INDENTATION OF THE PELLICLE OF *TETRAHYMENA* AT THE CONTRACTILE VACUOLE PORE BEFORE SYSTOLE

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The contractile vacuole of fresh-water protozoa contributes to the maintenance of constant cell volume by eliminating fluid at a rate equal to the passive entry of water into the hyperosmotic cell (5, 8). In addition, after changes in environmental osmolarity, adjustments in the rate of fluid output of the contractile vacuole minimize changes in cell volume (8). At least a few species of protozoa show a third aspect of water regulation, i.e., the ability to return to the original cell volume after passive volume changes (2, 3, 6, 11). These regulatory mechanisms imply a feedback control system relating cell volume (and/or cell osmolarity) to the cyclic activity of the contractile vacuole and its rate of fluid output. There is little concrete evidence on the nature of any aspect of this control system. Even less well understood, but perhaps equally important, is the mechanism by which the contractile vacuole is involved in the regulation of intracellular solutes (2).

We have observed that the pellicle of *Tetrahymena* at the contractile vacuole pore indents immediately preceding systole, the expulsion of the vacuolar fluid. In a cell in which the expulsions were arrested temporarily, the periodic indentations of the pellicle persisted. We suggest that the indentations are a reflection of one aspect of the control mechanism by which the vacuolar cycle is regulated.

An indentation of the pellicle at the vacuolar pore before systole appears in recently published cinemicrographs of *Paramecium multimicronucleatum* (13), but the authors do not comment on the indentation.

MATERIALS AND METHODS

Tetrahymena pyriformis strain W were cultured axenically in 2% proteose-peptone, and were harvested by gentle centrifugation after 2–4 days' growth. The cells were immobilized for observation in the culture

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The figures are cinemicrographs of *Tetrahymena*, showing stages of the cycle of the contractile vacuole. The frames were selected from sequences taken at 2 frames/second with phase contrast optics. The prints are in negative contrast.

FIGURES 1–4 Sequences of normal cycles of four different cells. × 2000. In Fig. 4 the contractile vacuole is between two food vacuoles. The times of the frames after frame (*a*) in each sequence:

