The Rate of Pulsation and the Function of the Contractile Vacuole in *Paramecium multimicronucleatum**).

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With 3 figures in the text.

Contents.

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I. Relative rate of pulsation of anterior and posterior contractile vacuoles.

1. Flourishing cultures.

Introduction.

UNGER (1926) states that in *Paramecium calkinsi* the rate of pulsation is the same in both the anterior and posterior vacuoles; that in *Paramecium aurelia* the rate is higher in the posterior vacuole, and that in *Paramecium caudatum* it is higher in the anterior vacuole. CHILD and DEVINEY (1926), EISENBERG (1925), PORT (1927) and PARK (1929) confirm his report in reference to *Paramecium caudatum*. STEMPELL (1914) and FORTNER (1924) maintain that the opposite obtains.

I have been unable to find any reference to the relative rate of pulsation of the vacuoles in *Paramecium multimicronucleatum*. Detailed observations on the relative rate of pulsation in this species and in *Paramecium caudatum* are presented below.

Materials and Methods.

The culture medium was prepared by adding 5 gms. of timothy hay to boiling tap water, and then boiling the mixture for eight minutes. The infusion was filtered through cotton, made up to a liter by the addition of tap water, left 24 hours, and then inoculated with approximately 10 cc. of an old mass culture, containing several hundred specimens.

All the individuals of *Paramecium multimicronucleatum* were descendants of a single clone, grown in mass culture, and subcultured every 10 days. No conjugation has been observed in this clone.

The observations were made as follows: one or two drops of the culture medium, containing from ten to fifteen animals and some food masses were transferred by means of a pipette to a square vaseline enclosure on a glass slide. A seven-eighths inch cover glass was then placed on the vaseline enclosure and pressed down sufficiently to seal the solution in the enclosure and yet permit the animals to move freely in all directions. The slides were then left for five to ten minutes to allow the animals to recover from the mechanical shock of manipulation before being studied.

Only resting animals were studied. The anterior and posterior vacuoles were observed simultaneously in each individual, and only those records, which were obtained in observations on at least ten consecutive pulsations for each vacuole were retained. The rate of pulsation was measured to the fifth of a second by means of stop-watches and electric stop-clocks. The rate of pulsation is always given in terms of the time interval between pulsations in seconds.

The hydrogen ion concentration of the cultures was measured by means of a potentiometer and a quinhydrone electrode just before the animals were transferred to the vaseline enclosures. The temperature at which the observations were made varied between 20 and 23° C, unless otherwise stated.

Results.

a) *Paramecium multimicronucleatum.*

Two sets of observations were made. In the first, individuals from a single mass culture were studied over a period of 16 days, a total of 55 individuals; in the second, individuals from five different mass cultures were observed, over a period of 20 days, a total of 30 individuals.

1. The results obtained in the first set of observations are given in table 1. This table shows that the average rate of pulsation of the anterior vacuole varied from 5.7 to 13.8 seconds, and that of the posterior vacuole from 5.1 to 13.4 seconds, but that the rate of the posterior vacuole was higher in all but 6, or 89% of the animals observed; it further shows that the rate varied on different days and in different individuals on the same day, but that it did not vary consistently with the age of the culture, or with the hydrogen ion concentration of the medium.

2. In the 30 animals studied the average rate of pulsation of the anterior vacuole varied from 5.72 to 18.80 seconds, and that of

Table 1.

Relation between the rate of pulsation of the anterior and of the posterior contractile vacuoles in *Paramecium multimicronucleatum* in flourishing cultures.

The anterior and posterior vacuoles were observed and timed simultaneously in each individual. Each of the series of averages is the result obtained by the continuous observation of consecutive pulsations of a single individual.

Age of culture in days	pH	Designation of individual	Number of pulsations observed		Average interval between pulsations in seconds		Difference in rate of the vacuoles in seconds	
			Anterior	Posterior	Anterior	Posterior	Posterior higher	Anterior higher
2	5.63	I	22	23	9.80	9.20	0.60	
		II	13	13	10.07	9.64	0.43	
		III	17	17	10.66	10.47	0.19	
		IV	28	30	9.82	9.12	0.70	
		V	23	23	10.04	9.91	0.03	
4	5.89	I	10	12	12.66	9.50	3.16	
		II	12	15	12.77	10.80	1.97	
		III	25	29	12.19	10.65	1.44	
		IV	23	21	11.41	13.47	—	2.06
		V	46	49	13.00	12.20	0.80	
6	6.26	I	31	40	9.67	7.31	2.64	
		II	19	25	8.95	7.17	1.78	
		III	20	20	7.05	6.89	0.16	
		IV	28	28	7.60	7.82	—	0.22
		V	41	55	13.80	10.50	3.30	
7	6.46	I	16	17	7.80	7.30	0.50	
		II	24	24	6.04	5.92	0.22	
		III	10	11	7.58	6.62	0.95	
		IV	10	10	8.54	8.90	—	0.46
		V	30	33	9.02	8.00	0.80	
9	6.82	I	28	31	9.18	8.31	0 87	
		II	11	13	8.09	6.69	1.40	
		III	16	19	8.75	7.37	1.38	
		IV	11	9	6.27	8.11	—	1.84
		V	35	38	7.34	6.63	0.71	
11	6.94	I	10	10	7.40	6.90	0.50	
		II	26	27	7.84	7.33	0.51	
		III	12	13	10.00	8.50	1.50	
		IV	10	11	8.52	7.48	1.04	
		V	25	27	9.20	8.40	0.80	
12	6.98	I	31	40	12.00	9.02	2.98	
		II	11	15	10.00	7.90	2.10	
		III	10	11	10.50	9.46	1.04	
		IV	29	38	11.66	8.50	3.15	
		V	16	18	8.00	7.11	0.99	
13	7.04	I	86	101	8.19	7.89	0.30	
		II	46	52	7.82	6.96	0.86	
		III	32	38	9.34	7.80	1.54	
		IV	12	14	9.08	7.66	1.42	
		V	11	11	8.63	8.18	0.45	

(Cont'd on next page.)

Table 1 cont'd.

Age of culture in days	pH	Designation of individual	Number of pulsations observed		Average interval between pulsations in seconds		Difference in rate of the vacuoles in seconds	
			Anterior	Posterior	Anterior	Posterior	Posterior higher	Anterior higher
14	6.88	I	25	24	7.40	7.71	—	0.31
		II	10	10	6.25	5.50	0.75	
		III	18	19	7.94	7.40	0.54	
		IV	22	23	7.27	6.70	0.50	
		V	13	15	6.30	5 30	1.00	
15	6.96	I	46	58	6.95	5.53	1.42	
		II	44	44	6.60	6.68	—	0.08
		III	12	16	6.80	5.50	1.30	
		IV	29	37	7.58	6.00	1.58	
		V	21	23	6.05	5.65	0.40	
16	7.12	I	34	43	8.00	6.20	1.80	
		II	10	11	7.87	6.87	1.00	
		III	12	13	8.00	7.30	0.70	
		IV	39	43	5.70	5.10	0.60	
		V	25	27	9.50	8.80	0.70	

the posterior vacuole from 5.34 to 21.4 seconds, but the rate of the posterior vacuole was higher in all but 3, or 90% of the animals observed. The hydrogen ion concentration of the five mass cultures varied from p_H 5.10 to p_H 7.75, and the temperature varied from 22.0 to 30.0° C, but neither of these factors affected the relative rate of the two vacuoles.

b) *Paramecium caudatum.*

Twenty-seven individuals from 3 different mass cultures were observed. The cultures varied in age from 2 to 12 days. The hydrogen ion concentration varied from p_H 5.5 to 7.0

The average rate of pulsation of the anterior vacuole varied from 6.75 to 13.6 seconds, and that of the posterior vacuole from 6.62 to 17.2 seconds, but the rate of the posterior vacuole was higher in all but 9, or 67% of the animals observed.

Conclusion.

Both in *Paramecium multimicronucleatum* and in *Paramecium caudatum* the rate of pulsation is usually higher in the posterior vacuole than in the anterior. The percentage of animals, in which the rate of the anterior is higher, is greater in *Paramecium caudatum*, but the contention of Child and Deviney and others, that the rate

of the anterior vacuole is usually higher in *Paramecium caudatum* is not confirmed. Neither the age nor the hydrogen ion concentration of the medium, nor the temperature seem to influence the relative rate, in flourishing cultures.

2. Depleted or depressed cultures.

CHILD and DEVINEY (1926) hold that in toxic solutions or under depressing culture conditions the rate decreases in *Paramecium caudatum* more in the anterior than in the posterior vacuole, so that it may become equal in both, or even lower in the anterior vacuole. EISENBERG (1925) offers some evidence that any factor, affecting the rate, equally affects both vacuoles. The effect of unfavorable culture conditions on *Paramecium multimicronucleatum* is considered below.

