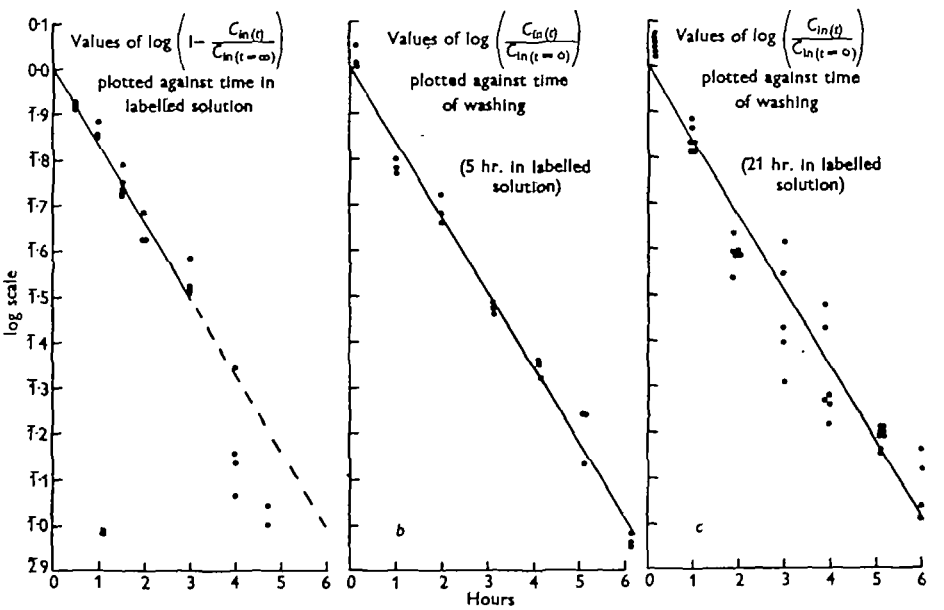

Fig. 2. The uptake and loss of ^{42}K from *Spirostomum* plotted according to equations (3) and (4).

Measurements of the transfer constants K_{in} and K_{out}

To obtain data for plotting according to equations (3) and (4), the rate of uptake of potassium was measured in a population of animals, and when the animals had taken up an appreciable amount of labelled ions they were washed for variable periods in unlabelled medium and the rate of loss measured. The values of

$\log\left(1-\frac{C_{in(t)}}{C_{in(t=\infty)}}\right)$ were calculated from the uptake data and they are plotted against time in Fig. 2*a*. The value of $C_{in(t=\infty)}$ is the mean of all measurements made whilst the animals were in a steady state with the medium. As $C_{in(t)}$ approaches $C_{in(t=\infty)}$ variation becomes greater, and as Solomon (1952) pointed out only the first few points describe a straight line. Following Treherne (1954) the line drawn through the data is the mean of the slopes calculated for lines adjoining the individual points to the origin. As there is a marked change of slope after 3 hr., only data for this first period are considered. The slope of this line is $-K_{out}/2{\cdot}303$.

The rate of loss of labelled ions from the animals was measured after 5 hr. and after 21 hr. in the labelled solution, that is, prior to and after reaching a steady state with respect to the labelled ion. The data plotted according to equation (4) are shown in Figs. 2*b* and *c*. The slopes of the regression lines through the data are equal to $-K_{out}/2{\cdot}303$.

The slopes of the lines in Figs. 2*a*, *b* and *c* are practically the same, and so it follows that the values of K_{out} measured by several methods must be almost identical. This, together with the results of other experiments listed in Table 2, is considered to be justification for the theoretical treatment and it may be concluded that *Spirostomum* behaves as a one-compartment system to potassium.

