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STUDIES ON FEEDING AND DIGESTION IN PROTOZOA II. FOOD VACUOLE CYCLE IN TETRAHYMENA CORLISSI

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Introduction

Holozoic feeding, *i. e.* intake and intracellular digestion of particulate food, living organisms or non living material, is common among the protozoa. Our knowledge of this process as shown by the thorough review of KITCHING [5], is rather incomplete and scanty in comparison to the rapid progress made in several other fields of protozoan physiology in the last two decades. Among others the absence of papers concerning the electron microscopy of the protozoan digestion is apparent. Recent papers on the fine structure of protozoa contain micrographs revealing details of food vacuoles in several groups (Rhizopoda [1, 4, 7, 15], Sporozoa [19, 20], Ciliata [16] etc.) but only one comprehensive paper has come to our attention which discusses the complete food vacuole cycle [17, 18]. This work on *Pelomyxa* emphasizes the changes in the food organisms *(Paramecium* or *Tetrahymena)* and in the membrane of the food vacuole.

For some years we have been studying certain aspects of protozoan digestion with the primary aim to obtain information on the mechanism of intracellular breakdown of the materials ingested [11, 12, 14]. The histophagous *Tetrahymena corlissi* [21] proved to be a favourable organism in this work. The food intake and digestion in histophagous ciliates has not been discussed in the literature in spite of the great possibilities they offer in the study of protozoan feeding [9]. We have been studying these processes both by light and electron microscopy. In the present paper only the electron microscopic data will be presented. Light microscopic findings and a general discussion will be presented in a forthcoming publication. A short abstract of this work has been reported elsewhere [13].

Materials and preparation

Tetrahymena corlissi THOMPSON, strain W[1] has been cultivated axenically in a solution of following composition: Bacto-Tryptone (Difco) — 1%, Bacto Yeastextract (Difco)[2] — 0,05%. Heavy cultures were harvested by a hand driven centrifuge and twice washed prior to feeding in Prescott solution.

[1] Obtained through the courtesy of Dr. J. O. CORLISS of Illinois University, Urbana, Ill., U.S.A.

[2] Kindly supplied by Difco Co., Inc.

The washed individuals were being fed on fresh frozen sections of rat or mouse spleen for different times (30 minutes to 4 hours). Some samples were starved after a feeding of 30—60 minutes. Unfed and fed samples as well as individuals starved after feeding were fixed in buffered osmic acid according to PALADE. The organisms were dehydrated, infiltrated and embedded in methacrylate. All steps were performed in conical centrifuge tubes. The early phases of ingestion were studied in animals fixed while feeding and embedded together with the food. Thin sections were cut on a Porter-Blum microtome and examined with a Tesla electron microscope using a 60 kV beam.

Observations

T. corlissi readily and rapidly ingests mammalian tissues, *e.g.* spleen sections. This results in the rapid formation of numerous food vacuoles. All the animals fixed at different times, with the exception of the early moments, contain vacuoles in different phases of digestion. Thus a critical timing could not be achieved and the food vacuole cycle was reconstructed on the basis of the relative frequencies of the different vacuolar types in the different samples.

Structure of the mouth parts[3] *participating in food vacuole formation.* The ciliated buccal cavity (Figs. 1 and 2, BC) ends in a narrow opening, the cytostome (Figs. 1 and 2, CS). This very conspicuous structure is formed by ridge-like protoplasmic processes. The pellicle of the buccal cavity does not end abruptly at the level of the cytostome but continues on the vacuolar side of the ridge (Fig. 2, P). Several fibrils or tubular structures strengthen this area (Figs. 1 and 2, F) which is most probably identical with the cytopharynx. The newly formed food vacuole, *i.e.* the ingestion vacuole follows distally (Figs. 1 and 2, IV) limited by a thin membrane (Fig. 2, VM).

Contents of food vacuoles. The very large ingestion vacuoles (Figs. 1 and 2, IV) contain more or less granular-fibrillar material evenly distributed in their lumen. The space between the individual particles seems empty. In no instances are traces of the original structure of the ingested tissues discernible in these vacuoles.

