Observations on the Initial Movements of the Food Vacuoles of *Paramecium multimicronucleata* Powers and Mitchell with Comments on Conditions in other Species of the Genus[1]).

By

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Since the work of Nirenstein (1905) and of Metalnikow (1912 and 1916) on *Paramecium caudatum*, little attention has been paid to the problem of what causes the food vacuoles of *Paramecium* to drop from the cytopharynx; yet this problem is fundamental to our understanding of the general physiology of Protozoa of this type. Does an increase in cyclosis carry the vacuole away? Is there, perhaps, a contraction at the end of the cytopharynx which pinches the vacuole off? Do both of these processes occur, either successively or simultaneously? Are all species essentially alike in the mechanism involved? These are some of the more important questions which need to be answered.

As was pointed out earlier, however, (Bragg, 1935) answers to all of these and similar questions would not solve the problem since, ultimately, the whole process involves deep-seated physico-chemical changes within the cell. But, since descriptive details of any physiological process must necessarily precede any real understanding of the physico-chemical conditions pertaining to it, any observations tending to throw light upon even the mere mechanics involved have some value.

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The present paper aims primarily to describe the movements of the food vacuoles of *Paramecium multimicronucleata* POWERS and MITCHELL as they pass from the cytopharynx into the cytoplasm and to compare these movements with those of other members of the genus already studied (NIRENSTEIN, 1905; BRAGG, 1935, 1935a).

The animals used came from two sources: (1.) some which were collected by myself from Crystal Lake, Norman, Oklahoma during the winter of 1935—1936 and (2.) a culture kindly sent to me by Dr. ROBERT KING of the State University of Iowa, to whom thanks are extended. Most of the observations were made on animals from the latter source although sufficient use of the former was made to determine that no visible differences in the manner of food vacuole formation existed in the animals from the two regions.

The Oklahoma animals were cultured in standard hay infusion, the Iowa animals in a medium made by boiling a small quantity of whole wheat flour in tap water. Both types of medium were allowed to ripen from twenty-four to forty-eight hours before introducing the animals to them. Observations were not made till the animals had had sufficient time (at least twenty-four hours) to become adjusted to the new medium. A small culture of the Iowa animals was also made with the hay infusion and another of the Oklahoma animals in the wheat medium to check whether any differences might be correlated with the medium used. Since no such differences were found, these small cultures were discarded and further observations were largely confined to the Iowa animals in the wheat cultures.

Observations.

The food vacuole of *P. multimicronucleata* forms at the inner end of the cytopharynx, essentially as in other species of *Paramecium*. There it grows constantly larger through the action of the peribuccal cilia and the membranelles of the buccal groove which force a constant stream of water down the cytopharynx. Often, when the outside medium contains relatively few particles, the vacuole holds largely water in which a few minute particles float. When the medium contains many particles of the proper size, the vacuolar contents often become relatively dense and the water content correspondingly less in proportion to the whole. The vacuoles as they fall vary in size, the more fluid ones, with some exceptions, tending to be somewhat larger than those containing more solid matter. No essential differences could be seen in the manner in which these two types of vacuoles leave the cytopharynx. Some slight differences in shape are noted below.

As the vacuole forms, cytoplasmic granules can be seen to move in a posteriorly directed stream toward the vacuole. This streaming motion of the granules (cyclosis) increases and decreases in rate from time to time with an approximate rhythmic periodicity, best seen by singling out one or more large granules for observation and following these with the eye toward the vacuole. The vacuoles pass from the pharynx into the endoplasm independently of these changes in the rate of cyclosis anterior to and about the forming vacuole. Each vacuole takes about 78.1 seconds to form (average of 29 readings at room temperature — range 60.0—147.1 seconds), but cyclosis may increase and decrease several times while a single vacuole is forming. Sometimes a vacuole falls just as cyclosis is increasing, sometimes just as it is decreasing, and all sorts of intermediate conditions exist. Out of an estimated number of some 500 vacuoles, no more than fifty were seen to fall just as the cyclosis increased.

