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Miscellaneum

Exocytosis in *Entamoeba*

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Abstract

Exocytosis in *Entamoeba* is described in which the vacuole moves posteriorly and comes out of the tail or the uroid. Sometimes the whole vacuole comes out but more often only a part of it is expelled. The remaining part gets detached and moves back into the cytoplasm.

Introduction

A considerable amount of information is available on the method of food ingestion by *Entamoeba* (ZAMAN, 1970; WESTPHAL & MICHEL, 1971) but little is known about the method of excretion by this cell. The fate of ingested food particles in *Entamoeba* is very intriguing as the parasite is capable of ingesting a variety of particulate matter including latex particles, various dyes and its own cysts (McCONNACHIE, 1955). The *amoeba* must, therefore, possess an effective mechanism of excretion or exocytosis for those substances which it is unable to digest. In a previous study it was suggested that the tail or uroid of *Entamoeba* probably has an excretory function (ZAMAN, 1961). In that study the actual mode of excretion, however, was not recorded. In this paper photographic evidence is presented to show how this process takes place.

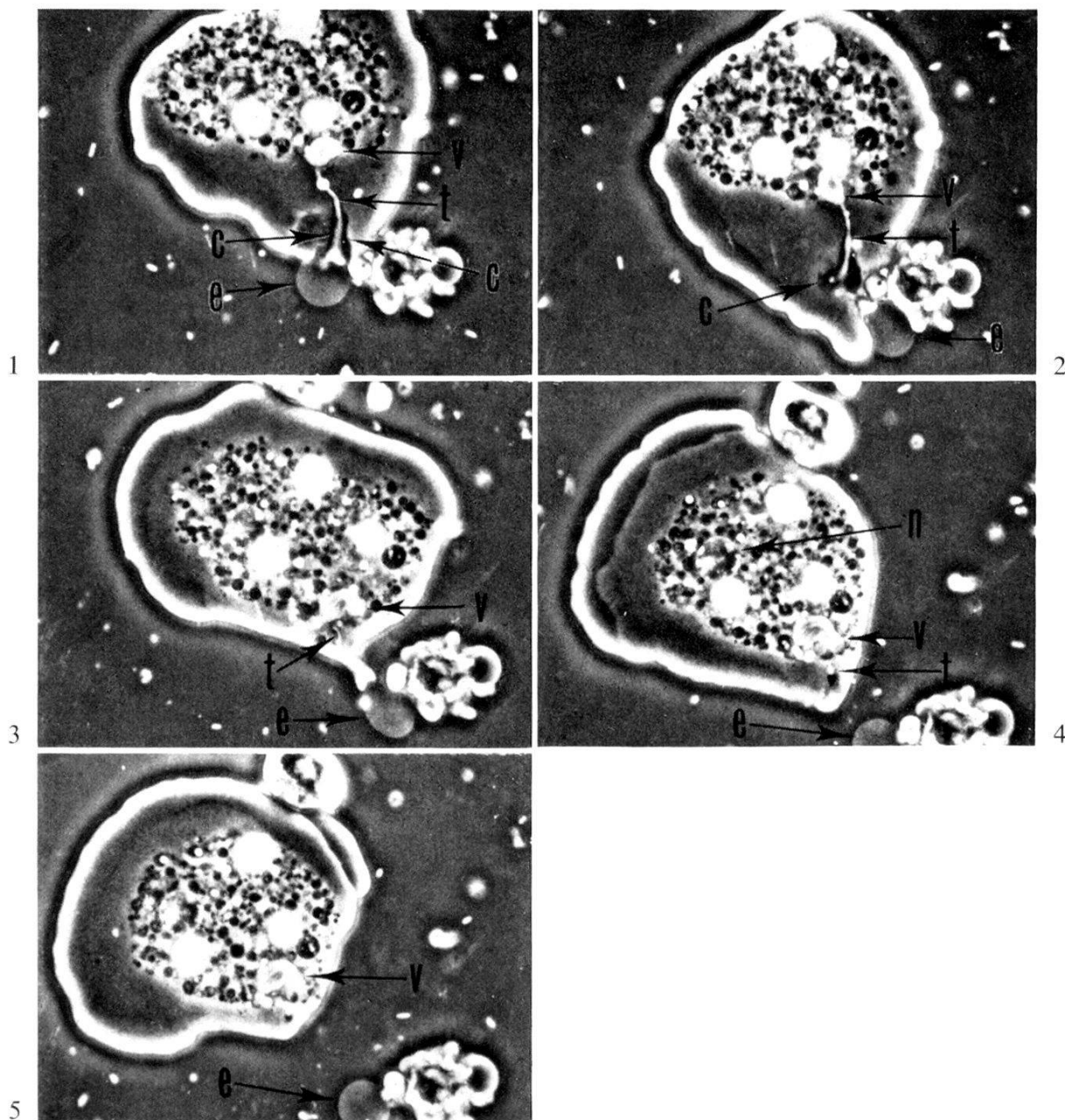
Material and Methods

Entamoeba invadens was used for this study as this species is active at room temperature and spreads out well on microscope slides. Light microscopy was done in Reichert "Zetopan" microscope using a negative (anoptal), and Leitz "Ortholux" microscope using a positive phase contrast. Photographs were taken in both instances with an electronic flash at time intervals of approximately 4 seconds.