The great majority of the digestion vacuoles is filled with a homogeneous dark material (Fig. 3), a condensation and dehydration product of the content of the ingestion vacuoles. In later vacuoles the homogeneous mass breaks up into dark granules and at the same time intergranular spaces reappear (Figs. 3 and 7). These granules disappear also from the older vacuoles and leave behind nothing but a finely dispersed, light, filamentous granular material (Figs. 5, 6 and 8, DV).

Vacuolar membrane. All food vacuoles are surrounded by a smooth vacuolar membrane (Figs. 1 to 8, VM) displaying identical fine structure in early and late vacuoles. In some micrographs (Fig. 7, insert) its doubleness is

[3] In the naming of mouth parts the usage recommended by CORLISS [2] has been followed.

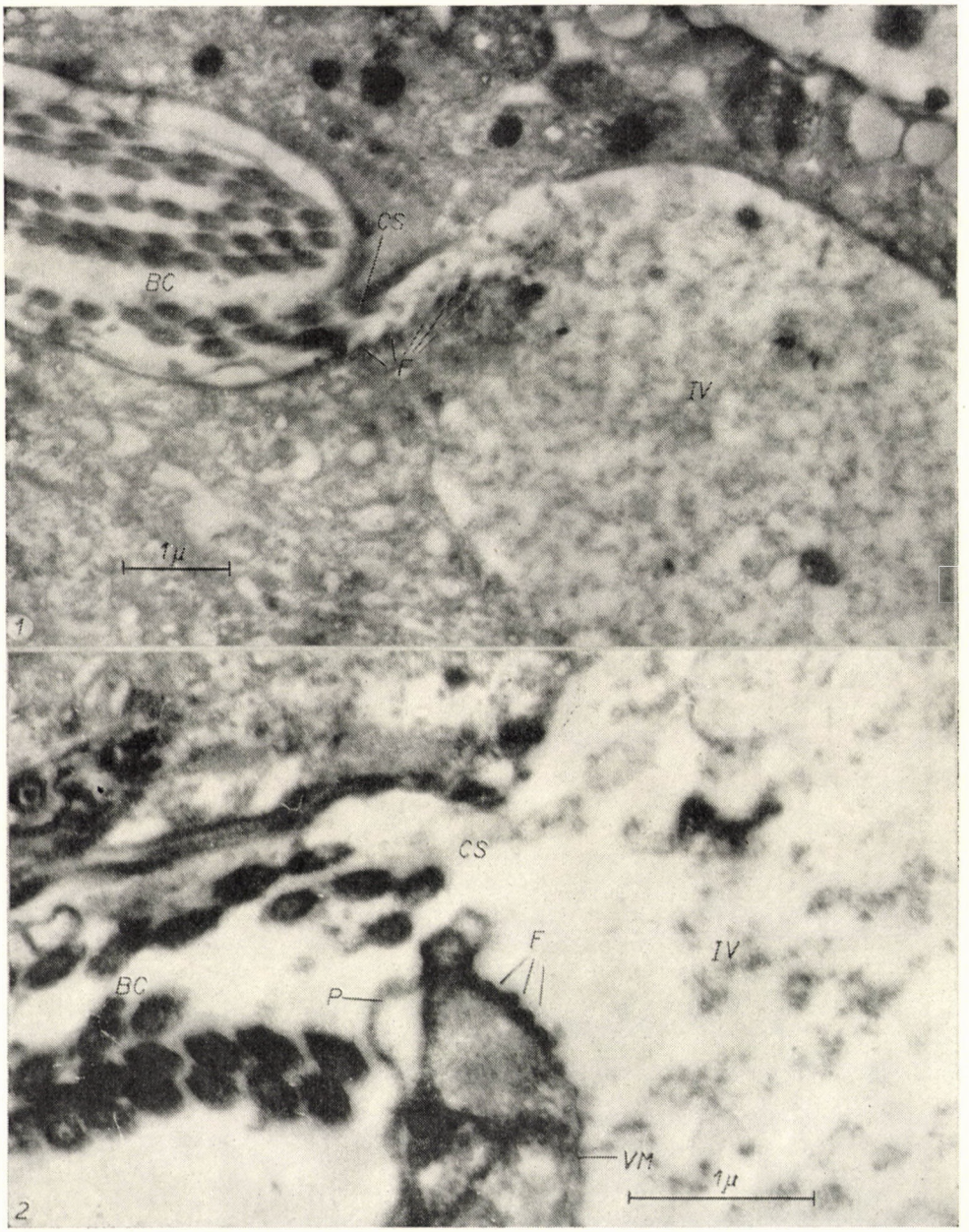

Fig. 1. Longitudinal section through mouth area of *Tetrahymena corlissi.* The ciliated buccal cavity (BC) and the ingestion vacuole (IV) are separated by the cytostome (CS). Note the oblique sections (F) of fibrils or tubular structures right of the latter. The ingestion vacuole (IV) contains granular material. ×14.500

Fig. 2. A similar section of *T. corlissi.* The pellicle (P) of the buccal cavity (BC) shortly continues on the right side of the cystostome (CS). The dark round structures (F) are possibly identical with the fibrils (F) in Fig. 1. VM — membrane of the ingestion vacuole (IV) containing less granular material. ×24.000