Table 2

Exp. no.	External potassium concentration (mM/l.)	Mean volume ± S.E. of mean ($mm.^3 \times 10^{-4}$)	Temp. (° C.)	Equation used for calculation of K_{out}	K_{out} ($hours^{-1}$)	C_{in} (mM/l.)	K_{in} ($hours^{-1}$)
1	0·475	48 ± 5·3	16·5	3	0·259	7·05	3·9
2	0·48	68 ± 3·4	18	3	0·317	5·7	3·8
				4	0·473		5·5
3	0·48	40 ± 1·6	16·5	4	0·296	8·75	5·4
4	0·49	47 ± 1·4	16·5	4	0·270	7·0	3·8
6	0·07	32 ± 4·0	22	3	0·393	7·0	39·3
12	0·45	91 ± 4·5	18	3	0·263	4·5	2·9
		95 ± 3·8	18	3	0·256	4·0	2·4
13	0·42	65 ± 8·6	18	3	0·29	4·5	3·1
22	0·5	55 ± 2·8	18	3	0·38	7·1	5·5
				4 (5 hr.)	0·38	7·1	5·4
				4 (21 hr.)	0·38	7·1	5·4
8	0·04	51 ± 1·7	18	3	0·35	3·0	38·7
	1·00	42 ± 1·5	18	3	0·27	9·4	3·5

It may be seen from Table 2 that when the concentration of the external medium is altered the values of K_{out} remain relatively constant, but K_{in} varies considerably. The significance of this point will be discussed later.

The effect of feeding on the uptake of potassium

Spirostomum feeds on bacteria which grow in the culture medium when boiled wheat grains are added. To obtain a source of food for these experiments the

common bacteria from a thriving *Spirostomum* culture were plated out and grown on agar slopes.

The uptake of potassium was measured in three groups of animals of common stock. Group 1 was fed throughout the experiment, whilst group 2 was fed until it was transferred to the labelled solution and group 3 was starved until it was transferred and then fed. After the initial separation of the animals feeding where necessary was carried out every 12 hr. until 6 hr. before placing the animals in the labelled solution. From then onwards it was carried out every 2 hr. in order to maintain the supply of bacteria in the medium.

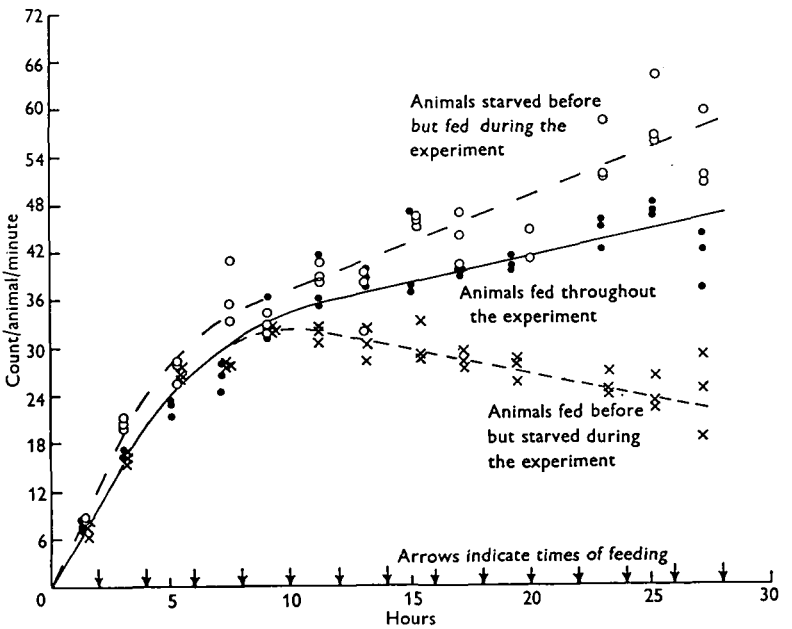

Fig. 3. The effect of feeding and starvation on the uptake of ^{42}K by *Spirostomum*.

The results of the experiment are shown in Fig. 3. The animals of group 1 do not reach a steady state with labelled potassium during the experiment; the value of C_{in} at 28 hr. is calculated as 9·8 mM/l. The animals of group 2 attain a maximum activity at 10 hr., i.e. the normal period, and then the level falls. This fall of activity is associated with a similar fall of volume so that the concentration of internal potassium remains fairly constant at 6·7 mM/l. The potassium activity in animals of group 3 continues to rise throughout the experiment at a greater rate than in animals of group 1. The mean volume of these animals does not appear to alter during the experimental period, and the value of the internal potassium at 27 hr. is 12·5 mM/l. Obviously the potassium concentration is greatly influenced by feeding, and this probably accounts for much of the variation in the experimental results.

