

Physiology of the contractile vacuole in ciliates¹⁾.

4. The effect of heavy water.

By

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With 6 figures in the text.

Introduction.

Heavy water in high concentration has been found to reduce the velocity of certain chemical and biological processes (cf. BARNES and JAHN, 1934) but no extensive study has been made of the effect of heavy water on the contractile vacuole in Protozoa. Although TAYLOR, SWINGLE, EYRING and FROST (1933) noticed the contractile vacuoles of *Paramecium caudatum* cease to contract in 92 % heavy water and "greatly increase in size until they appear as large bags of clear fluid almost completely filling the entire cell body", these workers did not study the rate of pulsation of the vacuole in different concentration of heavy water. Since the contractile vacuole in Protozoa is closely associated with the water metabolism of the organism and also since it may serve as an indicator of the general physiological activities going on in the cell, it is clear that the contractile vacuole is a particularly suitable indicator for the study of heavy water effects on living organisms (BARNES and GAW, 1935).

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.

P. caudatum and *Blepharisma undulans* were used in this study. They were taken from subcultures which were derived from the main cultures described in previous papers of this series. Organisms before being introduced into heavy water were repeatedly washed with distilled water. A very fine pipette was used to transfer the organisms to heavy water with as little water carried over as possible (approximately 1/250 cc.). The fine pipette did not seem to injure the organisms in any way. As soon as the organisms were trans-

Table 1.
Physical properties of 30 %, 50 % and 95 % heavy water ¹⁾.

	30 %	50 %	95 %
Mobility of ions			
H	284.75	264.45	218.78
K	61.29	59.35	54.98
Cl	62.23	60.25	55.89
Solubility of salts in 1.00 g. of D ₂ O			
NaCl	.3428	.3320	.3077 g. at 25 C.
BaCl	.3366	.3230	.2924
Density	1.0311	1.0530	1.1024
Viscosity	11.39	11.74	12.51
Surface tension	71.26	70.27	68.05 dynes/cm.

ferred to heavy water in a depression slide, the latter was sealed with a cover glass by vaseline. Observations were made from time to time under the dissecting and compound microscopes with the cover glass on. However, occasionally the cover glass was removed during observations on account of the moisture condensed on it. The rate of contraction was recorded as usual by a stop watch. Two or more of three successive complete systoles were taken for each vacuole. Observations were made after at least an hour or more in heavy water. Physical properties of heavy water are given in Table 1.

¹⁾ SELWOOD and FROST (1933) give the surface tension of 100 % heavy water as 67.80 dynes/cm. TAYLOR and SELWOOD (1934) give the density of 100 % heavy water as 1.1079 and the viscosity as 12.60.

Experimental Results.

The first experiments were performed on *Paramecium caudatum* and in 95% heavy water the contraction of the vacuole was completely inhibited. Continuous observation for 45 minutes failed to reveal any pulsation and the vacuoles were greatly enlarged (Fig. 1). This species did not live more than six hours in 95% heavy water. Three tests were run each involving about ten individuals. No great change in shape occurred but some of the specimens were slightly rounded at the ends. Movement was greatly reduced and after three hours had practically ceased. In one case the effect of

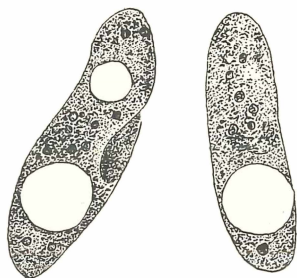


Fig. 1. *P. caudatum*, after 3 hours in 95% heavy water with vacuoles enlarged. All the drawings were made with camera lucida at a magnification of $500\times$ except Fig. 3 in which the magnification is $250\times$.

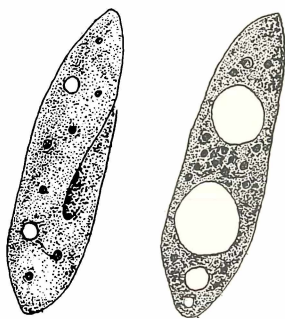


Fig. 2. At right, *P. caudatum*, after 48 hours in 50% heavy water with the vacuoles enlarged and, at left, 12 hours after being transferred to a mixture of half ordinary water and half hay infusion with the vacuoles returned to the normal condition.

the 95% heavy water was reversible; the vacuole became normal in both rate and contraction and shape after several hours in a mixture of equal parts of hay infusion and distilled water (Fig. 1). Three trials in 50% heavy water were run involving about thirty individuals which lived more than 24 hours in this concentration but the exact longevity was not determined. No change in shape was observed but the vacuoles were enlarged (Fig. 2). Movement was not greatly reduced at first but after 10 hours the movement was appreciably reduced. The rate of pulsation of the vacuole gradually declined. After 24 hours the time for a complete systole was 24.6 secs. at 19.9° (compared to 10.8 secs. under normal conditions at this temperature) (Table 2). With most individuals the effect was reversible on return to ordinary water (Fig. 2). In 30%

Table 2.