Methods.

Two sets of observations were made. In the first set a large number of vaseline enclosures, containing from five to thirty individuals were prepared. In twenty-seven of these preparations one or more animals survived for from 27 to 120 days. At first the number of individuals increased, but signs of malnutrition soon became evident and the number gradually decreased, until by the end of the month only one or two individuals were alive on each slide. The progressive emaciation indicated that death was due to starvation. Incidental to other studies, a record of the relative rate of pulsation was obtained for 20 such individuals.

In the second set of observations 35 individuals from a mass culture, subjected to a gradual increase in concentration of sea water up to 35 percent during 20 days, were studied. Observation showed that the rate of pulsation and the rate of feeding were markedly depressed.

Results.

1. The results obtained in the first set of observations are given in table 2. This table shows that the average rate of pulsation of the anterior vacuole varied from 16.3 to 134 seconds, and that of the posterior vacuole from 14.0 to 144 seconds, but that the average rate of the posterior vacuole was higher than that of the anterior in all but 6, or 70 % of the animals observed.

2. In the culture medium-sea water mixtures the average rate of pulsation of the anterior vacuole varied from 9.50 to 92.80 seconds,

Table 2.

Relation between the rate of pulsation of the anterior and of the posterior contractile vacuoles in *Paramecium multimicronucleatum* in depleted cultures.

The anterior and the posterior vacuole were observed and timed simultaneously in each animal. Each of the series of averages is the result obtained by continuous observation of consecutive pulsations of a single animal.

Age of slide in days	Designation of individual	Number of pulsations observed		Average interval between pulsations in seconds		Difference in rate of the two vacuoles in seconds	
		Anterior	Posterior	Anterior	Posterior	Posterior higher	Anterior higher
8	I	10	12	23.4	19.6	3.8	
	II	8	9	28.5	24.6	3.9	
	III	10	9	47.3	54.0	—	6.7
	IV	9	9	134.0	144.0	—	10.0
	V	12	13	26.5	22.8	2.3	
9	I	10	9	23.2	26.4	—	3.2
	II	10	10	35.3	32.8	2.5	
	III	7	10	28.4	20.2	8.2	
	IV	19	21	40.5	37.6	2.9	
	V	11	13	30.0	28.8	1.2	
10	I	11	11	41.3	42.0	—	0.7
	II	10	11	110.8	97.5	13.33	
	III	11	11	26.1	26.8	—	0.7
	IV	10	10	29.3	29.1	0.2	
	V	12	12	30.5	29.5	1.0	
11	I	9	10	75.6	62.5	13.1	
	II	10	11	27.1	24.6	2.5	
	III	10	10	25.4	25.8	—	0.4
	IV	13	13	91.3	89.8	1.5	
	V	10	10	36.5	34.1	2.4	

and that of the posterior vacuole from 8.50 to 134.3 seconds, but the average rate of the posterior vacuole was higher than that of the anterior in all but 14, or 60 % of the animals observed.

3. Table 3, compiled from some of the records obtained in the first set of observations, shows that e. g. in individual 26 A, the time interval between pulsations varied from 25.0 to 311.0 seconds in the anterior vacuole and from 20.0 to 311.0 seconds in the posterior vacuole, but that the variations consistently occurred simultaneously in both vacuoles and in the same direction; so that if the rate of one vacuole changed, that of the other vacuole changed simultaneously and in the same direction. This simultaneous variation was observed both in animals in which the rate of the anterior vacuole was higher and in animals in which that of the posterior vacuole was higher.

Table 3.

Simultaneous variation in the rate of pulsation of the anterior and posterior contractile vacuoles in single individuals of *Paramecium multimicronucleatum*. The rate is given in terms of the time interval between successive pulsations in seconds.

Designation of individuals									
34 A I		10 B I		8 A VII		35 A I		26 A I	
Anterior	Posterior	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior	Anterior	Posterior
36	36	47	69	144	143	308	327	311	311
33	30	141	91	125	145	187	187	33	34
40	32	152	132					47	32
37	35	124	98					29	19
36	31	90						38	55
34	35							45	43
40	40							143	138
42	37							187	185
								51	52
								200	200
								25	25
								20	20

Conclusion.

In depleted cultures the average rate of pulsation of the anterior vacuole varied from 16.3 to 134.0 seconds, and that of the posterior vacuole from 14.0 to 144 seconds, (table 2); the rate of the posterior vacuole was higher in only 70 % of the animals observed: in depressed cultures the variations were 9.50 to 92.80 seconds for the anterior vacuole and 8.50 to 134.3 seconds for the posterior vacuole, while the rate of the posterior vacuole was higher in only 60 % of the animals observed. In flourishing cultures the rate of the anterior vacuole varied at most from 5.7 to 18.8 seconds and that of the posterior only from 5.3 to 21.4 seconds, and the rate of the posterior vacuole was higher in 89 % of the cases. This indicates that in depleted and depressed cultures the rate of pulsation of both vacuoles was greatly decreased, and that the posterior vacuole was more depressed by the unfavorable culture conditions than the anterior vacuole, in opposition to CHILD and DEVINEY'S contention for *Paramecium caudatum*. Though variations in the rate of pulsation occur simultaneously in the two vacuoles, the relative rate of the two vacuoles may be changed by the culture conditions.

3. During fission.

a) Three vacuole stage.

Fourteen individuals were observed in the three vacuole stage of fission. The three vacuoles were observed simultaneously for an average of 10 consecutive pulsations for each vacuole. The rate of the posterior vacuole was higher than the rate of the anterior vacuole in 8, or approximately 57 % of the individuals studied; it was higher than the rate of the middle vacuole in 7, or in 50 % of the individuals.

b) Four vacuole stage.

Five individuals were observed in the four vacuole stage of fission. The rate of the posterior vacuole was higher in 4, or 45 % of the nine daughter cells observed.

Conclusion.

Fission decreased the percentage of animals in which the rate of the posterior vacuole was higher from 89 % to 57 and 45 % for the three and four vacuole stage respectively.

4. Non-feeding animals.

On two occasions a total of five individuals were observed which were not feeding and contained no food vacuoles in their bodies. Other individuals in the same enclosure were feeding and contained a large number of food vacuoles. There was no indication of fission in any of the five individuals; the feeding currents were in evidence, but all solid particles were ejected from the currents before they reached the cytostome.

The rate of the posterior vacuole was higher in none of the individuals.

Summary and Discussion.

Though the number of individuals observed is relatively small (187 animals and 7261 pulsations) and is different in the different observations, owing to the difficulty of obtaining a record of at least ten consecutive pulsations of the anterior and posterior vacuoles simultaneously, the results obtained are at least suggestive. When the animals were feeding under favorable culture conditions, the rate of the posterior vacuole was higher in approximately 89 % of the individuals; feeding in a depleted medium, or in a medium

containing sea water, decreased the percentage of specimens in which the rate of the posterior vacuole was higher to 70 and 60 % respectively; fission in the three vacuole stage decreased it to 57 %, and fission in the four vacuole stage decreased it to 45 %; and cessation of feeding without fission decreased it to 0 %.

The conditions, which influenced the relative rate, had one thing in common, decrease in the rate, or absence of feeding. This suggests that feeding was the main factor in determining the relative rate. Decrease in the rate of feeding decreased the percentage of animals in which the rate of the posterior vacuole was higher. This seems to confirm FORTNER's (1924) contention that the particular function of the posterior vacuole is the removal of water that enters with the food vacuole and by absorption from the oesophagus. It also confirms STEMPELL's (1914) argument that, since the water entering with the food vacuole reaches the posterior vacuole first, the posterior vacuole should have a higher rate of pulsation.

According to CHILD (1914, 1934), the metabolic gradient of *Paramecium* necessitates a higher rate of pulsation in the anterior contractile vacuole. The results obtained in this study with non-feeding animals, in which the rate of the anterior vacuole was always higher, may seem to agree with his theory. But the results obtained with feeding animals in which a higher rate of metabolism can be demonstrated, as will be shown later, indicate that the metabolic gradient, if actual, is not the only factor determining the relative rate of the vacuoles.

The relative rate depends directly on the amount of feeding, or on the amount of water taken in by the oesophagus. Any condition in the medium, influencing the feeding or the absorption of water, will influence the relative rate of pulsation. Most of the observations on the relative rate, made by investigators, have been made incidentally, while the animals were being studied under various experimental conditons and not in the normal culture medium, and hence not under normal feeding conditions, as in the studies of EISENBERG (1925); or they have been made on animals trapped in small spaces or held by mechanical pressure, or retarded by gelatin, agar, or, relatively low concentrations of HCl, as in CHILD's studies. All these conditions may interfere with the usual rate of feeding and water intake. This may account for the fact that most authors report a higher rate of pulsation for the anterior vacuole.

