The Initial Movements of the Food Vacuole of *Paramecium trichium* STOKES.

By

Arthur N. Bragg, Norman, Oklahoma, U.S.A.

Detailed observations on the cycle of events within the bodies of Infusoria as the food vacuoles pass through the endoplasm have been reported many times. Especially is this true of members of the genus *Paramecium* (NIRENSTEIN, 1905; KHAINSKY, 1910; METALNIKOW, 1912; and others).

NIRENSTEIN describes in detail the movements of the food vacuole in *P. caudatum* as it passes from the cytopharynx. According to this author cyclosis increases in the region of the forming vacuole just before it drops away. The vacuole then elongates somewhat often assuming a spindle-like form, leaves the end of the pharynx, and passes rapidly toward the posterior end of the animal while rotating. It then ceases its movement, rounds out to a sphere, and takes up its cycle through the cell.

While no one so far as I have determined has specifically said so, the assumption apparently has been that all species of *Paramecium* form their food vacuoles in essentially the same manner. Some time ago while working on the methods of ingestion of various species of this genus, I found this not to be true. So different were the various species studied that I was able to distinguish species solely upon the manner in which the food vacuoles formed (BRAGG, 1935). One species, especially, was so markedly different from the others studied that I gave it detailed attention. This was *P. trichium* STOKES. Search of the literature revealed no account of ingestive phenomena in this organism.

Materials and Methods.

The organisms used were collected from wild sources and cultured in standard hay infusion at room temperatures. A drop of culture fluid containing the organisms was placed on a slide, first ringed with vaseline, and a cover-slip was placed gently upon this. Observations were made as the animals became quiet about masses of zoöglea or moved slowly along the bottom while feeding. Often carmine powder and a drop of neutral red (0.5 mg. per 1 ccm. distilled water, Shipiro, 1927) were added to the preparation in order to make the processes more visible. In all, about 1500 vacuoles were seen to form and to pass into the endoplasm. Without exception all of these followed the cycle outlined below whether the above mentioned reagents were added to the culture or not.

Observations.

Preliminary observations in *P. caudatum* confirmed the essentials of the process of ingestion as described by Nirenstein but with this exception. I could demonstrate no increase in cyclosis except on the ventral (oral) surface of the cytopharynx before the vacuole dropped away. Also, the rate of the vacuole's passage to the posterior end of the animal appeared to me greater than the rate of movement of the endoplasmic granules about it. The significance of these things will be discussed later.

In *P. trichium* the cycle of events is as follows: just before the vacuole is about to fall from the cytopharynx cyclosis suddenly increases on all sides of the pharynx anterior to the forming vacuole. The granular stream passes posteriorly, pouring over and around the vacuole, and spreads into the posterior region of the cell. Just at the instant that the moving granules reach the vacuole the latter starts rotating while still maintaining its general position at the end of the cytopharynx. The vacuole makes about three very fast revolutions and then leaves its position and passes rapidly away while still rotating. Its path through the endoplasm is always dorsad as well as toward the posterior region; it never goes directly to the posterior end of the animal as it does in *P. caudatum* or *P. aurelia*. The path is, therefore, diagonal to that of the endoplasmic stream and the vacuole comes to rest outside this stream in the more solid endoplasm dorsal to it. The whole effect is as though the streaming granules twist the vacuole from the place of its formation at the posterior end of the cytopharynx and throw it violently posteriodorsad into the more solid endoplasm above.

Throughout this whole process the vacuole tends to retain its spheroidal form. Occasionally, especially with the larger sized vacuoles, gentle waves of motion can be seen to pass over the vacuolar membrane just as the vacuole leaves the pharynx or during its passage through the endoplasm. These movements are always very slight and are of the type which one would expect to result from the sudden movement of a thin-walled bag filled with fluid such as the vacuole essentially is. Such movements never resulted in a spindle-shaped vacuole in any of my specimens.

Discussion.

The real interest in these observations lies in their bearing upon the question of the forces involved in the production of infusorian food vacuoles. All observers are agreed that in *Paramecium* food organisms are wafted down the buccal groove and into the forming food vacuole by the action of the peribuccal cilia and membranelles and that through this action the vacuole forms appended as a drop on the caudal end of the cytopharynx. Here it grows gradually larger till, reaching its full size, it suddenly drops into the endoplasm. One of the crucial points in the whole cycle of the food vacuole concerns the forces which cause the vacuole thus to drop away.

Nirenstein (1905) was inclined to the view that the increased cyclosis carries the vacuole away. Others have suggested that an active contraction at the end of the cytopharynx pinches the vacuole off. Neither of these views has been certainly proved and even if either of them were we still would have to explain why an increase in cyclosis should always occur just at the right time and in the right place or, assuming the other theory, why the cytopharynx should contract. Ultimately, the whole question is, of course, bound up with the physico-chemical processes of the cell.