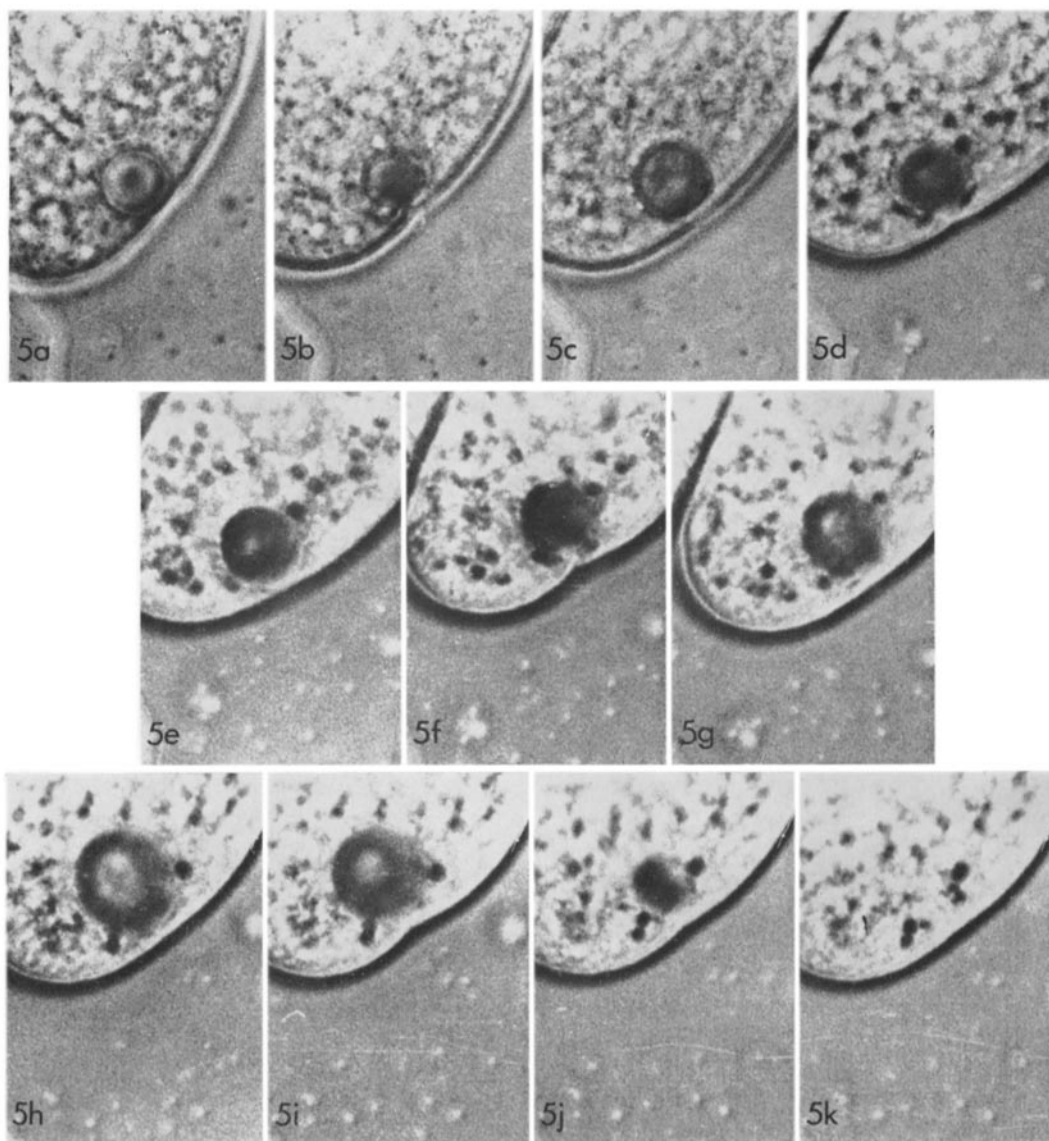| Frame | Seconds | | |
	(*b*)	(*c*)	(*d*)
Figure 1	3.5	5.0	6.5
2	2.0	2.5	—
3	1.0	1.5	—
4	2.5	4.5	5.0
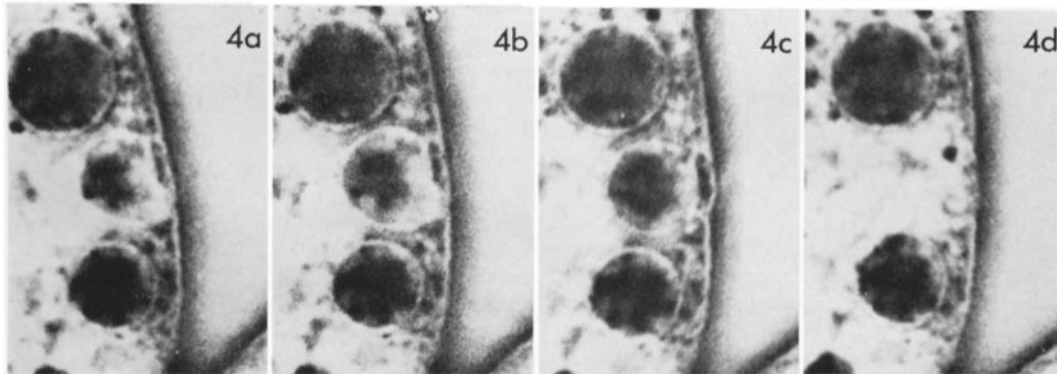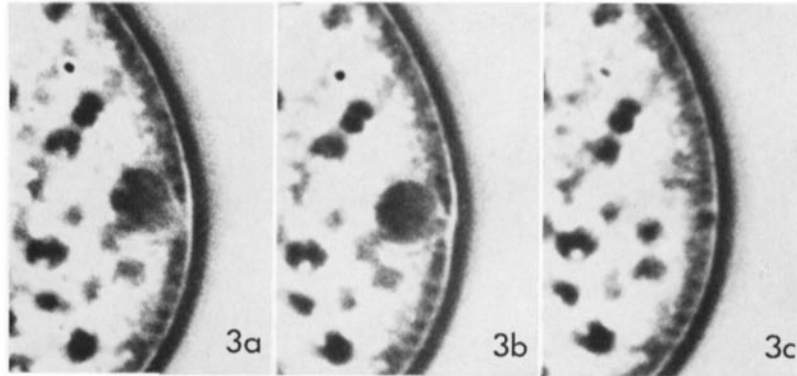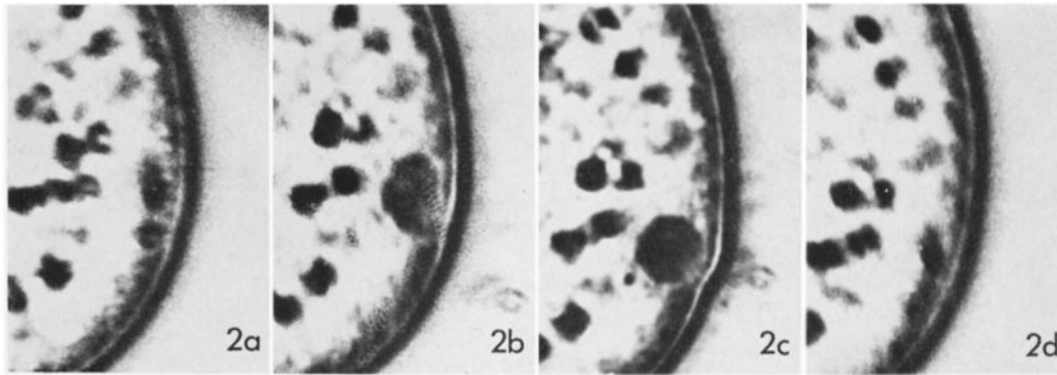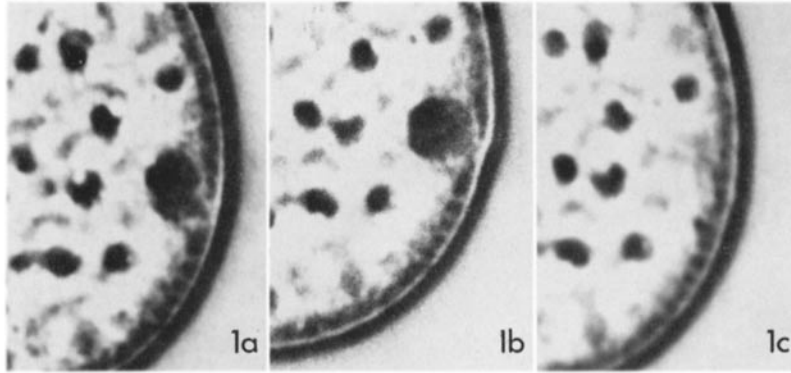
FIGURE 5 The contractile vacuole cycle in one cell in which systole was temporarily arrested. × 1200. Frames (b), (d), and (f) show three successive indentations of the pellicle in the sequence of six indentations during the period of malfunction. Frames (h) through (k) show the first normal cycle after the malfunction. The times of the frames after frame (a):