Observations on the contractile vacuole of *Paramecium caudatum* in 50% heavy water (10 individuals).

Date	Time in secs. for one comp. systole	T° C.	Remarks
4:15 p. m. (12/5/'34)	—	—	—
7:30 p. m.	12.7	20.1	—
10:00 a. m. (12/6/'34)	17.7	19.9	—
4:15 p. m.	20.7	19.8	—
9:15 a. m. (12/7/'34)	21.5	19.9	—
4:15 p. m. (48 hours in D ₂ O)	24.6	19.9	Three died and disintegrated. The rest swim around actively.

Table 3.

Observations on the contractile vacuole of *Paramecium caudatum* in 30% heavy water (10 individuals).

Date	Time in secs. for one comp. systole		T° C.	Remarks
	In 30% D ₂ O	In control		
10:30 p. m. (12/13/'34)	—	—	—	—
9:00 a. m. (12/14/'34)	16.4	10.9	19.5	—
10:30 p. m.	18.9	11.3	18.5	—
10:30 a. m. (12/15/'34)	17.3	9.3	21.0	—
10:30 p. m. (48 hours in D ₂ O)	21.1	11.2	19.2	Movement of the animals in 30% D ₂ O very slow.

Table 4.

Observations on the contractile vacuole of *Blepharisma undulans* in 50% heavy water (10 individuals).

Date	Time in secs. for one comp. systole	T° C.	Remarks
11:00 a. m. (12/6/'34)	—	—	—
4:45 p. m.	203.8	19.7	—
10:06 p. m.	232.6	19.8	Three died, and the others move slowly.
11:00 a. m. (12/6/'34)	390.2	19.8	Two more died, and the others hardly moving.
2:15 p. m.	All disintegrated.		

Table 5.

Observations on the contractile vacuole of *Blepharisma undulans* in 30% heavy water (10 individuals).

Date	Time in secs. for one comp. systole		T° C.	Remarks
	In 30%	In control		
11:05 a. m. (12/11/'34)	—	—	—	—
1:05 p. m.	194.6	98.1	18.8	—
4:05 p. m.	171.7	106.4	18.5	—
11:05 p. m.	204.9	86.2	18.1	—
11:05 a. m. (12/12/'34)	204.5	104.6	18.5	—
11:05 p. m.	226.5	106.2	18.5	—
11:05 a. m. (12/13/'34) (48 hours in D ₂ O)	—	105.2	19.0	Animals in 30% D ₂ O all disintegrated.

Table 6.

Rate of contraction of the anterior vacuole of *Paramecium caudatum* in 30% heavy water as affected by temperature.

Temp. C.	Time in secs. for one complete systole	Rate of contraction per minute
7.7	65.4	0.917
10.4	65.9	0.910
12.1	45.3	1.32
13.2	37.7	1.59
14.3	32.1	1.86
15.6	27.9	2.15
16.6	24.1	2.65
17.7	19.9	3.01
18.8	16.5	3.63
19.9	15.3	3.91
21.0	12.8	4.68

Temp. range of 13.3° C., μ = 23,200 calories.

heavy water (Table 3) the reduction in rate of the vacuole was also obtained and the effect was reversible in almost all cases.

The experiments were repeated with *Blepharisma undulans* and the same effects were observed (see Tables 4—5). However, a striking morphological change occurred in *Blepharisma* in 95% heavy water. The body becomes almost spherical and in one case this change was reversible on return to ordinary water. The enlargement of the vacuole was similar to that in *Paramecium* (Figs. 3 and 4).

The effect of temperature on the contractile vacuole of *P. caudatum* in 30% heavy water was investigated and controls treated exactly same way were also carried out in ordinary water. The same procedure was used as in the preceding paper on the effects of temperature. The temperature range was about 14°C. ($10 \pm 1^\circ$ to $24 \pm 1^\circ$) (Table 6). It is significant to note that the graph obtained in 30% heavy water appears to show an almost rectilinear



Fig. 3. At left, *B. undulans*, after 2 hours in 95% heavy water with the body form changed and the vacuole enlarged and, at right, 10 hours after being transferred to a mixture of half ordinary water and half hay infusion, with normal body form and vacuole.

