COMPARATIVE STUDIES OF THE ULTRASTRUCTURE OF ENTAMOEBA HISTOLYTICA AND ENTAMOEBA COLI

by

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CONTENTS

A. INTRODUCTION

Controversy on taxonomy and pathogenicity has persisted ever since the first description - almost a century ago - of the amoebae living in the intestine of man.

Intestinal amoebae are said to have been observed by Lewis (1870) and Cunningham (1871) and, according to Dobell (1919), they probably belonged to the species now known as *Entamoeba coli*. The first accurate description of an intestinal amoeba was that by Lösch (1875) who named the parasite Amoeba coli. He felt that it was not sufficiently well defined to give it species status. Lösch did not consider that these amoebae were directly responsible for his patient's dysentery and said "Es bleibt nur noch die Frage zu entscheiden, ob die ganze Krankheit durch die Amöben hervorgerufen worden, oder ob sie in Folge anderer Ursachen entstanden war und die Amöben erst nachträglich in den Darm gelangten und die Krankheit unterhielten".

Several other investigators - Koch & Gaffky (1887), Kartulis (1885), Osler (1890) - recorded the presence of amoebae, similar to those described by Lösch, in stools from patients with dysentery and in liver abscesses, but the relationship of these amoebae to dysentery remained in doubt. According to Councilman & Lafleur (1891) Baumgarten (1890), referring to the opinion expressed by Kartulis that the amoebae are the cause of dysentery, said "We will not contradict this view, although recent observations and some of earlier dates have shown that similar forms of amoebae are found in the faeces of healthy individuals".

Councilman & Lafleur (1891) in their classical clinical description of amoebic dysentery and amoebic liver abscess found amoebae similar to those described by Lösch and named them *'Amoeba dysenteriae'*. They concluded that "the *Amoeba dysenteriae* has been shown to be the causative agent from its constant presence in the stools and in the anatomical lesions".

Quincke & Roos (1893) were the first to observe the cystic

form and to deduce that it was involved in the transmission of disease. "Nicht nur durch Uebertragung amöbenhaltigen Stuhles in das Rectum lässt sich Amöbendysenterie auf Katzen ubertragen, sondern bei Gegenwart encystirter Amöben auch durch Einbringung per os".

Casagrandi & Barbagallo (1897) showed that the intestinal amoebae differ both morphologically and in their cultural characteristics from the free living species. They proposed the generic names *'Entamoeba'* and described various phases in the life cycle of what is now known as *Entamoeba coli*.

Although Schaudinn (1903) is credited with the distinction between the non-pathogenic *Entamoeba coli* and the pathogenic *Entamoeba histolytica*, his account of 'sporulation' in place of encystation in the latter, created much confusion. In the same year Huber rediscovered the cysts of *Entamoeba histolytica* which he showed to Schaudinn. The latter would not admit that they belonged to *E. histolytica* and said that they belonged to a different species which he would call *E. tetragena*. Viereck (1907) adopted Schaudinn's name and called the quadrinucleate cysts which he observed *Entamoeba tetragena* and Elmassian (1909) named the small trophozoites associated with the quadrinucleate cysts *Entamoeba minuta*.

The three species thus far labelled, *Entamoeba histolytica* Schaudinn 1903, *Entamoeba tetragena* Viereck 1907 and *Entamoeba minuta* Elmassian 1909, had all been implicated in the production of endemic tropical dysentery. Walker (1911) expressed the opinion that "*E. tetragena* Viereck is identical with *E. histolytica* Schaudinn and that the life cycle of *E. histolytica* includes the development of Tetragena cysts".

Walker & Sellards (1913), in an investigation into the relationship of the different species of amoebae to endemic tropical dysentery, demonstrated that *E. histolytica* is the cause of amoebic dysentery and that *E. coli* is a harmless commensal living in the intestine of man. They conducted 60 feeding experiments involving three groups of prisoners. Of the group of patients fed various strains of amoebae from water and other non-human sources in Manila,

none became infected and they concluded "that the amoebae play no part in the aetiology of endemic tropical dysentery". Of 20 men who were fed different strains of *E. coli* 17 became parasitized although none developed any sumptoms of dysentery and the conclusion was drawn that "*E. coli* is an obligatory parasite ... and that it is non-pathogenic, and consequently plays no role in the aetiology of entamoebic dysentery". As a result of their experiments with *E. tetragena* and *E. histolytica* they concluded "that *E. tetragena* Viereck is identical with *E. histolytica* Schaudinn, that tetragena cysts are produced in the life cycle of *E. histolytica*, and that *E. minuta* Elmassian is a pre-encysted stage of *E. histolytica*". Of 20 patients fed different strains of *E. histolytica* 18 became infected although only four developed dysentery. These experimental dysenteries all developed after ingesting *E. histolytica* isolated from healthy persons who were 'carriers' of this parasite. None developed from cases of acute amoebic dysentery or from amoebic liver abscess. They pointed out that the amoebae ingested were accompanied by the micro-organisms contained in the dysenteric stool and "consequently, the small percentage of dysenteries resulting from these findings has no bearing upon the aetiology, but is evidence of the frequent latency of this disease".

In the same year Kuenen & Swellengrebel (1913), working in Sumatra described three 'stadia' of what was then called *E. tetragena*. In the first phase they considered it to be a true tissue-parasite living in the tissues of the intestine or liver, and named it the 'histolytica phase'. In the second phase, the amoeba lived in the lumen of the bowel and behaved rather like *E. coli*. It was smaller than the histolytica phase and corresponded to Elmassian's *E. minuta*, and they accordingly called it the 'minuta phase'. The third was the 'cystic phase', which was usually found in conjunction with the minuta phase, and was responsible for the dissemination of the species. They differed from Walker & Sellards *(loc. cit)* in regarding the tissue phase as playing no part in the cycle.

Commenting on the work of Wenyon & O'Connor (1917) in which they demonstrated the presence of *E. histolytica* cysts in apparently healthy hosts, Dobell (1918) said "The explanation of all these

observations is now so obvious that it is almost difficult to believe that they ever appeared problematic; and it turns simply upon the proper appreciation of the habits of the amoeba. *E. histolytica* is - unlike most parasitic amoebae - a tissue parasite. It lives in and upon the living tissues of its host, and it can exist in no other way. This is the most important fact about the amoeba - as far as man is concerned. Now a parasite which feeds upon its host may obviously do so to a greater or less extent. The ideal condition for host and parasite alike is a state of equilibrium like that between Prometheus and the eagle - the former regenerating sufficient tissue each day to compensate for the ravages of the latter. The 'natural' condition of a man infected with *E. histolytica* is similar. His amoebae feed, grow and multiply at the expense of the living tissue lining his large intestine ... A human being in this Promethean state of equilibrium is now called a 'carrier': and there can be little doubt that this is the 'normal' or most common condition of infection of human beings with *E. histolytica* in nature. The carrier can only be known by the presence of the cysts of the amoebae in his faeces".

Dobell (1919) was the principal critic of the commensal mode of life in *E. histolytica* and said "there are still a few workers who find it difficult to believe that *E. histolytica* is a tissue-parasite always". He considered Walker & Sellards (1913) concept of the life cycle to be the only correct one and said of Kuenen & Swellengrebel, they "hold peculiar views concerning the life history of *E. histolytica*" and "have never proved that the 'minuta' forms are capable of living saprozoically, and it is more than probable that they are not".

Craig (1927) supported Dobell's Promethean view that *E. histolytica* is always a tissue parasite and said that "in every carrier of this parasite there must be numerous foci of infection present in the mucous membrane from which toxic material is absorbed, and whether symptoms are produced or not depends on the resistance of the individual carriers".

The issue was further confused by Wenyon & O'Connor (1917)

who stated that *E. histolytica* is a species which is composed of different races distinguishable by the size of their cysts. Dobell (1919) reached the same conclusion and said "we have demonstrated the existence of at least five. There is no evidence that the different races differ in their geographical distribution or in any character save size".

Brumpt (1925, 1949), in an attempt to explain why such a small proportion of people harbouring cysts actually developed disease, caused further confusion by splitting the amoebae producing quadrinucleate cysts into two morphologically indistinguishable species, *E. dysenteriae* and *E. dispar* on the basis of pathogenesis in kittens. In his Precis de Parasitologie (1949) he pointed out that the former is pathogenic and causes clinical symptoms in warm and tropical countries, to which it is restricted, though in carriers it may live as a commensal, in the minuta stage, but the latter has a cosmopolitan distribution, is non-pathogenic and represented exclusively by minuta forms living in the lumen of the gut and feeding on bacteria, without ever producing lesions.

Dobell (1931) had revised his earlier views and concluded "that *E. h. hominis* (i.e. the human strain of *E. histolytica*) sometimes lives in man as a harmless commensal - just as *E. h. macacorum* (i.e. the simian strain) typically does in its natural hosts". Unfortunately this statement appeared in a highly technical journal which did not reach the many physicians and others who had been influenced by Craig (1927).

Cysts of a small size appear to have first been described by von Prowazek (1912) when he observed an intestinal amoeba with cysts from 6-8 µm in diameter, which he called *Entamoeba hartmanni*. Dobell (1919) records that Mathis & Mercier (1916) "regard all cysts of *E. histolytica* with diameters of less than 10 µm as 'abnormalities' - a view which is certainly untenable". Sapero *et al.* (1942) in an extensive study of the data presented by Dobell and Jepps (1918) and Malins-Smith (1918) as well as their own survey of cysts from 283 patients "demonstrated the existence of two significantly different races" and stated "as a general rule that the 10 micron line for

measurements on living cysts of *E. histolytica* may be used to divide the organism into the large or small race". In summary they point out that "the small race trophozoites have not been observed to ingest red blood cells" and that "it has not yet been definitely established that any particular clinical syndrome is associated with the small race infections".

Freedman & Elsdon-Dew (1958, 1959) grew amoebae of different size and origin together and produced a bimodal size-distribution curve. Using size as a criterion of species they suggested that the "small amoebae with *histolytica* morphology should be a separate species, *Entamoeba hartmanni*, Prowazek, 1912".

Burrows (1959) in a morphological study of species of amoebae stated "*Entamoeba hartmanni* may be distinguished from the dwarf forms of *E. histolytica* by the smaller sizes of the trophozoites, by the smaller and more *E. coli*-type of nucleus, by the smaller sizes of cysts, by the smaller percentage of nuclear diameter to cyst diameter, and by the diffuse type of cyst vacuolation".

Hoare (1952) who had himself re-examined Dobell's material, in a review of the evidence for and against a commensal phase in the life cycle of *Entamoeba histolytica*, said "A critical analysis of the available data leaves no doubt that in symptomless carriers, as well as in latent periods of chronic infections, the amoebae live in the lumen of the gut and feed - among other faecal elements - on bacteria. Apart from their food habits, these trophozoites known as the minuta forms can be distinguished from the haematophagous amoebae, found in dysenteric patients, by their smaller size. Since they are the only amoebae capable of producing cysts, the minuta forms represent the essential stage of *E. histolytica* whereas the haematophagous amoebae have no place in its normal life cycle:".

Having established the existence of a commensal phase in the life cycle of *Entamoeba histolytica* and excluded *Entamoeba hartmanni* as a pathogen, the picture was further confused when in 1956 an amoeba was isolated by Dr. F.H. Connell from a patient with a history of diarrhoea. Although morphologically indistinguishable from

Entamoeba histolytica, it differed in its cultural characteristics. This strain was able to grow as well at room temperature as it did at 37°C and has been termed the 'Laredo' or *E. histolytica*-like strain. Several other strains, such as 'Huff' and 403, both of which were isolated from asymptomatic carriers, have also been shown by Richards *et al.* (1966) not only to be thermo-adaptable but also osmo-adaptable.

Brief mention should be made of *Entamoeba invadens*, the parasite associated with amoebiasis in certain reptiles and which lives as a commensal in turtles. It was isolated in 1934 by Rodhain from a python in the Antwerp Zoo. The ease of cultivation of this parasite, which grows at room temperature, and its marked resemblance to the trophozoites and cysts of *Entamoeba histolytica* have resulted in extensive studies of its ultrastructure.

A.1. THE PROBLEM OF PATHOGENICITY

It is apparent that appreciation of the host-parasite relationship in infections with *E. histolytica* has been confused by a number of issues.

The recognition of the small race of *E. histolytica* as a distinct species - *E. hartmanni*, and the Laredo strains has clarified the situation to some extent. This has left only the large race *E. histolytica* for consideration as a pathogen.

Some workers considered that *E. histolytica* is potentially always a pathogen, but that its virulence remains dormant until activated by extraneous factors. Others held the view that the large race comprised two types - the non-pathogenic, cosmopolitan *E. dispar* and the pathogenic *E. dysenteriae* confined to the tropical and sub-tropical regions.

It is, however, generally accepted that in many persons infection with *E. histolytica* does not result in symptoms or disease whereas in others the manifestations range from mild gastrointestinal disturbances to extensive invasion of the tissues. Is this dissimilarity

in behaviour of the amoebae in different hosts, one of species, race or strain difference?

Neal (1957) on the basis of experimental results of intracaecal infections in rodents "tentatively proposed that *E. histolytica* comprised two types differing only in virulence". However, some 13 years later (1971) he followed the concept proposed on epidemiological grounds by Elsdon-Dew (1958) that in individual hosts the invasive form may arise from commensal forms as a result of some environmental factor.

Speculation as to the mechanism of tissue invasion and the factors responsible for it coupled with the conflicting views, led to the present study.

As light microscopy affords no further clues, it is hoped that the ultrastructure may reveal some difference which might be related to pathogenicity.

Trophozoites of *E. histolytica* isolated from patients with clinical amoebiasis were studied and compared with those from asymptomatic patients. In addition trophozoites and cysts of the non-pathogenic *E. coli* were examined.

The various strains examined from culture and from human and animal tissue are shown in Table 1. A glossary of terms used throughout this study is presented in the appendix.

TABLE 1

DIFFERENT STRAINS VIEWED UNDER THE ELECTRON MICROSCOPE

Entamoeba histolytica:						*Entamoeba coli:*	
Human Colon	Human Liver Abscess	Hamster L.A.	Monoxenic	Axenic	Multixenic	Human Colon	Multixenic
AS 1	AN	AN	AN	HK9	H2	C 2	C 2
AS 2	AM	AX	AX	NIH	H3	C 4	C 5
AS 3	AP	HALA	HALA	AH	H 12		C 6
AS 4	AX	AK	AK	HALA	H 22		C 10
AS 5		N	N	AK	L 132		C 13
AS 6		AL	AZ				C 15
		AZ					C 16
							C 17

B. REVIEW OF THE LITERATURE ON ULTRASTRUCTURE

The first detailed account of the ultrastructure of *Entamoeba histolytica* was by Osada in 1959. He collected trophozoites from 48 hour cultures in Dobell-Laidlaw medium and fixed them in a buffered solution of Osmium tetroxide. The cytoplasm was shown to be finely alveolated and of a lower electron density than the nucleoplasm. The nucleus contained many electron-dense granules which he regarded as chromatin. It was bounded by a membrane consisting of two layers, interspersed with minute holes and separated by a thin clear space. He noted a lack of mitochondria, endoplasmic reticulum or Golgi's apparatus.

Deutsch & Zaman (1959), in a study of *Entamoeba invadens*, drew attention to electron-dense particles of about 20 nm in size. In trophozoites these particles were dispersed throughout the cytoplasm or concentrated into small masses, whereas in cysts they were compacted into larger bodies. They pointed out the striking resemblance of these bodies to certain virus particles. They studies the crystalline structure of these particles at high magnifications and identified them as 'chromatoid bodies'. The lack of mitochondria in this species was noted and it was postulated that the "enzymes which generate energy by transfer of electrons to oxygen might be lacking or dispersed in the cytoplasm".

Barker & Deutsch (1958), in a study of the chromatoid bodies, observed that the larger bodies are formed by aggregation of the smaller ones and that the total number of bodies decreases in the trophozoites until encystment.

Miller & Swartzwelder (1960) studied the nuclear components of *Entamoeba histolytica* from culture and observed particles approximately 40 nm in diameter, grouped in three rows near the nucleus. The size and structure was considered to be compatible with sectioned virus particles as seen under the electron microscope.

Miller, Swartzwelder & Deas (1961), employing several

different fixation techniques, demonstrated that the granular aggregates on the inner periphery of the nucleus were not artefacts of fixation but represented valid structures. They described a rudimentary endoplasmic reticulum and a pore system of the nuclear membrane.

Fletcher, Maegraith & Jarumilinta (1962), in their electron micrographs of trophozoites of *E. histolytica* from culture, showed that the parasite is bounded by "a double limiting or plasma membrane". Food vacuoles bounded by a limiting membrane with a double structure similar to that of the plasma membrane, indicated that vacuoles were formed by invagination of the plasma membrane. They rarely found more than one type of foodstuff within a vacuole and suggested that different physico-chemical conditions for the hydrolysis of specific substrates, are created within individual vacuoles.

For the next few years ultrastructural studies appear to have been concentrated on *Entamoeba invadens*. Barker (1962, 1963a, b) confined his attention to the structure and function of the chromatoid body. He showed that it was composed of ribonucleic acid and protein and that it arose by a process of aggregation of small groups of 25-30 nm units, which formed polycrystalline masses in precysts and early trophozoites. His results supported the hypothesis that the chromatoid body represented a crystalline store of ribosomes and were incompatible with a virus interpretation of the nature of the body.

Barker & Svihla (1964) advanced the hypothesis that in *E. invadens* "the large crystalloids, the chromatoid bodies, are a manifestation of a special parasite-host adaptive mechanism. Ribonucleoprotein is synthesised under favourable conditions, crystallised during the encystation perid, stored as aggregates of ribosome-like particles in the cyst, dispersed in the mature cysts, and finally shared by the eight young amoebae, which are thus supplied with previously synthesised 'ribosomes' for a period of quick growth to establish themselves in a new host".

Siddiqui & Rudzinska (1963, 1965) were the first to demonstrate the presence of the chromatoid body in axenic trophozoites of *E. invadens* and that it is composed of lamellae arranged in parallel

arrays, which at higher magnifications, appear to form a closely packed helix. They proposed the introduction of a new, more specific term 'helical ribonucleoprotein (RNP) bodies' for bodies of the same nature as those found in *E. invadens* and postulated that in cells actively synthesising protein, but having little or no endoplasmic reticulum, the free ribosomes aggregate to form either polyribosomes or helices. They demonstrated that the limiting membrane of vacuoles was approximately the same thickness and density as the plasmalemma, which suggested that they were formed from the plasmalemma. The nucleus was shown to have a cortical honecomb layer, resembling the structure in *Amoeba proteus*, but not observed in bacterised cultures of *Entamoeba invadens*.

Rondanelli *et al.* (1966, 1967, 1968) noted the presence of oval electron-dense bodies on the inner leaflet of the plasmalemma. These were in greater abundance in *E. invadens* than in *E. histolytica*, while their absence was noted in the Laredo or *E. histolytica*-like strain. It was at first thought that these bodies were connected with the contractile activity of the cytoplasm, but later it was suggested that they might be concerned with the histolytic enzyme activity of the pathogenic amoebae. The higher concentration of filamentous spirals in trophozoites from experimental infections than in those from culture, was attributed to a higher biosynthetic activity *in vivo*.

Whereas previously the studies had concentrated mainly on the fine structure of *E. invadens*, the latter half of the 1960's heralded a spate of interest in *E. histolytica*, particularly in experimental and human infections.

Miller & Nelson (1968) compared the chromatoid body of *E. histolytica* with that of the Laredo strain and confirmed that it was formed by condensation of ribosomal material, as had been observed in *E. invadens*. They observed, however, that this condensation occurred at different stages in the life cycles of the two strains.

Lowe & Maegraith (1969, 1970a) observed a 'rosette' of tubular bodies encircling a finely granular area in the cytoplasm of

trophozoites of *E. histolytica* from culture. They were the first to record the ultrastructure of trophozoites of *E. histolytica* from the tissues of experimentally infected hamsters and from axenic culture, strain HK9 (1970b & c). The rosette arrangements were seen in trophozoites from both sources and although originally thought to be viruses, it was subsequently suggested that they might be structural organelles of the parasite.

This rosette arrangement was observed by Trevino-Garcia Manzo *et al.* (1970a, b, c, 1971a, b, 1972) in monoxenic cultures of amoebae from the human colon, but not in cultures with mixed bacteria and was referred to as a centriole-like structure. Their ultrastructural observations showed that trophozoites from experimentally produced liver abscesses in hamsters, were identical to amoebae grown in different cluture media. Their study of the effects of various anti-amoebic drugs on the fine structure of trophozoites in culture, showed proliferation of the smooth endoplasmic reticulum, cytoplasmic elements similar to the Golgi apparatus and autophagic vacuoles. The latter were considered to be part of a self-destruction mechanism induced by the amoebicides. Surface-active lysosomes were observed in strains HK9 and ABRM, within which acid phosphatase was found. The ingestion of red blood cells, although observed more frequently in pathogenic amoebae, was not considered as an indication of increased virulence.

Eaton *et al.* (1969), in the course of work designed to study the behaviour of *E. histolytica* in cell-cultures, discovered a system of surface lysosomes, each of which appeared to be equipped with a 'trigger' mechanism. The following year, they confirmed their observations by scanning electron microscopy. They suggested that these lysosomes are responsible for the cell damage observed and that in pathogenic *E. histolytica* this represents a modification of an organelle possessed by other non-pathogenic members of the genus *Entamoeba*.

Magaudda *et al.* (1970) in a scanning microscope study of a thermo-adaptable strain of *E. histolytica* (Messina strain) suggested that the surface-active lysosome with its 'trigger' mechanism might be an artefact of fixation. Another interpretation was that the absence

of such organelles on the surface of their amoebae might be attributable to lack of pathogenic activity in these amoebae.

In a comparative study by El Hashimi & Pittman (1970) the only structural difference noted between trophozoites of *E. histolytica* from the human colon and from culture, was the presence of a fuzzy layer about 30 nm wide on the outer layer of the plasmalemma in the former. A similar comparative study by Kee-Mok Cho *et al.* (1972) did not reveal an extramembranous fuzzy layer in trophozoites from the intestinal mucosa. Nuclear pores were seldom observed in trophozoites from culture, whereas they were regularly seen in trophozoites from the human colon.

Ludvik & Shipstone (1970) reported the presence of subpellicular dark bodies with lens-shaped profiles immediately below the cytoplasmic membrane in trophozoites of *E. histolytica* from culture. It was suggested that they might be closely connected with the "new synthesis of cell membrane" or represent direction chemotactic centres. Osmiophilic 'button-like' bodies were observed in the nucleus, which were considered to be some form of nuclear virus-like parasite or symbiont.

Rosenbaum & Wittner (1970) noted an increased number of helical sub-fragments derived from the larger RNP bodies in amoebae grown in continuous association with bacteria and in axenic amoebae exposed to bacteria. Their observation of ribosomal helices in close association with digestive vacuoles, led them to suggest that the small helices might be related to increased levels of hydrolase activity present during digestion, and that filaments might contain or actually represent enzymic protein.

Griffin & Juniper (1971) did not observe the aggregation of ribosomal helices into chromatoid bodies in trophozoites of *E. histolytica* from the human colon. They suggested that they could not be sites of ribosomal functions in amoebae from human disease, but rather that they acted as stored aggregates of helices for use after encystment.

Kress, Wittner & Rosenbaum (1971) demonstrated the presence

of specific regions in the cytoplasm of trophozoites of *E. histolytica* from culture, which appeared to constitute sites for ribonucleoprotein helix formation.

Miller & Deas (1971) gave the first brief account of the fine structure of cysts of *E. histolytica* of the Laredo, Huff and W.B. strains. The trophozoites of these strains were grown in an encystment medium, which permitted the study of cysts in various stages of maturation. A fuzzy coat on the plasmalemma of rounded-up trophozoites, was the first indication of the formation of a cyst wall. They noted a reduction in the size of the chromatoid bodies as the number of nuclei increased. The manner in which RNA assumed a 'latent' form and was distributed to the cytoplasm which then gave rise to daughter amoebae, was demonstrated.

Feria-Velasco & Trevino (1972) in a study of trophozoites of *E. histolytica* under various cultural conditions, paid particular attention to the 'rosette' arrangements previously described. When the plane of sectioning was parallel to the longitudinal axis, the cylindrical units were seen to be bullet-shaped and limited by a unit membrane. The constant proximity of the rosette-like body to digestive vacuoles and to the plasmalemma, led them to speculate that it is probably related to the digestive processes. Feria-Velasco *et al.* (1972) demonstrated a glycocalyx made up of an amorphous layer, measuring 15 nm and a filamentous layer, 50 nm wide, in strains HK9 and NIH from axenic culture. Because of its mucopolysaccharide nature, the glycocalyx was thought to play an important role in the biology of vegetative forms, since glycoproteins are related to cellular recognition phenomena.

Griffin (1972) did not observe surface lysosomes in amoebae from the human colon, and suggested that the results obtained by Eaton *et al.* were due to special experimental conditions and were probably of no significance in the pathogenesis of the disease. It was thought that contact cytolysis and surface-active lysosomes might be false leads. He suggested that *E. histolytica* routinely eats inflammatory cells and considered that material from such cells might contribute to the pathogenesis of amoebiasis.

The observation by Takeuchi & Phillips (1972) in experimental amoebiasis, that epithelial damage precedes amoebic contact, would seem to be compatible with a chemical mechanism of damage.

Diamond *et al.* (1972) and Mattern *et al.* (1972) in companion papers, demonstrated the presence of two morphologically different viruses in axenic cultures of *E. histolytica*. One was a filamentous particle which replicated in the nucleus as multiple clusters of fine filaments and ultimately lysed the nucleus causing cell death. The other, a polyhedral virus was seen only in the cytoplasm and also resulted in lysis of the cell.

Westphal *et al.* (1972) studies the phagocytosis of a crithidia species by *E. histolytica*. They observed the formation in the amoeba of an area of fine reticular cytoplasm along the line of contact, which they termed the 'phagocollar'. This area was visible around the crithidia during invagination until phagocytosis was complete and the digestive processes began. Formation of the phagocollar took place after contact with other microorganisms, but not with amoebae of the same species.

Rondanelli *et al.* (1972) studied the fine structure of trophozoites of *E. coli* (London 1971 strain) from culture. This species had not been previously described. Most of the organelles which had been observed in *E. histolytica* were noted. The presence of surface lysosomal systems in *E. coli*, which had been regarded as peculiar to *E. histolytica*, was the main point of discussion.

Barker & Swales (1972) in a comparison of the fine structure and optical diffraction patterns of fibonucleoprotein microcrystals in cysts of *E. invadens* with those in trophozoites, demonstrated that they were composed of similar ultrastructural units.

Zaman (1972, 1973a, b) demonstrated the presence in trophozoites of *E. invadens*, of a tail or uroid. This is an ectoplasmic structure separated from the endoplasm by an electron-dense area, indicating the presence of a partly dissolved membrane between the two regions. A study of the intranuclear bodies showed that they were able to penetrate the nuclear membrane. The cysts of *E. invadens*

were seen to contain two types of vacuoles - large and small. The former contained remnants of food material while the latter, lying near the cyst wall, were thought to contain enzymes required for encystation. The glycogen formation occurred around the food vacuoles and the mass which ultimately formed was not surrounded by a membrane. The uroid had been demonstrated in *E. histolytica* on light microscopy by Bird (1956) as a fixed point on the surface which became the apex of a tail-like projection when the parasite was in active progression.

Bird *et al.* (1974) have maintained an interest over a number of years in the nature and origin of the granular bodies of the rosette arrangements seen by several authors. Attention was focused on the similarity between these particles and a rhabdovirus of plants, which has a louse as a vector.

While numerous studies have been conducted on the ultra-structure of the various species of amoebae and numerous hypotheses propounded, it would seem as Neal (1971) stated "that there is still no definite information as to the underlying basis of pathogenicity of *E. histolytica*".

C. MATERIALS AND METHODS

C.1. STRAINS

Unless otherwise stated all material was obtained from patients admitted to King Edward VIII Hospital, Durban.

C.1.a. *E. histolytica*.

C.1.a.i. from the human colon.

Six strains - AS1, AS2, AS3, AS4, AS5 and AS6 were isolated between 1971 and 1973, from material obtained at sigmoidoscopy from patients with amoebic dysentery.

C.1.a.ii. from human liver pus.

Four strains - AN, AM, AP and AX were isolated in 1973 from patients with amoebic liver abscesses.

C.1.a.iii. from monoxenic culture.

The following strains were isolated from amoebic liver abscess pus according to the method of Freedman *et al.* (1958). They were established in Locke-egg medium with *Clostridium welchii* (68) as the concomitant organism.

Strain N/68 isolated in 1960.
Strains HALA/68, AK/68 and AL/68 isolated in 1972.
Strains AN/68, AX/68 and AZ/68 isolated in 1973.

C.1.a.iv. from axenic culture.

Five strains - HK9, NIH 200, HALA, AH and AK have been maintained in this laboratory. Strains HK9 and NIH 200 were kindly supplied by Dr. L.S. Diamond. The other three were established by the writer in axenic culture from monoxenic culture.

C.1.a.v. from asymptomatic cyst passers.

Four strains - H2, H3, H12 and H22 were isolated from patients undergoing treatment for Tuberculosis at King George V Hospital, whose gel-diffusion tests were negative. They were maintained in Locke-egg medium together with their associated flora.

C.1.a.vi. *E. histolytica*-like strain.

Strain L 132 was isolated from a cyst-passer with no evidence of invasion and whose gel-diffusion test was negative.

C.1.a.vii. Cysts of *E. histolytica* from the human colon.

Cysts were isolated from a patient with amoebic liver abscess who had been given a course of Flagyl.

C.1.b. *E. coli*.

C.1.b.i. from the human colon.

Two strains - C2 and C4 were isolated direct from the human colon.

C.1.b.ii. Seven strains C5, C6, C10, C13, C15, C16 and C17 were established in Locke-egg medium from cysts of *E. coli*.

C.1.b.iii. Cysts of *E. coli* were isolated from the same source as the trophozoites of strain C2.

C.2. CULTIVATION TECHNIQUES

C.2.a. Establishment of trophozoites in culture from cysts of *E. histolytica*.

These were obtained either:

C.2.a.i. by inoculating faecal material containing cysts into Locke-egg medium with starch and incubating at 37°C. The cultures were examined at regular intervals for the presence of trophozoites,

which once established were subcultured every 48 hours; or

C.2.a.ii. by concentrating the cysts by the zinc-sulphate flotation technique (Craig & Faust, 1943) followed by several washes in distilled water and inoculation into Locke-egg medium. To compensate for any reduction of bacterial associates due to the zinc-sulphate solution, a few drops of a culture of *Clostridium welchii* was added to the tubes which were incubated at 37°C. Once established the cultures were transferred into new medium every 48 hours.

C.2.b. Isolation of trophozoites from human liver abscess.

The pus obtained from the liver aspirate, which in the majority of cases was sterile, was examined for the presence of trophozoites. A quantity of pus was centrifuged and the deposit inoculated into Locke-egg medium, to which was added a knife-point of sterile ground rice and *Clostridium welchii* as the concomitant organism. The cultures were examined at regular intervals over a period of ten days and once established, were subcultured every 48 hours.

C.2.c. Isolation of *E. histolytica* from asymptomatic cyst-passers.

A survey, involving the examination for the presence of cysts of *E. histolytica*, was conducted on faecal material from some 200 patients from King George V Hospital. When cysts were observed, serum was obtained from the patient and examined for the presence of antibodies to *E. histolytica* antigen, by the gel-diffusion technique (Maddison 1965). A total of four patients were found to have a negative gel-diffusion result and on this basis were considered to be asymptomatic cyst-passers (Maddison *et al.* 1965). The trophozoites were established in culture by the method described in C.2.a.ii. Little difficulty was experienced in establishing these strains in Locke-egg medium. However, attempts at monoxenization and axenization have proved fruitless.

C.2.d. Maintenance of axenic cultures.

The various strains were grown in 16 × 125 mm screw-capped Kimax tubes

containing TPS-1 medium (Diamond 1968b), incubated at 37°C and transferred every 48 hours. At each transfer blood-agar plates were set up to check for possible contaminants.

C.2.e. Axenization of multixenic and monoxenic strains of *E. histolytica*.

The monoxenic cultivation of *E. histolytica* with trypanosomatids is based on the techniques of Diamond (1968a, b).

C.2.e.i. To initiate amoeba-trypanosome cultures from cultures in Locke-egg medium with *Clostridium welchii* as the concomitant organism, the entire deposit of amoebae and starch was inoculated into a tube of TPS-1 medium. To each tube was added 1 ml from a 5-7 day stock culture of *T. cruzi* containing 1-2 million trypanosomes per ml and 0,25 ml of 50 000 units of Penicillin. The cultures were incubated at 37°C in wooden racks holding the tubes at an angle of 15-20 degrees. The initial transfer, after 72 or 96 hours incubation, was achieved by turning the surface of the tubes containing the amoebae and starch uppermost and pouring off the contents. This method permitted the disposal of the bulk of the starch without the loss of the amoebae which were left attached to the glass surface. Tubes were then overlayed with new TPS-1 medium containing trypanosomes and Penicillin. Subsequent transfers were invariably conducted by the conventional method (Diamond 1968b) of chilling, centrifuging and transference of the deposit. During the initial stages of establishing a culture the amoebae were scanty and the interval between transfers was frequently lengthened. The number of subcultures before a culture became established varied from one strain to another. The trypanosome debris transferred over with each subculture seemed to suppress the growth of some strains of amoebae. When this occurred the method of pouring off the supernatant and pouring on new medium was employed. This resulted in virtually no transfer of dead trypanosomes and the amoebae seemed to flourish. Axenization was achieved by transferring the cultures to new medium without trypanosomes.

C.2.e.ii. The initiation of monoxenic cultures from cultures with numerous bacterial associates proved more difficult and TPS-1 medium

was not suitable. Being such an enriched medium, the bacteria flourished and suppressed the growth of the amoebae, despite the addition of various antibiotics. Partial success was achieved with TTY-SB broth to which trypanosomes were added but the complete removal of extraneous flora was not achieved.

C.2.f. Establishment of trophozoites in culture from cysts of *E. coli*.

Considerable difficulty was encountered in the excystment of several strains of *E. coli* in Locke-egg medium. This prompted experimental use of various excystment agents, of which Difco Bile Salts No. 3 proved to be the most satisfactory. Cysts were concentrated by the zinc-sulphate flotation technique and washed several times in distilled water. After the final wash, the supernatant was removed, leaving approximately 1 ml over the cysts. An equal volume of a 1% solution of Bile Salts was added to the cysts which were left at 37°C for one hour. They were then transferred together with their bacterial associates into Locke-egg medium containing starch and incubated at 37°C. In some instances an equal volume i.e. 5 ml of TPS-1 medium was added to the cultures in an attempt to provide additional nourishment and encourage growth of the trophozoites immediately after excystment. Once established the cultures were transferred every 48 hours.

C.3. MEDIA

C.3.a. TPS-1 and TTY-SB.

These are both monophasic media devised by Diamond (1968a, b). The former is used for the maintenance of axenic cultures and the latter for the initiation of the amoeba-trypanosome cultures.

C.3.b. Locke-egg.

This is a modification of the diphasic medium originally devised by Boeck & Drbohlav (1925) and employed in the maintenance of monoxenic and multixenic cultures.

C.3.c. Tobie's medium.

This is a diphasic medium devised by Tobie *et al.* (1950) for the maintenance of stock cultures of trypanosome species.

C.4. PRODUCTION OF LIVER ABSCESSES IN HAMSTERS.

Liver abscesses were produced in golden hamsters *(Mesocricetus auratus)* by the method of Wiles *et al.* (1963). The inocula were:

a) sterile amoebae from human liver pus;

b) amoebae originally isolated from humal liver pus and maintained in Locke-egg medium with *Clostridium welchii* as the bacterial associate (Freedman *et al.* 1958);

c) amoebae from axenic culture.

Initially the series of experiments involved the inoculation of each strain into several hamsters with a view to establishing whether the strain was virulent or not. Subsequent series, however, necessitated the passage of suspensions of amoebae of each strain through several hamsters. To facilitate the frequent passaging of amoebae, a modification based on the technique of Jarumilinta (1966) was introduced. This involved the insertion of a piece of infected diaphragm or liver from the margin of an abscess between the diaphragm and liver of the hamster to be infected, rather than the inoculation of a suspension of amoebae. Animals were routinely sacrificed after five days and a portion of necrotic tissue prepared for electron microscopy as described in C.6.a.

C.5. FIXATION AND EMBEDDING.

Initial studies were conducted on trophozoites of an axenic strain of *E. histolytica* and fixation was aimed at causing minimal disturbance to the cultural conditions of the amoebae. To this end the medium TPS-1 (Diamond 1968b) was employed as a vehicle for the fixative. As

this proved most satisfactory all subsequent trophozoites were fixed in 1% glutaraldehyde in TPS-1 medium.

After 30 minutes of fixation at room temperature, the cells were centrifuged at 500 × g for three minutes, the fixative removed and the plug of amoebae washed three times with fresh medium, centrifuging for five minutes at 500 × g.

The supernatant was then removed and replaced with 1% osmium tetroxide in 0,2 M sodium cacodylate.

Between each of the phases of dehydration through ascending grades of ethyl alcohol and propylene oxide, the amoebae were resuspended and centrifuged for three minutes.

Impregnation with Araldite was accomplished at 50°C by two changes of resin at two hourly intervals. Finally the tube was spun at 2000 × g for 10 minutes and most of the resin pipetted off, leaving approximately 1 ml over the button of cells. The cells were resuspended in the remaining resin and a small quantity of the material pipetted into a Beem capsule, which was then filled with fresh resin. This was polymerized for 48 to 72 hours at 70°C, thus producing easily manoeverable blocks. This method resulted in a large number of cells remaining freely suspended in the resin.

Thin sections about 50-80 nm (silver/grey) were cut on a Cambridge Huxley manual ultramicrotome. These were mounted unsupported on 200 mesh copper grids and stained with lead citrate according to Reynolds (1963) for two minutes. Observations and photography were carried out on a Zeiss EM9A electron microscope.

As there was considerable variation in the composition of the different exudates, the necessary changes in the initial preparation of the specimens, aimed at obtaining maximum numbers of trophozoites, are detailed below.

C.5.a. Trophozoites of *E. histolytica* from axenic culture.

The supernatant was removed from the tube by running a Pasteur pipette

attached to a suction pump along the upper surface of the tube, thus leaving the amoebae still clinging to the lower surface. Freshly prepared 1% glutaraldehyde in TPS-1 medium was slowly poured into the tube which was gently agitated to resuspend the cell, which was then processed as above.

C.5.b. Trophozoites of *E. histolytica* from the human colon.

Exudate obtained at sigmoidoscopy was placed on a glass slide in a drop of physiological saline and examined under a cover slip by the light microscope, to ascertain whether trophozoites were present in sufficient numbers. The exudate was then carefully removed from the slide, with a wooden applicator, and suspended in fixative and processed as above.

C.5.c. Trophozoites of *E. histolytica* from human liver pus.

Having established the presence of trophozoites, a quantity of pus was centrifuged at 500 × g for three minutes. The deposit was withdrawn with a large bore pipette and placed in fixative. When the pus was thick, treatment with 10 units of Streptodornase per ml of pus for 30 minutes at 37°C with repeated shaking, followed by centrifugation (Lello, 1954) successfully broke down the congealed pus.

C.5.d. Trophozoites of *E. histolytica* from hamster liver pus.

In order to obtain sufficient numbers of amoebae free of liver cells, portions of liver tissue were transferred under aseptic conditions to tubes of TPS-1 medium and incubated at 37°C for periods of four to 12 hours. When sufficient numbers of amoebae had emerged, the tissue was removed and 0,5 ml of glutaraldehyde, to give a final concentration of 1%, was added to the tube. The trophozoites were then processed further.

C.5.e. Trophozoites established in culture from cysts of *E. histolytica* and *E. coli*.

The overlay of Locke-egg cultures, containing amoebae and starch, was

agitated and filtered through several layers of gauze to remove the large particles of starch, which make sectioning difficult. The filtrate was centrifuged, the supernatant removed and replaced by fixative. The deposit containing the amoebae and finer particles of starch was gently agitated to ensure adequate fixation and processed as above.

C.5.f. Trophozoites of *E. coli* from the human colon.

A quantity of faeces was mixed with physiological saline, filtered through several layers of gauze and allowed to settle for a few minutes. The supernatant was examined for trophozoites and then centrifuged at 500 × g for three minutes. The concentrated amoebae were then fixed and processed further.

C.5.g. Cysts of *E. histolytica* and *E. coli*.

Cysts of both species of amoebae were collected from faeces and concentrated by means of the zinc-sulphate flotation technique (Craig and Faust, 1943). The concentration of cysts on top of the zinc-sulphate solution was collected from 8-12 tubes and placed in a tube of distilled water. They were washed several times in distilled water and finally centrifuged at 500 × g for five minutes.

The cysts of *E. histolytica* were fixed in the normal manner and processed further, whereas the cysts of *E. coli* proved more difficult. It was necessary to employ various concentrations of glutaraldehyde from 2% to 20% in TPS-1 medium in an effort to obtain adequate fixation of the entire cell.

D. RESULTS

D.1. AXENIC STRAIN HK9.

A preliminary description of the ultrastructure of trophozoites of *Entamoeba histolytica* will be based on observations made on strain HK9. This strain was used throughout the preliminary studies which were aimed at establishing and perfecting techniques for electron microscopy.

Strain HK9 was isolated in 1951 in Korea by Dr. W.W. Frye from a patient with acute amoebic dysentery. It was established in axenic medium in 1964 by Dr. L.S. Diamond of N.I.A.I.D. (National Institute of Allergy and Infectious Diseases) Bethesda, U.S.A. and has been maintained at the Amoebiasis Research Unit, Durban, for the past four years. The ultrastructure of this strain has been described by several authors (Lowe & Maegraith 1970, Trevino-Garcia Manzo 1971, Feria-Velasco 1972).

D.1.a. Cytoplasm.

The trophozoite is irregularly shaped (Fig. 1) and bounded by a limiting membrane (the plasmalemma) which is made up of two apparently similar electron-dense lines separated by a space but which is regarded as a "unit" membrane (Robertson 1961). The plasmalemma has a thickness of approximately 10 nm and probably acts as a selectively permeable barrier between the cytoplasmic matrix and the exterior.

On the inner surface of the plasmalemma electron-dense bodies measuring 33-83 nm in diameter are frequently found (Fig. 2). In some cells they are few in number or absent, while in others they seem to be confined to certain parts of the cell. They are probably similar to the 'lens-shaped electron-dense bodies' seen by Lowe & Maegraith (1970) and to the sub-pellicular bodies noted by Ludvik & Shipstone (1970).

The cytoplasm is finely alveolated with a ground substance consisting mainly of electron-dense particles of varying sizes. Single particles approximately 15-30 nm in diameter and rosette-like units 60-90 nm in diameter (Fig. 3) are similar to the glycogen particles

described by Revel (1964) in various animal tissues. The former are also referred to as β particles and the latter, more complex units, as glycogen of the α configuration. According to Revel, the rigid classification of glycogen into two main types should be avoided as there is evidence that "the two different morphological types are merely two extremes of a graded spectrum".

Other less dense particles approximately 20 nm in diameter are seen throughout the cytoplasm and probably represent the particles containing ribonucleoprotein described by Palade (1955) in liver cells. Scattered in the cytoplasm are slender rods varying in length and about 25 nm in diameter (Fig. 4). Each rod consists of small particles stacked along its length in what appears to be a helical pattern. Numerous crystalline aggregates composed of these helical rods arranged in parallel arrays are present in the cytoplasm and have been termed the chromatoid bodies (Fig. 3). The structure of the ribonuclear material of chromatoid bodies has recently been shown to consist of two clearly separated populations of different mass (Morgan 1968), the one being two or three times the size of the other.

The literature contains many conflicting reports on the chemical nature and origin of the chromatoid and related bodies. Of particular importance is the work of Barker (1963a, b) and of Siddiqui & Rudzinska (1963) on *Entamoeba invadens*. The former author has shown that the chromatoid body contains mainly ribonucleic acid and protein and that it arises by a process of aggregation of small groups of particles measuring 25-30 nm. The latter workers demonstrated that it is composed of bands or lamellae arranged in parallel arrays and that at a higher magnification the lamellae appear to be composed of coiled fibrils which seem to form a closely packed helix. They proposed the introduction of a more specific term 'ribonucleoprotein' (RNP) bodies.

The endoplasmic reticulum first described and so named by Porter *et al.* (1945) in their ultrastructural studies of chick-embryo cells in culture, is poorly developed in trophozoites of *Entamoeba* species. In general, it comprises membrane-enclosed compartments ranging from vesicles and caniculi through extensive flattened sacs to large vacuoles. In this strain (HK9) it appears to consist mainly of

vesicles or fine tubules which frequently open out into vesicles at one end (Fig. 5).

Numerous vacuoles, bounded by a limiting membrane similar in structure and density to the plasmalemma, and varying considerably in shape and size, are present in the cytoplasm (Fig. 1 & 6).

Electron-dense cylindrical units which form rosette-like conglomerates surrounding a finely granular area are present in the cytoplasm and have been observed by several authors (Lowe & Maegraith, Feria-Velasco). These rosette-like structures are approximately 1 000 nm in diameter and are composed of 14-22 electron-dense cylindrical bodies (Fig. 7). These cylindrical units are also observed free in the cytoplasm without forming conglomerates and appear to be bound by a unit membrane enclosing a relatively clear central core.

Several electron-dense bodies 200-300 nm in length and 140-160 nm in width are present in the cytoplasm and are shown in Figure 6. Although similar in appearance to the dark oval microbodies recorded by Ludvik & Shipstone (1970), they are somewhat smaller.

Vesicles measuring 240-350 nm in diameter and similar to those observed by Lowe & Maegraith (1970) were seen in the cytoplasm of these trophozoites (Fig. 1 arrowed). Morphologically similar vesicles 300-400 nm in diameter were observed on the plasmalemma and are shown in Figures 1 and 8.

It is occasionally possible, during the process of pseudopod formation, to observe the differentiation into ectoplasm and endoplasm (Fig. 9). The ectoplasm is devoid of food vacuoles or large particulate matter and is separated from the endoplasm by an electron-dense area. A tail or uroid (Fig. 10) as first described by Bird (1956) is also occasionally seen. It is an ectoplasmic structure which assumes a posterior position giving polarity to the amoeba. Apart from rosettes of glycogen this region is usually devoid of structures. Between the uroid and the endoplasm there is frequently an accumulation of small vesicles.

D.1.b. Variation in cell pattern.

In the majority of trophozoites examined, the cellular inclusions described above were evenly distributed throughout the cell as seen in Figures 1 and 9. These cells appeared to be motile and usually showed a nucleus. However, in others there was a progressive variation in the density of the cytoplasm and location of the cytoplasmic organelles as shown in Figures 11, 12, 13 and 14. The endoplasmic reticulum increased both in size and quantity and became surrounded by a homogenous electron-dense material closely resembling chromatin as observed in mammalian cells undergoing mitosis (Fig. 12, 13 & 14). Two types of vacuoles were observed within the dense matrix, the smaller (SV) measuring up to 316 nm in diameter, appeared to form as a result of budding from the short rods of smooth endoplasmic reticulum. The larger vacuoles (V) measuring up to 1.6 µm frequently contained fragments of membranous material.

D.1.c. Nucleus.

The nucleus (Fig. 1 & 15) was bounded by a double unit membrane approximately 30 nm in overall width, the inner and outer leaflets being separated by a perinuclear space approximately 16 nm. Distinct nuclear pores were occasionally observed, though not as frequently as recorded by Miller *et al.* (1961). The nucleoplasm had a greater electron density than the cytoplasm and contained a karysome composed of fine electron-dense granules. Chromatin material comprising clusters of moderately electron-dense granules was seen to be irregularly deposited on the inner periphery of the nucleus. The chromatin granules have been shown by the use of various fixatives to be valid structures rather than artefacts of fixation (Miller *et al.* 1961).

Numerous osmiophilic inclusions (Fig. 1) ranging in diameter from 110-500 nm and similar to the 'button bodies' described by Ludvik & Shipstone (1970) were present in the nucleus. These are similar to the cytoplasmic inclusions shown in Figures 1 and 8. On many occasions throughout this study the 'button bodies' appeared to pull out or fall out during sectioning revealing a clear space when viewed under the electron microscope.

D.1.d. Other organelles.

Two structures, which to the writer's knowledge have not been previously described, have been seen in strain HK9.

A granular mass, unbounded by membranes and comprising granules of different electron densities, was frequently seen in close proximity to the nucleus (Fig. 16 arrowed). The granules (Fig. 17) vary in size from 47 to 180 nm. This structure will be termed the paranuclear body (Proctor & Gregory 1972a). In one trophozoite (Fig. 18), in addition to the paranuclear body, a mass of similar structure adhering to the nuclear membrane was seen.

A stack of elongated smooth-walled vesicles 20-24 nm in diameter and bearing some resemblance to the Golgi apparatus of mammalian tissue was observed in a single trophozoite and is shown in Figure 19. These vesicles were swollen at their distal ends to form sacs which ranged in diameter from 38-45 nm.

D.1.e. Discussion.

Although in general the morphological features of HK9 were similar to those previously described, there was a variation in structural pattern in which the cytoplasmic material appeared to become progressively more floccular with aggregation of the organelles. The only reference so far noted to any variation in cytoplasmic structure is that of Ludvik & Shipstone (1970) who commented "some cytoplasmic regions are characterized by the lack of any structure and by the absence of ribosomes".

No organized ribosomal helices were observed in the cells in which the cytoplasmic material appeared to be more floccular.

The similarity between the limiting membranes of the trophozoites and of the vacuoles suggest that the latter are formed from the plasmalemma.

The diameter of the tubule of the Golgi-like structure shown in Figure 19 falls within the normal range of the Golgi apparatus of

mammalian cells. At a magnification of 114 000 the boundaries of the structure appear to be single unit membranes.

The vesicles seen within the cytoplasm and on the plasmalemma were similar to each other and to the osmiophilic inclusions of the nucleus.

Although the chromatin-like material of the cytoplasm under the electron microscope had the appearance of DNA, attempts to demonstrate this, using histological techniques, were not successful.

The filamentous paranuclear body recorded by Rondanelli (1968) in *E. histolytica in vivo* differs somewhat from the paranuclear body seen in this strain. He considered the threadlike bodies to have a function similar to that of a contractile vacuole. What the nature and function of the compact non-filamentous body of strain HK9 is, is difficult to say.

Neither mitochondria nor Golgi bodies have ever been reported in *Entamoeba* species, and apart from the structure shown in Figure 19 none were seen in the present investigation.

It is difficult to substantiate whether the technique employed is responsible for what appears to be variation in cell pattern. Using similar processing techniques and employing Locke's solution as an alternative vehicle for the fixative, the same general structural variations and prominent para-nuclear body were observed, though the definition of morphological detail was slightly inferior.

D.2. AXENIC STRAIN NIH 200.

Strain NIH 200 was isolated by Dr. John E. Tobie in 1948 from material obtained at sigmoidoscopy from a U.S. merchant seaman suffering from amoebic dysentery. The geographical origin of this strain is unknown. It was established in axenic culture in 1961 by Dr. L.S. Diamond and has been maintained at the Amoebiasis Research Unit since 1969. The ultrastructure of this strain has not, to the writer's knowledge, been previously described in detail.

D.2.a. Cytoplasm.

The trophozoites examined from 48 hour culture contained most of the organelles that are present in strain HK9.

The majority of cells contained a finely granular cytoplasm with varying amounts of glycogen, mainly of the α configuration (Fig. 20). Lying free within the cytoplasm were numerous fine tubules, similar to those observed in strain HK9, which opened out at one end into a vesicle (Fig. 22). These will be termed 'hairclip' arrangements (Proctor & Gregory, 1973c) and are possibly part of the poorly developed endoplasmic reticulum.

Ribosomal helices were present in all the cells examined, either singly or in small bundles (Fig. 21). The individual helices measured approximately 30 nm in width and varied in length, while the space separating one helix from the next was consistently 5,6 nm. The crystalline aggregates of ribosomes contained between 7 and 46 strands of RNP helices. The largest observed was 1,5 × 0,6 µm, although the majority were smaller and measured approximately 0,6 × 0,3 µm. In trophozoites from 72 and 96 hour culture there was an apparent increase in the size of the crystalline aggregates and a reduction in the number of single helices. The cells containing the larger aggregates appeared to be more spherical and less motile (Fig. 21, 23, 24 & 25).

In addition to the numerous food vacuoles, large numbers of crescent-shaped vacuoles measuring up to 1,8 µm in width and approximately 210 nm in depth, were present below the plasmalemma (Fig. 26).

In about 8% of the cells viewed, the cytoplasm was more homogenous than in the remainder and there was very little glycogen present. These cells contained bundles of ribosomal helices within double-membraned vacuoles (Fig. 27) which ranged in size from 540 nm to 1 µm. Both transversely and longitudinally cut helices were found within a single vacuole (Fig. 28). These helices were of a similar dimension to the free, non-membrane-bound helices. There was no apparent increase in number or any obvious change in structure in the membrane-bound helices in cultures maintained for 72 and 96 hours. Non-membrane-bound crystalline aggregates were never seen in these cells.

Occasional fibrous bundles varying in size up to 2,3 µm in length and 40 nm in width were present in the cytoplasm (Fig. 29). A few short strands of beaded elements in close association with fibrous material were present in one of the cells examined (Fig. 30).

Viewed under the scanning electron microscope, there appeared to be a difference in the number and size of the craters or pores on the surface of strains NIH 200 and HK9 (Fig. 31, 32). The surfaces of both strains were dotted with small depressions with an average diameter of 300 nm. In addition to these the surfaces of most NIH 200 cells revealed one or more larger vacuoles or craters ranging in size from 650 nm to 1,78 µm. Only very occasionally was the relatively smooth surface of HK9 broken by a large depression within the range of 760 nm to 1,1 µm.

A para-nuclear body differing from that seen in HK9 was present in NIH 200. It comprised apparently empty vesicles within the dense matrix, ranging in size from 32 to 110 nm (Fig. 33, 34).

Dense bodies ranging in size from 120 to 580 nm were observed in most of the cells examined in a region of homogenous cytoplasm close to the nucleus (Fig. 35, 36). A comparison of normally processed particles with non-osmicated, unstained material showed that these bodies are electron-dense, and that they appear to form as the result of coalescence of smaller particles within the mass of dense cytoplasm. Similar particles were observed to be randomly distributed throughout

the cytoplasm and in contact with the plasma membrane. Figure 21 shows a particle apparently being extruded through the plasmalemma.

A number of smaller electron-dense bodies approximately 60 × 80 nm were observed in the 96 hour cultures (Fig. 37). They were situated at the tip of bud-like protrusions of the plasmalemma which were approximately 100 nm in width and 122 nm in length.

The examination of a large number of cells did not reveal the rosette arrangement of cylindrical units seen in HK9 and so frequently referred to by various authors (Lowe & Maegraith 1970, Feria-Velasco & Trevino 1972).

D.2.b. Nucleus.

The nucleus was bounded by a double membrane similar to that of HK9 and contained varying numbers of 'button bodies' which ranged in size from 120-580 nm (Fig. 20, 30).

D.2.c. Discussion.

Ribosomal helices both singly and in aggregates appeared to be present in greater abundance in strain NIH 200 than in HK9.

Chromatoid bodies were present in trophozoites of both HK9 and NIH 200 stained by haematoxylin and examined under the light microscope. Many were larger and more spindle-shaped than the characteristic blunt-ended bars of cysts of *E. histolytica*. Cells have also been observed in which no nucleus was seen and in which the chromatoidal bodies were extremely large, some measuring up to 4 µm. This substantiates the findings on electron microscopy in which very large chromatoidal bodies are seen in cells in which a nucleus was not demonstrable.

The hypothesis of Siddiqui & Rudzinska (1965) might account for the apparent increase in size of the crystalline aggregates in older cultures. They state that "in cells actively synthesizing protein but having little or no endoplasmic reticulum, the free ribosomes aggregate to form polyribosomes or helices".

The width of individual lamellae within the double-membraned

vacuole is approximately 30 nm. Comparison of these dimensions with those of individual helices lying free or in bundles in the cytoplasm removed any doubt that these are helices of ribonucleoprotein (RNP).

The membrane-bound vacuoles containing bundles of helices might be interpreted as autophagic vacuoles involved in the breakdown of some of the RNP helices at a particular phase in the metabolic cycle of the amoebae. Figures 27 and 29 show numerous vacuoles, the contents of which appear to be in different stages of lysis.

The width of the perinuclear space of both strains NIH 200 and HK9 falls within the 15-30 nm range of most eukaryotic organisms. Perhaps the region close to the nucleus, of electron-dense particles within a dense mass of cytoplasm, represents the active stage of a quiescent para-nuclear body?

There may be some relationship between the fibrous bundle seen in the cytoplasm and the filamentous virus described by Diamond *et al.* (1972). The beaded elements, however, bear no similarity to any virus particles previously described in *Entamoeba histolytica*. According to Diamond *et al.* (1972) indigenous virus has never been observed under the electron microscope in stable, healthy axenic cultures. However, strain NIH 200 frequently undergoes periods of diminished growth with spontaneous lysis of a small percentage of the cells. If these structures are, in fact, virus particles, it is possible that some of the cells were in a state of lysis caused by the viruses at the time of fixation.

D.3. OTHER AXENIC STRAINS.

Several strains of *E. histolytica*, isolated from patients with amoebic liver abscesses, were established in axenic culture.

In general the trophozoites contained most of the organelles seen in strains HK9 and NIH 200. However, a few interesting features were observed in the different strains and will be described below.

D.3.a. Strain AH.

This strain was isolated in 1971 from human liver pus and maintained in monoxenic culture with *Clostridium welchii* as the concomitant organism. A new method was employed to establish this strain in axenic medium. It involved the inoculation of the entire deposit of amoebae and starch from a Locke-egg tube in a tube of TPS-1 medium as described in C.2.e.i. In addition to antibiotics, to each tube was added 1 ml of supernatant from an axenic strain of *E. histolytica* which had been left at 4°C for half an hour and then passed through a 0,22 µm millipore filter. Plain tubes of TPS-1 medium were inoculated with 1 ml of the appropriate supernatant as controls.

Numerous crescent-shaped vacuoles were observed below the plasmalemma (Fig. 38). In many of the torphozoites lysosome-like vacuoles with extensive convolutions of the membranes were observed and are shown in Figures 39 and 40.

Numerous strands beaded along the entire length were seen in the cytoplasm of one of the trophozoites (Fig. 41). The average width of the beaded elements was 18-19 nm while the length varied considerably, probably due to the plane of sectioning. Similar beaded elements in a feathery arrangement were present in another trophozoite (Fig. 42, 43) in which the cytoplasm consisted mainly of empty vacuoles interspersed with bundles of ribosomal helices.

Almost the entire cell of one of the trophozoites examined was filled with glycogen and the nucleus was surrounded by bundles of ribosomal helices (Fig. 44).

The nuclear inclusions or 'button bodies' which ranged in size from 35-70 nm were very prominent and flanked by large masses of chromatin. They comprised a clear outer region with a more dense central core within which were electron-dense granules of varying sizes (Fig. 45).

D.3.b. Strain AK.

Strain AK was isolated in 1972 from human amoebic liver pus and maintained in Locke-egg medium with *Clostridium welchii* until 1973 when it was established, initially in TPS-1 medium with *Trypanosoma cruzi* and finally in axenic culture.

Small groups of beaded elements (Fig. 46) measuring 17-19 nm in width were seen in the cytoplasm of several trophozoites. Occasional bundles of filamentous material in close association with beaded elements (Fig. 47) and similar to those seen in strain NIH 200 were also observed. Regions of fibrous material or parallel lamellae interspersed with denser clumps of fibre are shown in Figure 48. These regions were frequently seen in close association with numerous small vacuoles (Fig. 49, 50). The denser regions may have been the result of sectioning through more tightly packed lamellae. Other regions of a fibrillar nature presenting a more whorl-like pattern were frequently observed (Fig. 51).

Several strands of fibres were observed in which banding was clearly visible (Fig. 54). Each fibre was approximately 10 nm in width and the distance between two fibres was approximately of the same order.

A bundle of spindle-like fibres which tapered towards each end was seen at one end of a nucleus (Fig. 55).

D.3.c. Strain HALA.

This strain was isolated in 1972 from human liver pus and maintained by passage through hamster livers. The following year it was inoculated from hamster liver into Locke-egg medium with *Clostridium*

welchii as the concomitant organism, then into TPS-1 medium with *Trypanosoma cruzi* and finally established in axenic culture.

Occasional electron-dense bodies and short strands of membranous material were observed in association with denser regions of cytoplasm (Fig. 52).

Apart from several areas of extensive membrane formation in close association with vacuoles of varying sizes (Fig. 53) nothing of particular interest was observed.

D.3.d. Discussion.

The dimensions of the beaded elements seen in strains AH and AK either alone or in close association with fibrous material, are similar to those observed in strain NIH 200.

The orderly arrangement of the beaded structures would suggest that they may be viral elements although they bear no resemblance to any virus particles previously described in *Entamoeba histolytica*. As previously mentioned, Diamond *et al.* (1972) have never observed indigenous virus in stable healthy axenic cultures. However, strain AK, rather like strain NIH 200, frequently undergoes periods of diminished growth with spontaneous lysis of a small percentage of the cells. If these elements are, in fact, virus particles their observation in a few cells is not unexpected in view of the unstable cultural characteristics of this strain.

Strains HALA and AH have always appeared to be extremely stable cultures and the observation of beaded elements in the latter strain is rather surprising. On the other hand it could be the result of the method employed to establish this strain in axenic medium. The method employed was prompted by the thought that the axenic cultures established and supplied by Dr. Diamond might contain some viral agent or metabolite which assisted in the establishment of the amoebae in axenic culture, particularly as most other workers had had little success in achieving this.

At the time of establishing this culture, the writer was not

aware of the findings of Diamond *et al.* (1972) which demonstrated the presence of viral agents indigenous to *E. histolytica* in axenic culture. The low level of associated virus particles in the supernatant of NIH 200 probably induced a slight lytic response in strain AH, which was not apparent when viewed under the light microscope but which resulted in the observation of the beaded elements under the electron microscope.

It is not certain whether the beaded structures and the fibrous material are different morphological manifestations of one type of virus. The trypanosomes which are employed in the monoxenic cultivation of the amoebae prior to axenization, should be investigated as a possible source of the beaded elements.

D.4. THE INDUCTION OF LYSOSOMAL VACUOLES IN STRAIN NIH 200.

When strain NIH 200 was initially examined the significance of the crescent-shaped vacuoles was not appreciated. It was only after the observation in material from sigmoidoscopy (D.6.a., Fig. 84) of surface-active lysosomes that attention was again focused on this strain. It was then that the resemblance of the crescent-shaped vacuoles to the 'surface-active lysosomes' first described by Eaton *et al.* (1969) was appreciated, except that the frond-like trigger mechanism was absent.

As trophozoites in axenic medium derive their nutrition by absorbtion and not by ingestion of particulate matter, it was thought that the trigger mechanism might have become redundant through lack of function. In an attempt to stimulate the production of this mechanism, particulate matter in the form of viable bacteria was added to the cultures.

Trophozoites of this strain were cultured in TPS-1 medium for 24 hours, after which they were inoculated into new medium. To each of four tubes was added 0,05 ml of *Clostridium welchii* from Robertson's Cooked Meat medium. To a further four tubes was added 0,05 ml of *Aerobacter aerogenes* in digest broth. After a further 16 hours incubation at 37°C the amoebae were fixed and embedded in the manner described in C.6.a.

The cultures with bacterial associates (Fig. 56) revealed numerous vacuoles, many with progressive stages of convolution immediately below the plasmalemma, which were deeper than those observed in amoebae from 48 hour axenic cultures (Fig. 57). The crescent-shaped vacuoles were from up to 1,8 µm in length and 210 nm in depth, while the distance between the lysosomal membrane and the plasma membrane was approximately 103 nm. As the trigger mechanism developed the vacuoles became rounder and progressively more D-shaped and the plasmalemma and lysosomal membrane appeared to be drawn together as shown in Figure 58, to form a double unit membrane of approximately 34 nm. The deeper vacuoles were approximately 740 nm in width and 560 nm in depth,

while the trigger mechanism itself and a maximum width of about 200 nm and was bounded by a single unit membrane of approximately 17 nm (Fig. 59).

Figures 60, 61 and 62 show the ingestion of bacteria by strain NIH 200 grown in association with *Clostridium welchii*. The formation of an area of finely granular cytoplasm along the line of contact is clearly seen in Figure 63. It is similar to the area observed by Westphal *et al.* (1972) during the phagocytosis of crithridia by *E. histolytica* and termed a phagocollar.

D.4.a. Discussion.

On addition of particulate matter in the form of bacteria, it was possible to show the progressive development of the complete organelle (Proctor & Gregory, 1973a). There was a definite diminution in width and an increase in depth of the crescent-shaped vacuoles, coupled with a reduction in distance between the lysosomal membrane and the plasmalemma. The drawing together of the two membranes might be the final stage before the development of the trigger mechanism.

Though certain authors (Magaudda *et al.* 1970) consider that the surface-active lysosome with its 'trigger' mechanism described by Eaton *et al.* (1969, 1970) might be an artefact of preparation or fixation, the present ultrastructural observations would indicate that this organelle is a real entity.

The surface-active lysosome with its trigger mechanism might be regarded as an organ of attack.

D.5. TROPHOZOITES OF *E. HISTOLYTICA* FROM MONOXENIC CULTURE.

Six strains of *E. histolytica* designated N/68, HALA/68, AK/68, AZ/68, AN/68 and AX/68, where '68' refers to the concomitant organism, were examined after 48 hours in culture. All six strains were originally isolated from human liver pus and maintained in Locke-egg medium in association with *Clostridium welchii*.

D.5.a. General description.

The cytoplasm was bounded externally by a single unit membrane and contained considerable quantities of glycogen, mainly of the α configuration. As a rule the glycogen was evenly distributed throughout the cell, but in some amoebae it was restricted to certain areas, the remainder of the cytoplasm being relatively devoid of such reserve material.

Numerous vacuoles containing substances derived from the culture medium, such as starch granules, bacteria in various stages of digestion, lipid globules and membranous fragments were present (Fig. 64). In the majority of cells viewed, ribosomal helices were observed to occur either singly or in bundles of varying sizes. In addition to the chromatin, the nucleus contained vesicles of variable size similar to the 'button bodies' recorded by Ludvik & Shipstone (1970) (D.1.a. Fig. 1). Interesting features observed in some strains will be discussed in greater detail.

D.5.b. Strain N/68.

At the time that it was fixed and embedded for electron microscopy this strain had been in monoxenic culture for over 10 years.

The rosette-like conglomerates of electron-dense cylindrical bodies, previously described in D.1., were seen in almost every cell of this strain. They were frequently observed in close proximity to digestive vacuoles and to the plasmalemma (Fig. 65). Examination of this strain after 72 and 96 hours in culture did not reveal an increase in the number of rosette-like structures or individual components which make

up the rosette. The cylindrical bodies which form the rosette-like structure were approximately 150 nm in length and 56 nm in width.

Structures comprising six or seven particles surrounding a central particle, the whole bounded by a membrane, were observed (Fig. 65 arrowed). The diameter of these structures was approximately 120-130 nm and that of each particle approximately 34 nm. In one cell six of these structures were seen in close proximity to a bundle of ribosomal helices (Fig. 66).

Small groups of parallel lamellae which appeared to be composed of coiled fibrils, were seen between membranes (Fig. 65, 67). The apparent space between the membranes was approximately 120-150 nm, while the diameter of each individual lamella was approximately 25-30 nm. These bore a close resemblance to the ribosomal helices.

D.5.c. Strain AX/68.

This strain was fixed and embedded for electron microscopy 25 days after initial inoculation into Locke-egg medium.

Regions of parallel lamellae composed of coiled fibrils and bounded by membranes were present in the cytoplasm (Fig. 68). They were similar in dimensions to those seen in strain N/68 though not as clearly defined. However, this was probably due to the orientation of these structures through the plane of section.

