THE PHYSIOLOGY OF CONTRACTILE VACUOLES

III. THE WATER BALANCE OF FRESH-WATER PERITRICHA

By J. A. KITCHING

From the Departments of Zoology of Birkbeck College, London,
and of the University, Edinburgh

(With Three Text-figures)

INTRODUCTION

STRONG evidence has been brought forward (Kitching, 1934, 1936 a) to show that in euryhaline marine peritrich ciliates the contractile vacuole prevents excessive swelling of the body when the organism is placed in dilute sea water. From this it has been inferred (Kitching, 1936 a, p. 24; and 1937) that in fresh-water Protozoa the contractile vacuole normally controls the body volume. In order to test this inference experiments have now been carried out on fresh-water Peritricha. The effects of sulphide and cyanide on fresh-water Peritricha have been investigated for comparison with results already obtained on marine Peritricha. It was found that cyanide could for a short time completely inhibit the actions of the contractile vacuoles of fresh-water Peritricha; therefore experiments were also carried out with solutions of cyanide and sucrose together, with a view to discovering the magnitude of the difference of osmotic pressure between the two sides of the surface membrane of the organism.

MATERIAL AND METHODS

Fresh-water Peritricha proved to be difficult material for experiment. Most of the species examined either were too opaque for continuous observation of the contractile vacuole, or were not sufficiently regular in shape for measurement of the body volume, or failed to remain in the same optical plane. Eventually I obtained on the legs of *Asellus aquaticus* a suitable organism apparently of the genus *Zoothamnium*, and somewhat similar to (although by no means identical with) *Zoothamnium affine* Stein (see Kent, 1880–1). Solitary individuals were used in these experiments. A few experiments were carried out on *Rhabdostyla brevipes* C. & L. and *Vorticella nebulifera* Ehr.

The organisms were subjected (1) to London tap water, (2) to a solution of sulphide, or cyanide, or sucrose, or sucrose and cyanide, in London tap water,

* Kindly collected for me from the Wanstead Park ponds, London, by Mr C. Styles.

and (3) to London tap water again. The effects on the contractile vacuole, body volume, and general activity of the organism were observed.

The methods used were in all respects similar to those already described (Kitching, 1934, 1936 a). Sulphide and cyanide solutions as before were made up with sodium sulphide and sodium cyanide, and were brought to the same pH as London tap water. The experiments were carried out in a cellar in which the temperature fluctuated between 14·5 and 16·0° C. During any one experiment the temperature range was much less than this.

RESULTS

Experiments with sulphide

(1) In London tap water the rate of vacuolar output and the ultimate (or maximal) diameter of the contractile vacuole remained approximately constant, although there were small fluctuations.
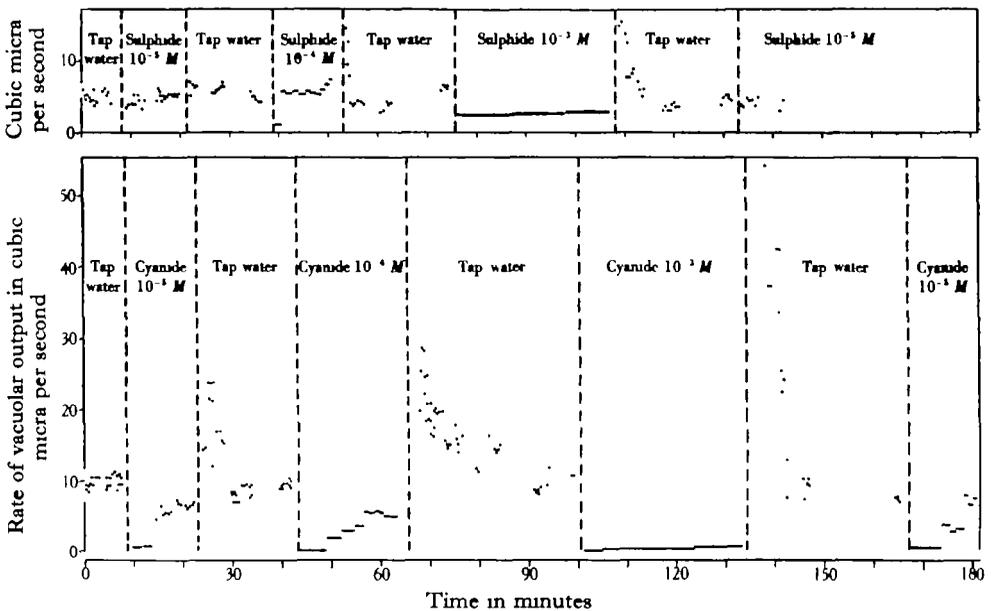