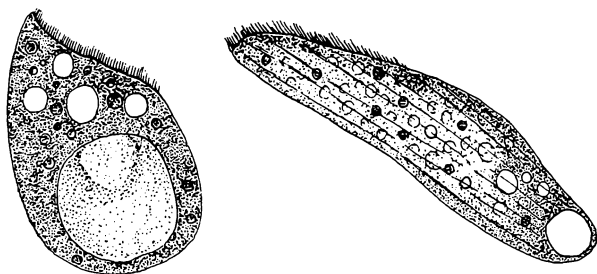


Fig. 4. At left, *B. undulans*, after 24 hours in 30% heavy water and, at right, 3 hours after being transferred to the mixture of half ordinary water and half hay infusion.

relation over the entire temperature range (Figs. 5—6). The average temperature characteristic is 22,000 calories (23,000, 25,000, 22,000, and 20,000). On the other hand, the curve obtained in ordinary water shows two decisive breaks at the critical temperatures $15 \pm 1^\circ$ and $21 \pm 1^\circ$ C. (Fig. 5). The average temperature characteristic below $15 \pm 1^\circ$ is 23,000 calories; between $15 \pm 1^\circ$ and $21 \pm 1^\circ$ is 16,500 calories, and above $21 \pm 1^\circ$ is 8,700 calories. These data from ordinary water (Table 7) agree quite well with those obtained in hay infusion medium.

In this case the reduction in the rate of contraction of the vacuole was not as great as in previous experiments in 30% heavy water in which the animals remained over 24 hours in the heavy water (Table 3).

Discussion.

The most significant result of the study of heavy water effects on the contractile vacuole of *Paramecium caudatum* and *Blepharisma undulans* is the marked reduction in the rate of contraction as compared with that of the controls (in ordinary water) at identical

Table 7.

Rate of contraction of the anterior vacuole of *Paramecium caudatum* in ordinary water as affected by temperature.

Temp. C.	Time in secs. for one complete systole	Rate of contraction per minute
12.1	38.9	1.54
13.2	30.1	1.99
14.3	25.7	2.33
15.6	22.2	2.70
16.6	19.1	3.14
17.7	17.7	3.38
18.8	17.1	3.50
19.9	15.1	3.97
21.9	13.4	4.45
22.2	12.0	5.00
23.3	11.1	5.40
24.4	10.7	5.56
25.5	9.9	6.03

12.1°—15.6°, μ = 23,000 calories; 15.6°—22.2°, μ = 16,500 calories;
22.2°—25.5°, μ = 8,700 calories.

temperatures (BARNES and GAW, 1935) and the indication that the rate of contraction in a given period of time is proportional to the concentration of heavy water. The fact that the frequency of pulsation in the contractile vacuole was reduced in heavy water indicates that the metabolism in the cell is correspondingly decreased.

In his recent paper on heavy water in chemistry, POLANYI (1935) points out that heavy hydrogen and light hydrogen have different permanent energy or different zero point energy (for explanation, see EYRING, 1933). The heavy hydrogen compounds have lower reactivity due to the lower permanent energy of heavy hydrogen. BERNAL and TAMM (1935) report that the vibrations of heavy water molecules are considerably less than those of ordinary water molecules. It seems probable that the effect of the heavy water on the contractile vacuole observed may be explained on the basis of these facts. When heavy water replaces the ordinary water as the medium, the metabolism is correspondingly slowed down, due to the lower reactivity of heavy hydrogen in heavy water. This corroborates the observation mentioned above that the rate of slowing down at a given period of time is proportional to the concentration of heavy water.

It is possible that the effects are due to the stabilizing action of the heavy water as suggested by BARNES (1933). This may be an effect on the colloids in the organism in which the bound water is known to be of greater density than "free" water. Also, enzymes

may be partially inhibited as a result of deuterium exchange in a key position (BARNES and LARSON, 1933).

