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

2. The effect of hydrogen ion concentration.

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With 5 figures in the text.

Introduction.

The literature on the effect of hydrogen ion concentration on the contractile vacuole in Protozoa is meager and contradictory. Thus, STEMPELL (1924) showed that variation of hydrogen ion concentration does not have any effect on the rate of contraction of the vacuole of *Paramecium*. EISENBERG (1929) found in *P. caudatum* and *Balantidium entozoon* that the rate of contraction of the vacuole is influenced by change of hydrogen ion concentration with an optimum around p_H 7.0. These results are apparently conflicting and it appears possible that the difference may be due to the salts used in the medium and furthermore, STEMPELL did not control the omotic pressure. Neither STEMPELL nor EISENBERG controled the temperature in their experiments.

The present work was undertaken, with controlled temperature and osmotic pressure of the medium, to determine the effect of the hydrogen ion concentration on the rate of contraction of the vacuole in four species of *Paramecium* (*P. caudatum*, *P. aurelia*, *P. multimicronucleata* and *P. polycaryum*) and *Blepharisma undulans* and also to compare the effect among different species of *Paramecium* and different types of contractile vacuole as represented in *Paramecium* and *Blepharisma undulans*.

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Material and Methods.

All the cultures used in this work were subcultures from the same stock cultures used in experiments reported in the first paper of this series.

Only the anterior vacuole was studied in *Paramecium* through out the entire work. The same experimental solution was used as in the experiments on osmotic pressure except the amount of KNO_3 was varies in each solution so as to maintain the same osmotic pressure when buffered with standard buffer salts for the desired hydrogen ion concentration. The hydrogen ion concentration of the solution was determined colormetrically by the LaMotte roulette comparator. The osmotic pressure of the solution was determined by the same method as in the preceding investigation. The organisms were washed with redistilled water before being introduced into experimental solutions and studies were made after two hours or more.

The temperature during observations of the vacuole was controlled by the method used in the third series of experiments of the previous paper. The rate of contraction of the vacuole was recorded as usual by a stop watch. Two or more readings of three successive complete systoles were taken for each vacuole. More that fifteen animals were studied in each solution of different hydrogen ion concentration and the average value is taken as the representative one for that particular p_H.

Experimental Results.

In the first series of experiments the solutions were buffered with Sørensen's phosphates over a p_H range of 5.8 to 8.0. It was found that all the forms investigated with the exception of *P. multimicronucleata* were unable to live normally at p_H 5.8. After an hour or less at this p_H reduced movement and finally disintegration were observed. Even in *P. multimicronucleata* at p_H 5.8 there were some abnormal forms but these were not studied.

It will be seen from Figs. 1 to 4 (Tables 1—6) that the rate of contraction of the vacuole is markedly affected by changes in the hydrogen ion concentration. All the species of *Paramecium* showed an optimum p_H within the range investigated although the optima are different in each species; namely, *P. caudatum* and *P. polycaryum* at p_H 7.0, *P. multimicronucleata* at p_H 7.5 and *P. aurelia* at p_H 6.4.

On the other hand, as is shown in Fig. 5, *B. undulans* does not show any optimum within this p_H range as the garph is a straight line. The data for *P. caudatum* are in fair agreement with those

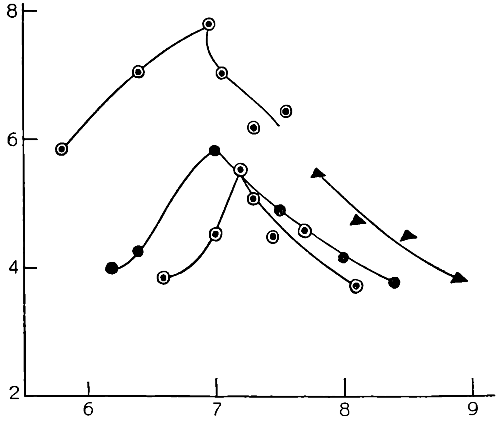

Fig. 1. Average rate of contraction of the anterior vacuole of *P. caudatum* as effected by p_H; two series of experiments (● first series and ▲ second series) and Eisenberg's data (1929) (⊙). The rate of contraction of the vacuole per minute is plotted against the hydrogen ion concentration (abscissae).