The cylindrical electron-dense bodies in a rosette arrangement and the individual components which made up the rosette were present in the cytoplasm (Fig. 69).

D.5.d. Strain HALA/68.

Strain HALA was established in monoxenic culture from hamster liver pus and had been in culture for seven months when it was prepared for electron microscopy.

Extensive membrane formation either in concentric whorls or in parallel arrays were present in the cytoplasm (Fig. 70). These were

particularly prominent in digestive vacuoles containing lipid globules. On the inner leaflet of the vacuolar membrane were electron-dense bodies with a foamy appearance (Fig. 71).

D.5.e. Strains AK/68 and AZ/68.

Examination of these two strains which had been in culture for 10 months and 17 days respectively at the time that they were embedded, revealed numerous fibrillar regions (Fig. 72) which are probably the microfilaments recorded by Michel & Schupp (1974) in *E. histolytica*.

D.5.f. Strain AN/68.

This strain had been in Locke-egg medium for two months when it was prepared for electron microscopy.

Apart from the rosette arrangement of dense bodies frequently seen in close proximity to vacuoles, the regions of fine fibrous material surrounded by glycogen and a zone of homogenous dense cytoplasm around the nucleus, no special differences from the other strains were observed.

D.5.g. Discussion.

The trophozoites were in general similar to those from axenic culture although the concentration of glycogen appeared to be greater. According to von Brand (1966), it has been shown (Morita 1938) that *E. histolytica* taken from starch-containing cultures are very rich in glycogen.

There was considerable variation in size among the vacuoles of trophozoites from axenic and monoxenic culture. In the former, in which there was a lack of particulate matter, they were smaller and invariably appeared empty although some vacuoles contained membranous fragments. In the latter they were larger and contained bacteria and other ingested material in different stages of digestion.

Ribonucleoprotein helices either singly or in bundles of varying sizes were present in trophozoites both from monoxenic and from

axenic culture.

The electron-dense cylindrical bodies in a rosette arrangement were observed in five of the six strains examined. This structure seemed to be more frequently encountered in cells from monoxenic than in those from axenic culture. The observation in strain N/68 of the rosette arrangement or the individual components which make up the conglomerate in almost every cell after 48 hours in culture, led to the speculation that it might be part of the lysosomal system of the cell. However, further examination of this strain after 72 and 96 hours in culture did not reveal an increase in the number of rosettes per cell. In a detailed study of the osmiophilic bodies Feria-Velasco & Trevino (1972) speculated that in view of their constant proximity to digestive vacuoles and the plasmalemma, they might be part of the digestive process of the parasite.

Sub-pellicular bodies though present seemed to be less frequently observed than in axenic trophozoites. Hollow membranous structures, some of which resembled the 'hairclip-like' strands of smooth endoplasmic reticulum, were present in the cytoplasm of a few cells.

The structures seen in strain N/68 comprising particles bounded by a membrane as shown in Figure 66 when first observed were thought to bear some resemblance to the transverse section through the longitudinal fibres of a flagellum, although they did not exhibit the 9 + 2 pattern. However, on further examination and measurement of these particles it was felt that they might be ribosomal helices in transverse section. A comparison of the diameter of these structures with the distance between the membranes bounding the parallel lamellae shown in Figures 65 and 67, suggested that they were differently orientated sections of the same organelle. If in fact these structures are ribosomal helices bounded by a membrane, they were seen in a more orderly arrangement than those observed in strain NIH 200.

The elaborate membrane formations observed in vacuoles of strain HALA/68 may have some association with increased enzyme activity for the digestion of the lipid globules. The electron-dense bodies seen

in close proximity to the membranes bore a close resemblance to the electron-dense bodies which have been observed in the cytoplasm of various trophozoites and to the foamy bodies observed by Szubinska (1971) in the study of new membrane formation in *Amoeba proteus*.

The fibrous regions observed in the cytoplasm of some of the trophozoites were similar to those seen in axenic strain AK (D.3.b., Fig. 51) and may correspond to the "double tracks" 7-8 nm in width observed by Eaton *(pers. comm.)* and to the microfilaments 2-6 nm in width seen by Michel & Schupp (1974) in *E. histolytica* and thought to be concerned with cytoplasmic streaming and cell motility.

It was possible to compare trophozoites of strains AX/68 and AN/68 with their antecedents taken directly from human liver pus (D.7.b. and d.). The most noticeable difference observed was in the arrangement of the cylindrical electron-dense bodies in strain AX. In trophozoites from human pus they were seen in a most disorganised form whereas in those from monoxenic culture they formed the rosette arrangement so frequently observed. The nuclei of strain AN from either source were surrounded by regions of dense homogenous material, suggesting that this is a characteristic of the strain which does not alter under different environmental conditions.

D.6. TROPHOZOITES OF *E. HISTOLYTICA* FROM THE HUMAN COLON.

Six strains of *E. histolytica*, which were designated AS1-AS6, obtained at sigmoidoscopy from patients with amoebic dysentery, were examined. Table 2 shows the essential differences between these trophozoites and those from axenic culture.

D.6.a. Cytoplasm.

The trophozoites were irregularly shaped and bounded by a plasmalemma on the outside of which was a "fuzzy" layer (Fig. 73, 74). Pseudopodia were more frequently observed than in trophozoites from axenic culture and the differentiation into ectoplasm and endoplasm was more marked.

The electron-dense bodies seen on the inner surface of the plasmalemma of axenic trophozoites were less frequently observed in these trophozoites. Glycogen of the α configuration was fairly evenly distributed throughout the cytoplasm. Crystalline aggregates previously described in axenic trophozoites of *E. histolytica* were more frequently observed than single helices and as many as 20 aggregates comprising from three to 11 ribosomal helices were found in a single plane of sectioning (Fig. 75).

Scattered throughout the cytoplasm were numerous fine tubular structures which opened out at one end to form a vesicle (Fig. 76) and are probably part of the poorly developed endoplasmic reticulum.

The presence of 'hairclip'-like organelles was noted on the outer surface of some of the trophozoites examined (Fig. 77). These took the form of a tubular structure lying parallel to the surface of the cell which opened out at one end to form a vesicle (Fig. 78). In some instances, the structure was at right angles to the cell surface and attached at the vesicle end by an electron-dense area (Fig. 79). These structures were remarkably uniform in length, measuring approximately 0,45 µm. They bore a close resemblance to the short 'hairclip' arrangements of smooth endoplasmic reticulum which are present in the cytoplasm of these trophozoites and in those from axenic culture

(D.2.a. Fig. 22) and which measure approximately 0,42 µm in length.

Numerous vacuoles were present in the cytoplasm, each with a limiting membrane similar in width and density to the plasmalemma. A fuzzy layer was noticeable on the inner surface of many of the vacuoles, particularly those in close proximity to the plasmalemma. Although some of the vacuoles appeared empty, the majority contained either whole ingested erythrocytes or particulate matter which probably represented the remnants of red blood cells (Fig. 77 & 80).

The structure depicted in Figure 81 (arrowed) has, as previously stated, been recorded by several authors (Lowe & Maegraith 1970, Feria-Velasco & Trevino 1972). It comprised electron-dense rods in a typical rosette fashion about a central homogenous dense area of cytoplasm and was present in trophozoites from patients with amoebic dysentery. The arrangement of the structure appeared to be much more regular in trophozoites from axenic culture (D.1.a., Fig. 7) than in those from sigmoidoscopy material. In exudate from endoscopy, the components were seen mainly in the form of rods rather than as the granules which were noted in axenic trophozoites.

Microtubules approximately 18 nm in diameter were present in the cytoplasm of a few cells and are shown in Figure 82, in which their size may be compared to the small bundle of helices present. The tubules appeared to be arranged in bundles approximately 59 nm in diameter and although there seemed to be a discrepancy in the size, they are probably similar to those recorded in axenic trophozoites by Rosenbaum & Wittner (1970) who recorded their diameter as being 36 nm. Similar structures were observed by Lowe & Maegraith (1970b) also in trophozoites from axenic culture, although no measurements were recorded.

Figure 83 shows an amoeba containing a phagocytosed white cell in which the nucleus of the cell is clearly visible. This cell measures 11,3 µm, whereas the amoeba has a diameter of 20,5 µm.

This work has revealed an organelle (Fig. 84) similar to that observed by Eaton *et al.* (1969 & 1970) in the course of their studies

into the behaviour of *E. histolytica* in cell cultures. They described its complex structure and termed it "a surface-active lysosome equipped with a trigger mechanism". It takes the form of a surface depression in the plasmalemma beneath which is a cup-shaped, membrane-bound vacuole. At the distal end, where the plasmalemma and outer membrane of the lysosomal vacuole meet, the width is approximately 47 nm. This becomes progressively narrower until it is about 33 nm at the neck or region from which the frond or 'trigger' as labelled by Eaton *et al.* (1969 & 1970) originates. Granules measuring 11-12 nm in diameter are seen on both surfaces of the double-layered membrane of the trigger and on the inner surface only of the lysosomal vacuole. Within the trigger is an electron-dense mass which is approximately 149 nm in width and 226 nm in length. It appears to be composed of granules similar in size to those on the surface of the double-layered membrane (Proctor & Gregory 1972b).

D.6.b. Nucleus.

The nucleus (Fig. 73) is frequently irregular in shape and bounded by a limiting membrane. The moderately electron-dense peripheral chromatin was arranged in clusters similar to those of axenic trophozoites, although it appeared to be fine than the latter. Button bodies, although present, appeared to be fewer in number than in axenic trophozoites. No well defined karysome was observed.

D.6.c. Strain AS6.

The cell pattern of this strain varied from that of the other colonic strains examined. Although only a few cells were present they were extensively viewed at different levels of sectioning.

The general appearance of many of the cells differed in that the glycogen was arranged in clumps of varying sizes and did not present the appearance usually observed in glycogen of the α configuration, but was seen as an amorphous mass (Fig. 85). The cytoplasm comprised mainly glycogen and ingested red blood cells. In general, the glycogen was sparse in the cells containing newly ingested erythrocytes, whereas the cells in which the red blood cells were in various stages of

digestion were filled with glycogen. Numerous small vacuoles were observed, many in close proximity to the plasmalemma.

In contrast to the cells examined from other strains, ribosomal helices, either singly or in bundles, were not observed. The 'hairclip' arrangements so frequently seen in the cytoplasm of other trophozoites were either absent or rare. Extensive examination of the plasmalemma, which was very crenated, did not reveal a 'fuzzy' coat (Fig. 86).

The majority of cells examined contained bundles of fibrous material of varying sizes. These varied in length, measured approximately 24-29 nm in diameter, and were always in close association with glycogen (Fig. 87). In one of the cells examined these fibrous elements were observed randomly arranged in an area of cytoplasm surrounded by glycogen (Fig. 88). This area had the appearance of a large vacuole, but no membrane was observed. Examination of a deeper level of the same cell revealed these fibrous elements in a more definite parallel arrangement (Fig. 89).

Figure 90 shows a bacterium which has been ingested by the cell.

D.6.d. Discussion.

Although the majority of specimens were fixed within an hour and a half after removal from the rectal ulcers, it was some three hours before strain AS6 was dealt with. The crenation of the plasmalemma of these trophozoites may be due to the time which had elapsed between removal from the tissues and fixation, or it may be the result of pinocytosis in the physiological saline in which they were left during this period. Chapman-Andresen (1963) showed that a 0,125 M solution of sodium chloride induced pinocytosis in free-living amoebae. The changes observed under the light microscope included immobilization of the cell, wrinkling of the plasmalemma channel formation and pinching off of vesicles. Brandt (1958) demonstrated that the first phase of pinocytosis consists of absorption of the solute to the outer surface of the plasmalemma. This phase corresponded in time to the appearance of surface wrinkling.

The present study confirms the observations of Zaman (1970) that the digestion of erythrocytes commences within a short time after ingestion. However, there was little evidence of digestion of the red blood cells in strain AS6 fixed after three hours. It is possible that the structural differences observed were due to the slowing down of the metabolic processes of the cell as a result of prolonged exposure to oxygen after removal from the tissues.

An interesting phenomenon was the presence of 'hairclip' arrangements on the plasmalemma and in the cytoplasm of some cells. McLaughlin & Meerovitch (1973) have shown that the antigenic properties of *Entamoeba invadens* are associated with the "cytosol" and the smooth endoplasmic reticulum. If these structures, which appear to move through the cytoplasm to the surface of the cell, originate in the endoplasmic reticulum, they may be associated with the transportation of antigenic material. Although it is generally accepted that the release of antigens only occurs to any extent upon the death or lysis of the cell, the intact amoebae may become somewhat antigenic by virtue of the 'hairclips' on their surfaces. On the other hand, they may be concerned with some other function such as penetration of the tissues. The infrequent observation of these structures, however, would not appear to favour either hypothesis. The rarity of their presence may be the result of destruction of these delicate structures during fixation, or to the low concentration of cells in many of the final preparations. A number of micrographs have revealed certain unusual areas on the plasmalemma which could be related to the base of the 'hairclips'.

The presence of a'fuzzy'coat on the outer surface of the plasmalemma has been recorded by El-Hashimi & Pittman (1970) and Griffin & Juniper (1971) in their study of trophozoites from the human colon. A'fuzzy'layer on the inner surface of the limiting membrane of vacuoles suggest that they are derived from the plasmalemma. Contrary to the observations of these authors, crystalline aggregates were frequently seen in trophozoites from the human colon. Sub-pellicular bodies, though present, appear to be less numerous than in trophozoites from axenic culture. The observation of an ingested bacterium together with red cells in one of the trophozoites viewed was of particular

interest as it is generally accepted that the invasive forms of *E. histolytica* do not ingest bacteria.

A mode of action of the surface-active lysosome similar to that proposed by Eaton *et al.* (1970) is envisaged. An electron-dense mass separates the spaces S1 and S2 which are in equilibrium (Fig. 84). As a result of mechanical contact or some form of chemical stimulation, the exceedingly fragile double-layered membrane enclosing space S2 is ruptured, with a resultant inequality of pressure. The increased pressure within the lysosomal vacuole (S1) leads to the ejection of the granular mass. Whereas Eaton *et al.* (1970) regard the modified lysosome as an agent of external aggression, it seems more likely that the granular mass within the trigger is involved in the attack. This granular mass may contain an enzyme which, as Eaton *et al.* (1970) have suggested, is involved in the type of plasmalemma damage which they have observed.

TABLE 2

ESSENTIAL DIFFERENCES BETWEEN AXENICALLY CULTIVATED TROPHOZOITES AND THOSE FROM THE COLON.

	Axenic		Colonic
	HK9	NIH 200	
Cytoplasmic hairclips	rare	numerous	numerous
Surface hairclips	absent	absent	present
Vacuoles	empty or containing fragments of membranous material		partially digested erythrocytes and particulate matter
Surface vacuoles	rare	numerous crescent-shaped	surface-active lysosome with trigger
Fuzzy coat	absent	absent	present
Paranuclear body	present	present in two forms	absent
Rosette arrangement	present	individual components	present
Button bodies	present	present	present but less numerous
Membrane-bound helices	absent	present	absent
Sub-pellicular bodies	present	present	scanty

D.7. TROPHOZOITES OF *E. HISTOLYTICA* FROM HUMAN LIVER ABSCESS.

Four strains - AN, AM, AP and AX were obtained from pus aspirated from patients with amoebic liver abscesses. In general, the morphology under the electron microscope was similar to that of trophozoites from sigmoidoscopy material and from axenic culture. However, there were individual differences within the strains, the details of which follow on the brief general description.

D.7.a. General description.

The trophozoites varied in shape from rounded to irregular forms and many showed pseudopodia (Fig. 91). Most of the cells contained vast quantities of glycogen of the α configuration, either in large clumps or evenly distributed throughout the cytoplasm. A'fuzzy'coat was present on the outer surface of the plasmalemma (Fig. 92). At right angles to the plasmalemma of most of the cells numerous strands of unit membrane, which varied in length were observed. Ribosomal helices ranged from a few single helices scattered throughout the cytoplasm to numerous small bundles of up to six strands. Occasional pale globules resembling lipid globules of liver cells were seen in the cytoplasm. Erythrocytes in different stages of digestion were present. Many of the small vesicles and vacuoles were empty while others contained tightly-wound membrane whorls or partially digested liver material. Scattered throughout the cytoplasm in many of the cells were hollow membranous structures, some of which resembled the 'hairclip'-like strands of smooth endoplasmic reticulum previously described (D.2.a., Fig. 22). Electron-dense rosette arrangements similar to those observed in trophozoites from the colon (D.6.a., Fig. 81) and from axenic culture (D.1.a., Fig. 7) were present in most of the amoebae examined.

D.7.a.i. Strain AN.

A thin layer of ectoplasmic material was seen immediately below the limiting membrane of many of the cells (Fig. 93). Within this region, which separated the plasmalemma from the mass of glycogen

which occupied the remainder of the cell, were numerous clear vacuoles. A layer of similar material surrounded many of the nuclei (Fig. 94) in which there were large concentrations of 'button bodies', here seen as holes (D.1.c.).

Adjacent to the nucleus of one of the cells examined was a rosette arrangement of electron-dense bodies (Fig. 94). The layer of material surrounding the nucleus continued around this arrangement, which was considerably larger than those previously observed. Within this layer and surrounding the rosette arrangement were numerous clear vacuoles.

D.7.b. Strain AM.

Ribosomal helices were not observed, although a vacuole containing several helices was present in one of the cells (Fig. 95). Rosette arrangements similar to those seen in strain AN were present although the individual components were in a less orderly array (Fig. 96). They were not surrounded by vacuoles but were in close proximity to large masses of glycogen.

Two rather interesting nuclei were observed in this strain. Masses, probably representing chromatin material, were seen at opposite poles of one of the nuclei (Fig. 97). The remainder of the nucleoplasm was rather devoid of structure. In the other, the ground substance was in the form of a fine lattice, with granular material in small clumps at opposite sides of the nucleus (Fig. 98).

D.7.c. Strains AP and AX.

These two strains had several features in common. The electron-dense bodies which usually make up the rosette, were frequently observed in disorderly arrangement (Fig. 99). In one plane of sectioning three rosette arrangements seemed to have combined to form a pattern similar to that of a clover leaf (Fig. 100). In another section of the same area the components were more spread out forming a U shaped pattern (Fig. 101). Large concentrations of vesicles of varying sizes and tubular structures, many resembling the 'hairclip' arrangements previously mentioned

(D.2.a., Fig. 22), were frequently seen in close proximity to these electron-dense bodies. A few dense strands were seen in one of the cylindrical units sectioned parallel to the longitudinal axis (Fig. 102) and probably correspond to the longitudinal lines described by Feria-Velasco and Trevino (1972). A few of the cells examined possessed what appeared to be a double plasmalemma in places (Fig. 103).

The nuclei again proved to be most interesting; several were observed which had recently divided. Electron-dense bodies measuring 32-34 nm in diameter and arranged in two rows were observed between a pair of newly divided nuclei (Fig. 104). Though they appeared to be outside the nuclear membrane, the two rows of particles were separated from each other by another membrane. In two other nuclei the chromatin was interspersed with crystals (Fig. 105, 106). Several inclusions, in the form of vacuoles containing what appeared to be ribosomes and other cytoplasmic inclusions, were seen within the nuclei.

D.7.d. Discussion.

The rosette arrangement of electron-dense bodies were more frequently observed in trophozoites direct from human liver pus than in any other cells examined. They were considerably larger and had an irregular form.

The narrow layer of ectoplasm separating the plasmalemma from the remainder of the glycogen-filled cells was a more constant feature in strain AN than in the other three strains. The material from which this strain was obtained had been treated during the initial preparation with a digestive enzyme - streptokinase/streptodornase. It is possible that this enzyme, to which the plasmalemma is no doubt permeable, may have had some effect on the ultrastructure of these trophozoites.

In general, these trophozoites contained considerably more glycogen than those from the human colon or from axenic culture. The large number of clear vacuoles around the periphery of many cells were probably pinocytic vacuoles taking in fluid containing glucose from the surrounding necrotic tissue. If this contained saccharide it might be converted by the amoebae to the polymer glycogen and account for the

greater concentration of this component in these trophozoites compared with others.

The subpellicular bodies, so frequently observed in trophozoites from the human colon and from axenic culture, were not seen in amoebae from liver pus. Occasional lysosome-like vacuoles were seen immediately beneath the plasmalemma, but a trigger mechanism was never observed. Although 'hairclip'-like arrangements of smooth endoplasmic reticulum were frequently observed in the cytoplasm, they were not seen on the surface of the cells, despite extensive viewing. The fuzzy coat on the outer surface of the plasmalemma was more prominent on these trophozoites than on those from sigmoidoscopy material.

The observation of crystals associated with the dense-chromatin in only two nuclei throughout this entire study is difficult to interpret. They could be the result of staining with lead citrate but one would have expected to encounter stain deposit more frequently considering the number of cells which have been viewed. On the other hand the most interesting nuclei have been seen in trophozoites from human pus and these crystals may be associated with some form of nuclear activity.

The ultrastructure of trophozoites of *E. histolytica* obtained direct from human liver abscess has not, to the writer's knowledge, been previously described.

D.8. TROPHOZOITES OF *E. HISTOLYTICA* FROM HAMSTER LIVER ABSCESS.

The difficulty experienced in obtaining trophozoites of virulent strains from human patients in sufficient numbers for ultrastructural study led to the experimental production of liver abscesses in golden hamsters *(Mesocricetus auratus)*. Seven strains of *E. histolytica* originally isolated from human liver pus were employed.

D.8.a. General description.

The ultrastructural appearance of the trophozoites were in general similar to those from the human colon (D.6.a.) and from human liver pus (D.7.a.). A 'fuzzy' coat was present on the outer surface of the plasmalemma of all the cells examined (Fig. 107) and also on the inner surface of vacuoles in close proximity to the plasma membrane. Glycogen of the α configuration was present in great abundance and evenly distributed throughout the cytoplasm (Fig. 108). In the majority of the cells viewed, ribosomal helices both singly and in bundles were observed. Occasional helices within a double-membraned vacuole were present. Osmiophilic bodies were seen on the inner surface of the plasmalemma and in the cytoplasm of some of the strains. Occasionally these dense bodies were observed apparently being extruded by the cell.

Erythrocytes in different stages of digestion were observed as well as pale grey globules, resembling the lipid globules of liver cells. Crescent-shaped vacuoles were observed, many of which resembled lysosome-like vacuoles without a trigger mechanism. 'Hairclip' arrangements of smooth endoplasmic reticulum were present in varying amounts in the cytoplasm of several of the strains examined. A few special structures were observed in the different strains and will be discussed in greater detail.

Apart from strain HALA, every other strain had been in culture in Locke-egg medium with *Clostridium welchii* as the concomitant organism at the time of primary inoculation into hamster liver. By the second or third passage a bacteriologically sterile lesion was obtained.

Shown in parenthesis for each strain are the number of passages through hamster liver at the time of fixation of the trophozoites for electron microscopy.

D.8.b. Strain HALA (33, 1).

This strain was inoculated direct from human liver pus into hamster liver and first viewed after the 33rd and subsequently after the first passage. In trophozoites from the 33rd passage a tubular structure which opened out at one end to form a vesicle was observed on the plasmalemma lying parallel to the cell (Fig. 109). It closely resembled the'hairclip'arrangements seen on the surface of trophozoites from the human colon (D.6.a., Fig. 78).

Numerous electron-dense 'blebs' were seen on the outer surface of the plasmalemma (Fig. 110). They measured approximately 80 nm in diameter and were surrounded by a unit membrane (Fig. 111). There was a slight thickening of the plasmalemma at the point of attachment. A 'bleb'which seemed to have forced its way through the plasmalemma was observed (Fig. 112). It was separated from the cytoplasm by a less dense area within which were longitudinal lines. A unit membrane extending down one side and into the cytoplasm where it folded back on itself was clearly visible.

Material from this strain which had been fixed and embedded immediately after the first passage through hamster liver was thereafter examined but did not reveal'hairclip'arrangements or'blebs'on the surface of the plasmalemma.

D.8.c. Strain AK/68 (1, 12).

Several regions of membranous material (Fig. 113, 114) with dimensions similar to those of a unit membrane were seen in close association with the plasmalemma. On the surface of one of the cells a structure was observed almost at right angles to the plasmalemma and in close proximity to cellular material probably of host origin (Fig. 115). It bore a resemblance to the'hairclip'arrangement seen in strain HALA, although the detail was somewhat distorted. In addition to the normal

cytoplasmic organelles, occasional unbound masses of fibrous material were seen (Fig. 116).

D.8.d. Strain AX/68 (1).

Several electron-dense bodies measuring approximately 90-130 nm in diameter were observed in the cytoplasm (Fig. 117, 118, 119). They had a foamy appearance similar to the dense droplets described by Szubinska (1971) in *A. proteus*. Many of these bodies were seen in close contact with the plasmalemma and appeared to be passing to the exterior.

An orderly arrangement of particles was observed in the cytoplasm of one of the cells viewed (Fig. 120). The particles, with an average diameter of 25 nm were arranged around almost the entire periphery of what appeared to be a vacuole although a membrane was not apparent. Within this region was a pear-shaped mass of cytoplasmic material surrounded by similar particles. Within the border of particles was a second incomplete ring and exterior to the entire arrangement was a cluster of similar particles.

D.8.e. Strain AZ/68 (1, 6).

Apart from a region on the surface of the cell of lamellae showing a trilaminar structure (Fig. 121) nothing of interest was observed in trophozoites from the first passage. There appeared to be a break in the plasmalemma from which arose two strands of unit membrane in close contact with each other. These in turn gave rise to several parallel lamellae which branched out in all directions. In the cytoplasm immediately beneath this region were two strands of unit membrane.

In material from the sixth passage a trophozoite and a leucocyte were observed in close proximity to one another (Fig. 122, 123). Between the amoeba and the leucocyte and surrounding the greater part of the latter were concentrations of cellular debris. Although the cell membrane of the leucocyte seemed to be intact, regions were observed where bud-like processes appeared to be extruded by the cell. A small concentration of bud-like processes were seen in close proximity to the uroid

region of the amoeba whose plasmalemma was intact.

D.8.f. Strain N/68 (1).

Several regions corresponding to the tail or uroid described in *E. histolytica* by Bird (1956) and in *E. invadens* by Zaman (1970) were observed. This region appeared as a projection at the posterior end of motile amoebae (Fig. 124). The cell surface formed a number of irregular protrusions around which the plasma membrane could be traced. The irregularity of the protrusions was evident from the fact that some have been cut at various angles, and appear separate from the main body.

Large numbers of erythrocytes had adhered to the surface of the trophozoites in this region and the commencement of the ingestion process in which the erythrocytes are drawn into the cell was clearly seen (Fig. 125).

A region of parallel lamellae immediately beneath the cell surface was observed (Fig. 126). Although similar to the region of lamellae seen in strain AZ/68, it differed in that the concentration of membranes was within the cytoplasm and not protruding from the plasmalemma. This region appeared to be continuous with a granular mass of low electron density projecting to the exterior, the details of which were not clearly visible.

D.8.g. Strain AL/68 (12).

Apart from numerous crescent-shaped vacuoles beneath the plasmalemma and cytoplasmic vacuoles containing membranous whorls, little of interest was seen in the trophozoites of this strain. No ribosomal helices were observed.

D.8.h. Strain AN/68 (12).

The cytoplasm contained large numbers of ribosomal helices singly, in small bundles and within vacuoles. An interesting arrangement of these helices in which they seemed to fan out from both sides of a dense band was seen in the cytoplasm of one of the trophozoites

(Fig. 127). The central band may be a unit membrane but clearer observation was impeded by the limited resolution of the microscope.

At the base of a pseudopod containing a recently ingested red blood cell, which had a spherical shape, were numerous small vesicles and attached to the membrane of the erythrocyte were several electron-dense bodies (Fig. 128). These resembled the sub-pellicular bodies frequently seen in trophozoites. A region of dense fibrous material was seen in the cytoplasm of a few of the trophozoites viewed (Fig. 129).

An organelle similar to the surface-active lysosome seen in trophozoites from the human colon (D.6.a., Fig. 84) and induced in strain NIH 200 (D.4., Fig. 59) was observed. The frond-like outgrowth bounded by the plasmalemma and the lysosomal membrane was clearly visible (Fig. 130). The dense mass seen within the trigger mechanism in trophozoites from the colon was not apparent and might have been destroyed during fixation.

A large vacuole containing lipid globules in different stages of digestion and considerable quantities of membranous material was present in the cytoplasm (Fig. 131). A group of small vacuoles continuous with this large food vacuole and surrounded by ectoplasm was seen protruding to the exterior of the cell.

D.8.i. Discussion.

It was initially intended to establish hamster liver abscesses with the different strains of *E. histolytica* and study the morphology of the trophozoites resulting from such abscesses. A portion of liver containing trophozoites of strain HALA which was being maintained in hamsters and routinely passaged every five days was embedded for comparison with newly established strains.

In these trophozoites from the 33rd passage through hamster livers the observation of 'hairclip' arrangements on the surface of the cell, which was similar to those seen on the trophozoites from the human colon, altered the aim of the experiments. It was thought that the hairclip arrangements on the surface of the trophozoites might be

associated with increased virulence. It was thus decided to examine the various strains both from the initial abscess and from the 12th passage. Unfortunately, several of the specimens prepared from the initial abscess were only sectioned for viewing under the electron microscope some weeks later, when it was discovered that there were too few cells available for examination. It was not possible to repeat many of the experiments as the cultures had died.

Contrary to the observations of Lowe & Maegraith (1970c) numerous trophozoites were observed in which there was obvious differentiation of ectoplasm and endoplasm. The 'rosette' arrangement of tubular bodies around a dense area of cytoplasm was only observed in strain N/68, in which it was frequently seen in a less orderly arrangement than in axenic HK 9. That it was not found in the other strains may simply mean that insufficient material was viewed, particularly as it was seen in every one of these strains apart from AZ/68 and AL/68 from monoxenic culture. The small group of vacuoles protruding to the exterior and shown in Figure 131 might be interpreted as waste material being extruded to the exterior. It has been shown (Hopkins and Warner, 1946) in a study of the cytology of *E. histolytica* that there is no specific site for elimination of excretory vacuoles.

Jarumilinta & Kradolfer (1964) using light microscopy described the leucocytocidal effect of *E. histolytica* trophozoites *in vitro* and pointed out that contact between the amoeba and the leucocyte appeared to be essential. Eaton *et al.* (1970) demonstrated the presence of an enzyme containing organelle with a trigger mechanism at the amoebic surface. The cytolytic activity of *E. histolytica* was shown to be contact dependant and the primary effect was on the cell membrane. Both groups of observers reported that the maximum time that elapsed between contact and initial appearance of cytolytic response was at least 10 minutes.

Although surface-active lysosomes were not observed beneath the plasmalemma of the amoeba in the region in close contact with the leucocyte, the large concentration of debris in this region would suggest that the amoeba had exerted some toxic effect on the leucocyte. Although the primary effect is said to be on the cell membrane which in

these micrographs appears to be intact, it might be that contact had not been of sufficient duration at the time of fixation to observe the extensive cytolytic response described by the above-mentioned workers.

The trophozoites in material from hamster liver abscess produced by strain AZ/68 were so numerous that they were fixed on the slides used for light microscopy and not by the conventional method which involved the transfer of a portion of liver into TPS-1 medium and subsequent fixation of trophozoites some hours later. The trophozoites of strain HALA in which surface hairclips were observed were fixed *in situ* in a small piece of liver, whereas the other strains, apart from strain AZ/68 were fixed by the conventional method.

The process of ingestion of erythrocytes commencing with the adhesion of the cells to the surface of the trophozoite, the elongation of the cell while being drawn into the cytoplasm and the final digestion within the cytoplasm, was clearly seen in many of the trophozoites examined. The small profiles of smooth endoplasmic reticulum along the base of newly ingested erythrocytes seems to demarcate the ectoplasm from the endoplasm. The similarity between the particles shown in Figure 120 and ribosomes, suggests that this may be a region actively engaged in protein synthesis.

This study confirms the observations first made by Bird (1956) that particles, cells and bacteria adhere to the external surface of the uroid of *E. histolytica*. The electron-dense clumps of mucoid material observed by Zaman (1961) at the uroid of *E. invadens*, were not seen in these trophozoites. The vacuoles observed outside the cell region of the uroid probably represent the undigested food which Hopkins & Warner (1946) observed were eliminated in this area, either by being pushed through the surface or by the breaking off of cytoplasmic protrusions. The fixation methods aimed at minimal disturbance of the cells were comparatively successful as the uroid was frequently demonstrated.

The production of bacteriologically sterile abscesses at the second or third passage confirms the observation of Wiles *et al.* (1963) that the hamster liver appears to have a bacteriostatic or bacteriocidal effect on *Clostridium welchii*.

D.9. TROPHOZOITES OF *E. HISTOLYTICA* FROM ASYMPTOMATIC CYST PASSERS.

Five strains designated H2, H3, H12, H22 and L132 were isolated from patients with no evidence of invasion and negative serology by gel-diffusion using the technique of Maddison (1965). On this basis they were considered to be asymptomatic cyst-passers or 'carriers'.

Strain L132 was able to grow at room temperature and to withstand considerable lowering of osmolarity and was thus considered to be an amoeba of the Laredo type. The other four strains were sensitive to low temperature and to reduced osmolarity and on present criteria must be regarded as *E. histolytica*. Attempts were made to establish some of these strains in either monoxenic or axenic culture with a view to establishing liver abscesses in hamsters.

D.9.a. Hamster inoculation.

Several attempts were made under varying conditions to produce an abscess with strain H22. The growth of the other strains of amoebae in Locke-egg medium was too poor to provide adequate numbers for inoculation.

A large concentration of trophozoites from Locke-egg medium was inoculated into the liver of each of two hamsters and 100 000 units of Penicillin and 0,5 gms Streptomycin was introduced into the peritoneum of one of them. Both hamsters died within 24 hours, probably as a result of peritonitis. Examination of the livers did not reveal an abscess and only dead trophozoites were found at the site of injection.

On another occasion 1 ml of medium containing trophozoites was soaked up in sterile cotton wool which was placed on top of the liver. At autopsy five days after inoculation the liver was disintegrated and large numbers of haematophagous amoebae were present. Subsequent passage of these trophozoites into two hamsters failed to produce an abscess in either.

Further experiments were conducted in which trophozoites were soaked up in cotton wool to which antibiotics had been added. These

were applied to the livers of two hamsters and resulted in the death of one after 24 hours. Examination of the other, five days after inoculation, did not reveal either an abscess or the presence of trophozoites.

D.9.b. Electron microscopy.

D.9.b.i. Cytoplasm.

The trophozoites were irregularly shaped and bounded by a plasmalemma approximately 8 nm wide. The cytoplasm contained glycogen mainly of the α configuration. Numerous vacuoles of varying sizes containing bacteria in different stages of digestion, starch and membranous fragments were present (Fig. 132). Ribosomal helices, mainly in bundles of varying sizes, were observed frequently in close association with vacuoles or with the nucleus. Electron-dense bodies in a rosette arrangement around a homogenous dense area of cytoplasm were observed in close proximity to vacuoles in strain H22. The individual components measured approximately 54 nm in width and 130 nm in length (Fig. 133).

Structures similar to the individual components which make up the rosette arrangement, though differing somewhat in dimensions, were observed in strain H2. In one trophozoite they were seen in very close association with a vacuole (Fig. 134) and in another in close proximity to the nuclear membrane and several small vacuoles (Fig. 135). In the former they measured approximately 40-43 nm in width and approximately 126 nm in length. In the latter they were of a similar length and approximately 55 nm in width.

D.9.b.ii. Nucleus.

The nucleus was similar to those of trophozoites from other sources and, in addition to chromatin contained vesicles similar to the 'button bodies' described by Ludvik & Shipstone (1970). Small dense intranuclear bodies concentrated in groups within the chromatin but, with no apparent central core, were seen in two of the nuclei of strain H12 (Fig. 136).

In this strain numerous dense bodies presenting a fine mesh appearance and bounded by membranes were observed (Fig. 132). In one of the cells examined these structures were seen in close proximity to the nucleus and their density was similar to that of the nucleoplasm (Fig. 137, 138). Two nuclei were present in this cell and in close proximity to each was a small stack of smooth-walled vesicles which appeared to be slightly swollen at their distal ends (Fig. 137, 139).

D.9.b.iii. Discussion.

The trophozoites were in general similar to those of all the other strains examined although they appeared to be somewhat smaller, their average diameter being 16 μm. There was no apparent difference between trophozoites of *E. histolytica* and those of the Laredo type.

The subpellicular bodies observed in other trophozoites were infrequently observed, although only a few cells from each strain were examined. Silard *et al.* (1974) in a study of a strain isolated from a "carrier", commented on the "rare occurrence of 'subpellicular osmiophilic bodies' as against their infrequent presence in pathogenic amoebae".

The discrepancy between strains H22 and H2 in the dimensions of the cylindrical electron-dense bodies may be due to the plane of section. That they were not seen in strains H3, H12 and L132 is probably the result of inadequacy of the numbers of cells available for viewing. Their observation in strain H22 in the typical rosette arrangement so frequently encountered in the present study, leaves no doubt that they are also present in trophozoites from asymptomatic cyst passers.

The dense bodies (Fig. 137) seen in strain H12 were thought to be derived from, or to have budded off from, the nucleus. However, examination of these structures at high magnifications did not reveal a double membrane.

The lack of a typical Golgi apparatus in *E. histolytica* recorded by several workers apparently also applies to amoebae from asymptomatic cases. However, the stack of smooth-walled vesicles

observed in Figure 139 in close association with the nuclei in strain H12 bore a superficial resemblance to the Golgi apparatus. It is possible that vesicles may bud off from this stack of membranes to provide substances for the formation of new plasma membrane when the cell divides, thereby performing a function analogous to the Golgi apparatus of higher organisms.

The inability thus far, to establish these trophozoites in monoxenic or axenic culture precluded any assessment of their invasiveness in hamster livers. Although other workers (Bos 1973, Thompson *et al.* 1954 and De Carneri 1958) have succeeded in producing liver abscesses in hamsters with amoebae associated with mixed bacterial flora, it was not achieved in the present study.

The disintegrated liver in which trophozoites were observed did not have the appearance of the abscesses normally produced by *E. histolytica* from monoxenic cultures. The presence of haematophagous trophozoites in this liver is evidence that they had invaded although the negative results of subsequent passages is rather surprising.

Further studies employing various antibiotics aimed at establishing strain H22 in monoxenic culture in Locke-egg medium with *Clostridium welchii* or *Escherichia coli* as the concomitant organism are in progress.

D.10. ULTRASTRUCTURE OF CYSTS OF *E. HISTOLYTICA*.

Miller & Deas (1971) gave a brief account of the fine structure of cysts of *E. histolytica*. This was based on organisms of the Laredo, Huff and W.B. strains of *E. histolytica* grown in Nelson's encystment medium (Nelson & Jones, 1964). More recently Rondanelli *et al.* (1973) compared the ultrastructure of a strain of *E. coli* tending to undergo encystment in culture with that of *E. histolytica* encysted under similar conditions. As far as the writer is aware this present description is the first account of the ultrastructure of cysts of *E. histolytica* direct from the human colon (Proctor & Gregory, 1973).

D.10.a. Cytoplasm.

The ground substance contained glycogen and interspersed vacuoles containing particles in various stages of digestion (Fig. 140). Ribosomal helices single, in bundles lying free in the cytoplasm and also membrane-bound, were present (Fig. 140, 141). The non-membrane-bound bundles are the chromatoid bodies of light microscopy and were frequently observed close to the nucleus. The individual helices within the cytoplasm measured 34 nm in diameter, whilst the diameter of a single helix from both membrane and non-membrane-bound bundles was 33 nm. The space separating one helix from another within a bundle was approximately 4 nm. The average width of a bundle within a double-membraned vacuole was 600 nm whilst that of bundles lying free in the cytoplasm was 2 μm.

Within the cytoplasm were numerous electron-dense bodies between 50 nm and 100 nm in diameter which bore a close resemblance to the nuclear inclusions (Fig. 142). Many of these bodies appeared to comprise a dense outer region and a less dense central core.

The cyst wall, which was approximately 120 nm thick, appeared to be made up of two layers (Fig. 140, 143). The outer amorphous layer of low electron density measured about 40 nm. The inner fibrous layer was more electron-dense and measured approximately 80 nm.

Immediately below the fibrous layer and bounding the cytoplasm was a unit membrane, similar to the plasmalemma of trophozoites and measuring approximately 10 nm in width. Osmiophilic bodies, ranging in size from 30 nm to 80 nm, and similar to those observed in trophozoites were seen at intervals on the inner leaflet of the plasma membrane (Fig. 143).

D.10.b. Nucleus.

The nucleus with an average size of 1,7 × 1,6 µm was bounded by a double membrane. Within the chromatin were numerous 'button bodies' measuring approximately 175 nm in diameter and similar to those observed in axenically cultivated trophozoites by Ludvik & Shipstone (1970) and in the present study (Fig. 144).

D.10.c. Discussion.

The cytoplasm contained many of the organelles observed in trophozoites of *E. histolytica* both from culture and from human tissue. However, neither the 'hairclip'-like structures of smooth endoplasmic reticulum nor the rosette arrangement of electron-dense granules surrounding a homogenous area of cytoplasm were observed. As cysts from only one source were examined it cannot be categorically stated that cysts of *E. histolytica* do not possess this rosette arrangement.

The glycogen was seen to be evenly distributed throughout the cell, except in those in which large crystalline aggregates were observed. In the latter, the glycogen was in the form of a large unbound mass and a nucleus was often not demonstrable in sections. This would appear to substantiate the observations of light microscopy, which have shown the presence of a large glycogen mass and chromatoid bodies in cysts which contained only one nucleus and were regarded as being immature.

Although cysts in various stages of maturation were observed under the light microscope, it was not possible to demonstrate more than two nuclei in the prepared material. As a result, neither the reduction in size and ultimate disappearance of the chromatoid bodies, nor the separation of the plasmalemma from the amorphous coat in the

mature cysts as described by Miller & Deas (1971) were observed.

Apart from the observation in the present study in strain NIH 200, in monoxenic culture and occasionally in trophozoites from hamster liver abscess of membrane-bound ribosomal helices, these have, to the writer's knowledge, only been previously reported *in vivo* from experimentally infected rats by Rondanelli *et al.* (1968). As observed in strain NIH 200 the crystalline aggregates and membrane-bound helices were never seen in the same cells.

The presence of vacuoles containing particles in various stages of digestion would indicate, as observed by Zaman (1972a) in his study of the cysts of *I. butschlii* that the digestive processes continue after encystation. As will be seen in the section D.12 on cysts of *E. coli* the digestive processes do not appear to stop with the commencement of encystation.

D.11. TROPHOZOITES OF *ENTAMOEBA COLI* (Grassi 1879, Cassagrandi & Barbagallo 1895) FROM CULTURE AND FROM THE HUMAN COLON.

Entamoeba coli is one of the largest amoebae found in the human colon. It occurs as a harmless commensal and its distribution is world-wide. The trophozoites are found in the upper part of the large intestine. Nine strains of *E. coli* were obtained, the trophozoites being examined either from the colon or after excystment in Locke-egg medium.

Many of the organelles recorded in *E. histolytica* (Ludvik & Shipstone 1970, Griffin & Juniper 1971, Lowe & Maegraith 1970a, b, c) and in *E. coli* (Rondanelli *et al.* 1972) were also noted in this study. They include the presence of ribosomes, helical bodies, crystalline aggregates, glycogen and the lack of mitochondria and Golgi-complex. Other features of interest as well as some variation between strains of organelles were observed.

D.11.a. Cytoplasm.

The trophozoites were irregularly shaped and bounded by a limiting unit membrane similar to that of *E. histolytica* (Fig. 145). Osmiophilic subpellicular bodies were observed on the inner surface of the plasmalemma. They were more frequently observed in trophozoites from the colon than from culture and appeared to be larger and less numerous that those in *E. histolytica*. In some instances the plasmalemma appeared to be stretched at this point, as though the body was being extruded (Fig. 146).

A fuzzy layer (Fig. 147) was seen on the outer surface of the plasmalemma of newly excysted trophozoites from culture and from the colon, although it appeared to be more dense in the latter. It was, however, not observed on trophozoites which had been in culture for several months (Fig. 148). Large numbers of vacuoles were present in the cytoplasm which in trophozoites from the colon, contained partly digested bacteria, while those in trophozoites from culture contained, in addition, partially digested starch.

In many of the trophozoites from the colon the cytoplasmic organelles tended to be aggregated in the centre of the cell, leaving a clear outer region (Fig. 149). In one cell the ectoplasm was separated from the endoplasm by a concentration of glycogen (Fig. 150) and in another by a region of closely packed vesicles (Fig. 151). These phenomena were not observed in trophozoites from culture medium (Fig. 152) in which the cytoplasm was more homogenous and contained considerably more glycogen. There appeared to be greater numbers of riboxomal helices, both singly and in small bundles, in trophozoites from culture (Fig. 153).

Lysosome-like vacuoles, similar to those seen in the present study in trophozoites of *E. histolytica* from the colon and described by Eaton *et al.* (1969) in *E. histolytica* in culture were observed below the plasmalemma, both in excysted trophozoites of *E. coli* and in those from the colon. However, a trigger mechanism was observed in only one instance in a trophozoite from culture (Fig. 154). An irregularly shaped region walled off from the remainder of the cytoplasm by a pair of unit membranes, was observed in a single trophozoite from the colon (Fig. 155). Within this region was a vacuole containing remnants of digested particles, glycogen of the α configuration and numerous small vesicles.

Large concentrations of hollow membranous structures, bounded by unit membrane and bearing a close resemblance to smooth endoplasmic reticulum, were observed in some of the trophozoites from several of the strains examined both from the colon and from culture. Many were of considerable length with an average internal diameter of approximately 50 nm. They were seen either in stacks or lying randomly in the cytoplasm, invariably surrounded by glycogen and in close proximity to vacuoles of varying sizes (Fig. 156-159). In strains C2 and C5 double-walled tubules with an internal diameter of approximately 84 nm were seen (Fig. 160). The distance between the strands was constant, although the length appeared to vary. Numerous strands of unit membrane (Fig. 161) were seen on the surface of many cells. They varied in length and in most instances were at right angles to the plasmalemma and frequently in close proximity to cytoplasmic vacuoles. Variations in the arrangement of membranes in which the surface organelle was associated

with a complex arrangement of unit membrane within the cytoplasm (Fig. 162, 163) were observed in one of the cells examined.

D.11.b. Nucleus.

Although there was considerable variation in the arrangement of the chromatin between trophozoites of the same and different strains, in general it appeared denser in *E. coli* than in *E. histolytica*. In some instances it was fairly evenly distributed on the inner surface of the nuclear membrane and interspersed with intranuclear bodies, which varied in size and structure. In others it was confined to a few clumps entirely filled with intranuclear bodies (Fig. 164, 165). In many of the cells the outer membrane of the nuclear envelope seemed ill-defined, while the entire nucleus was frequently surrounded by a clear region or halo. In one of the cells examined an apparently empty spherical body was observed traversing the nuclear membrane possibly through one of the nuclear pores (Fig. 166).

The most interesting feature was a filamentous virus-like structure initially seen in the nucleus of trophozoites of strain C2, one of the two strains examined direct from the colon (Fig. 145, 167). In the trophozoites which had been established in culture from cysts of the same strain, smaller fragments of the filamentous structure were present.

Seven other strains of *E. coli* were established in culture from cysts and examined after set intervals of time in culture (Table 3). The filamentous material was present in varying amounts in the nucleus of three of these strains.

A strange phenomenon was noted in strain C16, which was embedded and examined after 9 and 21 days in culture. After 9 days in culture a nucleus almost entirely filled with well-defined strands of filamentous material was observed. The nuclear membrane was not apparent and small bundles of filamentous material were seen in the cytoplasm close to the nucleus (Fig. 168). One of the nuclei of a binucleate trophozoite was in an apparently similar state with no nuclear membrane, and the nucleoplasm was surrounded by numerous

TABLE 3

TIME-DISTRIBUTION OF THE FILAMENTOUS MATERIAL IN THE NUCLEUS

Strain	No. days in culture at time of fixation	Filamentous material
C 2	0	++
	2	+
C 4	0	-
C 5	4	-
C 6	40	++
C 10	19	+
C 16	9	+++
	21	-
C 17	6	-
C 13	120	-
C 15	120	-

ribosomal helices (Fig. 169). That the nuclear membrane was not observed in the latter may be due to the plane of sectioning. However, after 21 days in culture extensive examination of numerous trophozoites did not reveal any filamentous structures.

D.11.c. Discussion.

Many of the structures described in trophozoites of *Entamoeba histolytica* were seen in the trophozoites of *E. coli*. Neither mitochondria nor a typical Golgi complex were observed and the smooth endoplasmic reticulum was in general poorly developed by comparison with other organisms.

The fuzzy coat on the outer surface of the trophozoites from the colon and those newly excysted from culture is of particular interest in view of its resemblance to the layer observed in the present study on trophozoites of *E. histolytica* from the human colon and from human liver abscess. It has been described by El Hashimi & Pittman (1970) and Feria-Velasco *et al.* (1972) in trophozoites of *E. histolytica*. The former workers noted a fuzzy layer about 30 nm wide on the surface of amoebae from the human colon, although no extra-membranous layer was seen in trophozoites from axenic and monoxenic culture. However, the latter investigators demonstrated the presence of a glycocalyx comprising an amorphous and a filamentous layer totalling 65 nm in width on the surface of two strains grown in axenic medium.

The lysosomal vacuoles are similar to those observed in trophozoites of *E. histolytica* from the human colon (D.6) and induced in strain NIH 200 (D.7). They were observed by Rondanelli *et al.* (1972) in trophozoites of *E. coli* from culture. Although first described by Eaton *et al.* (1969) in *E. histolytica*, these authors had observed such systems in other members of the genus *Entamoeba* and did not claim, as has been suggested by Rondanelli *et al.* (1972), that they are unique to *E. histolytica*.

The intranuclear bodies are similar to those seen in trophozoites of *E. histolytica* and which have been described by several authors (Ludvik & Shipstone 1970, Lowe & Maegraith 1970a, b, Feria-Velasco & Trevino 1972, Zaman 1973a) in other members of the genus

Entamoeba. Feria-Velasco & Trevino (1972) on the results of cytochemical studies suggested that these bodies may be part of the lysosomal system of the parasite. Zaman (1973a) on the other hand, from observations of occasional nuclei of *E. invadens* which were completely free of these bodies, suggested that they were not essential for the survival of the amoebae. It has been shown (Lowe & Maegraith 1970, Zaman 1973a) and in the present study, that the vesicles are able to pass through the nuclear membrane, though whether this happens through a nuclear pore is not known.

The clear region around the nucleus might be regarded as an artefact of fixation, though its frequent observation in several of the strains examined would suggest otherwise. This phenomenon has often been seen under the light microscope in trophozoites stained by haematoxylin.

Although Rondanelli *et al.* (1972) recorded a sparsity of endoplasmic reticulum, the present study has revealed that some amoebae have a greater concentration of membranous structures with diameters approximating those of smooth endoplasmic reticulum of various mamalian cells. It is possible that the increase in the elongated smooth-surfaced profiles is associated with increased glycogen metabolism under different cultural conditions. The double-walled tubules seen in two strains appear as individual structures although it may be that they form some sort of network in the cytoplasm. It should be emphasised that trophozoites of *E. coli* in even relatively formed faeces are in unfavourable osmotic conditions and the apparent aggregation of organelles in the centre of the cell may be a result.

The observation of the filamentous virus-like structures in the nuclei of trophozoites of strain C2 from the colon prompted the examination of other strains of *E. coli*. Subsequent trophozoites examined were from strains established in culture from cysts with the aid of Bile salts No. 3. The presence of filamentous material in smaller units in the trophozoites in culture 48 hours after excystment, would suggest some form of reassembly in the newly excysted amoebae. The conflicting results obtained from strain C16 point to a possible breakdown and reassembly at some stage in the life cycle of the trophozoites.

Electron-dense cylindrical units lying free in the cytoplasm or in a rosette arrangement, so frequently observed in trophozoites of *E. histolytica*, were not seen in any of the 9 strains of *E. coli*.

D.12. ULTRASTRUCTURE OF CYSTS OF *E. COLI*.

Previous studies on the ultrastructure of cysts of *E. coli* (Rondanelli *et al.* 1973) were confined to strains of *E. coli* tending to undergo encystment in monoxenic culture medium. As far as the writer is aware this is the first account of the fine structure of cysts of *E. coli* direct from the human colon (Proctor & Gregory 1974). The cysts were isolated from the same source as the trophozoites of *E. coli* which were designated C2 in the previous chapter.

D.12.a. Immature cysts.

D.12.a.i. Cyst wall: In the newly encysted amoebae or immature cyst, the endoplasm was clearly differentiated from the ectoplasm and both regions are bound by a unit membrane (Fig. 170). Convolutions of the outer membrane resulting in a structure with a thickness of approximately 12 nm were occasionally observed (Fig. 171). In other sections a dense mass surrounded by a convoluting plasmalemma (arrowed) with a thickness of approximately 10 nm was seen (Fig. 172).

D.12.a.ii. Cytoplasm: The cytoplasm contains many of the organelles observed in trophozoites of *E. histolytica* and *E. coli*. There was usually a single irregularly shaped nucleus in which a karyosome was often demonstrable. The glycogen was evenly distributed throughout the cell which also contained numerous digestive vacuoles and bundles of ribosomal helices (Fig. 170).

D.12.b. Intermediate stage.

D.12.b.i. Cyst wall: With maturation of the cyst there was a progressive thickening of the cyst wall. The intermediate stage (Fig. 173, 174) in the development of the wall apparently comprised:

1) a pale granular region (G) 50 nm in width immediately above the plasmalemma (P);

2) a dense fibrous layer (F) measuring approximately 190 nm; and

3) a relatively pale external region (E) measuring approximately 100 nm.

D.12.b.ii. Cytoplasm: There was an apparent increase in the number of electron-dense bodies on the inner leaflet of the plasmalemma in the intermediate stage. At this stage five nuclei were seen in the plane of section. There was a reduction in the size of the vacuoles, fragmentation of the crystalline aggregates into smaller bundles and an apparent reduction in the quantity of glycogen.

D.12.c. Final stage.

D.12.c.i. Cyst wall: In what appeared to be the final stage (Fig. 175) in the development of the wall, the pale granular inner region was no longer observed and the fibrous layer was closely adherent to the plasmalemma. The outer region was granular in appearance and was not a continuous membrane. It measured approximately 570 nm at its thickest point, where there were groups of parallel lamellae. These varied in number from two or three to 12 strands, each with a thickness of approximately 11 nm and a trilaminar appearance (Fig. 176, 177). In the more mature cells there appeared to be an increase in volume in the region of the parallel lamellae, followed by an explosion of this region and the subsequent release or ejection of these lamellae to the exterior (Fig. 178-180).

D.12.c.ii. Cytoplasm: The cytoplasm had a more homogenous appearance and contained occasional small bundles of ribosomal helices and very little glycogen (Fig. 175). In several of the cells observed, an area of cytoplasm was separated from the rest of the cell by two unit membranes (Fig. 181 arrowed). Within this region there were invariably small bundles of ribosomal helices, vacuoles which frequently contained glycogen and numerous small vesicles. Several other similar regions were observed some of them bounded by three unit membranes as shown in Figure 182.

Numerous vacuoles containing one or two strands with a diameter of approximately 11 nm were observed in several of the cells

examined and are shown in Figure 183.

D.12.c.iii. Nucleus.

In many of the more mature cells the outer region of the nucleus was rather ill-defined and it was difficult to observe the membrane separating the nucleoplasm from the cytoplasm. Numerous osmiophilic inclusions (Fig. 184) which ranged in size up to 240 nm and bore a close resemblance to the button bodies seen in trophozoites of *E. histolytica* and of *E. coli* were present. Many were bound by a unit membrane apprximately 14 nm in width and an electron-dense central core was frequently observed.

D.12.d. Discussion.

As the cysts became pregressively more mature there was a definite reduction in the quantity of glycogen, a fragmentation of the chromatoidal bodies or ribonucleoprotein helices into smaller bundles and the complete disappearance of the digestive vacuoles.

The regions of cytoplasm separated from the rest of the cell by two or three unit membranes and containing bundles of helices and glycogen within vacuoles, were more commonly observed in mature cysts. Their presence deep in the cytoplasm would imply that they are laid down within the cell and are not the result of invagination of the plasmalemma prior to encystment. One might speculate that the assortment of organelles within these membranes are to be utilised at a later stage or have no further use and are to be broken down and discarded by the cell.

The osmiophilic bodies on the inner surface of the plasmalemma were similar to those observed in the present study in trophozoites and cysts of *E. histolytica* and in trophozoites of *E. coli*. Ludvik & Shipstone (1970) in their study of trophozoites of *E. histolytica* suggested that these bodies might be closely connected with the "new synthesis of cell membrane". The apparent increase in the number of these bodies in the intermediate stage of development of the cyst wall suggests that they may be associated with the laying down of the fibrous layer of the wall.

In a study of new membrane formation in *A. proteus* upon injury of cells, Szubinska (1971) suggested that the new membrane is formed from material normally present in the cytoplasm in the form of dense droplets. The droplets measure 10-120 nm in diameter and have a foamy appearance which she considers to be an artefact produced during electron bombardment. Under certain circumstances the material is extruded to the exterior where it appears to hydrate forming myelin figures and spread over the surface.

James (1914) is of the opinion that external conditions contribute to the thickening of the cyst wall. If further thickening does occur on exposure to the external environment, it is possible that the parallel lamellae although they seemed to be ejected in some cells, are new membrane formations produced in preparation for such thickening. Although no dense bodies were seen in the region of the parallel lamellae they were occasionally observed in the fibrous layer.

The area of convolution on the plasmalemma (Fig.171) and the dense mass below the outer layer (Fig. 172) in immature cysts seem to correspond to the areas of parallel lamellae in the mature cysts. The appearance and dimensions would suggest a similarity between the plasmalemma and these trilaminar structures.

The strands within the vacuoles bounded by a unit membrane bore a close resemblance on dimensions and appearance to the unit membrane of the plasmalemma.

With the deposition of the fibrous layer of the cyst wall, the gap was gradually closed between this layer and the plasmalemma, while the outer membrane appeared to be broken down. The ultrastructural appearance, particularly of the intermediate stage of the cyst wall, would seem to confirm the light microscopy observation by McConnachie (1969) that the inner layer is comparatively thick and rigid and the outer layer thin and elastic. She has reported that the cysts of several species of amoebae are irreversibly damaged by drying, but those of *E. coli* are apparently able to resist this to some degree.

The arrangement of small bundles of ribosomal helices in a

ring adjacent to the cyst wall, as observed by Barker (1963a) in his studies on the cysts of *E. invadens*, was not a feature of the mature cysts of *E. coli*. This might, however, be related to the degree of maturity as Ray & Sen Gupta (1954) reported by Barker (1963a), state that this was observed in mature cysts preparing to excyst.

The irregularly shaped nucleus so frequently seen in the immature cysts could possibly be in the early stages of division. The button bodies were scattered around the periphery within the chromatin and not necessarily 'grouped in complexes' as observed by Rondanelli *et al.* (1974). The filamentous structure seen in the nucleus of trophozoites isolated from the same source, was not observed in the nuclei of these cysts.

Rondanelli *et al.* (1973) expressed the view that the digestion phase does not stop at the moment of encystment and suggested that the products of digestion could be used as the precursor of the cyst wall.

The difficulty encountered in obtaining satisfactory fixation of the cytoplasm necessitated the use of various concentrations of glutaraldehyde. The fixation of the cytoplasm in early stages and of the cyst wall of more mature cysts was very satisfactory with 1% glutaraldehyde in TPS-1 medium. However, with progressive thickening of the cyst wall it was necessary to increase the concentration of glutaraldehyde for satisfactory fixation of the cytoplasmic organelles. 15% glutaraldehyde in TPS-1 medium proved to be the most satisfactory concentration, although there was a degree of shrinkage of the cytoplasm from the cyst wall in some cells.

E SUMMARY AND DISCUSSION

The present study has shown that the cytoplasm of the trophozoites of *E. histolytica* and *E. coli* contained glycogen of the α configuration and ribosomal helices either singly or in bundles of varying sizes. In contrast to the findings in mammalian cells and certain free-living amoebae, the endoplasmic reticulum was, in general, poorly developed and neither mitochondria nor typical Golgi complexes were present. The cytoplasm was bounded by a unit membrane - the plasmalemma - approximately 10 nm thick. On the inner surface of the plasma membrane of most cells were dense ovoid bodies measuring 30-50 nm in diameter. The nucleus was bounded by a double unit membrane approximately 30 nm in overall width and perforated by nuclear pores. The inner and outer leaflets of the nuclear membrane were separated by a perinuclear space of approximately 16 nm. The nucleoplasm was more electron-dense than that of the cytoplasm and contained chromatin and spherical intranuclear bodies.

The cysts of *E. histolytica* and *E. coli* contained most of the organelles seen in trophozoites. Immediately exterior to the plasmalemma of both species was the cyst wall which in *E. histolytica* was made up of two layers. The outer amorphous layer was of low electron density and measured approximately 40 nm in width. The inner fibrous layer was more electron-dense and measured approximately 80 nm in width. The cyst wall of *E. coli* was considerably thicker than that of *E. histolytica*. In the intermediate stage of development it comprised three layers with an overall width of approximately 340 nm. In what was considered to be the final stage of development the wall comprised two layers and measured approximately 570 nm at the thickest point.

There was, however, considerable divergence from the basic pattern in the amoebae studied and Table 4 shows the distribution of organelles in the various strains examined.

'FUZZY' COAT.

A 'fuzzy' coat was observed in the trophozoites in 14 out of

TABLE 4

DISTRIBUTION OF ORGANELLES IN VARIOUS STRAINS

	Entamoeba histolytica							*Entamoeba coli*		
	TROPHOZOITES						CYSTS	TROPHOZOITES		CYSTS
ORGANELLE	AXENIC	MONO-XENIC	COLONIC	HUMAN LIVER	HAMSTER LIVER	ASYMPT-OMATIC		COLONIC	CULTURE	
FUZZY COAT	-	-	+	++	+	-	-	+	-	-
SUB-PELLICULAR BODIES	++	+	+	-	+±	±	+	+	+	±
RIBOSOMAL HELICES:										
Single	+	+±	+	+	+	±	±	±	±	±
Bundles	+	+	++	+	+	+	+	-	±	+
Membrane-bound	+	-	-	-	±	-	±	-	-	±
ROSETTE	+	+	+	++	+	±	-	-	-	-
HAIRCLIPS:										
Cytoplasm	+	+	+	±	+	±	-	±	±	-
Surface	-	-	+	-	±	-	-	-	-	-
LYSOSOMAL VACUOLES:										
Without Trigger	+	+	+	+	+	±	-	±	±	-
With Trigger	-	-	+	-	+	-	-	-	-	-
GLYCOGEN	+	++	+	++	+±	+	+	+	+±	+
PARANUCLEAR BODY	+	-	-	-	-	-	-	-	-	-
INTRANUCLEAR BODIES	+	+	±	+	+	±	±	+	+	±
VIRUS-LIKE FILAMENTS:										
Cytoplasm	±	-	-	-	-	-	-	-	-	-
Nucleus	-	-	-	-	-	-	-	+	+	-

15 strains of *E. histolytica* examined from human and animal tissue but not in those from culture. Surface coats have been described for many protozoan and metazoan parasites and there is considerable evidence (Vickerman 1974) that it has a role in assisting the adhesion of the cell to its substrate or to other cells, in the binding of particles for pinocytosis or phagocytosis and in determining the antigenic character of the cell.

Feria-Velasco *et al.* (1972) and Lushbaugh & Miller (1974) using cytochemical staining techniques demonstrated the presence of what they called a glycocalyx in *E. histolytica*. The former workers observed this in axenic trophozoites and the latter reported a morphological difference between the coats of axenic amoebae and those of monoxenic and *in vivo* trophozoites. On the basis of their experimental data Lushbaugh & Miller (1974) considered this glycocalyx to be of parasite origin. They suggested that a factor or factors which are common to both monoxenic and *in vivo* growth conditions are correlated with the amount of filamentous material in the coat of *E. histolytica* and that perhaps the presence of bacteria which have been related to virulence by many workers (Phillips & Gorstein 1966, Vincent & Neal 1960 and Wittner & Rosenbaum 1970) might be a factor. The observation in the present study of a greater concentration of 'fuzzy' layer in trophozoites from human liver abscesses, which were bacteriologically sterile, would suggest that some other factor is involved. As their glycocalyx was demonstrated by the use of special staining techniques, it may of course not be the same material as that observed in the present study.

SUB-PELLICULAR BODIES.

Osmiophilic sub-pellicular bodies were more frequently observed in trophozoites from axenic culture than in either those from monoxenic culture or those from human and animal tissue. They were not observed in trophozoites from human liver abscess. Although Rondanelli *et al.* (1968) did not observe them in trophozoites of the Laredo or *E. histolytica*-like strain, they were occasionally seen in the single Laredo-like strain examined in the present study. These authors suggested that

the bodies might be concerned with the histolytic enzyme activity of the pathogenic amoebae, while Ludvik & Shipstone (1970) suggested that they might be closely connected with "the new synthesis of cell membrane or represent direction chemotactic centres".

The diameter of the sub-pellicular bodies and many of those observed in the cytoplasm in the present study fell within the range of the bodies with a foamy appearance observed by Szubinska (1971) in a study of "new membrane" formation by *Amoeba proteus* upon injury. She has shown that material which gives rise to new membrane can be visualized by conventional methods when it is present in the cytoplasm but, when it is released to the outside, special techniques are necessary.

The apparent increase in the concentration of the sub-pellicular bodies in the intermediate stage of development of the cyst wall of *E. coli*, might be associated with the laying down of the layers outside the plasmalemma. If these bodies are similar to those observed by Szubinska (1971) and are associated with new membrane formation their greater concentration in trophozoites from axenic culture than in trophozoites from other sources is strange. If, on the other hand, they are concerned with histolytic enzyme activity in pathogenic strains, as suggested by Rondanelli *et al.* (1968), it is rather surprising that they were not observed in the four strains examined direct from human liver abscess.

RIBOSOMAL HELICES.

Numerous studies have been conducted on the structure and function of the chromatoid bodies of *Entamoeba* spp. Barker (1962, 1963a) studies the structure of the particles isolated from the chromatoid body of *E. invadens* and showed that this contained mainly ribonucleic acid and protein and that it arose "by a process of aggregation of small groups 25-30 nm units which form polycrystalline masses in precysts and early cysts". He considered that the results "support the hypothesis that the chromatoid body represents a crystalline store of ribosomes and are incompatible with a virus interpretation of the nature of the body". He pointed out that the "RNA chromatoid bodies seem to occur when cells are preparing for a period of rapid production of new cells or quick

growth, as in cysts of the parasitic protozoa before hatching, and in spermatogenic cells before the production of sperm". Barker & Svihla (1964) advanced the hypothesis that "the large crystalloids, the chromatoid bodies, are a manifestation of a special parasite-host adaptive mechanism".

Siddiqui & Rudzinska (1963, 1965) demonstrated the presence of chromatoid bodies in axenically grown trophozoites, where there is no cyst formation and proposed the term 'helical RNP bodies' for such chromatoid bodies. They commented on the similarity between the RNP helices and the helical arrangement of ribosomes observed in the differentiating muscle cell of the somites in the tail of *Rana pipiens* by Waddington & Perry (1963) and in the differentiating epithelial and connective tissue cells of the small intestine of the rat foetus by Behnke (1963). They drew attention to the fact that all these cells were in a phase of active protein synthesis and growth and that their endoplasmic reticulum was poorly developed.

Rosenbaum & Wittner (1970) demonstrated the filamentous core nature of helices following ribonuclease digestion and also observed that "while short RNP helices approach digestive vacuoles and even appear to attempt penetration, helical fragments themselves could not be detected within vacuoles". They could, "however, detect the presence of single and double filaments within vacuoles and they may represent the filamentous core from previously intact helices, suggesting that vacuolar penetration of helical core material may occur as the helix becomes denuded of RNP particles". On the basis of their observations they speculated "that enzyme protein synthesis may be occuring in association with the larger RNP bodies themselves, and that newly synthesised protein may be carried via the short helices to digestive vacuoles".