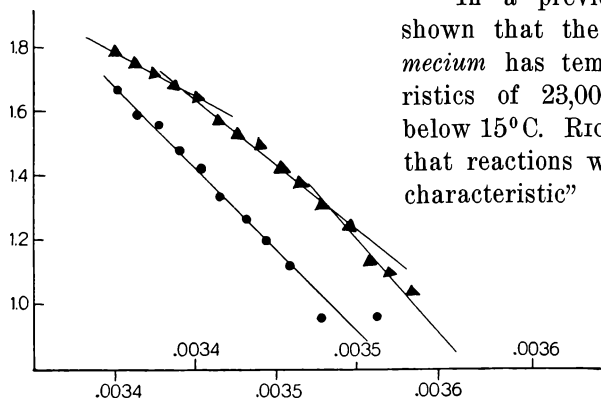


Fig. 5. The relation between the log of the rate of contraction of the vacuole in *P. caudatum* and the reciprocal of the absolute temperature, plotted according to the ARRHENIUS equation (see text). Triangles indicate the graph for the experiment conducted in ordinary water; circles indicate the results in 30% heavy water (see text). The reciprocals of the absolute temperature (abscissae) above the line are those for the graph indicated by triangles.

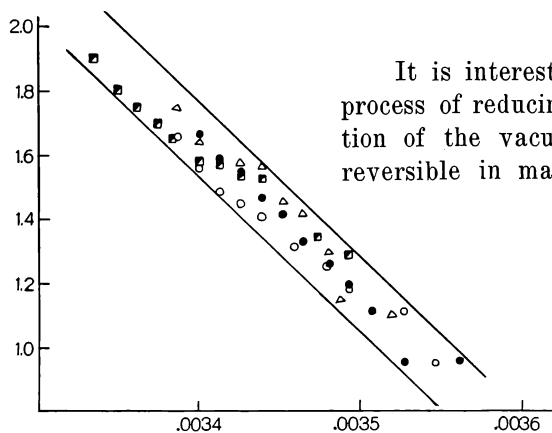


Fig. 6. The results of four series of temperature studies of the rate of contraction of the vacuole of *P. caudatum* in 30% heavy water (see text).

In a previous paper it was shown that the vacuole in *Paramecium* has temperature characteristics of 23,000—25,000 calories below 15°C. RICE (1923) points out that reactions with a "temperature characteristic" of approximately 20,000 calories involve hydrolysis. It is possible that the reduction of the rate of pulsation of the contractile vacuole may be due to the action of heavy hydrogen which takes part in hydrolysis.

It is interesting to note that this process of reducing the rate of contraction of the vacuole in heavy water is reversible in many cases, especially in lower concentration (30%). The vacuole becomes normal in every respect, size as well as the rate of contraction, after the organism was returned to a mixture of half ordinary water and half hay infusion for one to

several hours. This shows, I believe, that the effect of the heavy water on the contractile vacuole is perhaps purely a chemical one. It is significant that these results agree with the recent work of

BARNES and WARREN (1935) on the pulsation of the frog heart in heavy water (from the same sample which was used in my experiments).

As already mentioned, TAYLOR, SWINGLE, EYRING and FROST (1933) noticed the enlargement of the contractile vacuole in 92 % heavy water and its persistence without contraction for hours. This was confirmed in the present work in both *Paramecium caudatum* and *Blepharisma undulans*. These investigators did not make any attempt to explain this effect, but it would appear that this enlargement of the vacuole without contraction may be due to a change in the ectoplasm (after hours in heavy water) so that it prevents the vacuole from contracting. YOCOM (1934) reports a similar effect in the vacuole of *Euplotes* when the animal is transferred to 65 % sea water. Thus an enlargement of the contractile vacuole may be a consequence of the arrest of contraction brought about by the increased osmotic pressure of the medium in the case of *Euplotes* or by the inhibition of the contractile mechanism in the case of *Paramecium* in 95 % heavy water.

In 95 % heavy water, the change of the body form of *Blepharisma undulans* was remarkable (see Fig. 3). The reason for this is unknown except that the heavy water may effect the contractile elements in the cell thus producing the spherical shape. In the cells of *Spirogyra* in 95 % heavy water (from unpublished results of BARNES) the protoplasts shrink to $\frac{2}{3}$ or $\frac{1}{2}$ their original size, suggesting that concentrated heavy water may remove ordinary water from living cells.

EYRING and SHERMAN (1933) in their theoretical consideration of separation of isotope state that since "all chemical reactions having a positive temperature coefficient will go about half as fast or less, at ordinary temperature with the heavy as with the light hydrogen isotope; it will be possible to single out such reactions in biological process". The present investigation of the activity of the contractile vacuole of *Paramecium caudatum* seems to support this view. The temperature characteristic of *P. caudatum* in 30 % heavy water over a range of 14° C. (11°—25°) has been determined, and the temperature characteristic was found to be around 22,000 calories, which is similar to the value of 24,000 calories which occurs in this organism in culture medium and ordinary water, below 16° C. PACSU (1933) found that the velocity of mutorotation of glucose in heavy water at 20° C. is identical with that in ordinary water at 10° C. The effect is the same as that produced by low temperature.