Fig. 1. The effects of various concentrations of sulphide (above) and cyanide (below) on the rate of output of the contractile vacuole of *Zoothammum* sp. from fresh water.

(2) In solutions of sulphide of strength not less than $M/1000$ the rate of output was diminished; sometimes it was checked very considerably at first but recovered a little; in either case it finally attained a steady value which was lower than the original value in ordinary tap water. The ultimate diameter was greater and the duration of each vacuolar cycle longer than in (1).

(3) On return of the organism to tap water there was at first a rapid rise in the rate of output to a value considerably above the original (i.e. in (1)), but later the

rate of output and the ultimate diameter dropped approximately to their original values.

Solutions of sulphide of less concentration than $M/1000$ had little effect on the rate of vacuolar output, except that sometimes they produced a short temporary check; but the ultimate diameter and the duration of the vacuole were usually considerably increased. The effects of sulphide are shown graphically in Fig. 1. The body volume was not measured in any of these experiments with sulphide, but it appeared that in the stronger solutions of sulphide there was an increase in body volume which was followed by a slow decrease when the animal was returned to ordinary tap water.
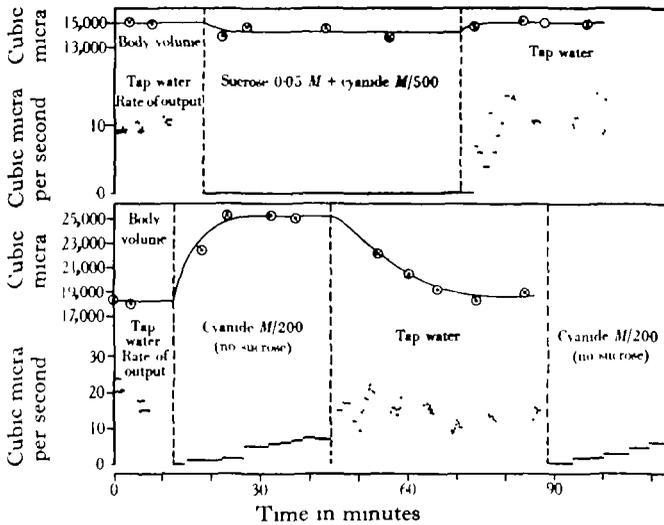

Fig. 2 The effects of 0 05 M sucrose together with cyanide (above) and of cyanide alone (below) on the rate of output of the contractile vacuole and on the body volume of *Zoothammum* sp. from fresh water.

Experiments with cyanide

(1) In London tap water the rate of vacuolar output, the ultimate vacuolar diameter, and the body volume remained constant as before.

(2) Cyanide had marked effects in concentrations as low as $M/100,000$, but the effects were stronger in $M/1000$ solution. Organisms transferred to cyanide solutions (see Figs. 1 and 2) at first showed a great reduction in rate of vacuolar output (to zero in $M/1000$ solution). During this period the body swelled. Then in the course of a short time (up to about 10 min.) the contractile vacuole began to function more rapidly, and assumed a steady rate of output which, however, was still below the original (in (1)). The body volume also became steady at a value considerably above the original. The ultimate diameter of the contractile vacuole was greater than originally.

(3) On return of the organism to tap water without cyanide there was a great increase in the rate of vacuolar output to a value much above the original value

(in (1)). The rate of output and the ultimate diameter then gradually decreased until they attained approximately their original values. At the same time the body volume decreased approximately to its original value.

Experiments with sucrose or with sucrose and cyanide

As before, the rate of vacuolar output and the body volume remained constant while the organism was in tap water.

