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Ingestion of Erythrocytes by Entamoeba

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The ingestion of erythrocytes by *Entamoeba* is a well known phenomena. The actual method of ingestion, however, has not been clearly recorded. The purpose of this study, therefore, was to observe and record this process with the phase-contrast and electron microscopy.

Entamoeba invadens were used for the study as in our laboratory it was observed that this species readily ingest sheep erythrocytes, and unlike *E. histolytica,* remain active at room temperature.

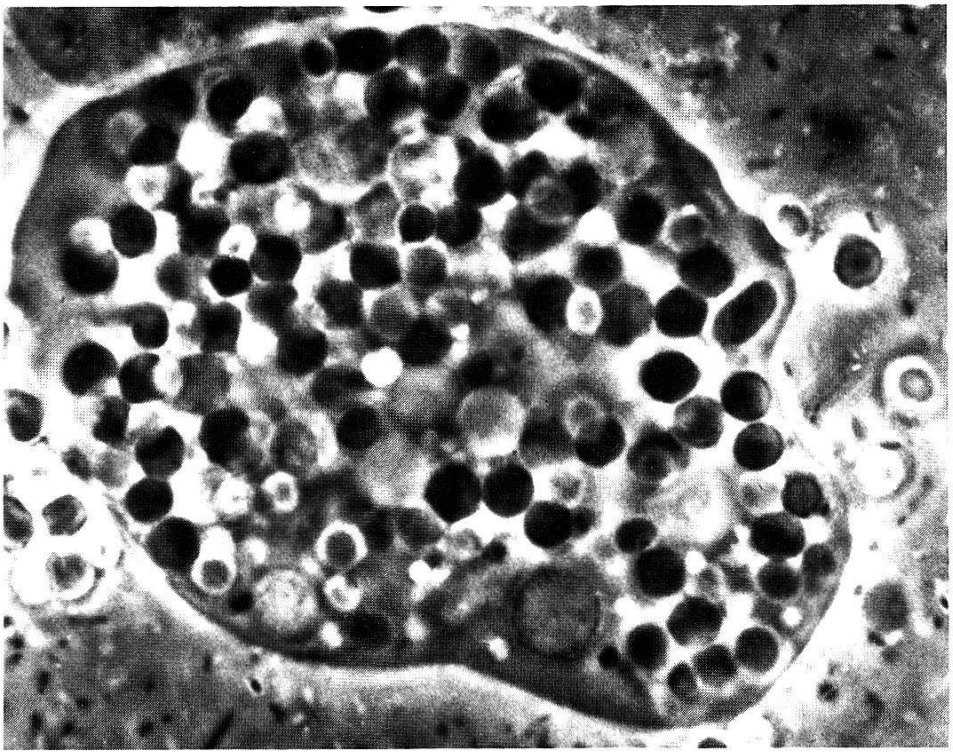

Fig. 1. Entamoeba invadens trophozoite containing a large number of sheep erythrocytes. Phase contrast.

Materials and Methods

Entamoeba invadens were cultured in Jones' medium (1946) at 25°C. This is a monophasic medium in which the amoebae grow luxuriantly. They become generally free of starch in about six days time from the date of innoculation. These starch-free trophozoites were used for the study. The erythrocytes were prepared from sheep blood collected in Alsever's solution which was later washed in physiological saline to remove all plasma and free haemoglobin. 0.1 ml of sheep cell concentrate was then added to tubes of six-day-old cultures containing 5 ml of medium. The tubes were gently shaken to disperse the red cells and the sediment examined after ten minutes. For light microscopy, Leitz phase-

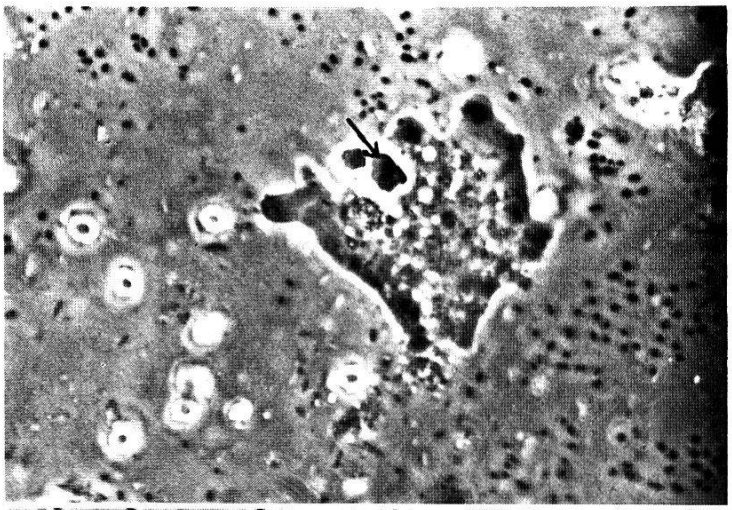

Fig. 2. Arrow marks an erythrocyte prior to its entry into the cytoplasm of the trophozoite. Phase contrast.