Frame	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
Seconds	4.5	10.0	15.5	20.5	25.5	28.5	54.5	56.5	57.5	58.5

medium under agar-coated coverslips or in tiny drops of culture medium under mineral oil. Sliding the coverslip permitted manipulation of the animals' orientation. The cells were observed and photographed in a Zeiss microscope equipped with phase-contrast optics at 500 or 1000 x magnification. Cine-micrographs were made on Kodak tri-X or plus-X reversal film with a Sage model 500 cinephotomicrographic apparatus and a Bolex H16 M camera. In all the records shown here, the framing speed was 2/sec, and the exposure time was 0.25 sec. The films were viewed and the frequencies of the various events were

determined with a LW photo-optical data analyzer. Frames were selected and printed in negative contrast.

OBSERVATIONS

When a *Tetrahymena* is oriented under the microscope with the light path tangential to the surface of the cell at the vacuolar pores, an indentation of the pellicle at the pores can be seen just prior to systole, which followed the onset of indentation within 1 sec. Figs. 1–4 and 5 *h–k* show selected frames from sequences of cinemicrographs of five different cells in which the indentations in the normal vacuolar cycle could be observed. That the indentation is at a pore is seen best in Fig. 4 *c*. The indentations in the five cells vary somewhat in form and extent, but they clearly represent the same phenomenon. When cells were held tightly under a coverslip or under oil, the indentations were less pronounced than when the cells were just partially immobilized.

The normal vacuolar cycle in *Tetrahymena* consists of five stages readily distinguished with the light microscope: 1) Filling of the vacuole from collecting canals during which the shape of the vacuole is somewhat irregular. The collecting canals are seen most clearly in cells in dilute medium when a high rate of fluid output is necessary. 2) A period during which there is no apparent increase in volume of the vacuole. This period is best seen in cells in a medium of high osmolarity in which the cycle is slow. 3) A change in shape of the vacuole from irregular to spherical. 4) Indentation of the pellicle, prior to any indication of the beginning of expulsion. 5) Systole and the simultaneous return of the pellicle to its unindented configuration.