In 0·05 *M* sucrose solution (without cyanide) made up in London tap water there was a rapid shrinkage of the body, accompanied by a wrinkling of the pellicle,

Table I

External medium	Rate of output in μ^3 per sec.	Body volume in μ^3
Rhabdostyla brevipes		
Tap water	10·4	8,160
Cyanide $M/400$	4·60	10,110
Tap water	9 41	8,390*
Zoothamnium sp.		
Tap water	13·4	12,800
Cyanide $M/400$	4·91–6·91	19,100
Tap water	8 64	13,230
Tap water	17 85	18,130
Cyanide $M/200$	5 09–7 78	25,130
Tap water	13·10	18,550
Cyanide $M/200$	2·81–5·9	—
Tap water	6·70	11,480
Cyanide $M/500$ + sucrose 0 01 M	2·18	15,670
Tap water	6 33	12,590*
Tap water	8 02	8,110
Cyanide $M/500$ + sucrose 0·035 M	0·00	10,440
Tap water	6·58	9,580*
Tap water	5 81	11,660
Cyanide $M/500$ + sucrose 0·035 M	0·019	12,510
Tap water	10·35	21,000
Cyanide $M/500$ + sucrose 0·05 M	0 00	21,800
Tap water	11·53	21,400
Tap water	9 66	15,000
Cyanide $M/500$ + sucrose 0·05 M	0 16	14,300
Tap water	10 77	14,940
Tap water	6 50	12,220
Cyanide $M/500$ + sucrose 0·075 M	0 00	11,500†
Tap water	8·30	13,000
Tap water	7·83	13,080
Sucrose 0·05 M (no cyanide)	0 87	10,650†
Tap water	8·45	14,540
Tap water	12·6	—
Sulphide $M/200$	4·0	—
Tap water	10·4	—
Sulphide $M/5000$	8·2	—
Tap water	11 2	—
Sulphide $M/50,000$	11 5	—
Tap water	12·4	—

    * The body was probably still shrinking.
    † The pellicle became wrinkled, and therefore the value given is probably too large.

until a steady value was reached; and at the same time there was a decrease in the rate of vacuolar output. However, the contractile vacuole continued to function, although at a reduced rate (see Fig. 3). Numerous food vacuoles were formed. On return of the organism to plain tap water there was an increase in body volume and in rate of output to values slightly above the original (tap water) ones.

In solutions of $M/500$ cyanide $+ 0.035\ M$ sucrose there was a slight increase in body volume; the rate of output decreased and remained very low. After return of the organism to tap water the original values were regained.

In solutions of $M/500$ cyanide $+ 0.05\ M$ sucrose there was very little change in body volume, and the rate of output remained at zero or at a very low level. On return of the organism to plain tap water the contractile vacuole resumed its normal
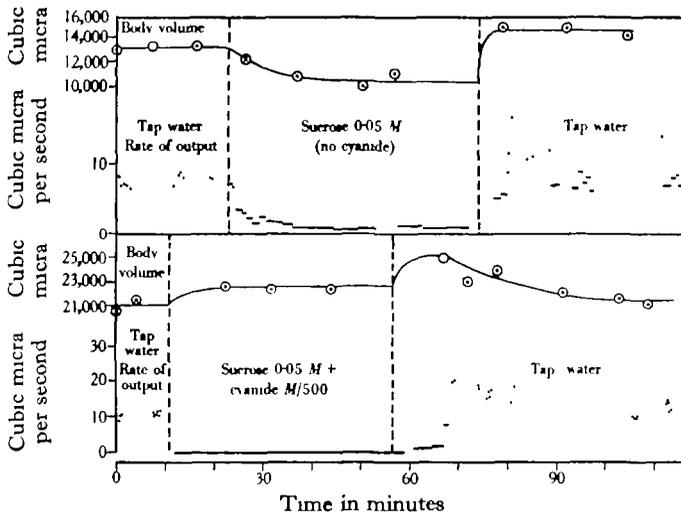

Fig. 3. The effects of 0 05 M sucrose without cyanide (above) and with cyanide (below) on the rate of output of the contractile vacuole and on the body volume of *Zoothamnium* sp. from fresh water. N.B. The values given in the upper graph for the body volume during treatment with 0·05 M sucrose are probably too high, as the pellicle became wrinkled.

rate of output. In one case, however (Fig. 3), the contractile vacuole did not start again for some minutes, and during this time the body swelled visibly. The contractile vacuole then started again and maintained a rate of output much above the original. However, the body volume then decreased, and the rate of output fell to its original level.