Although core material was not observed in the present study, ribosomal helices bounded by membranes and which have been interpreted as autophagic vacuoles were observed in trophozoites from both axenic and monoxenic culture and to a lesser extent in trophozoites from hamster liver abscess. The only other reference to such structures was by Rondanelli *et al.* (1968) who observed them in trophozoites from

experimentally infected rats. It is worthy of note that all these trophozoites were in unusual conditions. On the other hand, bundles of helices were observed within vacuoles in cysts of *E. histolytica* and to a lesser extent in cysts of *E. coli* from the human colon, which is the usual location for cyst formation.

Individual ribosomal helices were present in the cytoplasm of all the trophozoites of *E. histolytica* examined, although they appeared to be increased in number in trophozoites from monoxenic culture. In aggregated form these helices were most numerous in trophozoites of *E. histolytica* from the human colon which is not in accord with the findings of Griffin & Juniper (1971) or those of El Hashimi & Pittman (1970). The former workers did not observe aggregated helices in amoebae from four cases of human amoebic dysentery and considered "that chromatoid crystals are not formed and cannot be the site of ribosomal function in amoebae from human disease". The latter found only a single crystalloid aggregate whereas in the present study up to 20 aggregates comprising from 3 to 11 helices were seen in a single plane of section in many of the trophozoites from the colon.

In trophozoites of *E. coli* from both the colon and from culture individual ribosomal helices appeared to be less abundant than in trophozoites of *E. histolytica* from any source. Bundles of helices were present in varying numbers in cysts of *E. coli*, to a lesser degree in trophozoites from culture and were apparently absent from trophozoites from the colon.

The frequent observation of RNP helices in close association with digestive vacuoles in trophozoites from all sources would favour the hypothesis that they are the site of protein synthesis and that the individual helices are concerned with the transportation of newly synthesised protein. The presence of bundles of helices in trophozoites of *E. histolytica* from the colon and their apparent absence in trophozoites of *E. coli* also from the human colon suggests some association with an active stage of protein synthesis in sites where the amoebae are concerned with rapid multiplication and possibly penetration of the tissues.

There may be some correlation between the disappearance of

digestive vacuoles and the fragmentation into smaller bundles and eventual disappearance of chromatoid bodies with increasing maturity of cysts. On the other hand, it may be that the RNA has assumed the 'latent' form first described by Hakansson (1936) from light microscopy and demonstrated under the electron microscope by Miller & Deas (1971). As bundles of ribosomal helices are also present in trophozoites from axenic culture it would appear that chromatoid bodies are not directly related to encystment or excystment.

ELECTRON-DENSE CYLINDRICAL UNITS (ROSETTES).

The electron-dense cylindrical units so frequently observed in a rosette arrangement around a finely granular area of cytoplasm, were seen in most of the strains of *E. histolytica* examined. When, despite extensive viewing, a rosette arrangement had not been observed in strain NIH 200 it was felt that this might be a differential diagnostic feature from HK9 in which it had been frequently seen. However, in another later study the rosette formation was observed in strain NIH 200 after transfer of a lysate of HK9. Such transfer studies are continuing.

These bodies were observed in almost every amoeba from human liver abscess though in a more disorderly arrangement than in trophozoites from axenic or monoxenic culture. They were frequently observed in trophozoites from the human colon and occasionally in trophozoites cultivated from asymptomatic cyst passers. In the latter and in the Laredo strain they were frequently seen as isolated units in the cytoplasm and often in association with vacuoles. However, only a few cells were viewed from each of these five strains.

In a detailed description of the electron-dense cylindrical bodies which form the rosette-like conglomerates, Feria-Velasco & Trevino (1972) showed that when sectioned parallel to the longitudinal axis the unit has a bullet shape and is limited by a unit membrane. The substructure comprised 6-7 longitudinal lines and a spherical dense body up to 8-10 nm in diameter. Although the plane of section of the cylindrical bodies was in many instances parallel to the longitudinal axis in the material examined in this study, the longitudinal lines were observed in only one section - in trophozoites of *E. histolytica* from human liver

abscess. This may be the result of the method of fixation as Feria-Velasco & Trevino (1972) have shown that the substructure was better observed in trophozoites initially fixed with osmium tetroxide than in those fixed with glutaraldehyde and post-fixed with osmium. These workers speculated that the rosette arrangement is probably related to the digestive processes by virtue of its constant proximity to digestive vacuoles.

Bird *et al.* (1974) have demonstrated the similarity in morphology between these cylindrical bodies and that of rhabdoviruses with hosts ranging through plants, insects and mammals. They believe that viral material carried by *E. histolytica* could be one of the main factors involved in the pathogenesis of amoebiasis.

The electron-dense cylindrical bodies were not observed in the trophozoites of 9 strains of *E. coli* examined. They were not seen in either cysts of *E. histolytica* or in cysts of *E. coli*. However, only cysts from a single source were examined in each case.

LYSOSOMAL VACUOLES.

Numerous crescent-shaped vacuoles were observed immediately beneath the plasmalemma in many of the trophozoites of *E. histolytica*. Although it proved possible to induce the formation of a 'trigger' (Eaton *et al.* 1969) by the addition of particulate matter (Proctor & Gregory 1973a) such a 'trigger' was observed in *E. histolytica* only in trophozoites from human and animal tissue. However, a lysosomal vacuole with a 'trigger' was observed in one cell of *E. coli* from culture. Rondanelli *et al.* (1974) observed surface-active lysosomes with 'triggers' with identical appearance in both *E. coli* and *E. histolytica* but did not consider them to be an aggressive mechanism. Eaton *et al.* (1970) had observed similar lysosomes in other members of the genus *Entamoeba* and did not consider them to be specific to *E. histolytica*. However, they felt that "in pathogenic strains of *E. histolytica* this organelle has been adapted to function at the levels of pH and Eh obtaining at the intestinal mucosal surface, enabling the amoeba to attack the host tissues and to achieve both penetration and a food supply at the same time".

Miller *et al.* (1972) said that the numerous surface lysosomes they observed in *E. histolytica* were "usually much smaller than reported" and thus they are probably smaller than those seen in the present study. On the basis of serial sections they concluded "that lytic enzymes are not discharged by rupture of the lysosomal membranes as previously reported. Instead, there are filiform extensions from the plasmalemma above the lysosome (previously termed the 'trigger') into which the membranes of the latter extend. From these extensions, vesicles are formed and detached at the host parasite interphase".

'HAIRCLIPS'.

Irregularities and extensions of the cell membrane, many of which appeared to be derived from the plasmalemma, featured prominently in several of the strains examined. However, the 'hairclip'-like structures seen on the surface of the trophozoites from hamster liver abscess and from the human colon took on a more orderly form and did not appear to be derived from the plasma membrane. As previously stated, they were remarkably uniform in size and bore a close resemblance to some components of the poorly developed endoplasmic reticulum. Although they do not resemble the apocrine processes observed by Miller *et al.* (1972) and were never seen in association with a lysosomal vacuole, they may perform a similar function.

These surface 'hairclips' were not observed on the surface of amoebae from human liver pus nor on the surface of *E. coli* trophozoites from the colon. 'Hairclip'-like structures were, however, observed in the cytoplasm of *E. coli* in which the endoplasmic reticulum particularly from culture, consisted of hollow membranous structures with diameters approximating those of smooth endoplasmic reticulum of various mammalian cells.

INTRANUCLEAR AND PARANUCLEAR BODIES.

The presence of intranuclear bodies has been recorded by many workers. These were seen in both trophozoites and cysts of both species in the present study. The observation, in the cytoplasm close to the nucleus in strain NIH 200, of a diffuse mass containing dense bodies

with dimensions (120-150 nm) similar to those seen in the nucleus would suggest that these may have originated in the nucleus.

Many of the dense bodies seen in the cytoplasm of other strains were similar in dimensions and appearance to the 'button bodies' (Ludvik & Shipstone 1970) of the nucleus. Feria-Velasco & Trevino (1972) showed that they gave a positive acid-phosphatase reaction and suggested that they might be part of the lysosomal system of the parasite. Zaman (1973a) is, however, of the opinion that they are not essential for the survival of the amoebae. Their function remains unknown.

VIRUS-LIKE STRUCTURES.

Diamond *et al.* (1972) demonstrated the presence of viral agents indigenous to *E. histolytica* in axenic cultures. One was shown to replicate in the nucleus as multiple clusters of fine filaments; the other is a polyhedral virus seen only in the cytoplasm. Both types of agent produce lysis of susceptible strains of *E. histolytica*. Neither the polyhedral particles nor the filamentous virus were observed in the present study in either the strains received from Dr. Diamond nor in trophozoites from other sources. However, bundles of filamentous material interspersed with beaded elements were observed in the cytoplasm of strains NIH 200, AH and AK from axenic culture but were not morphologically similar to those described by Diamond *et al.* (1972). However, in a companion paper Mattern *et al.* (1972) recorded that the "small bundles of filaments are found diffusely scattered throughout the cytoplasm. These bundles are often associated with densely staining beaded structures which are about 30 nm in diameter and which clearly differ morphologically from individual filaments as seen in the nucleus. Their role, if any, in the infection process is not known".

Filamentous virus-like structures were observed in the nuclei of trophozoites from several of the strains of *E. coli* examined. They were not observed either in trophozoites of *E. histolytica* or in the cysts of either species and differed morphologically from the filamentous virus observed by Diamond *et al.* (1972) in *E. histolytica*.

DOUBLING OF THE PLASMALEMMA.

The double plasma membrane seen in places in trophozoites from human liver abscess may be a response to membrane damage by the immune system of the host, with which the amoebae in the liver are in close contact. Double plasma membranes were not seen in any of the trophozoites from hamster liver abscess. If such a double plasmalemma is indeed a reaction to damage by the immune system, it is possible that antibodies had not had time to develop in the hamsters which were sacrificed after only five days.

INGESTION OF HOST CELLS.

Griffin (1972) suggested that *E. histolytica* routinely "eats" inflammatory cells and that material from such ingested cells might contribute to pathogenicity. In only one instance was any evidence of ingested leucocytes seen in the trophozoites examined, though partially digested erythrocytes were observed on numerous occasions. If, indeed, material from ingested inflammatory cells does contribute to pathogenicity more frequent observation of such material would have been expected in the fifteen strains examined from human and animal tissue.

FIBRILLAR STRUCTURES.

The fibrillar regions seen in the cytoplasm of several of the trophozoites may represent the lamellae described by Eaton *(personal communication)* and the microfilaments seen by Michel & Schupp (1974). Eaton considered these to be lipo-protein lamellae which are present throughout the cytoplasm but concentrated in certain specific regions of active motility such as the ectoplasm of freshly extruded pseudopodia, phagocytic activity and the uroid. He advanced a theory of amoeboid motion by aggregation of these lamellar particles under the influence of a polarising process. Michel & Schupp (1974) considered the bundles of microfilaments to be 'actin-like' and their parallel arrangements were regarded as support for the proposal that an active contractile system exists. They considered that twenty-four hour treatment with uranyl acetate was a prerequisite for clearer observation of these microfilaments. The infrequent observation of such regions in the

present study may be due to a difference in fixation techniques.

These authors consider that the combination of fibrillar bundles (microfilaments) with ribonucleoprotein helices which they observed in the pre-cyst stage, indicates a transportation function which probably plays some role in cyst formation, in the course of which chromatoid bodies up to a few μm in size are formed. Their acceptance of Lowe & Maegraith's (1970a) concept of a 'pre-cyst' stage in cultures merely on the basis of the presence of chromatoid bodies is untenable in view of the frequent observation by other workers and in the present study of chromatoid bodies in axenic trophozoites which do not encyst.

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This study has confirmed the presence of a number of structures in the *Entamoeba* for which the functional purposes have not been defined although many authors have speculated as to their significance.

When one considers the almost infinitesimal part of an amoeba which a thin section reveals, it is not surprising that different workers observe a variety of organelles or that the structures present a variety of appearances.

The differing interpretations of the function of many of the organelles given by various authors is indicative of our lack of understanding and it is apparent that further studies are necessary to determine the exact nature and significance of such organelles as the intranuclear bodies, the 'fuzzy' coat, the lysosomal vacuoles and many others.

The structure of the surface-active lysosome suggests that it is an aggressive mechanism despite the opinions of Miller *et al.* (1972) and Magaudda *et al.* (1970). The observation of the apparent disorganization of a leucocyte on contact with an amoeba suggests that some such mechanism is involved. This is parallelled by the well-known observation, under the light microscope, that the pus cells in amoebic dysentery are usually necrotic in contrast to those encountered in

bacillary dysentery. The finding of such an organelle in trophozoites of *E. coli* suggests that the presence of this organelle *per se* is not an indication of invasiveness though it may well still be aggressive.

The presence of surface 'hairclips' has no immediate explanation and continued investigation is indicated. Why they were not seen on the surface of trophozoites from culture and in only some situations in invasive amoebae is difficult to explain. It should be recalled that they were seen on the surfaces of trophozoites from the colon and from hamster liver abscess but not on trophozoites from four cases of human liver abscess.

The presence of a paranuclear body is also a mystery. It was only observed in axenic trophozoites of strain HK9 and NIH 200 but not in the other axenic strains - AH, HALA and AK, nor was it encountered in trophozoites from any other source.

The most striking feature of the invasion of the human tissue by the amoebae is the apparent lack of response on the part of the host (Elsdon-Dew 1958). It would almost seem that the host does not recognise the intact amoeba as foreign. Considerable interest has been displayed in the mechanism by which certain parasites protect themselves against the defence mechanism of the host. Smithers & Terry (1967, 1969) and Smithers, Terry & Hockley (1969) demonstrated that the rhesus monkey is highly susceptible to an initial infection with *Schistosoma mansoni*, but strongly resists reinfection. They suggested that this "concomitant immunity" may be explained if the adult worms are covered by host antigens which would afford them an 'immunological disguise' against the immune mechanism of the host, which would be able to kill new invading forms lacking the disguise. The existence of host antigens at the surface of the worms was demonstrated by transferring worms, grown to maturity in mice, directly into the portal circulation of rhesus monkeys immunized against mouse erythrocytes. These worms were rapidly destroyed by an immune response directed against sites on the surface of the worm, whereas mouse worms survived well on transfer to normal non-immunised monkeys.

The 'fuzzy' coat of *E. histolytica* from tissue may be analogous

to the host material incorporated on to the integument of schistosomes. It may be of host origin and provide protection against the host's immune response. The high level of humoral antibodies found in invasive amoebiasis does not appear to provide the human host with protection against further invasion. Possibly the corresponding antigens are intra-amoebic. It must be recalled that a 'fuzzy' layer was observed on the surface of trophozoites of *E. coli* from the human colon! However, whether this 'fuzzy' coat is of host or parasite origin remains open to speculation.

The observation of filamentous virus-like structures in the cytoplasm of three strains from axenic culture is strange, particularly as, according to Diamond *(personal communication)*, indigenous virus has never been observed by electron microscopy in stable, healthy axenic cultures of *E. histolytica*. If, in fact, these structures are virus elements, it is possible that they were observed because of the lysis of a small percentage of the cells, as Diamond *(personal communication)* has said that indigenous virus has been observed in host cells which have undergone spontaneous lysis. Studies involving the transfer of lysate from trophozoites of *E. coli* containing the filamentous material into other strains of *E. coli* and *E. histolytica* in axenic culture, are at present being undertaken.

The disorderly arrangement in which the electron-dense cylindrical bodies (the rhabdovirus) were observed in trophozoites of *E. histolytica* from human liver abscess, would indicate an active state of these structures in trophozoites invading the liver. Their presence in trophozoites of *E. histolytica* from all sources and their apparent absence from trophozoites of non-pathogenic *E. coli* would suggest that they may be associated with invasiveness.

Despite the fact that in this study several hundred sections of a large number of samples of many strains of amoebae were examined it is only too clear that the amount of material is minute in comparison with the extent of the problem being studied.

F

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KEY TO LETTERING USED IN PLATES

A	Amoeba
B	Bud-like protrusions
BB	Button bodies
Bm	Bacterium
C	Cellular material
CB	Crystalloid aggregates - chromatoid body
Ch	Chromatin
CW	Cyst wall
Db	Dense body
DF	Fibrous layer of cyst wall
E	Electron-dense body
EC	Ectoplasm
EL	External region of cyst wall
EN	Endoplasm
ER	Endoplasmic reticulum
F	Fibrous bundle
FB	Foamy body
FM	Fibrous material
FV	Food vacuole
G	Granules in paranuclear body
GL	Golgi-like complex
Gly	Glycogen
IF	Intranuclear filaments
L	Lysosomal vacuole
LA	Parallel lamellae
Lc	Leucocyte
LG	Lipid globule

Mb	Microbodies
MH	Membrane-bound helices
N	Nucleus
OT	Osmiophilic tubules
P	Plasmalemma
PG	Pale granular region of cyst wall
Ph	Phagocollar
PNB	Paranuclear body
Ps	Pseudopodium
R	Rosette
Rbc	Erythrocytes
Rh	Ribosomal helices
S	Sub-pellicular bodies
St	Starch granule
SV	Small vacuoles
Sv	Small vesicles
T	Trigger
U	Uroid
V	Vacuole

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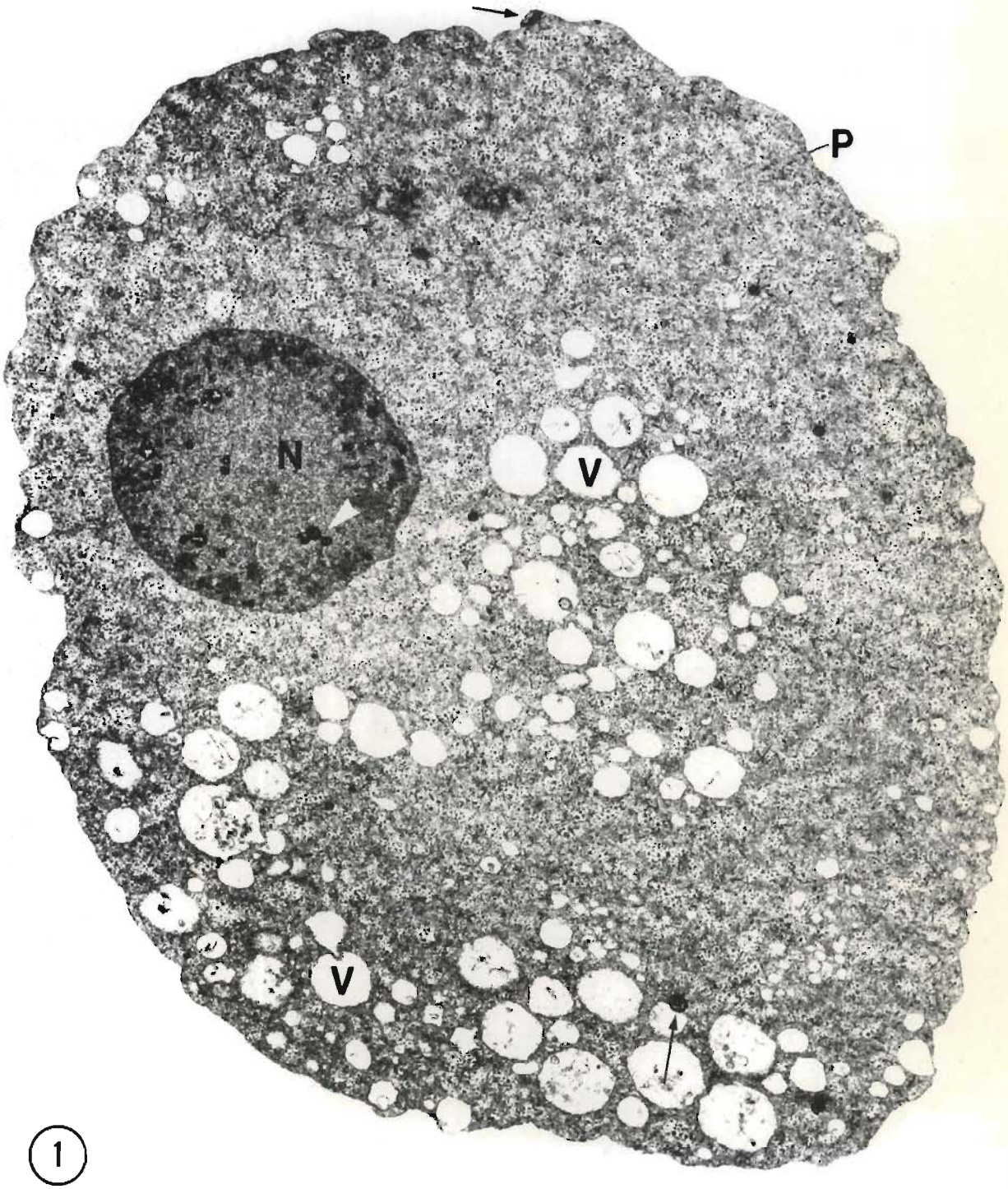
APPENDIX - GLOSSARY OF TERMS

'Fuzzy' coat	Filamentous material approximately 30 nm wide on the outer surface of the plasmalemma as first described by El Hashimi & Pittman (1970) in trophozoites of *E. histolytica* is shown in Figure 74.
Osmiophilic sub-pellicular bodies	Oval electron-dense bodies measuring 33-83 nm on the inner leaflet of the plasmalemma noted by Rondanelli *et al.* (1966, 1968) and shown in Figure 2.
Ribosomal helices	These are slender rods on which small particles 25-30 nm in width are stacked in what appears to be a helical pattern. They are composed of RNA and protein. Packed helical arrays of ribonucleo-protein particles form the chromatoid bodies of light microscopy and have been termed 'helical ribonucleoprotein bodies' by Siddiqui & Rudzinska (1963). These are shown in Figures 3 and 4.
Rosette arrangement of osmiophilic bodies	Electron-dense cylindrical units which invariably form a rosette arrangement surrounding a finely granular area of cytoplasm are shown in Figure 7.
'Hairclip'-like structures	Fine tubular structures within the cytoplasm which open out at one end into a vesicle are shown in Figure 22.
Lysosomal vacuoles	First described by Eaton *et al.* (1969) are surface depressions beneath which is a cup-shaped membrane-bound vacuole. A frond-like outgrowth or 'trigger' mechanism which is continuous with the interior of the surface lysosome may be present and is shown in Figure 84.

Para-nuclear body	An unbound mass in close proximity to the nucleus and comprising granules of different electron densities, and first described by Proctor & Gregory (1972a), is shown in Figures 16 and 17.
'Button bodies'	First described by Ludvik & Shipstone (1970) in the nucleus of *E. histolytica* as having a button-like structure. The bodies have a peripheral membrane with considerable variation in their internal structure. They range in size from 100 to 500 nm and are shown in Figure 1.

Trophozoites of *E. histolytica* from axenic culture

Fig. 1 Shows a trophozoite from strain HK9 bounded by a plasmalemma (P) and containing vacuoles (V) and a nucleus (N) within which are the 'button bodies' (arrowhead). Vesicles on the plasmalemma and within the cytoplasm are indicated by arrows. x 8 640



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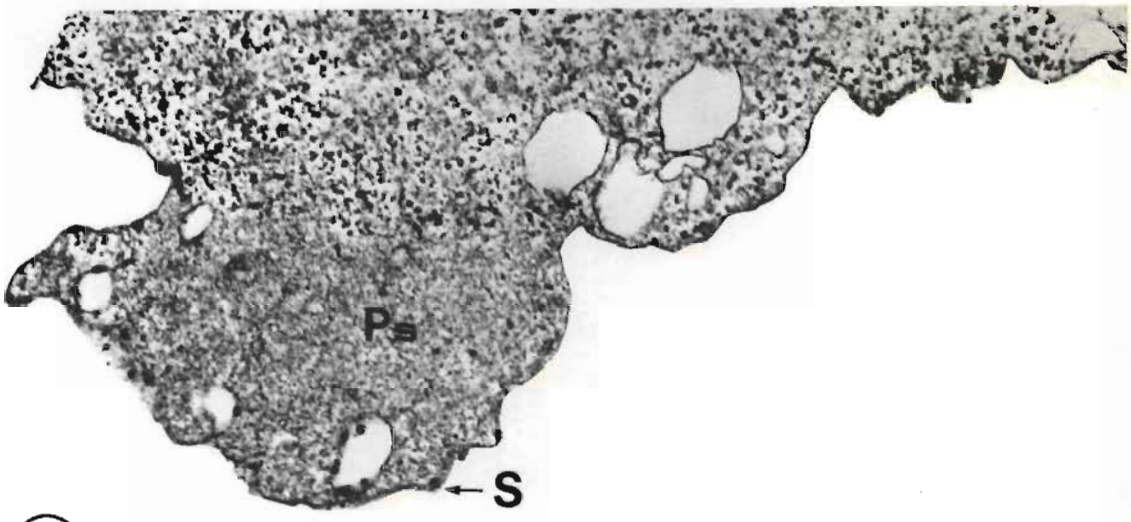
Trophozoites of *E. histolytica* from axenic culture

Fig. 2 Part of a trophozoite showing the sub-pellicular bodies (S) and a pseudopodium (Ps). x 25 500

Fig. 3 Portion of the cytoplasm containing a nucleus (N) glycogen (Gly) and chromatoid bodies (CB). x 16 150

Fig. 4 Note the microbodies (Mb) and the individual ribosomal helices (Rh). x 31 280

Fig. 5 A region of endoplasmic reticulum (ER) surrounded by homogenous electron-dense material. x 20 500



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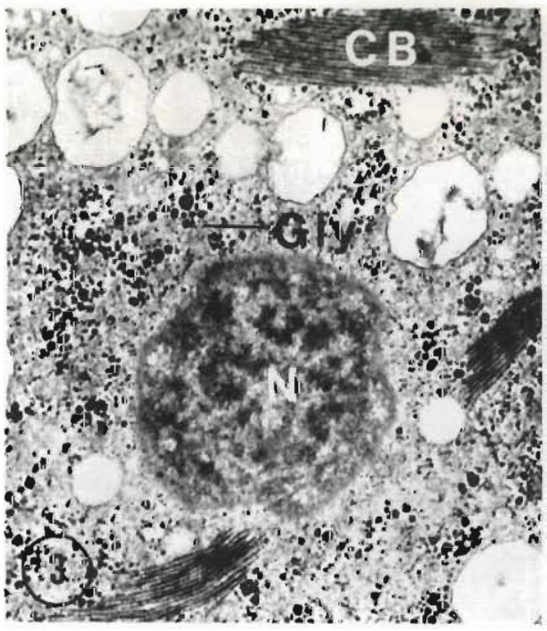

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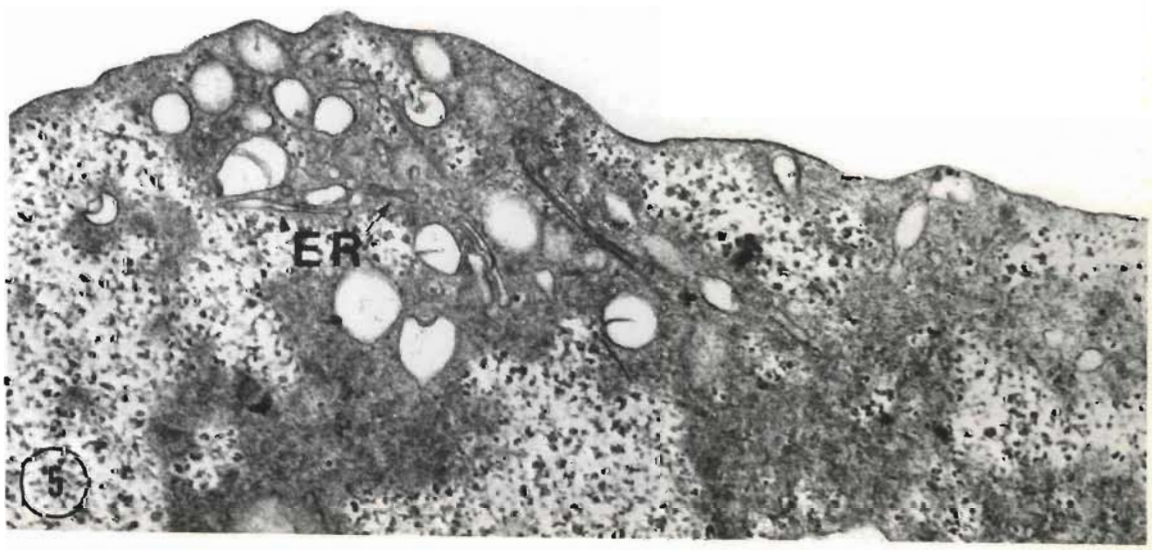

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Trophozoites of *E. histolytica* from axenic culture

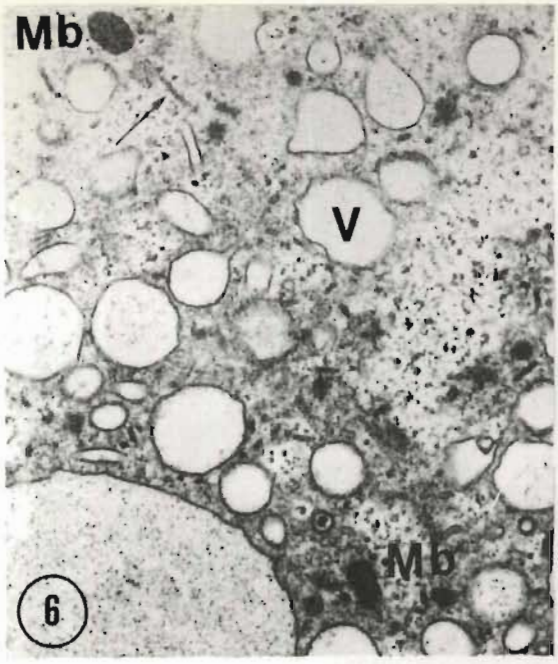
Fig. 6 Part of the cytoplasm containing numerous vacuoles (V) short helical rods (arrow) and electron-dense micro-bodies (Mb). x 24 700

Fig. 7 Electron-dense cylindrical bodies (arrowheads) arranged in a rosette around a finely granular area of cytoplasm. x 33 800

Fig. 8 Note the vesicles (arrowed) in close association with the plasmalemma and a vacuole. x 34 000

Fig. 9 Note the lack of inclusions in the pseudopodium (Ps) of the trophozoite. x 2 700

Fig. 10 Shows the uroid region of a trophozoite (U). x 14 400



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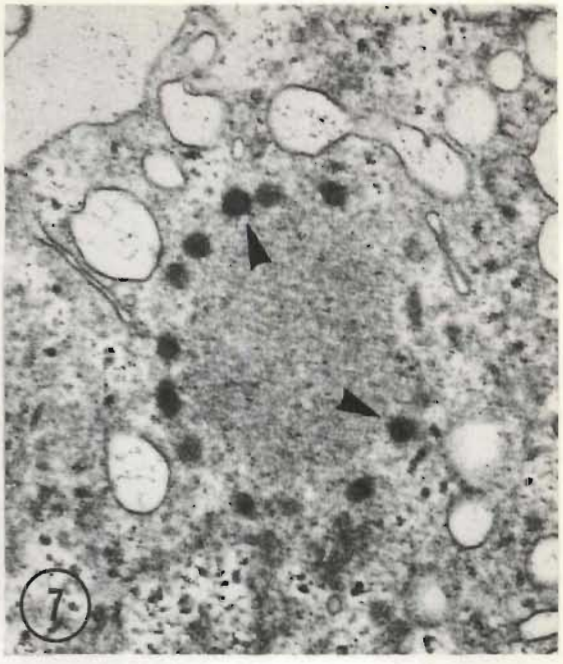

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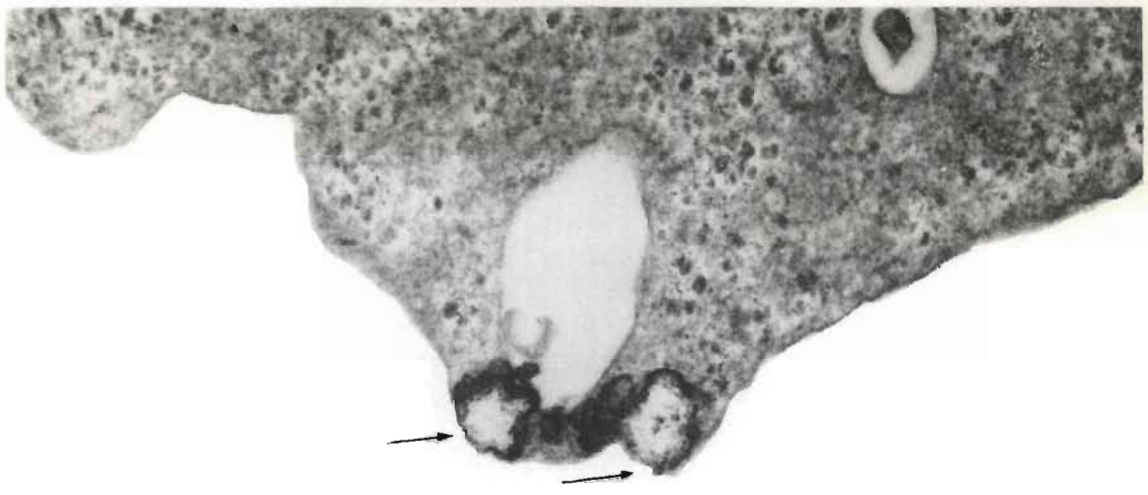

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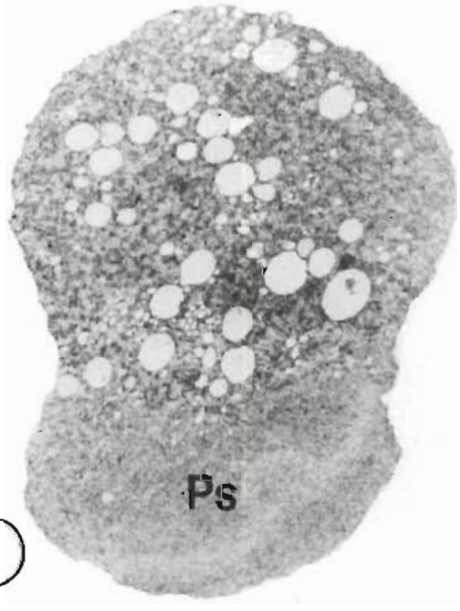

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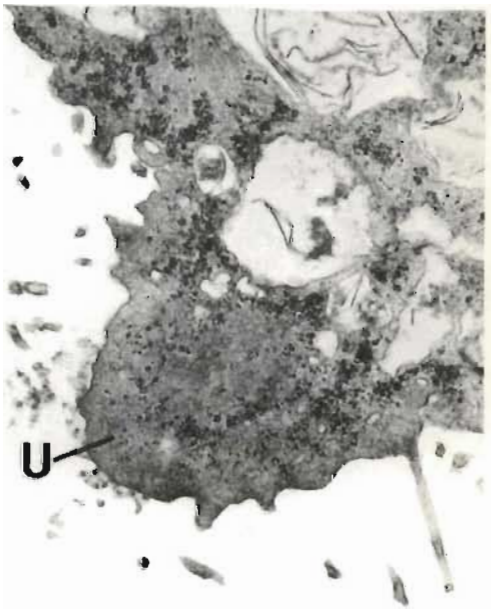

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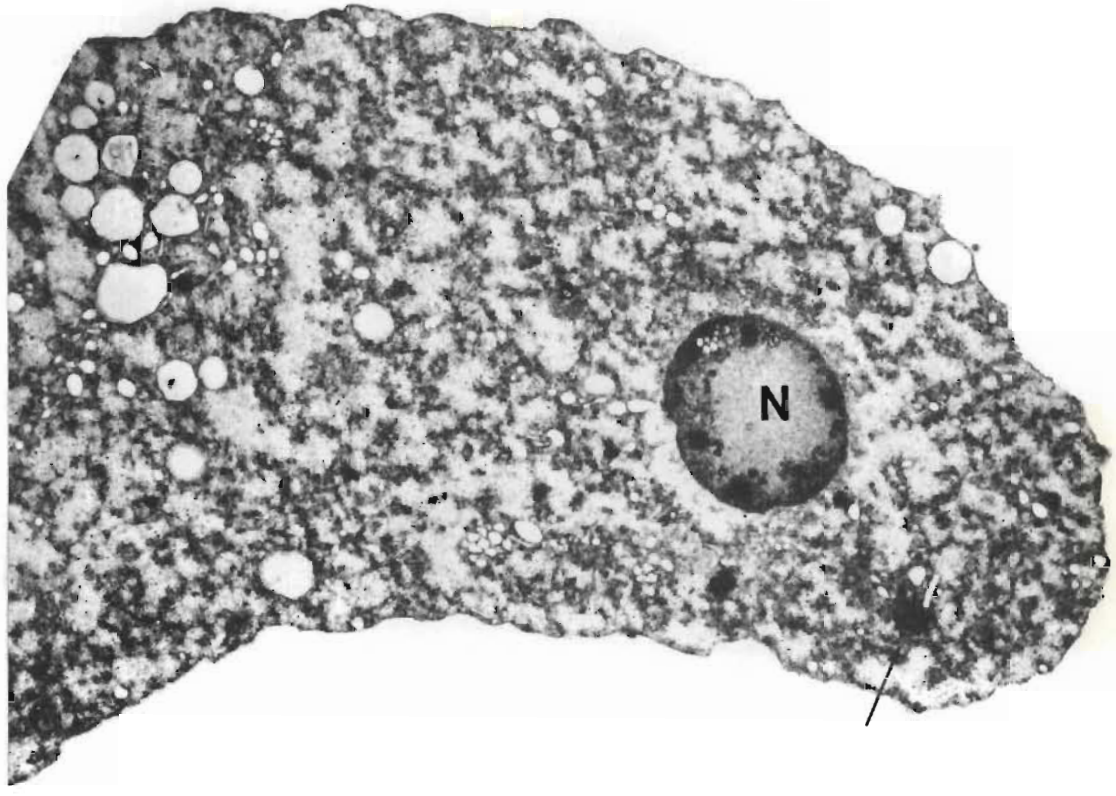
Trophozoites of *E. histolytica* from axenic culture

Fig. 11 Part of a trophozoite showing the electron-dense bodies in a rosette arrangement (arrow) the nucleus (N) and the early stages of cytoplasmic flocculation. x 4 680

Fig. 12 A trophozoite showing greater flocculation of the chromatin-like material (Ch). x 4 680



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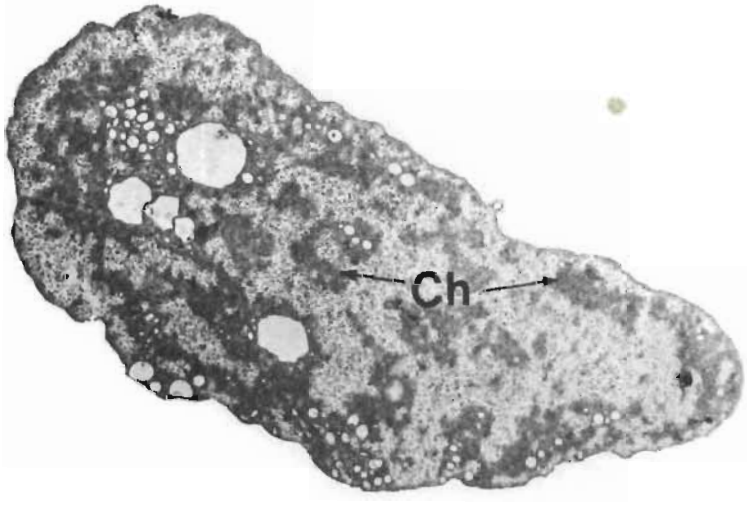

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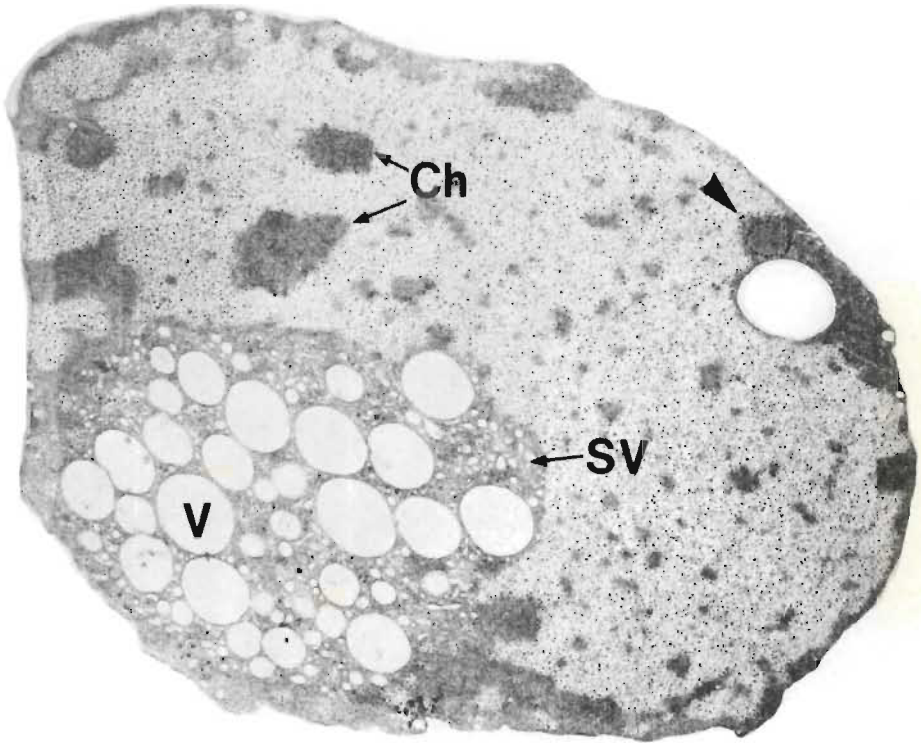
Trophozoites of *E. histolytica* from axenic culture

Fig. 13 A trophozoite showing the variation in the density of the cytoplasm. The large and small vacuoles (V and SV) are aggregated within electron-dense material and the remainder of the cytoplasm contains material closely resembling chromatin (Ch). Note the rosette arrangement of electron-dense bodies (arrowhead) in close proximity to a vacuole. x 4 680

Fig. 14 Trophozoite showing an apparent reduction of the organelles and flocculation of the chromatin-like material (Ch). x 4 680



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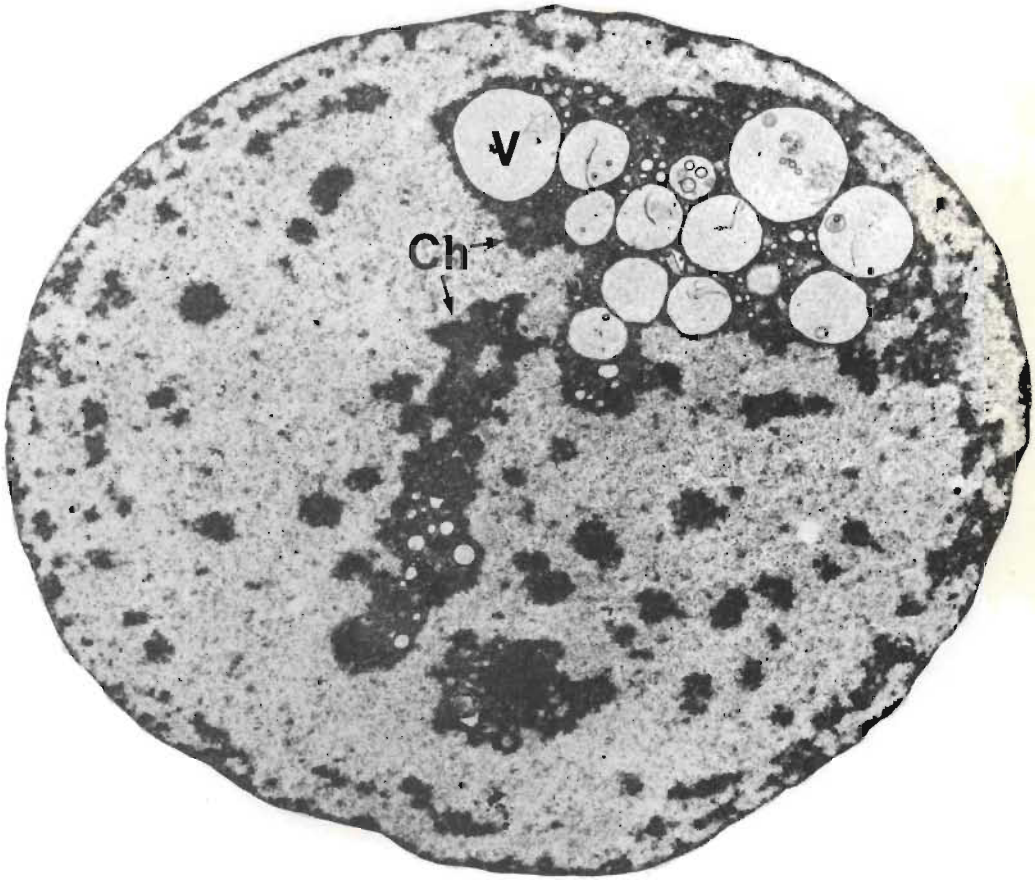

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Trophozoites of *E. histolytica* from axenic culture

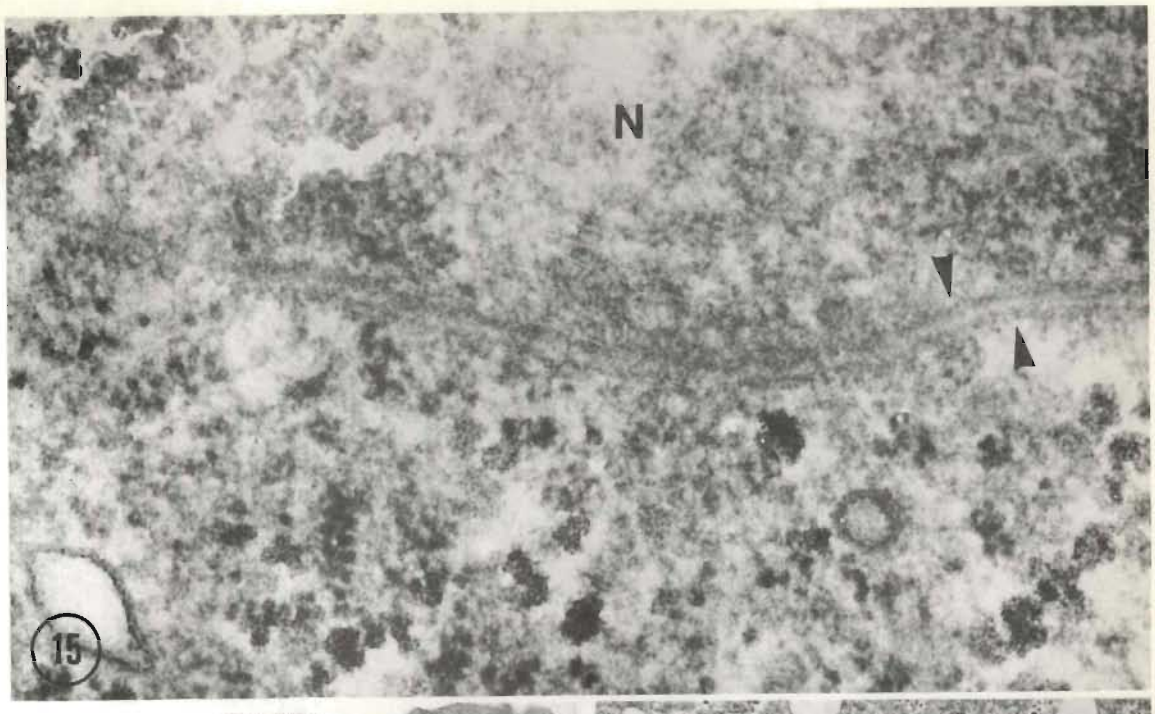
Fig. 15 Note the two unit membranes (arrowheads) and the perinuclear space of the nucleus (N). x 125 300

Fig. 16 Part of a trophozoite showing the nucleus (N) and the para-nuclear body (PNB). x 6 120

Fig. 17 The para-nuclear body showing the variation in size of the granules (G). x 31 680

Fig. 18 Note the mass (arrowed) similar to the para-nuclear body apparently adherent to the nucleus (N). x 20 160

Fig. 19 Part of a trophozoite showing the Golgi-like complex (GL). x 28 560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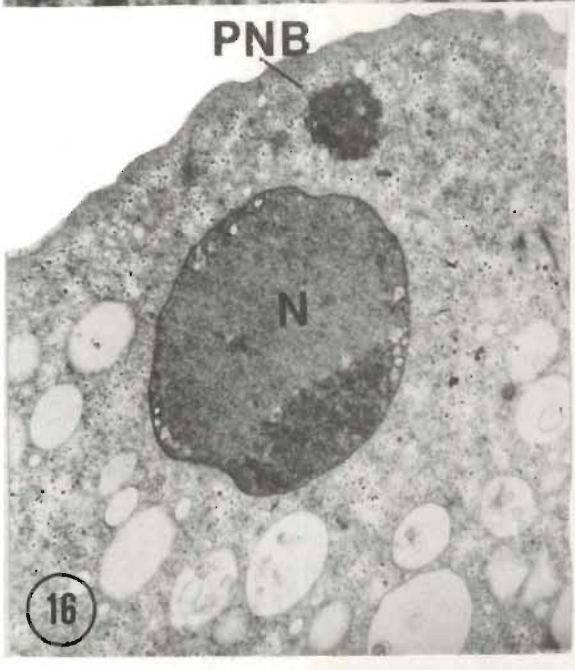
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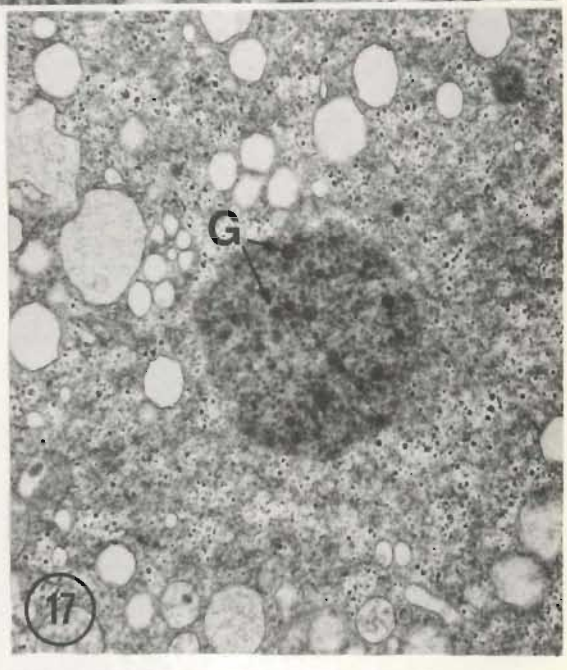
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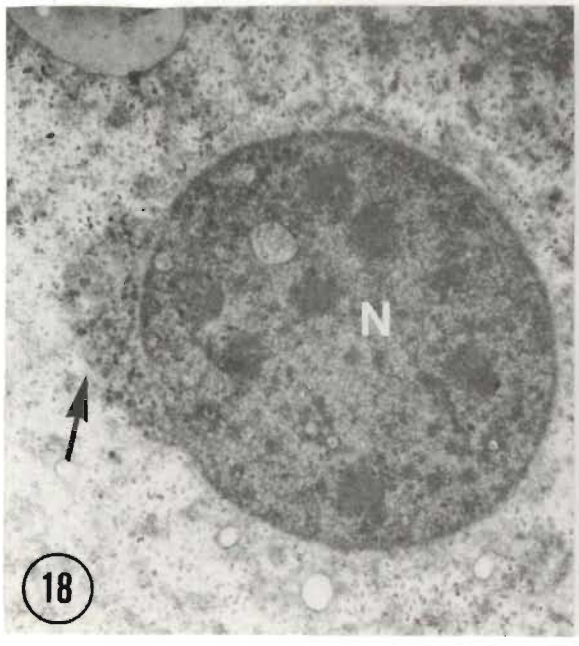
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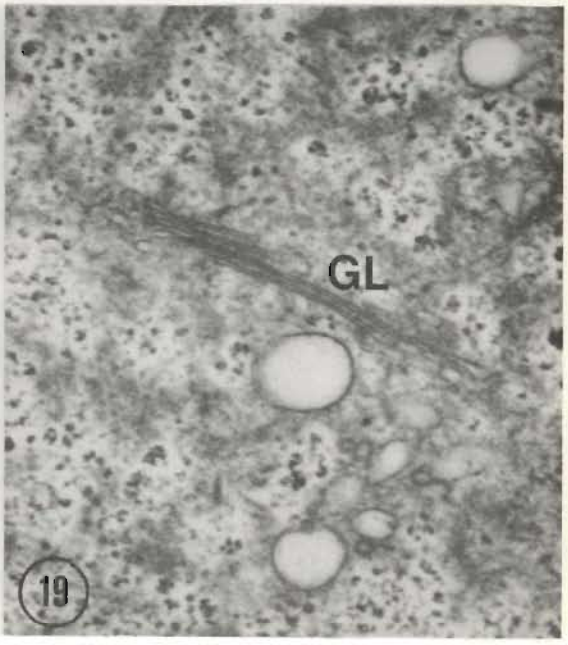
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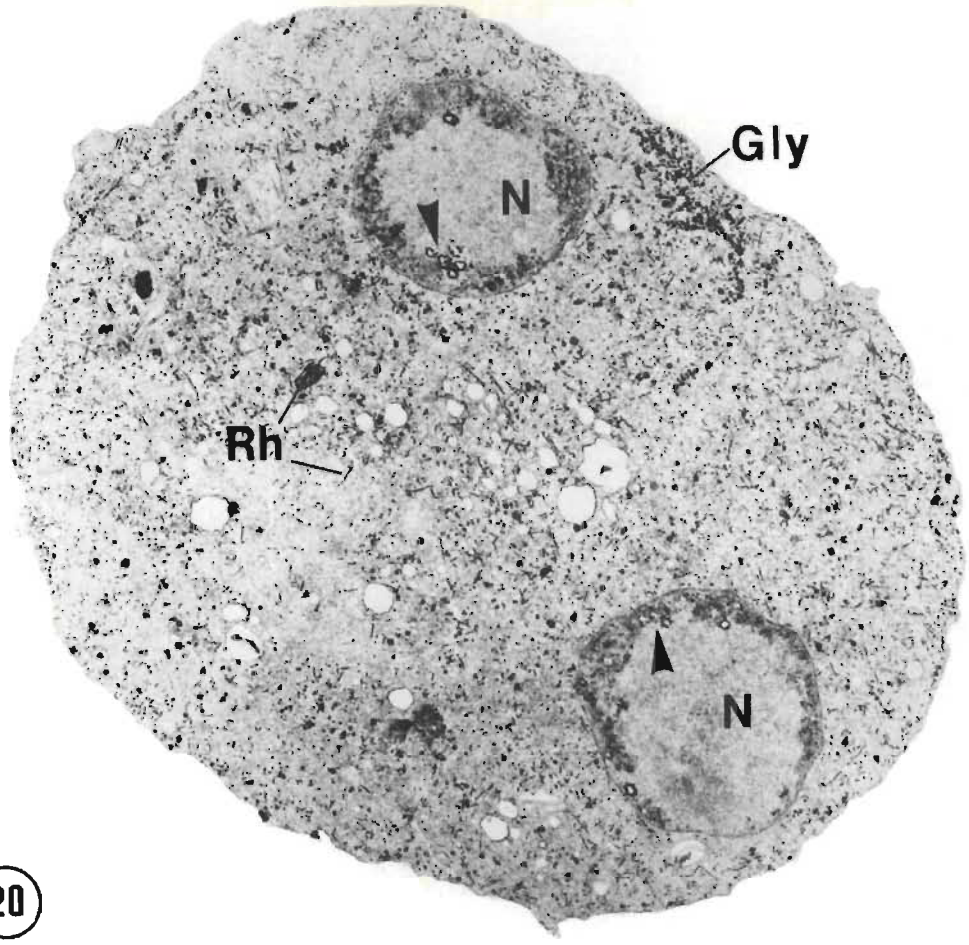
Trophozoites of *E. histolytica* from axenic culture

Fig. 20 Binucleate trophozoite containing glycogen (Gly) ribosomal helices (Rh). Within the nuclei (N) are button bodies (arrowheads). x 8 400

Fig. 21 Trophozoite containing aggregates of ribosomal helices - the chromatoid bodies (CB). Note the electron-dense particle (arrowed) apparently being extruded through the plasmalemma. x 8 800



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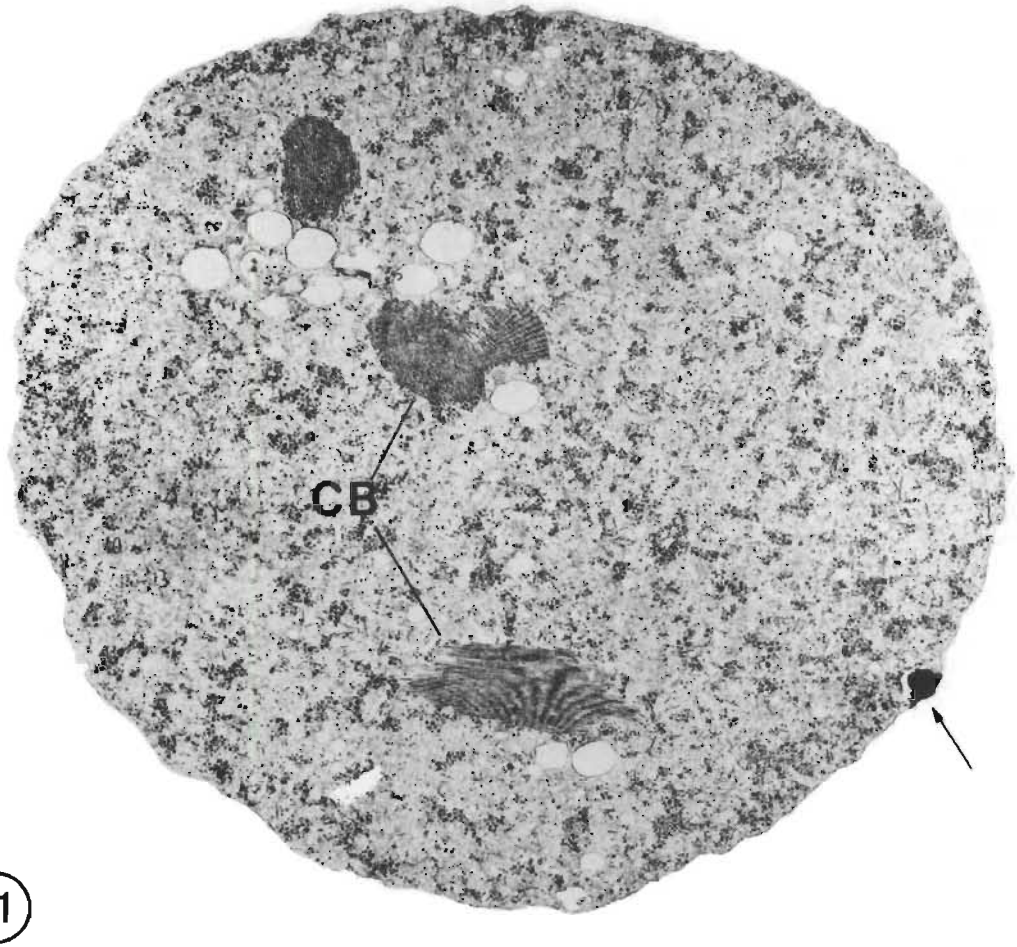

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Trophozoites of *E. histolytica* from axenic culture

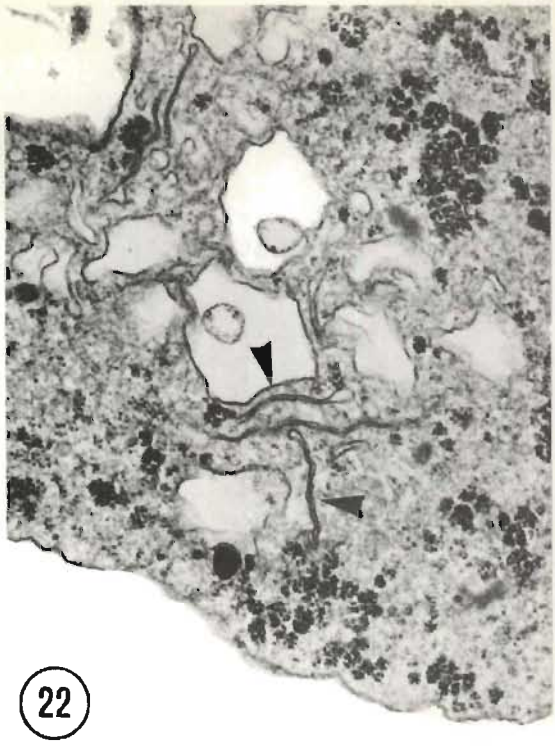
Fig. 22 Part of a trophozoite showing the 'hairclip' arrangements of smooth endoplasmic reticulum (arrow-heads). x 36 960

Fig. 23 High power of a chromatoid body or aggregate of ribosomal helices. x 28 500

Fig. 24 Transverse section through a crystalloid aggregate. x 93 000

Fig. 25 Longitudinal section through a crystalloid aggregate x 93 000

Fig. 26 Part of a trophozoite showing the crescent-shaped vacuoles beneath the plasmalemma. x 28 350



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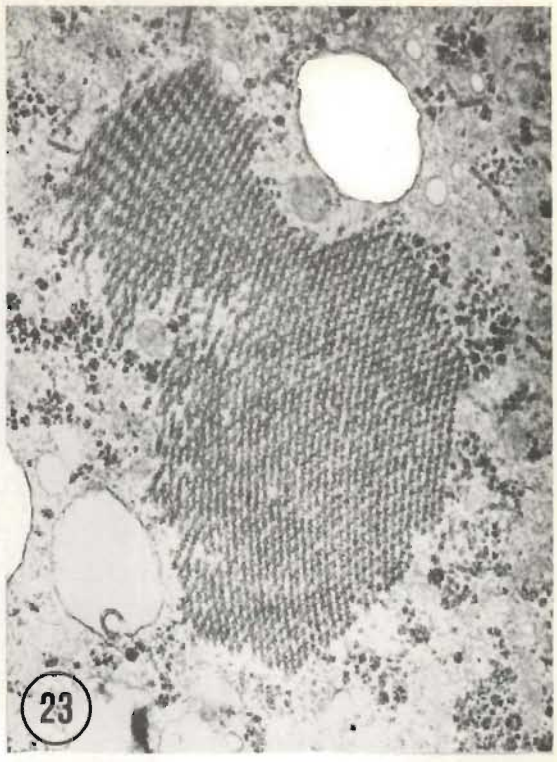

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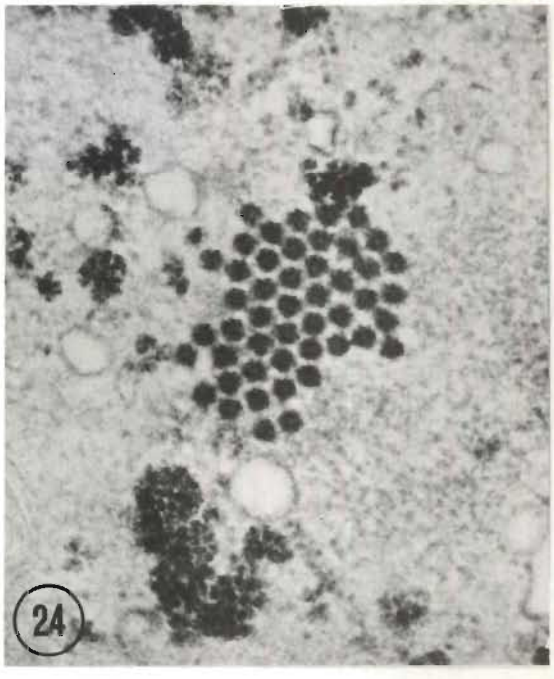

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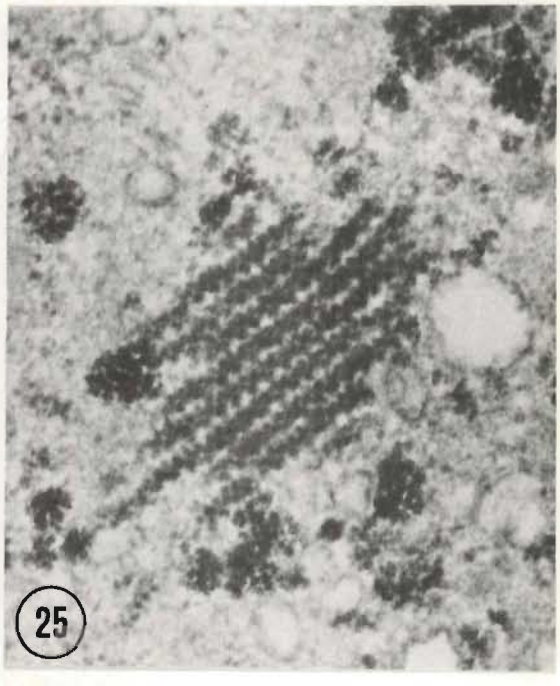

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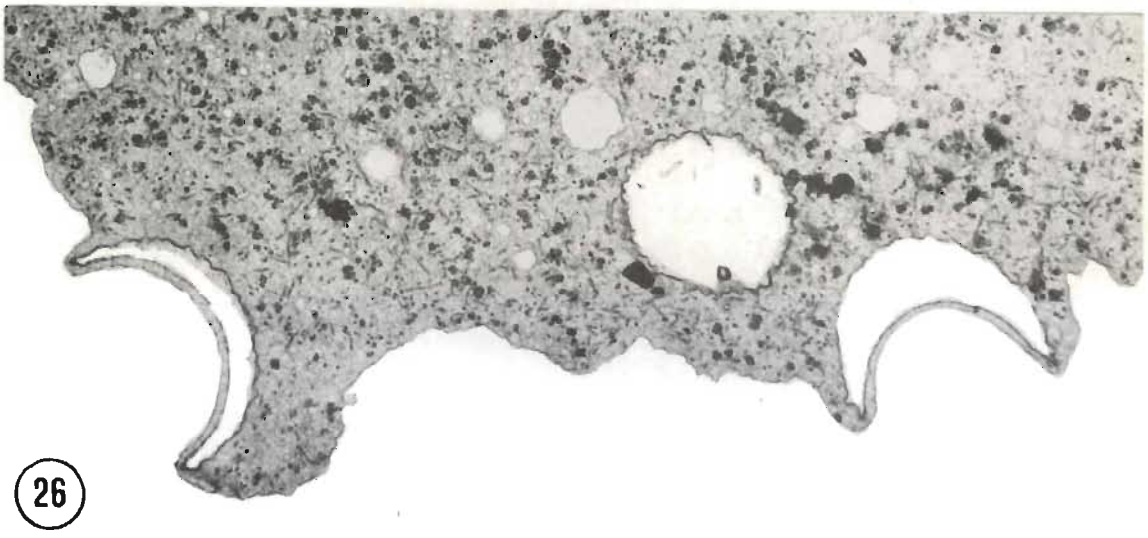

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Trophozoites of *E. histolytica* from axenic culture

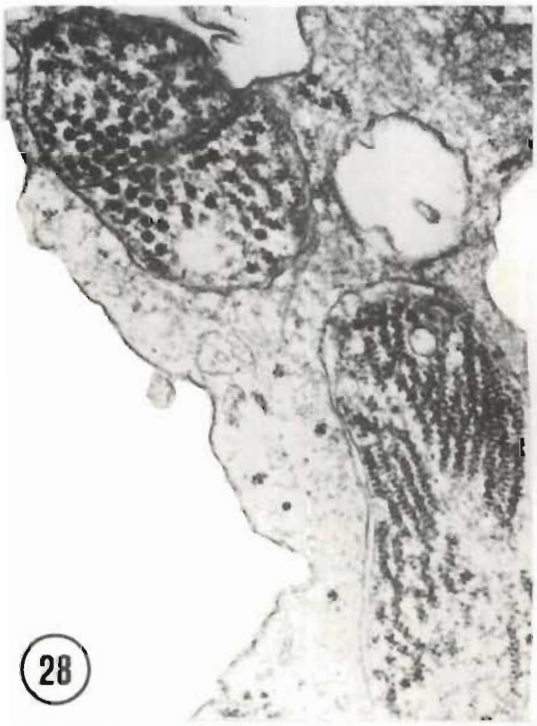
Fig. 27 A trophozoite of NIH 200 from 48 hour culture showing the numerous double-membraned vacuoles containing ribosomal helices (MH). x 5 580