Preparations for electron microscopy were made by washing 7-day old cultures of *Entamoeba* in physiological saline and fixing the heavy suspension of trophozoites in ice cold 4% glutaraldehyde in phosphate buffer (pH 7.4, MILLONIG, 1962). The glutaraldehyde fixed cells were washed once in Millonig phosphate buffer without additives and then post fixed for one hour in ice-cold 1% Osmium tetroxide, also in the same phosphate buffer. Specimens were dehydrated in ethanol series and with two additional 15 minute changes in propylene oxide. Embedding was done in "Araldite" according to standard technique. Sections were cut with a Porter Blum microtome and examined after staining with uranyl acetate and lead citrate (REYNOLDS, 1963) at 50 kv in a Hitachi HS8 electron microscope.

Results

Observations in the light microscope showed that during excretion one of the vacuoles moved posteriorly and came into the tail region. Subsequently the whole of it came out of the tail. The tail during this process showed an elongated cleft through which the expulsion occurred. When only part of the vacuole was



Abbreviations: n = nucleus, v = vacuole, t = tubular structure connecting the internal with the external vacuole, e = external vacuole, c = cleft in the tail.

Fig. 1. *E. invadens* trophozoite "Anophtral" phase. Showing the two portions of the vacuole connected by a tubular structure. Also note the small clump of "free" vacuoles lying near the tail.

Fig. 2. Same cell after 4 seconds.

Fig. 3. Same cell after 4 seconds. The cleft in the tail has disappeared but the remnants of the tubular connection is still visible.

Fig. 4. Same cell after 4 seconds. The external vacuole has separated completely.

Fig. 5. Same cell after 4 seconds. The cell has reverted to normal and has moved away from the external vacuole which is left behind.



Fig. 6. Section has gone through the tail of *Entamoeba*, which shows a cleft (marked by arrows). The broad arrow shows the direction in which the excretory products are coming out. 21,000 \times .