The dependence of the relative rate of pulsation on the extent of feeding may explain the difference in the relative rates of *Para-*

mecium multimicronucleatum and *Paramecium caudatum*. MÜLLER (1932) states that the cytostome of *Paramecium multimicronucleatum* is larger, and that it forms, in a given time, at least two and a half times as many food vacuoles as does *Paramecium caudatum*. It is likely that the food vacuoles are also generally larger and that more water is absorbed from the oesophagus, so that it is quite probable that the posterior vacuole disposes of at least three times as much water in *Paramecium multimicronucleatum* as in *Paramecium caudatum*. Under such conditions, a larger number of individuals of *Paramecium multimicronucleatum* can be expected to have a higher rate of pulsation in the posterior vacuole.

II. Relation between the rate of feeding and the rate of pulsation of the contractile vacuoles.

STEMPELL (1914) asserts that most of the variations in the rate of pulsation of the contractile vacuoles in different animals, and in the same animal are due to variation in the number of food vacuoles formed, and in the amount of water taken in with the food vacuoles. FORTNER (1926) expresses the same opinion, but they present no evidence in support of this opinion. UNGER (1926) finds depression rythms in the life-cycle of *Paramecium*, during which the presence of a small number of food vacuoles in the animals is associated with a decrease in the rate of pulsation of the contractile vacuoles. ANDREJEWA (1931) offers some evidence that the rate of pulsation is directly related with the number of food vacuoles present in the animals, but the evidence is not conclusive. PORT (1927), maintains that the rate of pulsation is the same in well nourished animals and in animals which have been starved by isolation in pure water for 36 to 40 hours. The following paragraphs contain results obtained in detailed observations concerning this problem.

Methods.

Mass cultures of *Paramecium multimicronucleatum* were used. Three of these cultures were studied until the food supply became greatly diminished or exhausted, one for 21 days, one for 22 days, and one for 29 days. Eleven other mass cultures were studied for periods of time varying from two days to sixteen days. In all 790 individuals were observed, forming a total of 4464 food vacuoles, an average of 5.7 food vacuoles for each individual. The study consisted in removing approximately 10 animals and particles of

food to the usual vaseline enclosure, selecting a stationary, feeding individual, simultaneously observing and timing the rate of pulsation of the posterior contractile vacuole and the rate of formation of the food vacuoles, and repeating this with other specimens on several slides until a record was obtained for 10 to 12 individuals each day. By means of an ocular micrometer measurements were made of the length and width of each animal, of the diameter of the food vacuoles just before ingestion, and of the diameter of the contractile vacuoles when at maximum distention.

Results.

a) Variation in the number and the size of the food vacuoles.

The number of food vacuoles present in the animals varied from day to day and often in different animals on the same day. During the first three or four days all the animals were usually filled with food vacuoles, which sometimes numbered a hundred or more. From then on to about the eighth day the number varied in different cultures and also in different individuals of the same culture, from 10 to 50. After that the number decreased irregularly, varying first from 5 to 15 for different individuals, then 3 to 10 and finally 2 to 3 or none by about the twenty-first day.

The size of the food vacuole varied considerably. When the culture was flourishing the average size of the food vacuole, just prior to its release into the endoplasm, varied from 17.25 microns to 25 microns, though vacuoles of the following sizes were observed: 27.60, 31.00, 34.50, 37.95, 41.40, 44.85, 51.75, and 65.55 microns. Though the animals varied in size from 330 microns to 227 microns, their size bore no relation to the size of food vacuoles formed. As the cultures began to decline on about the fourteenth day, and thereafter became gradually depleted, the animals became more and more emaciated and the size of the food vacuole decreased, first to 13.80 microns and then progressively to 10.35, 6.90 and 3.45 microns, though some larger vacuoles were formed at the same time. This decrease in size will be referred to again presently.

b) Variation in the rate of formation of food vacuoles.

The time required for the formation of the food vacuole also varied considerably. The shortest time observed was 17 seconds and the longest 365 seconds. This variation did not depend upon

the size of the animal nor usually upon the size of the food vacuole formed, but probably upon the quantity and quality of the bacteria present in the immediate vicinity of the animals. Unusually large food vacuoles, however, often required an unusually long time for their formation. Thus one food vacuole, 41.40 microns in size, required 141 seconds for its formation.

There was a minimum variation at the middle of the culture period, and a greater variation both at the beginning and at the end of the culture period. This fact is correlated with the rate of formation of the food vacuoles. By referring to Figs. 1 and 2 it will be seen that the rate of food vacuole formation is highest at the middle of the culture period and lower at the beginning and at the end. This shows that, the higher the rate of food vacuole formation, the less the variation in the rate of formation.

c) Variation in the size of the contractile vacuoles.

During the first two or three days of the culture the size of the contractile vacuoles measured approximately 17.25 microns, the posterior vacuole being nearly always slightly smaller. Occasionally on the third, and usually on the fourth day, and for two or three days after that, the size of the contractile vacuoles in many individuals increased to 18.20 or even to 20.70 and occasionally to 21.50 microns. After this increase in the size of the contractile vacuoles, there occurred, on the tenth day in some cultures, and on the twelfth or fourteenth day in others, a gradual decrease in the size of the vacuoles to 17.25 microns, though the posterior vacuole often measured only 15.52 microns. With succeeding days the vacuoles became progressively smaller, the anterior and posterior vacuoles measuring respectively 15.52 and 13.80, 13.80 and 13.80, 14.00 and 10.35, 10.35 and 8.62, 8.62 and 8.62, and finally 8.50 and 7.90 microns. These changes in the size of the contractile vacuoles will be referred to again presently.

d) Relation between the rate of feeding and the rate of formation of the food vacuoles.

Reference to figures 1 and 2 reveals that usually the rate of pulsation paralleled the rate of formation of the food vacuoles, increasing and decreasing with it on different days. This indicates that there is a direct relation between the two rates. However figure 1 shows that a decrease of 6.0 seconds in the rate of feeding (from 59.0 seconds on the 4th day to 65.0 seconds on the 5th day)

was accompanied by a decrease of only 0.1 second in the rate of pulsation (from 8.6 to 8.7 seconds); and that an increase of 5.4 seconds in the rate of feeding (from 65.0 seconds on the 5th day to 59.6 seconds on the 6th day) was accompanied by an increase of 0.7 seconds in the rate of pulsation (from 8.7 to 8.0 seconds). This indicates that the increase or decrease in the rate of pulsation is not always proportional to the increase or decrease in the rate of feeding. Figure 2 shows that an increase of 2.3 seconds in the rate of feeding (from

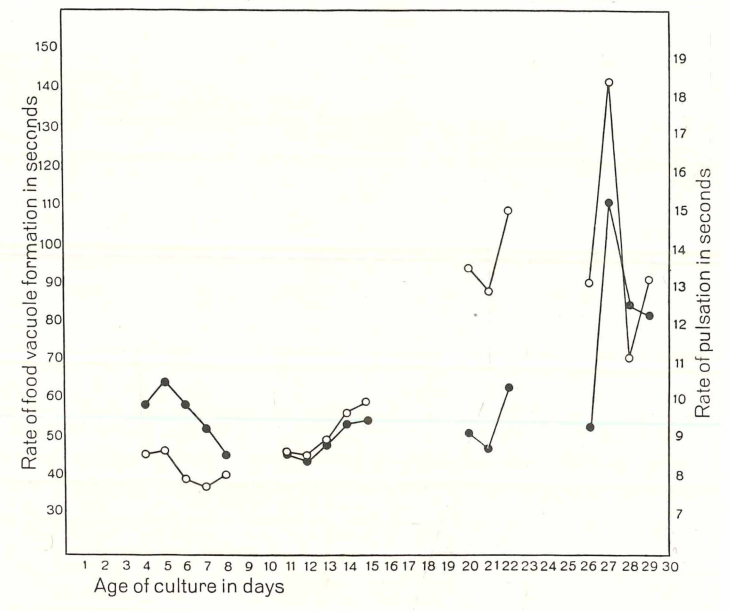

Fig. 1.

Figs. 1 and 2. Relation between rate of feeding and rate of pulsation of the contractile vacuoles on successive days, in individuals taken from a mass culture, on the days noted. Each solid circle represents the average rate of feeding of approximately ten individuals on the day noted; the average of the individuals was ascertained by noting the time required to form from 5 to 10 or more food vacuoles. Each clear circle represents the average rate of pulsation of all the consecutive pulsations of the contractile vacuole which occurred during the entire time the rate of formation of the food vacuoles was being observed.