My observations of *P. caudatum* leave me in doubt as to whether increase in cyclosis pulls the vacuole away. No such increase could be seen in my specimens except a very slight one and this on one side of the cytopharynx only. If an increase in granular streaming were to pull the vacuole away one would expect the path taken by the vacuole in its initial course to be a markedly curved one unless the same rate of movement were maintained by the granules on all sides of the cytopharynx. The path should not be merely posterior in direction but posterio-dorsad. As a matter of fact, the path of the vacuole is but slightly curved. It follows a line very nearly parallel to the body wall till well into the posterior portion of the body. Also, the rate of the vacuole's passage through the endoplasm as judged by the eye is greater than that of the endoplasmic stream. Often during the vacuole's backward passage granules may be seen to move to the side from in front of the oncoming vacuole as though pushed aside.

On the other hand, the rotation of the vacuole during its passage is just what one would expect if the granular stream were to pull the vacuole away. This is also true of the spindle-like shape so often assumed by the falling vacuole.

P. trichium presents results which are clearer. Here the vacuole behaves almost exactly as though pulled away by cyclosis. Its rotation during its passage, the direction of its path, and its rate of movement in relation to that of the endoplasmic granules all point to this conclusion.

There is one point, however, which is not clear. As noted above, the first movement of the vacuole is a sudden rotation while the vacuole still maintains its essential position at the end of the cytopharynx. What is the relation of the vacuole to the cytopharynx at this time? If it be still attached, the whirling motion must twist the pharynx about itself till the vacuole is pulled away. If it be not attached during this time, what causes it to remain in this position and not be immediately carried away in the cytoplasmic current? Despite much diligence I never could clearly see the end of the cytopharynx during the fraction of a second that the vacuole was rotating there.

What ever the forces may be which cause the dropping of the vacuole from the cytopharynx, the initial stimulus which sets these forces in motion is often a large particle striking against the inside of the vacuolar membrane. I observed this many times and never saw an exception to it. Whenever a large carmine grain or a large mass of zoöglea was ingested increase in cyclosis followed by the falling of the vacuole started just at the instant that the particle hit the vacuolar membrane. It made no difference what time had elapsed since the preceding vacuole had fallen; for example, in one individual the average time taken for the formation of twenty-six individual vacuoles was 18.1 secs. (time taken with a stop-watch). When the next vacuole had been forming 8.4 secs. a large carmine grain was swept into it. The vacuole immediately fell. Twenty-two subsequent readings on this same individual averaged 16.1 secs. and no vacuole took less than 12.4 secs. to form.

Although when such a stimulus is applied the vacuole always falls, it should be noted that such a stimulus appears not to be necessary for the falling of the vacuole. Hundreds of vacuoles were under observation during the total time of their formation and no such large particles entered. Yet these vacuoles left the pharynx in the typical manner. It should also be noted that *P. trichium* differs in this whole matter from *P. caudatum.* Large particles entering the food vacuoles of the latter species have no effect which I could find on the time when the vacuole will leave the cytopharynx. In one case, for example, I saw the end of a long plant filament become so closely appressed to the inner side of the vacuolar membrane that the latter was bowed out sharply, yet the vacuole continued to form for some seconds. Apparently, the vacuolar membrane or the cytoplasm immediately surrounding it is less sensitive in this species than in *P. trichium.*

Summary and Conclusions.

The food vacuoles of *P. trichium* drop from the cytopharynx during a definite cycle of events. This cycle differs in several fundamental particulars from the cycle in *P. caudatum* so ably outlined by NIRENSTEIN (1905). The initial stimulus which sets in motion the mechanism which causes the vacuole to drop is often a large particle striking the inner surface of the vacuolar membrane. Such a stimulus is not necessary, however.

The evidence presented favors the view that an increased cyclosis pulls the vacuole from the cytopharynx in *P. trichium.* In *P. caudatum* the evidence is not clear, my interpretations differing somewhat from those of NIRENSTEIN (1905).

Nothing suggesting a contraction at the end of the cytopharynx of *P. caudatum* could be seen. The end of the cytopharynx in *P. trichium* during the crucial moment before the vacuole left it was not observed.

Bibliography.

BRAGG, ARTHUR N. (1935): Proc. Okla. Acad. Sci. Vol. 15 (in press).

KHAINSKY, A. (1910): Physiologische Untersuchungen über Paramecium caudatum. Biol. Zentralbl. Bd. 30 p. 267.

METALNIKOW, S. (1912): Contributions à l'etude de la Digestion intracellulaire chez les Protozoaires. Arch. de Zool. Exp. et Gen., 5e Ser. T. 9 p. 376.

NIRENSTEIN, E. (1905): Ernährungsphysiologie der Protista. Ztschr. f. Allg. Physiol. Bd. 5 p. 434.

SHAPIRO, NOAH (1927): The Cycle of Hydrogen-ion Concentration in Food Vacouoles of Paramecium, Vorticella, and Stylonychia, Trans. Am. Micros. Soc. Vol. 46 p. 45.