Fig. 28 High power showing the membrane-bound helices both in transverse and longitudinal section. x 45 220

Fig. 29 Part of an amoeba from 72 hour culture showing the membrane-bound helices (MH) in different stages of lysis, an electron-dense body (E) and a fibrous bundle (F). x 32 560

Fig. 30 Shows the short strands of beaded elements in close association with fibrous material. x 31 200

27



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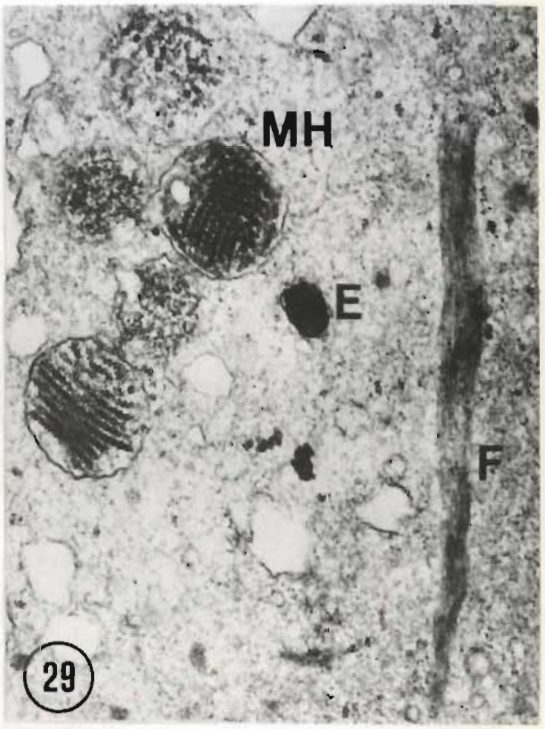

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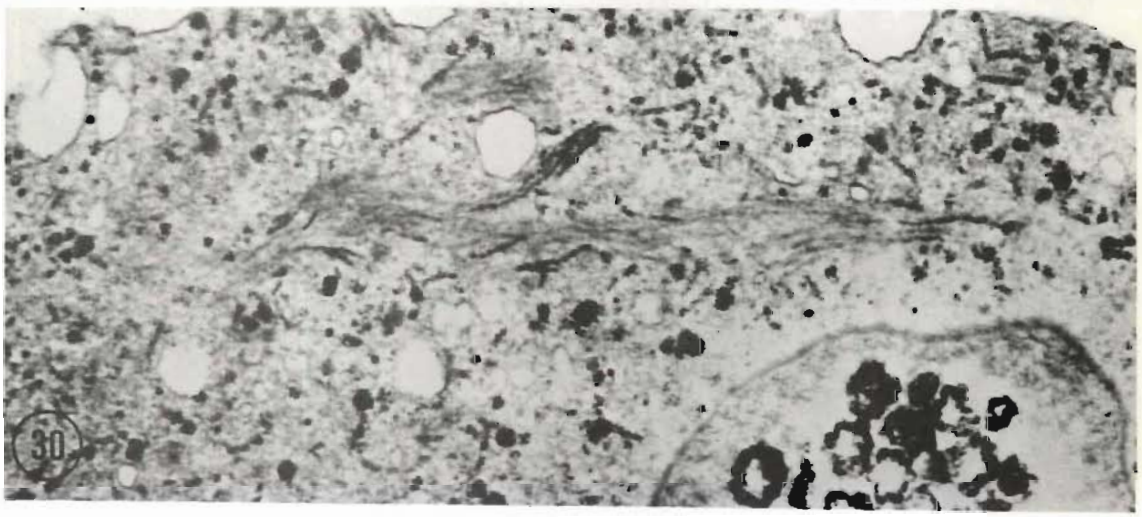
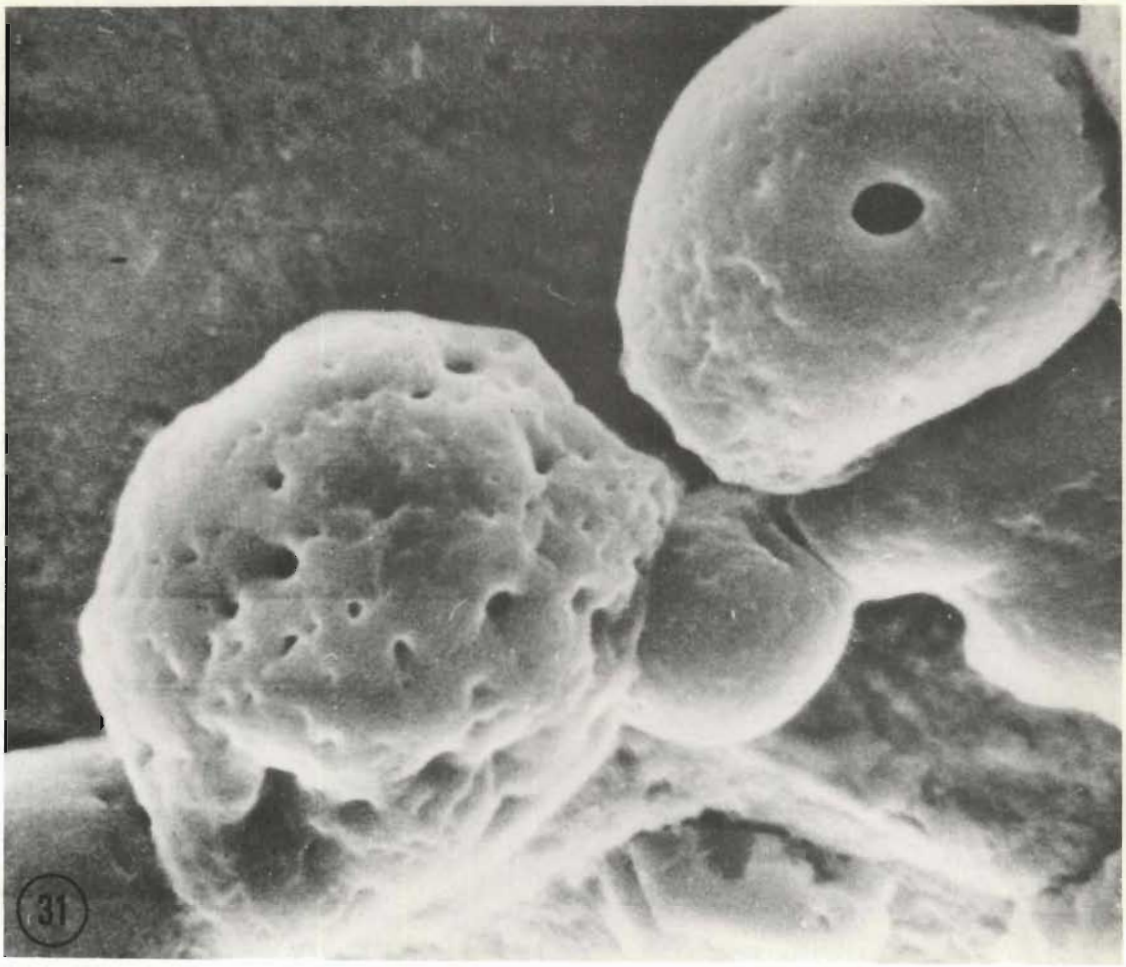


30

Trophozoites of *E. histolytica* from axenic culture

Fig. 31 Stereoscan of NIH 200 showing the large craters on the surface. x 4 800

Fig. 32 Stereoscan showing the relatively smooth surface of HK9. x 4 800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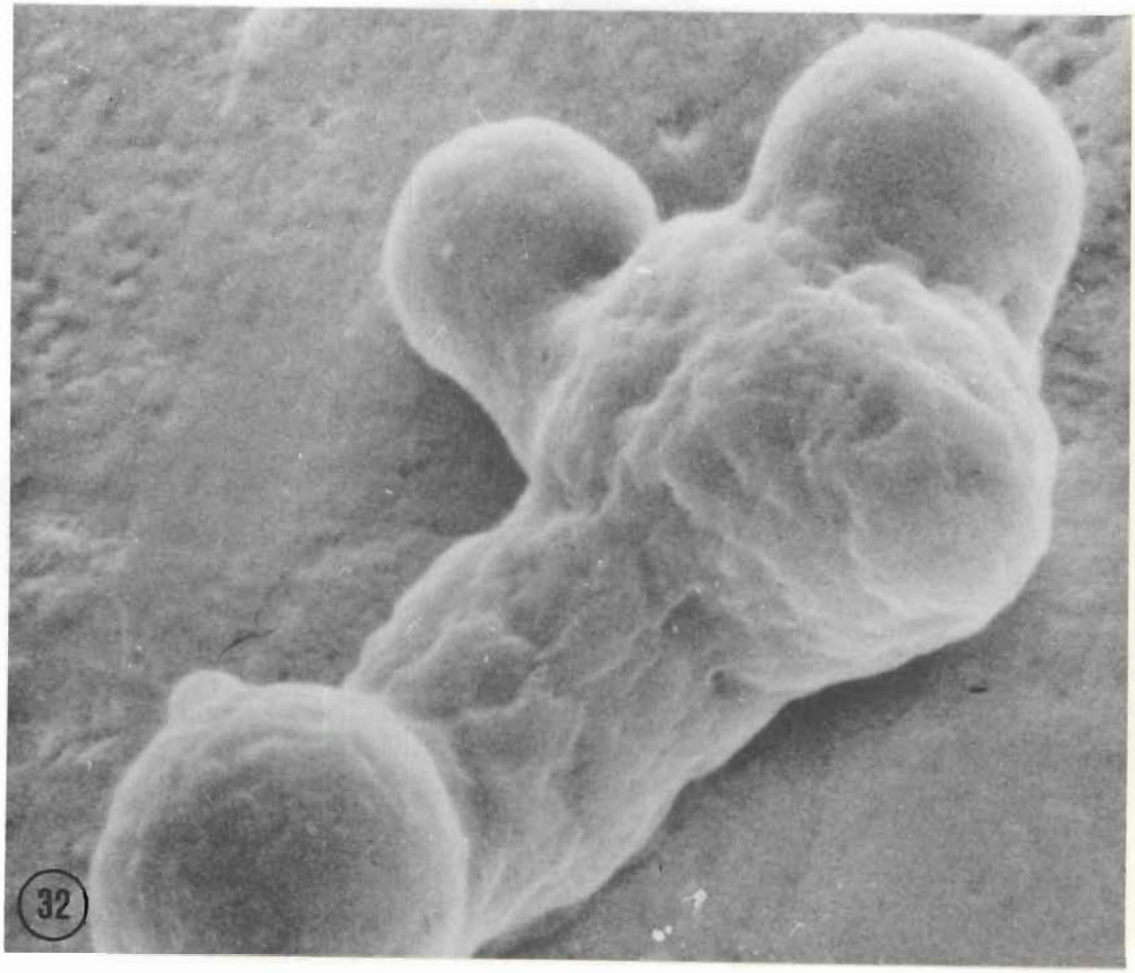
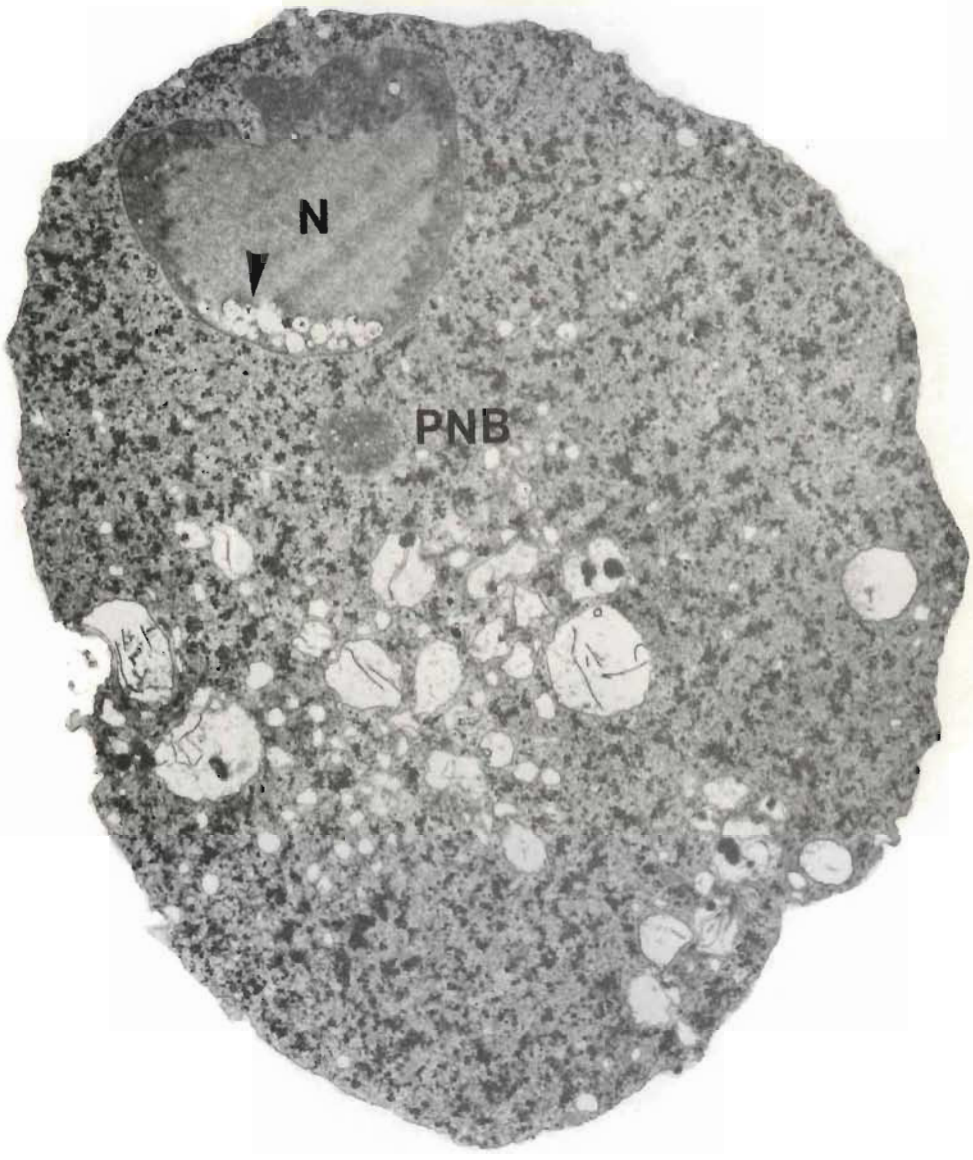

32

Trophozoites of *E. histolytica* from axenic culture

Fig. 33 A trophozoite showing the nucleus (N) button bodies (arrowhead) and the para-nuclear body (PNB). x 7 830

Fig. 34 Shows part of the nucleus containing button bodies (arrowhead) and the para-nuclear body (PNB). x 31 200



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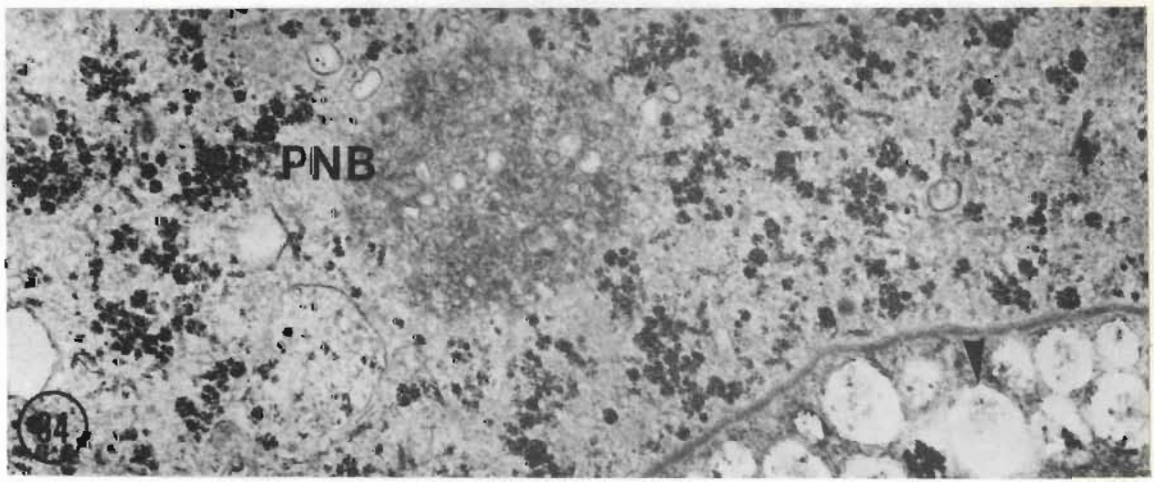


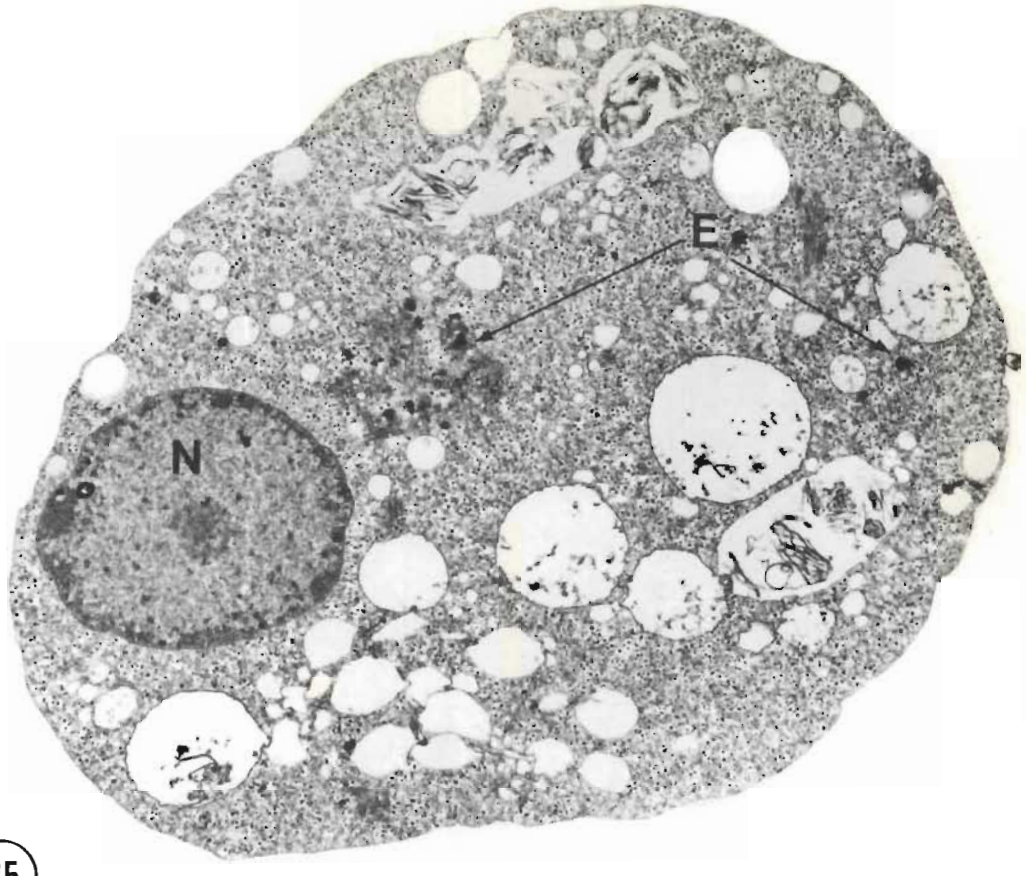
34

Trophozoites of *E. histolytica* from axenic culture

Fig. 35 A trophozoite of strain NIH 200 showing electron-dense bodies (E) in a region of homogenous cytoplasm close to the nucleus (N). x 5 400

Fig. 36 High power showing the electron-dense bodies (E) within the dense cytoplasm. x 15 360

Fig. 37 Part of an amoeba showing the electron-dense bodies at the tip of bud-like protrusions of the plasma membrane (B). x 25 650



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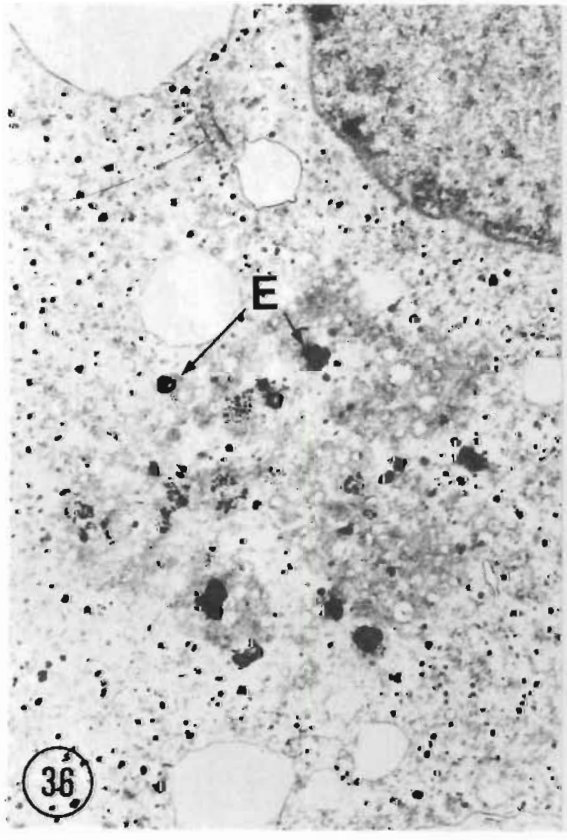

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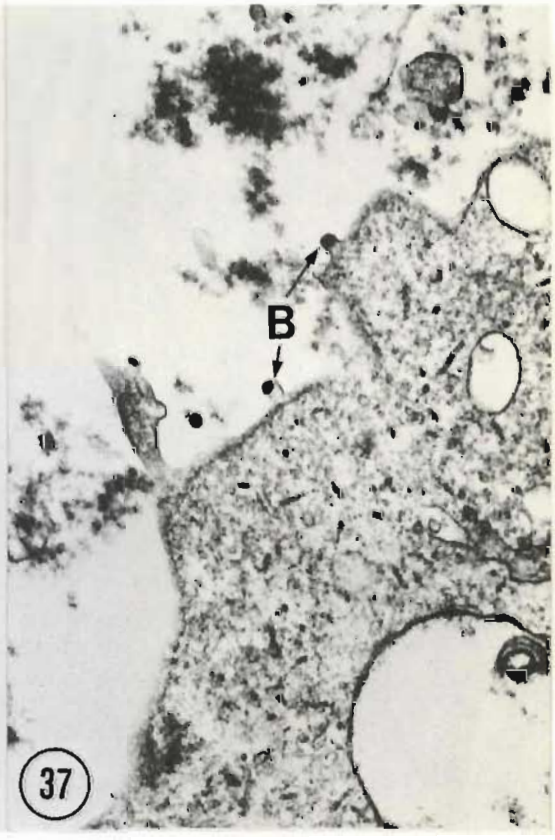


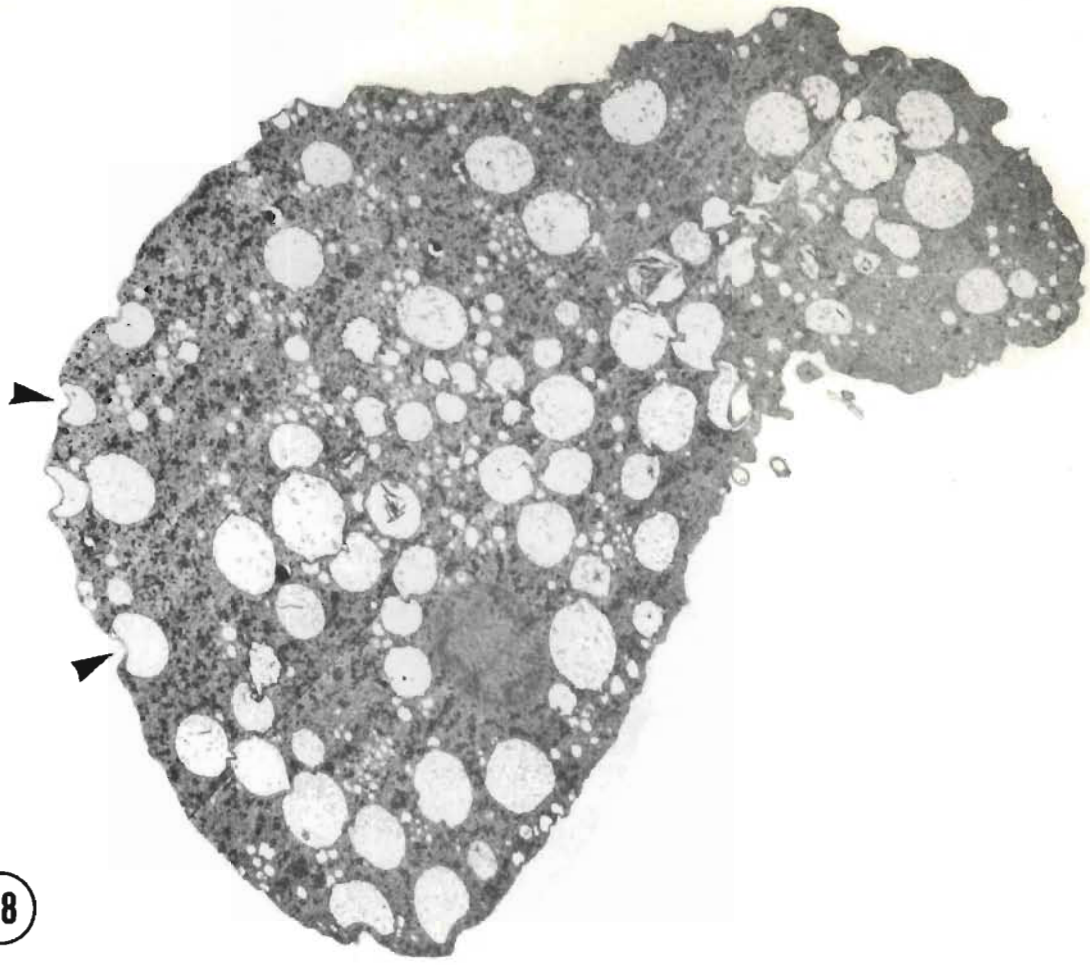
37

Trophozoites of *E. histolytica* from axenic culture

Fig. 38 A trophozoite showing numerous crescent-shaped vacuoles (arrowheads) beneath the plasmalemma. x 3 080

Fig. 39 Shows a lysosome-like vacuole. x 12 000

Fig. 40 Shows the extensive convolutions of the membranes of a lysosome-like vacuole. x 40 850



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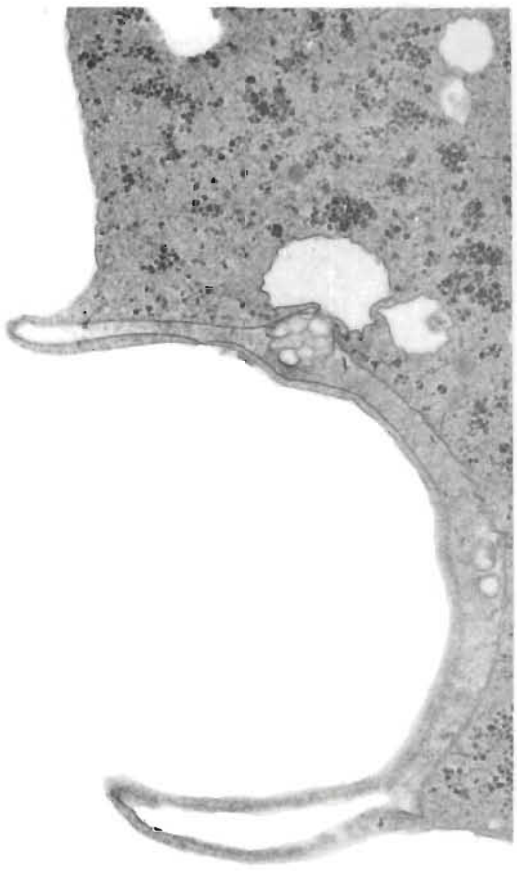

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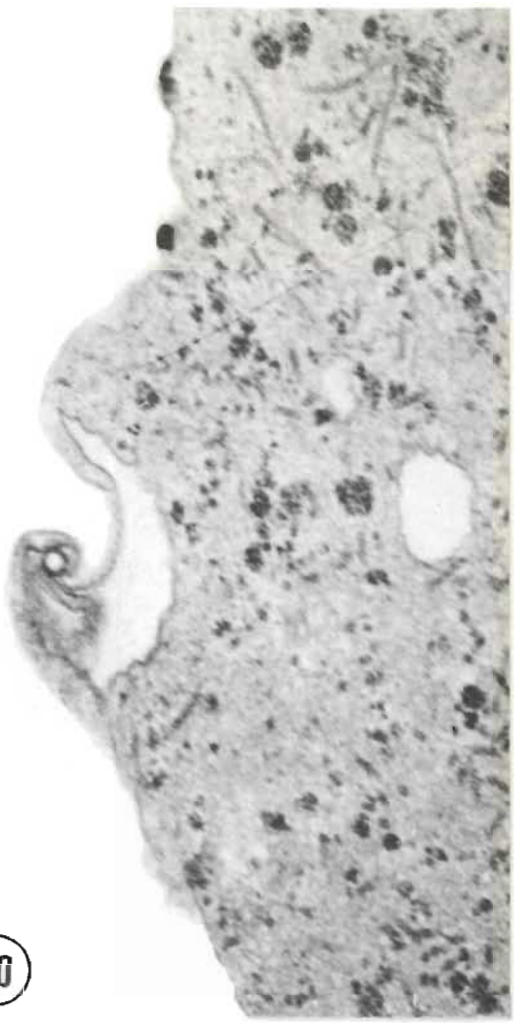


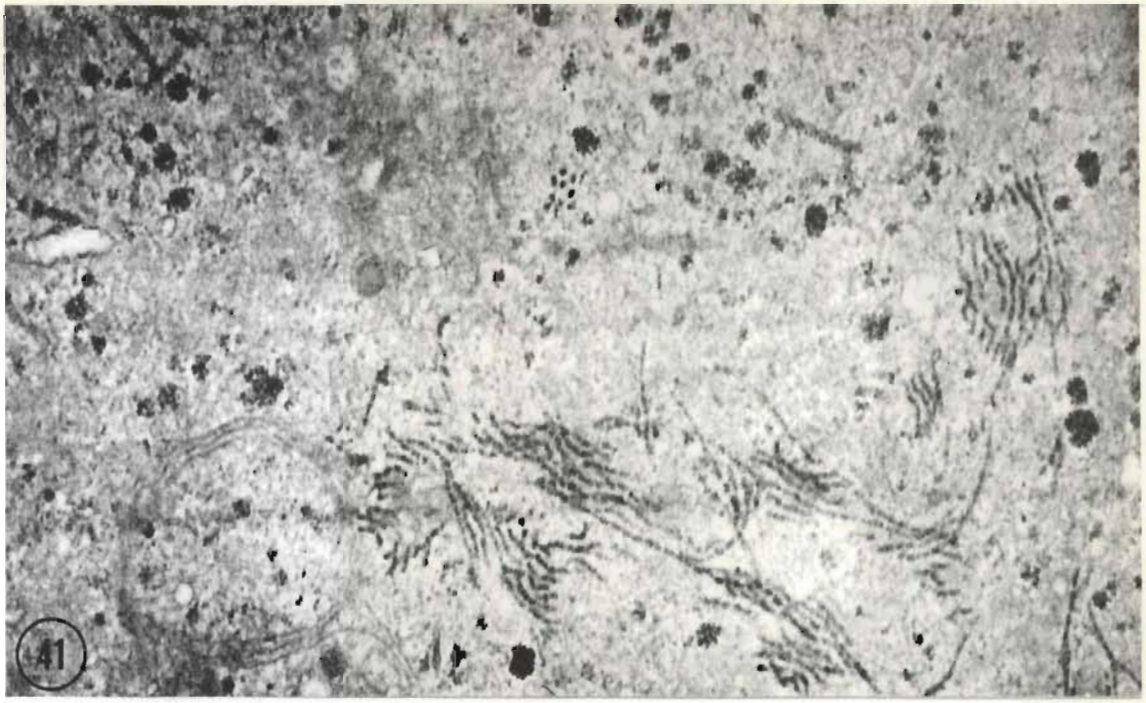
40

Trophozoites of *E. histolytica* from axenic culture

Fig. 41 Shows strands beaded along the entire length in the cytoplasm of strain AH

Fig. 42 Beaded elements in a feathery arrangement (arrowhead) in close proximity to vacuoles (V) and crystalloid aggregates (CB). x 31 720

Fig. 43 High power of the beaded elements in a feathery arrangement. x 57 200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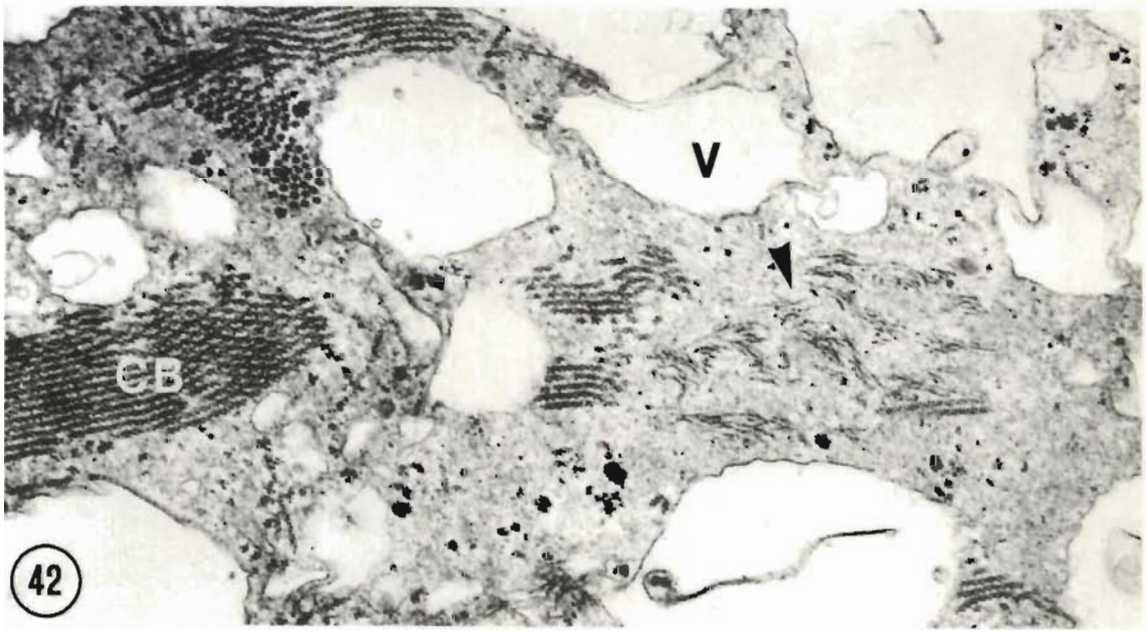
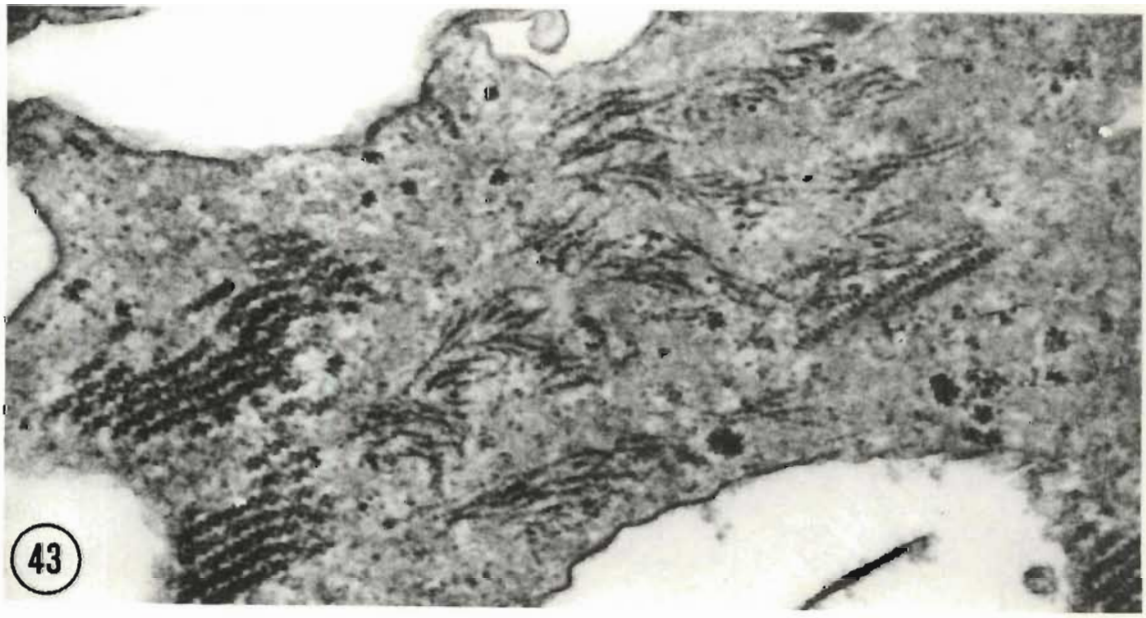

V
CB
42

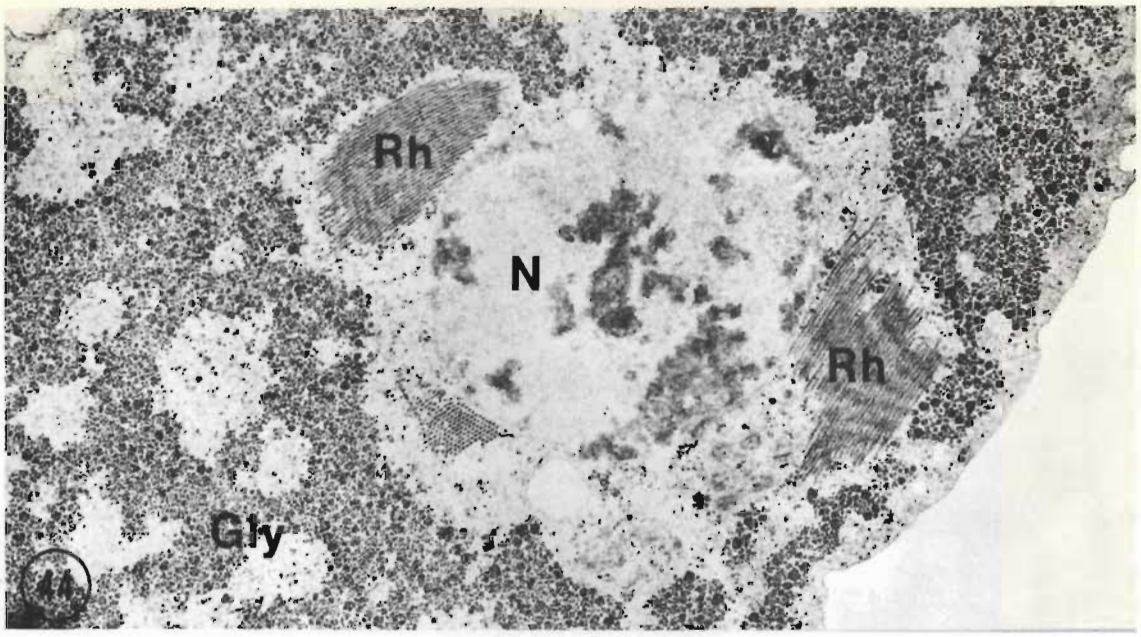

43

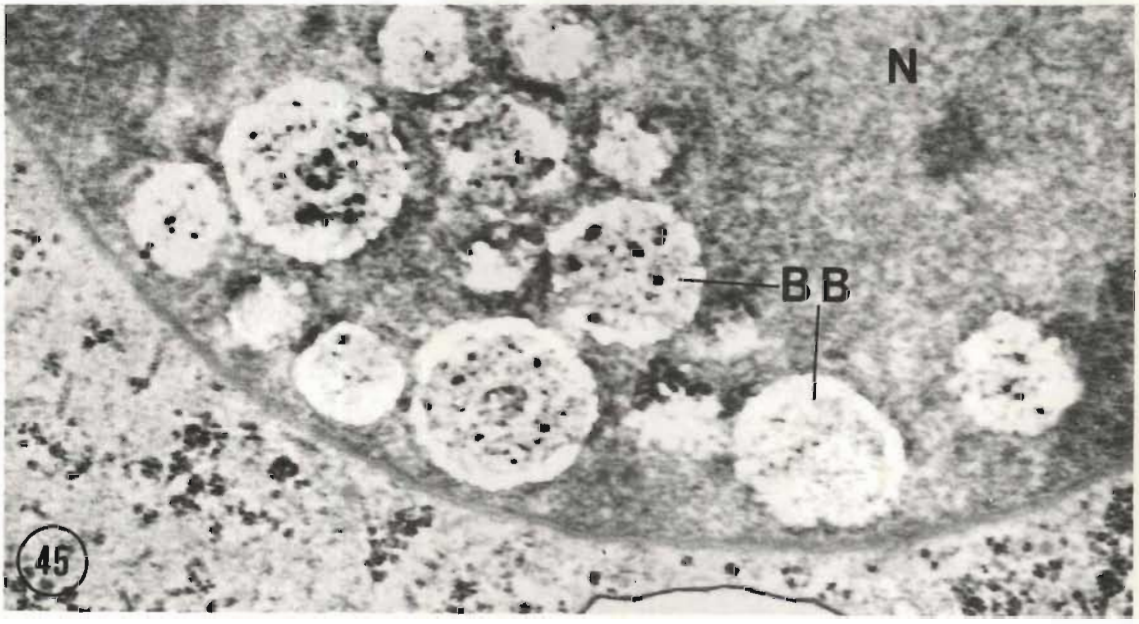
Trophozoites of *E. histolytica* from axenic culture

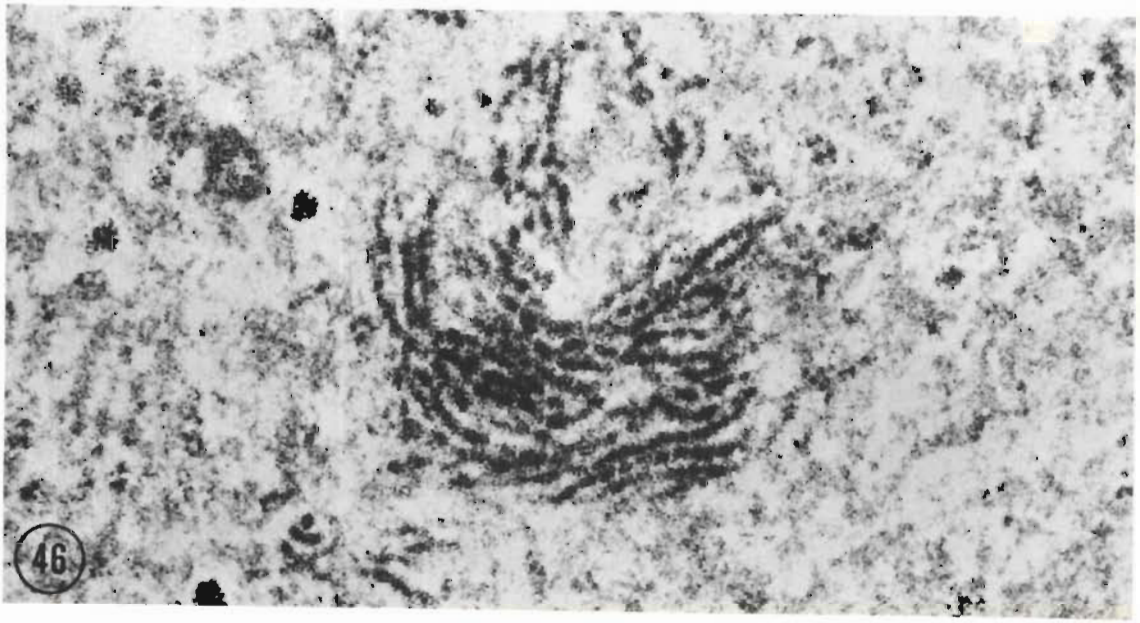
Fig. 44 Shows a nucleus (N) bounded by bundles of ribosomal helices (Rh) and glycogen (Gly). x 12 480

Fig. 45 Shows the electron-dense granules within the button bodies (BB) of the nucleus (N). x 38 480

Fig. 46 Part of the cytoplasm showing the group of beaded elements. 80 600


Rh
N
Rh
Gly
44


N
B
B
45

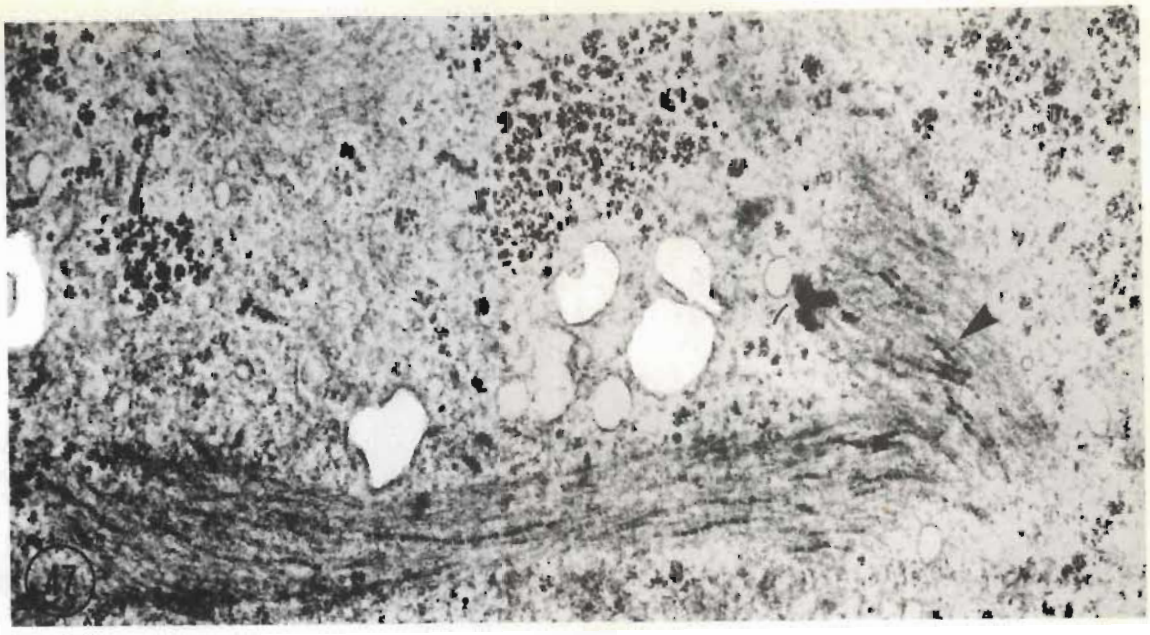

46

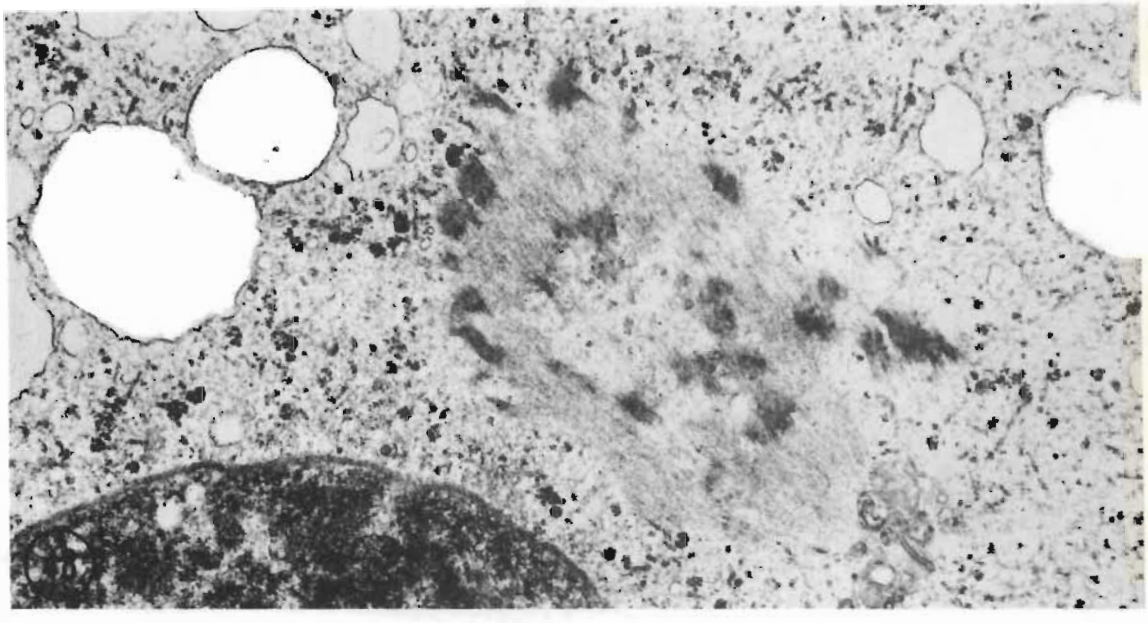
Trophozoites of *E. histolytica* from axenic culture

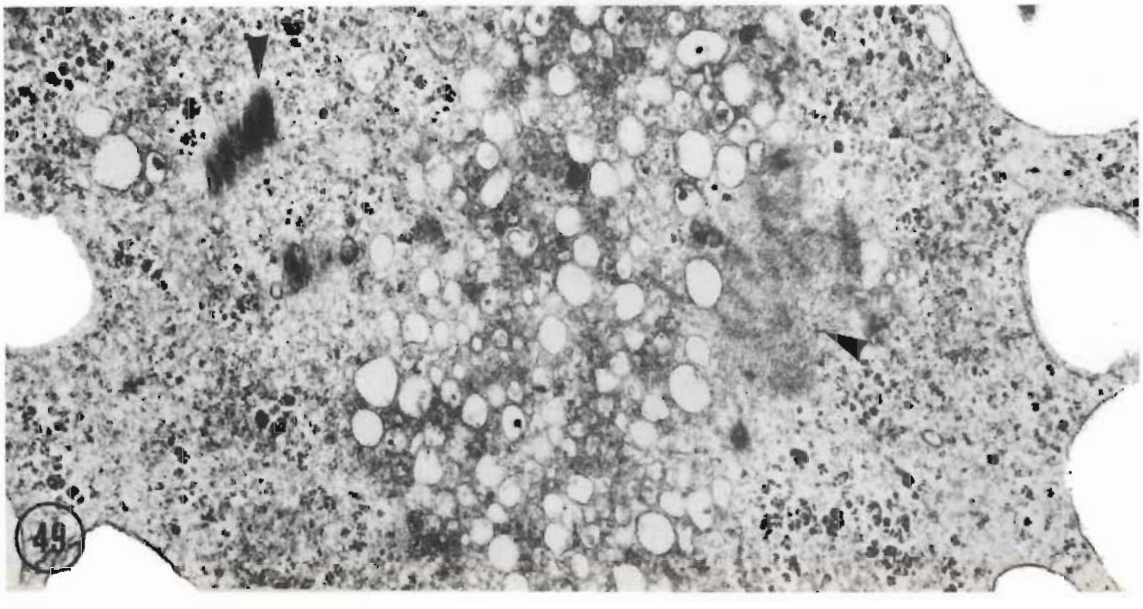
Fig. 47 Shows bundles of fibrous material in close association with beaded elements(arrowhead). x 38 480

Fig. 48 A region of fibrous material interspersed with denser clumps of fibre. x 24 700

Fig. 49 Part of the cytoplasm showing the fibrous regions (arrowheads) in close association with numerous small vacuoles. x 24 700







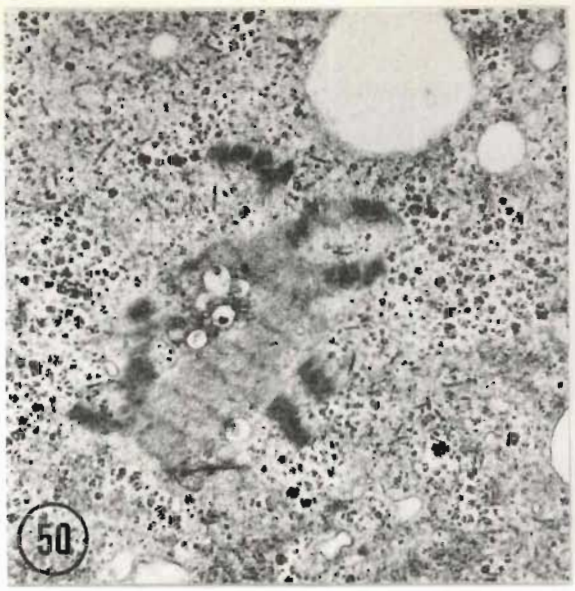
Trophozoites of *E. histolytica* from axenic culture

Fig. 50 Shows a region of fibrous material interspersed with vacuoles. x 17 280

Fig. 51 A fibrillar region (arrowhead) in strain AK presenting a whirl-like pattern in close proximity to vacuoles and electron-dense bodies (E). x 17 100

Fig. 52 Part of the cytoplasm of strain HALA showing the electron-dense bodies (E) in close association with strands of membranous material. x 33 250

Fig. 53 An area of extensive membrane formation in close association with vacuoles. x 33 250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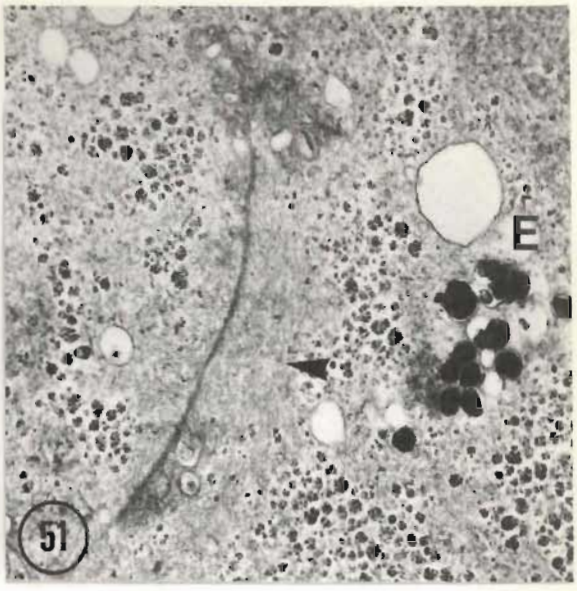
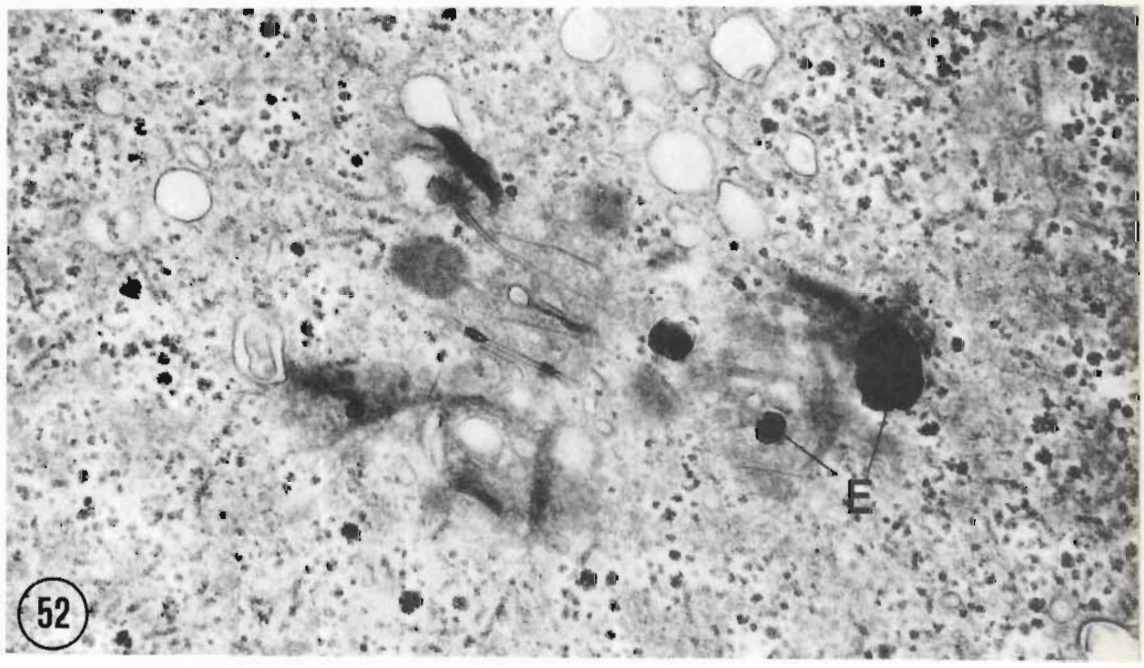
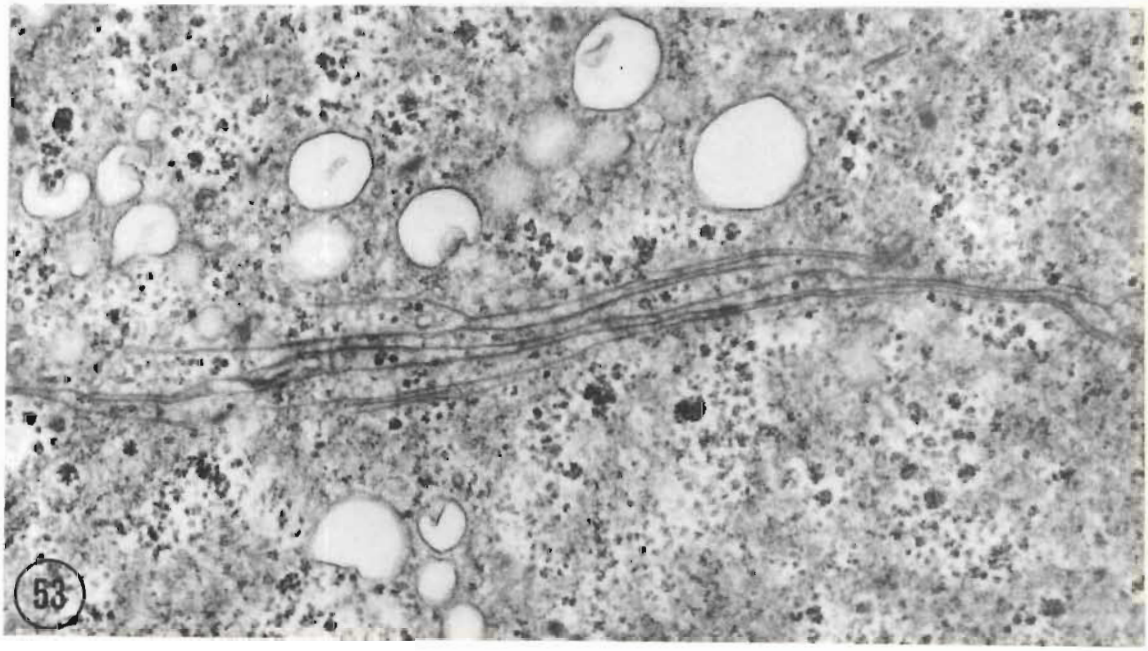
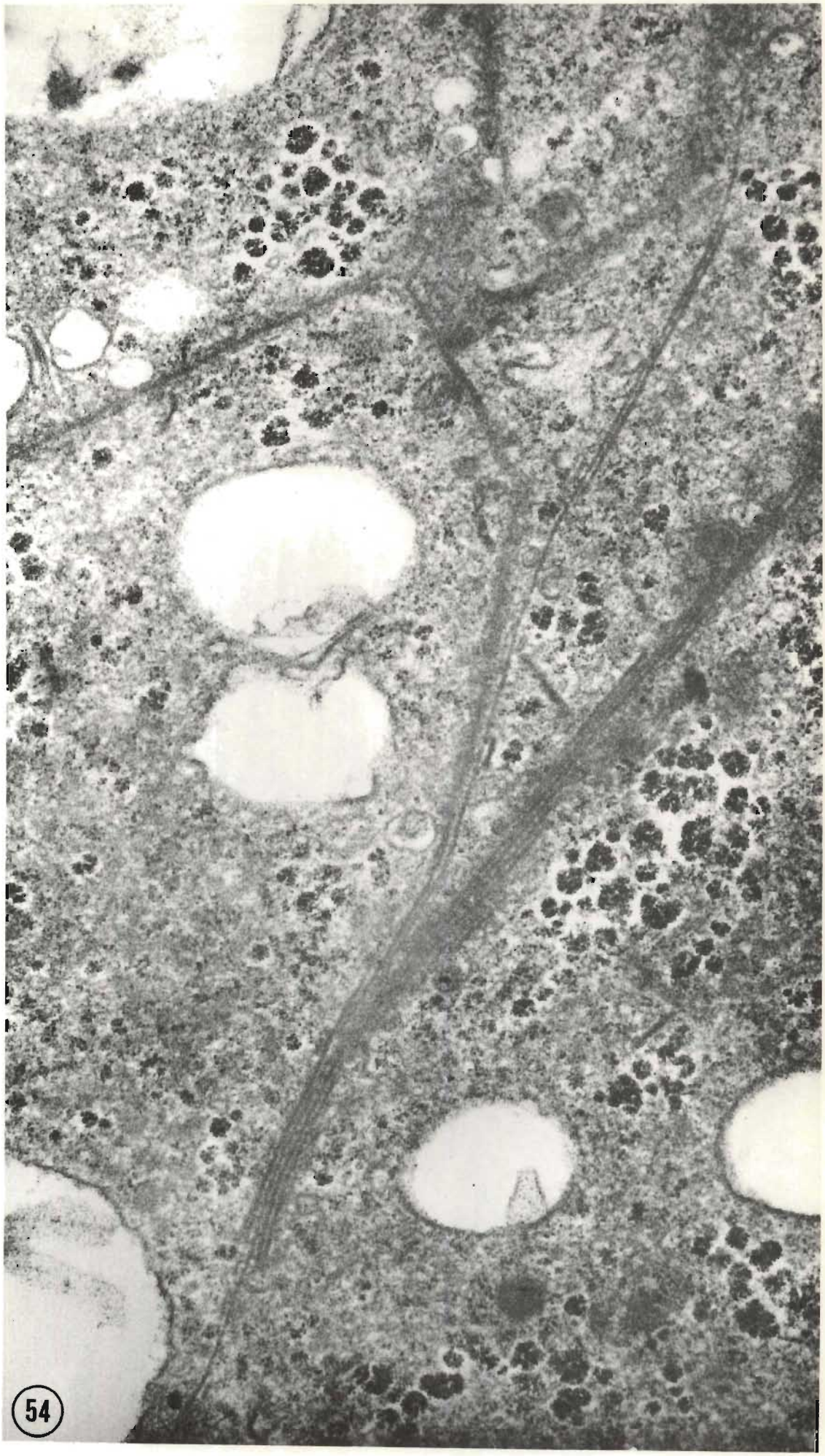

E
51


E
52


53

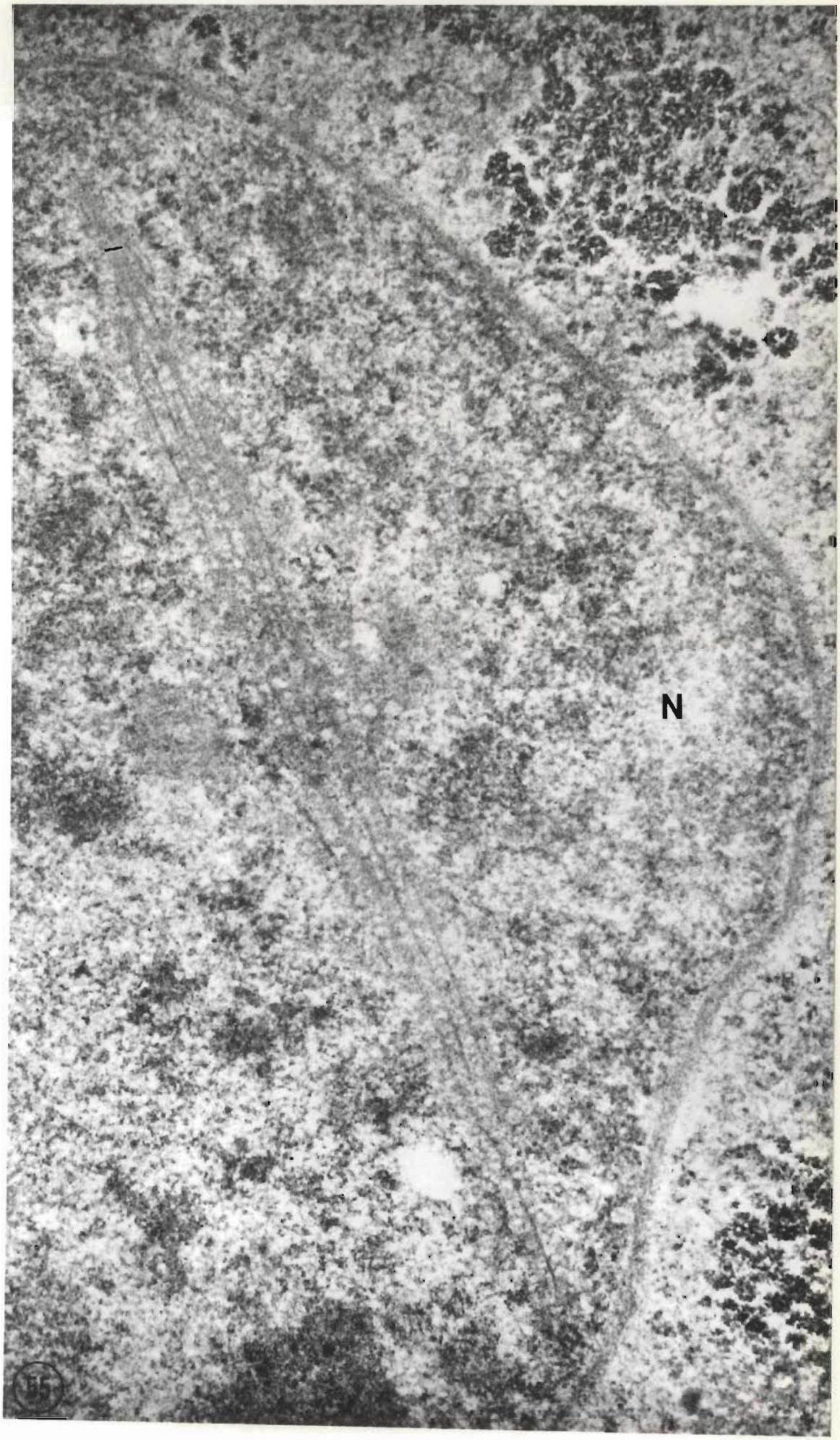
Trophozoites of *E. histolytica* from axenic culture

Fig. 54 Part of the cytoplasm of a trophozoite of strain AK showing the banding of fibres. x 59 200


54

Trophozoites of *E. histolytica* from axenic culture

Fig. 55 Part of a nucleus (N) of a trophozoite of strain AK showing the spindle-like fibres tapering towards each end. x 73 600


