

Physiology of the contractile vacuole in ciliates¹⁾.

3. The effect of temperature.

By

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With 6 figures in the text.

Introduction.

It has long been known that the rate of pulsation of the contractile vacuole in Protozoa increases as the temperature rises. KANITZ (1907), KHAISKY (1911), and COLE (1924) showed this relationship in *Paramecium caudatum* and other Infusoria. COLE, using the ARRHENIUS equation, obtained μ values of 2,600, 18,600 and 25,600 calories for the temperature ranges of 22—31°, 16—22°, and 9—16° C. respectively. This indicates the presence of a catenary set of three reactions which were affected differently by temperature change.

This paper contains observations on the contractile vacuole of four species of *Paramecium* (*T. caudatum*, *P. aurelia*, *P. multimicro-nucleata* and *P. polycaryum*) and of *Blepharisma undulans* and *Spirostomum ambiguum* as affected by temperature. The investigation was undertaken to determine the effect of temperature on the vacuoles of different species of *Paramecium*, and on morphologically different types of contractile vacuoles as are represented in *Paramecium*, *Blepharisma* and *Spirostomum*.

¹⁾ Part of a dissertation submitted to the Faculty of the Graduate School of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Material and Methods.

All the cultures were subcultures from the stock cultures which were used in the experiments reported in previous papers of this series. All of the data on *Paramecium* in this work were obtained only from the anterior vacuole and the same individual was used at different temperatures. Although the actual rate of contraction varies in different individuals taken from the same culture, the temperature characteristics are constant for the species (except in the case of *Blepharisma undulans* noted below). The hanging drop method was used and the general procedure was the same as previously reported.

The preparation was placed in a micro-incubator covered with a piece of thin mica which was fitted around the microscope objective with a rubber diaphragm and held tightly in place with rubber bands. The microscope was placed in a wooden box with glass windows and the micro-incubator was not connected to the source of electricity. Instead, two electric lights of different sizes were used inside the box. All the experiments were performed in a room with a temperature of about 10.5° C. By turning on first the smaller, then the larger, and then both of the light bulbs, it was possible to raise the temperature very slowly and to make several readings for the same or at slightly different temperatures. The thermometer was placed in the microincubator and was carefully shielded from radiant heat from the light bulbs. The fact that some reversible curves may be superimposed (*Blepharisma* series) shows that the thermometer readings indicated the temperature of the drop of medium quite accurately. The rates of pulsation recorded for *Paramecium* are the means of three to six readings taken at the, same temperature, while those for *Blepharisma undulans* and *Spirostomum ambiguum* are single readings. The prolonged diastole of the latter two organisms (as long as several minutes at low temperatures) prevented the measurement of more than one contraction at each temperature. For this reason, two stop watches were used in some series and every pulsation was recorded as the temperature slowly rose. In all experiments, the first readings were taken after the preparation had been in the micro-incubator an hour or more at room temperature (10.5° C.). The temperature characteristics are calculated from the following form of the ARRHENIUS equation:

$$\mu = \frac{4.606 (\log k_1 - \log k_2) T_1 T_2}{T_1 - T_2}.$$

Experimental Results.

Paramecium caudatum was first investigated in order to compare the results with those obtained by COLE (1924). I was able to repeat COLE's results which indicate the presence of a catenary set of three reactions that were affected differently by temperature change (Fig. 1). The other three species of *Paramecium* (*P. aurelia*, *P. multimicronucleata* and *P. polycaryum*) were also studied over a temperature range of about 16° C. and the results were similar to those obtained with *P. caudatum* (Fig. 2). In the first few experiments, individuals of *Blepharisma undulans* from a mass culture of about three months old were used. There was considerable variation in the rate of pulsation and in the μ values for different individuals (Figs. 3 and 4). Later, it was realized that the age of the culture might have something to do with these variations. Accordingly, organisms from a six-day old culture started from a single animal in fresh hay infusion were used. The results were far more satisfactory as the rate of pulsation was more constant, and the data yielded more or less the