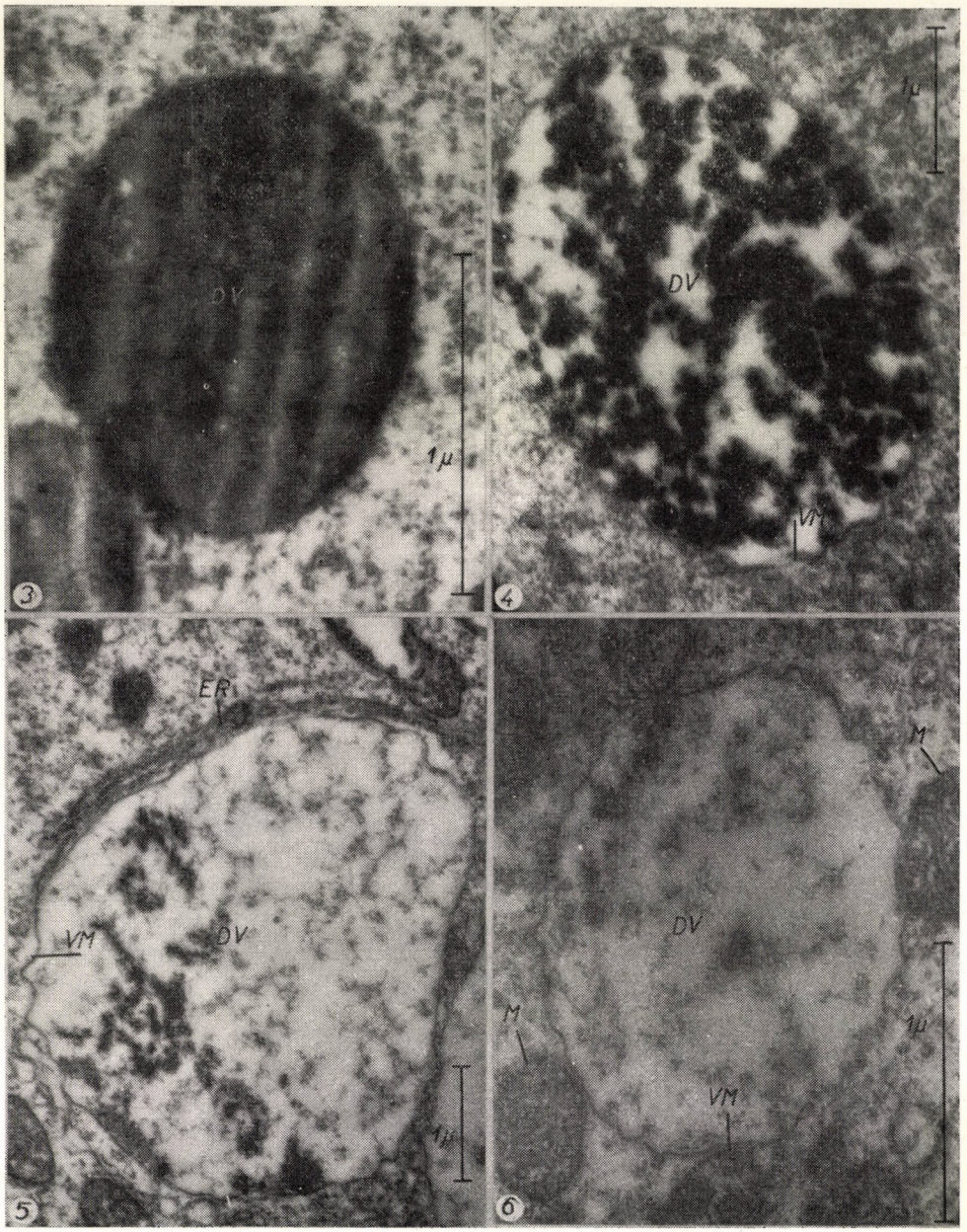

Fig. 3. Section of digestion vacuole (DV) in its most compact form. ×44.000
Fig. 4. Digestion vacuole (DV) containing large dense granules. Note the distinct vacuolar membrane (VM). ×19.200
Fig. 5. Food vacuole (DV) with dark, granular and light, more fibrillar, content. Endoplasmic reticulum (ER) is adhering the vacuole membrane (VM). ×15.750
Fig. 6. Digestion vacuole (DV) in a later phase of digestion. M — mitochondria. ×36.000