N

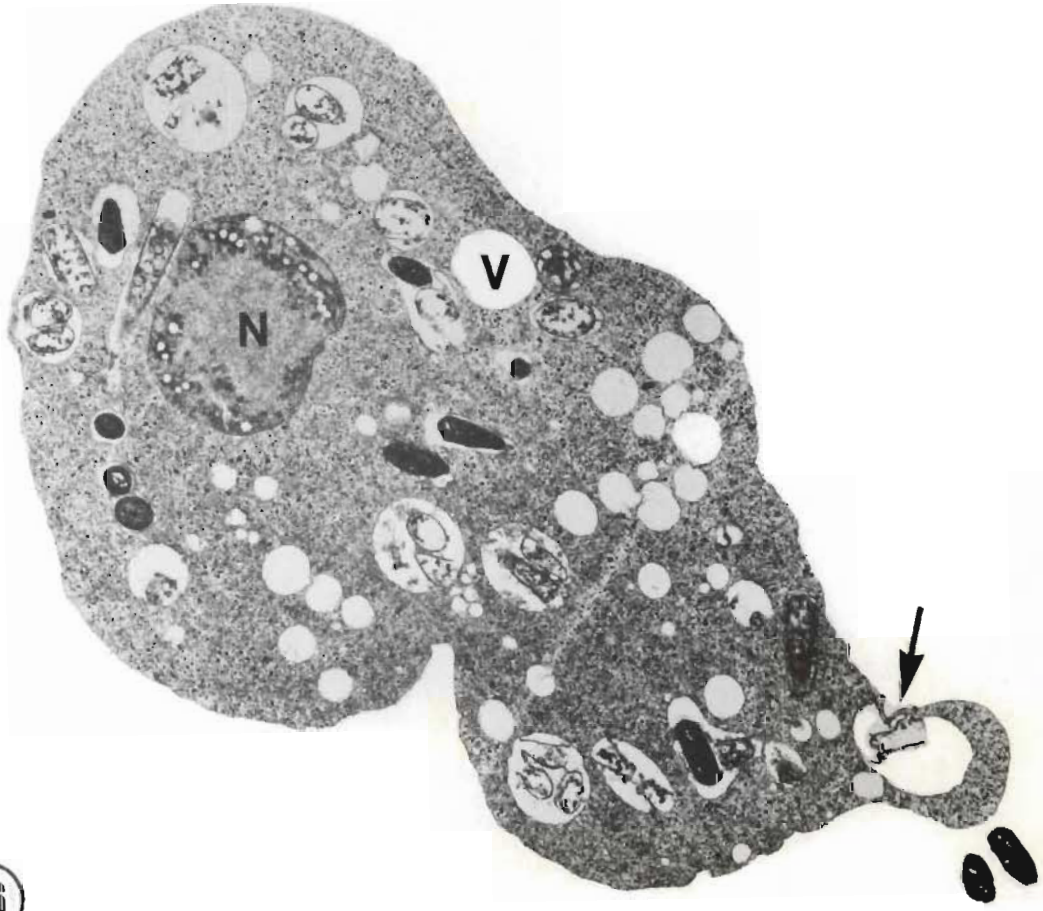
Trophozoites of *E. histolytica* from monoxenic culture

Fig. 56 A trophozoite showing a nucleus (N) numerous vacuoles (V) and the commencement of convolution of a lysosome-like vacuole (arrowed). x 5 340

Fig. 57 Crescent-shaped vacuoles observed below the plasma membrane of axenic trophozoites of strain NIH 200. x 25 500

Fig. 58 Part of a trophozoite of strain NIH 200 grown in association with bacteria showing the commencement of convolution of the lysosomal vacuole (L). x 14 400

Fig. 59 Shows the final stage of convolution of the lysosomal vacuole (L) and the formation of the trigger mechanism (T). x 42 320



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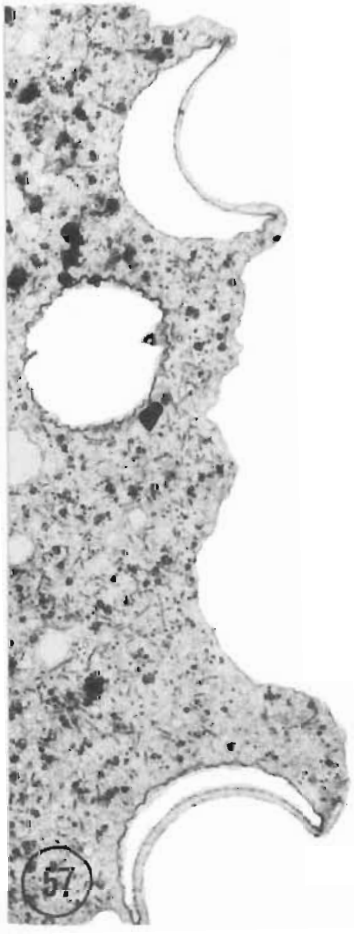

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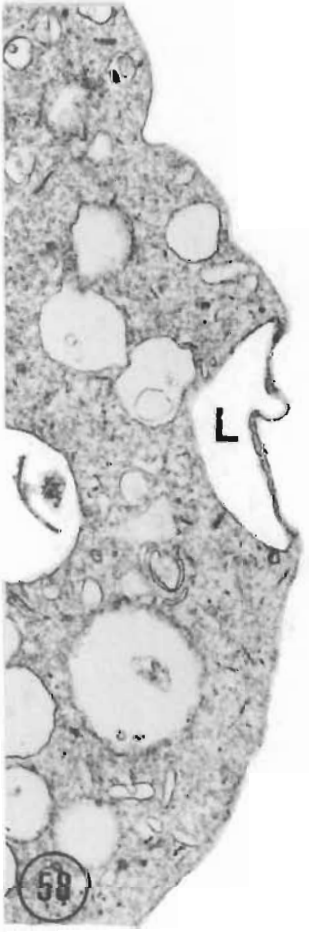

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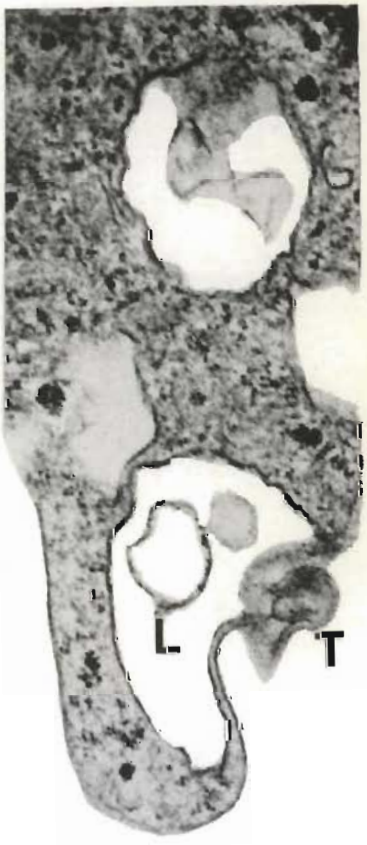


59

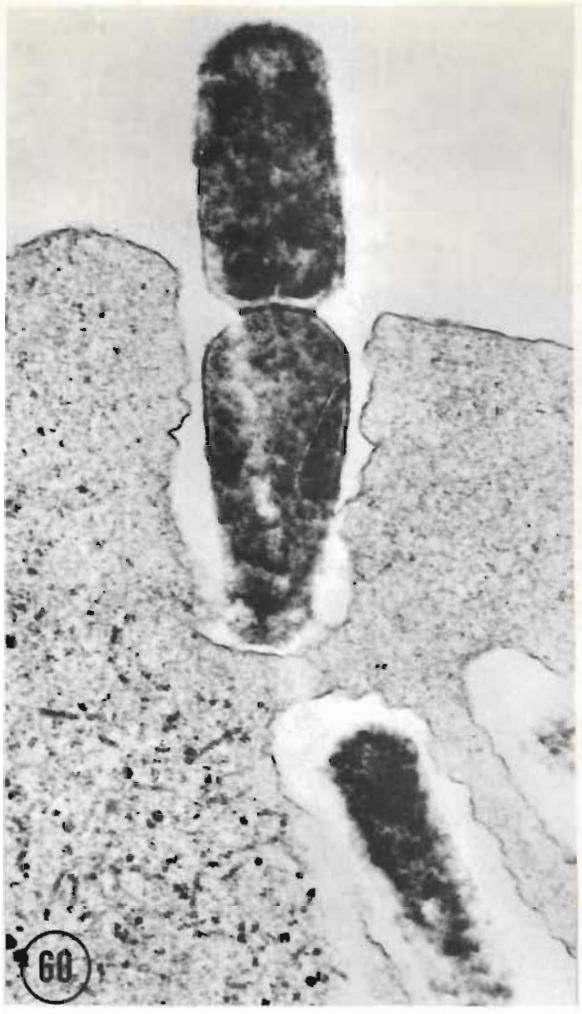
Trophozoites of *E. histolytica* from monoxenic culture

Fig. 60 Shows the commencement of the ingestion of bacteria. x 29 450

Fig. 61 Shows the pseudopodia (Ps) surrounding a bacterium. x 29 450

Fig. 62 Shows a bacterium within a vacuole formed by the process of phagocytosis. x 29 450

Fig. 63 Part of a trophozoite showing the phagocollar (Ph). x 14 880



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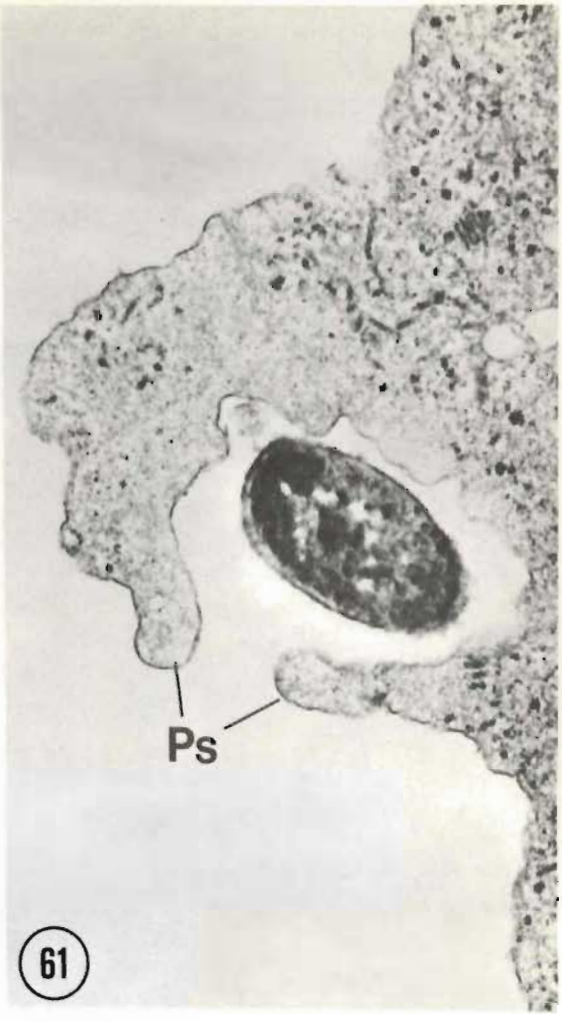

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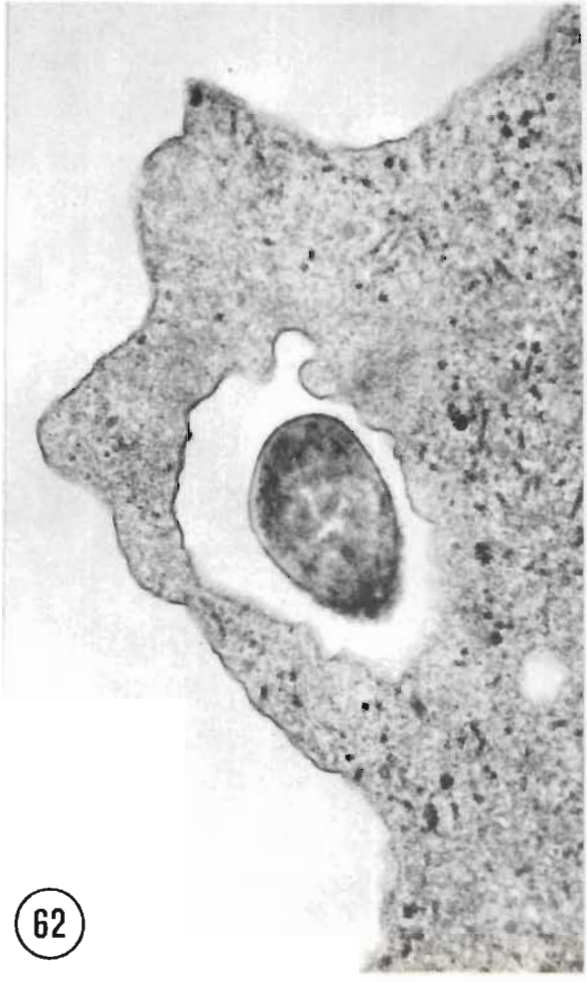

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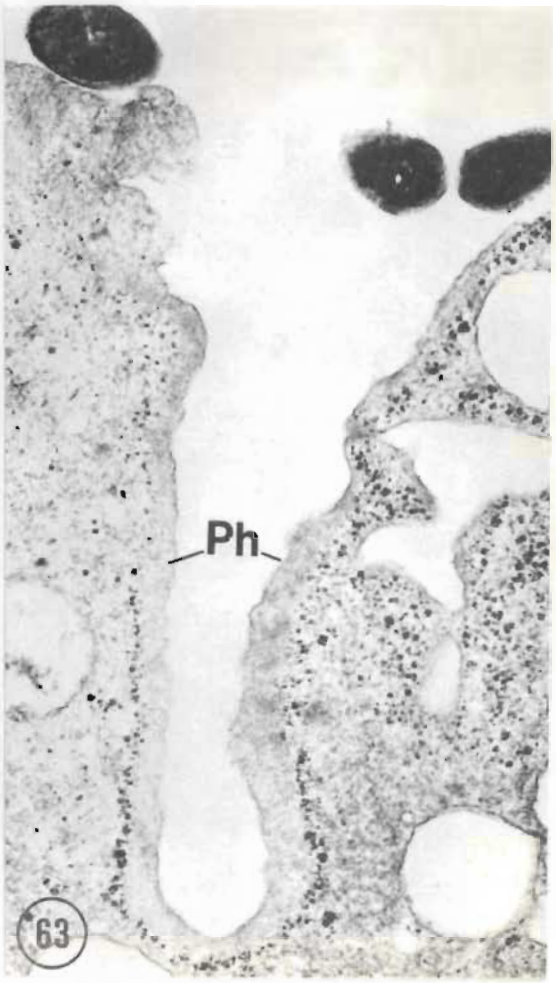
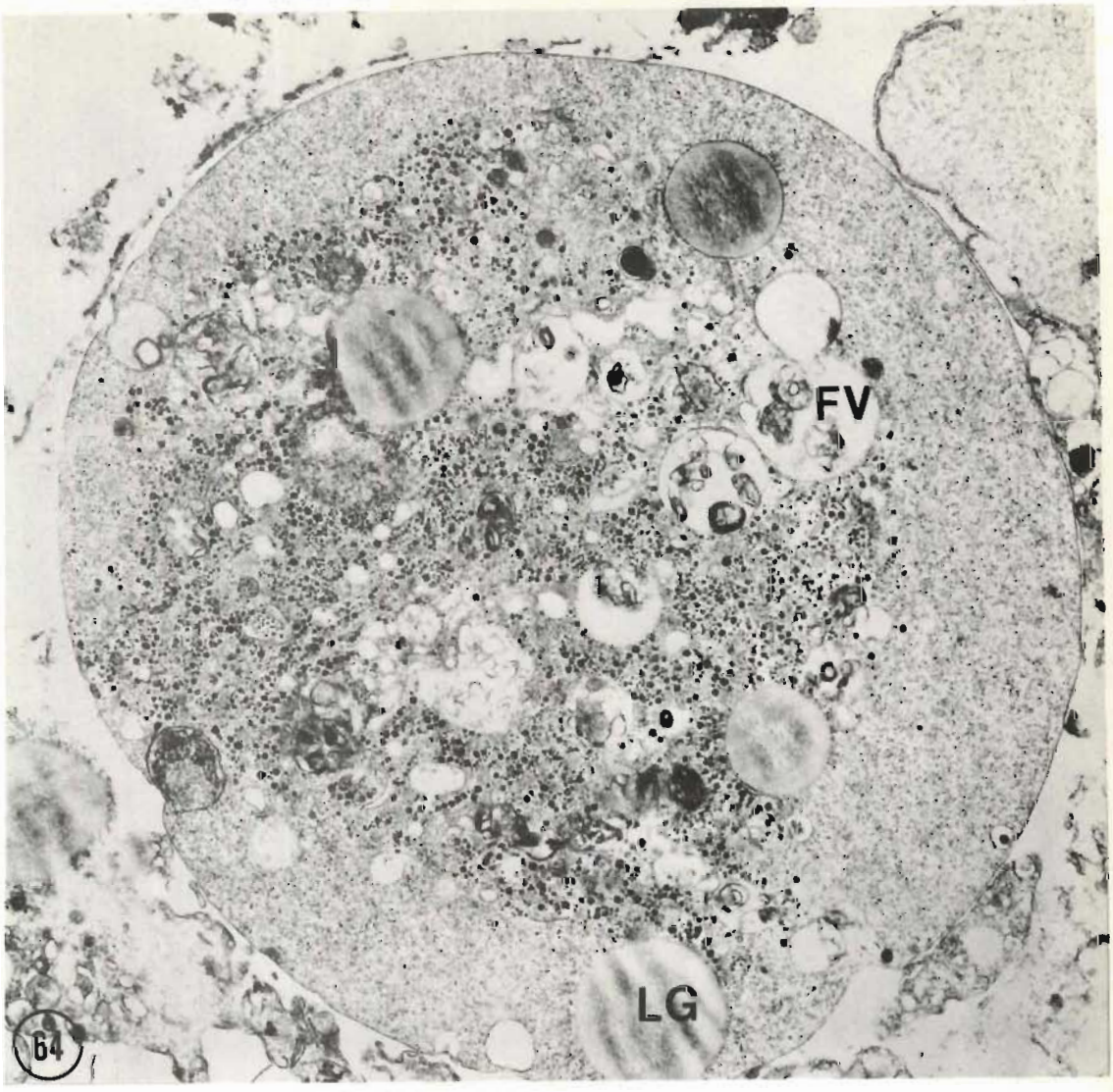


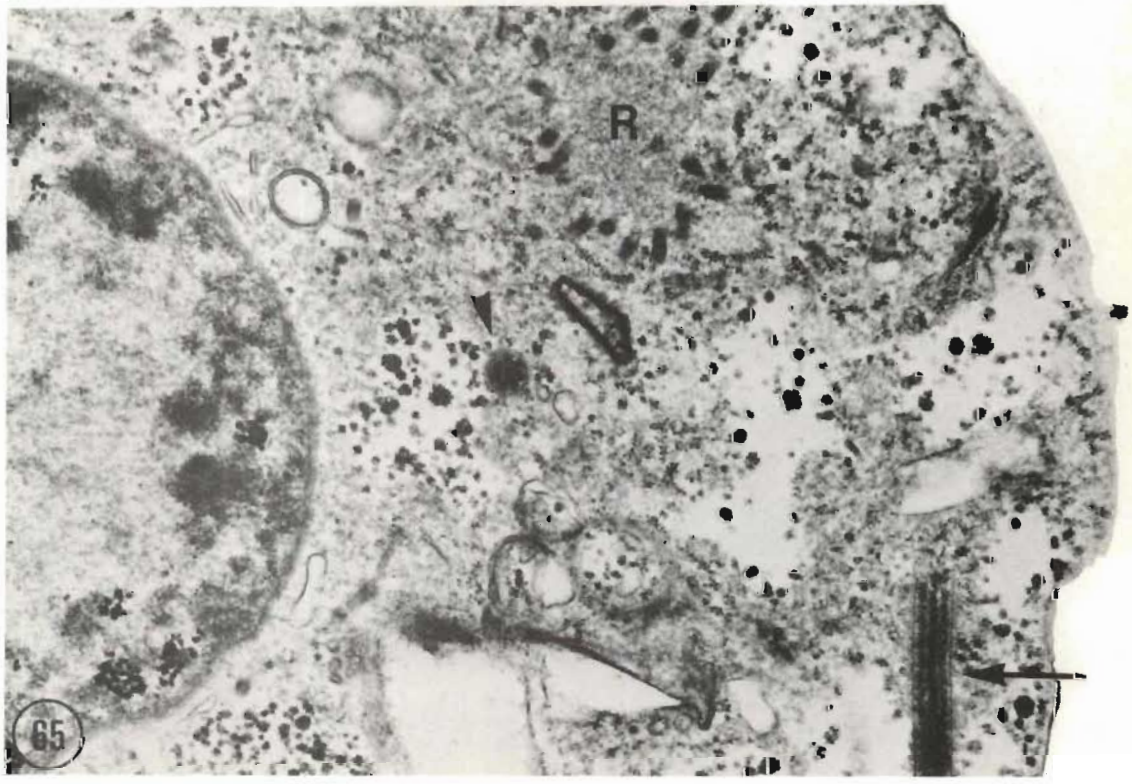
63

Trophozoites of *E. histolytica* from monoxenic culture

Fig. 64 A trophozoite from locke-egg culture showing numerous vacuoles containing membranous fragments (FV) and lipid globules (LG). x 10 800

Fig. 65 Part of a trophozoite of strain N/68 showing the rosette of electron-dense bodies (R), small particles bounded by a membrane (arrowhead) and the parallel lamellae between membranes (arrowed). x 35 380


FV
LG
64

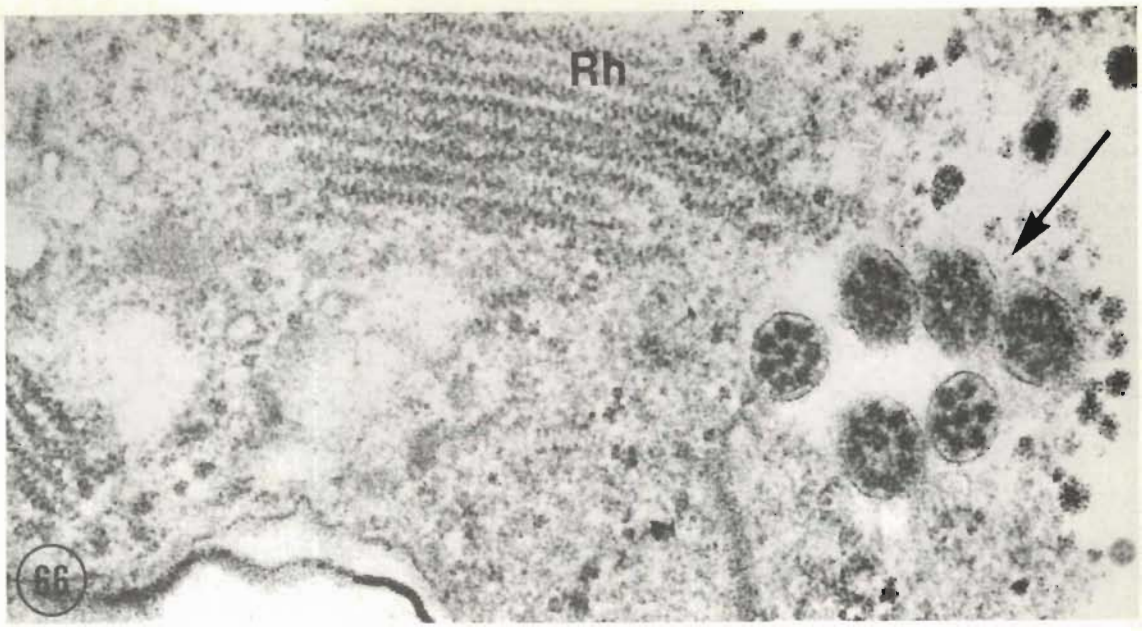

R
65

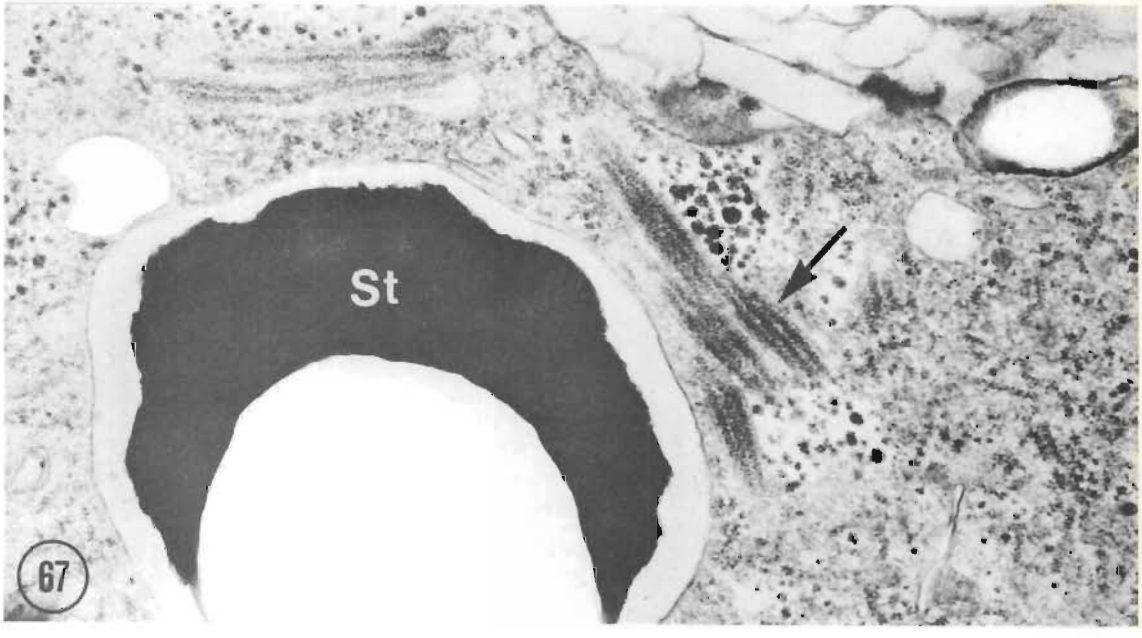
Trophozoites of *E. histolytica* from monoxenic culture

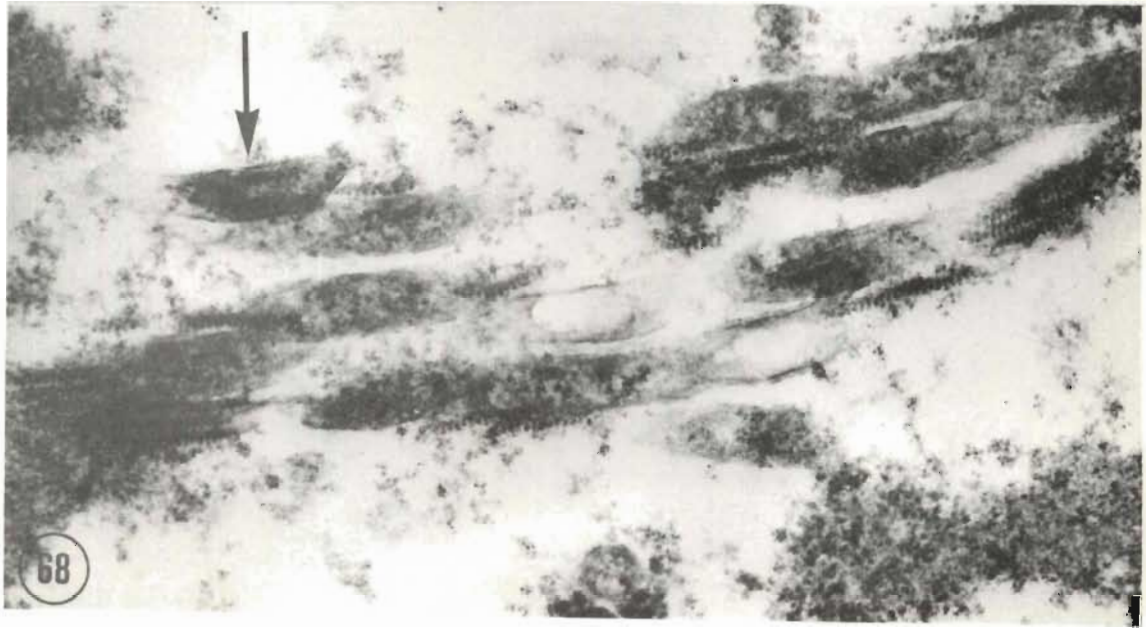
Fig. 66 Shows a group of structures (arrowed) each of which comprises six or seven particles bounded by a membrane, in close proximity to a bundle of ribosomal helices (Rh). x 89 900

Fig. 67 Part of a trophozoite of strain N/68 showing the coiled fibrils between membranes (arrowed) in close proximity to a vacuole containing a starch granule (St). x 43 500

Fig. 68 Shows the coiled fibrils between membranes (arrowed) in the cytoplasm of strain AX/68. x 64 380


Rh
66

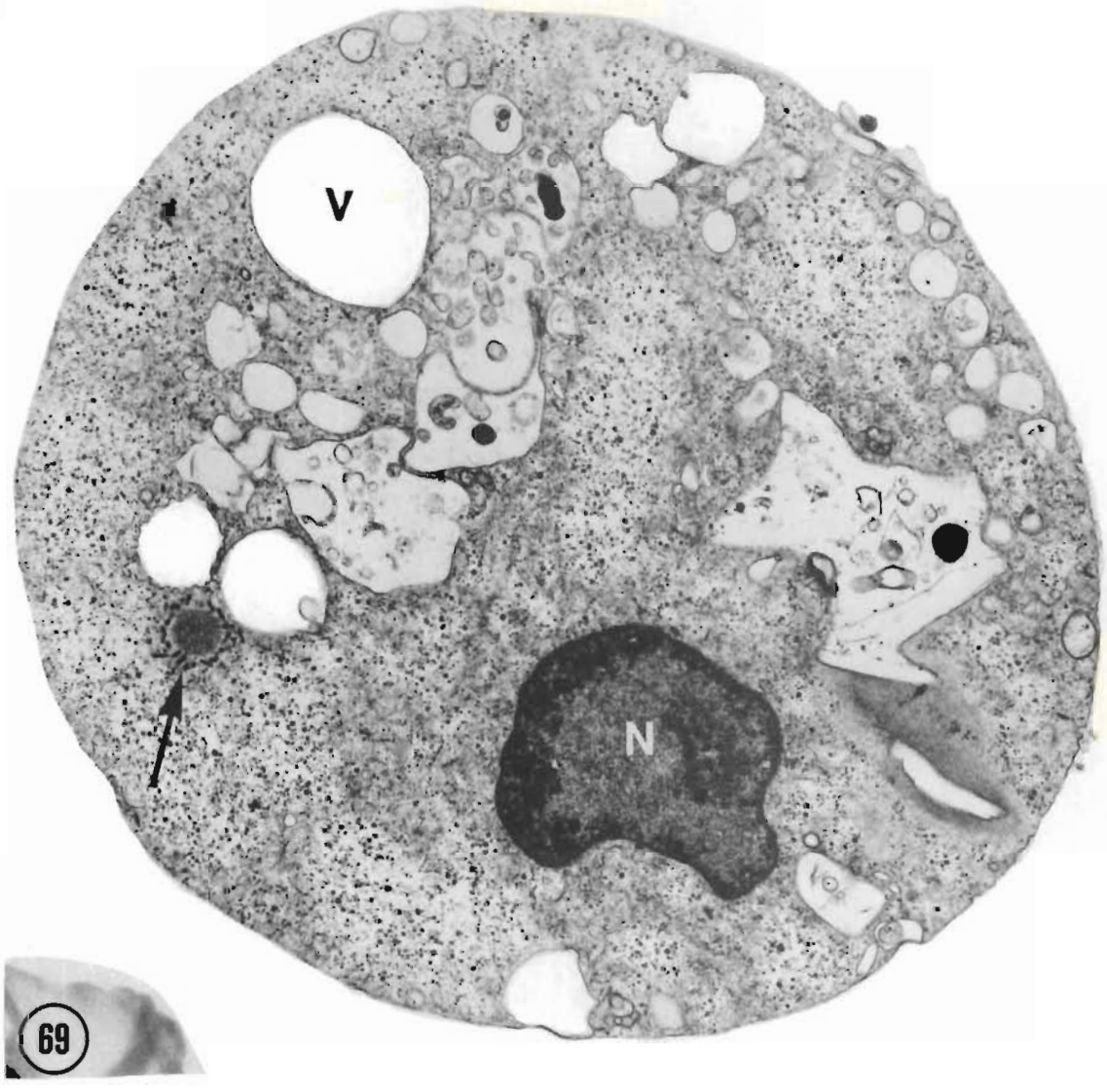

St
67


68

Trophozoites of *E. histolytica* from monoxenic culture

Fig. 69 A trophozoite of strain AX/68 showing a rosette of electron-dense cylindrical bodies (arrowed) numerous vacuoles (V) and the nucleus (N). 9 600

Fig. 70 A region of extensive membrane formation in concentric whorls (arrowed) and in parallel arrays (arrowheads) in the cytoplasm of strain HALA/68. x 93 000



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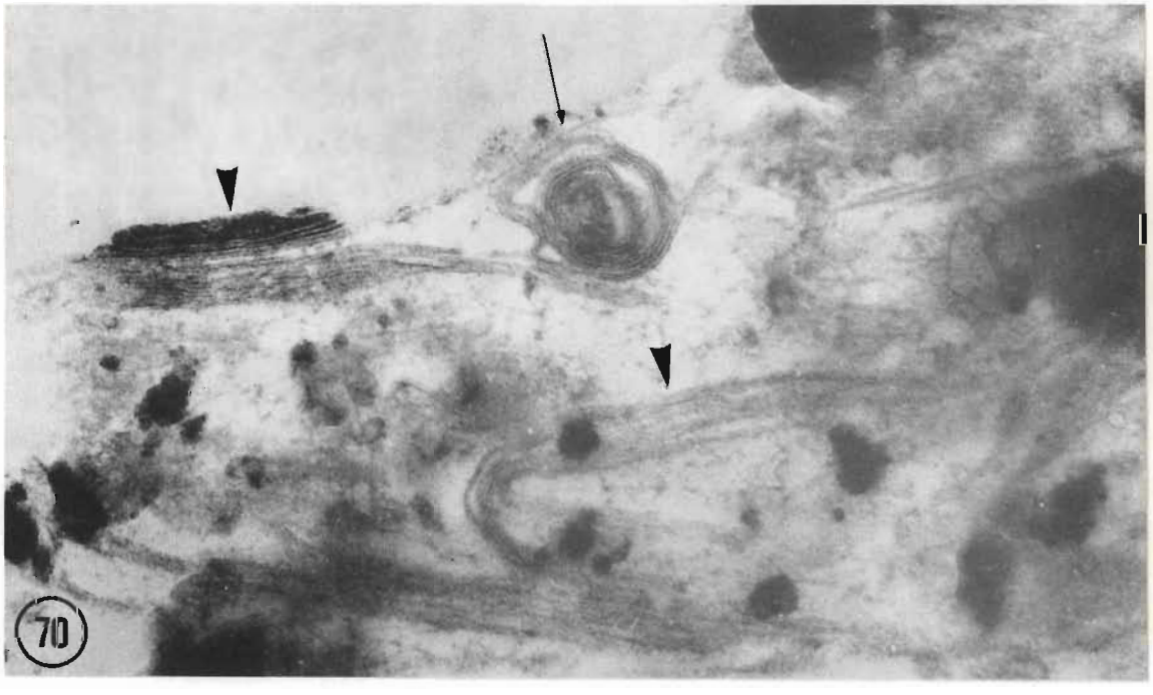
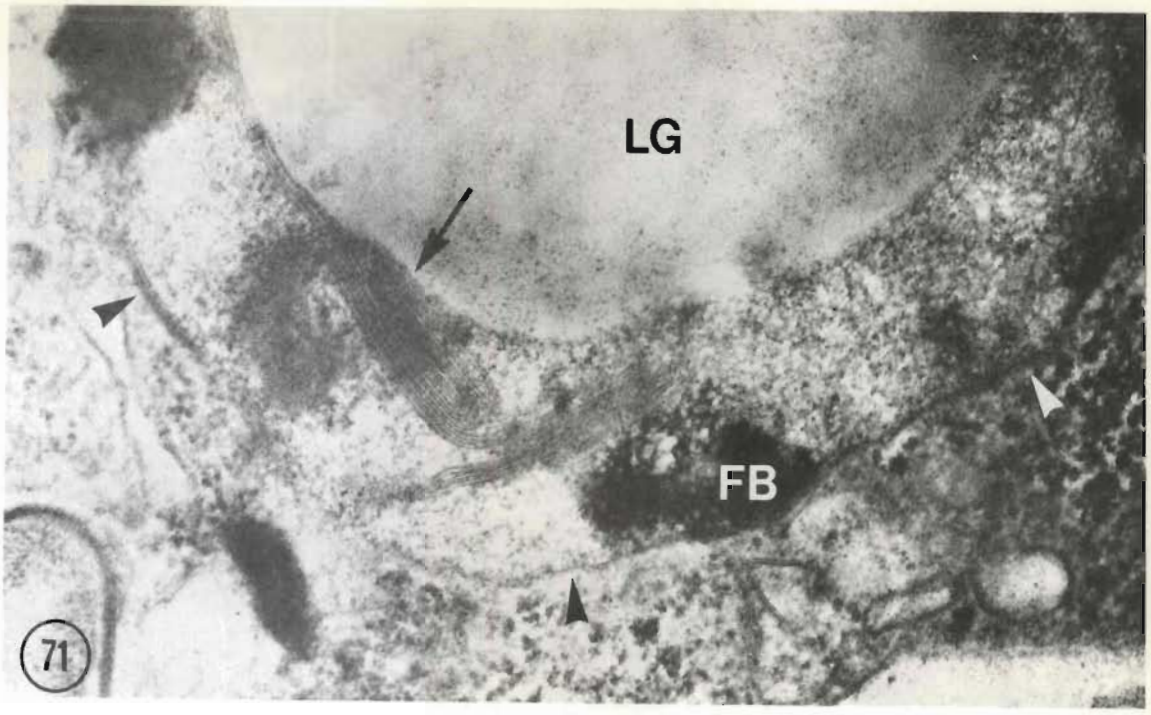


70

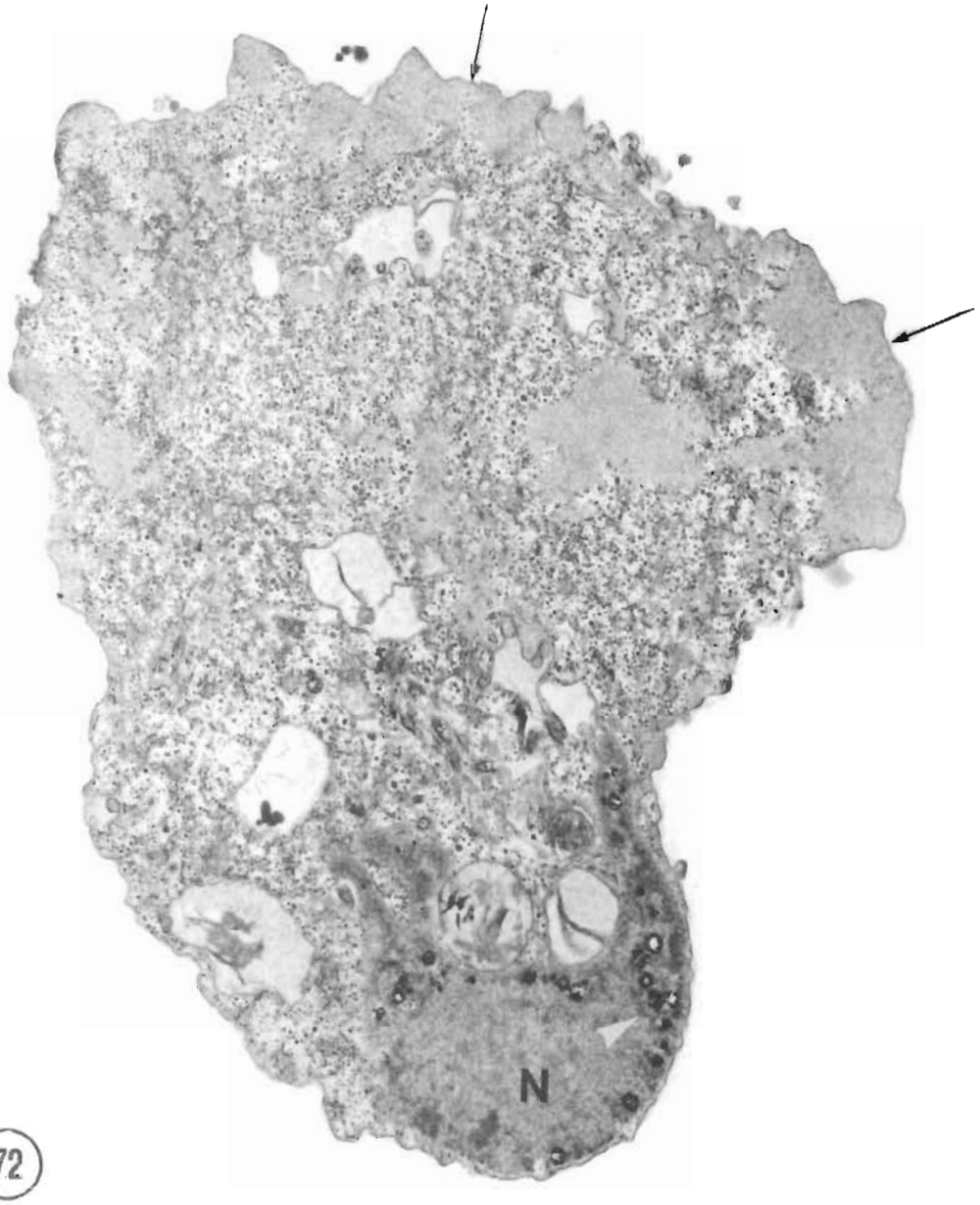
Trophozoites of *E. histolytica* from monoxenic culture

Fig. 71 Shows the foamy electron-dense body (FB) the extensive membrane formation (arrowed) and a lipid globule (LG) all within a digestive vacuole, the membrane of which is indicated by arrowheads. x 78 000

Fig. 72 A trophozoite of strain AK/68 showing the numerous fibrillar regions (arrowed) and a nucleus (N) containing button bodies (arrowhead). x 11 530



71

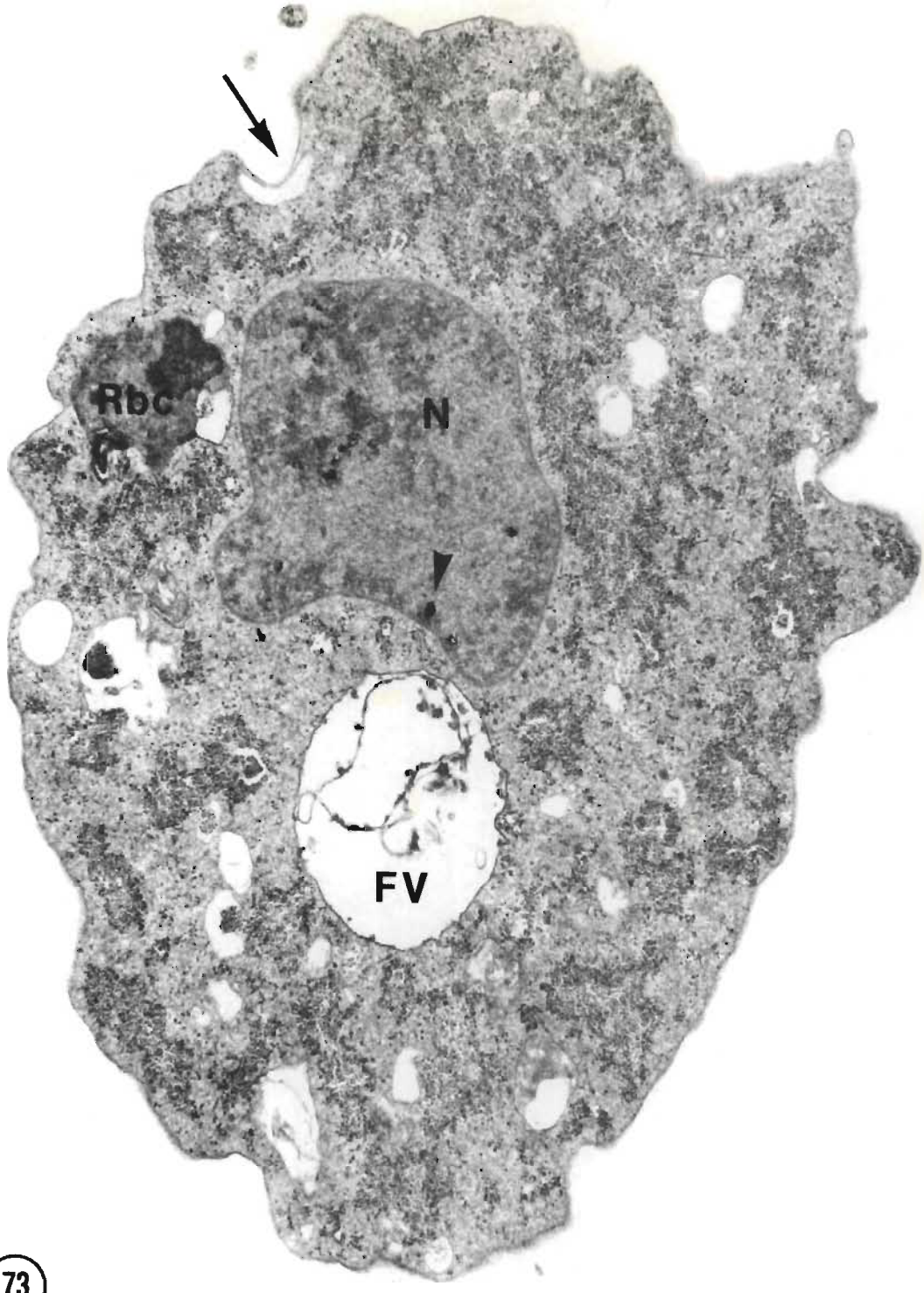


72

Trophozoites of *E. histolytica* from the human colon

Fig. 73 A trophozoite showing the nucleus (N) with its button bodies (arrowhead) and vacuoles containing partially digested food material (FV) and red blood cells (Rbc). Arrow denotes possible site of partially sectioned surface lysosome. x 7 128

Fig. 74 Shows the 'fuzzy' coat on the outer surface of the plasmalemma. (Figure 148 shows the smooth plasma membrane of trophozoites of *E. coli*). x 117 800



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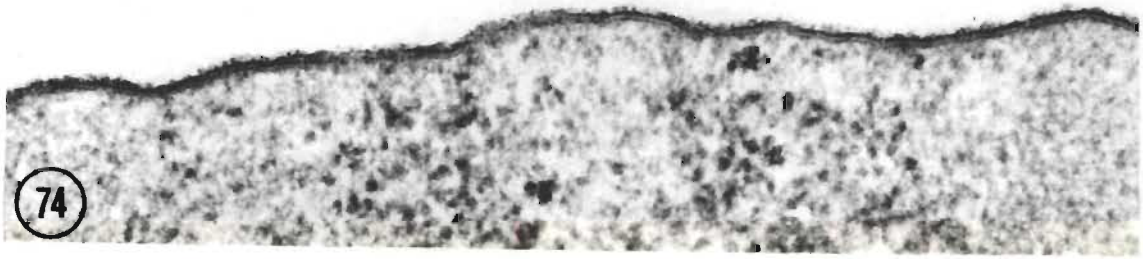
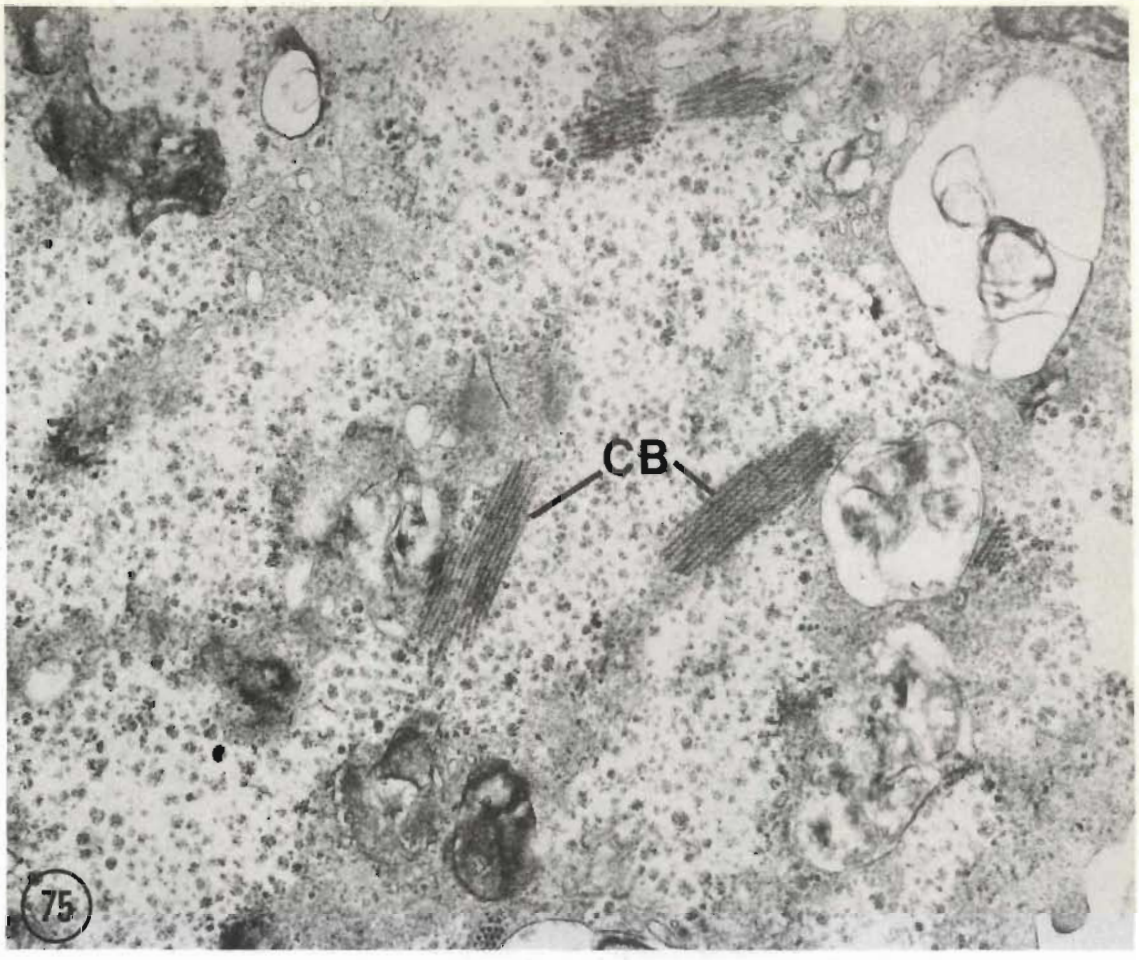


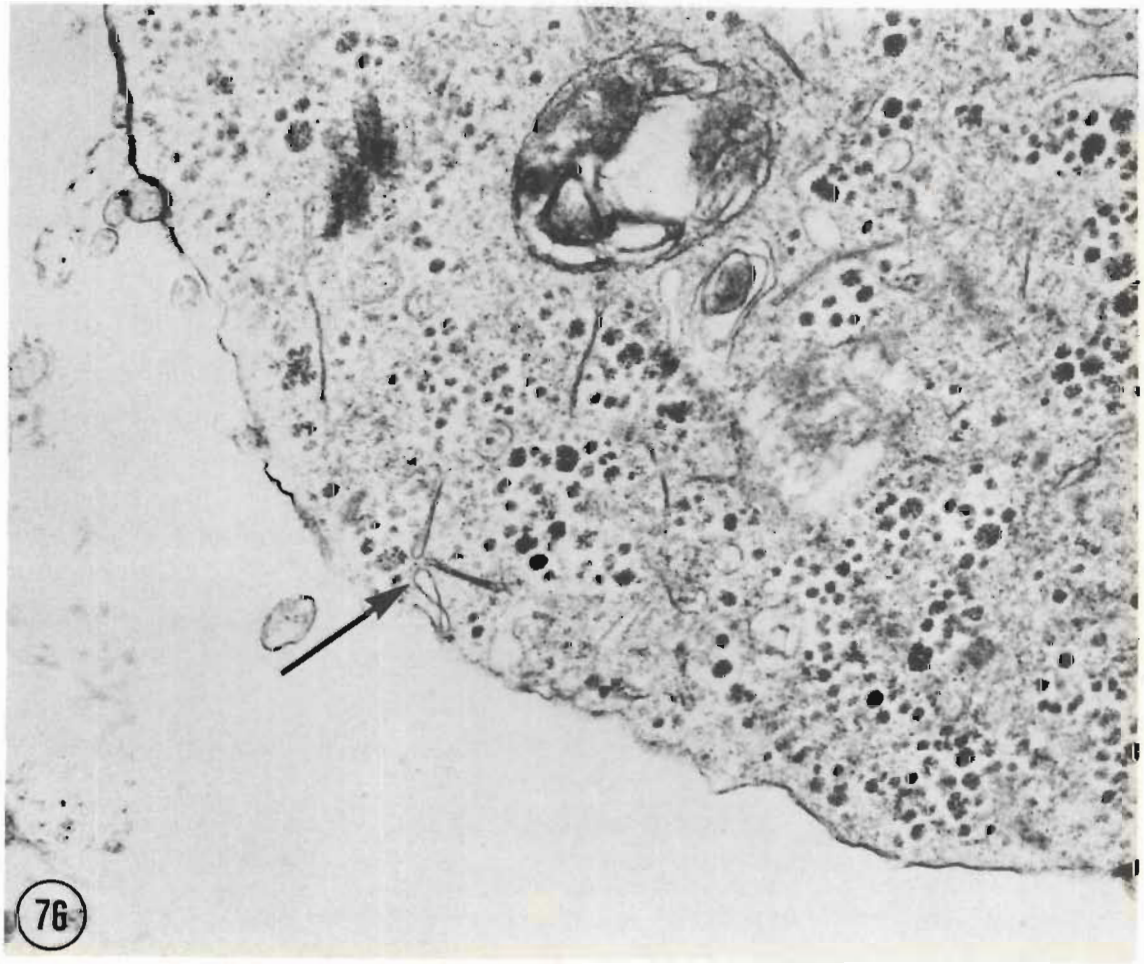
74

Trophozoites of *E. histolytica* from the human colon

Fig. 75 Part of the cytoplasm showing numerous crystalloid aggregates (CB). x 18 200

Fig. 76 Shows the 'hairclip-like' arrangements of smooth endoplasmic reticulum (arrowed). x 29 640


CB
75

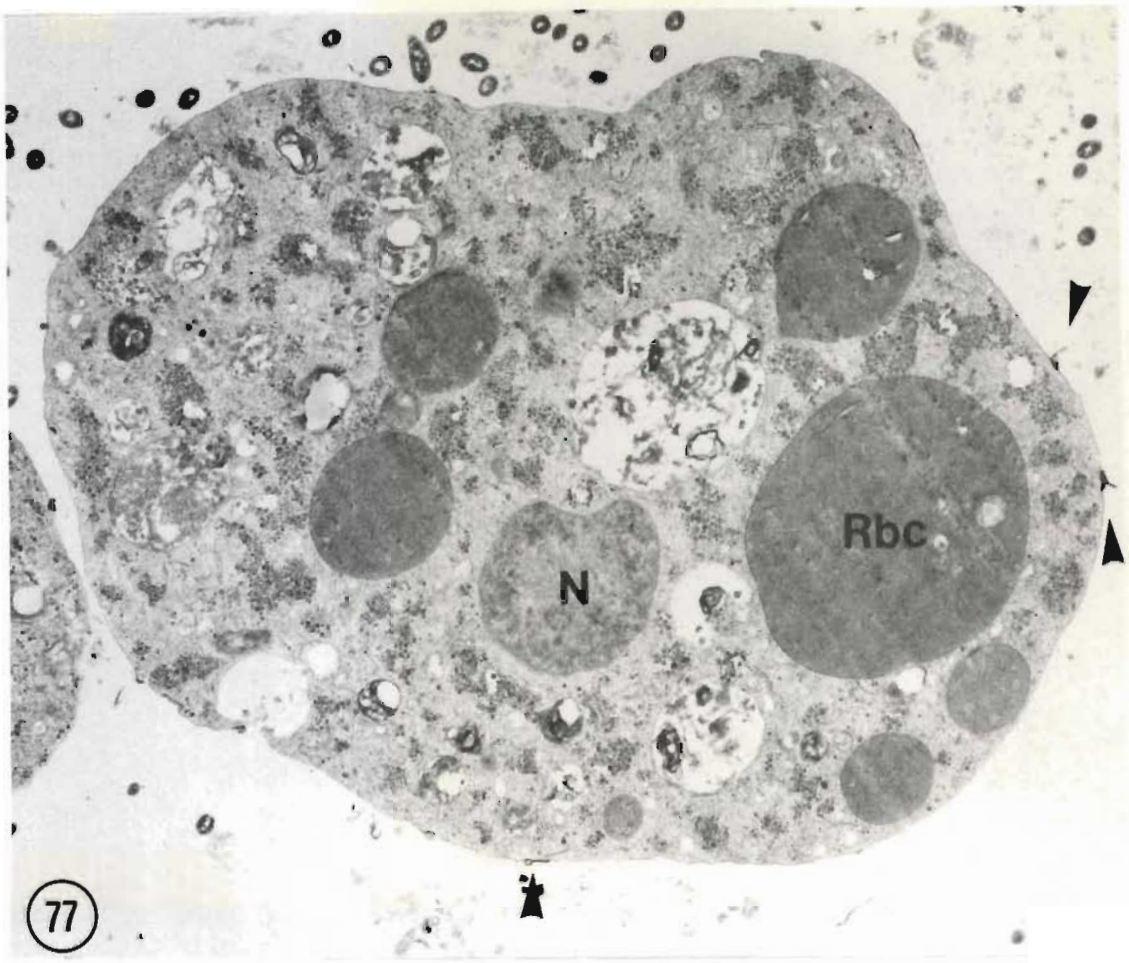

76

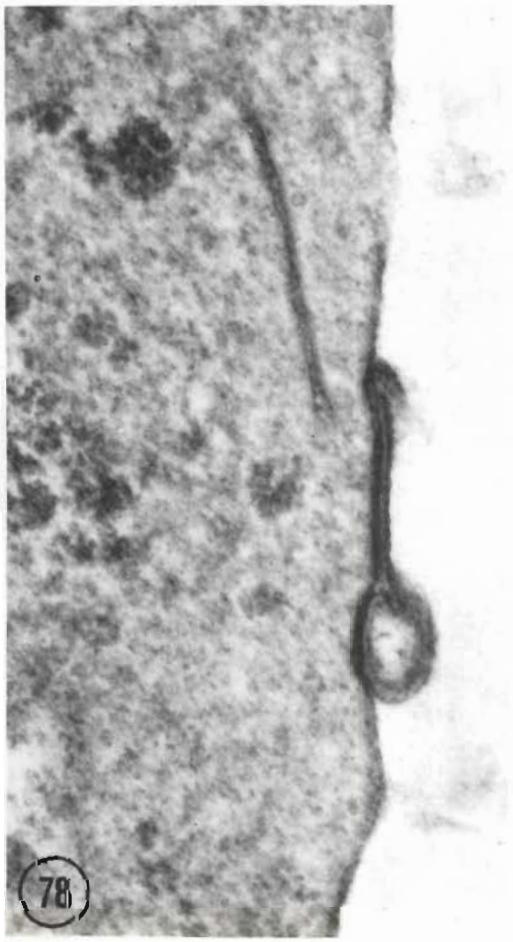
Trophozoites of *E. histolytica* from the human colon

Fig. 77 A trophozoite showing the hairclip-like structures on the outer surface of the cell (arrowheads). Within the cytoplasm are several red blood cells (Rbc) in different stages of digestion and a nucleus (N). x 7 128

Fig. 78 Shows the hairclip-like structure lying parallel to the surface of the cell. x 97 920

Fig. 79 Shows a hairclip-like structure at right angles to the surface of the cell. Note the electron-dense region at the base of the structure. x 45 600


Rbc
N
77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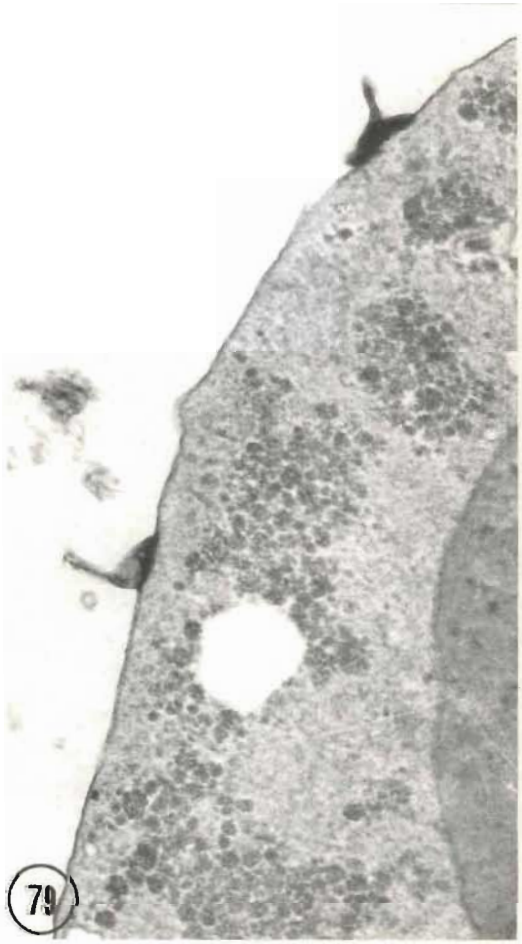
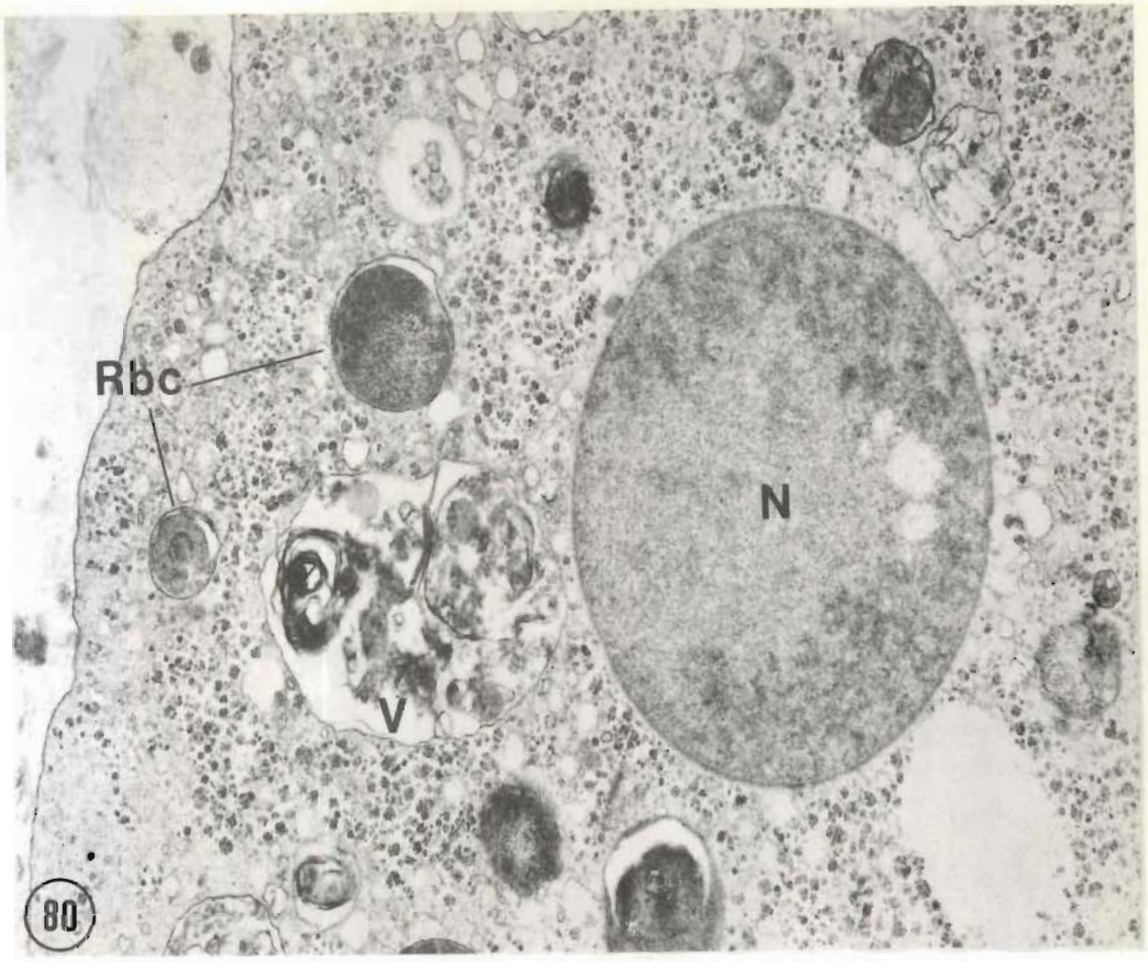

79

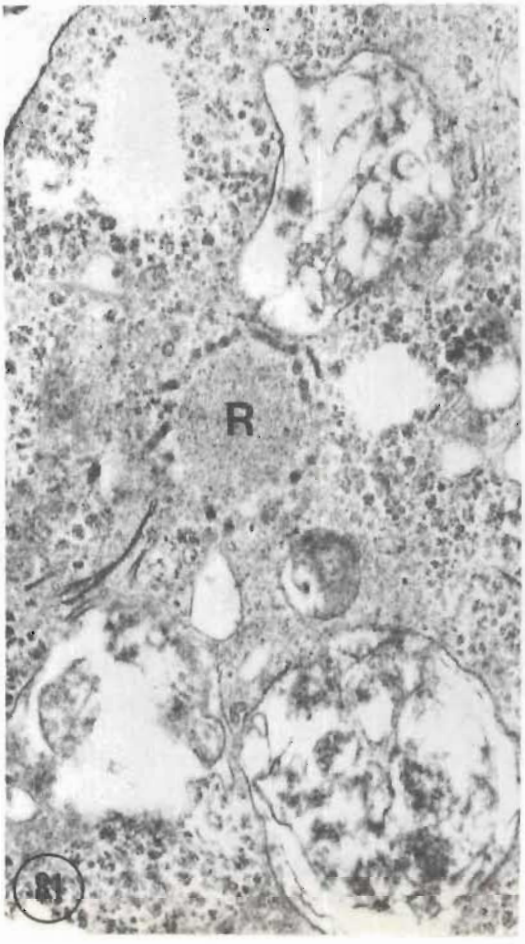
Trophozoites of *E. histolytica* from the human colon

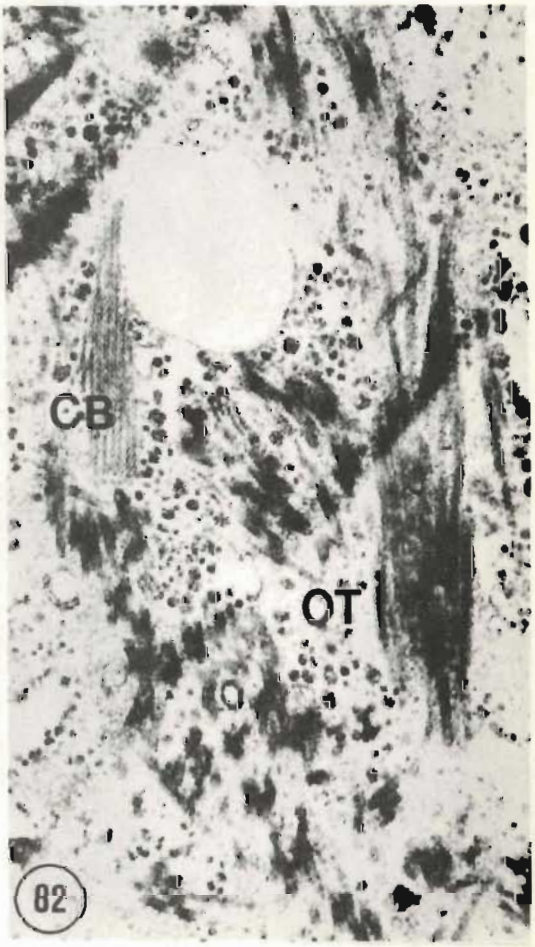
Fig. 80 Part of the cytoplasm containing a nucleus (N) particulate matter within vacuoles (V) and erythrocytes (Rbc) in different stages of digestion. x 15 500