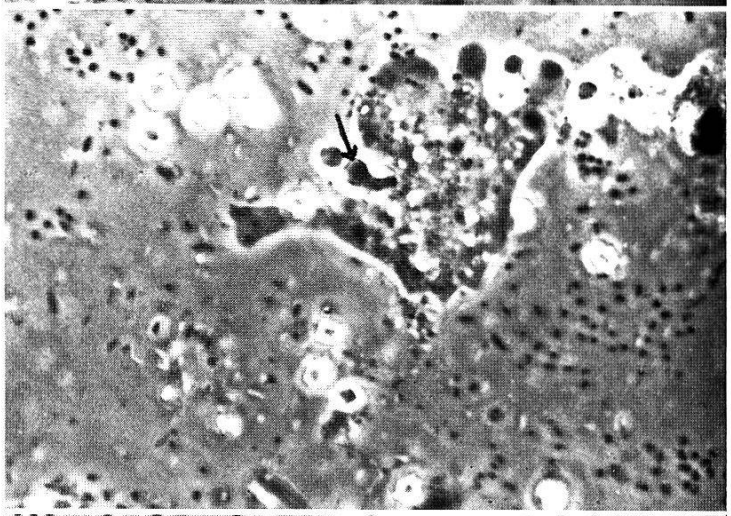

Fig. 3. Arrow marks the same erythrocyte during its entry into the cytoplasm. Phase contrast.

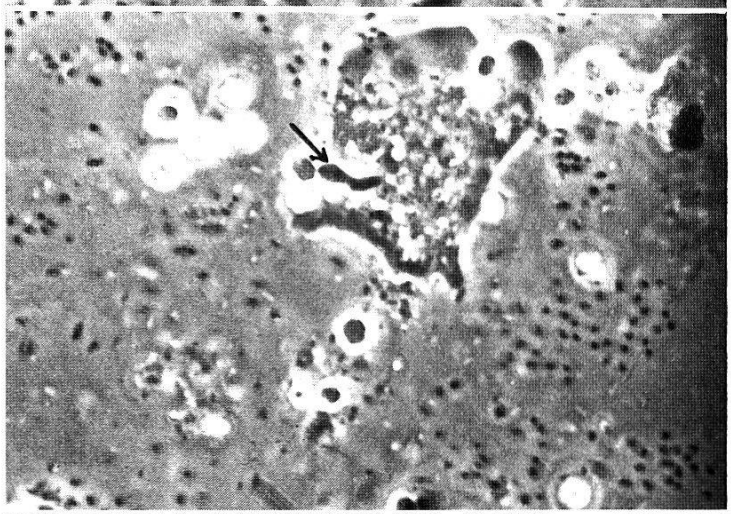

Fig. 4. Arrow marks the same erythrocyte which appears elongated as it enters the cytoplasm. Phase contrast.

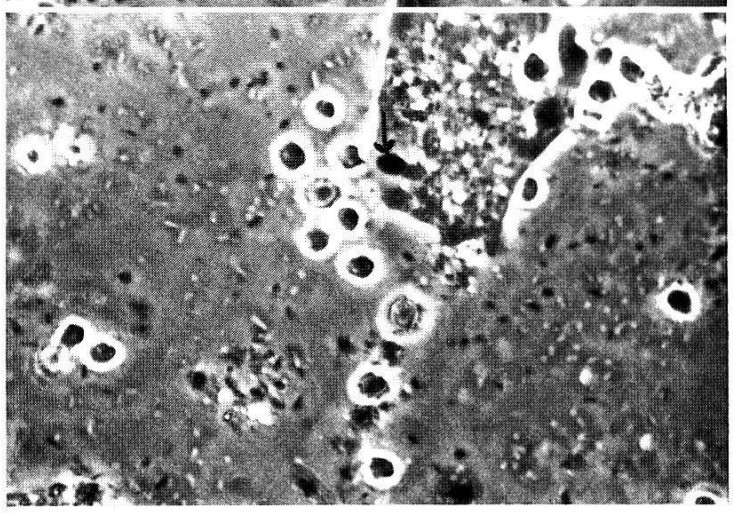

Fig. 5. Arrow marks the same erythrocyte having entered the cytoplasm. Phase contrast.