CROZIER and HOAGLAND (1934) claim that the magnitudes of temperature characteristics have been found to be highly significant in that they are not scattered at random but are found to be grouped in definite modes. The difference between 22,000 calories and 24,000 calories might be significant as CROZIER and HOAGLAND consider them as different modes. But on the other hand, this difference may be due to the difference in viscosity of 30 % heavy water and ordinary water as the viscosity of the former is 11.4 and that of the latter 10.87 (SELWOOD and FROST, 1933). The change in value may be one of the modifications of the temperature characteristic discussed by CROZIER and STIER (1926). There are several instances in which a normal temperature characteristic is slightly increased or decreased under changed conditions. HINSHELWOOD, WILLIAMSON and WOFFENDEN (1934) studied the reaction between oxygen and deuterium and found that the energy of activation of the branching process is the same for both isotopes of hydrogen, i. e., 26,500 calories. However, POLANYI (1934) states that the activation energy of compounds containing deuterium should be greater than that of hydrogen compounds. UREY and TEAL (1935) have recently discussed this interesting problem of temperature coefficients in heavy water. They state that if there is no difference in the energy of activation of a given process in the two types of water, one may conclude that the slowest reaction does not involve deuterium exchange. In the formation of hydrogen bromide, however, the temperature characteristics are 17,740 and 19,870 calories for ordinary and heavy hydrogen and the difference is nearly equal to the difference in zero point energy; i. e., 2.1 K calories (K calorie = 1000 cal.). In the case of the vacuole, if one ignores the critical temperatures, the energy of activation over the entire temperature range is greater in the heavy water which agrees with the interpretation of POLANYI and UREY.

The apparent obliteration of the break in the temperature graph for the vacuole in heavy water resembles the effect of increased oxygen pressure on the temperature graph for oxygen usage by leeches (CROZIER and STIER, 1926) in which the μ at lower temperature of 24,000 calories extends over the entire temperature range and the μ of 7,900 calories (occurring above 19° C.) is wiped out.

The experiments on the temperature characteristic in heavy water seem to support the view that ARRHENIUS equation may be applied to biological processes. As we have noted, the graph of the temperature characteristic of the vacuole of *Paramecium* in heavy water appears to be a straight line over a temperature range of

about 14° C. The slowest master reaction of the catenary set controls the rate of water discharge from the vacuole, and it appears that the catalyst in control at lower temperature in water is so slowed down in heavy water that it governs the rate at all temperatures. However, as the experiments were conducted in such a way that they always started from the lowest temperature, it is conceivable that the heavy water acts on the catalyst at the lower temperature first. In the first paper of this series the vacuole was shown to function in osmoregulation. In 30 % heavy water the rate of pulsation is reduced but the organism does not swell suggesting that the permeability to heavy water is reduced as in the erythrocyte (PARPART, 1935). However, the sea urchin egg is freely permeable to D₂O (LUCKÉ and HARVEY, 1935).

In conclusion, it should be pointed out that the first biological experiments involving a newly-discovered isotope will obviously be open to many conflicting explanations, but it is encouraging to note that the general effect of heavy water on the rate of contraction of the contractile vacuole is in harmony with the lower energy content of systems containing deuterium. It is clear that the use of heavy water will afford a new and effective method of controlling the rate of physiological processes.

Summary.

1. The rate of pulsation of the contractile vacuole in *Paramecium caudatum* and *Blepharisma undulans* was reduced considerably in 30 %, 50 %, and 95 % heavy water, and there is indication that the rate of reduction is proportional to the concentration of heavy water.

2. In both *Paramecium* and *Blepharisma* the vacuoles are greatly enlarged in 95 % heavy water and in *Blepharisma* the shape of the body becomes almost spherical.

3. The rate of pulsation of the anterior contractile vacuole in *P. caudatum* in 30 % heavy water has been determined over a temperature range of 14° C. (10°—24° C.). The temperature characteristic is about 22,000 calories over the entire range, which resembles that of 24,000 calories found in this organism in ordinary water below 16° C.

I wish to express my thanks to Dr. T. CUNLIFFE BARNES under whose direction the experiments were carried out. I am also indebted to Professor L. L. WOODRUFF for helpful advice.

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