The exact behavior of the vacuole as it passes from the cytopharynx into the endoplasm varies slightly depending upon its contents. Large vacuoles, especially those containing a large proportion of fluid, elongate very slightly as though a gentle pulling action had been applied to the posterior region of the vacuolar wall. Distortion from the typical spheroid shape is the result. The distortion, however, is never great and in small vacuoles and even in some large ones may not be visible at all. In no case does the vacuole take on a spindlelike shape such as that commonly seen in *P. caudatum*: at most, a slight temporary protuberance makes its appearance on the pharyngeal side of the vacuolar wall.

Just as the vacuole leaves the pharynx, the latter often lengthens slightly and momentarily narrows at its posterior end. This phenomenon, which may be interpreted as a form of contraction, was seen on several occasions but was not observed in all cases studied. Therefore, there may still be some doubt as to whether or not it always occurs. I am inclined to believe it typical, since it is sometimes difficult to be certain that one sees all that goes on in so small a space where the processes being studied happen each time in the fractional part of a second.

As the pharynx lengthens and the vacuole begins its movement preparatory to dropping off, cytoplasmic granules rush in from all sides behind (that is, anterior to) the falling vacuole, between this and the posterior end of the cytopharynx. As the vacuole leaves the pharynx, these streams of granules come together behind it and the granules come to rest in the region immediately posterior to the cytopharynx where the next vacuole is now forming.

This movement of the granules as the vacuole falls may be interpreted in either of two different ways: (1.) it may be the visible expression of cytoplasmic forces which take the vacuole from the place of its formation. Such would be the interpretation of those who believe that the vacuole leaves the cytopharynx under the influence of cyclosis. Or (2.) it may be merely the mechanical result of the vacuole's passage into the endoplasm, since this type of movement is just what one would expect to occur in a fluid cytoplasm if a relatively large object (the vacuole) suddenly started to move through it. I am inclined to accept the latter interpretation for the following reason: as noted above, the vacuole often tends to elongate slightly just as it starts to fall. If the vacuole were carried away from the cytopharynx by cyclosis from behind (anterior to) it, the force applied would be in the nature of a push rather than a pull. If the vacuole were pushed off, it should not elongate as it is sometimes observed to do; if it were distorted at all, the distortion would be more likely to flatten the spheroid vacuole in a plane approximately perpendicular to the axis of the path of the vacuole's fall. This is all the more evident since the vacuolar membrane appears to be very thin in this species (as is also evident in *P. aurelia*) and the vacuolar shape easily distorted. Many times I have seen two recently formed food vacuoles come lightly into contact in the endoplasm and in nearly all cases both vacuoles were temporarily bowed out of shape as the result. Incidently, this is more likely to occur in the larger, more fluid vacuoles than in smaller denser ones, though I can see no evident reason why this should be so.

The path of the vacuole through the endoplasm is almost straight; it curves mesiad only sufficiently to conform with the shape of the animal. Each vacuole moves slowly (as compared with *P. caudatum*) approximately to the point where the posterior end of the animal narrows markedly and there eventually comes to rest. During its passage through the endoplasm it may rotate slowly for about one revolution or it may rotate little or none. When it reaches the posterior region of the cell, however, it usually rotates at least once (never in my observations more than three times) before coming to rest.

Comparison of the Initial Movements of the Food Vacuoles in Different Species of *Paramecium.*

The initial movements of the food vacuoles apparently have, thus far, been discussed in detail in but two species of *Paramecium*, *P. caudatum* (Nirenstein, 1905) and *P. trichium* (Bragg, 1935). A summary of the conditions in these species together with a short discussion of the initial movements of the food vacuoles in *P. aurelia* is also available (Bragg, 1935 a). To facilitate comparisons, Table 1 is presented which summerizes the conditions so far as known in the different species.

