Physiology of the contractile vacuole in ciliates[1]).

1. The effect of osmotic pressure.

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With 5 figures in the text.

Introduction.

The contractile vacuole in Protozoa, according to HARTOG (1888) is an organelle for regulating the osmotic pressure within the cell. EISENBERG (1925, 1929) showed that the rate of contraction of the vacuole is a function of the osmotic pressure, but neither the temperature nor the hydrogen ion concentration were controlled in her experiments.

This study presents observations of the contractile vacuole of four species of *Paramecium* (*P. caudatum*, *P. aurelia*, *P. multimicronucleata* and *P. polycaryum*) and *Blepharisma undulans* as affected by solutions of different osmotic pressure. The investigation was undertaken to determine the effect of osmotic pressure on the vacuole in different species of *Paramecium* and the effect of osmotic pressure on morphologically different types of contractile vacuoles, as represented in *Paramecium* and *Blepharisma*. Later papers in this series will deal with the effect of hydrogen ion concentration, temperature and heavy water on the vacuole.

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Material and Methods.

All the cultures used in this investigation were derived from Professor Woodruff's stock cultures except *P. caudatum* which was isolated from a wild culture. As Cole (1924) points out, there is a considerable variation in the rate of contraction for different individuals of the same species and there are also differences between the anterior and posterior vacuole of the same animal. Therefore, in order to obviate this difficulty, all of the data on *Paramecium* in this paper were obtained from animals closely related by descent and only from the anterior vacuole.

In order to control the osmotic pressure accurately, it is necessary to find a chemical medium in which the organisms will live normally at least for 12 hours. The following solution meets the purpose for both *Paramecium* and *Blepharisma*:

KNO_3	1.5 gram
K_2HPO_4	0.18 „
$MgSO_4 \cdot 7H_2O$	0.06 „
$FeCl_3$	0.003 „
H_2O	1000 cc.

Different dilutions were made from this solution in order to get solutions of different osmotic pressure. The osmotic pressure of the solutions was obtained by determining the lowering of the freezing point (by the method described by Johlin (1929)). The hydrogen ion concentration was determined by La Motte's H-ion roulette comparator.

The organisms from the mass cultures were repeatedly washed several times in redistilled water before being introduced into the experimental solutions. Study of the contractile vacuole was made after two hours or more in the experimental solutions. The hanging drop method was used in observing and recording the rate of contraction.

The rate of contraction of the vacuole was recorded by a stop watch. Three or more readings of three successive complete systoles were taken for each vacuole. More than twenty animals were used in each concentration and the average value is taken as the representative one for that particular concentration.

After the completion of the first two series of experiments, in which the temperature was controlled only within two degrees, it appeared that more accurate control is important. In the third series, therefore, the temperature during observation was controlled

by the following method: A large glass dish filled with water was placed on the stage of the compound microscope. The cover glass with the hanging drop containing the animal to be studied was sealed to the depression slide with vaseline. This preparation was placed under water in the glass dish and a water immersion objective was used to study the vacuole. By this method, the temperature during observation did not change more than 0.6° C.

Experimental Results.

The first experiments were made with *P. caudatum* (Tables 1—2) as a basis for comparision with EISENBERG's results with this species. The rate of concentration of the vacuole is modified by the osmotic pressure of the medium and the data are in close agreement with those of EISENBERG when plotted together as in Fig. 1. Three other species of *Paramecium* were also studied and in each case the rate of contraction of the vacuole is decreased by increased osmotic pressure of the solution (Figs. 2, 3 and 4).

Table 1.

Paramecium caudatum.

△ Value	p_H	Temp. C.	Time in secs. for one complete systole	Rate of contrac. per minute
.009	7.2	$21.4 \pm 1°$	7.9	7.60
.026	7.2	„	8.5	7.05
.036	7.4	„	9.0	6.66
.049	7.4	„	10.3	5.83
.066	7.2	„	12.5	4.80
.075	7.4	„	14.0	4.26
.080	7.4	„	16.3	3.68

Table 2.

Paramecium caudatum.