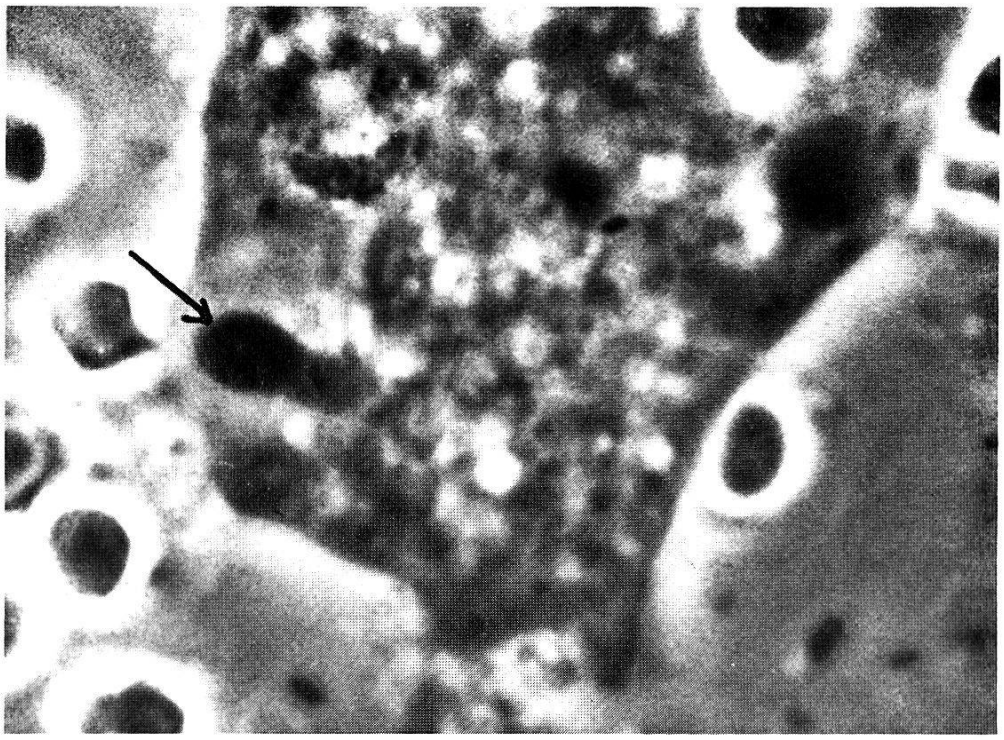

Fig. 6. An enlargement of Fig. 5 showing position of the erythrocyte in the cytoplasm. Phase contrast.

contrast set up was used, and the sequence of events relating to the process of ingestion was recorded on Kodak Tri X film using an electronic flash.

Preparations for electron microscopy were made by fixing the amoebae-erythrocytes suspension in buffered 2.5% Glutaraldehyde (pH 7.4) five minutes, half an hour and three hours after the introduction of erythrocytes into the culture tubes. The parasites were then post-fixed in buffered 1% Osmium tetroxide (pH 7.4). They were washed, dehydrated in graded series of cold ethanol and embedded in Araldite according to the standard technique. Thin sections were cut on a Poter-Blum ultra microtome with glass knives. The sections were collected on formvar-coated grids and examined after staining with uranyl acetate followed by lead citrate (REYNOLD's, 1963). Micrographs were made in a Hitachi HS 8 microscope.

Results

E. invadens rapidly ingested sheep erythrocytes and within ten minutes some trophozoites were observed packed with erythrocytes (Fig. 1). However, like the observations made by MCCONNACHIE (1955) with rat erythrocytes, the number of cells ingested varied greatly from one trophozoite to another. The actual process of ingestion was seen clearly on a number of times under the phase-contrast microscope. In this process the erythrocyte first adhered to the surface of the trophozoites and then was quickly drawn into the cytoplasm. The whole process was not taking more than a few seconds. No encircling pseudopodia were formed during the ingestion. As the erythrocyte entered the cytoplasm it appeared elongated (Figs. 2, 3, 4, 5 and 6).