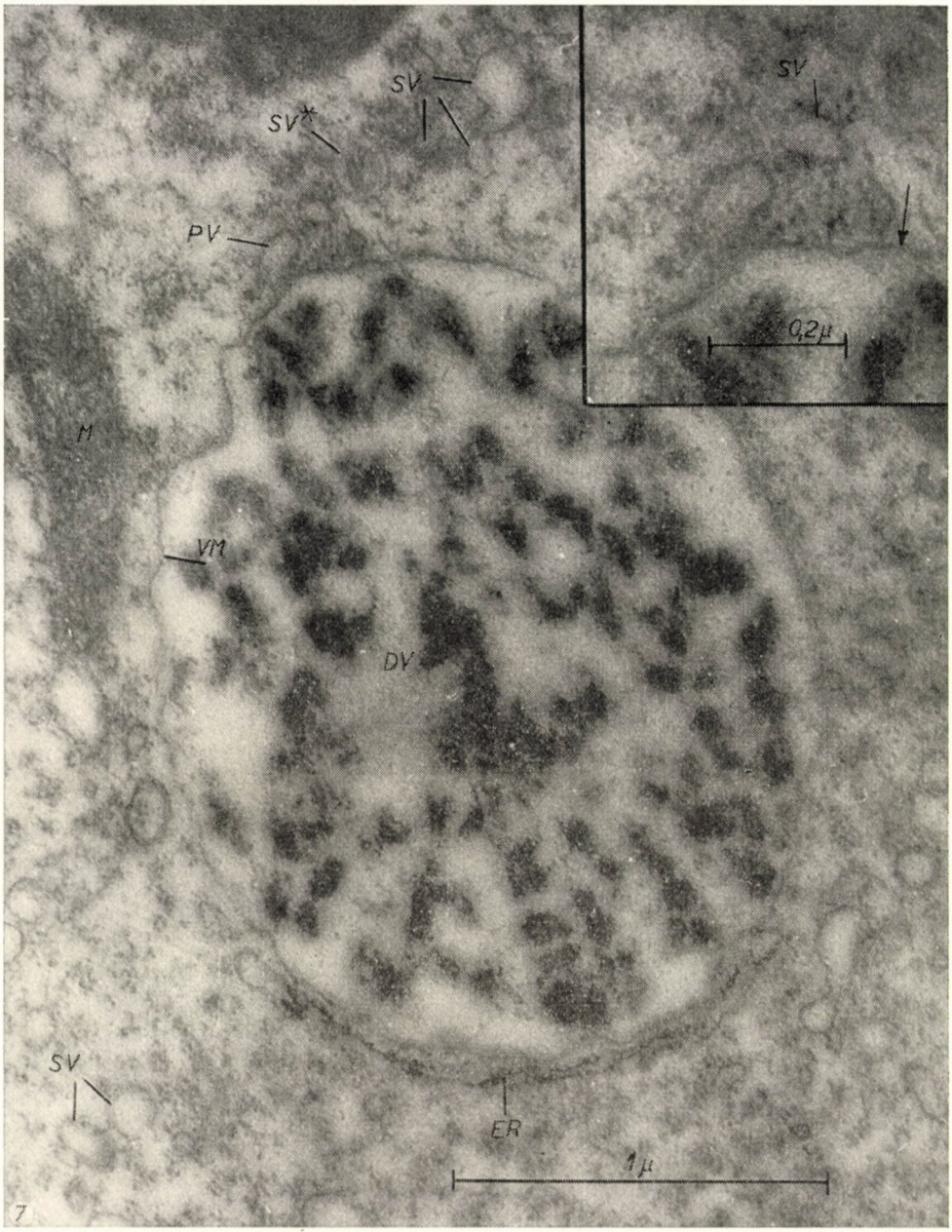

Fig. 7. Late digestion vacuole (DV) with dense granular content. The cytoplasm contains several small vacuoles (SV), some of which with particulate material (SV*). At PV the membrane forms a small vacuole by pinocytosis. ×49.600
Insert. Higher magnification of pinocytotic area (PV). Arrow points to double vacuolar membrane. ×88.000