53.2 seconds on the 4th day to 50.9 seconds on the 5th day) was accompanied by a decrease of 0.8 seconds in the rate of pulsation (from 9.7 to 10.5 seconds). This indicates that occasionally the rate of pulsation actually decreased while the rate of feeding increased.

Occasionally the opposite happened; the rate of pulsation increased while the rate of feeding decreased.

These facts find a probable explanation in the gradual increase in the diameter of the contractile vacuoles during the first half of the culture period, from 17.25 to 21.50 microns, as mentioned previously. At the same time that the rate of food vacuole formation

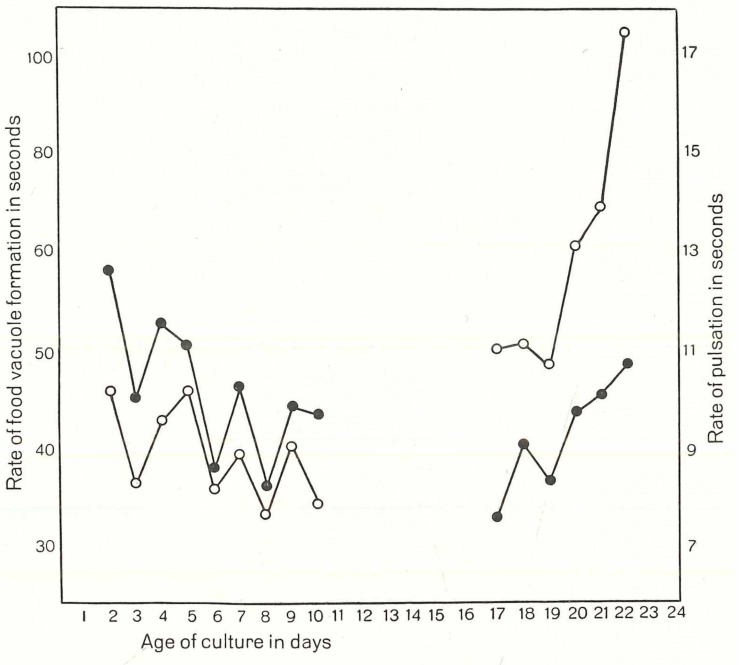

Fig 2.

was increasing, the size of the contractile vacuoles was increasing. The relatively larger amount of water taken in by the increased rate of feeding was compensated for, not merely by a higher rate of pulsation, but also by an increase in the size of the contractile vacuoles, which enabled the animal to eliminate a larger amount of water per unit pulsation. As a result, the rate of pulsation needed to be increased only slightly in most instances, and could even be decreased if the increase in the size of the contractile vacuoles was sufficiently great. As a further result there was no definite or unit rate of pulsation for a definite or unit rate of food vacuole formation. The rate of pulsation was often the same for two different rates of food vacuole formation, and was often different for the same rate

of food vacuole formation, due to the difference in the size of the contractile vacuoles on different days.

From the 11th to the 29th day in figure 1, and from the 17th to the 22nd day in figure 2 the rate of pulsation decreased relatively much more than the rate of feeding. Moreover, during this period of the cultures, as mentioned above, the size of the contractile vacuoles decreased in diameter from 17.25 to 7.90 microns. This relatively larger decrease in the rate of pulsation despite a decrease in in the size of the contractile vacuoles is explained by a still greater decrease, during this period, in the diameter of the food vacuoles, namely from 25.0 to 3.45 microns. The contractile vacuoles gradually decreased in size to 0.76, 0.53, 0.23, 0.13, and 0.1of their original volume; the food vacuoles decreased gradually in size to 0.29, 0.17, 0.07, 0.02 and 0.002 of their volume. This indicates that the food vacuoles decreased in size simultaneously with the contractile vacuoles, but that the decrease in the size of the food vacuoles was relatively much greater. This relatively much greater decrease in the size of the food vacuoles explains the relatively greater decrease in the rate of pulsation, even though the size of the contractile vacuoles decreases.

The results obtained in the study of the other eight mass cultures were the same as those presented in figures 1 and 2. During 107 out of the 123 days of observation, the rate of pulsation paralleled the rate of formation of the food vacuoles, increasing and decreasing with it. During the remaining 16 days, when the rate of pulsation did not parallel the rate of formation of the food vacuoles, there was probably a change in the size of one of the vacuoles without a corresponding change in the size of the other, due probably to the conditions prevailing in the cultures on those days.

Conclusion and Discussion.

The rate of pulsation of the contractile vacuoles varies directly with the rate of formation of the food vacuoles. There is no definite fixed rate of pulsation for a fixed definite rate of formation of food vacuoles. The rate of pulsation depends not only on the number of food vacuoles formed per unit time, but also on the size of the food vacuoles and on the size of the contractile vacuoles. The size of the contractile vacuole is apparently adjusted to the number and size of the food vacuoles. The variations in the size of the contractile vacuoles and of the food vacuoles on different days accounts

for the varying relations of the rate of pulsation and the rate of formation of food vacuoles on different days.

UNGER (1926) and ANDREJEWA (1931) maintain that the rate of pulsation of the contractile vacuole varies directly with the number of food vacuoles present in the animal. The results presented above do not support their contention. When the rate of feeding and the rate of pulsation were observed simultaneously, it was found that animals containing only a few vacuoles or even none, e. g. animals which have just completed fission and contain no food vacuoles, may have as high a rate, and even a higher rate of pulsation than animals with a very large number of food vacuoles.

I have been unable to confirm PORT's (1927) statement that the average rate of pulsation is the same in well fed and in starving animals, though the rate of isolated and individual pulsations may be the same. Even when emaciated animals from declining cultures are transferred to a vaseline enclosure with masses of food, it is found that the rate of pulsation is lower in them than in well fed animals, though both are forming food vacuoles at the same rate. However, if the underfed animals continue to feed, the rate of pulsation is gradually increased. Thus in one slide in which all the animals were emaciated, the time interval between pulsations in the first animal studied was 15.5 seconds; that of the second animal studied, ten minutes later, was 12.00 seconds; that of the third animal, ten minutes later, was 9.8 seconds; and that of the animals studied still later varied between 9.5 and 8.5 seconds. In these animals the rate of pulsation is related not only to the rate of feeding, but also to the amount of feeding which preceded the time of observation.

III. Relation between locomotion and the rate of pulsation of the contractile vacuoles in *Paramecium multimicronucleatum.*

PÜTTER (1900) and KAMADA (1935) state that locomotion increases the rate of pulsation of the contractile vacuoles in *Paramecium caudatum.* ANDREJEWA (1931) maintains that locomotion increases the rate of formation of food vacuoles and thus results in an increased metabolism and an increased rate of pulsation of the contractile vacuoles. Evidence opposed to the contentions of these authors is presented here for *Paramecium multimicronucleatum.*

Methods.

The observations were made on animals immediately after having been put into vaseline enclosures and on animals which had been in such enclosures for from 5 hours to 38 days. In all 39 animals were observed for a total of 1387 pulsations. As many consecutive pulsations as possible were observed for each animal, the average number being 35, and the highest being 118. Of these 1387 pulsations 401 occurred during locomotion or immediately after cessation of locomotion, an average of 10 per animal.

The observation of a large number of consecutive pulsations of the contractile vacuole during locomotion was made possible by the use of critical illumination and a mechanical stage. Practice in the rapid adjustment of the focus of the microscope according to the movements of the animal, and in automatic manipulation of the mechanical stage, greatly facilitated the observations on moving animals. As the contents of the contractile vacuole were expelled, and the cytoplasm flowed in from all sides to fill the space formerly occupied by the vacuole, the critical illumination produced the effect of a shadow passing over this region. Even though the vacuole was only obscurely visible, its contraction could be detected from this shadow effect. Familiarity with this phenomenon made it possible to follow the pulsations in moving animals even when the vacuole was not clearly visible. Even so it was very difficult to follow swimming animals during the first hour after the slide had been set up, and several hours were often spent on several preparations to obtain a single record. As the slide cultures became older the rate of locomotion of the animals decreased more and more until it became a leisurely forward movement with slow rotation, sometimes alternately clockwise and counter-clockwise. Shortage of food caused a gradual emaciation of the animals and resulted in the cytoplasm becoming very clear and transparent, so that the contractile vacuoles were sharply visible.

Results.

a) Types of Locomotion.

Resting animals often fed for fifteen minutes or longer. When they did move, they either (a) swam off and continued swimming for a minute or more before they again settled down to feed, or (b) they moved off but stopped every few seconds to sample the medium and continued to do this for several minutes, or (c) they

interrupted their feeding only occasionally by very short periods of locomotion of only a few seconds duration. In (a) the animal moved on a spiral course while the body rotated on its long axis. This method of locomotion obtained in covering long distances; it will be designated "swimming". In (b) the animal sometimes moved on a spiral course, but much more frequently it moved forward in a straight or curved line in contact with the substratum, and hence without any rotation of its body on its long axis. This method obtained in covering shorter distances, as when moving between neighboring masses of food; such interrupted movement, whether spiralling or not, will be designated "crawling". In (c) the usual method of locomotion was the same as in (b), but since it occurred only at relatively long intervals and for only a few seconds, it will be designated "spasmodic movement".