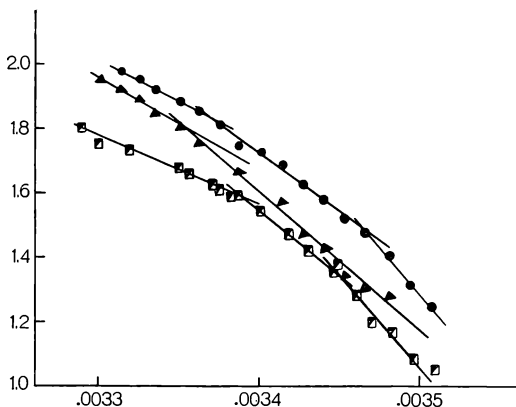


Fig. 1. Average rate of contraction of the anterior vacuole of *P. caudatum* at different temperatures (two series). The data of COLE (1925) are indicated by the points marked \blacksquare . In this and the following figures the log of the rate of contraction of the vacuole is plotted against the reciprocal of the absolute Temperature as required by the ARRHENIUS equation (see text).

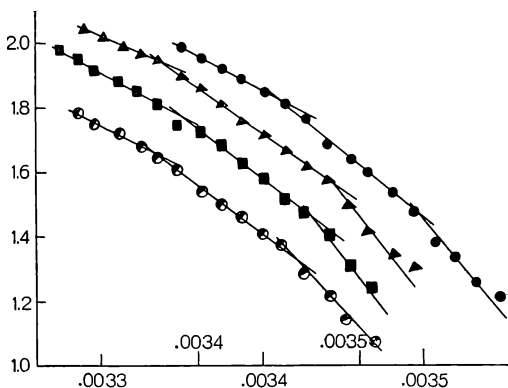


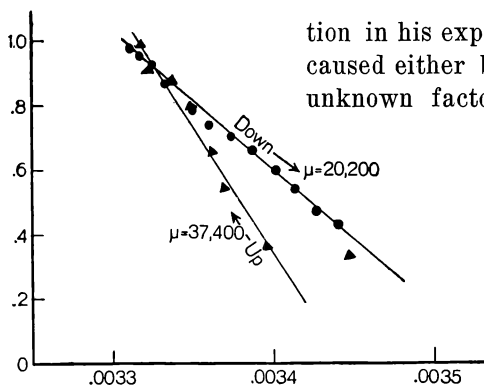
Fig. 2. Average contraction rates of the anterior vacuole of four species of *Paramecium* (*P. polycaryum* \circ , *P. multimicronucleata* \blacktriangle , *P. aurelia* \bullet , and *P. caudatum* \blacksquare) at different temperatures.

same μ values (16,700 calories) for different individuals (Fig. 5). The experiments on the effect of temperature on the vacuole of *Spirostomum ambiguum* were also carried out over a temperature range of about 20° C. It was found that the graph is a straight line indicating a single master reaction with a μ value of about 12,000 calories (Fig. 6). All the μ values are recorded in Table 1.

Discussion.

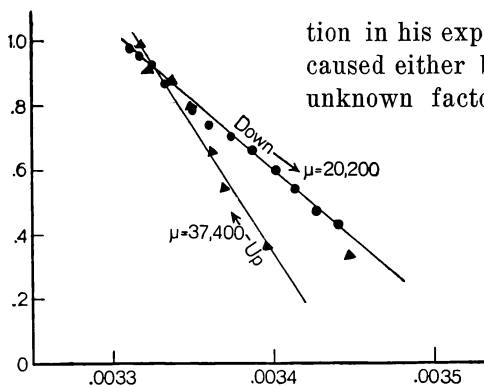
The temperature characteristics obtained in the present experiment on *P. caudatum* agree in general with those of COLE (1924) and KHAINSKY (1911). Since COLE used chloretonized animals in his experiments, the differences in the absolute rate of pulsa-

Fig. 3. Contraction rates of the vacuole of *Blepharisma undulans* at different temperatures (three series from old culture).



tion in his experiments and mine might be caused either by the anesthetic or by other unknown factors in the medium (osmotic pressure, hydrogen ion concentration, etc.). A comparable difference is also observed between the data of COLE and KHAINSKY. The curves, however, when plotted together, are almost parallel to each other below 23° C., and the breaking points practically coincide. Accordingly, the μ values are nearly the