Fig. 81 Shows the electron-dense cylindrical bodies in a rosette arrangement (R). x 19 840

Fig. 82 Part of an amoeba showing the bundles of osmiophilic tubules (OT) and a crystalloid aggregate (CB). x 19 600


Rbc
N
V
80

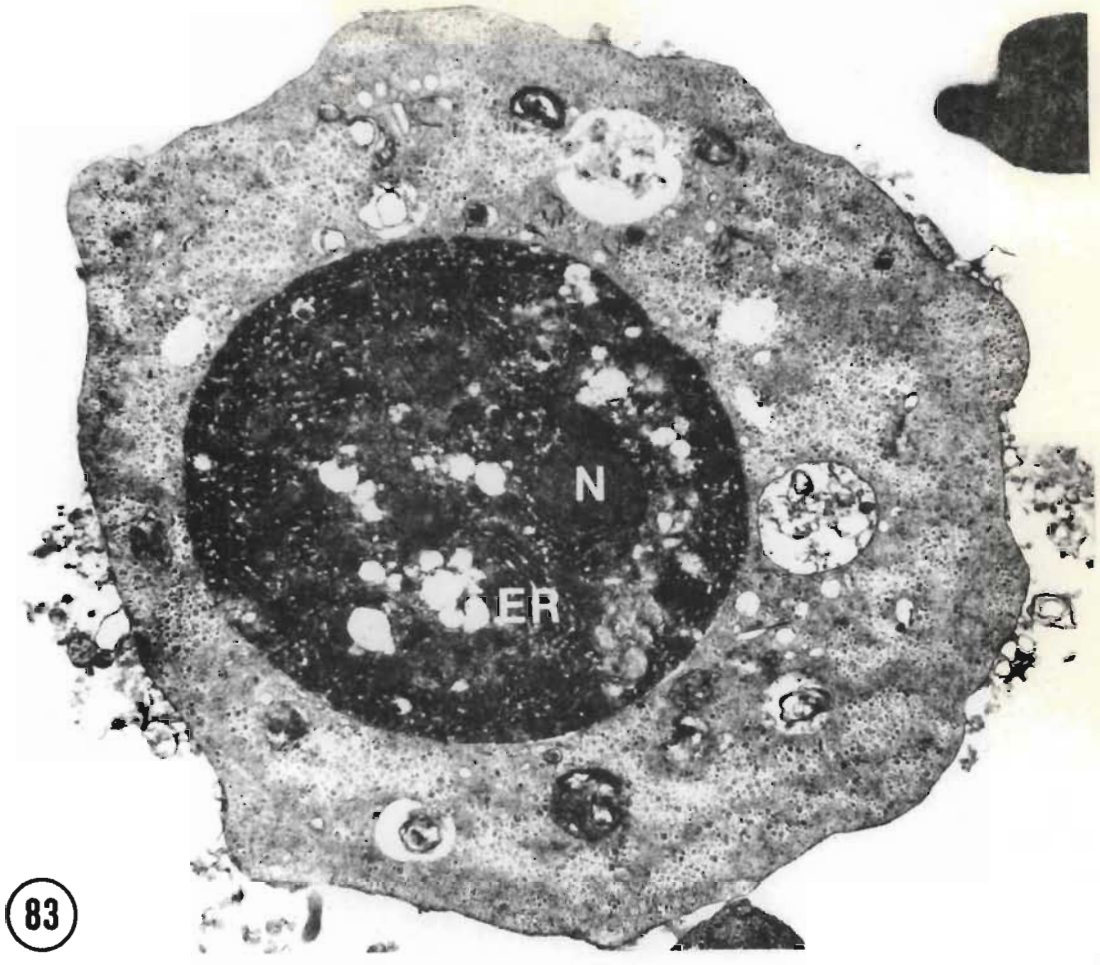

R
81


CB
OT
82

Trophozoites of *E. histolytica* from the human colon

Fig. 83 A trophozoite containing a phagocytized white cell. Note the nucleus (N) and endoplasmic reticulum (ER) clearly visible within the ingested cell. x 6 240

Fig. 84 Section through a surface-active lysosome showing the trigger mechanism (T) and the electron-dense granular mass (D). x 85 840



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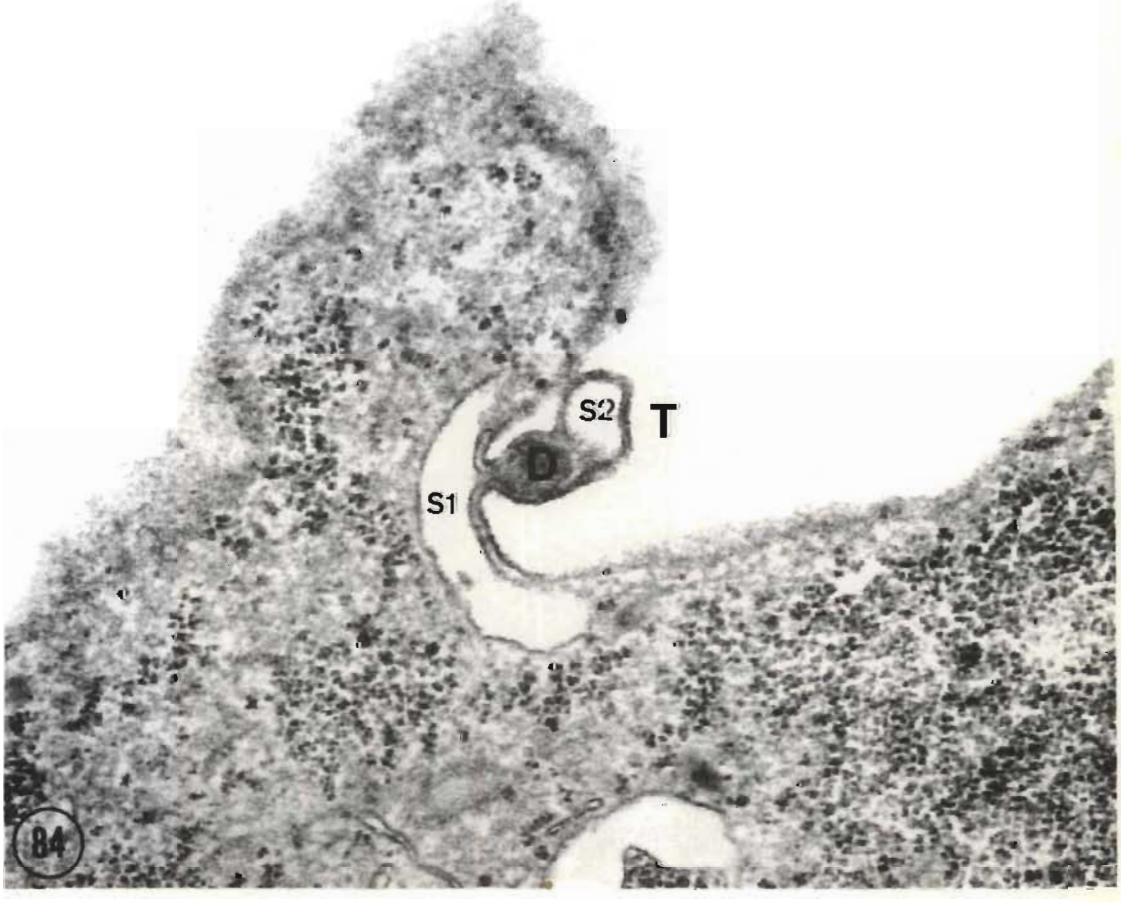
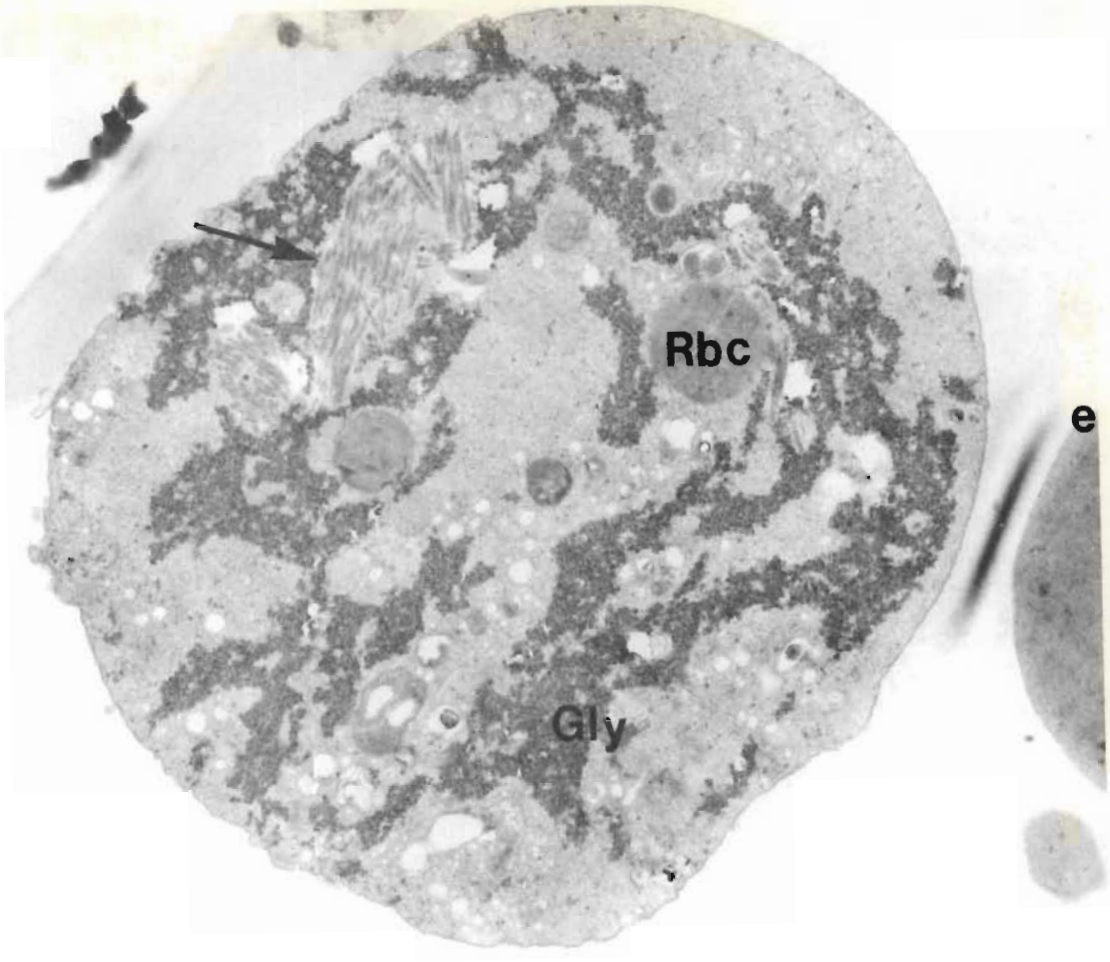


84

Trophozoites of *E. histolytica* from the human colon

Fig. 85 A trophozoite from strain AS 6 showing the glycogen arranged in clumps (Gly) erythrocytes (Rbc) and a bundle of fibres (arrowed). x 8 400

Fig. 86 Shows the crenation of the plasmalemma of strain AS 6 which did not have a 'fuzzy' coat. x 80 600



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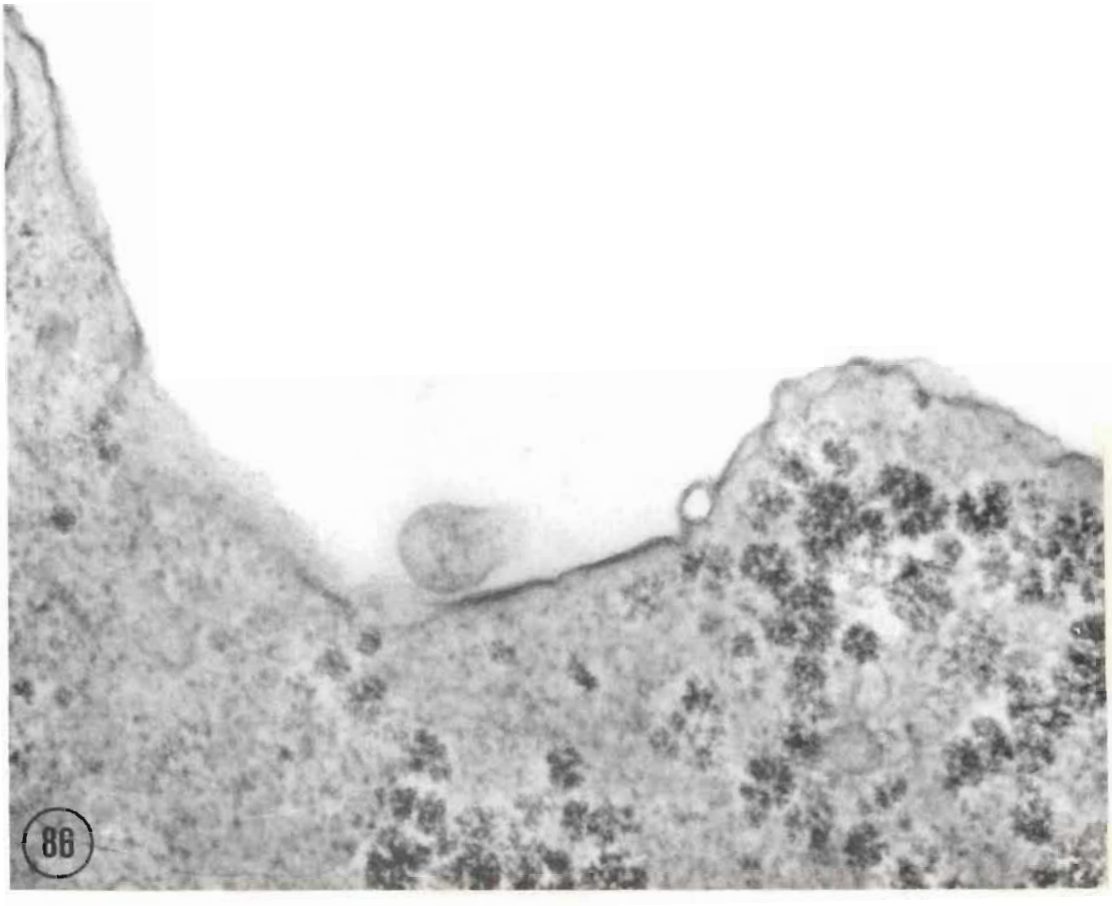
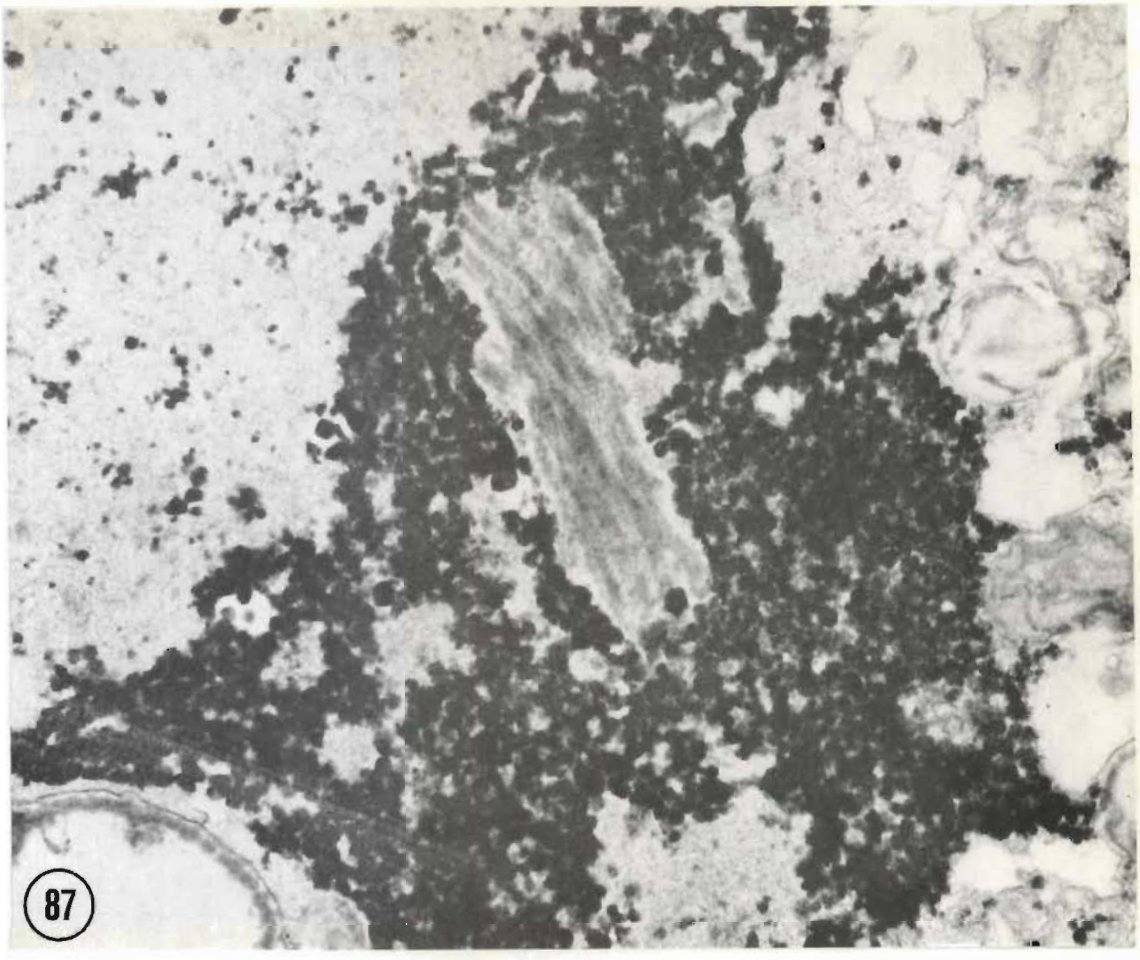


86

Trophozoites of *E. histolytica* from the human colon

Fig. 87 Shows a bundle of fibrous material surrounded by glycogen. x 34 320

Fig. 88 Shows the randomly arranged bundles of fibrous material within a clear region of cytoplasm. x 21 400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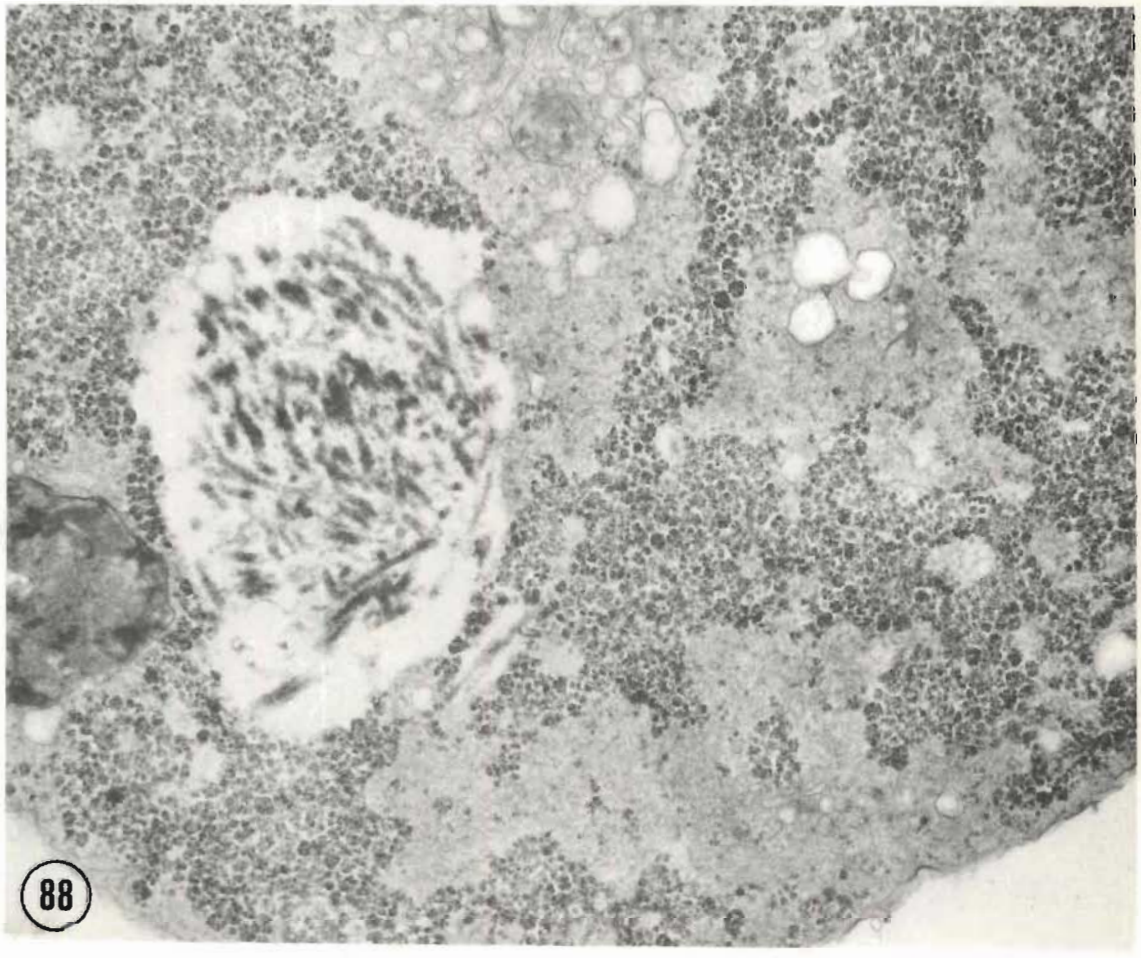
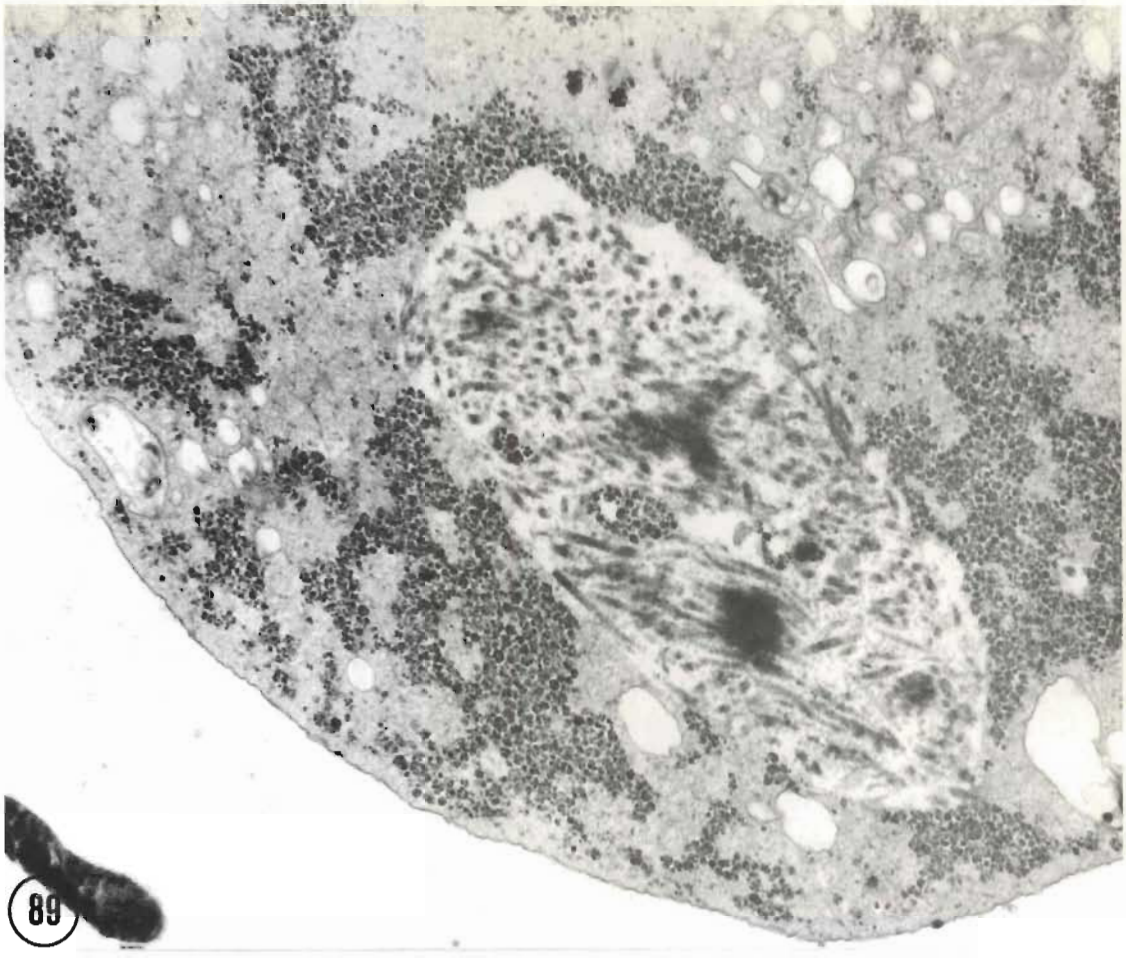

88

Trophozoites of *E. histolytica* from the human colon

Fig. 89 Shows a section through the bundles of fibrous elements at a deeper level than those shown in Fig. 88 x 16 640

Fig. 90 Part of the cytoplasm showing an ingested bacterium (Bm) and erythrocytes (Rbc). x 34 320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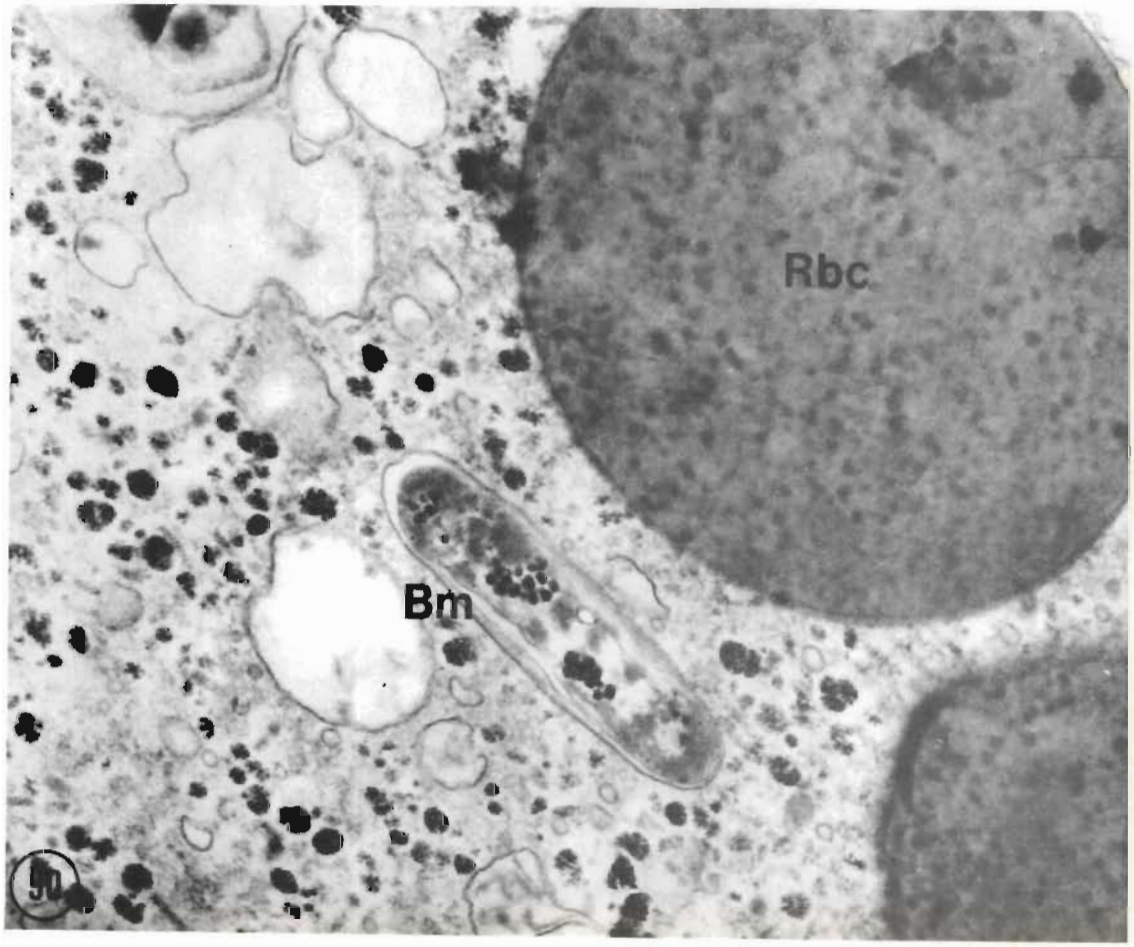
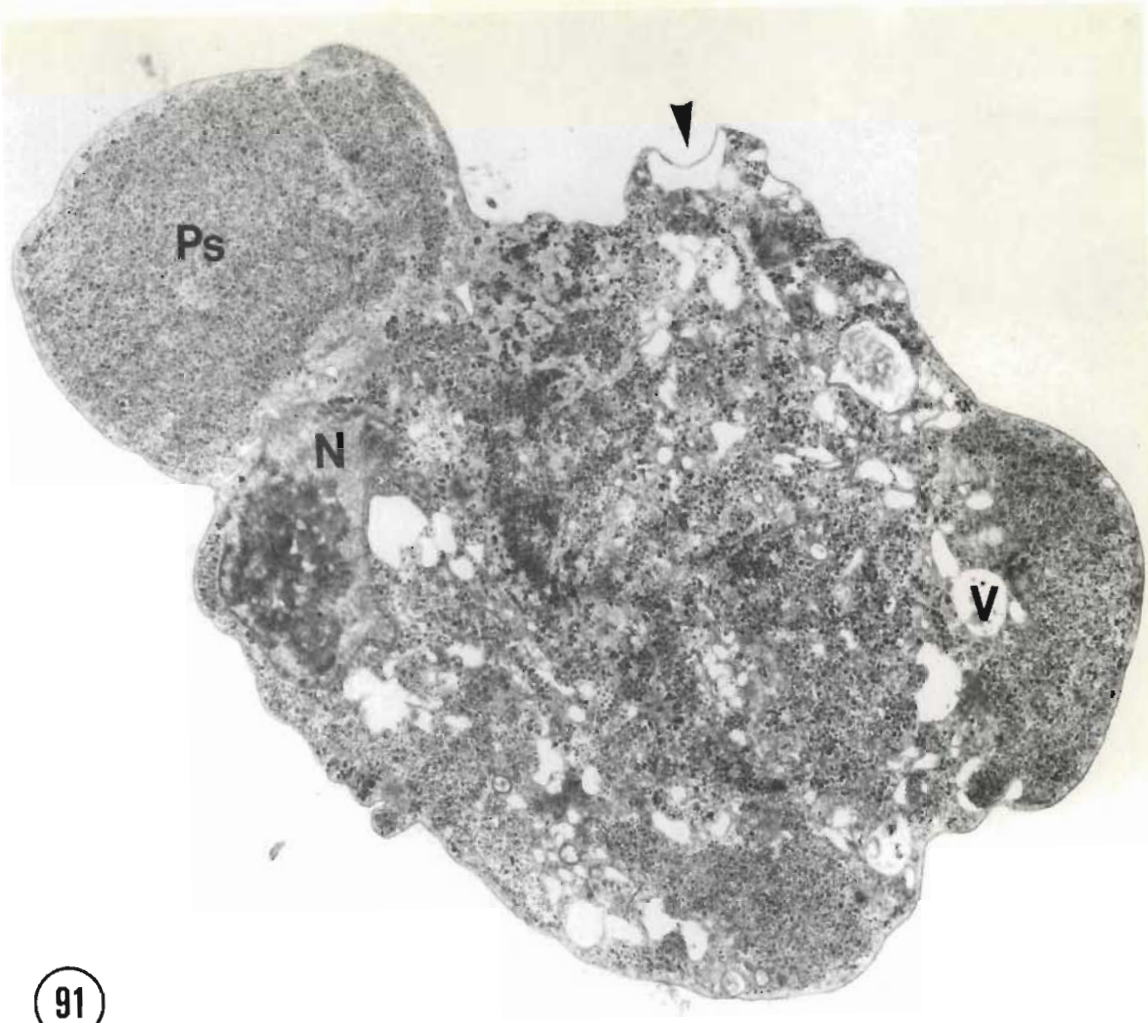

Rbc
Bm
90

Trophozoites of *E. histolytica* from human liver abscess

Fig. 91 A trophozoite showing a nucleus (N) and a glycogen filled pseudopodium (Ps) and numerous vacuoles (V). Note the crescent-shaped vacuole (arrowed) beneath the plasmalemma. x 9 128

Fig. 92 Shows the 'fuzzy' coat on the outer surface of the plasmalemma and on the inner surface of the vacuole (V). x 86 800



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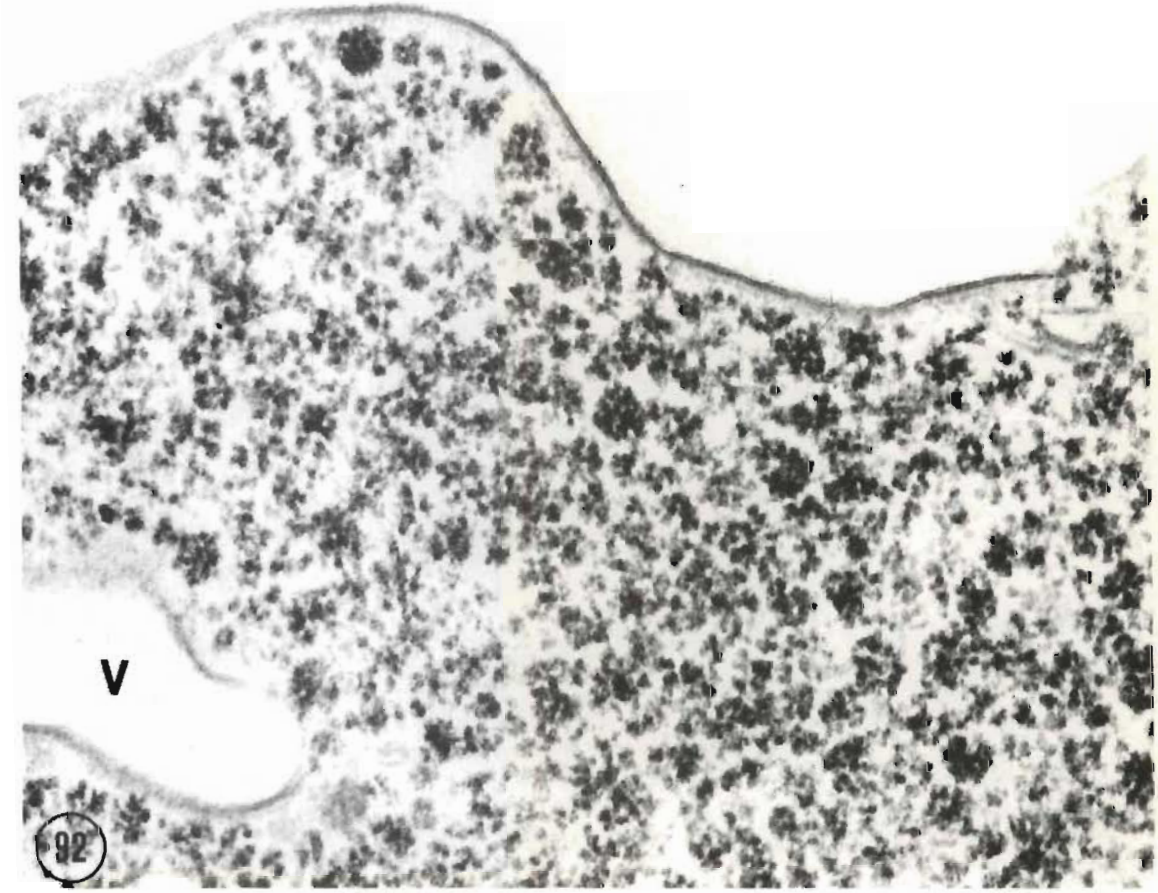
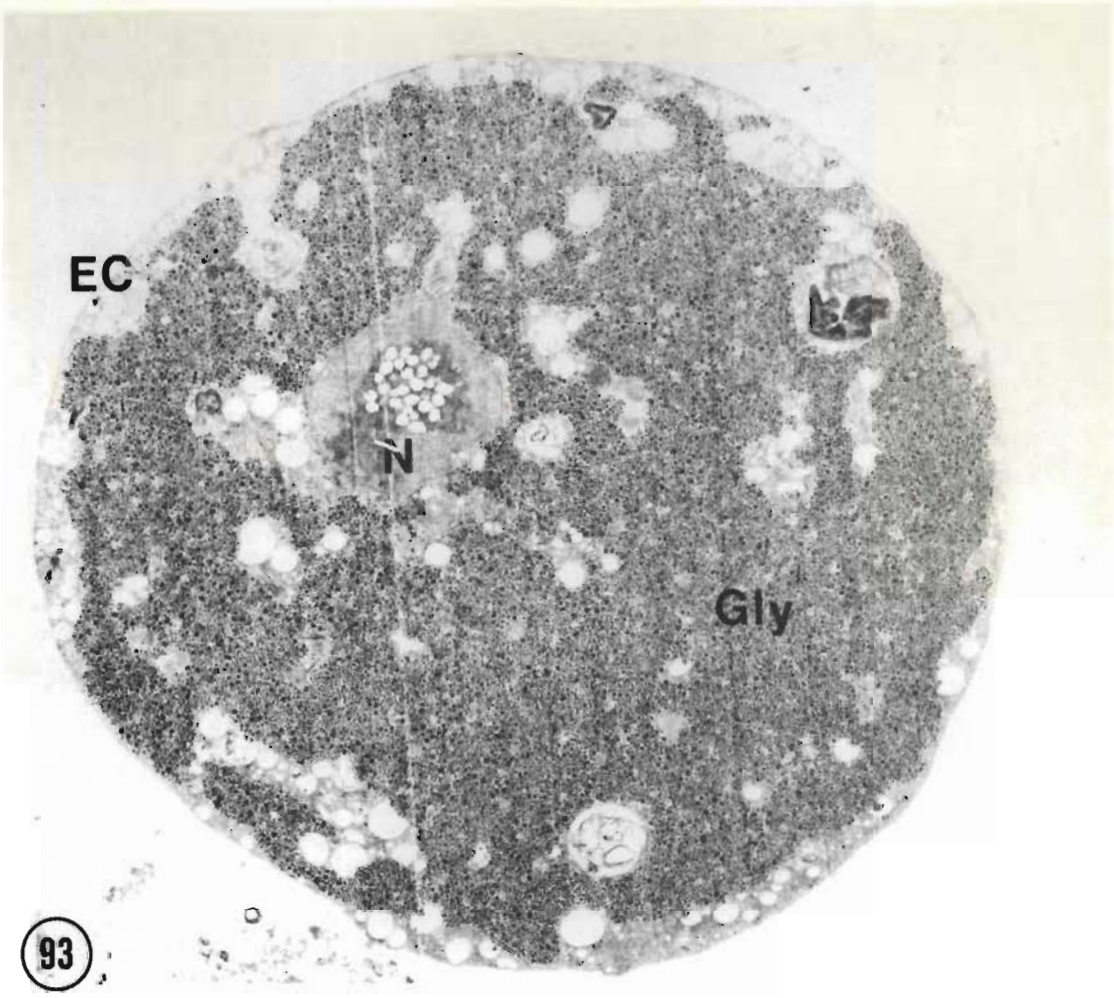


92

Trophozoites of *E. histolytica* from human liver abscess

Fig. 93 A trophozoite of strain AN showing the thin layer of ectoplasm containing numerous small vacuoles (EC) the nucleus (N) and glycogen (Gly). x 7 800

Fig. 94 Shows a rosette of electron-dense cylindrical bodies (R) surrounded by numerous vacuoles (V) and in close proximity to the nucleus (N) containing button bodies (BB). x 25 480



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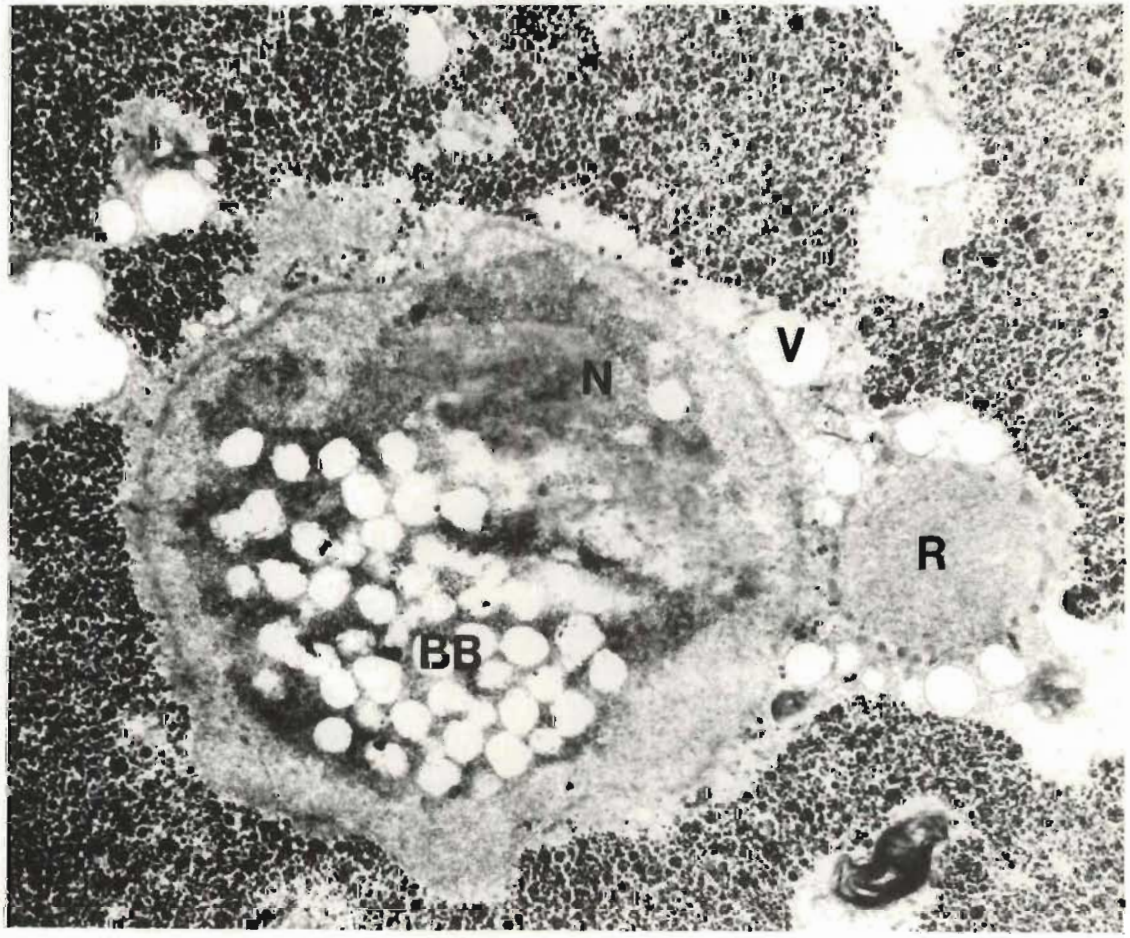
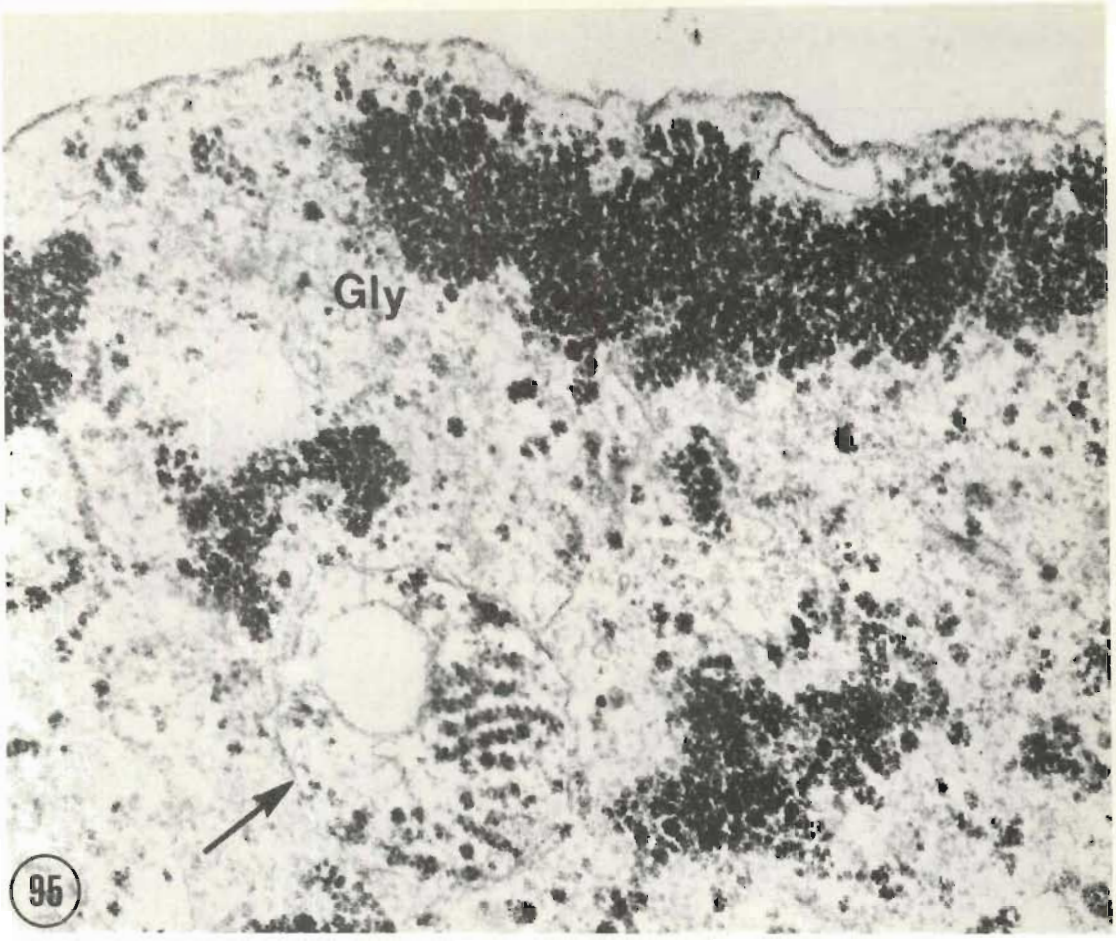
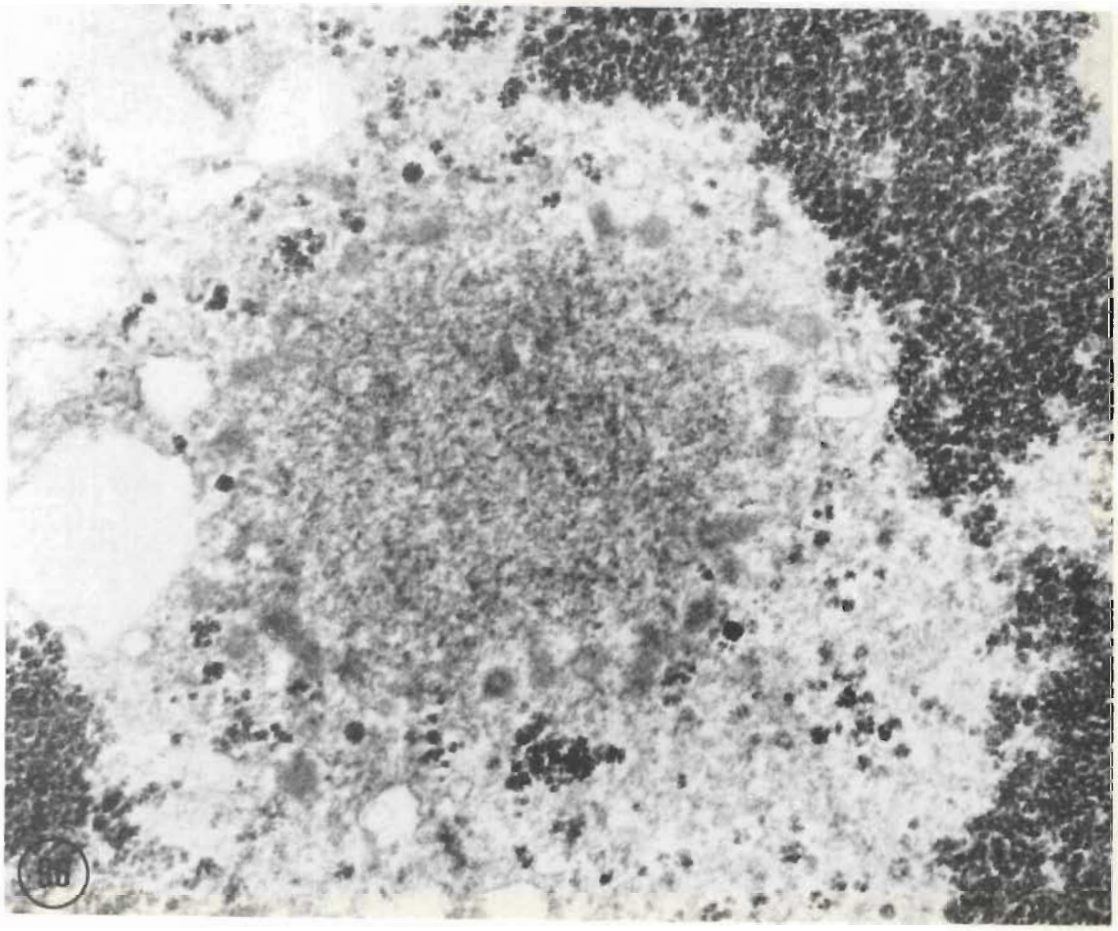


Trophozoites of *E. histolytica* from human liver abscess

Fig. 95 Part of an amoeba of strain AM showing a vacuole containing several helices (arrowed) in close association with glycogen (Gly). x 53 040

Fig. 96 Shows a less orderly arrangement of the electron-dense cylindrical bodies around a finely granular area of cytoplasm. x 53 040

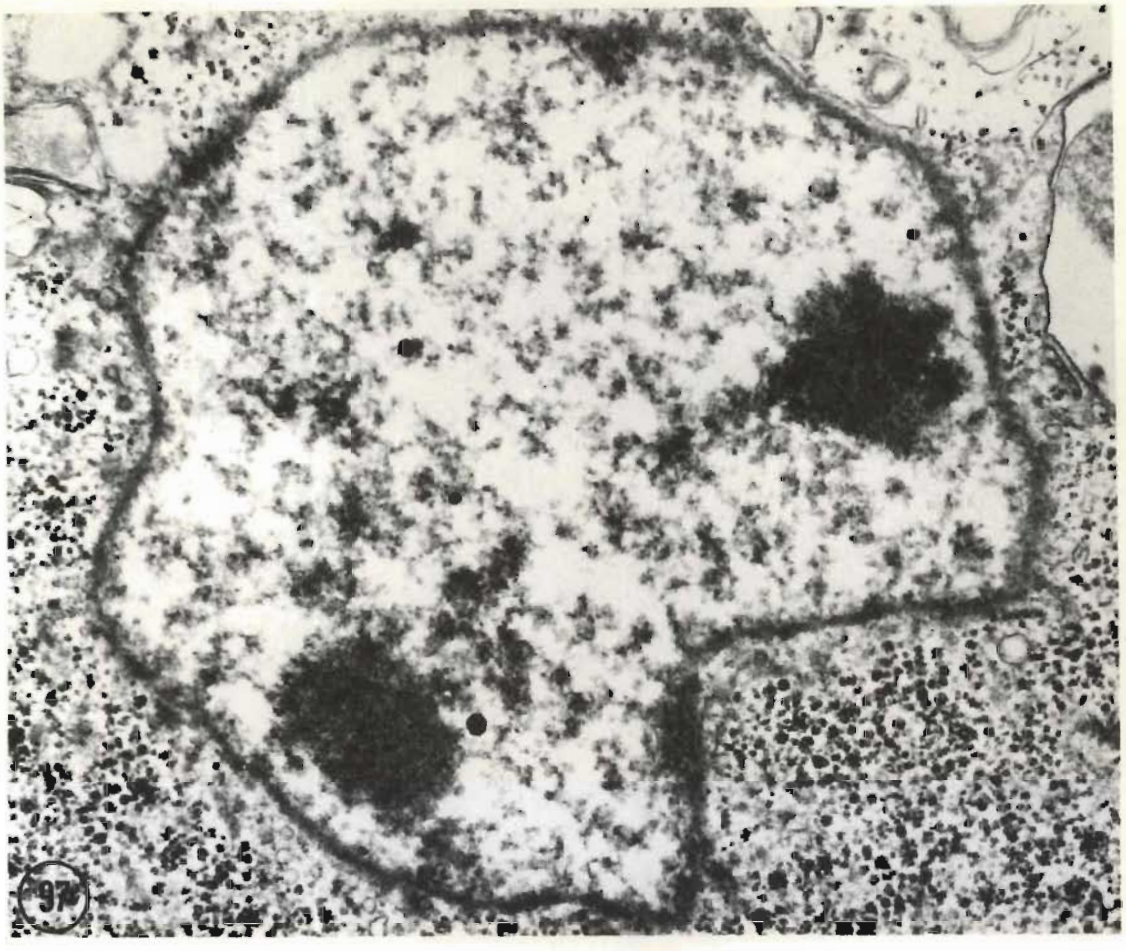

Gly
95



Trophozoites of *E. histolytica* from human liver abscess

Fig. 97 Shows a nucleus containing masses, probably representing chromatin material, at opposite poles. x 34 320

Fig. 98 Shows the lattice-like ground substance of a nucleus (N) with clumps of amorphous material at opposite sides. x 34 320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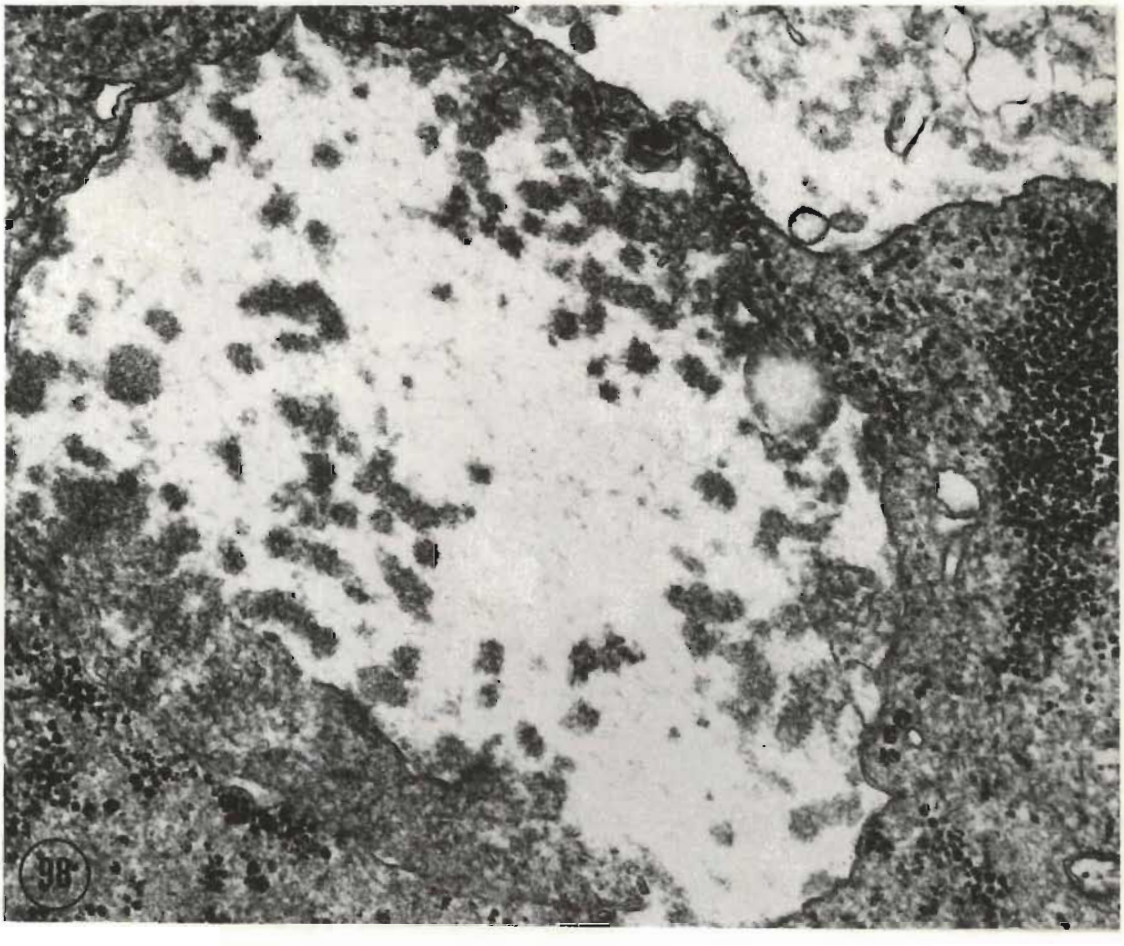
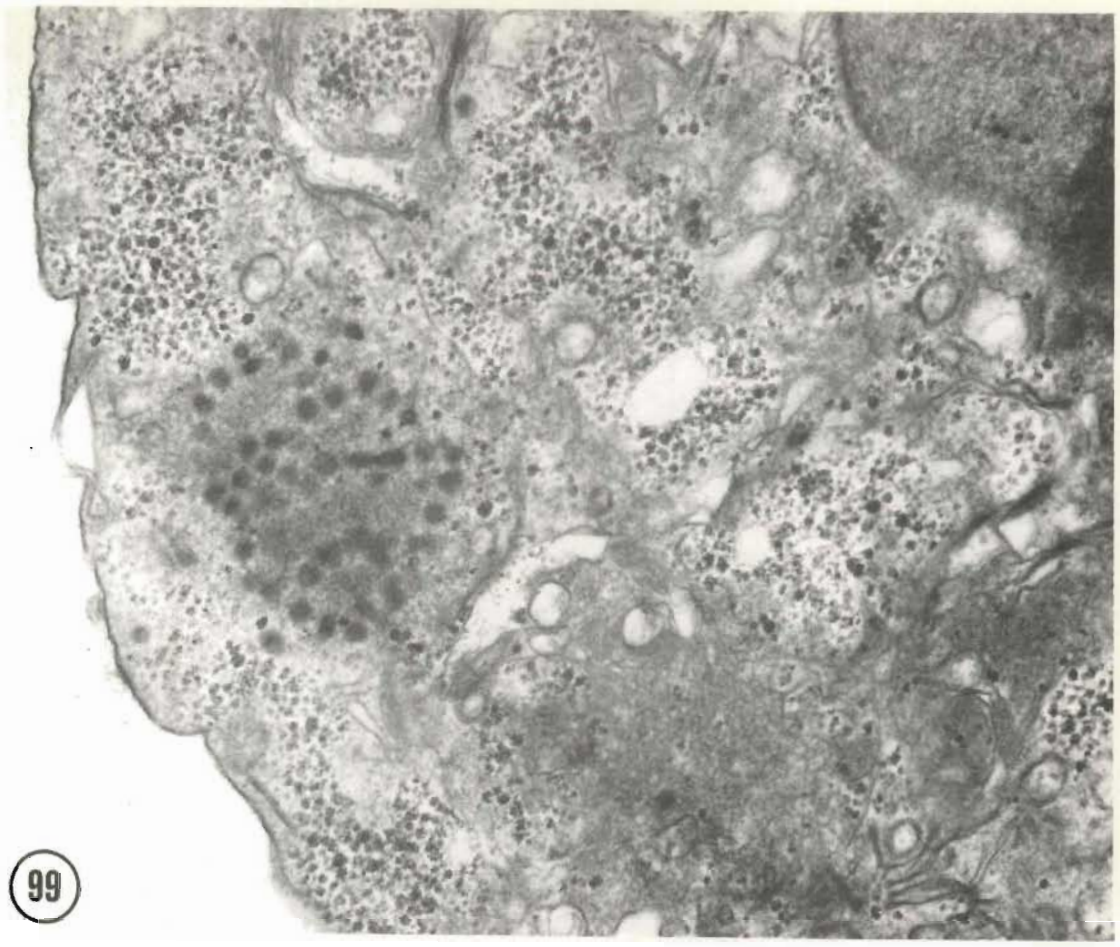

98

Trophozoites of *E. histolytica* from human liver abscess

Fig. 99 Part of an amoeba from strain AP showing the disorderly arrangement of the electron-dense cylindrical bodies. x 32 860

Fig. 100 Shows the clover leaf pattern formed by the electron-dense cylindrical units. x 74 400



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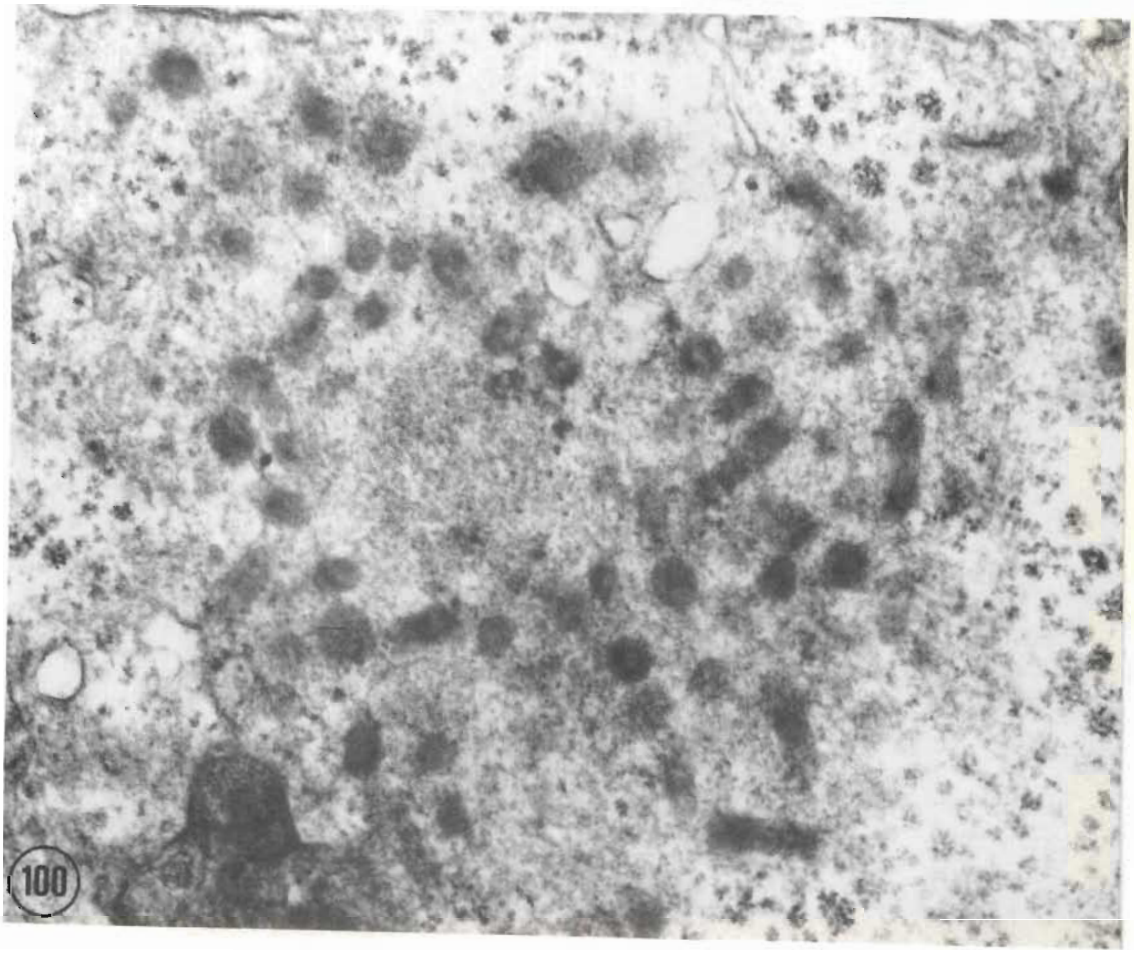
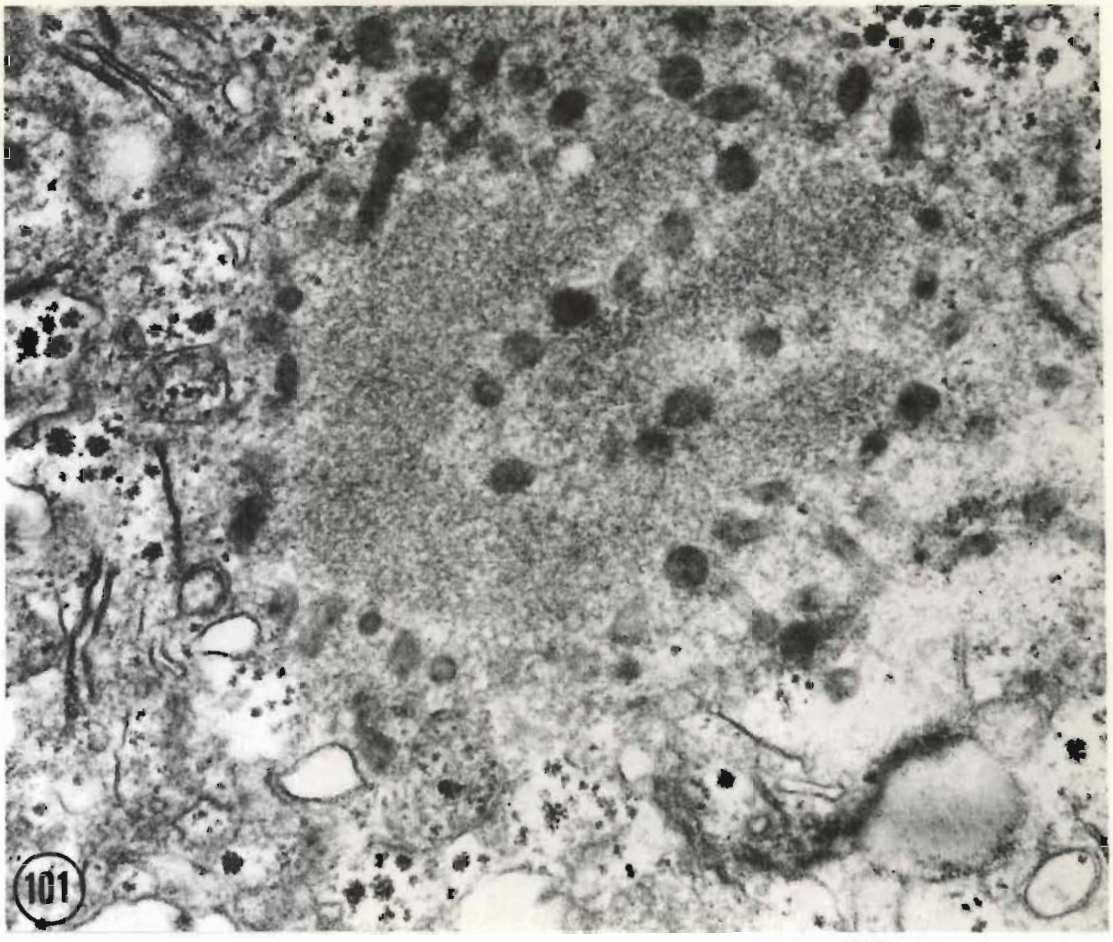


100

Trophozoites of *E. histolytica* from human liver abscess

Fig. 101 Section through a deeper level of the electron-dense cylindrical units in which they form a U-shaped pattern. x 97 600

Fig. 102 Shows the longitudinal lines in one of the electron-dense cylindrical bodies sectioned parallel to its longitudinal axis. x 198 240