The effects of changes of the external potassium concentration

Animals of the same population were acclimatized to culture media containing 0·02, 0·08, 0·32 and 1·28 mM/l. potassium for 3 days. They were then transferred to similar but labelled media, and after 12 hr. the concentrations of potassium in the animals were measured. The means of the volumes of the animals in the various concentrations were practically the same and the internal potassium concentrations were calculated as 3·2, 4·5, 5·7 and 7·8 mM/l. respectively.

It is concluded that the animal is capable of regulating its potassium content even when the external concentration is very low.

The uptake of potassium at different temperatures

The potassium activities were measured in groups of animals of the same population kept at different temperatures. It was found that animals kept at 15° C. contain three times the amount of labelled ions found in animals at 25° C. This difference in activity was associated with a difference in volume so that the potassium concentrations were practically the same. Owing to the volume difference, comparable values of transfer constant were not obtained and a Q_{10} for potassium transport could not be calculated.

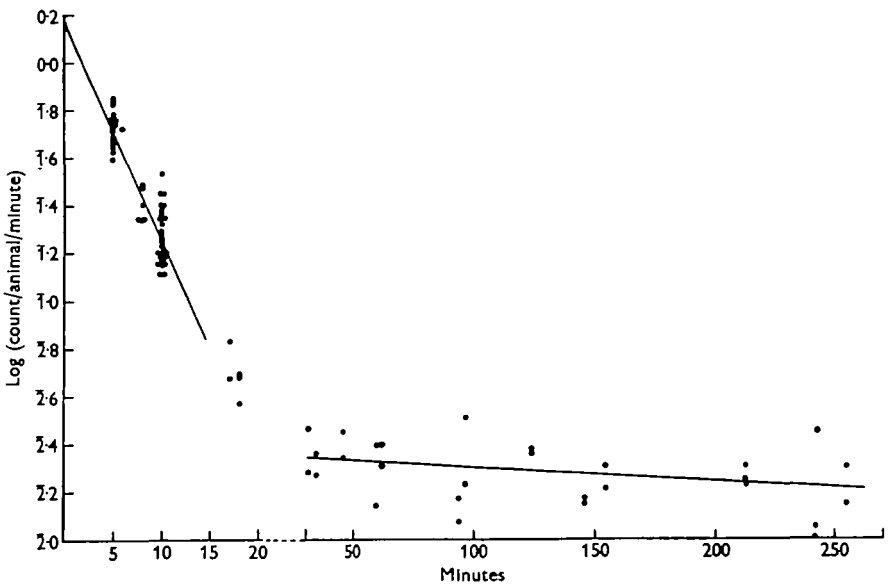

Fig. 4. The rate of loss of ^{22}Na from *Spirostomum*.

Experiments with ^{22}Na

The uptake of sodium and measurements in K_{in} *and* K_{out}

A preliminary experiment with labelled sodium indicated that the animals reached a steady state with the ion in a matter of minutes and that the apparent internal concentration was very low. Therefore the rate of loss of sodium was

measured from animals which were in a steady state with the labelled ion. The logarithms of the activities ($C_{in(t)}$) measured during Exp. 16 are plotted in Fig. 4 against time in unlabelled medium. It is clear from the figure that the washing out process may be divided into two components: a fast component which is calculated to account for 98% of the sodium in the animal and a slow component which for practical purposes may be disregarded. The animal is considered to behave in the main as a one-compartment system to sodium. The concentration of sodium in the animals shown in Table 3 was calculated from the activity in the animals at zero time which was found from the regression of log C_{in} against time, only the first 10 min. being considered. A plot of this data according to equation (4) is equivalent to altering the scale of the ordinate so that log $C_{in(t=0)}$ is zero. The slope of the regression line is equal to $-K_{out}/2{\cdot}303$.

Table 3

All experiments carried out at 18° C.