△ Value	p_H	Temp. C.	Time in secs. for one complete systole	Rate of contrac. per minute
.012	7.2	$20.4 \pm .3°$	8.4	7.14
.031	7.2	„	9.3	6.45
.050	7.2	„	11.4	5.26
.062	7.3	„	12.7	4.72
.075	7.2	„	15.7	3.82

It was also found that the rate of the morphologically different vacuole of *Blepharisma undulans* can be controlled by osmotic pressure (Fig. 5, Tables 3—4). There is considerable variation of the vacuole

in organisms from old and new cultures. Accordingly, only organisms from new cultures (5—7 days old) were studied.

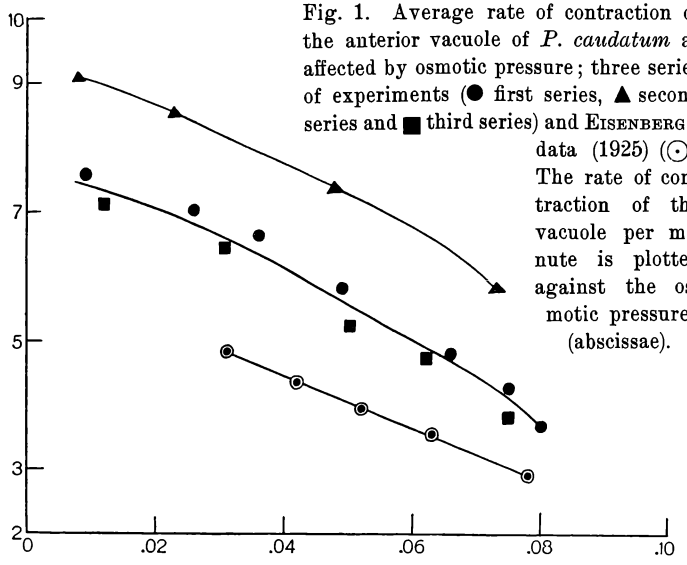

Fig. 1. Average rate of contraction of the anterior vacuole of *P. caudatum* as affected by osmotic pressure; three series of experiments (● first series, ▲ second series and ■ third series) and EISENBERG's data (1925) (⊙). The rate of contraction of the vacuole per minute is plotted against the osmotic pressure (abscissae).

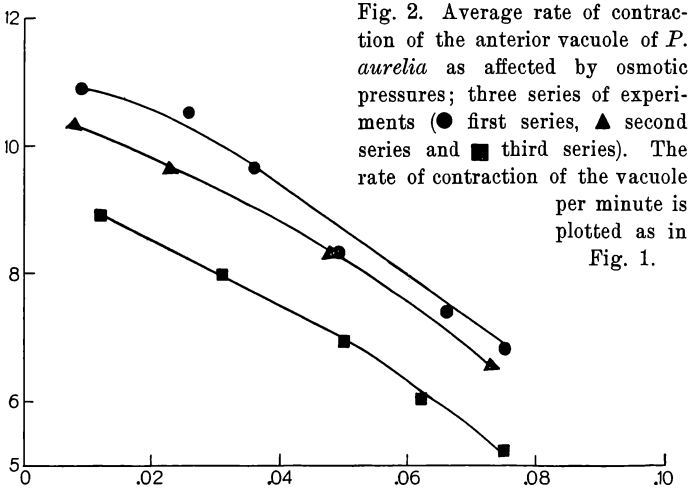

Fig. 2. Average rate of contraction of the anterior vacuole of *P. aurelia* as affected by osmotic pressures; three series of experiments (● first series, ▲ second series and ■ third series). The rate of contraction of the vacuole per minute is plotted as in Fig. 1.

Finally the same results were obtained in the last series of experiments on these forms when the temperature was controlled as outlined above (Figs. 1—5, third servies).

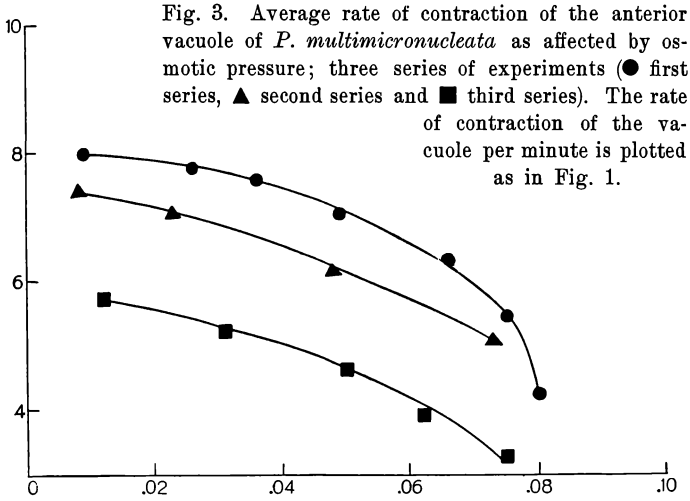

Fig. 3. Average rate of contraction of the anterior vacuole of *P. multimicronucleata* as affected by osmotic pressure; three series of experiments (● first series, ▲ second series and ■ third series). The rate of contraction of the vacuole per minute is plotted as in Fig. 1.