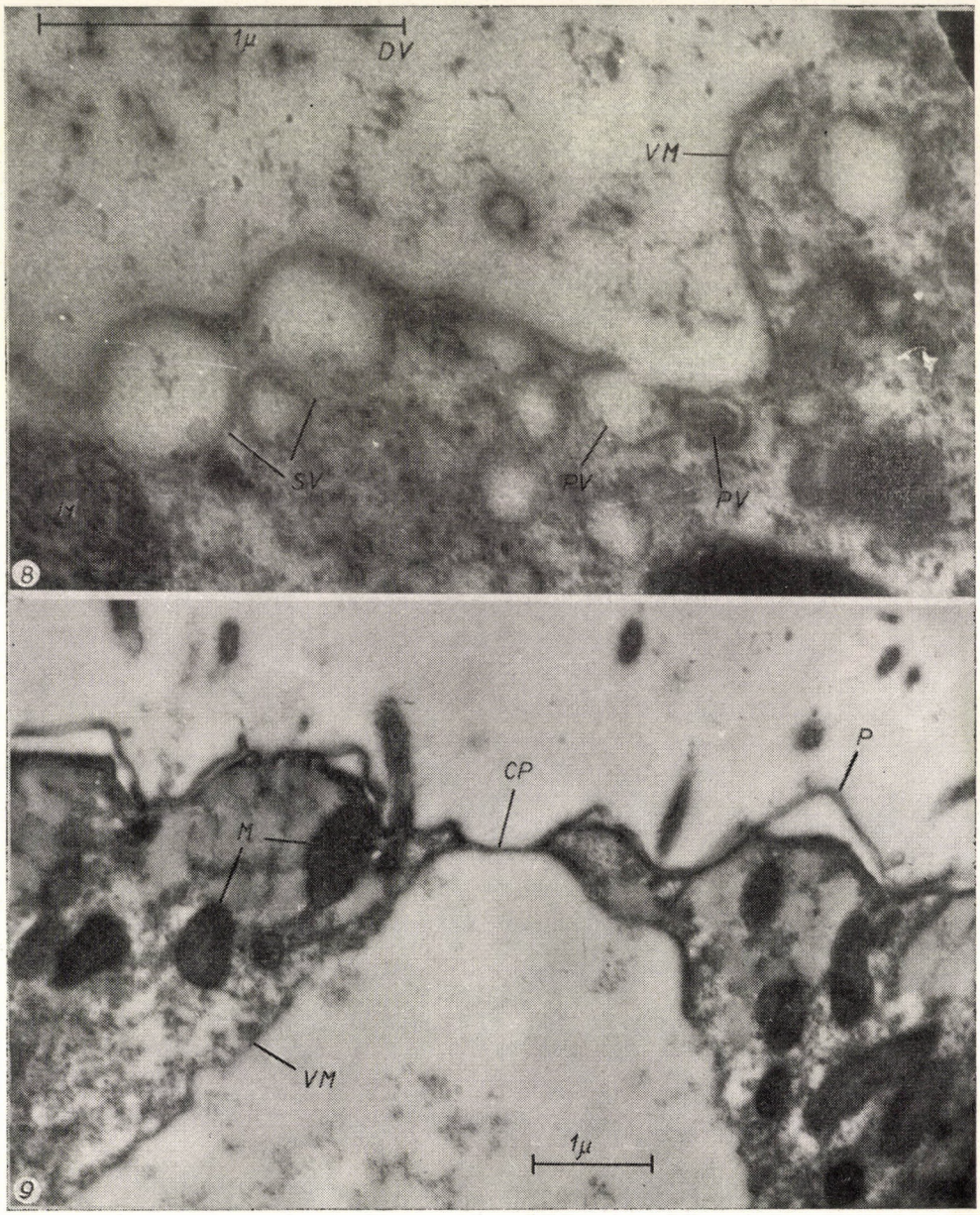

Fig. 8. Late digestion vacuole (DV). Pinocytotic formation (PV) of small vesicles (SV) some of which contain particles. ×46.500

Fig. 9. Surface area of *T. corlissi* representing a structure (CP) possibly identical with the cytoproct. ×15.000

conspicuous. This membrane does not show in the most dense early vacuoles because it is close-fitting to the food mass (Fig. 3). The membrane of the late vacuoles containing dense granules or fibrillar material has an appearance which suggests the involvement of intracellular pinocytosis. Small vacuoles are observed closely associated with the membrane (Figs. 7 and 8, SV). While some of them contain no visible structures, others are filled with granules (Fig. 7, SV*). Here and there the vesicles are connected with the food vacuole (Figs. 7 and 8, PV) budding off from it. Most probably the membrane of the small vacuoles is a derivative of the food vacuole membrane.

Cytoproct. In some micrographs (Fig. 9, CP) points of the animal's surface are to be found where a large vacuole with fibrillar material adheres to the surface pellicle. This point is perhaps the cytoproct but we cannot yet exclude the possibility that it represents the contractile vacuole pore. To differentiate these structures further investigations are required.

Discussion

Micrographs of the mouth parts could not be taken in sufficient number to obtain a clear picture of their organisation. All typical parts [2, 3] of a hymenostome mouth, *i. e.* buccal cavity, cytostome and cytopharynx have, however, been found. At the level of the cytopharynx fibrils or tubular structures are conspicuous which may be identical with the pharyngeal fibrils seen in silver preparations [3] and with the fan of fibres described by METZ and WESTFALL [8]. No structural details have been found