Exp.	External sodium concentration (mM/l.)	Mean volume ± S.E. of mean ($mm^3 \times 10^{-4}$)	K_{out} ($hours^{-1}$)	C_{in_0} (mM/l.)	K_{in} ($hours^{-1}$)
16	2·0	79 ± 3·8	13·7	1·05	7·2
17	1·0	59 ± 2·2	12·2	1·02	12·4
	2·0	59 ± 2·7	14·0	1·57	11·1
	4·0	66 ± 3·0	14·6	1·62	5·9
18	1·0	62 ± 2·9	15·6	0·83	12·9
	6·0	67 ± 3·5	12·4	0·91	1·9

Two further experiments, 17 and 18, were carried out to determine the values of C_{in} and K_{out} for animals kept in media with different sodium concentrations. Fortunately, the volumes of the animals were not significantly different in the two experiments, so that the transfer constants listed in Table 3 may be compared. There is no correlation between the concentration of the medium and the values of K_{out}, but the values of K_{in}, calculated from equation (2), decrease with increasing C_{out}. Allowing for the variability of the material and experimental error it is considered that C_{in} and K_{out} are constant when C_{out} is varied from 1 to 6 mM/l. The significance of constant values for C_{in} and K_{out}, but a variable K_{in} when C_{out} is altered, will be discussed later.

Experiments with ^{22}Na *and* ^{82}Br

The concentration of sodium and bromide in the animals

Before discussing the significance of the concentration of cations in the animals it is necessary to know the concentrations of the anions. Chloride ions are likely to be the most concentrated inorganic anions in the animal, but it was impracticable to use a radioactive isotope of chlorine for their measurement. As an alternative

^{82}Br was used. Preliminary experiments showed that the animals would live in a culture medium where the sodium and potassium chlorides were replaced by bromides.

The uptake of labelled bromide is illustrated in Fig. 5. The scatter of results at the beginning of the experiment is attributed to contamination of the ^{82}Br by ^{80}Br. The concentration of bromide in the animal is calculated as 0·34 mM/l., whilst in the medium it was 2·5 mM/l. It is clear that the animals maintain a lower bromide concentration in their bodies than is found in the medium.

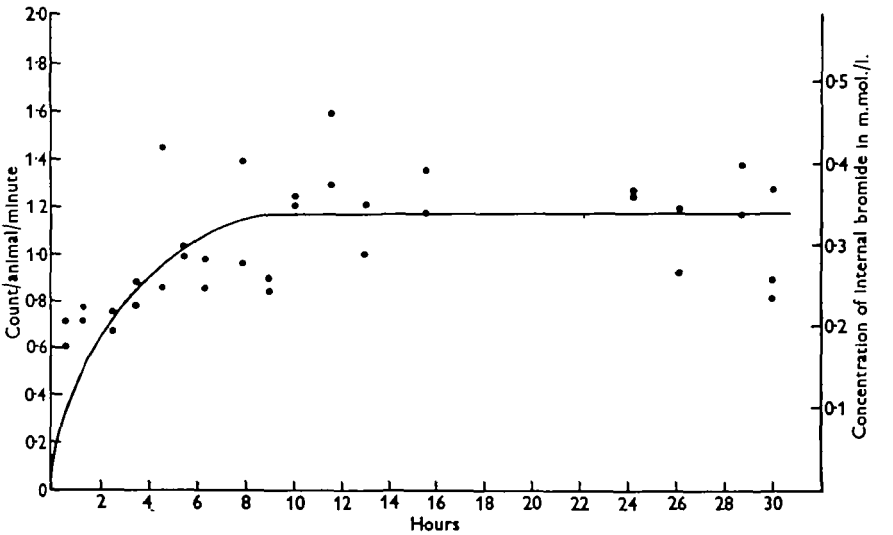

Fig. 5. The uptake of ^{82}Br by *Spirostomum* from culture medium containing 2·5 mM bromide.