of Eisenberg (see Fig. 1). In the second series of experiments the solutions were buffered with borate-hydrochloric acid mixtures over a p_H range of 7.8 to 9.0. It was again evident that the hydrogen ion concentration influences the rate of contraction of the vacuole

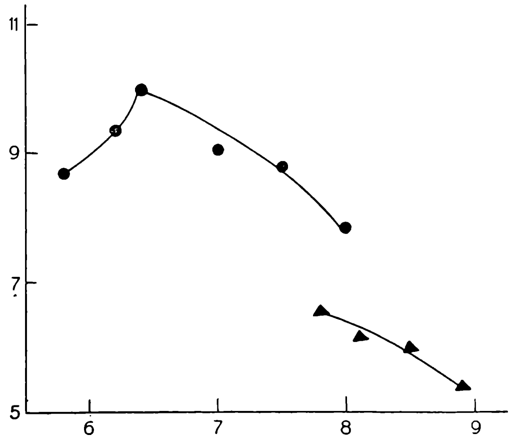

Fig. 2. Average rate of contraction of the anterior vacuole of *P. aurelia* as affected by p_H; two series of experiments (● first series and ▲ second series). The rate of contraction of the vacuole per minute is plotted as in Fig. 1.

(Figs. 1 to 4) but none of the graphs for species of *Paramecium* show optima due to the fact that the p_H range in this series was above 7.5. However, this more alkaline range served to show the optimal p_H for *B. undulans* at 8.5 (Fig. 5).

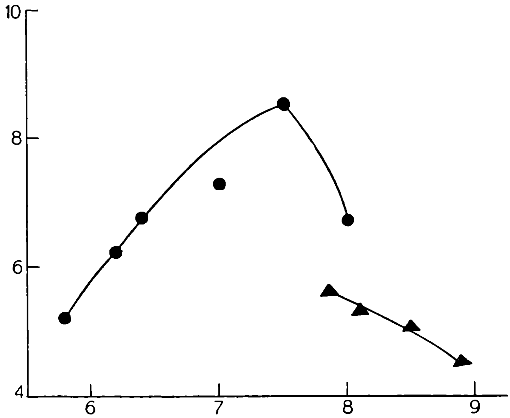

Fig. 3. Average rate of contraction of the anterior vacuole of *P. multimicronucleata* as affected by different p_H; two series of experiments (● first series and ▲ second series). The rate of contraction of the vacuole per minute is plotted as in Fig. 1.

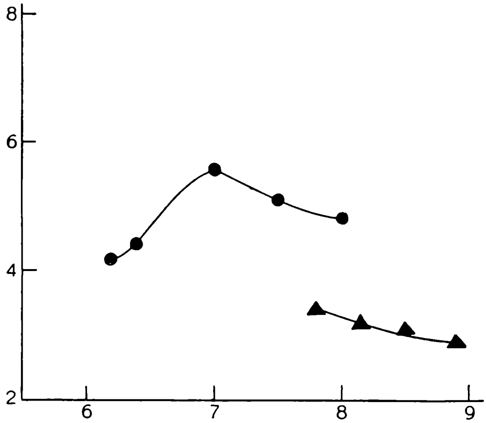

Fig. 4. Average rate of contraction of the anterior vacuole of *P. polycaryum* as affected by different p_H; two series of experiments (● first series and ▲ second series). The rate of contraction of the vacuole per minute is plotted as in Fig. 1.

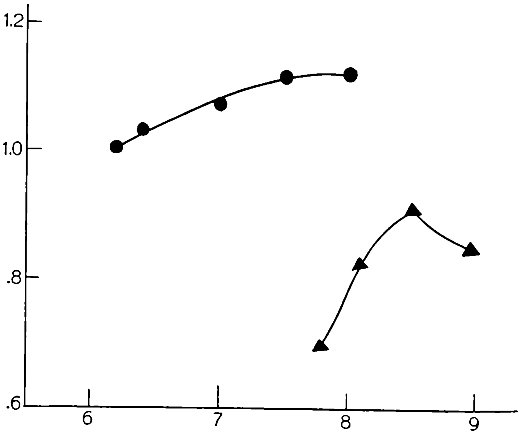

Fig. 5. Average rate of contraction of the vacuole of *Blepharisma undulans* as affected by different p_H; two series of experiments (● first series and ▲ second series). The rate of contraction of the vacuole per minute is plotted as in Fig. 1.

Table 1.