which would account for the peculiar feeding mechanism. As in other *Tetrahymena* species, the feeding of *T. corlissi* can only be accomplished in a "Strudler" mode which necessitates the extracellular breaking down of tissues prior to ingestion. An extracellular gelatinase may play a definite role in this process [10].

This view is further supported by the fact that no original structure of the food is preserved even in very early ingestion vacuoles. Their content is represented by irregular fibrillar-granular material. These particles are probably the products of the assumed extracellular "predigestion". They are absorbed together with a considerable amount of water, thus forming the rather large ingestion vacuoles.

The ingestion vacuoles are rapidly transformed into early digestion vacuoles found in great number in all individuals except those starved for a longer time. The change is accomplished by the dehydration and condensation of the content. The early vacuoles are filled with a dark, homogeneous material. The disappearance of discernible structures during early digestion has been noted also in light microscopical work on *Amoeba proteus* [11, 12]. Attacked by digestive fluids this material is broken down into dark, rather large granules,

a process accompanied by the rehydration of the vacuole, and later into finely dispersed, filamentous material. The latter is to be found in the late vacuoles. No light amorphous mass has been found adhering to the late vacuole membrane which is regarded as a digestion product by some authors [7, 15]. The changes observed clearly point to the role of de- and rehydration and the production of digestive enzymes in the digestion of the food praticles [18]. The decreasing amount of material in the vacuoles is explained by the intake of digestion products in the cytoplasm.