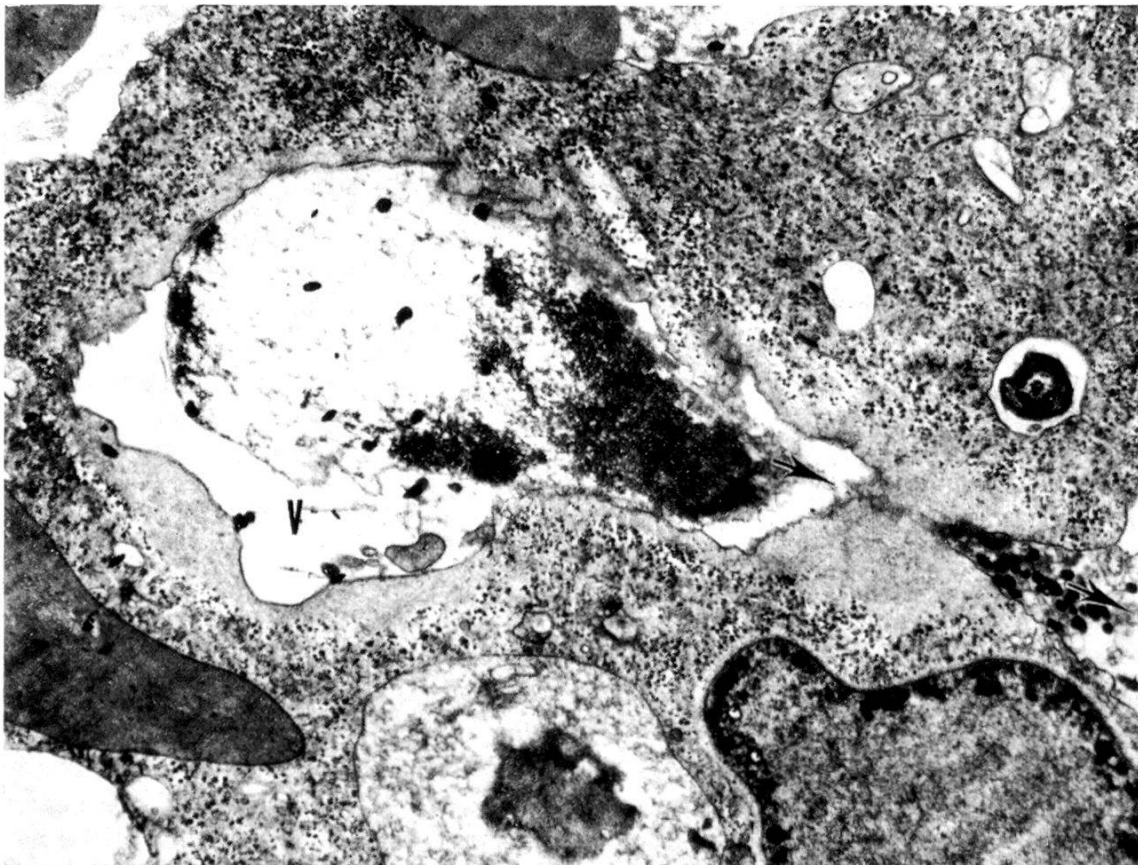


Fig. 7. Section has gone through the vacuole which is involved in excretion. Arrows mark the direction in which the excretory products have come out. The cleft in the tail has closed but the margins of the cleft are still visible. 21,000 \times .



Fig. 8. The cleft of the tail has completely disappeared, but there is a clear ectoplasmic band running from the vacuole (v) to the tail probably indicating the area through which the excretion had taken place. Note the posterior portion of the vacuole is elongated and the vacuolar membrane is interrupted at this point (marked by an arrow). 5,600 \times .

expelled, the two portions, one inside the cytoplasm and the other outside were momentarily connected by a thin tubular structure (Fig. 1). As these two portions of the vacuole moved away from each other the tubular structure ruptured (Fig. 2). Finally the cleft in the tail closed and the expelled vacuole was left behind as the *Entamoeba* continued its forward movement (Fig. 3, 4, 5).

In the electron microscope it was not possible to see all the stages involved in the process. However, some sections were showing different stages of excretion. In Fig. 6 an elongated cleft is seen in the tail of an *amoeba*. The section shows the portion of tail through which the excretory products are coming out, the connection with the vacuole is not seen. Fig. 7 shows the closure of the cleft with parts of the excretory products still retained in the vacuole. Fig. 8 shows a large vacuole with an elongated posterior end and a clear ectoplasmic band running from the vacuole to the tail.

Discussion

The process described here may not be the only method used by the cell for excretion. There may be other methods which are not known. It is clear, however, that excretion does occur in the region of the tail. In some free living *amoebae* the water expulsion vesicle or the contractile vacuole also moves towards the tail before discharging its contents (BOVEE, 1970). However, the phenomenon described here in which an intact vacuole or a part of it is expelled out of the tail has not been observed in any other *amoeba*. The phenomenon also explains why free vacuoles are generally seen outside the cytoplasm near the tail of the *Entamoeba*. The vacuole involved in the excretory process does not seem to be morphologically distinct from other food vacuoles. In the electron microscope it

generally appears larger than other vacuoles, but the size of the vacuole is a variable character and is not a reliable criterion for differentiation. Unless further studies show that there is a specific excretory vacuole I would assume all food vacuoles are capable of excreting their contents by the method described in this paper.

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