In solutions of $M/500$ cyanide $+ 0.075\ M$ sucrose the body shrank and the rate of output fell to zero. The original values were regained when the organism was returned to tap water.

Examples of these experiments are shown graphically in Figs. 1 and 2. In Table I are summarized the results of the more complete experiments; the values given are averages and refer only to those parts of experiments during which steady readings were obtained—the initial unsteady part of each period of treatment being

discarded. In many other experiments changes of body volume were observed qualitatively similar to those described above; but accurate measurement was often impossible owing to changes in the inclination of the organism with regard to its substratum. Such changes were very obvious without the help of a micrometer. During an increase in body volume the neck of the organism under experiment swelled out so as to be entirely obscured, and the outer (unattached) end of the organism, which originally was nearly flat, rose up as a hemispherical dome. Conversely, shrinkage was made obvious by the recovery of a neck and of a flat outer end. In extreme cases of shrinkage the pellicle became wrinkled.

DISCUSSION

The rate of vacuolar output of fresh-water Protozoa is reduced when the external concentration of non-penetrating solutes is raised considerably. Agreement on this matter is general as regards ciliates (Kitching, 1934; Yocom, 1934; Kamada, 1935; Müller, 1936). In the case of amoebae, Adolph (1926) alone has claimed that the contractile vacuole is independent of the external osmotic concentration, but he used comparatively dilute solutions; the results of Zuelzer (1910), Hogue (1923), and Müller (1936) are in opposition to Adolph's conclusions.

The contractile vacuole of Protozoa may continue to function, although at a reduced rate, even after the organism has undergone shrinkage as a result of an increase in the osmotic concentration of the external medium (Kitching, 1934; Kamada, 1936). It has been pointed out (Kitching, 1936b) that after an increase in the external osmotic pressure, the contractile vacuole may raise the internal osmotic pressure above the new external osmotic pressure, so that water is still drawn in through the body surface by osmosis; but such an increase of internal osmotic pressure can only be brought about by a corresponding elimination of internal water and so involve a shrinkage of the body. This conception of the contractile vacuole as an osmo-regulatory pump is vital to the discussion which follows.

As a result of treatment with cyanide, the contractile vacuole of fresh-water Peritricha was temporarily stopped; the body then swelled, and the contractile vacuole started again with a reduced rate of output. On recovery in tap water the contractile vacuole maintained at first an unusually high rate of output, which gradually fell off as the body shrank to its normal size. There is clearly a close correlation between vacuolar activity and body volume, and it may reasonably be concluded that the contractile vacuole normally controls the body volume. However it might be objected that in these experiments the increase in body volume on treatment with cyanide was due, not to a checking of the mechanism for body volume control, but to an increase of internal osmotic pressure consequent on the inhibition of some oxidative process. For instance, if large quantities of glycogen were to be broken down to lactic acid, or some analogous process take place, without the possibility of removal of the end-products, then a swelling of the body might conceivably be observed. However, in marine Peritricha kept in sea water and

treated with cyanide no such swelling was detected (Kitching, 1934). Marine Peritricha treated with cyanide only swelled when they were in dilute sea water— a medium in which the contractile vacuole normally maintains a high rate of output, but in which, owing to the swollen state of the body, the formation of such end-products would have the least effect relative to the body volume. It can only be concluded that this objection is invalid in the case of marine Peritricha, and that it is therefore a most unlikely explanation of the observations recorded for fresh-water Peritricha.