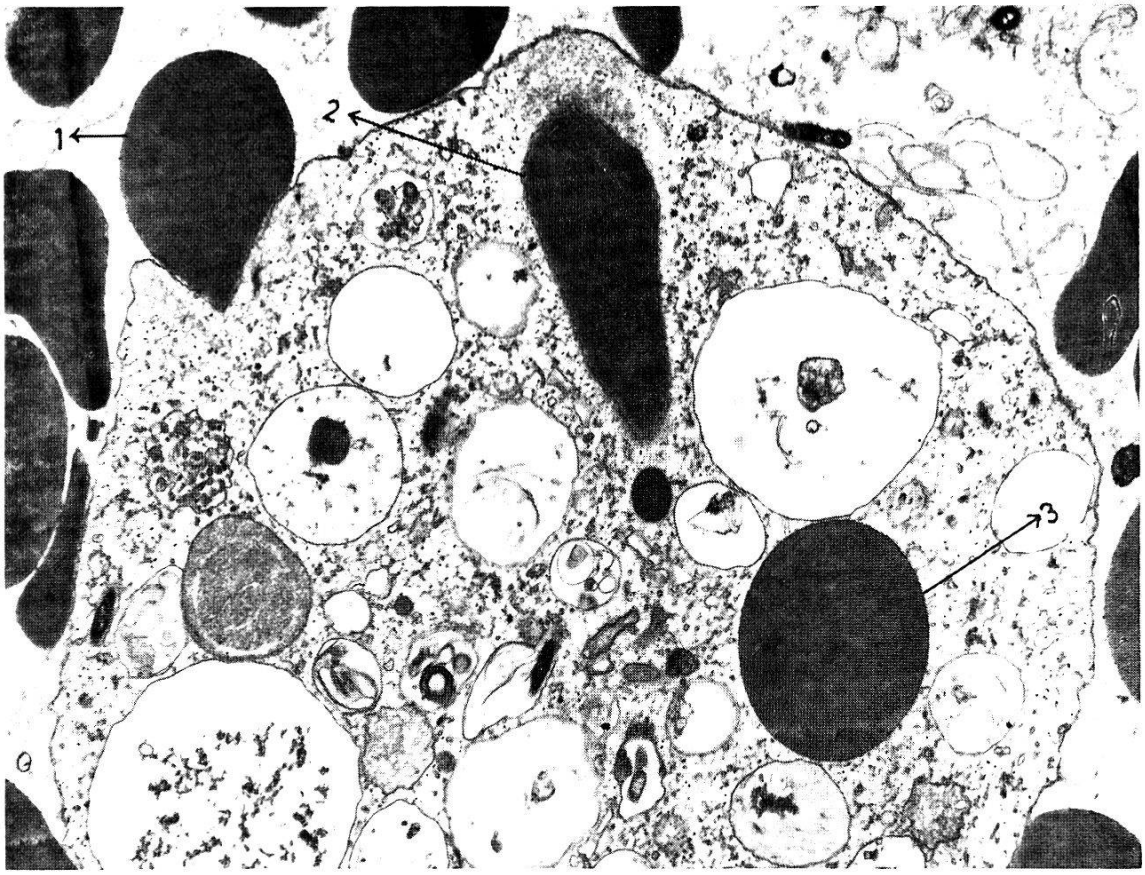

Fig. 7. An electron micrograph showing erythrocyte before entry (1), during entry (2) and after entry (3) into the cytoplasm of the trophozoite. During entry the erythrocyte appears elongated as in the light microscope. The plasma membrane is closely applied to the body of the erythrocyte as it enters the cytoplasm. At this stage no vacuolar space is observed around the ingested erythrocyte. × 6,300.

In the electron microscope a similar process was observed in which the erythrocyte first came in contact with the surface of the trophozoite, indented the plasma membrane and then entered the cytoplasm. As in the light microscope it appeared elongated during the process of entry but resumed its spherical shape after the completion of the process (Fig. 7).

The trophozoite fixed after half an hour showed the erythrocytes in different stages of digestion (Fig. 8). The erythrocytes, as they became digested and haemolysed appeared lighter in colour and showed detachment of their cell membrane. After haemolysis, clumps of electron dense material, probably haemoglobin, appeared within the food vacuole. Some of the trophozoites fixed after three hours showed completely haemolysed erythrocytes appearing as electron dense circles with clear interiors (Fig. 9).