The relation between the various locomotor movements and the rate of pulsation of the contractile vacuoles is presented in tables 4 to 7.

In the tables, "Designation of individual" e. g. 11 B II, indicates the number of days of enclosure (11), the number of the slide (B), and the number of the individual observed on that day (II).

b) The effect of continuous swimming on the rate of pulsation of the contractile vacuoles.

Table 4 shows that during continuous swimming there were three types of pulsations of the contractile vacuoles.

Type I. The vacuoles contracted just before the animal began to swim, then, a small, clear area appeared in their place, after which the animal began to swim. While the animal was swimming, this minute clear area either did not enlarge or did so only very slowly, even though the animal swam about for several minutes. During this time the radial canals were clearly visible as distinct channels often extending more than half the length of the body of the animal. As soon as the animal came to rest, the minute vacuoles began to enlarge, and then soon reached normal size and contracted after which the rate of pulsation was nearly the same as it was before the animal began to swim. (Table 4, I.)

Type II. The vacuoles contracted just before the animal began to swim but they slowly and gradually enlarged to normal size and contracted before the animal came to rest. Several such pulsations could occur before the animal came to rest. When the animal came to rest, the vacuoles again pulsated at the rate which obtained before the swimming. (Table 4, II.)

Table 4.

Relation between the rate of pulsation of the contractile vacuoles and swimming. Each line of the table contains intervals between consecutive pulsations in the order given and under the conditions indicated by the headings.

Designation of individual	Interval between consecutive pulsations in seconds		
	At rest	Swimming	At rest
	Type I		
11 B II	27	148	13
	6	150	15
	17	253	15
16 B II	20	138	30
13 C I	25	80	18
	29	111	12
	25	120	10
	32	170	14
	15	140	16
13 C III	23	132	15
24 B	36	100	32
26 A I	42	253	55
	38	311	33
	22	200	25
26 B	20	155	20
29 A	41	120	35
	45	215	82
	45	164	46
31 A I	21	128	22
31 A II	21	67	25
Total average	27.5	157.7	28.1
	Type II		
24 A		123, 144, 105, 103, 365, 150	
	30	300, 210, 60	
22 B	27	93, 50	27
24 B		190, 145, 90	30
26 A I	48	288, 110	45
	45	143, 187	51
29 A	34	146, 80	23
	24	103, 78	22
Total average	32.4	141.7	34.8
	Type III		
29 A	32	26, 26, 27, 24, 165	45
	34	36, 34, 158	30
30 A	20	50, 190	50
30 A	19	25, 110, 145	44
31 A I	20	20, 20, 50, 120	20
Total average	26.5	66.8	28.5

Type III. The vacuoles continued to function at the resting rate for one or more pulsations after the animal had begun to swim. But they then contracted and remained contracted or grew only very slowly and did not pulsate again until the animal came to rest after which the pulsation proceeded at the former resting rate. (Table 4, III.)

Table 4, Type I and II shows that swimming produced a very marked decrease in the pulsation rate. This decrease began suddenly when swimming commenced and stopped suddenly when swimming ceased. The pulsation rate after the period of swimming was approximately the same as it was before the swimming began.

Table 4, Type III shows that though the first few pulsations that occurred while the animal was swimming might function at the normal resting rate, continued swimming produced as in Type I and II, a very marked decrease in the pulsation rate.

c) The effect of "crawling" on the rate of pulsation of the contractile vacuoles.

Table 5 shows that either no pulsations occurred while the animals were "crawling", or that the time intervals between the pulsations that occurred while the animals were "crawling" were usually longer than those which occurred while the animals were resting; that if they were not longer, they gradually became so, if the animals continued "crawling", and that, after "crawling" had ceased, the rate returned to that which obtained before "crawling".

d) The effect of "spasmodic movements" on the rate of pulsation.

Table 6 shows that "spasmodic movements" could decrease or increase the rate of pulsation.

Conclusion.

The frequency of the pulsations of the contractile vacuoles varies inversely with the nature and extent of the locomotor movements. Both swimming and "crawling" decrease the rate of pulsation, but swimming decreases the rate more than "crawling" does. The extent of locomotion necessary to produce this effect varies at different times, but usually the effect is produced immediately. "Spasmodic movements" may decrease or increase the rate of pulsation. This increase in the rate of pulsation by "spasmodic movements", and

Table 5.

Relation between the rate of pulsations of the contractile vacuoles and "crawling" Each line in the table contains intervals between consecutive pulsations in the order given and under the conditions indicated by the headings.

Designation of individual	Interval between consecutive pulsations in seconds		
	At rest	"crawling"	At rest
11 B II	17	80, 53, 70, 58, 32	15
16 B I	24	30, 53, 40, 45	20
	20	23, 29, 40, 35	29
16 B II	27	41, 37, 31	21
24 B	35	57, 65, 82	22
22 C	32	54, 84	25
29 A	45	90	42
	35	65	48
	38	168	58
31 A I	—	54, 85, 58, 133, 22	20
	26	27, 28, 160	47
	28	115	20
	21	94	25
	28	87	25
	24	127, 43	—
32 A II	50	580, 235	—
32 A III	—	68, 62	33
Total average	30.0	81.4	29.4

occasionally also by swimming and crawling for the first few pulsations, might be explained as follows.

According to JENNINGS (1904), attached, feeding animals are much less sensitive to external stimuli than swimming animals. It is probable that they are also less sensitive to internal stimuli, such as the internal hydrostatic pressure. If so, a higher internal pressure would be tolerated while feeding. If the animal then started to swim, it would become sensitive to the pressure and would continue to evacuate water for some time, even though it were not taking in any water. The swimming frequencies might be equal to or even higher than the resting frequencies. In the same manner, if the animal had quickly eliminated all of the excess water while swimming, and then settled down to feed, it might require some time for the system to fill up with water to such an extent as to cause a pulsation in the now less sensitive animal. Under such conditions the feeding frequencies would, for a time, be lower than the swimming frequencies.

Table 6.

Relation between the rate of pulsation of the contractile vacuoles and "spasmodic movement".

Each line in the table contains intervals between consecutive contractions in the order given and under the conditions indicated by the headings.

Designation of individual	Interval between consecutive pulsations in seconds		
	At rest	Spasmodic movement	At rest
	A. Increases pulse rate		
1/8 D	35	15,10	10
1/8 E	25	22	10
16 A	27	23	27
29 A	34	33,35	34
34 A	31	29,29	29
38 C	26	25	33
38 D	36	25	27
38 E	45	39	26
4 F	33	27,23	36
4 G	56	38,56	30
Total average	34.8	28.6	26.2
	B. Decreases pulse rate		
1/8 D	12	15	12
1/8 E	10	15	10
1/8 F	9	14	10
1/8 G	13	20	15
3 F	12	16	12
3 H	11	20	13
3 C	12	21	13
16 A	25	44	29
29 C	16	20,22	16
30 D	17	45,24	22
30 E	16	20,22	16
31 A I	21	24,22	28
38 C	26	33	36
Total average	15.3	23.3	17.8

Table 7 contains the average rate of pulsation in resting, "crawling" and swimming animals, both under favorable and unfavorable culture conditions, the number of animals studied and the total number of pulsations observed. It again shows that "crawling" and swimming greatly decrease the rate of pulsation, usual in resting animals, and that swimming decreases it more than "crawling" does.

Discussion.

Ludwig (1929) states that in swimming, the peristome cilia of *Paramecium* lash less forcibly than the body cilia, since they function in feeding and not in swimming. Mast and Lashley (1916) maintain that the feeding currents usually are interrupted during swimming.

Table 7.

Relation between locomotion and the average rate of the pulsation of the contractile vacuoles under various conditions.

The rate is given in terms of the time interval between pulsations in seconds.

Condition of animals	Favorable culture conditions (within 2 hours of enclosure)			Unfavorable culture conditions (after 5 hours to 38 days of enclosure)		
	Average rate	Number of pulsations observed	Number of animals observed	Average rate	Number of pulsations observed	Number of animals observed
Resting	9.9	17 881	724	28.2	1990	175
Crawling	34.6	150	14	68.1	179	29
Swimming	41.6	135	13	94.6	195	28

I have repeatedly confirmed these observations. Critical study showed that the same thing happened, though to a less degree, when the animals were engaged in "crawling" or in "spasmodic movements". All these locomotor activities therefore, either stop the feeding, or decrease the extent of feeding. This indicates that the effect of locomotion on the rate of pulsation is indirect; it is due to the fact that locomotion interrupts the feeding process, and thus diminishes the amount of water taken into the system.