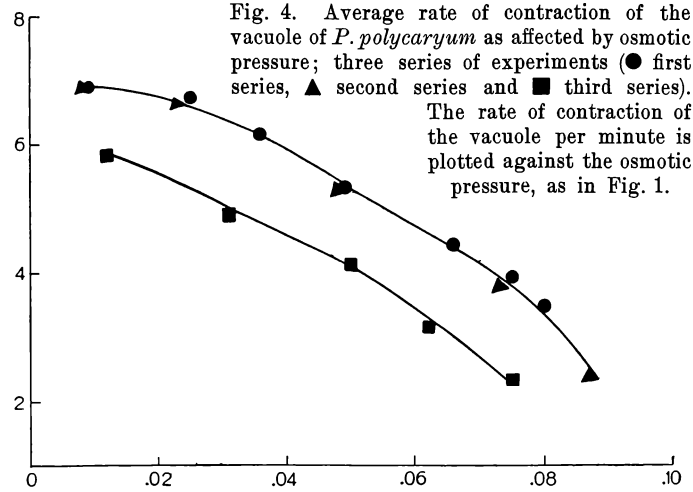

Fig. 4. Average rate of contraction of the vacuole of *P. polycaryum* as affected by osmotic pressure; three series of experiments (● first series, ▲ second series and ■ third series). The rate of contraction of the vacuole per minute is plotted against the osmotic pressure, as in Fig. 1.

Table 3.
Blepharisma undulans.

△ Value	p_H	Temp. C.	Time in secs. for one complete systole	Rate of contrac. per minute
.009	7.2	23.0 ± 1°	46.7	1.284
.026	7.2	„	49.8	1.204
.036	7.4	„	52.0	1.155
.049	7.4	„	57.6	1.041
.066	7.2	„	66.0	.952
.075	7.4	„	70.2	.854
.080	7.4	„	85.1	.705

Table 4.
Blepharisma undulans.

△ Value	pH	Temp. C.	Time in secs. for one complete systole	Rate of contrac. per minute
.012	7.2	20.2 ± 1°	99.6	.602
.031	7.2	"	111.7	.537
.050	7.2	"	142.1	.422
.062	7.3	"	162.1	.370
.075	7.2	"	194.0	.309

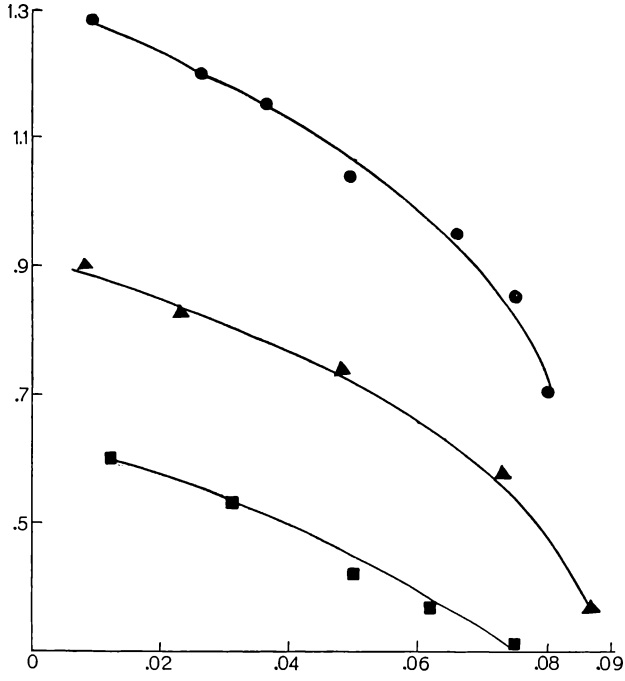

Fig. 5. Average rate of contraction of the vacuole of *Blepharisma undulans* as affected by osmotic pressure; three series of experiments (● first series, ▲ second series and ■ third series). The rate of contraction of the vacuole per minute is plotted as in Fig. 1.