Paramecium caudatum.

pH	△ Value	Temp. C.	Time in secs. for one complete systole	Rate of contrac. per minute
8.0	.012	21.5 ± .3°	14.3	4.19
7.5	.012	"	12.2	4.91
7.0	.012	"	10.3	5.82
6.4	.011	"	14.0	4.28
6.2	.012	"	15.0	4.00
5.8	.013	"	*died within an hour	

Table 2.

Paramecium caudatum.

pH	△ Value	Temp. C.	Time in secs. for one complete systole	Rate of contrac. per minute
8.0	.012	22.2 ± .3°	7.6	7.89
7.5	.012	"	6.8	8.82
7.0	.012	"	6.5	9.08
6.4	.011	"	6.0	10.00
6.2	.012	"	6.4	9.37
5.8	.013	"	6.9	8.70

Table 3.

Paramecium multimicronucleata.

pH	△ Value	Temp. C.	Time in secs. for one complete systole	Rate of contrac. per minute
8.0	.012	22.1 ± .3°	8.9	6.74
7.5	.012	"	7.0	8.57
7.0	.012	"	8.2	7.31
6.4	.011	"	8.7	6.89
6.2	.012	"	9.6	6.26
5.8	.013	"	11.5	5.21

Table 4.

Paramecium polycaryum.

pH	△ Value	Temp. C.	Time in secs. for one complete systole	Rate of contrac. per minute
8.0	.012	22.3 ± .3°	12.4	4.83
7.5	.012	"	11.7	5.12
7.0	.012	"	10.7	5.60
6.4	.011	"	13.4	4.47
6.2	.012	"	14.2	4.21
5.8	.013	"	*died within an hour	

Table 5.
Blepharisma undulans.

p_H	△ Value	Temp. C.	Time in secs. for one complete systole	Rate of contrac. per minute
8.0	.012	21.0 ± .3°	52.9	1.134
7.5	.012	"	53.6	1.119
7.0	.012	"	55.8	1.076
6.4	.011	"	57.9	1.035
6.2	.012	"	59.4	1.009
5.8	.013	"	*died within an hour	

Table 6.
Blepharisma undulans.

p_H	△ Value	Temp. C.	Time in secs. for one complete systole	Rate of contrac. per minute
7.8	.015	20.9 ± .3°	85.7	.700
8.1	.015	"	72.3	.829
8.5	·014	"	65.8	.911
8.9	·015	"	70.3	.853

Discussion.

Recent developments in the theory of the molecular structure of water have thrown new light on the nature of the hydrogen ion in aqueous solutions, in fact, it is now known that free hydrogen ions do not exist in biological solutions or in living matter. The hydrogen ion being a free proton, forms part of a water molecule the oxonium ion OH_3 (cf. Barnes and Jahn 1934 p. 305). The hydrogen nucleus which may be taken as the hydrogen ion at any moment is constantly changing and the water molecules affected are then in an unstable or loosened condition particularly favourable for participation in hydrolytic reactions. Thus, the catalytic effect of the hydrogen ion on hydrolysis in general receives a new interpretation. Now the action of the hydrogen ion in hydrolysis is indicated by a temperature characteristic or energy of activation of 20,000 calories or slightly above and it is interesting to note that these values were obtained for the rate of pulsation of the contractile vacuole (as will be discussed in the third paper of this series). It is not surprising therefore, that the rate of contraction of the vacuole is markedly influenced by a change in p_H.

Aside from its action in influencing hydrolysis which may affect the vacuole, the hydrogen ion may modify the peripheral protoplasm which may change the permeability to water. It is interesting to note that the p_H optima for the vacuole in each species are near the optimal p_H for growth in these species which perhaps indicates a rather indirect mode of action of p_H on the vacuole.

Summary.

1. The rate of contraction of the anterior contractile vacuole of four species of *Paramecium* (*P. caudatum*, *P. aurelia*, *P. multimicronucleata* and *P. polycaryum*) has been studied over a p_H range of from 5.8 to 8.9. It was found that the rate of contraction varies with the p_H, with the occurrence of an optimum around the neutral point, p_H 7.0.

2. The rate of contraction of the contractile vacuole of *Blepharisma undulans* has been studied over a p_H range of 5.8 to 8.9). It was found that the rate of contraction varies with the p_H. There is an optimum around p_H 8.5.

3. The action of the hydrogen ion in influencing the contractile vacuole is discussed.

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