PÜTTER (1900), and KAMADA (1935) did not observe the animals in the usual culture medium. PÜTTER added JENSON's jelly to slow up the motion of the animals. KAMADA's observations were made during a study of the effects of varying osmotic pressures on the rate of pulsation. Both methods can be presumed to have an effect on the pulsation rate, independent and different from the effect produced by locomotion alone. PÜTTER found only a difference of 4 seconds in resting and moving animals; KAMADA an average difference of 2 to 3 seconds, and some of the rates in resting animals were higher than those in swimming animals. Neither mentions the number of animals, nor the number of pulsations observed, nor whether the pulsations studied were consecutive. From the slight difference in the rates while resting and while swimming it is evident that both confined their observations to those locomotor activities which I have called "spasmodic movements". During such movements the rates while swimming may be either higher or lower than the rates while resting as was shown in table 6, especially if only isolated pulsations are observed.

ANDREJEWA (1931) did not observe the rate of locomotion and the rate of pulsation simultaneously. She first ascertained the rate of locomotion by the distance travelled in a given time, then

entangled the animals in cotton fibers and ascertained the rate of pulsation while the animals were resting, and inferred that the same rate of pulsation obtained while the animals had been swimming. Her conclusions are therefore the result of suppositions and not of direct observation. Only the simultaneous observation of the rate of locomotion and the rate of pulsation of the vacuoles can establish the relation between these two activities.

ANDREJEWA (1931) argues that swimming increases metabolic activity and LUDWIG (1929) calculates that this increase is equal to 1 %. Both conclusions are very doubtful. The relative expenditure of energy when swimming and resting, can be inferred from the amount of water displaced by each activity. India ink added to the medium showed that the swimming animal displaced very little water: only the particles of India ink in the immediate vicinity of the animal were disturbed; and that a resting, feeding animal displaced a much larger amount of water as was evidenced by the two extensive vortices created in the water, one on each side of the animal, and by the strong current rushing by the cytostome. Moreover, the animal actively retained its position while feeding, which required a further expenditure of energy. Apparently much more energy is expended in feeding than in swimming. If the rate of pulsation is dependent upon the rate of metabolism, as ANDREJEWA and most investigators assert, we should expect a higher rate of pulsation in feeding animals, than in swimming animals, which I have found to be true.

IV. Variation in the rate of pulsation of the contractile vacuoles.

PÜTTER (1900) reports that in a total of over 1100 pulsations the time interval between pulsations varied in different individuals from 6 to 43 seconds, and that the maximum variation in single individuals was 21 seconds. He probably observed only resting animals. My observations show that "crawling" and swimming result in much greater variations than those recorded by PÜTTER. The extent of the variations under all conditions is presented in Table 8. This table has been compiled from the records of all the animals observed by me, a total of 983 animals and 20530 pulsations.

This table shows the following: that the maximum variation in the rate of pulsation, both in single individuals and in different individuals was relatively slight in resting animals under favorable

Table 8.

Maximum variation in the rate of pulsation of the contractile vacuoles under various conditions.

The rate of pulsation is given in terms of the time interval between pulsations in seconds; the range of the variations in terms of the time interval between pulsations in seconds is enclosed in brackets.

Condition of animals	Under favorable culture conditions (within 2 hours after enclosure)			Under unfavorable culture conditions (after 5 hours to 38 days of enclosure)		
	In the same individual	In different individuals	In average rate	In the same individual	In different individuals	In average rate
Resting	12 (15—27)	21 (6—27)	5 (8—13)	100 (20—120)	14 (6—120)	72 (10—82)
"Crawling"	62 (25—87)	73 (14—87)	22 (34—56)	144 (22—168)	158 (10—168)	127 (11—138)
Swimming	94 (10—104)	98 (10—120)	43 (35—63)	262 (103—365)	355 (10—365)	175 (12—187)

culture conditions, namely 12 and 21 seconds respectively; but that the variation was greatly increased by crawling, namely 62 seconds for single individuals and 73 seconds for different individuals, and more by swimming, namely 94 and 98 seconds respectively; that, though the maximum variation in resting animals under unfavorable conditions was relatively great, namely 100 seconds for single individuals and 114 for different individuals, it was further increased by crawling, namely 144 and 158 seconds, and still more by swimming, namely 262 and 355 seconds respectively; that even under unfavorable culture conditions individual pulsations occurred at a rate of 10 seconds which is only slightly lower than the usual rate under favorable conditions. The table also shows that the maximum variation in the average rate of pulsation of resting individuals was similarly increased progressively by "crawling" and swimming in favorable culture conditions and more so in unfavorable culture conditions. This demonstrates that the extent of the variations in the rate of pulsation depends upon the culture conditions and on the locomotor activities of the animals.

Figure 3 contains a graphic representation of the variations in the rate of 118 consecutive pulsations in a single individual after 10 days enclosure. It shows that the resting rates varied from 15 to 63 seconds; the crawling rates from 66 to 123 seconds; the swimming rates from 30 to 152 seconds. It also shows very definitely that, in order to obtain even an approximately correct idea of the

rate of pulsation in this individual it was necessary to observe as many consecutive pulsations as possible; that isolated, individual pulsations would have been of little value, and that the rate ascertained by observing only a few of the pulsations of this individual would depend entirely upon which of the 118 pulsations had been observed.

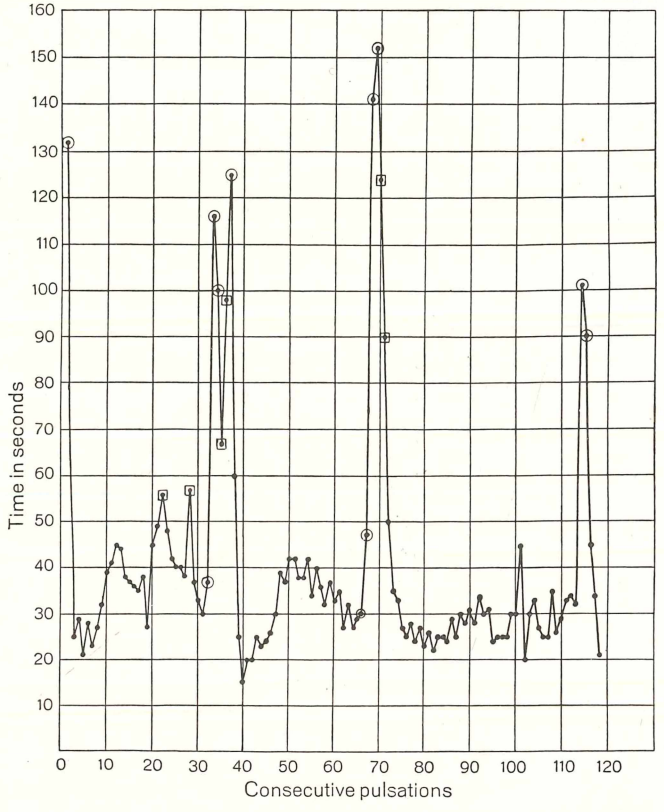

Fig. 3. Time intervals in seconds between 118 consecutive pulsations of individual 10 B I, showing the relation between the rate of pulsation and the time of observation. ●, at rest; ⊡, crawling; ⊙, swimming.

Table 9 shows that relatively high rates and very low rates of pulsation occurred, as early as 3 hours after enclosure, and at all times later and that there was no direct relation between the age of the culture and the variation in the rate of pulsation, except that the variations tended to become greater as the culture grew older. This is probably due to the fact that, as the culture grows

older, the food becomes more scarce, which results in less feeding and more search for food and consequently greater variation in the intake of water.

V. Relation between the rate of pulsation and absorption of water from the oesophagus.

Introduction.

The results obtained in the simultaneous observations on the rate of feeding and the rate of pulsation, indicate that the rate of pulsation is closely correlated with the number and size of the ingested food vacuoles. However, during the course of this work it was observed that in some animals which were at rest but did not form food vacuoles, pulsation of the contractile vacuoles continued for a period of over an hour, though at a greatly reduced rate, the average rate being 60 seconds; and that in dividing individuals, which did not form food vacuoles for half an hour, and in conjugating individuals, which did not form food vacuoles for 8 hours or more, the pulsations of the contractile vacuoles continued at the same or only slightly lower rate than obtains when food vacuoles are being formed. This indicates that the ingestion of food vacuoles is not the sole factor determining the rate of pulsation; and that it at most occasions variations in the rate.