Discussion.

The graphs representing the rate of contraction of the vacuole as influenced by the osmotic pressure, obtained in the present experiments on *P. caudatum*, do not differ considerably from those of Eisenberg (1925). However, I was not able to extend the graphs beyond the △ value 0.1 as Eisenberg did either with my own ex-

perimental solution or with solutions of glucose that she used in her experiments. None of the species of *Paramecium* nor *Blepharisma undulans* are able to live normally in solutions with △ value above 0.1. This is probably due to the fact that the osmotic pressure in the surrounding medium is so high that it causes dehydration, or possible the concentration of potassium is too high. EISENBERG did not state that she controlled the temperature and hydrogen ion concentration. If she did not do so, this might account for the difference of results, as it is a well known fact that temperature has considerable effect on the rate of contraction of the vacuole in ciliates.

The contractile vacuole of all the forms investigated reacted in the same way to changes in osmotic pressure in the medium. It seems that in these organisms, the rate of contraction of the vacuole is influenced by osmotic pressure below △ value 0.1 and further the morphologically different types of contractile vacuoles as represented by those of *Paramecium* and *Blepharisma* are affected very much the same by osmotic pressure.

Although the exact mechanism and function of the contractile vacuole is unknown, this investigation shows definitely that the rate of contraction may be controlled by variation in the osmotic pressure of the medium and provides additional support for HARTOG'S theory. HARTOG maintained that in the interstices of protoplasm, there are substances of high osmotic pressure. Therefore, when the protoplasm is immersed in water, water from outside possess into the protoplasm in order to maintain the osmotic equilibrium. Unless there is a means to eliminate the excess water, the protoplasm may eventually burst or disintegrate. The fact that the majority of marine protozoa do not possess contractile vacuoles is significant from the point of view of our present consideration. It is obvious that sea water has a higher osmotic pressure that fresh water, perhaps not very different from that within the protozoan cell. Hence the water from outside will not pass into the cell as the osmotic relationship outside and inside is evenly balance and consequently there is no need for the contractile vacuole to eliminate excess water. KITCHING (1934) has recently questioned this theory of osmoregulation by the contractile vacuole in certain marine ciliates.

Perhaps the most convincing evidence indicating that the contractile vacuole is an osmoregulator is afforded by the work of LLOYD on Spirogyra (cf SCARTH and LLOYD 1930, p. 38). In all cells where there is a rigid cell wall to oppose the internal pressure

excretion of water is unnecessary but Spirogyra gametes during conjugation are under the same necessity as a naked cell and develop contractile vacuoles. It will be recalled the Just (1930) found that the eggs of Nereis after treatment with hypotonic sea water eliminate water by the formation of vacuoles and he suggested that this may be a general phenomenon in many types of cells.

It is interesting to note that in the experiments with 30 % heavy water, which will be reported in a later paper of this series, the contractile vacuole was pulsating at half the normal rate but no swelling of the animals occurred. It is difficult to explain how the animals can maintain a low rate of contraction without change in osmotic pressure and without great disturbance in water metabolism. These results will be discussed in the later paper but they are mentioned here to indicate that further work with heavy water may require considerable modification of our view considering the contractile vacuole. However, the experiments in ordinary water reported in this paper support the theory that the contractile vacuole functions in osmoregulation.

Summary.

1. The rate of pulsation of the anterior contractile vacuole of four species of *Paramecium* (*P. caudatum*, *P. aurelia*, *P. multimicronucleata* and *P. polycaryum*) has been studied under different osmotic pressures. It was found that the rate of pulsation is decreased by increase in the osmotic pressure of the medium and the effect in general is the same in different species of *Paramecium*.

2. The morphologically different contractile vacuole of *Blepharisma undulans* in influenced by osmotic pressure in the same way as that of *Paramecium*.

3. The experiments indicate the contractile vacuole in ciliates may take part in osmoregulation.

I wish to express my thanks to Dr. T. Cunliffe Barnes for valuable advice. I am also indebted to Prof. L. L. Woodruff and Dr. T. L. Jahn for helpful criticism.

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