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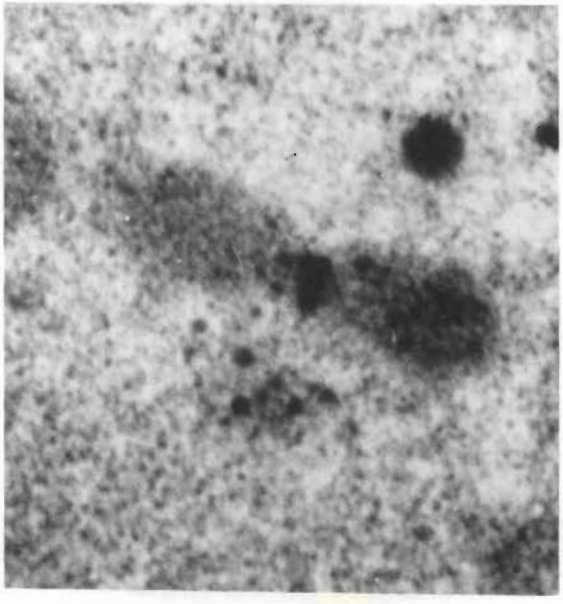
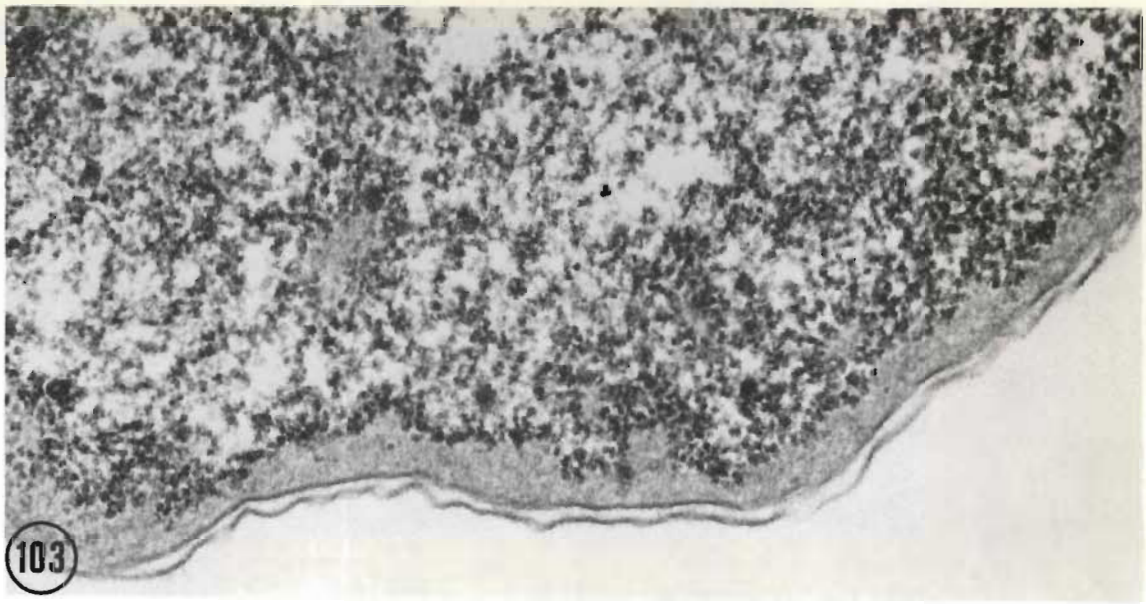


102

Trophozoites of *E. histolytica* from human liver abscess

Fig. 103 Shows the double plasmalemma seen in a few of the amoebae of strains AP and AX. x 53 760

Fig. 104 Shows the electron-dense bodies (arrowed) between two newly divided nuclei. Note the chromatin (Ch) in both nuclei and the concentration of button bodies (BB) in the nucleus on the right. x 43 500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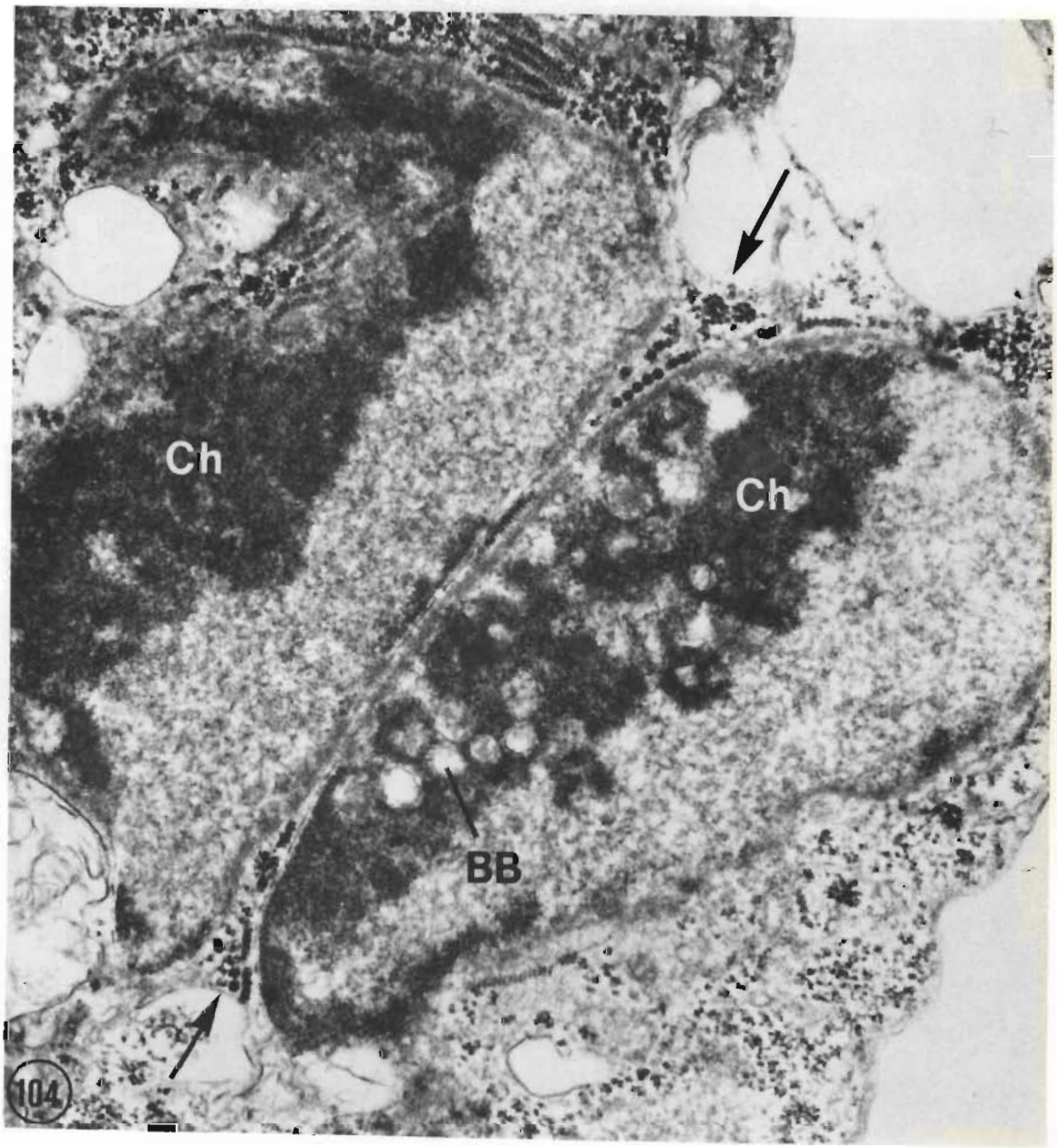
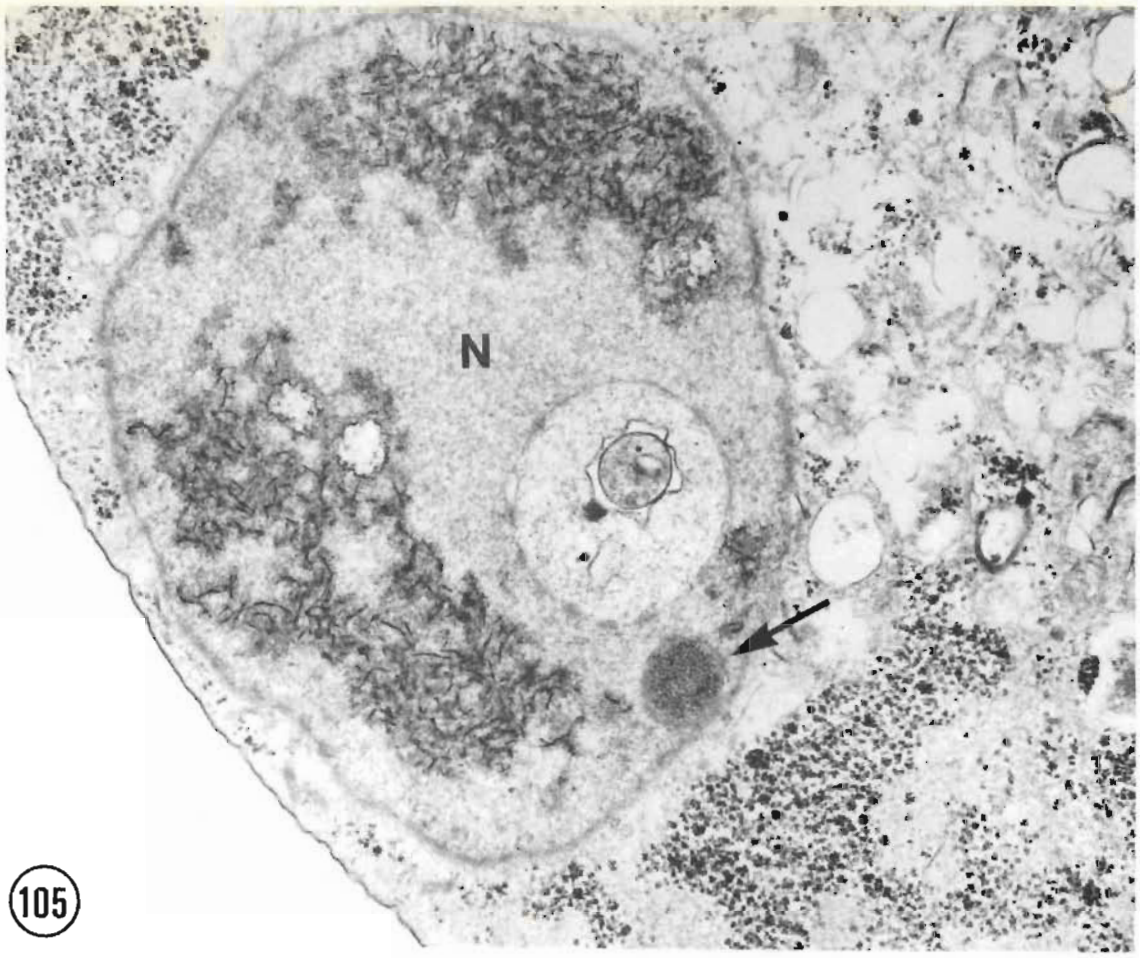

Ch
Ch
BB
104

Trophozoites of *E. histolytica* from human liver abscess

Fig. 105 Shows the chromatin of the nucleus (N) interspersed with crystals. Note the inclusion (arrowed) in close association with the nuclear envelope. x 28 420

Fig. 106 Note the chromatin interspersed with crystals within the nucleus (N) which is surrounded by smooth walled profiles of endoplasmic reticulum(ER). x 27 550



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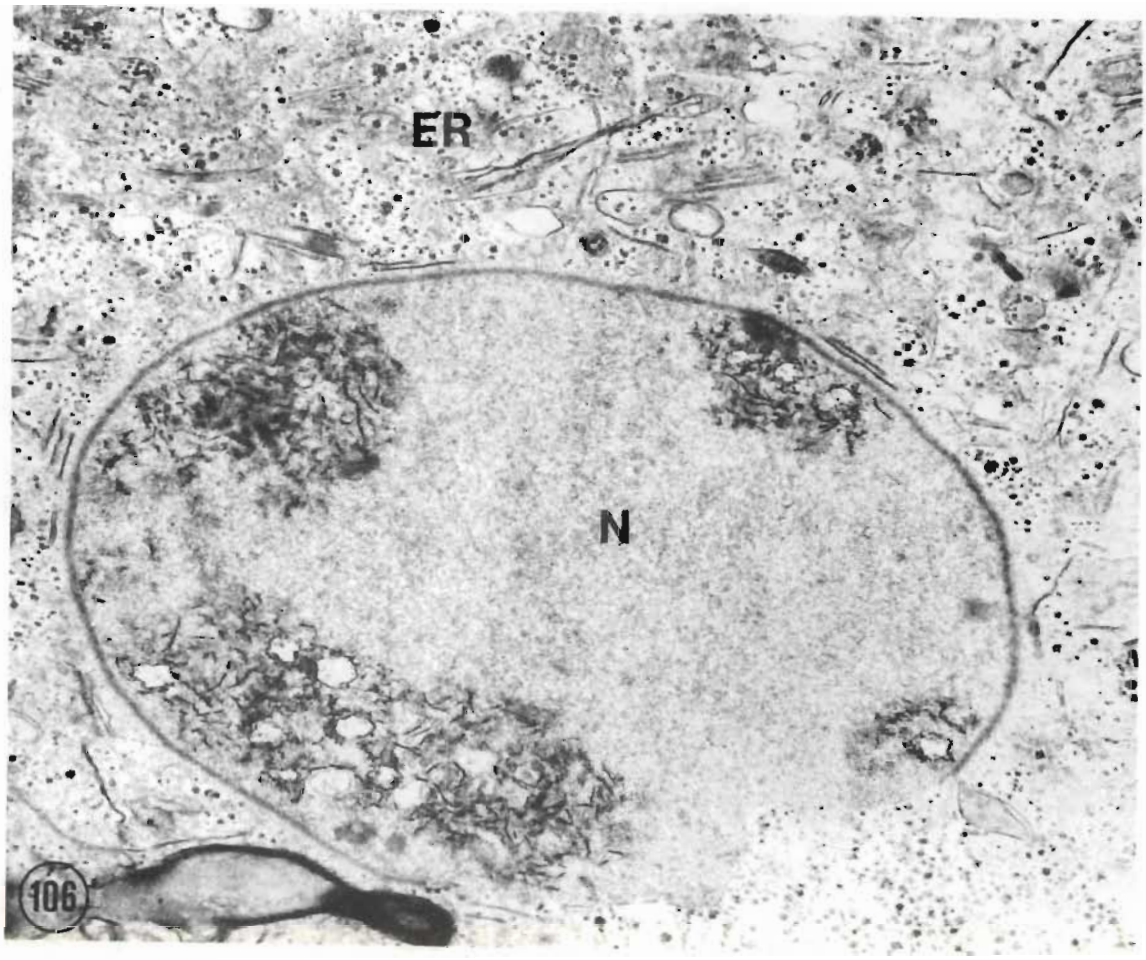
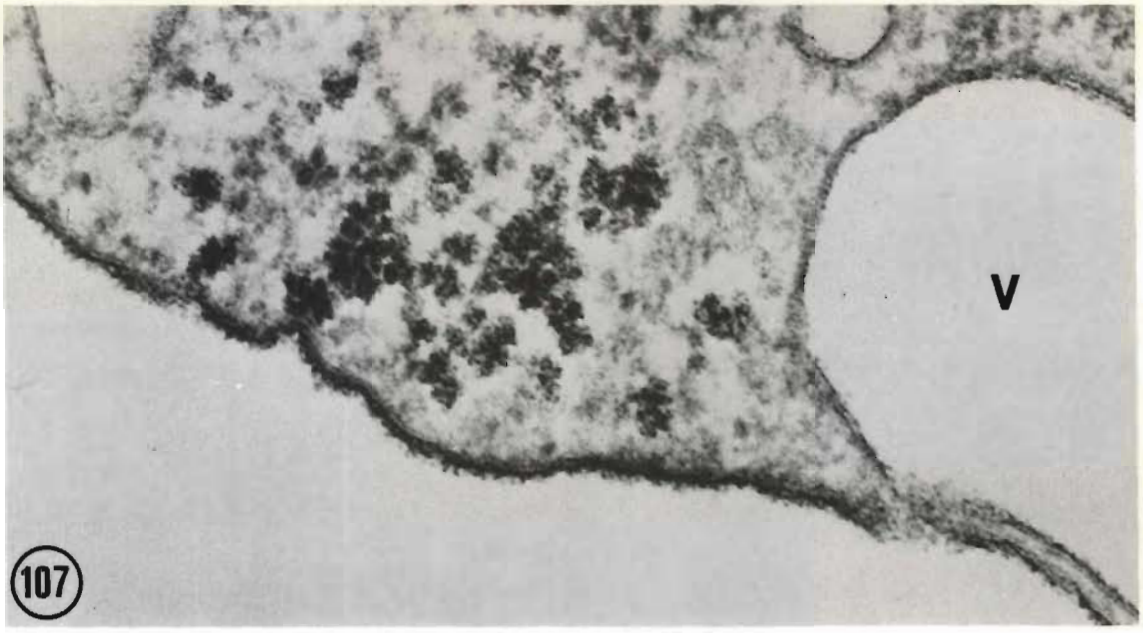


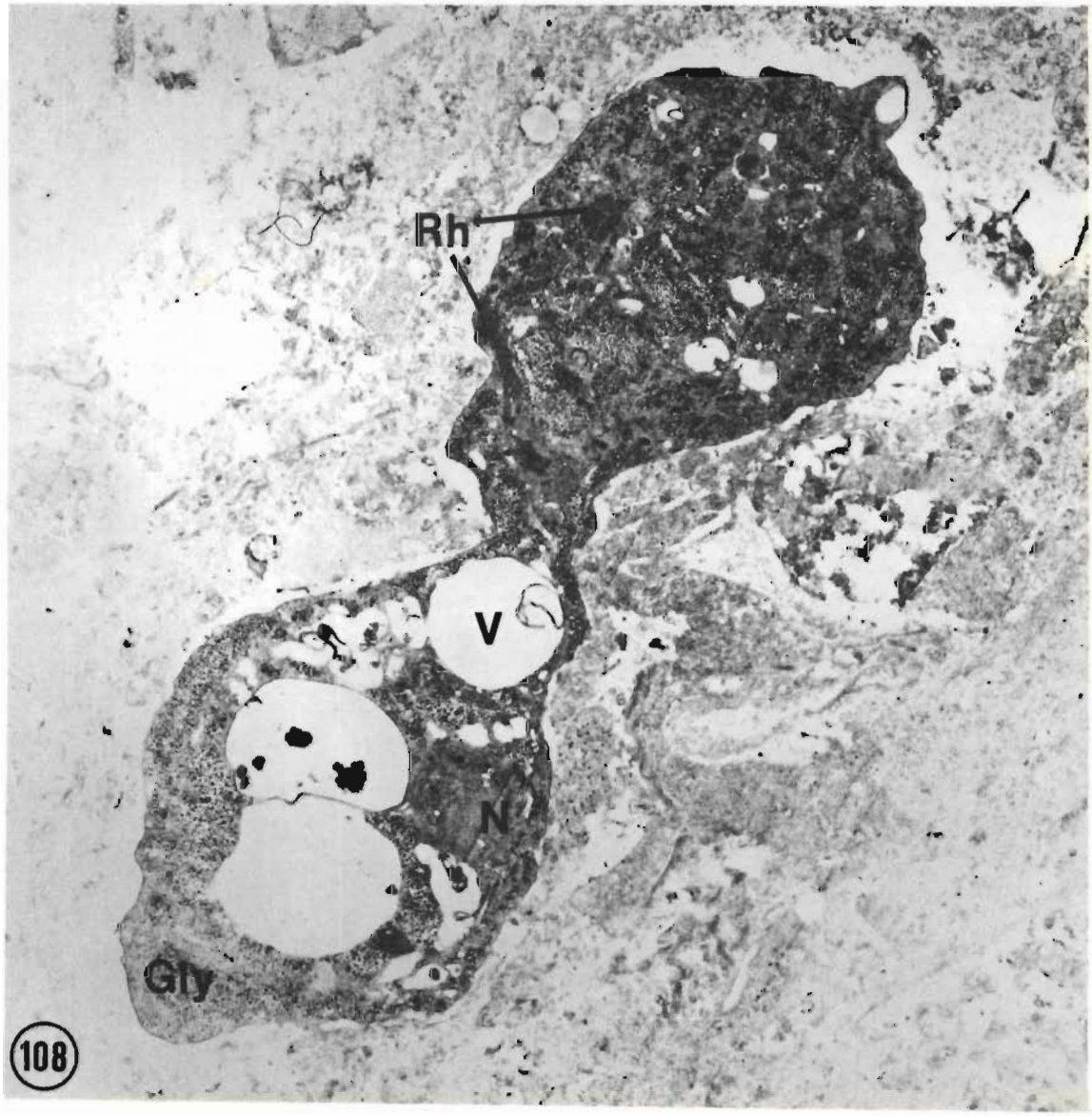
106

Trophozoites of *E. histolytica* from hamster liver abscess

Fig. 107 Shows the fuzzy coat on the outer surface of the plasmalemma and on the inner surface of the vacuole (V). x 131 660

Fig. 108 A trophozoite showing the bundles of ribosomal helices (Rh) numerous vacuoles (V) the nucleus (N) and glycogen (Gly). x 6 090


V
107


Rh
V
N
Gly
108

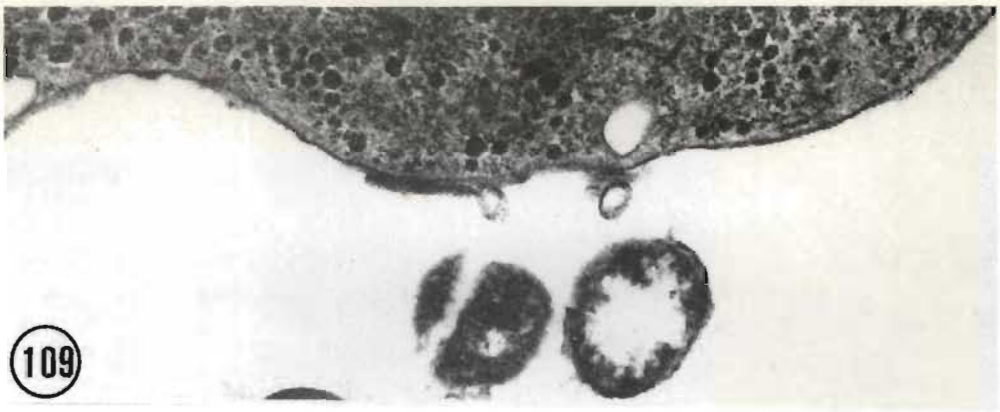
Trophozoites of *E. histolytica* from hamster liver abscess

Fig. 109 Shows a tubular structure which opens out at one end to form a vesicle lying parallel to the surface of a trophozoite of strain HALA from the 33rd passage through hamster liver. x 32 560

Fig. 110 A trophozoite from strain HALA showing numerous blebs on the surface of the plasmalemma (arrowed). x 11 000

Fig. 111 High power showing the unit membrane surrounding a surface bleb. x 89 760

Fig. 112 Shows an electron-dense bleb apparently being extruded by the cell. Note the unit membrane extending from the bleb into the cytoplasm (arrowed). x 68 200



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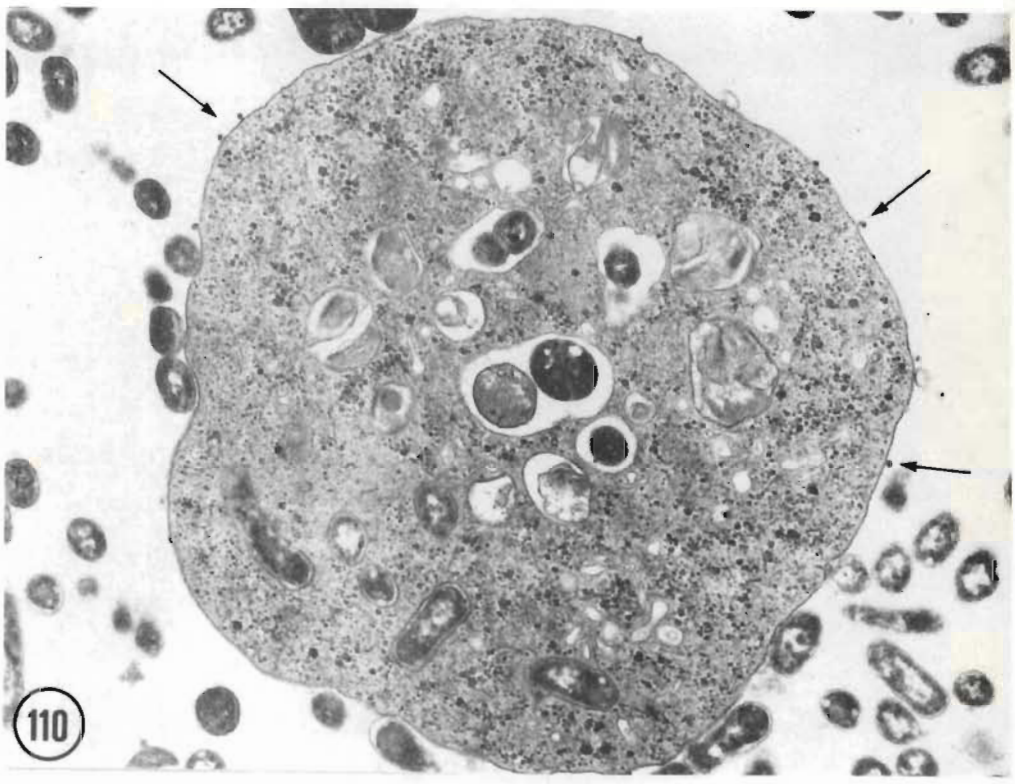

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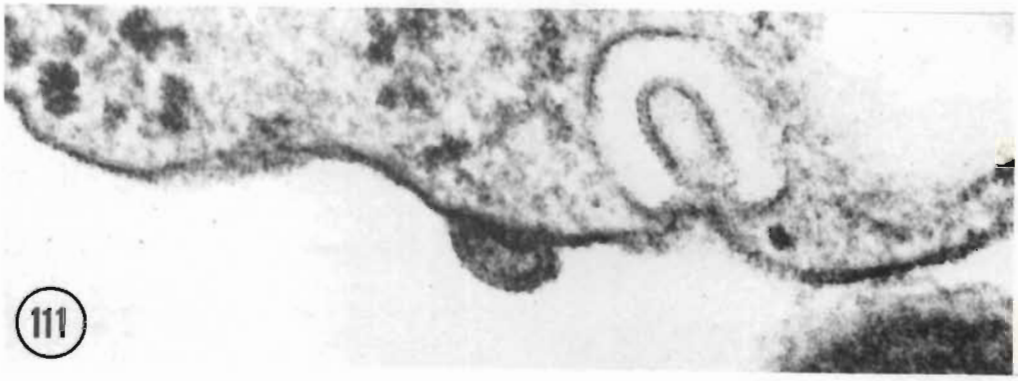

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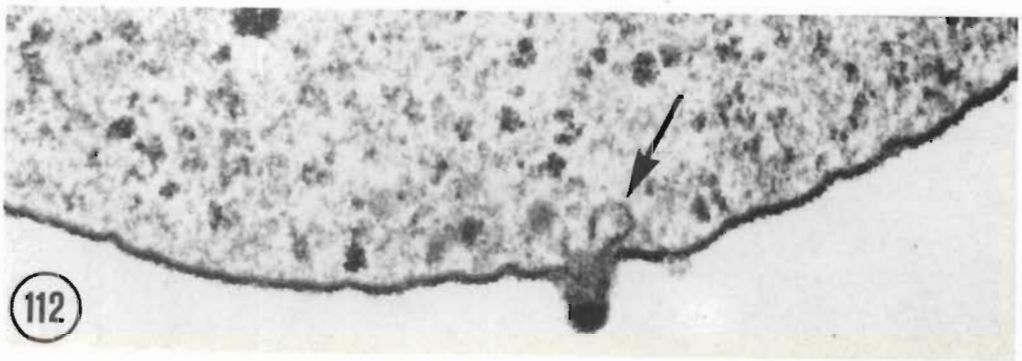


112

Trophozoites of *E. histolytica* from hamster liver abscess

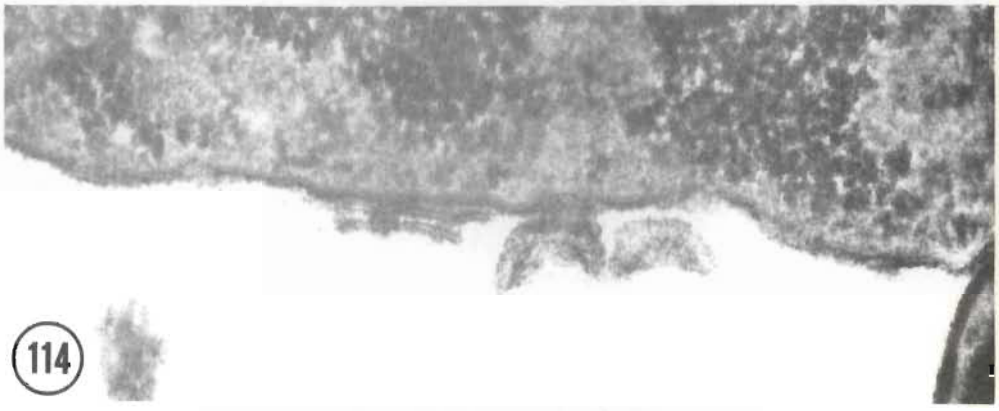
Fig. 113 A region of membranous material in close association with the plasmalemma. x 99 200

Fig. 114 Shows strands of unit membrane lying parallel to the plasmalemma. x 87 680

Fig. 115 Shows a structure similar to the surface hairclip arrangements shown in figure 79, on the surface of a trophozoite of strain AK/68. Note the cellular material (C) probably of host origin. x 64 640

Fig. 116 A trophozoite of strain AK/68 showing a region of fibrous material occupying a large portion of the cytoplasm (FM). x 9 600

113



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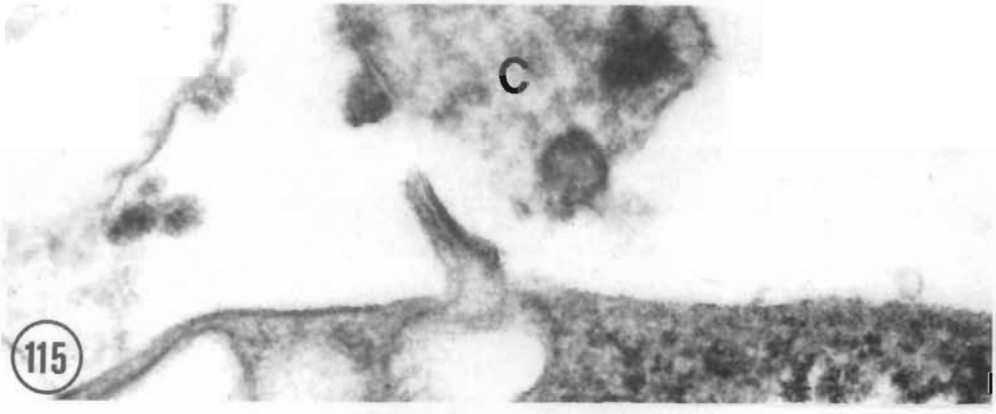

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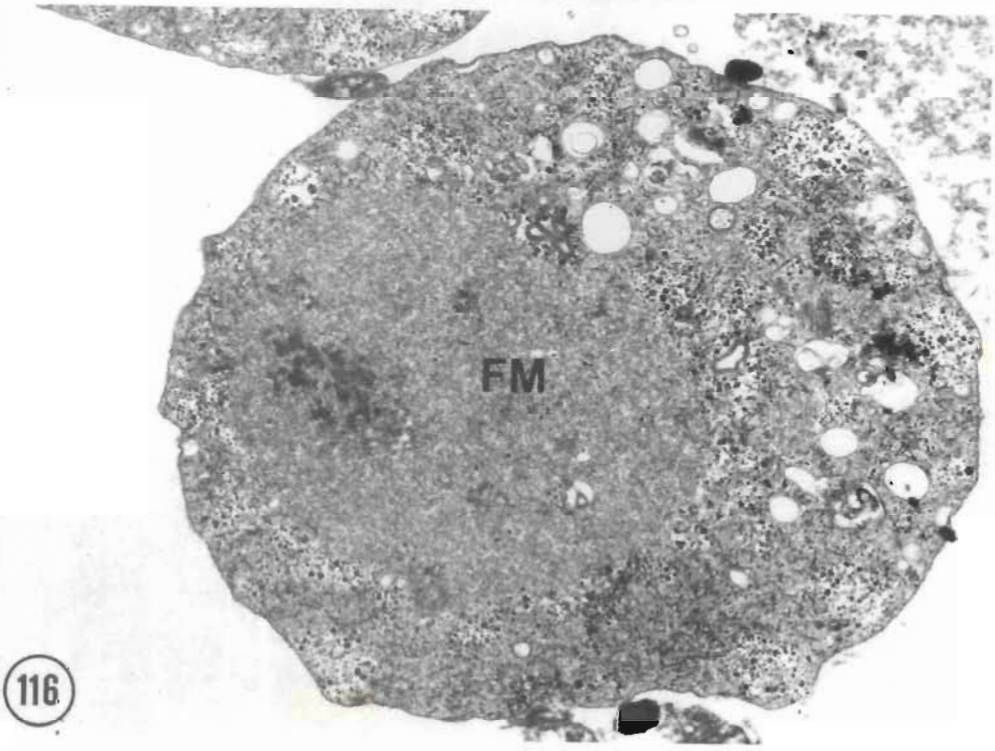


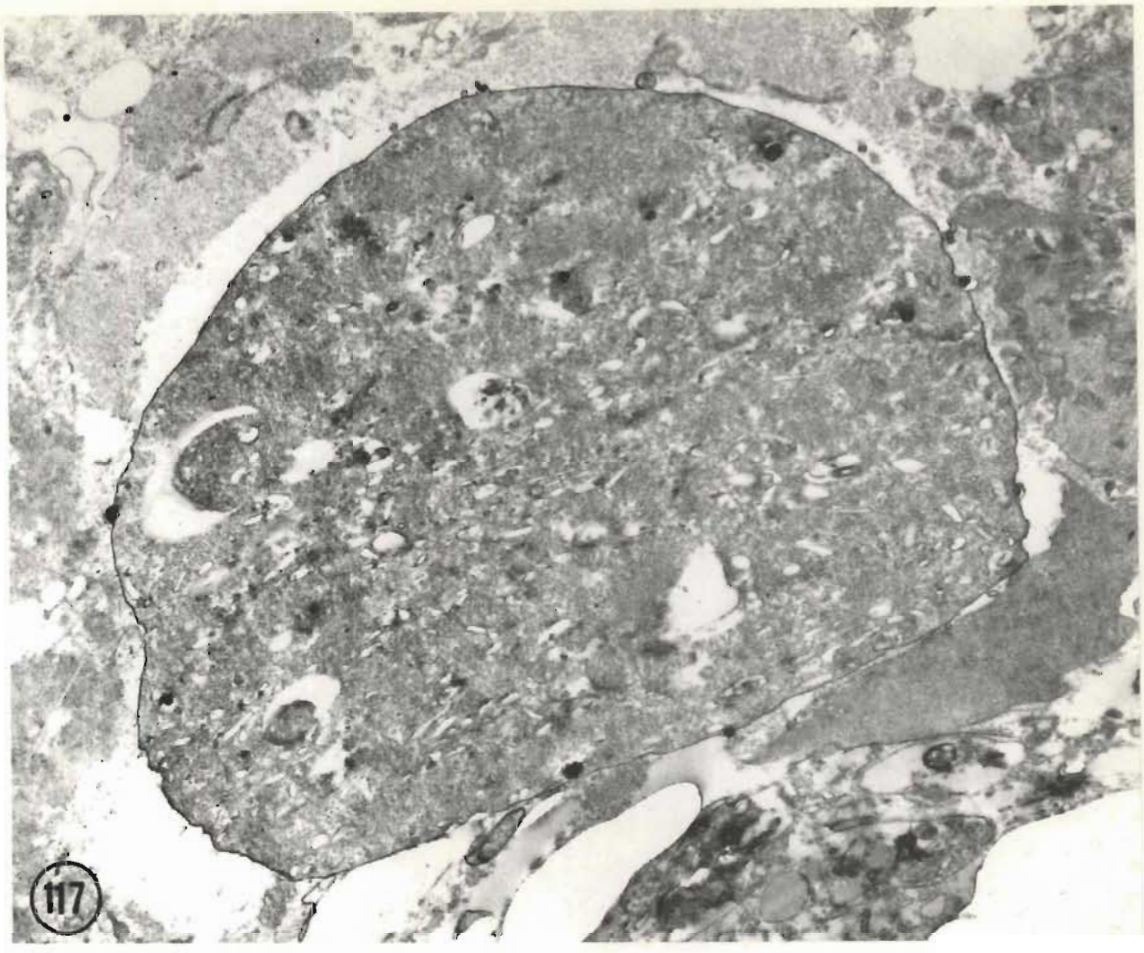
116

Trophozoites of *E. histolytica* from hamster liver abscess

Fig. 117 A trophozoite showing several electron-dense bodies immediately beneath the plasmalemma and in the cytoplasm. x 10 140

Fig. 118 An electron-dense body with a foamy appearance within the cytoplasm. x 137 280

Fig. 119 Shows an electron-dense body apparently being extruded by the cell. x 94 640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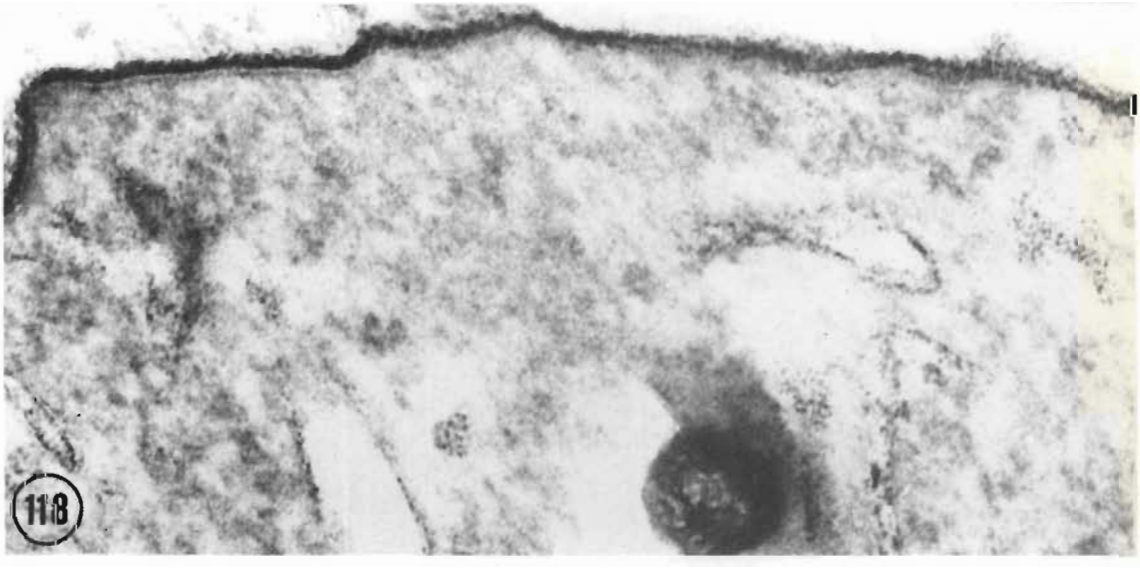
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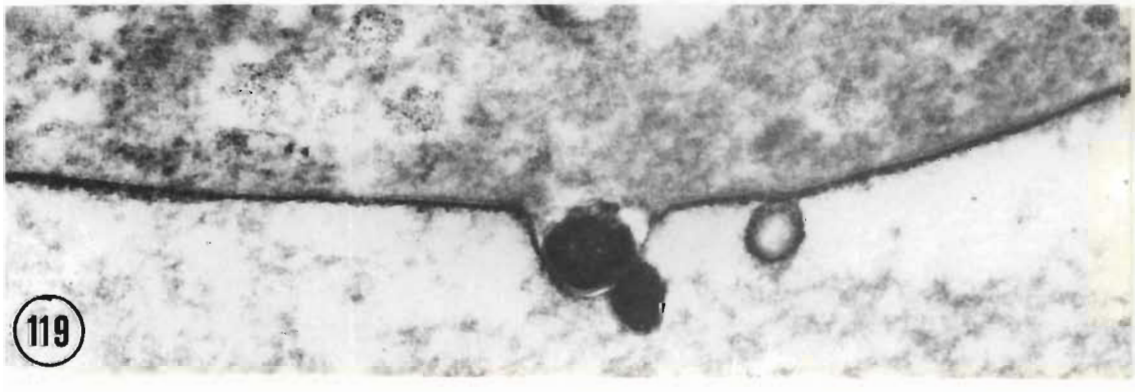
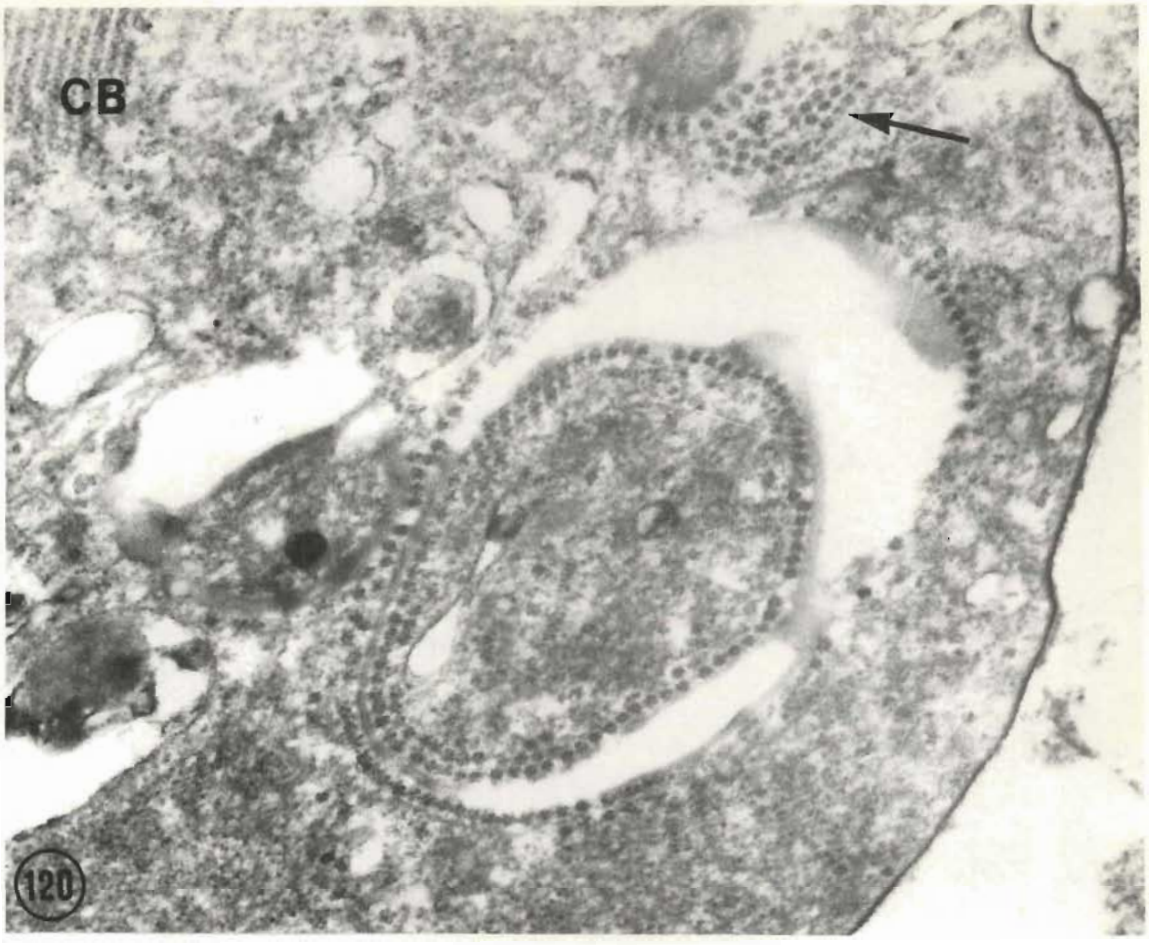
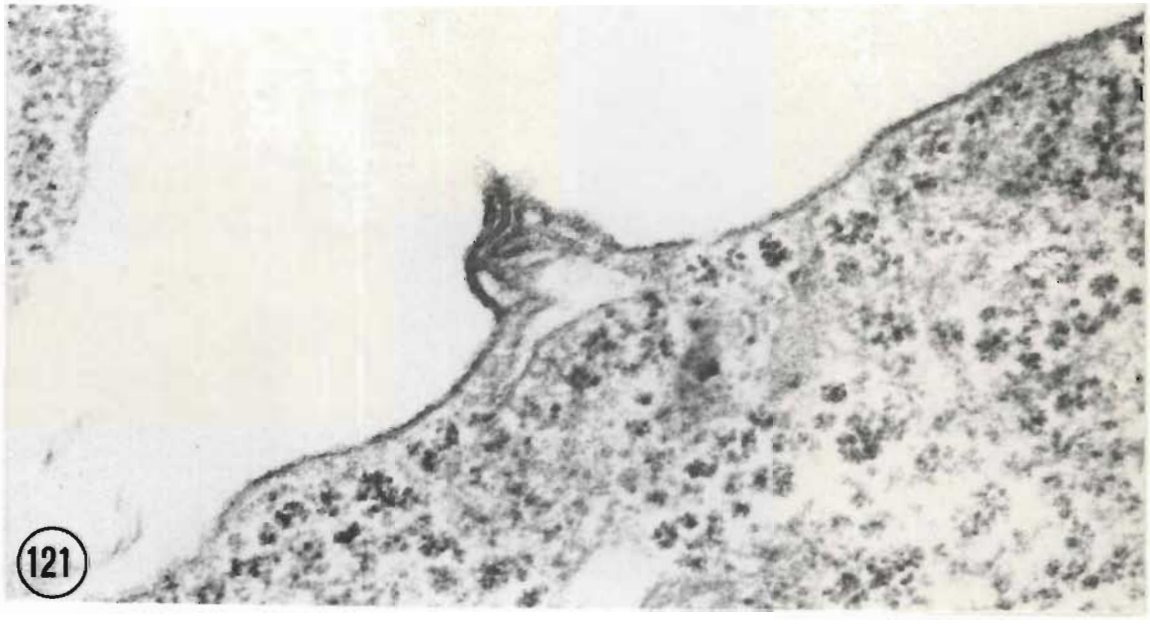

119

Trophozoites of *E. histolytica* from hamster liver abscess

Fig. 120 Shows the orderly arrangement of particles similar to ribosomes in the cytoplasm of a trophozoite from strain AX/68. Note the crystalloid aggregate (CB) and the group of particles (arrowed) exterior to the pear-shaped arrangement. x 53 040

Fig. 121 Shows the strands of unit membrane branching out to form parallel lamellae and in close association with unit membranes within the cytoplasm. x 80 600

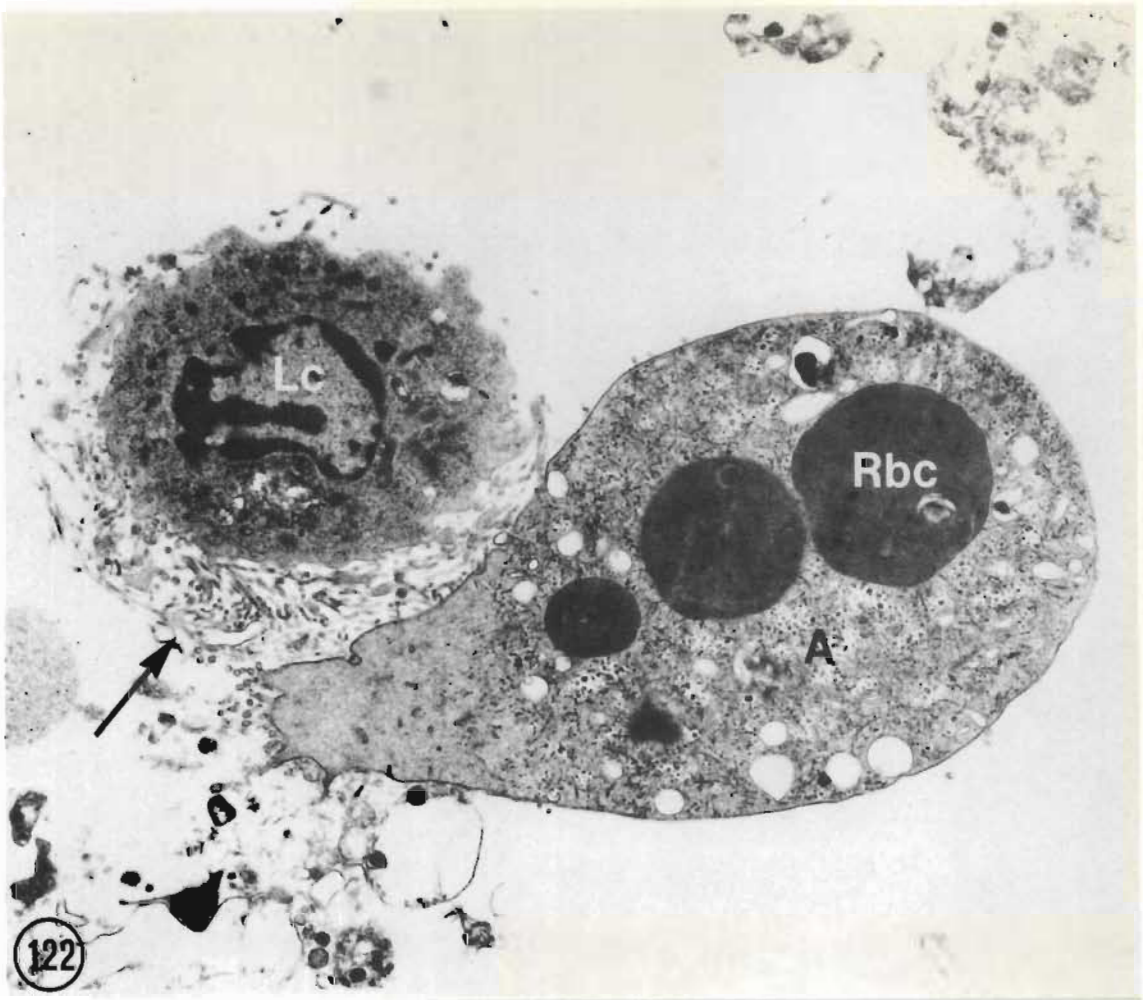

CB
120

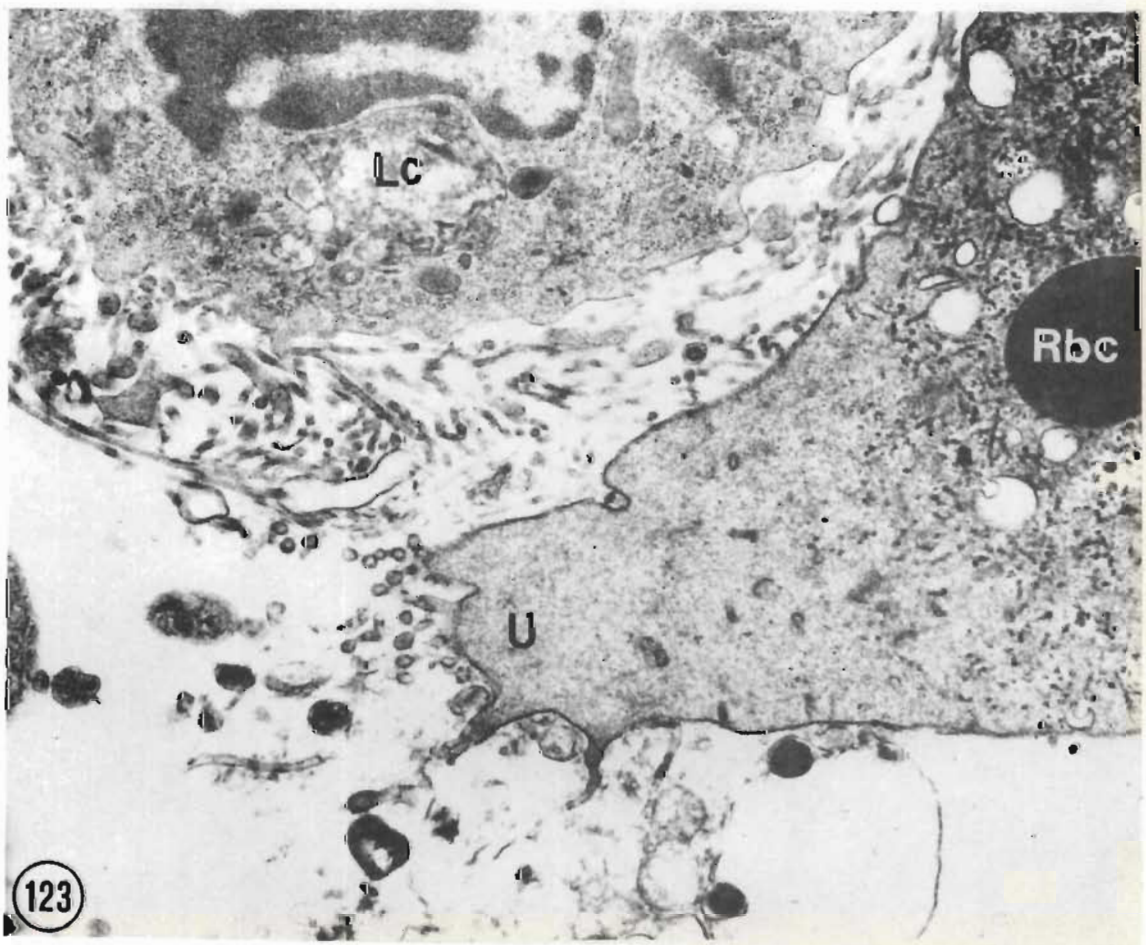

121

Trophozoites of *E. histolytica* from hamster liver abscess

Fig. 122 Shows a trophozoite (A) containing erythrocytes (Rbc) and a leucocyte (Lc) in close proximity. Note the concentration of cellular debris (arrowed) between the two cells and surrounding the leucocyte. x 10 100

Fig. 123 High power showing the concentration of cellular debris between the amoeba containing ingested erythrocytes (Rbc) and the leucocyte (Lc). Note the uroid region (U) of the amoeba. x 21 320

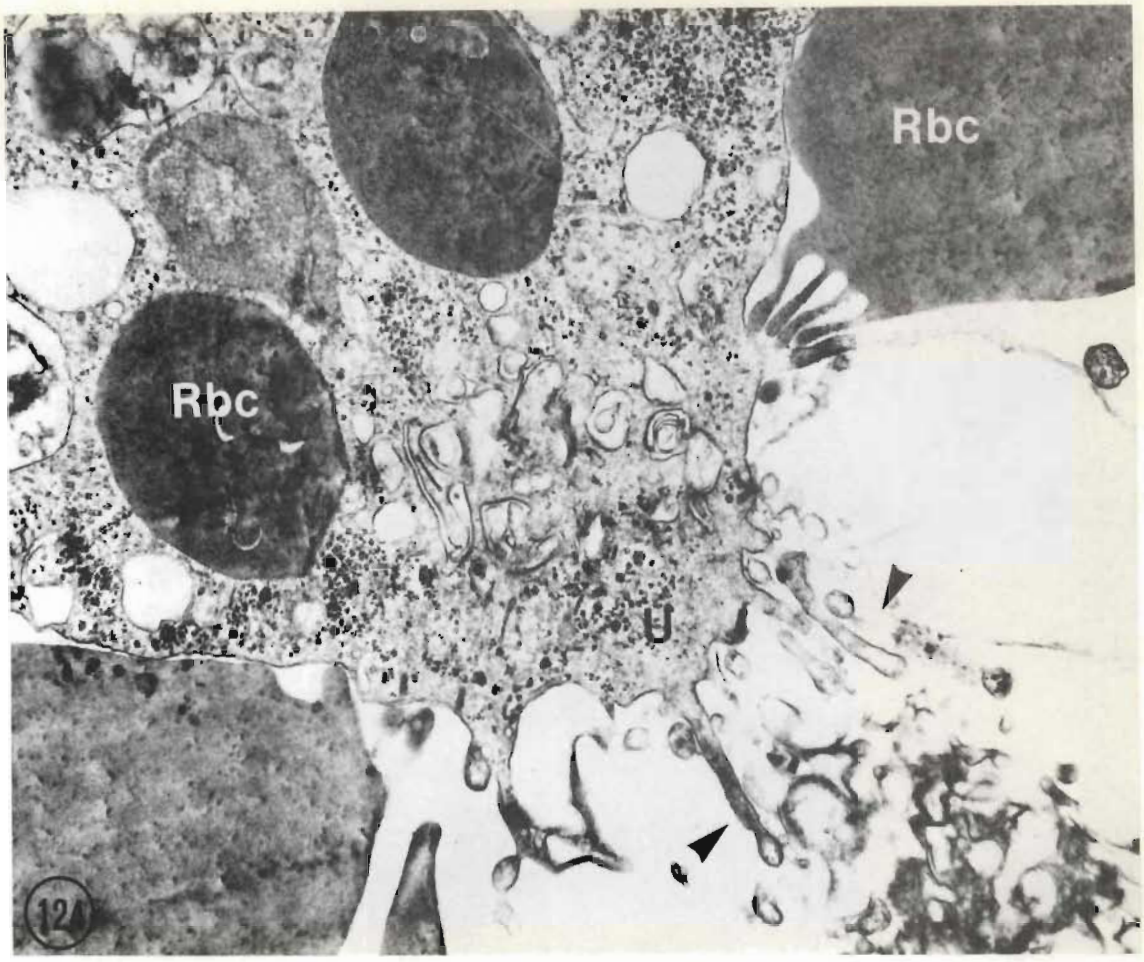

Lc
Rbc
A
122

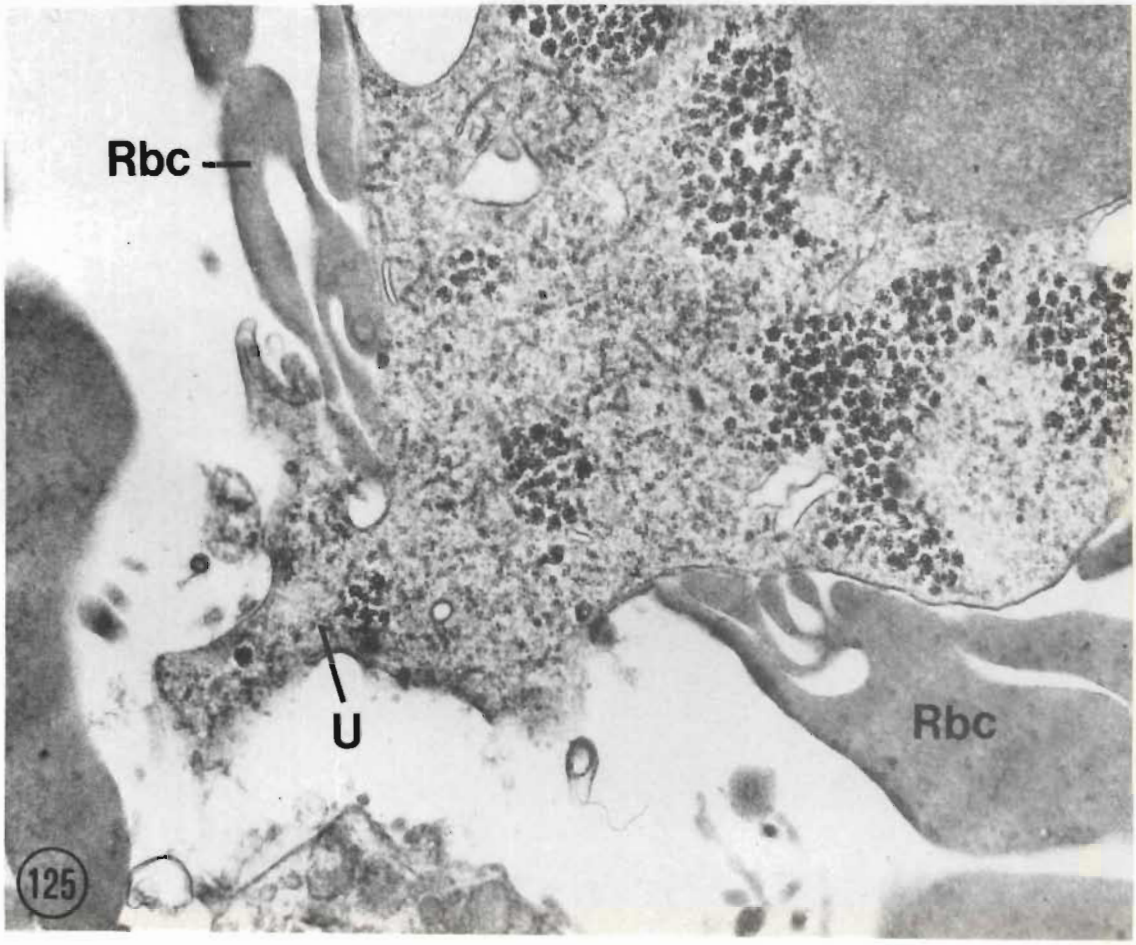

Lc
Rbc
U
123

Trophozoites of *E. histolytica* from hamster liver abscess

Fig. 124 Shows the uroid region (U) of an amoeba with numerous protrusions of the plasmalemma (arrowheads). within the cell and in the process of being ingested are several erythrocytes (Rbc). x 20 800

Fig. 125 Shows the adherence of erythrocytes (Rbc) to the uroid region (U) of an amoeba. x 34 320


Rbc
Rbc
U
124

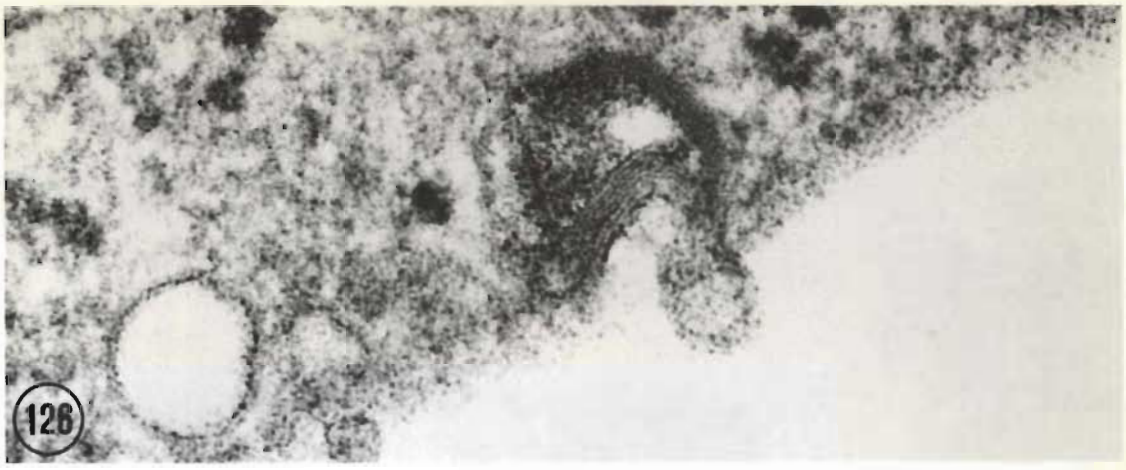

Rbc
U
Rbc
125

Trophozoites of *E. histolytica* from hamster liver abscess

Fig. 126 A region of parallel lamellae beneath the surface which appeared to be continuous with a mass of low electron density projecting to the exterior of a trophozoite from strain N/68. x 153 400

Fig. 127 A region of helices in strain AN/68 fanning out from both sides of a central band which may be a unit membrane. x 61 880

Fig. 128 Shows the numerous small vesicles (arrowhead) beneath the pseudopodium containing an ingested erythrocyte (Rbc). Note the electron-dense bodies (arrowed) attached to the membrane of the red blood cell. x 38 480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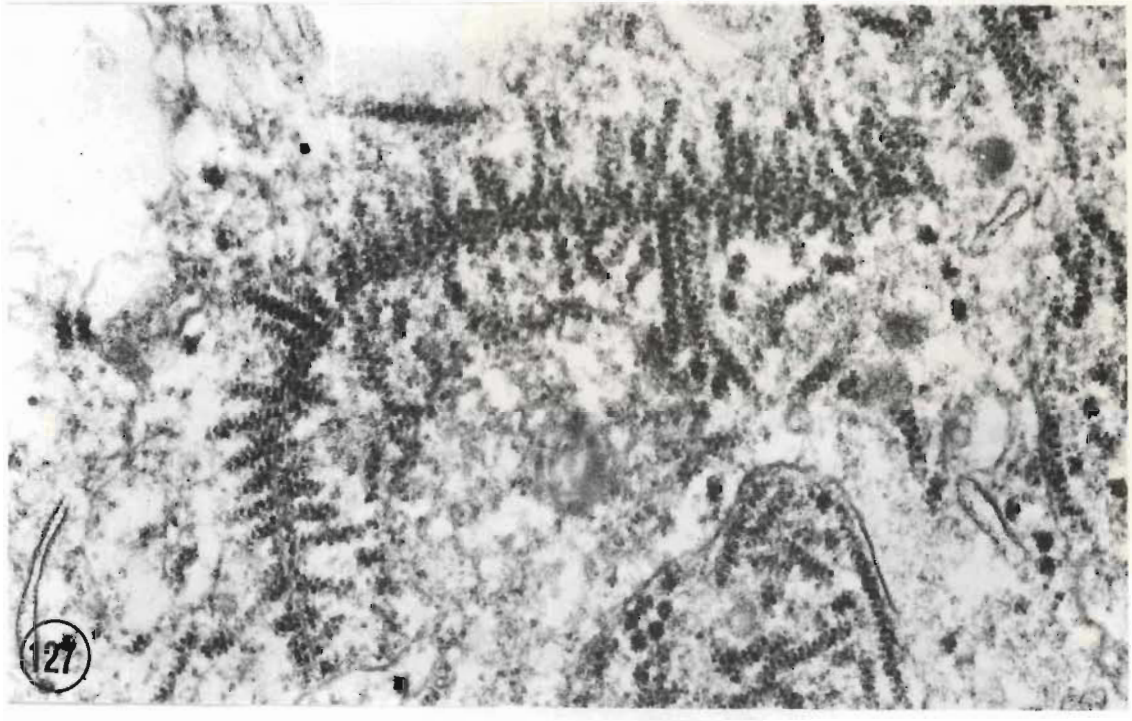
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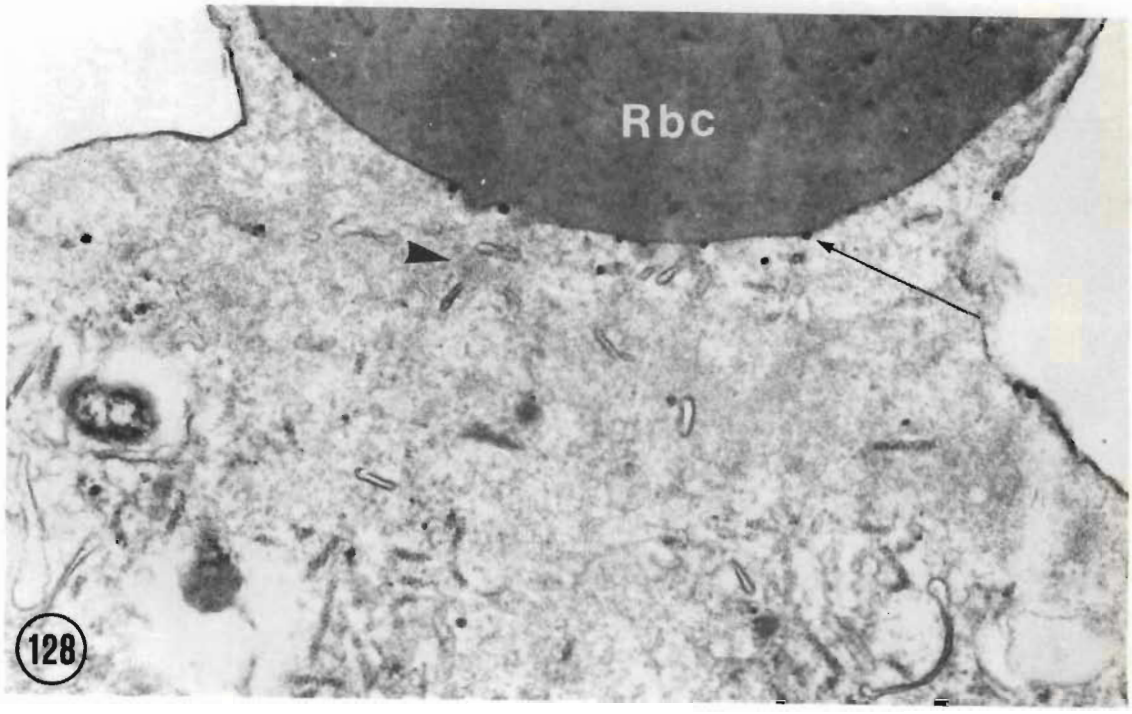
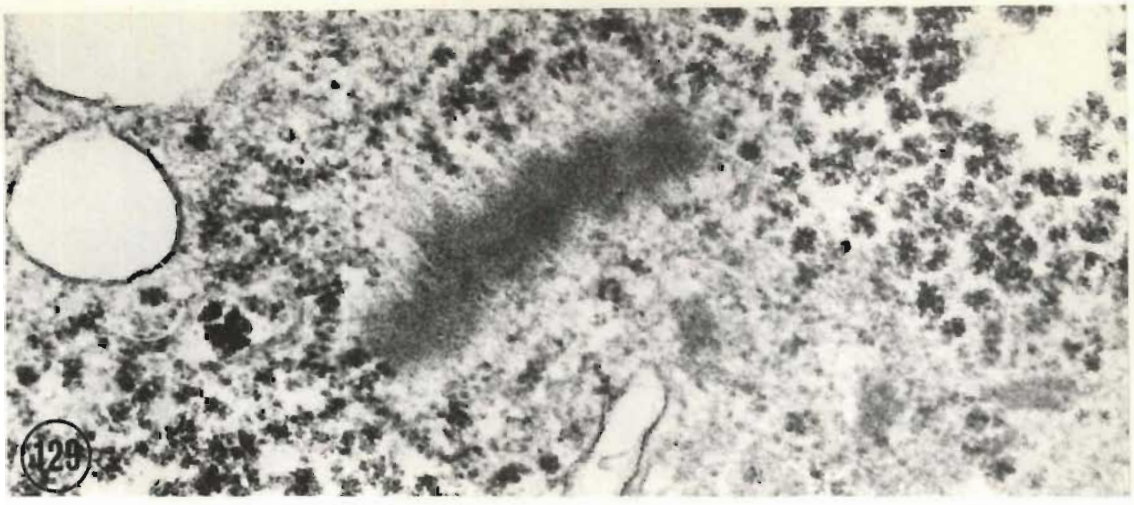