Fig. 4. Contraction rates of the vacuole of *B. undulans* at different temperatures (individuals from old culture). Different graphs were obtained by raising and by lowering the temperature (see text).



same; namely, 25,000, and 25,600 calories below 16° C.; 16,700 and 18,600 calories between 16° and 23°. It is possible that COLE'S data for the line giving a μ value of 18,600 calories might be fitted equally well by a line indicating about 17,000 calories

if it is drawn between the points at 22.75° and 22.25° and at 17° and 17.25° (as shown by the broken line in Fig. 1) instead of passing through the points at 22.75° and 17.25° . However, above 23° the difference between the μ values is conspicuous, the value from my data being 14,500 calories and that from COLE'S data only 8,600 calories. I have no explanation to offer save the fact that chloretonized animals might react differently at higher temperatures. KHAINSKY obtained a higher μ value than COLE above 23° and mine is still higher than KHAINSKY'S. Also, it will be recalled that the critical increment may indicate the master reaction controlling the rate at the time of the particular experiment; and CROZIER has shown that value of μ may change with the age or metabolic condition of the particular organism.

The other three species of *Paramecium*, *P. multimicronucleata*, *P. aurelia* and *P. polycaryum*, all show the same type of graph with practically the same μ values, especially between the temperatures $16.5 \pm 1^{\circ}$ and $22.5 \pm 1^{\circ}$ C. However, as shown in Fig. 2, the breaking point at higher temperatures in *P. multimicronucleata* is 26.8° instead of $22.5 \pm 1^{\circ}$. There is another series with the breaking point at 25° . Other series of this species were not carried as high as these two but none of them shows any sign of breaking around $22.5 \pm 1^{\circ}$. However, the data for *P. multimicronucleata* between 17.7°

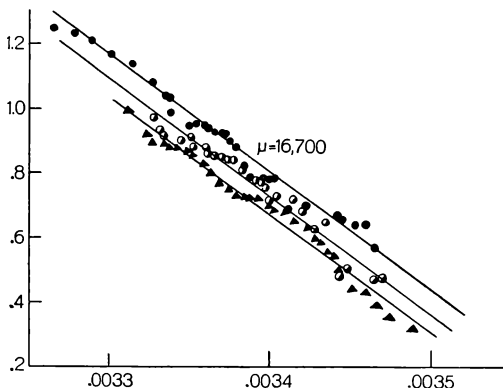


Fig. 5. Contraction rates of the vacuole of *B. undulans* at different temperatures (from new culture, three series).

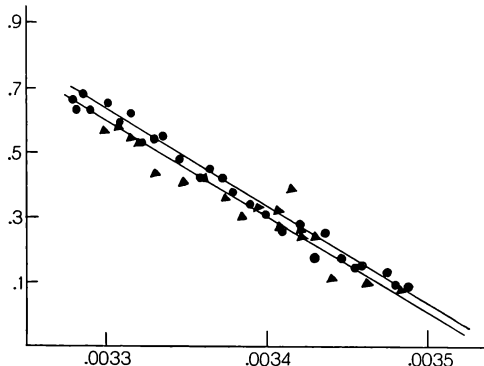


Fig. 6. Contraction rates of the vacuole of *Spirostomum ambiguum* at different temperatures (two series).

Table 1.

Comparison of the effect of temperature on the contractile vacuole of different species of ciliates as shown by the application of the VAN 'T HOFF-ARRHENIUS equation.