Discussion

The method of ingestion of food particles in *Entamoeba* has been previously described by WENRICH (1941). According to him starch is ingested by the formation of the "food cups" which are large enough to take in the starch granules with no vacuolar space between the food body and the cytoplasm. A similar study was made by ZAMAN (1962), using the electron microscope, in which it

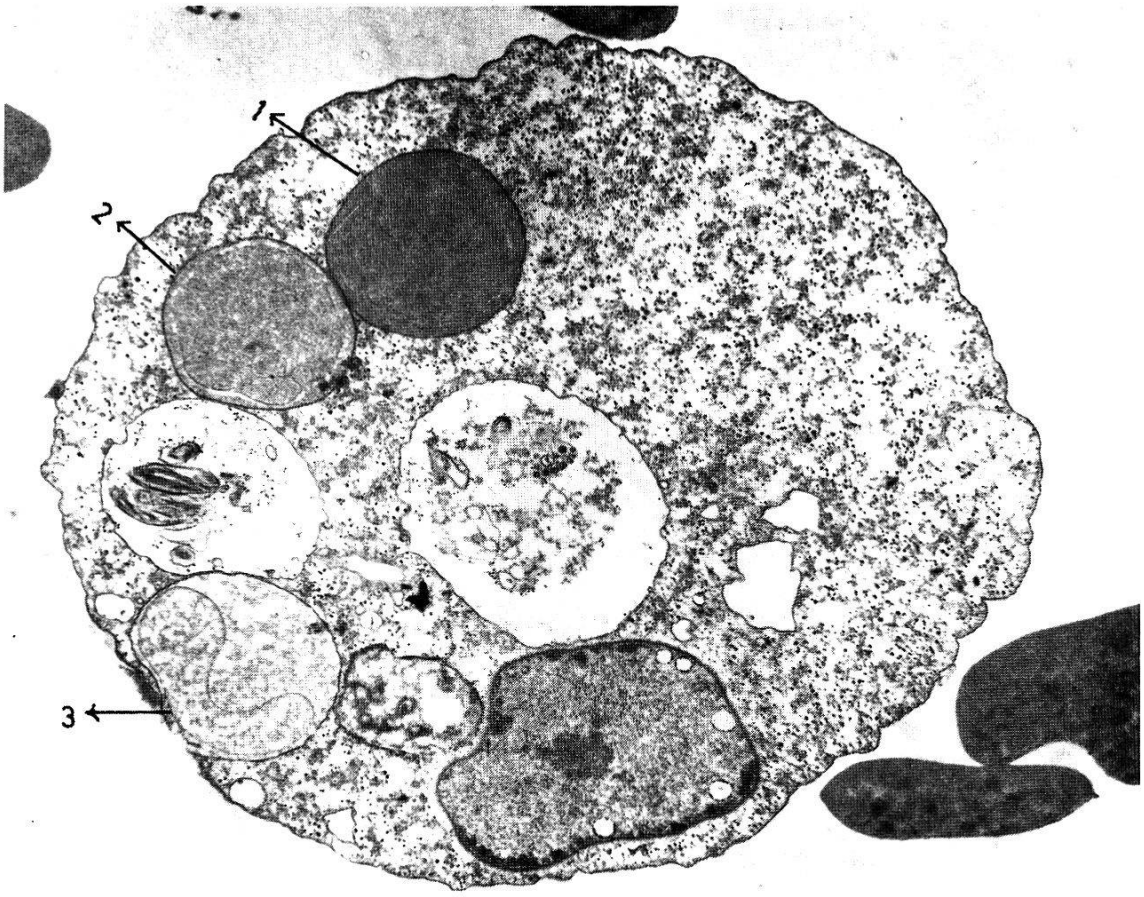

Fig. 8. Electron microscopy showing various stages of digestion of erythrocytes: (1) The erythrocyte has begun to haemolyse and an irregularity of its membrane can be made out. (2) The erythrocyte appears lighter as further haemolysis occurs and the irregular outline of its membrane is also more obvious. (3) The erythrocyte is now haemolysed and shows curled up membrane with clumps of electron dense material, probably haemoglobin lying in its vicinity. × 6,300.

was observed that the pseudopods were thrown around the starch grains during ingestion and that the plasma membrane of the trophozoite was continuous with the lining of the so-called "food cups". The plasma membrane was, however, so closely applied to the starch grains that it was not possible to observe the vacuolar space in the light microscope. The vacuolar space became visible in the light microscope after digestion had progressed for some time. Similarly, when the erythrocytes were ingested the plasma membrane was closely applied to the surface of the cell and the vacuolar space could not be observed in the light microscope. In the electron microscope, however, the plasma membrane could be seen around each erythrocyte ingested. The digestion of erythrocytes started within ½ an hour of ingestion as indicated by the changes observed in the electron microscope. In 3 hours, at least some of the erythrocytes were completely haemolysed. The erythrocyte membrane was last to be digested and could still be seen as circular bodies after 3 hours of ingestion.