The vacuolar cycle of *Tetrahymena* is typical for ciliates. For example, there are reports in other species of a time interval between attainment of maximum volume and systole (10, 16), and of the change in shape from irregular to spherical just before systole (7, 9, 10, 15).

The frames in Figs. 1–4 and 5 *h–k* were selected to show 1) the unindented pellicle adjacent to the vacuole after maximum volume is attained; 2) the indentation before the beginning of systole; 3) systole; and 4) return of the pellicle to the unindented configuration. In addition, Figs. 1–4 show the change in the shape of the vacuole from irregular to spherical. The collecting canals appear in Fig. 5, frames *h–k*. Also observed during the vacuolar cycle were changes in the collecting canals, an apparent reduction in vacuolar volume accompanying the change in shape, and a slight movement of the vacuole toward the cell surface just before systole (cf. 1, 9).

Fig. 5 shows selected frames from a sequence of cinemicrographs of a cell in which the contractile vacuole had been functioning normally after immobilization of the cell. For unknown reasons the systoles ceased for about a minute, but during this time the periodic indentations persisted. After six indentations the systoles resumed. Fig. 5 shows three successive indentations without subsequent systole (5 *a–g*) and the first normal vacuolar cycle after the period of malfunction (5 *h–k*). The mean length of the 12 vacuolar cycles before the malfunction was 9 sec (range 8–15 sec). The mean length of the 4 cycles after resumption of normal activity was also 9 sec (range 7–12). The mean interval between the onsets of the indentations during malfunction was 12 sec (range 8–16). Thus the frequency of indentations was not much affected by the cessation of systole.

DISCUSSION

The temporal relationship between the indentation of the pellicle at the contractile vacuole pore and systole suggests that the indentation is associated with triggering systole. Two events are necessary for systole: generation of a force on the contents of the vacuole and the opening of a pore. [There are usually two vacuolar pores in *Tetrahymena pyriformis*, but it is possible that only one of them opens at each systole (1). Fig. 4 *c* suggests that the indentation is at just one pore.]

The change in shape of the vacuole to spherical very likely reflects the exertion of a mechanical force on the contents of the vacuole. This occurs before indentation and systole, indicating that the force is not the immediate cause for the indentation or systole. Apparently the final event allowing systole is opening of a pore (cf. 1). We suggest that indentation, which precedes systole by about 1 sec, accompanies an event associated with the opening of a pore.

That the force on the vacuolar contents is manifested by a change in shape to spherical rather than a flattening may be inconsistent with the proposal that in *Tetrahymena* (12) and *Paramecium* (13) the force is generated in the adjacent cytoplasm, and not by the wall of the vacuole (cf. 14). A further consideration of this question is beyond the scope of the present paper.

The cause of the indentation may be a short-

ening of the "pore fibrils." These fibrils, observed in electron micrographs of *Tetrahymena* (1, 4), are arranged radially around the discharge canal [a cylindrical structure about 1 μ in diameter and length between the vacuole and the cell surface (1, 4)]. They are attached to the canal and to the surface of the contractile vacuole in such a way that their shortening might result in the indentation and opening of a pore by rupturing the membrane between the vacuole and the discharge canal. Fibrils similarly located have also been seen in *Tokophrya*, a Suctorian (15). An association of the fibrils in both *Tetrahymena* and *Tokophrya* with opening of a pore was suggested, but no indentations of the pellicle during the vacuolar cycle were reported (1, 4, 15). However, Cameron and Burton (1) observed, in electron micrographs, an apparent shortening of the discharge canal which may coincide with the indentation. As mentioned above, an indentation has been seen in the vacuolar cycle of *Paramecium* (13).

Fig. 5 shows that the indentation of the pellicle can occur without a subsequent expulsion of fluid, and that these indentations can occur with essentially unaltered periodicity, at least for a short time. Therefore, the indentations are separate

events, and are not simply caused by the beginning of systole.

The factor which determines when a systole is to be triggered is not a particular volume of the vacuole, since the maximum volume varies with external osmolarity (2, 7). Furthermore, there is usually a significant delay between the time when maximum volume is reached and systole. Frequency of systoles is also variable, and thus rate of fluid output is not dependent on a fixed systolic periodicity. Nevertheless, the persistent periodicity of the indentations during vacuolar malfunction suggests an inherent timing mechanism which triggers systole. Such a mechanism was postulated by Kitching (7). The rate of fluid output would be regulated by modulating the periodicity of the trigger for systole. The nature of the trigger, its relationship with the volume and/or the osmolarity of the cell, and most other aspects of the regulatory system remain unknown.

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