Species	Morphological type of contractile vacuole	Temperature characteristics in calories with temperature limits indicated											
		5° C.	10°	15°	20°	25°	30°						
<i>Paramecium caudatum</i>	radial canals			24,900	16,700	14,600							
<i>P. caudatum</i> (COLE)	"			25,600	18,600 (17,000?)	8,600							
<i>P. caudatum</i> (KHAINSKY)	"				18,900	10,200							
<i>P. polycaryum</i>	"			23,300	16,600	12,400							
<i>P. multimicronucleata</i>	"			25,000	16,500		9,500						
<i>P. aurelia</i>	"			25,000	17,000	10,900							
<i>Spirostomum ambiguum</i>	collecting canal with accessory vacuoles				12,000								
<i>Blepharisma undulans</i>	accessory vacuoles				16,700								
<i>Euplotes charon</i> (after CLARK)	"			13,500	5,800								
<i>Stylonychia pustulata</i> (after CLARK)	"			9,000	9,900								
<i>Glaucoma colpidium</i> (after CLARK)	"		29,000	21,000	9,300								

and 27.7° show a μ value of 16,500 calories, which is very close to the value of 16,000 calories found for *P. polycaryum* and 17,000 calories for *P. aurelia*.

These temperature characteristics for the mid-portion of the data for all four species of *Paramecium* (16,500—17,000 calories) are very close to one of those CROZIER (1924) found to be characteristic of oxidative processes (7,900, 11,500 16,500 calories). An oxidation reaction, therefore, might be the process which controls the vacuole pulsation in *Paramecium* in this temperature range. This value of about 16,600 calories, also agrees with one found by McCUTCHEON and LUCKÉ (1932) for the entrance of water into the Arbacia egg (in endosmosis it is 16,600 calories, and in exosmosis 12,000 calories to 16,000 calories). The similarity of these temperature characteristics is strongly suggestive of the possibility that the rate of pulsation of the contractile vacuoles in *Paramecium* might be influenced by permeability to water. With an excess of water in the cell, the rate of pulsation of the contractile vacuole might increase and thus tend to maintain the water content of the cell constant. Such an explanation seems logical if we accept HARTOG's theory (1888) that the contractile vacuole is an osmotic pressure regulator, especially with his assumption that the whole surface membrane is permeable to the water of the surrounding medium. However, EISENBERG (1925) considered that in *P. caudatum*, most of the water enters the body by means of the protoplasm bordering the peristome. Since the author did not give any experimental evidence for this view, this localized permeability is entirely theoretical. Furthermore, according to my own experience in working on various species of *Paramecium*, in staining with neutral red, etc., the stains penetrate almost instantly, and the granules in the cytoplasm are stained at once. Therefore, it does not seem unreasonable to consider that the entire membrane in *Paramecium* is permeable to water. It seems to me that permeability to water controlled by an oxidative reaction might determine the rate of pulsation in *Paramecium* above 16° C.

Temperature characteristics of 20,000 calories and slightly above are usually indicative of hydrolysis, RICE (1923) stating that the hydrogen ion as a catalyst in hydrolytic reactions has an energy of activation of 20,000 calories. For *Paramecium* in the lower temperature range in ordinary water and over the entire range in heavy water (to be described in a later paper) the μ values suggest that hydrolytic processes may determine the rate of contraction of the vacuole. As with the oxidative value this mechanism may act

indirectly through the control of the passage of water through the plasmatic membrane, for McCUTCHEON and LUCKÉ (1932) have shown that the permeability to water of the *Arbacia* eggs has a temperature characteristic of 20,000 calories under certain conditions. As was discussed in the preceding paper the modification of the rate of pulsation of the vacuole by changes in the p_H of the medium may be brought about by the action of the hydrogen ion on hydrolysis.

DEGEN (1905) reported the effect of temperature on the rate of pulsation of the contractile vacuole in *Glaucoma colpidium* over a range of 26° (6° — 32°), and he described *Glaucoma colpidium* as having accessory vacuoles instead of canals as in *Paramecium*. From his data CLARK (1933) calculated the μ values according to the ARRHENIUS equation. The curve for *Glaucoma* shows two breaking points (at 11° and 17°) and, as COLE stated in the case of *P. caudatum*, this indicates a catenary set of three reactions affected differently by temperatures. Since neither the data of COLE for *P. caudatum* nor the data here presented for that and other species are extensive for the temperature range below 11° C., it is impossible to state whether or not these forms (*Paramecium*, *Blepharisma* and *Spirostomum*) have a critical temperature at 11° . However, *Glaucoma* does not have a critical temperature at 23° or at 27° as does *Paramecium*, but the temperature characteristic for the higher temperature range is the same as that for some but not for all species of *Paramecium* (9,300 calories for *Glaucoma* above 17° ; 10,900 calories for *P. aurelia* above 23° ; 9,500 calories for *P. multimicronucleata* above 26.8° ; 8,600, 10,200 and 15,000 calories for *P. caudatum* above 23° in the data of COLE, KHAINSKY and the present work respectively; 12,400 calories for *P. polycaryum* above 23° C.). The temperature characteristic for the 11 — 17° range is different in *Glaucoma* and *Paramecium*, being 21,000 calories in the former and 25,000 calories in all species of the latter. Therefore, the effect of temperature on the vacuole of *Glaucoma* and *Paramecium* is different, and this seems to have some relationship to the morphology of the contractile vacuoles.