References

JONES, W. R. (1946). The experimental infection of rats with *Entamoeba histolytica;* with a method for evaluation the anti-amoebic properties of new compounds. – Ann. trop. Med. Parasit. *40,* 130–140

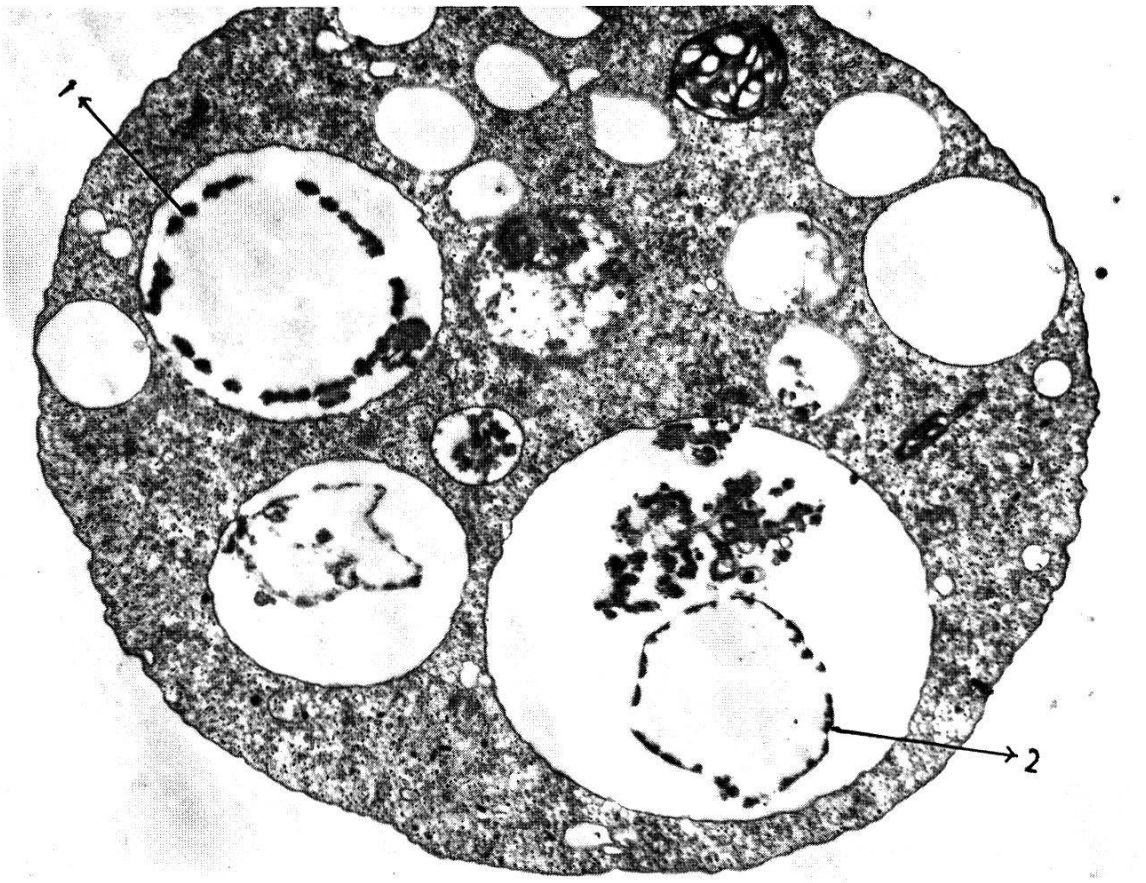

Fig. 9. An electron microscopy showing 2 completely haemolysed erythrocytes (1) and (2) lying in its vicinity. The vacuolar space is now clearly visible. × 6,300.

McConnachie, Elspeth W. (1955). Studies on *Entamoeba invadens* Rodhain, 1934, *in vitro,* and its relationship to some other species of *Entamoeba.* – Parasitology *45,* 452–481

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. – J. Cell Biol. *17,* 208–212

Wenrich, D. H. (1941). The morphology of some protozoan parasites in relation to microtechnique. – J. Parasit. *27,* 1-28

Zaman, V. (1962). An electron microscopic observation of starch ingestion by *Entamoeba invadens.* – Trans. roy. Soc. trop. Hyg. *57,* 535-537