It is readily seen from the table that the initial movements of the food vacuoles of *P. multimicronucleata* are closely similar to those of *P. aurelia.* The vacuoles of the latter species are usually considerably smaller and form at a faster rate (average, 59.3 seconds per vacuole; range, 4.3 to 58.7 seconds on the basis of 21 readings at room temperature in a flourishing standard hay infusion). These differences are probably correlated with the size of the animals, since *P. aurelia* is a much smaller species than *P. multimicronucleata.* Also the path of the food vacuoles through the endoplasm is likely to be more curved in *P. aurelia*, due possibly to the difference in the shapes of the posterior ends in the two forms, that of *P. aurelia* being usually more rounded than that of *P. multimicronucleata.*

P. caudatum differs from these in that its food vacuoles move very fast through the endoplasm, in that they rotate more, and in that they usually take on a spindle-like shape as they fall from the cytopharynx. There is some variation in this last mentioned characteristic, however, as was first recognized by Nirenstein (1905).

P. trichium differs radically from all of the above species in several particulars: (1.) increased cyclosis always occurs prior to the dropping of the vacuole (Bragg, 1935), (2.) rotation of the vacuole starts just as the cytoplasmic stream reaches it and continues for some time before the vacuole falls, and (3.) the path of the vacuole is markedly curved, due possibly in part to the shape of the posterior end of the animal.

It is of some interest to note that *P. aurelia*, *P. caudatum*, and *P. multimicronucleata* are all members of the "caudatum form group" of Woodruff (1921) whereas *P. trichium* belongs to the "busaria form group". May not the differences recorded in the last named species as compared with the others indicate a fundamental physiological difference in the two groups? This possibility seems indicated by the differences recorded but must remain hypothetical till others members of the bursaria form group are studied.

The basic physiological causes of the food vacuole's dropping from the cytopharynx may eventually be proved to be the same in all species of *Paramecium.* As it appears now, however, this does not seen to be the case. In *P. caudatum*, *P. aurelia*, and *P. multimicronucleata*, increase in cyclosis either does not occur near the time of the vacuole's fall or has been shown not to correlate with it. The path taken by the vacuole through the endoplasm in *P. caudatum* is not what one would expect if the observed movements of cytoplasmic granules were to pull or to push the vacuole away. In this species, also, the rate of the vacuole's fall is much too fast to be accounted for on the basis of cyclosis alone (Bragg, 1935). In *P. aurelia* and *P. multimicronucleata*, both the path taken by the vacuole and its rate of fall are such that they could easily be correlated with the cytoplasmic movements observed, even though in *P. aurelia* no special increase in cyclosis has been demonstrated. But even if we grant that cyclosis can and does carry the vacuole posteriorly in these species we still cannot explain on the basis of cyclosis alone why the vacuole leaves the cytopharynx in the first place. Therefore, in all members of the caudatum group of *Paramecium* cyclosis fails to explain the fall of the vacuole. Other factors, at present unrecognized, must be at work.

On the other hand, it has been shown clearly (Bragg, 1935) that in *P. trichium* all of the details of cytoplasmic movements anterior to and about the vacuole, as well as the path taken through the endoplasm and the rate of the vacuole's fall, are just what one would expect if a sudden increase in cyclosis were to carry the vacuole away.

There is another set of facts which seem pertinent to our discussion. Metalnikow (1912) noted that in *P. caudatum* food vacuoles containing nutritious particles circulate in the endoplasm for a longer time than those containing innutritious particles. I have confirmed this in *P. caudatum* and have also seen it in *P. aurelia* and *P. trichium* (Bragg, 1936). Furthermore, a food vacuole of any of the species of *Paramecium* discussed in this paper while apparently floating freely in the endoplasm may pass another vacuole in the same stream. If cyclosis alone were carrying these vacuoles along merely as logs might be carried by a stream of water, this phenomenon would not be likely to occur just as it does nor so often. In one specimen of *P. multimicronucleata* under observation, two vacuoles were passing slowly anteriorly in the ventral portion of the animal. When they had reached a point about half way to the anterior end of the cell, they suddenly shifted their direction of movement, passed to the dorsal portion of the body, and there continued anteriorly for a short distance. From the present point of view it is noteworthy that when these vacuoles moved dorsad, the anteriorly directed endoplasmic stream in the ventral part of the animal continued, so far as was visible, substantially as it had been. Of course, minute changes in gelation and solation which were not discernible may have occurred. The important thing to note, in any case, is that these vacuoles behaved differently than one would expect them to do if they were being carried passively by the cytoplasmic current of the cell. Observations tending to show the same sort of thing have also been made as the vacuoles are about to leave the animal. Vacuoles ready for defaecation pass anteriorly to the region of the cytopyge and pause there despite the fact that the cytoplasmic stream flowing around them may continue with no visible change in rate. Other vacuoles in the stream, not yet ready for egestion, may continue to circulate and have often been seen to pass the cytopyge region while other vacuoles, ready for egestion, remained stationary there.