Table 4

External concentration		Mean volume ± s.e. of mean ($mm^3 \times 10^{-4}$)	Internal concentration		
Sodium (mM/l.)	Bromide (mM/l.)		Sodium (mM/l.)	Bromide (mM/l.)	Halide (mM/l.)
1·19	1·69	53 ± 1·9	1·08	0·30	0·58
2·54	3·04	66 ± 3·5	1·01	0·41	0·58
4·09	4·59	55 ± 3·0	1·49	0·47	0·63

Animals were transferred from culture media to similar but labelled culture solutions containing labelled sodium and labelled bromide at three different concentrations. After 12 hr. samples were taken and washed for periods ranging from 5 min. to 4 hr. and they were assayed for radioactivity before and after the bromide had decayed. It is calculated from the regression of the bromide activity against time that animals in a medium containing 3·0 mM/l. bromide lose 4·3% of the labelled ion during the washing period of 5 min.

The calculated concentrations of sodium and bromide in the animals are summarized in Table 4. On the assumption (to be discussed later) that the proportion

of bromide to total halide is the same inside as outside the animal, the concentrations of total halide in the animals have been calculated and are shown in the last column of Table 4. Once again there is no correlation between the sodium concentrations in the animal and those in the medium and the values are similar to those of previous experiments.

Table 5. *The concentrations of inorganic ions in* Spirostomum ambiguum *compared with the medium. All concentrations are expressed as milli-osmoles/litre*

Ion	Medium	Cell	Medium	Cell	Medium	Cell
Na	1·19	1·08	2·54	1·01	4·09	1·49
K	0·65	7·0	0·65	7·0	0·65	7·0
Mg	0·2	?	0·2	?	0·2	?
Ca	0·5	?	0·5	?	0·5	?
Total cations measured	2·54	8·08	3·89	8·01	5·44	8·49
Br	1·69	0·30	3·04	0·41	4·59	0·47
Cl	1·4	0·28	1·4	0·17	1·4	0·16
PO_4	0·1	?	0·1	?	0·1	?
Other anions	0·05	??	0·05	??	0·05	??
Total anions measured	3·24	0·58	4·59	0·58	6·14	0·63
Total ions measured	5·78	8·66	8·48	8·58	11·58	9·12

DISCUSSION

The mean gross volume has been used in calculating the concentrations of ions in the animals as no accurate method is readily available for measuring the water content of *Spirostomum*. Grobicka & Wasilewska (1925) estimated that *Paramecium caudatum* has a water content of 89%, whilst Iida (1940) estimated it as 79%. By analogy it is expected that *Spirostomum* would have a high water content, and this is borne out by a series of histochemical tests which failed to find any appreciable quantities of fat or glycogen present in the experimental animals. It is suggested that the body water is at least 75% of the body volume, so that the calculated ionic concentrations may be subject to a correction not exceeding 33% to allow for the non-aqueous content of the animals.

Even allowing for this correction it is clear that the concentration of bromide is much lower in the cell than in the medium. Experiments with other animals suggest that cells do not discriminate between bromide and chloride ions (e.g. Koch (1938), Krogh (1938), Frey (1937)). Therefore it is assumed that when *Spirostomum* is in a steady state with the labelled ion the concentration of bromide may be used to calculate the total halide content of the cells, but the rate of uptake of bromide is not a measure of the rate of entry of halide.

In table 5 the concentrations of inorganic ions inside the animal are compared with the corresponding concentrations in the media. It may be seen that anions other than halide must be present inside the cells to balance the charges of cations. Conway (1945) suggested that an anion deficit in frog's muscle may be made up by

low molecular weight compounds suc has creatine phosphate, carnosine and adenosine triphosphate. If similar compounds make up the deficit in *Spirostomum* then it becomes clear that the animals are hypertonic to the medium.