In *Blepharisma undulans*, which has accessory vacuoles (MOORE, 1935) somewhat similar to those described by DEGEN (1905), the critical temperatures are not the same as in either *Glaucoma* or *Paramecium*. In *Blepharisma* there is a considerable difference in the rate of pulsation between individuals at the same temperature in old and new cultures and a great deal of variation in the former. Also the μ values in old and new cultures differ widely, and there is a large variation in different individuals from the same old culture.

Such large differences in the temperature characteristics were unexpected in view of the previous constant results obtained with *Paramecium*; for example, in one of the series the μ value for *Blepharisma* is 51,000 calories (which is perhaps one of the highest temperature characteristics known for a normal physiological process), while it is only 16,700 calories in individuals from the new culture. However, individuals of *Blepharisma* from a new culture (about six days old) gave results which were quite constant. This difference is probably due to the physiological difference between the individuals in old and new cultures. The sensitivity of this organism to light might explain part of this variation. STOLTE (1924) showed that under different conditions the form and structure of *Blepharisma* might be considerably varied. However, the data from the new culture show that the value between 14.9° and 28.8° is 16,700 calories. This value is the same as one CROZIER (1924) identified for oxidative processes, and it is also associated with water permeability (McCUTCHEON and LUCKÉ, 1932).

In *Spirostomum ambiguum*, which has a longitudinal collecting canal leading to the vacuole, the graph is one straight line with a temperature characteristic of 12,000 calories (11,700 and 12,800 calories) for the temperature range 13° to 30° C. This resembles CROZIER's 11,500 calories for oxidation processes and McCUTCHEON and LUCKÉ's, value of 12,000 calories for water permeability. CLARK (1933) computed from the data of ROSSBACH (1872) a value of 9,000 to 9,900 calories for *Stylonychia pustulata* for the range of 5° to 31° C. This is indicative of the possibility that in *Blepharisma*, *Spirostomum* and *Stylonychia* a single reaction might control the rate of contraction instead of a catenary set of three reactions as COLE has postulated for *Paramecium caudatum* and as evidence here presented indicates for the three other species of *Paramecium* studied. On this basis, the vacuole of *Euplotes charon* ($\mu = 13,500$ at 15.27° and 5,800 at 10—15° as computed by CLARK from data of ROSSBACH, l. c.) would be controlled by two reactions, and that of *Glaucoma colpidium* (data of DEGEN, 1905) by three reactions.

It should be pointed out that all of the temperature graphs obtained in the present study are not of equal validity due to the scattering of the points in one or two cases. Thus the graphs for the species of *Paramecium* (Figs. 1 and 2) are more uniform than those for *Blepharisma* and *Spirostomum* (Figs. 5 and 6). This must be kept in mind when considering the possible significance of the assigned μ values.