The vacuole membrane is a derivative of the mouth parts. As to the mode of its formation nothing definite can be said as yet. We may, however, assume that the depth of the mouth is limited by an undifferentiated plasma membrane which produces the vacuolar wall by extension and constriction [6]. Most authors [4, 7, 15, 16, etc.] do not differentiate a finer substructure in this membrane and regard it as single layered. RUDZINSKA and TRAGER [20] found a double membrane around the food vacuoles in *Plasmodium berghei*. In some of our micrographs the wall appeared double.

The membrane surrounding the early vacuoles is smooth or wavy without any indication of greater activity [18]. The late vacuoles containing granular-fibrillar material have a membrane exhibiting an intense pinocytotic activity. Regarding this phenomenon we may accept the view of ROTH [18] that the results of this process are the modification of the vacuolar membrane and the increased surface area for diffusion. Thus the benefit derived by the organism from this process is chiefly the rapid uptake of digestion products.

Summary

Axenically grown *Tetrahymena corlissi* cells readily ingest mammalian tissues, *e. g.* spleen. The ingestion and digestive vacuoles are surrounded by a (double) membrane 75 ÅU in thickness, differing from the differentiated cell membrane. The original structure of the ingested tissues is unrecognisable, a fact pointing to a certain extracellular "predigestion". The early ingestion vacuoles contain granular material, rapidly condensated to a dark homogeneous mass. Later the vacuole walls are lifted from the dark vacuolar content. The latter slowly breaks up and then almost disappears. At the same time a great number of small secondary vacuoles are budding off from the wall of the digestion vacuole. This process of intracellular pinocytosis must play a prominent role in the uptake of digestion products.

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ЗАХВАТ ПИЩИ И ПИЩЕВАРЕНИЕ У ПРОСТЕЙШИХ II. ЦИКЛ ПИЩЕВАРИТЕЛЬНЫХ ВАКУОЛЕЙ У TETRAHYMENA CORLISSI

М. МЮЛЛЕР и П. РЕЛИХ

Аксеническая Tetrahymena corlissi охотно заглатывает ткани млекопитающих, напр. селезеночную ткань. Захватывательные и пищеварительные вакуоли выстиланы тонкой (75 Å) двойной оболочкой, имеющей иную структуру, чем дифференцированная пелликула. Пищевой материал теряет исходную структуру уже к моменту заглатывания. Этот факт, вероятно, объясняется известным внеклеточным «предпереариванием». Ранние вакуоли заполнены зернистым веществом, которое быстро конденсируется и превращается в гомогенную темную массу. Стенки вакуоли потом отдаляются от этой массы, который постепенно разрыхляет и почти полностью исчезает. Одновременно отшнуровываются многочисленные мелкие вторичные вакуоли от стенки пищеварительной вакуоли. Этот процесс внутриклеточного пиноцитоза играет, наверно, большую роль в деле всасывания продуктов переваривания.

ÜBER DIE NAHRUNGSAUFNAHME UND VERDAUUNG BEI DEN PROTOZOEN II. ZYKLUS DER VERDAUUNGSVAKUOLEN VON TETRAHYMENA CORLISSI

M. MÜLLER und P. RŐHLICH

Axenisch gezüchtete *Tetrahymena corlissi* nimmt Säugetiergewebe, z. B. Milzgewebe sehr leicht auf. Die Einverleibungs- und Verdauungsvakuolen sind von einem 75 Å dicken (doppelten) Häutchen umgegeben, dessen Struktur von dem Aufbau der differenzierten Zellmembran abweicht. Das Nahrungsmaterial hat seine ursprüngliche Struktur schon zur Zeit des Einverleibens verloren, was wahrscheinlich mit einer bestimmten extrazellulären »Vorverdauung« zu erklären ist. Die Vakuolen sind zuerst von granulärem Material ausgefüllt, welches schnell in eine homogene dunkle Masse kondensiert wird. Die Vakuolenwände heben sich später von der dunklen Masse ab, welche sich allmählich auflockert und fast vollständig verschwindet. Gleichzeitig schnürt sicht eine große Anzahl von kleinen Vakuolen von der Wand der Verdauungsvakuole ab. Diesem Vorgang der intrazellulären Pinozytose kommt wahrscheinlich eine führende Rolle in der Aufnahme der Verdauungsprodukte zu.

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