Spirostomum is permeable to potassium ions, and the body concentration which is largely independent of the outside concentration is at least ten times as high as the concentration in the media. The concentration of sodium is much lower than that of potassium, and in media of higher sodium concentration this ion is kept out against the concentration gradient. In common with studies of the distribution of ions in other tissues there is no evidence to show that ion binding is responsible for this unequal distribution of sodium and potassium ions. If, for the purpose of discussion, it is assumed that *Spirostomum* is virtually impermeable to sodium owing to active transport directed outwards, then the high potassium concentration of the cell might be explained by a Donnan equilibrium as proposed by Boyle & Conway (1941) for frog muscle. If the cell is impermeable to sodium, potassium must enter to balance the charges of the indiffusible anions. The conditions are such that

$$\frac{\text{Potassium inside}}{\text{Potassium outside}} = \frac{\text{Halide outside}}{\text{Halide inside}}.$$

The ratios of the concentrations of ions in the cells, calculated from data taken from Table 4, are tabulated below:

$\frac{\text{Potassium inside}}{\text{Potassium outside}}$	10·8	10·8	10·8
$\frac{\text{Halide outside}}{\text{Halide inside}}$	5·3	7·6	9·5

It appears that a Donnan equilibrium might account for the high concentration of potassium and low concentration of halide in the cell if due allowance is made for experimental error and the variability of the animals.

Without a knowledge of the electrical potential gradient across the cell membrane of *Spirostomum* it cannot be conclusively proved that active transport of any ion is taking place, since the only certain criterion, defined by Rosenberg (1948), is that the ion in question is transported against the electrochemical gradient. There is no direct evidence to show that sodium is actively transported, but it does seem likely since otherwise it would be difficult to account for the low content of the cell when the external concentration is high. The fact that the exchange of sodium is much more rapid than that of potassium points to sodium passing through the cell membrane as an undissociated complex, since in the ionic state sodium diffuses more slowly than potassium.

The concentration of sodium in the cell is in some circumstances the same as the medium. Therefore osmotic equality between cell and medium cannot be obtained by opposing concentration gradients of sodium and potassium. However, osmotic influx of water into *Spirostomum* could be counterbalanced by the contractile vacuole pumping out water. Where the concentration of sodium in the medium

is high there are opposing concentration gradients so that the osmotic entry of water into the animal will be slowed down.

The values of K_{out} and C_{in} for potassium ions (Table 2) remain relatively constant when C_{out} varies greatly, and as an approximation it may be said that K_{in} varies inversely with C_{out}. K_{in} is a measure of the proportion of the external potassium concentration entering the animal in unit time, so it appears at first sight that an important part of the regulation of potassium is the result of the animals taking it up at the constant rate in spite of changes of the concentration in the medium. Similarly, in the case of sodium ions, C_{in} and K_{out} remain relatively constant when the concentration in the medium is altered (Table 3). The apparent movement of these ions independent of their concentration gradients could readily be explained by a system of exchange diffusion similar to that proposed by Ussing (1947) to account for the rapid sodium exchange in frog muscle. However, exchange diffusion cannot bring about changes of the total amount of any ion in the cell, and since the animals grow and multiply they must take up sodium and potassium. It is unlikely that the concentration of ions could be maintained constant indefinitely without the intervention of some mechanism other than exchange diffusion.

SUMMARY

1. The concentrations of potassium, sodium and bromide in the ciliate *Spirostomum ambiguum* have been measured by equilibration with radioactive media.

2. The potassium concentration in the animal is at least ten times greater than the concentration in the experimental media.

3. At the higher concentrations of the experimental media, sodium is kept out of the animal against the concentration gradient. The rate of uptake and loss of sodium is largely independent of the external concentration.

4. The time of half exchange of cell sodium is approximately 3 min., whereas for potassium it is between 2 and 3 hr.

5. After equilibration of the animals in culture medium containing bromide the cell concentration of bromide is found to be lower than the external concentration.

6. The effects of temperature changes on the uptake of potassium have been investigated. The size of the animal decreases with increased temperature but the internal potassium concentration remains fairly constant.

7. Feeding the animals increases the rate of uptake and the internal concentration of potassium.

8. It is concluded that the cytoplasm of *Spirostomum* is hypertonic to the medium.

9. The experimental results for cation exchange might largely be explained by exchange diffusion, but actively regulating processes seem necessary to account for the maintenance of constant internal concentrations when the animals grow and multiply.

10. It is suggested that potassium is concentrated in the cell in accordance with a Donnan equilibrium arising from the presence of indiffusible anions in the cell and an active process which keeps out sodium.

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