The temperature characteristics for the vacuoles of all species of ciliates known to have been studied are shown in Table 1. It can be seen from this table that the different morphological types of contractile vacuoles are affected differently by temperature in that their temperature characteristics are not the same. This difference in temperature characteristics, according to the interpretation of CROZIER (1924) indicates that the underlying chemical reactions which control the rate of contraction are different. Possible interpretations of some of these temperature characteristics in terms of oxidation and water permeability has been given above. However, another factor which must be considered in interpreting the possible meaning of temperature characteristics for the rate of contraction is that the rate of pulsation in *Paramecium* is probably directly proportional to the volume output, for no change in size of the vacuole with temperatures was noticed. This, however, is not the case in either *Blepharisma undulans* or *Spirostomum ambiguum*. Especially in *Blepharisma*, the decrease in the size of the main vacuole is very noticeable as the temperature rises. Due to the fact that it is extremely difficult to measure the size of the vacuole accurately while the organism is moving around, no actual measurements were made, but special notice was taken of the approximate size of the vacuole during the course of the experiment. In all of the species of *Paramecium*, as far as my observations go, there does not seem to be any noticeable change of the size of the vacuole within the temperature range studied.

The whole problem of temperature effects on biological processes is not as simple as it formerly seemed (BELEHRADEK, 1935) and this paper is by no means an attempt to clear up the situation. However, it seems to the writer that the data obtained in this investigation can be interpreted in terms of CROZIER'S view that the ARRHENIUS equation holds in biological processes.

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 determined over a temperature range of about 16° C. The temperature characteristics calculated by means of the VAN 'T HOFF-ARRHENIUS equation are nearly the same. Below 16.1°, the temperature characteristic is 25,000 calories for *P. caudatum*, *P. aurelia* and *P. multimicronucleata*, and 23,300 calories

for *P. polycaryum*. Between 16.1° and 25.1° , 16,600 calories for *P. polycaryum*, 16,700 calories for *P. caudatum*, 16,500 calories for *P. multimicronucleata* (between 17.7° and 26.8°) and 17,000 calories for *P. aurelia*. Above $22.5 \pm 1^{\circ}$, 14,500 calories for *P. caudatum*, 12,400 calories for *P. polycaryum*, 10,900 calories for *P. aurelia* and 9,500 calories for *P. multimicronucleata* (above 26.8°).

2. The rate of pulsation of the contractile vacuole of *Blepharisma undulans* has been determined over a temperature range of about 18° C. There is considerable difference between individuals from six-day old and three-month old cultures, and between individuals in the same three-month old culture in the rate of pulsation and in the temperature characteristics. The temperature characteristic of the individuals from a six-day old culture is constant; namely, between 14.9° and 28.8° it is 16,700 calories. In some individuals in a three-month old culture, the temperature characteristic was found to be as high as 44,000 calories and 51,000 calories. One reversible case from a three-month old culture showed a value of 18,000 calories above 21° C. and 34,000 calories below 21° C. Another individual showed a temperature characteristic of 37,400 calories as the temperature rose from 21° to 29° but only 20,200 calories as it was lowered again to 17.8° C.

3. The rate of pulsation of the contractile vacuole of *Spirostomum ambiguum* has been determined over a temperature range of about 18° C. The temperature characteristic is 12,000 calories between 13.6° and 32.1° C.

4. The above data on four species of *Paramecium*, together with those of other workers on *P. caudatum* can be interpreted to indicate that in all four species of *Paramecium*, the rate of pulsation within the temperature ranges studied, is controlled by a catenary set of three reactions. In *Spirostomum ambiguum* (present data) and *Stylonchya pustulata* (data of ROSSBACH) the rate of contraction seems to be controlled by only one reaction, and in *Euplotes charon* and *Glaucoma colpidium* above 11° (data of ROSSBACH) by two reactions. However, in *Blepharisma undulans* (present data) the rate of contraction seems to be controlled by one, two or three reactions dependent upon the age of culture from which the individuals are taken, and in individuals from about three-month old cultures these reactions do not seem to be the same in all cases.

5. The possible significance of the values of the temperature characteristics obtained is discussed.

I wish to express my thanks to Dr. T. CUNLIFFE BARNES and Dr. T. JAHN for valuable advice. I am also indebted to Professor L. L. WOODRUFF for helpful criticism.

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Digitale Literatur/Digital Literature

Zeitschrift/Journal: [Archiv für Protistenkunde](#)

Jahr/Year: 1936

Band/Volume: [87_1936](#)

Autor(en)/Author(s): Gaw H.Z.

Artikel/Article: [Physiology of the contractile vacuole in ciliates. 3. The effect of temperature. 201-212](#)