All of these observations tend to show that the circulation of the food vacuoles in the endoplasm of *Paramecium* is more than the mechanical result of the streaming of the cytoplasm. This being the case, it is not difficult to believe that forces other than cyclosis may also be involved in taking the vacuole from the cytopharynx. One factor may be the contraction of the cytopharynx. As noted above, this is probably typical of *P. multimicronucleata.* I have seen rare indications of it in *P. caudatum,* also. In *P. aurelia,* I have never seen anything which I could interpret as a contraction at the time the vacuole's fall but I am not sure that it does not occur.

The assumption that the same general result as observed in two species of animals must necessarily have the same essential cause seems to me to be unjustified, although this assumption is often tacitly made in most branches of biology, as pointed out by JENNINGS et al. (1932). In regard to the forces concerned in the formation of the food vacuoles of the different species of *Paramecium,* the differences which *P. trichium* shows as compared to the other species which have been studied seem to me to be a clear indication that the basic physiological mechanism may well be different in this species. At least, this possibility should be recognized in all further work on the subject, not only in *Paramecium* but in other species of Infusoria as well.

Table 1.

Comparisons of Conditions in Different Species of *Paramecium* as the Food Vacuoles Leave the Cytopharynx.

	Typical vacuolar shape	Rate of movement	Nature of path	Contraction of the cytopharynx	Cyclosis	Authority
P. caudatum	Often a spindle, sometimes otherwise	fast	—	—	increase	NIRENSTEIN, 1905
	Usually a spindle	fast	Approximately parallel to body wall	not typical	slight increase ventrally	BRAGG, 1935
P. aurelia	Usually Spheroid	slow	Curves slightly dorsad	None observed but may occur	No increase	BRAGG, 1935 a
P. multimicronucleata	Spheroid	slow	Approximately parallel to body wall	typical (?)	Rhythmical increase with no influence on vacuole	—
P. trichium	Spheroid	fast	curves markedly dorsad	Conditions unknown	Definite increase associated with the fall of the vacuole	BRAGG, 1935

Summary.

Of the eight or ten recognized species of *Paramecium* (Wenrich, 1928; Ludwig, 1930) the details of how the food vacuoles fall from the cytopharynx have hitherto been carefully studied in but two species, *P. caudatum* (Nirenstein, 1905) and *P. trichium* (Bragg, 1935). The conditions in *P. aurelia* have been studied in less detail (Bragg, 1935 a). In the present paper, this process in *P. multimicronucleata* is described and a summary of the conditions so far as known in the genus is presented and discussed.

The factors recognized as possibly influencing the fall of the food vacuole are (1.) the action of cyclosis and (2.) a contraction of the cytopharynx. It is also recognized that both of these processes working together may be involved.

In all species of the "caudatum form group" of Woodruff (1921) cyclosis alone cannot explain the fall of the vacuole. In *P. trichium*, this one factor may be sufficient so far as the mere mechanical forces are concerned. Contraction of the cytopharynx is very probably a factor in *P. multimicronucleata* and it may be in *P. caudatum* and in *P. aurelia*. The conditions in *P. trichium* are unknown.

All of the facts suggest that the various species differ in the manner in which the food vacuoles fall and it is possible that they differ correspondingly in the ultimate physiological processes involved. The facts further suggest that the complex of factors concerned may well be more nearly alike in any two species belonging to the same form group than in any two species belonging to a different form group.

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