HARTOG in 1888, influenced by the apparent absence of contractile vacuoles in marine protozoa, proposed the osmoregulatory theory of the origin and function of the contractile vacuoles, which has been almost universally accepted and defended by succeeding investigators, among them YASUDA (1900), DEGEN (1905), PÜTTER (1905), KANITZ (1907), KHAINSKY (1910), STEMPELL (1914, 1924), HERFS (1924), LLOYD (1928) and ANDREJEWA (1931). According to this theory the pellicle of *Paramecium* is a semipermeable membrane; there is, owing to the hypertonicity of the cytoplasm, a constant endosmosis of water from the medium into the cytoplasm which necessitates the development of contractile vacuoles to prevent undue distension and injury to the animal; the rate of pulsation of the contractile vacuoles is directly and mechanically determined by the rate of endosmosis through the pellicle; any factor which increases or decreases the endosmosis, e. g. change in the osmotic concentration of the medium, or changes in the metabolism of the animal, owing to change in temperature, locomotion or rate of feeding, mechanically changes the rate of pulsation; the amount of water ingested with the food va-

Table 9.

Relation between variation in the rate of pulsation of the contractile vacuole and the age of culture.

Only the maximum and minimum variations observed each day are listed.

Time under cover glass in days	Designation of individual	Range of variation in seconds	
		Maximum	Minimum
1/8	B II	10—104	
	B I		6—9
1	A III	15—110	
	C I		10—15
2	A I	18—123	
	C II		9—10
3	A III	10—80	
	C II		10—12
4	A V	20—120	
	B IV		12—17
5	A IV	12—74	
	A II		8—18
8	A III	19—75	
	B III		11—28
9	A IV	60—115	
	B II		15—80
11	B III	6—253	
	B I		7—13
13	C I	10—170	
	C III		15—142
16	B II	11—138	
	A II		20—115
22	C I	15—102	
	B I		13—93
24	A I	25—365	
	B I		10—190
26	A I	20—311	
	A II		13—26
29	A I	21—215	
	B I		12—25
30	A I	19—190	
	C I		15—45
31	A I	20—160	
	B I		10—16
32	A I	49—210	
	A III		33—68
37	A I	33—244	
	A II		14—55

cuole amounts at most to one-third of the total volume of water excreted by the contractile vacuoles; the remaining two-thirds of the water excreted in feeding animals, and all of the water excreted in non-feeding animals enters the animal by endosmosis through the pellicle.

Bozler (1924), Fortner (1924, 1926), and Müller (1932) admit the permeability of the pellicle, but contend that a part of the

water excreted is absorbed from the oesophagus. EISENBERG (1925) maintains that the pellicle is impermeable to water, and that all the water excreted enters by the cytostome, with the food vacuoles, and by absorption from the oesophagus during the formation of the food vacuoles.

The following paragraphs concern this problem.

Results.

a) Absorption of water from the oesophagus in feeding animals.

As demonstrated above, the pulsation of the contractile vacuoles often ceases during swimming, but is quickly resumed after the animal comes to rest and begins to feed. Close observation reveals that the contractile vacuoles, which contract immediately before the animal begins to swim and remain contracted during swimming, quickly enlarge after the animal comes to rest, and within 10 or 15 seconds begin to pulsate again regularly; but that the first and usually several succeeding pulsation of the vacuoles precede the actual ingestion of the first food vacuole. This indicates that the water expelled by the first few pulsations entered the animal, not by the ingestion of food vacuoles, but either by endosmosis or by absorption from the oesophagus while the food vacuole was forming.

b) Absorption of water from the oesophagus in non-feeding animals.

Observations on individuals which were not feeding, and on individuals in the process of fission or conjugation, but in which, as stated above, the contractile vacuoles continued to function, though no food vacuoles were formed, revealed the following facts: the feeding currents which, according to JENNINGS (1904) and others, are created solely by the cilia of the oral groove, were continuously present in all these individuals, but the solid particles were forcibly ejected from the currents before they reached the cytostome; and the cilia within the pharynx were in constant vibration. When a dilute solution of neutral red was added to the medium, the stain appeared first in the endoplasm adjoining the oesophagus, then in the posterior end of the animal, and from there it spread slowly through the entire animal; but the stain was confined to the peripheral layer of the endoplasm, as described by MÜLLER (1932) for feeding animals, and it never appeared in the ectoplasm. This indi-

cates that, though no food vacuoles were being formed, water was drawn into the oesophagus, and absorbed from the oesophagus, as in feeding animals; and that the regular and constant pulsations of the contractile vacuoles was due to the constant absorption of water from the oesophagus.

c) The cytostome and its relation to the rate of absorption of water from the oesophagus.

The complete cessation of pulsation during swimming for as long as five minutes, demonstrated above, indicates that the absorption of water from the oesophagus can be controlled by the animal. Evidence concerning the mechanism of such a control is contained in the following observations.

The cytostome of *Paramecium* is a narrow slit in the floor of the oral groove, bounded by a raised, thickened border in the form of an elongated oval, somewhat like lips.

An exconjugant individual, which had just separated from its partner was kept under constant observation under the oil-immersion lens for one and a half hours. During all this time it did not form any food vacuoles. The cilia of the oral groove were creating the usual feeding currents, and the cilia within the pharynx were in constant vibration. The body of the animal seemed turgid and the cytoplasm appeared very dense and viscous. Strong convulsive movements were evident in the endoplasm in the region of the oesophagus. At the same time the lips of the cytostome were rising and falling, opening and closing. Sometimes the closing of the lips began at one corner of the cytostome and passed like a peristaltic wave to the other corner This shows that the size and shape of the opening of the cytostome varies, and that the opening can be closed. The whole performance of the lips and of the endoplasm around the oesophagus had the appearance of an attempt to take water in through the cytostome and to force it into the body, though no vacuoles of any kind were formed. With the continued activity of the cytostome and the endoplasm the viscosity of the endoplasm gradually decreased and about an hour and a half after separation from its partner, the animal began to ingest food vacuoles. This seems to indicate that the decrease in the viscosity of the endoplasm was due in part at least to the active ingestion of water by the cytostome. Similar movements of the cytostome have been observed by me in animals still conjugating. METALNIKOFF (1912) states that

the cytostome is permanently open, but he gives no proof for this assertion.

The ability to open and close the cytostome affords an explanation of the variation in the action of the contractile vacuoles described above in swimming animals. For example, in what was called type I, in which no pulsations occurred during the entire period of swimming, sometimes for as long as 5 minutes, the cytostome was closed during the entire time, and no absorption of water took place. In type II, in which the pulsations continued during swimming, though at a greatly reduced rate, the cytostome was open part of the time, probably to test the surrounding medium for food, the amount of water absorbed, and therefore the rate of pulsation, depending upon the amount of testing.

d) Impermeability of the pellicle of *Paramecium* to water.

According to the adherents of the osmoregulatory theory at most one third of the water expelled by the contractile vacuoles is ingested with the food vacuoles; the remainder enters by endosmosis. The rate of pulsation due to endosmosis alone should, therefore be equal to two-thirds of the usual rate, unless the water ingested with the food vacuoles lowers the osmotic pressure of the cytoplasm and decreases endosmosis, in which instance the rate of pulsation should be the same in feeding and non-feeding animals. My results show that when the rate of feeding is greatly decreased or when feeding stops, as it does in most swimming individuals, the rate of pulsation is decreased not by one third, but to one-tenth or still less, and that often the pulsations cease entirely. This proves that if the osmoregulatory theory holds and endosmosis of water through the pellicle occurs, great and almost instant changes in osmotic gradient, correlated with feeding and non-feeding must be postulated.

All investigators hold that the rate of metabolism is higher during swimming, and results in a greater quantity of osmotically active substances in the cytoplasm, and that therefore, according to the osmoregulatory theory, the rate of pulsation should be higher in swimming than in resting animals. My results show that during swimming the rate of pulsation is greatly decreased, and that often the pulsations cease entirely. Even though, as has been shown above, the rate of metabolism is lower in swimming animals, this fact would not account for the great decrease in the rate of pulsation if endosmosis occurs to the extent postulated by the osmo-

regulatory theory, unless great and almost instant changes in osmotic gradient, correlated with rest and activity are postulated. Such changes in osmotic gradient are highly improbable. The facts presented warrant the conclusion that endosmosis does not occur in *Paramecium* and that all the water expelled by the contractile vacuoles enters by the cytostome. The conclusion that the pellicle is impermeable to water is supported by the following considerations.