The experiments with sucrose and cyanide were designed to discover the magnitude of the difference of osmotic pressure across the cell surface. Treatment with 0.05 M sucrose alone led to a decrease in body volume and in rate of vacuolar output; the contractile vacuole, however, did not stop. This is in accordance with expectation (see above, p. 147). Treatment with 0.05 M sucrose + cyanide led to a stoppage of the contractile vacuole from which there was little or no recovery while the organism remained in this solution; at the same time the body volume remained practically unaltered. Only when sucrose was added in this particular concentration (see Table I, p. 145) did the body volume continue unchanged. It may be concluded that the external concentration of sucrose—0.05 M—was just sufficient to prevent the swelling which would have taken place if there had been no sucrose in the external solution. The difference of osmotic pressure normally maintained across the body surface is therefore approximately equivalent to that of a 0.05 M sucrose solution. This estimate is dependent on the assumption that cyanide has no influence on the internal osmotic pressure of the organism except in so far as it inhibits the osmo-regulatory mechanism; this possibility has been discussed above. This estimate is not influenced in any way by tension on the body surface, unless the myonemes are caused to relax by the action of cyanide. However, this tension is probably relatively small. The hydrostatic pressure inside *Spirostomum ambiguum* has been estimated by Picken (1936) as 3–7 cm. of water.

The maintenance of the difference of osmotic pressure across the body surface is attributed to the contractile vacuole. It might be suggested that there is the possibility of active osmotic control at the body surface. This view has indeed been supported by Picken (1936) for purposes of a theory of the mechanism of contractile vacuoles which has recently been criticized (Kitching, 1937). Evidence which suggests that possibly the surface membrane may offer active resistance to the osmotic entry of water has been discussed by Schlieper (1930, p. 327). While it is possible that changes in the permeability of the body surface may have an important influence on the water balance of the organism, it appears that the contractile vacuole is more immediately responsible for the control of the water content of the protoplasm.

It is of interest that the contractile vacuole of a fresh-water peritrich which is treated with cyanide recovers slightly while the organism is still in cyanide solution. This recovery only takes place after swelling of the body has occurred, and does not occur if swelling is prevented by the addition of sucrose to the outside medium. It must be pointed out on the one hand that this swelling possibly provides extra

stimulus to vacuolar activity, and on the other that the separation of a given quantity of water from an internal salt solution more dilute than usual would require less than the usual expenditure of energy.

The estimate given above of the difference of osmotic pressure which is normally maintained across the surface membrane makes it possible to calculate very roughly the permeability of this membrane to water. Under normal conditions when the body volume is maintained at a constant value, the rate of entry of water through the surface membrane must be equal to the rate of vacuolar output. The surface area can be estimated (Kitching, 1936 a, p. 12), and the permeability to water expressed in cubic micra per square micron per atmosphere per minute. The results for four experiments are 0·247, 0·149, 0·128 and 0·125. The permeability of the body surface to water may therefore be deemed to lie between 0·125 and 0·25 cubic micra per square micron per atmosphere per minute. This is slightly greater than the value for marine Peritricha (0·05—0·1), but of the same order of magnitude. These estimates are of course very crude, but it is of interest that the fresh-water peritrich has a permeability of body surface greater if anything, and certainly not appreciably less, than that of its marine relative.

The nature of the influence of sulphide and cyanide—the latter in such very low concentrations—on the organism cannot be discussed until experiments on the effects of lack of oxygen have been carried out. Although *Paramecium* (Lund, 1918) is known to be remarkably insensitive to cyanide as regards its respiration, this does not necessarily apply to all Protozoa.

SUMMARY

1. The rate of output of fluid from the contractile vacuoles of fresh-water peritrich ciliates was reduced in solutions of sulphide of concentration not less than $M/1000$. This effect was reversible.

2. Cyanide depressed the rate of vacuolar output in a concentration of $M/100,000$, but was more effective in higher concentrations ($M/1000$). In cyanide solutions there was (a) either a severe initial check or a temporary stoppage of the vacuole, and then later (b) a partial recovery of the rate of output to a value which was still much below the normal. During the initial check the body swelled.

3. Return of the organism from cyanide solution to tap water was followed by a sharp increase in the rate of vacuolar output to an abnormally high value. The body then shrank gradually to its normal size, and the rate of output also fell slowly to normal.

4. The swelling of the body which followed treatment of the organism with cyanide could be exactly counteracted by the addition of sucrose to the outside medium in a 0·05 M concentration.

5. It is concluded that the normal difference of osmotic pressure across the body surface is equivalent to that of a 0·05 M sucrose solution, and that this difference is maintained by the contractile vacuole.

6. The permeability of the body surface to water is estimated as 0·125–0·25 ubic micra per square micron per atmosphere per minute.

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