SCHLIEPER (1930), in a critical review of the osmoregulatory theory of contractile vacuoles, states that, as yet, not a single convincing proof has been offered for the semipermeability of the cell walls of Protozoa, and that the constant stream of water through the animal may also be due to active intake and output on the part of the animal. HAYES (1930) asserts that 90 percent of marine Protozoa have contractile vacuoles. HERFS (1922) notes that *Opalina*, which has no mouth, also lacks a contractile vacuole, while *Balantidium*, which has a mouth, also has a contractile vacuole. TAYLOR (1923) shows that in *Euplotes* the accessory vacuoles, which form the contractile vacuole, most commonly arise near food vacuoles, and for this reason he considers the oral region the main point of entry of water. MACLENNAN (1933) in a critical study of the contractile vacuoles in ciliates from the stomach of cattle (*Ophryoscolecidae*) concludes: "The vacuolar fluid is mainly derived from excess fluid taken in by the cytostome during feeding. The general pellicular covering of the body is relatively impermeable and admits little if any fluid. Excretion by diffusion through the pellicle is unlikely." SPEK (1921) and HÖBER (1926) show that the pellicle of Protozoa is impermeable to balanced salt solutions, and that penetration of the pellicle by single salt solutions, often used in ascertaining the relation between osmotic pressure and the rate of pulsation of the contractile vacuoles, is a pathological process. EISENBERG (1925, 1929), FORTNER (1925), KAMADA (1935) and CHEJFEC (1935) maintain that in *Paramecium* increase in the osmotic pressure of the medium does not immediately affect the rate of pulsation as it should if the rate were mechanically determined by the osmotic gradient between the medium and the cytoplasm of the animal; that it requires at least a triple increase in osmotic pressure to produce any noticeable decrease in the rate of pulsation; and that, even if the animal is put into a medium, whose osmotic pressure produces a very marked decrease in the rate of pulsation, it adapts itself gradually to the changed osmotic conditions, and the rate of pulsation gradually returns to the former rate. CHEJFEC concludes that the recovery

of *Paramecium* when put into strongly hypertonic media is not a mere adaptation to changed environmental conditions, but an active resistance and a conquest of the environment, so that the animal makes itself independent of the environment, and its life processes return to normal despite the changed environment.

The evidence presented indicates strongly that the pellicle of *Paramecium* is impermeable to water.

VI. The function of the contractile vacuole.

The direct correlation, observed in this work, between the rate of pulsation and the amount of water ingested by the cytostome, proves quite clearly that one of the functions of the contractile vacuole is the regulation of the water content of the cytoplasm of the animal. Most investigators contend that the contractile vacuole also functions as an excretory organ in the removal of the katabolic wastes. WEATHERBY (1927, 1929) finds that only 1 % of the nitrogenous wastes actually excreted leaves the body by way of the fluid expelled by the contractile vacuoles, and concludes that the contractile vacuole is solely an osmoregulator. The excretion of nitrogenous wastes by the contractile vacuole is supported by the following considerations.

If the pellicle of *Paramecium* is impermeable to water, as demonstrated above, it is also very likely, under the usual environmental conditions, impermeable to chemicals dissolved in water; and katabolic nitrogenous wastes do not leave the body by diffusion as WEATHERBY postulates; but they leave the body either by the contractile vacuole or by the anus. The latter is very unlikely, since only a small amount of water is eliminated by the anus. The elimination by the pellicle of neutral red in the form of droplets (MÜLLER, 1932) indicates that under abnormal conditions the pellicle may act as an excretory organ.

NASSONOW (1924, 1925), v. GELEI (1925, 1928), FAURÉ-FREMIET (1925) and FORTNER (1926) have demonstrated an osmophilic plasma, identical in staining properties with the nephridial plasma of the kidney cells of amphibians (KALMUS, 1931), enveloping the radial canals of *Paramecium* and other Protozoa; this they interpret as an excretory structure, active in the elimination of the nitrogenous katabolic products.

The rate of pulsation, necessary for the removal of the excretory products is difficult to ascertain, since nitrogenous wastes are, ordin-

arily, not excreted by organisms the instant they are formed, and according to LUDWIG (1928), are tolerated in very high concentrations.

However, decrease in the rate of metabolism, e. g. in swimming, and in starving animals, resulted in a decrease in the rate of pulsation largely due to decrease in the amount of water ingested, but probably also due to decreased metabolism.

The absorption of water, observed by me in resting, non-feeding individuals and in fission and conjugating individuals suggests a positive function for the water absorbed. All of these animals were creating the usual feeding currents, though they were forming no food vacuoles, and the energy expended by them was very probably equal to that of feeding animals. If the contractile vacuole functions as an excretory organ and the rate of pulsation is correlated with rate of metabolism, the rate of pulsation in these individuals should be practically equal to that of feeding animals, which I found to be true.

WEATHERBY'S contention that the contractile vacuoles do not function as excretory organs is based on the fact that NESSLER'S reagent, when injected into the contractile vacuoles of *Paramecium*, gave no test for ammonia, and that the contents of two vacuoles of *Spirostomum*, when treated with urease and NESSLER'S reagent gave an estimated content of urea to the amount of only 1 part in 100,000. Much more delicate tests have been devised since then, and until these tests have been applied, the evidence presented very strongly indicates that the contractile vacuole also functions in the removal of the nitrogenous metabolic wastes; that the absorption of water from the oesophagus is an active process, necessary for the removal of the nitrogenous wastes; and therefore occurs even in non-feeding animals.

Most investigators, beginning with SPALLANZANI in 1776, hold that respiration is also a function of the contractile vacuole. LUDWIG (1928) maintains that the amount of water that enters the animal is too small to supply the oxygen for its respiratory needs, and that most of the oxygen enters by diffusion through the pellicle. This problem needs further investigation.

The constant absorption of water, except for relatively short periods of swimming, even in non-feeding animals; its confinement to the surface layer of the endoplasm, as was shown above, from which an exchange of oxygen and carbon dioxide could readily take place; and its rapid elimination, suggest that the water may supply the oxygen needed for respiration, and remove the carbon dioxide.

The contractile vacuoles would then, together with the nephridial canals and the oesophagus, constitute a mechanism by which a regulated stream of water is passed through the animal, which supplies the oxygen for respiration and removes the carbon dioxide and the nitrogenous katabolic wastes. In another paper, evidence indicating that LUDWIG'S results are inconclusive, and that the contractile vacuole probably also has a repiratory function will be presented.

VII. Summary.

1. Observations.

In flourishing cultures the rate of pulsation of the posterior vacuole is usually higher than that of the anterior vacuole.

The percentage of animals in which this obtains decreases progressively as the culture conditions become unfavorable and during fission, and is zero in non-feeding animals.

The size, the rate of formation, and the variations in the rate of formation of the food vacuoles, and the size of the contractile vacuoles vary directly with the condition and the age of the culture.

The rate of pulsation of the contractile vacuoles varies directly with the rate of feeding; the relation between the rate of pulsation and the rate of feeding, at any particular time, depends upon the relative size of the food vacuoles and the contractile vacuoles.

The rate of pulsation of the contractile vacuoles is lower in active than in resting animals; the magnitude of the difference depends upon the kind and the extent of the locomotion.

"Spasmodic movements", a few seconds in duration, may either increase or decrease the rate of pulsation.

"Crawling", or locomotion, usually in contact with the substratum, between neighboring masses of food, and sometimes for longer distances, decreases the rate and sometimes stops pulsation.

Swimming on a spiral path over relatively long distances greatly decreases the rate and usually stops pulsation instantly.

The relation between locomotion and the rate of pulsation is indirect, i. e. the pulsations decrease in rate or stop because feeding decreases or stops.

Variation in the rate of pulsation in one vacuole is accompanied by simultaneous and similar variation in the other; the magnitude of the variations, both in single individuals and in different individuals, varies with the culture conditions and the activities of the animal, being greater in unfavorable culture conditions and during locomotion.

The size of the opening of the cytostome can be varied by the animal.

In a solution of dilute neutral red the endoplasm adjoining the oesophagus stains first. This obtains no matter whether the animals are feeding or not, or dividing or conjugating.

2. Conclusions.

The pellicle of *Paramecium* is impermeable to water and salts and probably to gases.

Absorption of water from the oesophagus of the cytopharynx occurs during the formation of food vacuoles, and also continuously in non-feeding, in dividing and in conjugating individuals; it can be regulated by changes in the size of the aperture of the cytostome and may cease, e. g. during swimming; it is the main factor determining the rate of pulsation of the contractile vacuoles.

The effect of many experimental factors on the rate of pulsation is probably indirect, i. e. they affect the rate of feeding.

The activities of the contractile vacuoles and the radial canals are correlated with the activities of the cytostome and the oesophagus to create a constant stream of water through the animal, which serves for the intake of the oxygen required for respiration, and for the elimination of the nitrogenous metabolic wastes and carbon dioxide; besides regulating the water content of the cytoplasm, the contractile vacuoles have a respiratory and excretory function.

Paramecium is a semi-isolated organism having, within limits, a control over its environment.

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