Rbc
128

Trophozoites of *E. histolytica* from hamster liver abscess

Fig. 129 Part of the cytoplasm of strain AN/68 showing a region of dense fibrous material. x 80 600

Fig. 130 A surface-active lysosome (L) with a trigger mechanism (T) was seen in trophozoites from strain AN/68. x 57 200

Fig. 131 Shows a large vacuole containing lipid globules (LG) in different stages of digestion and membranous material. Note the group of vacuoles (arrowed) protruding to the exterior. x 21 600



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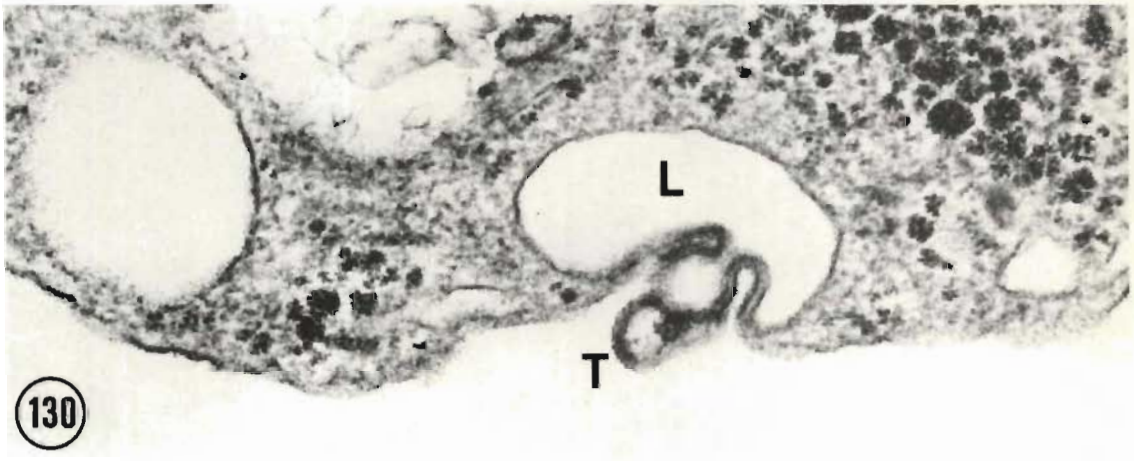

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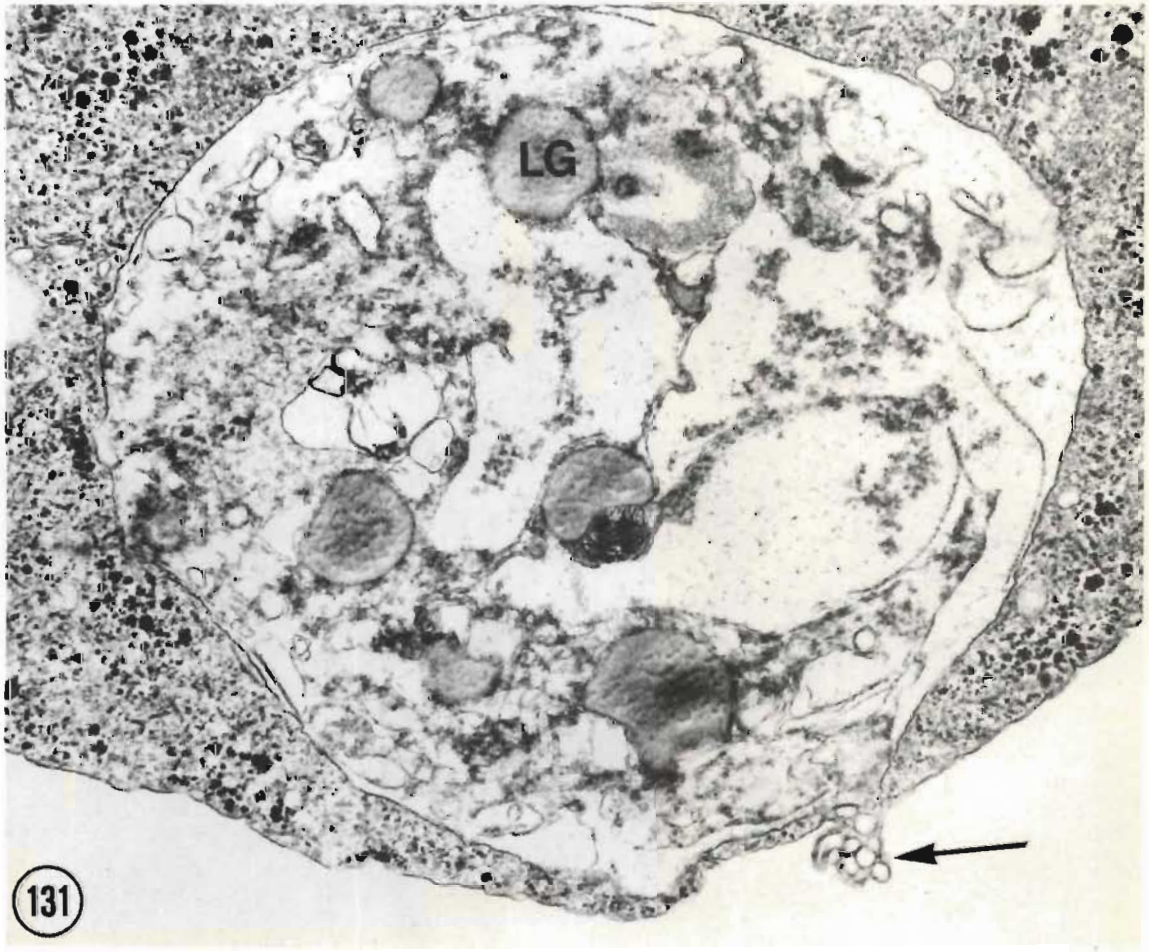
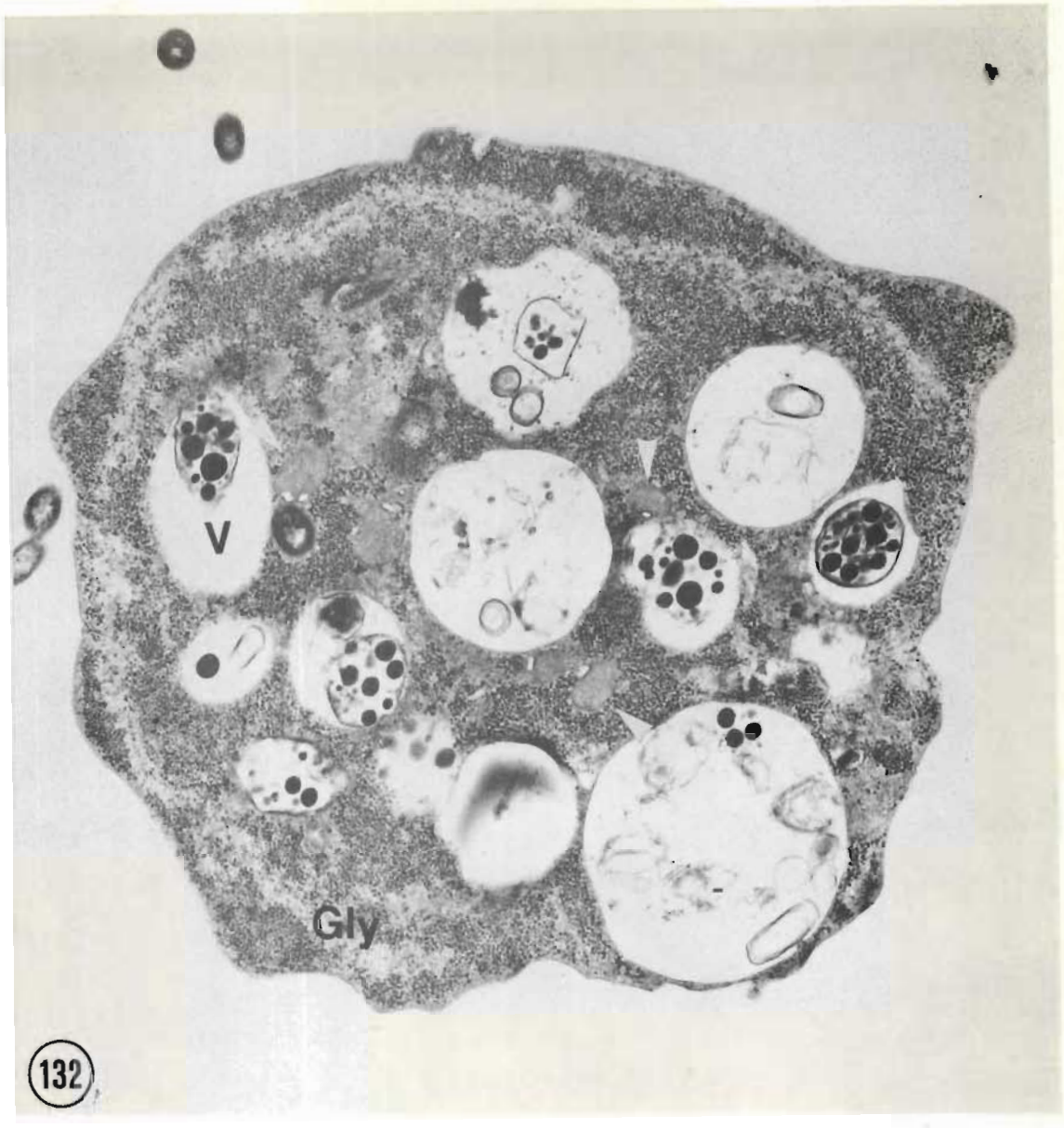


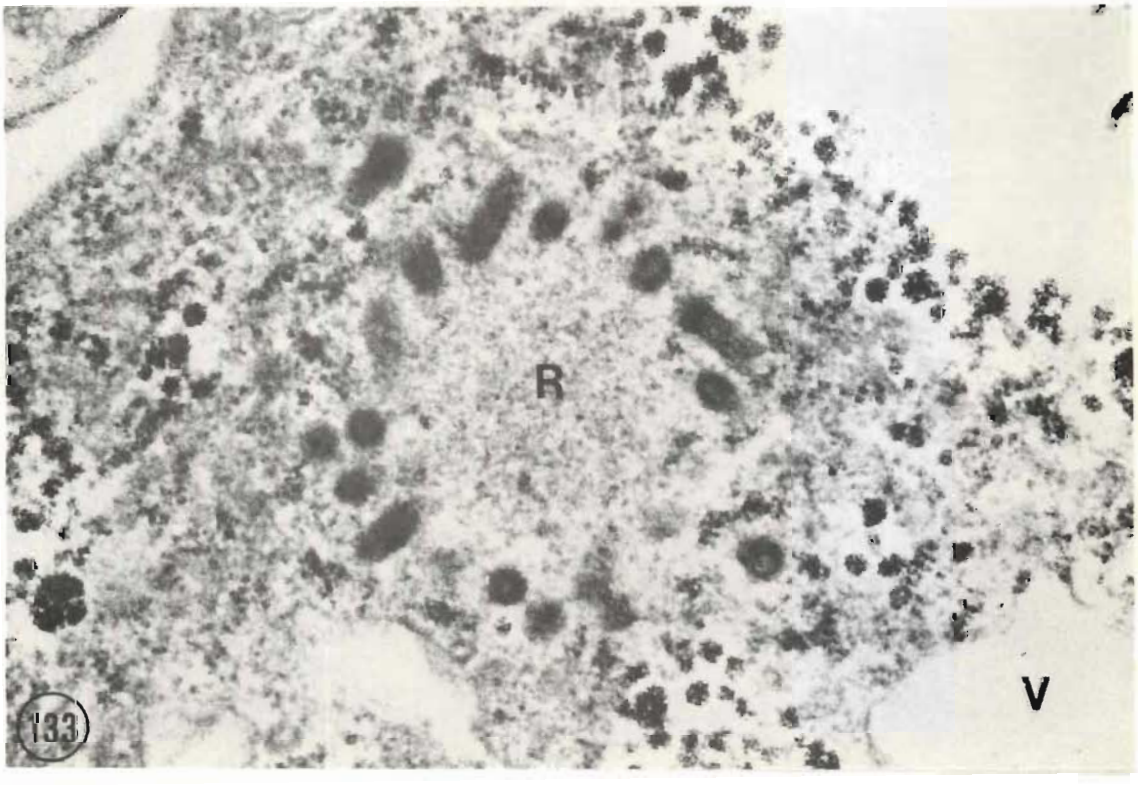
131

Trophozoites of *E. histolytica* from asymptomatic cases

Fig. 132 A trophozoite containing bacteria in different stages of digestion within the vacuoles (V), glycogen (Gly) and several dense bodies with a fine mesh appearance (arrowheads). x 12 150

Fig. 133 Shows the electron-dense cylindrical bodies in a rosette arrangement (R) in close proximity to vacuoles (V) in strain H 12. x 76 680


V
Gly
132

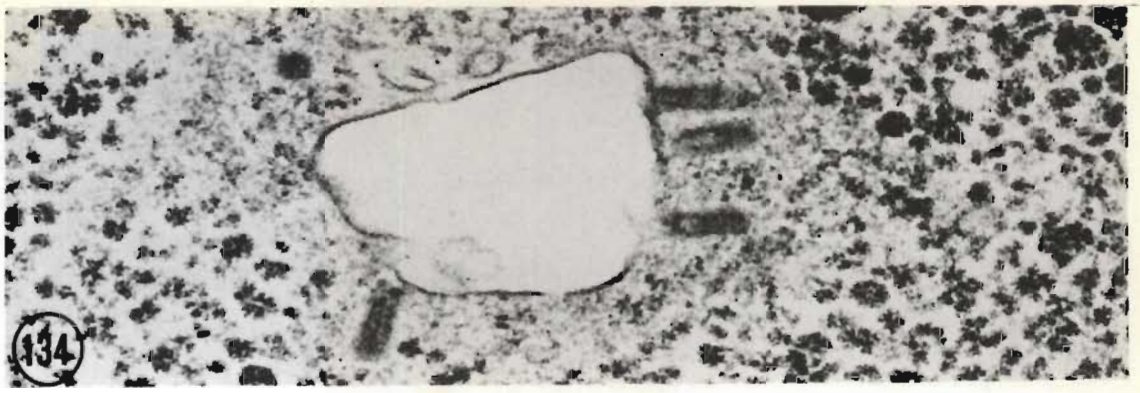

R
V
133

Trophozoites of *E. histolytica* from asymptomatic cases

Fig. 134 Shows several structures similar to the components which make up the rosette arrangement in close association with a vacuole. x 71 820

Fig. 135 Components similar to those shown in figure 134, in close proximity to the nuclear membrane (arrowed). x 66 560

Fig. 136 Shows the small dense bodies with no apparent clear central core (arrowed) within the nucleus (N) of strain H 12. Note the dense body (Db). x 64 960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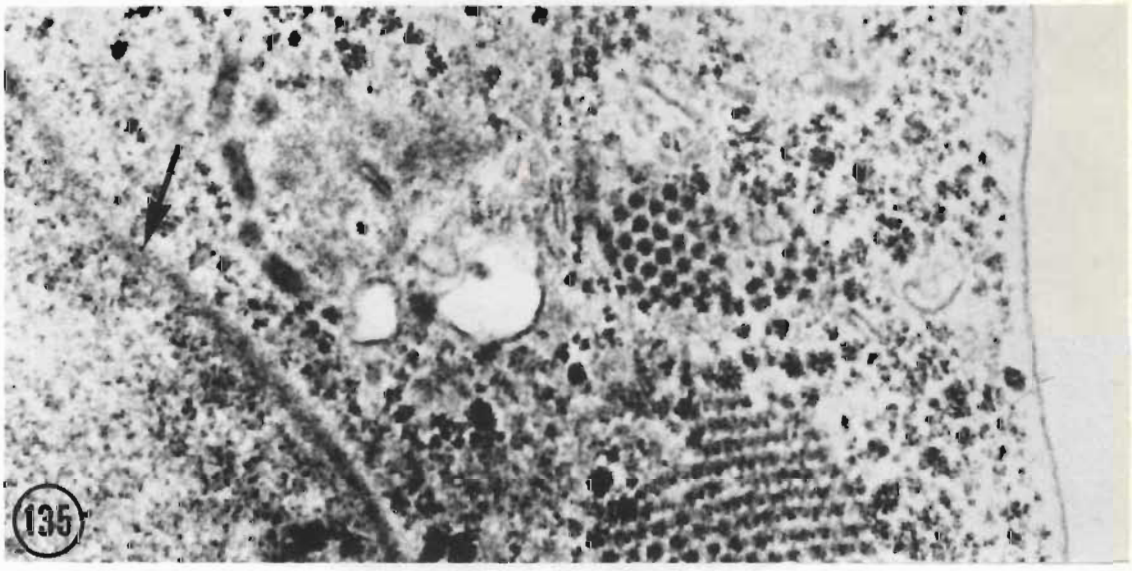
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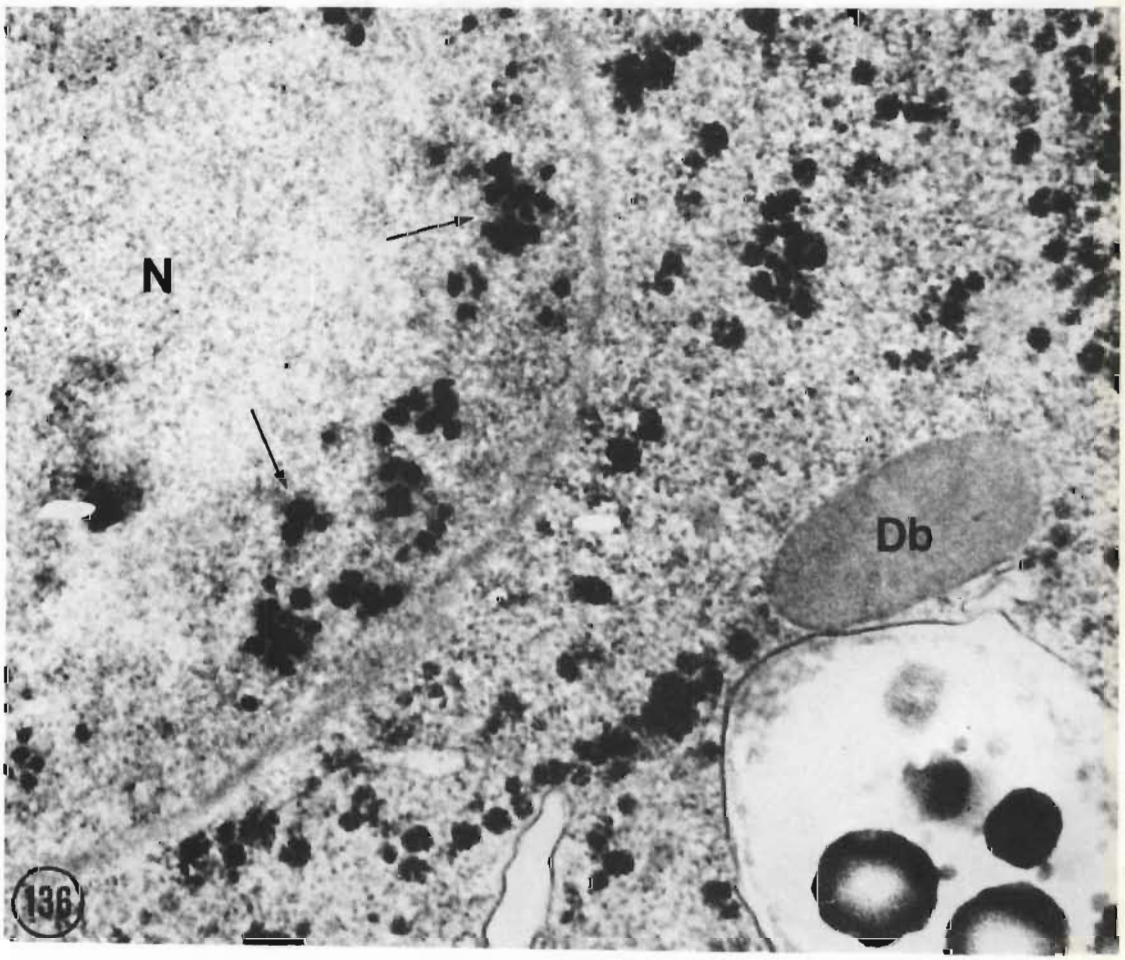
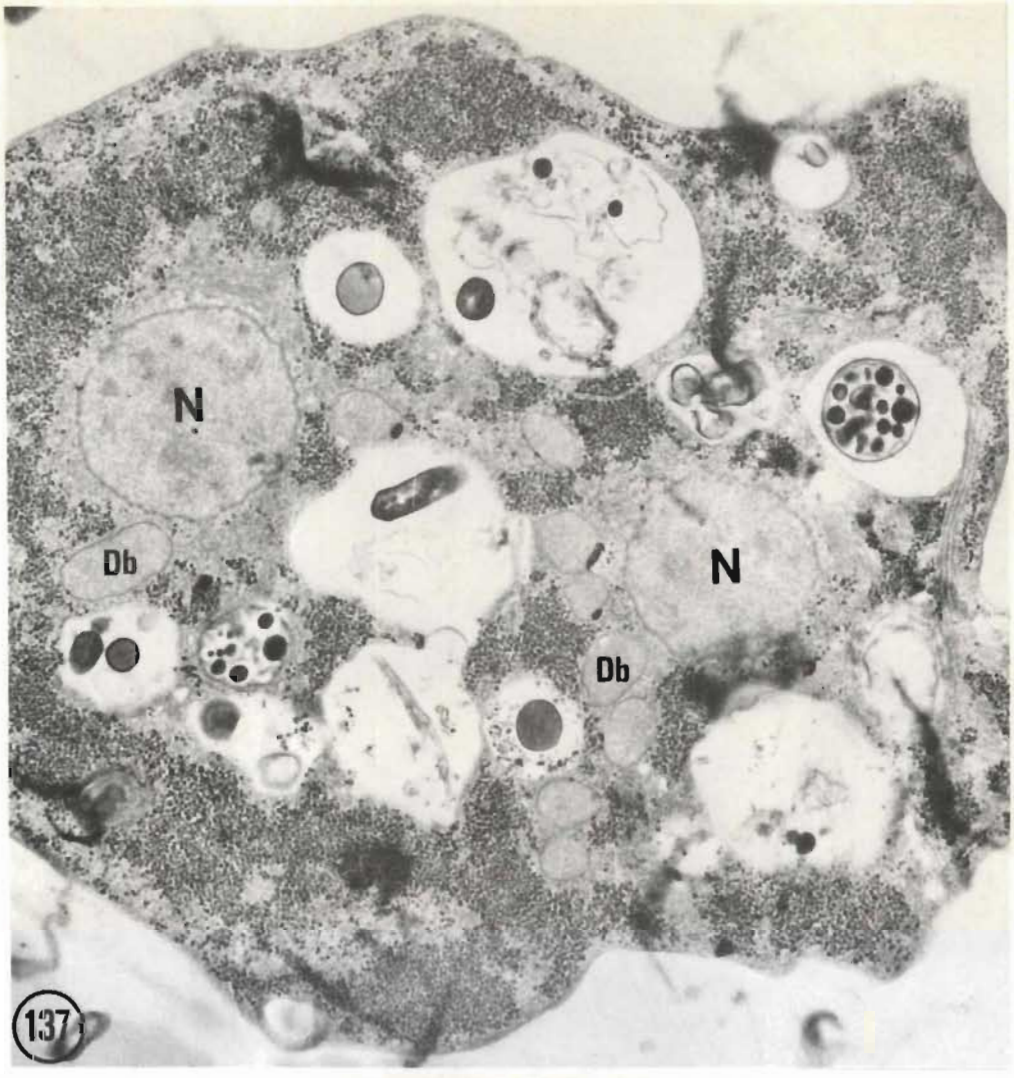

N
Db
136

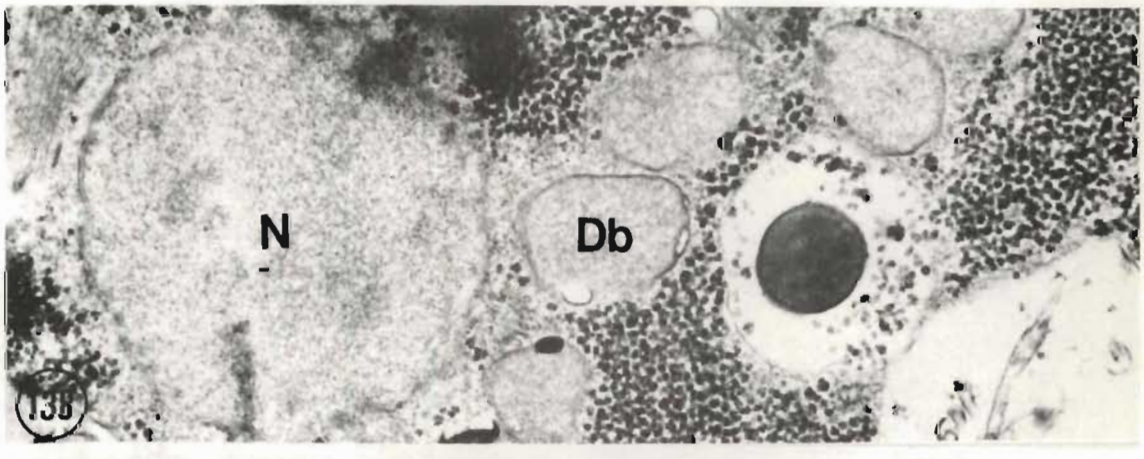
Trophozoites of *E. histolytica* from asymptomatic cases

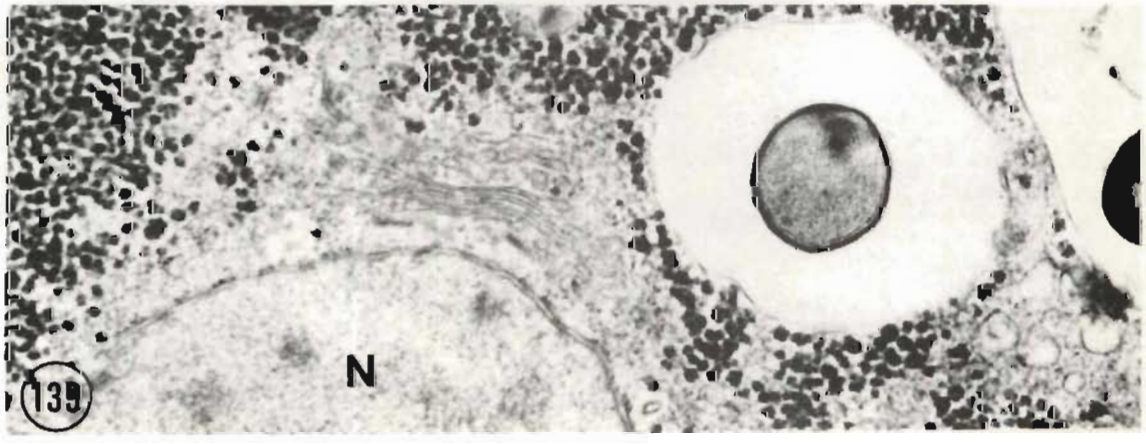
Fig. 137 A binucleate trophozoite containing numerous dense bodies (Db) bounded by membranes. The density of the bodies was similar to that of the nuclei (N). x 15 080

Fig. 138 High power showing the dense bodies (Db) in close proximity to a nucleus (N). x 33 800

Fig. 139 Shows a stack of smooth walled vesicles in close proximity to the nucleus (N). x 42 900


N
Db
N
Db
137

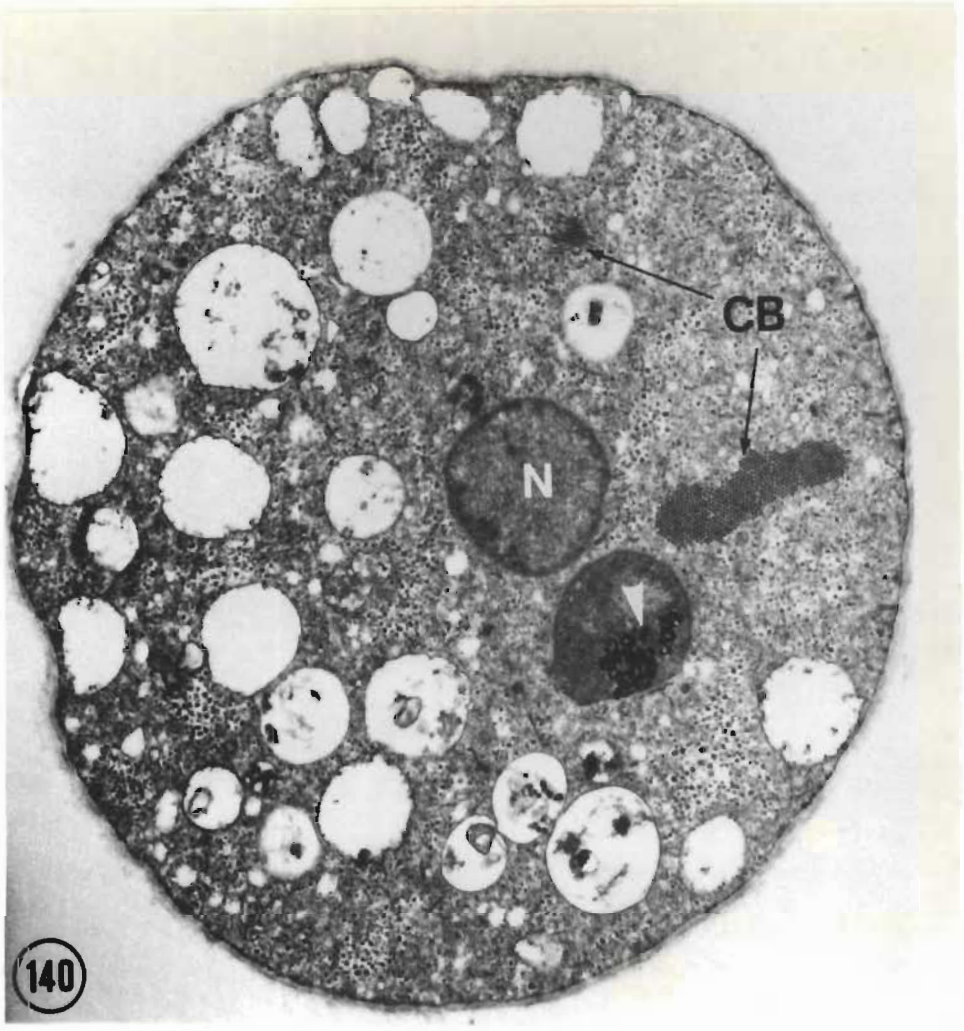

N
Db
138

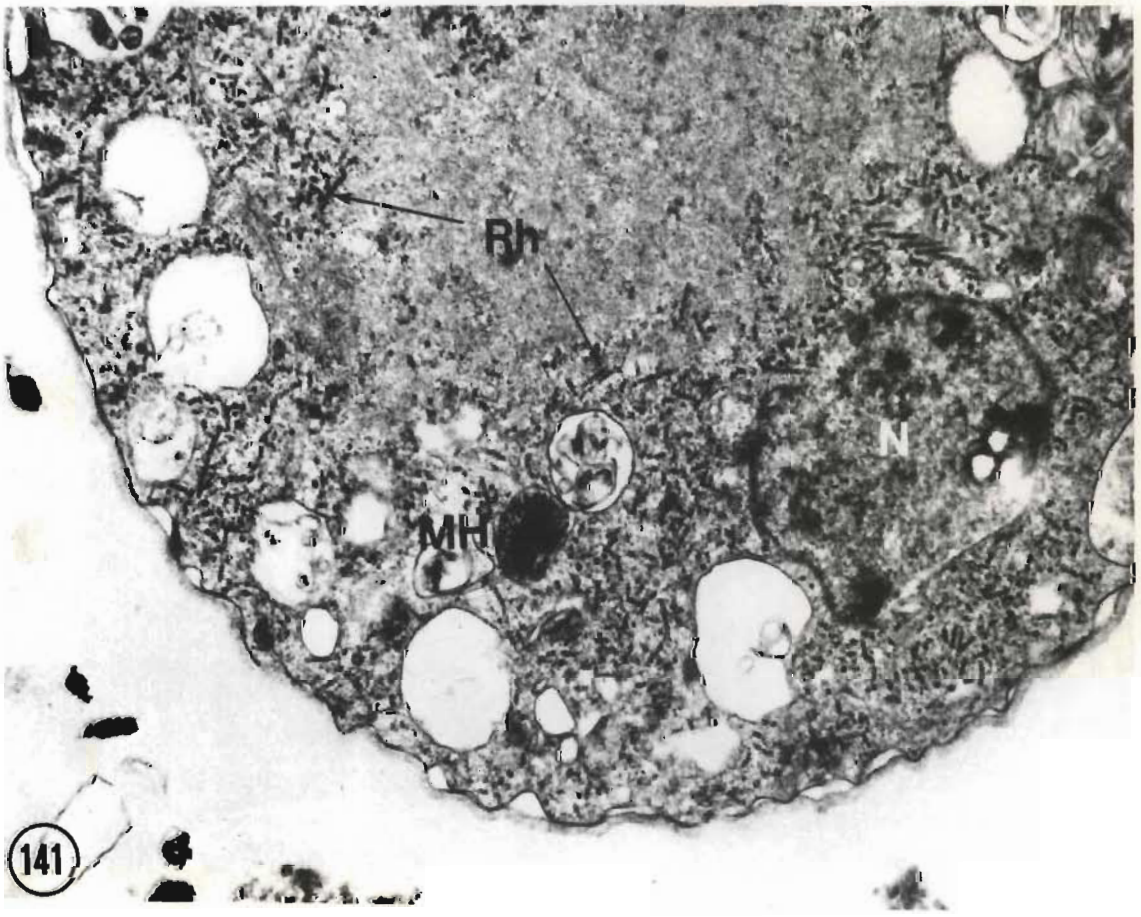

N
139

Cysts of *E. histolytica*

Fig. 140 A cyst showing two nuclei (N) one of which contains button bodies (arrowhead), numerous vacuoles and ribosomal helices in transverse and longitudinal section (CB). x 11 040

Fig. 141 Part of the cytoplasm showing individual helices (Rh) membrane-bound helices (MH) numerous vacuoles and a nucleus (N). x 21 850


CB
N
140

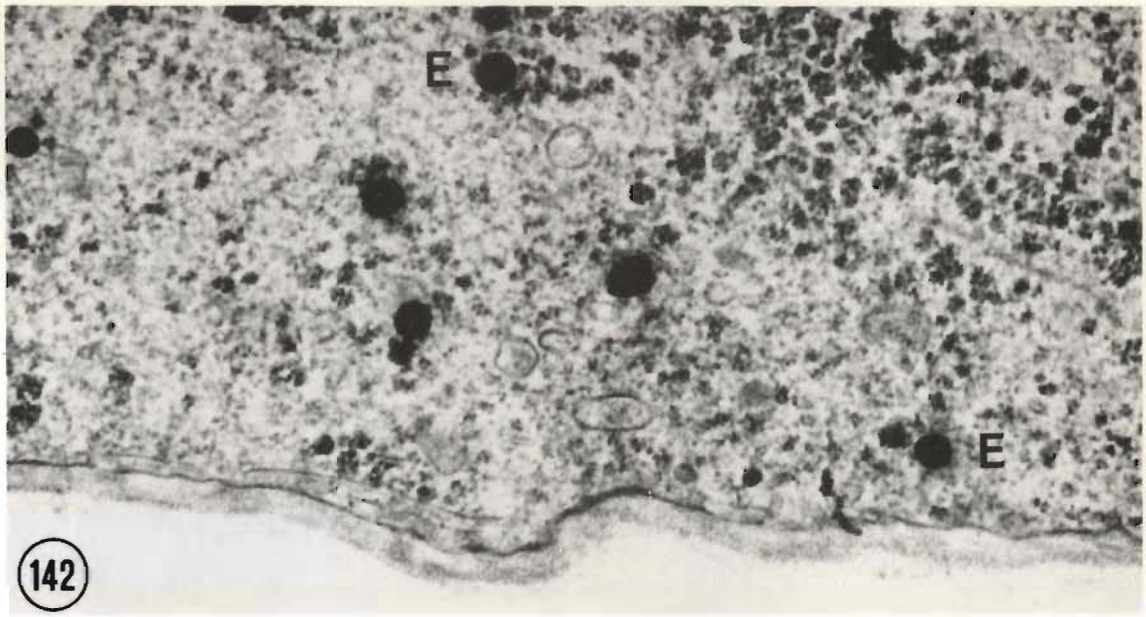

Rh
N
MH
141

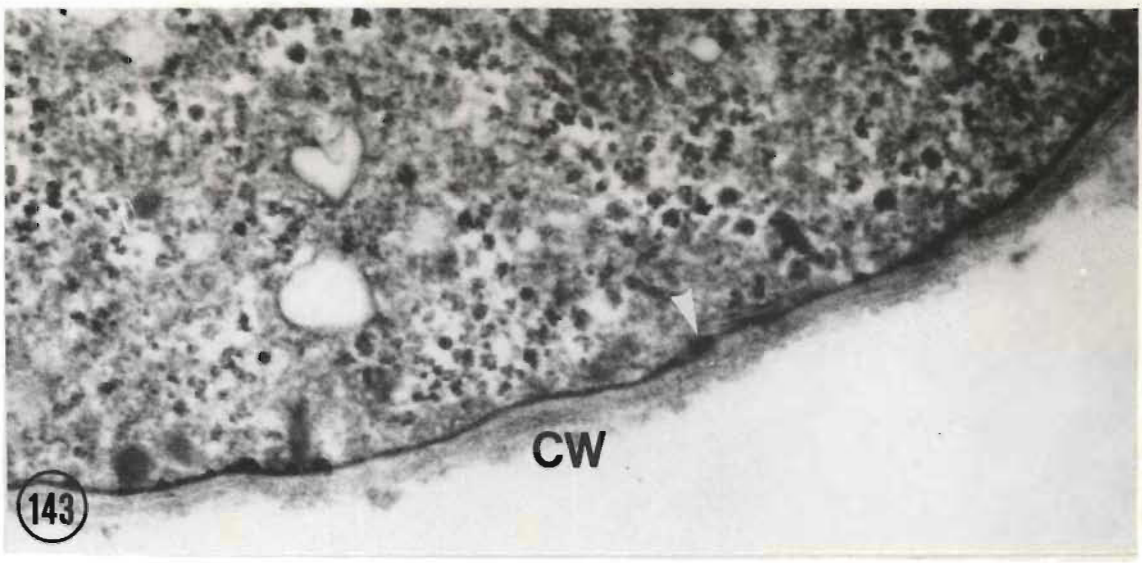
Cysts of *E. histolytica*

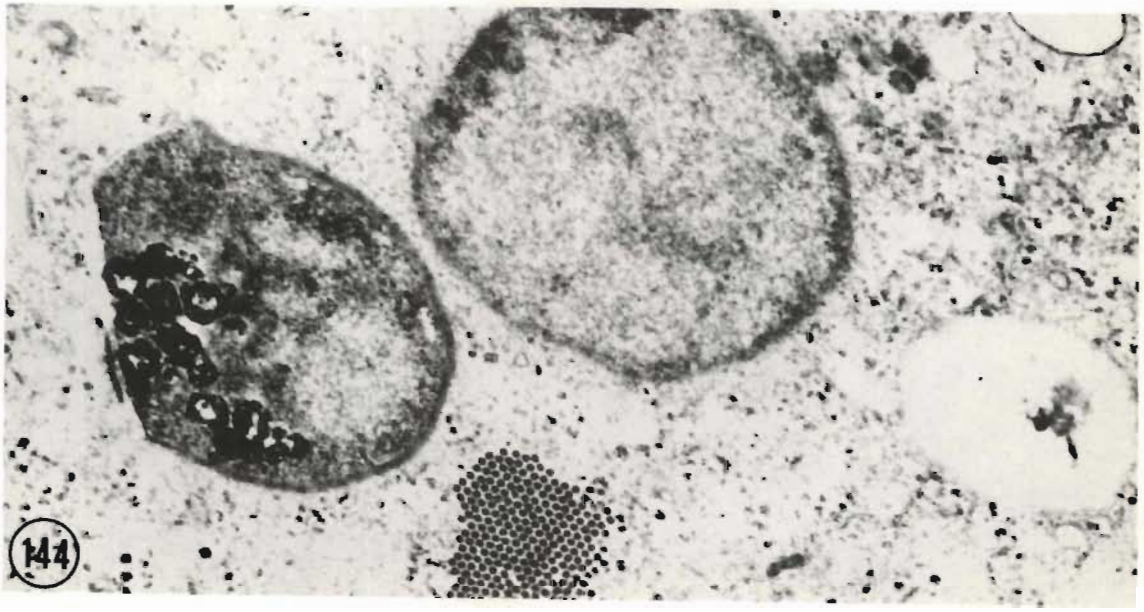
Fig. 142 Part of the cytoplasm showing the electron-dense bodies (E) comprising a dense outer region and a less dense central core. x 49 950

Fig. 143 High power of a portion of the cyst wall (CW) showing the outer amorphous layer and the inner fibrous layer. Note the osmiophilic bodies (arrowhead) on the inner leaflet of the plasmalemma. x 51 480

Fig. 144 Shows the button bodies within the nucleus. x 29 640


E
E
142


CW
143


144

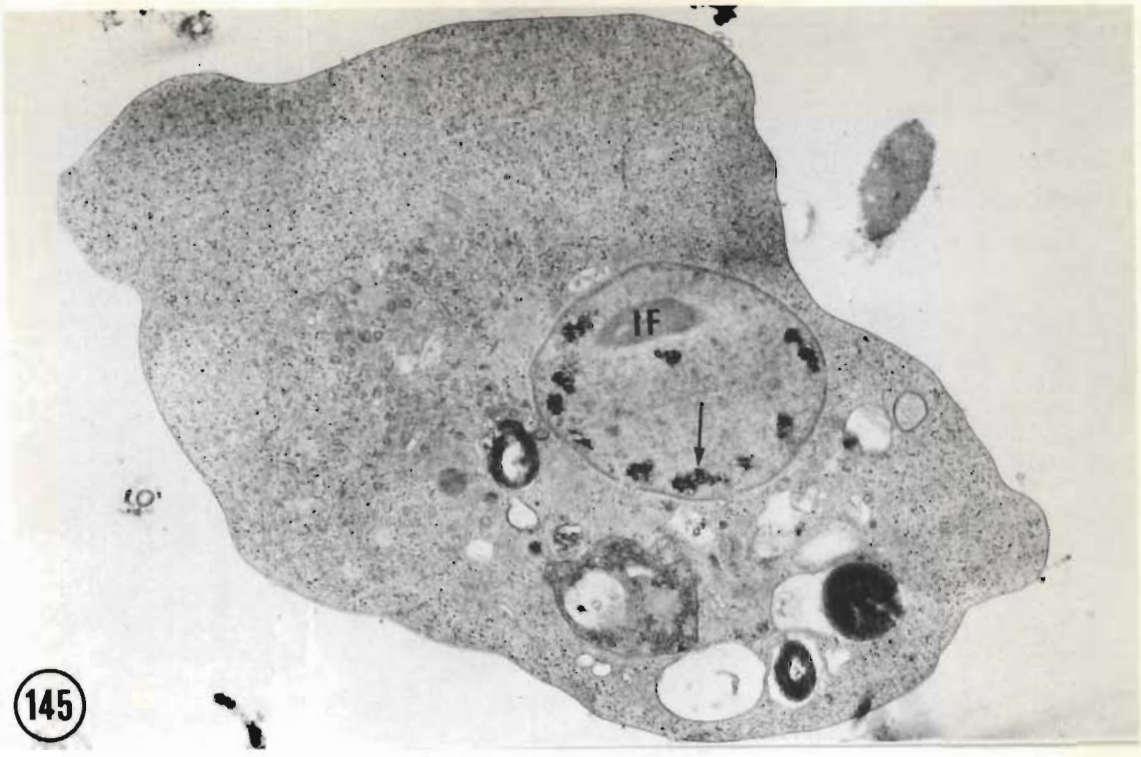
Trophozoites of *E. coli*

Fig. 145 A trophozoite from the human colon showing a nucleus containing button bodies (arrowhead) and a filamentous virus-like structure (IF). x 7 830

Fig. 146 Shows an osmiophilic body similar to those seen in trophozoites of *E. histolytica*. x 59 360

Fig. 147 Shows the 'fuzzy' coat on the outer surface of the plasmalemma of a trophozoite from the colon. x 89 960

Fig. 148 Shows the smooth surface of the plasmalemma of trophozoites from culture. 76 440



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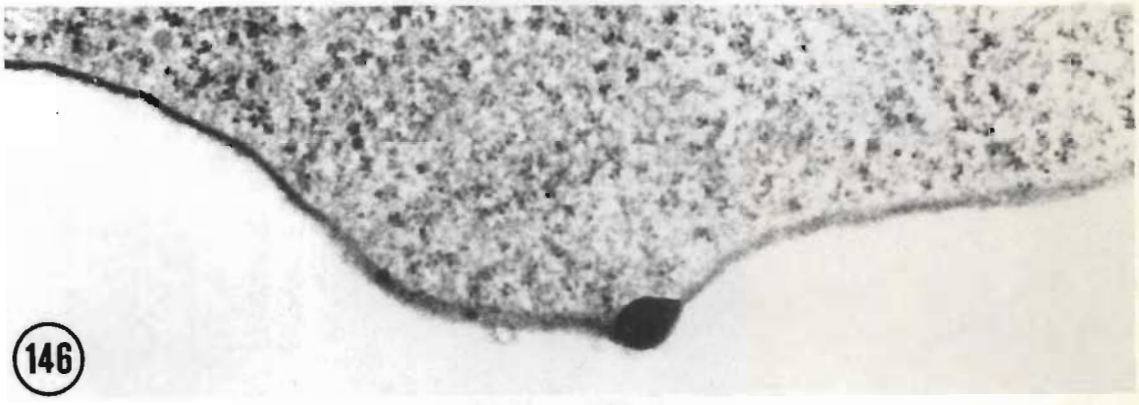

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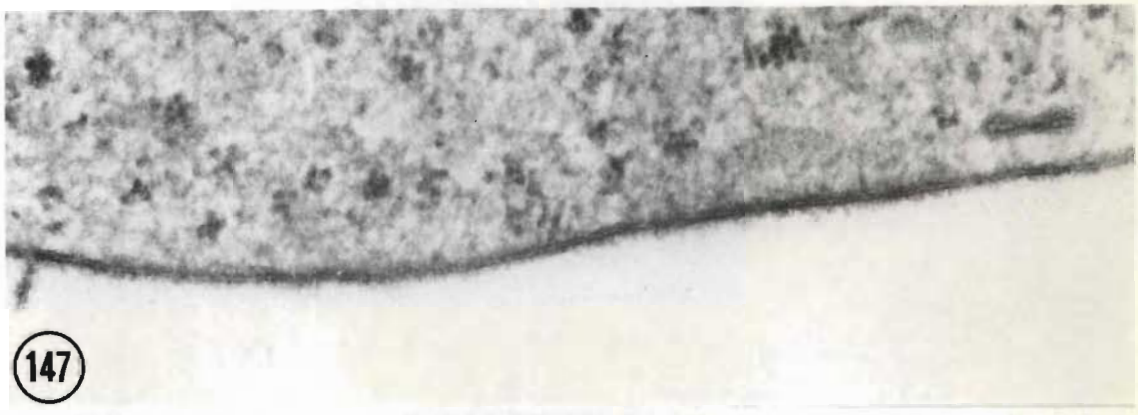

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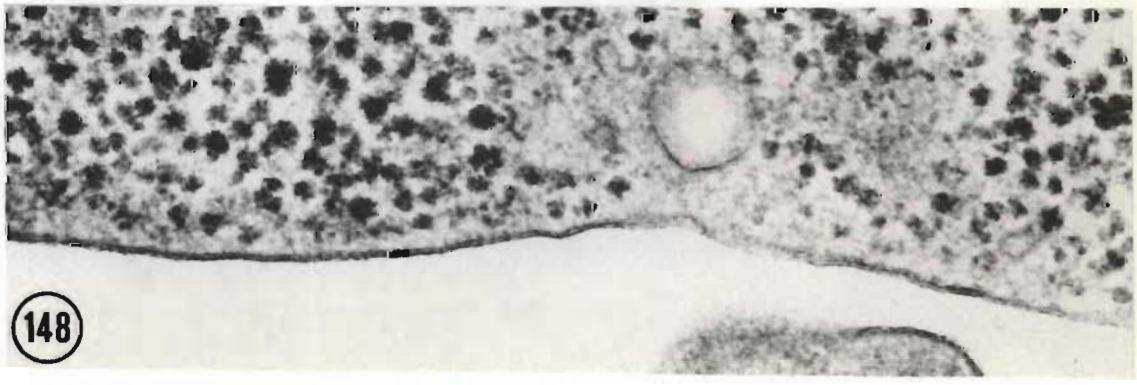
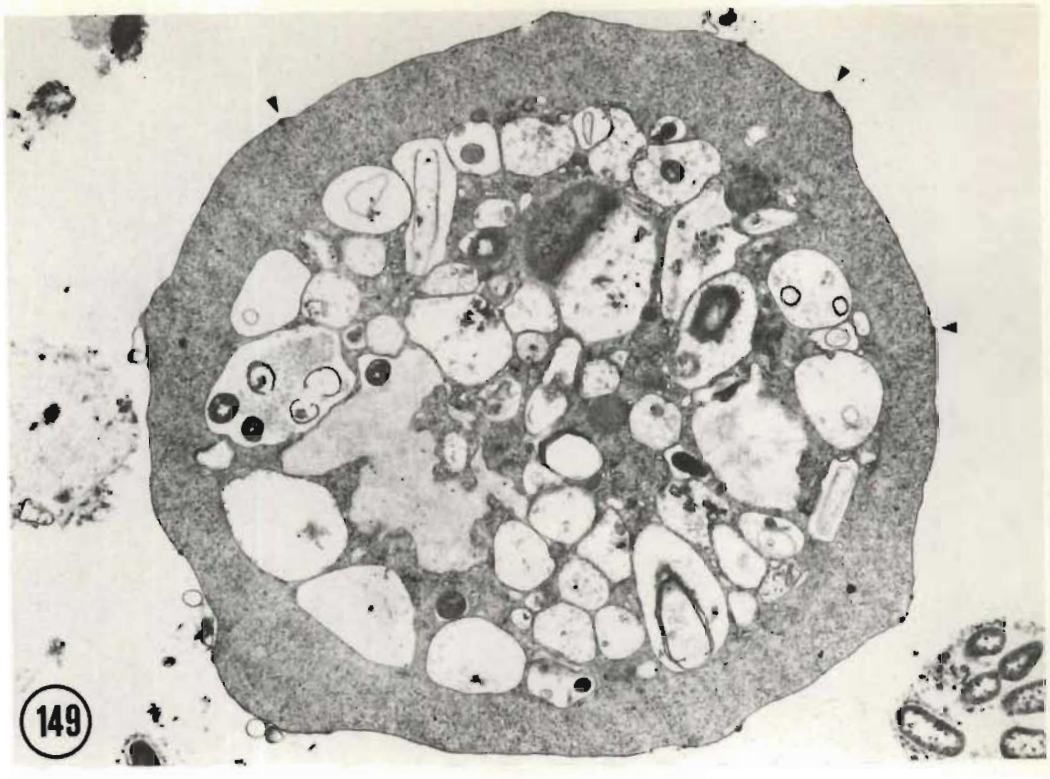


148

Trophozoites of *E. coli*

Fig. 149 A trophozoite from the colon in which the organelles are aggregated in the centre. Note the osmiophilic bodies beneath the plasmalemma (arrowheads). x 7 560

Fig. 150 Shows the concentration of glycogen (Gly) separating the ectoplasm (EC) from the endoplasm (EN). x 33 600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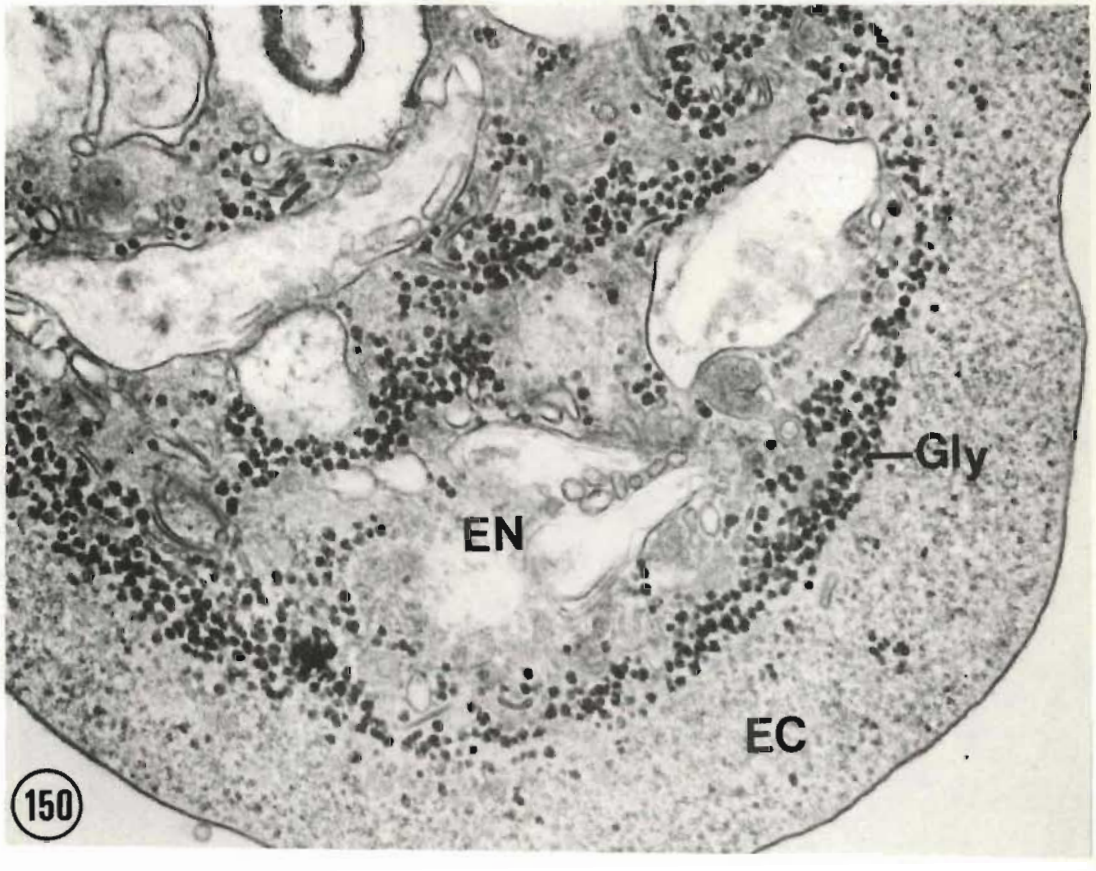
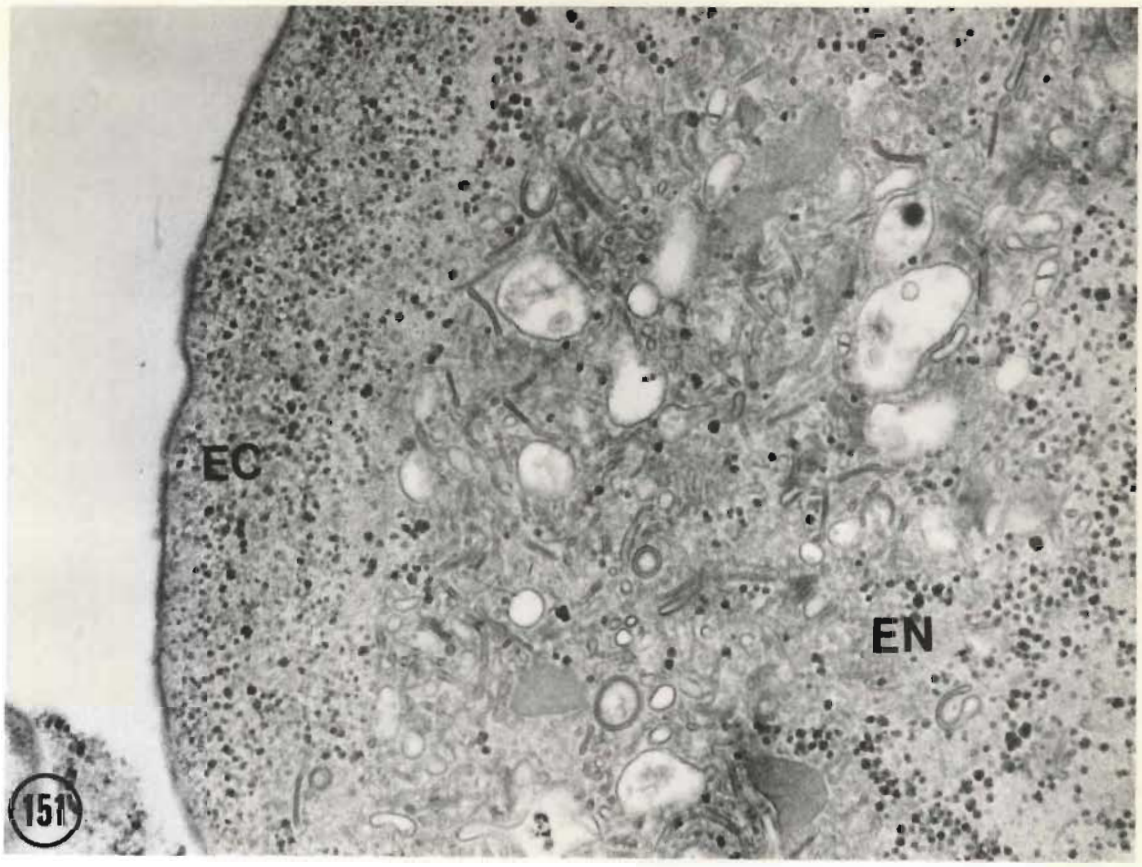
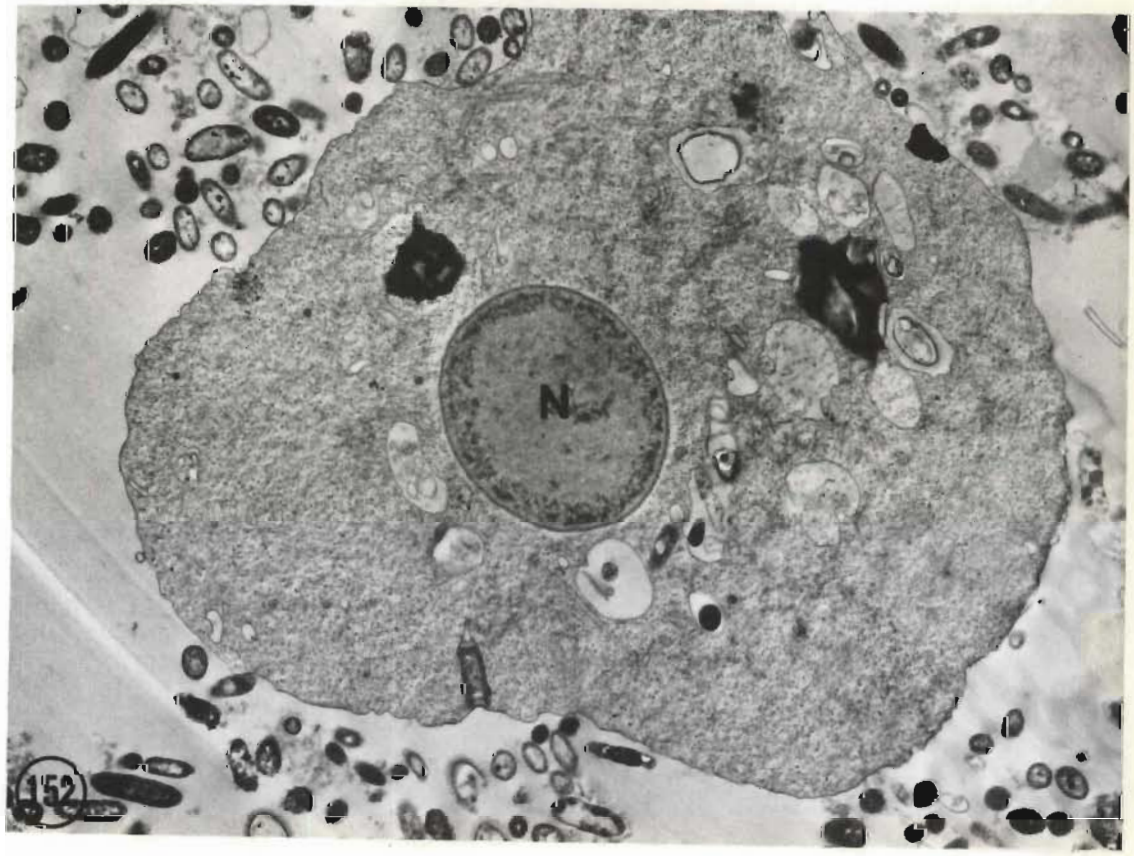

Gly
EN
EC
150

Trophozoites of *E. coli*

Fig. 151 Shows a concentration of small vesicles separating the ectoplasm (EC) from the endoplasm (EN). x 28 500

Fig. 152 A trophozoite from culture containing a nucleus (N) and showing the even distribution of the organelles. x 6 600


EC
EN
151

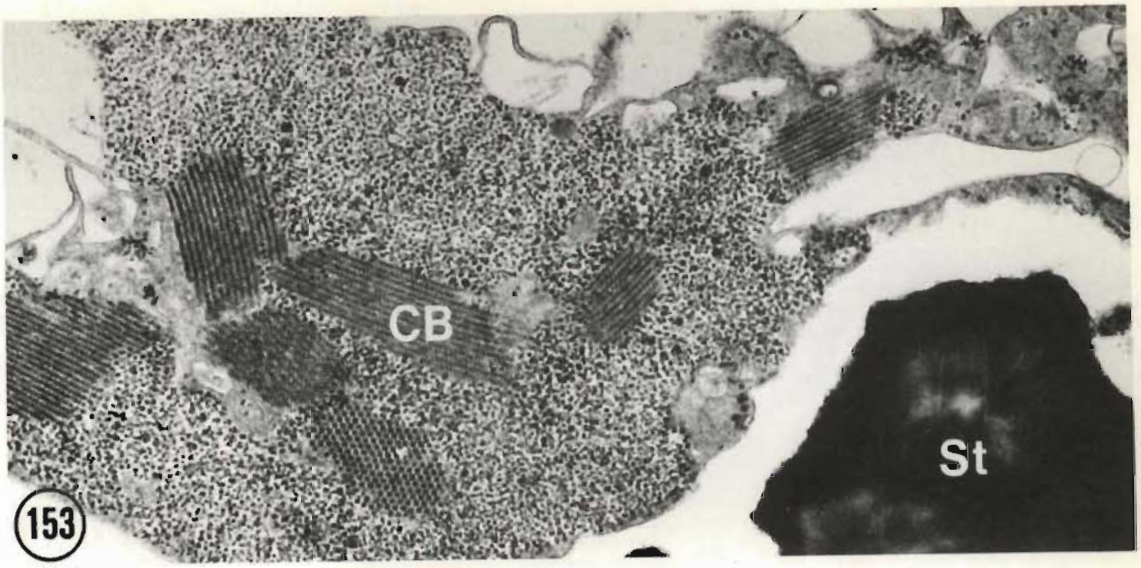

N
152

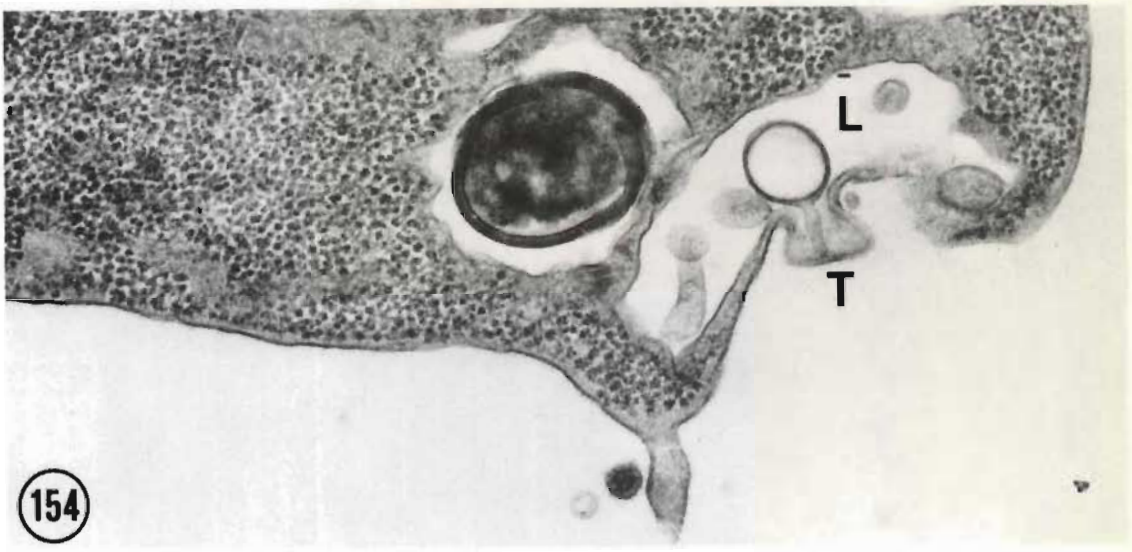
Trophozoites of *E. coli*

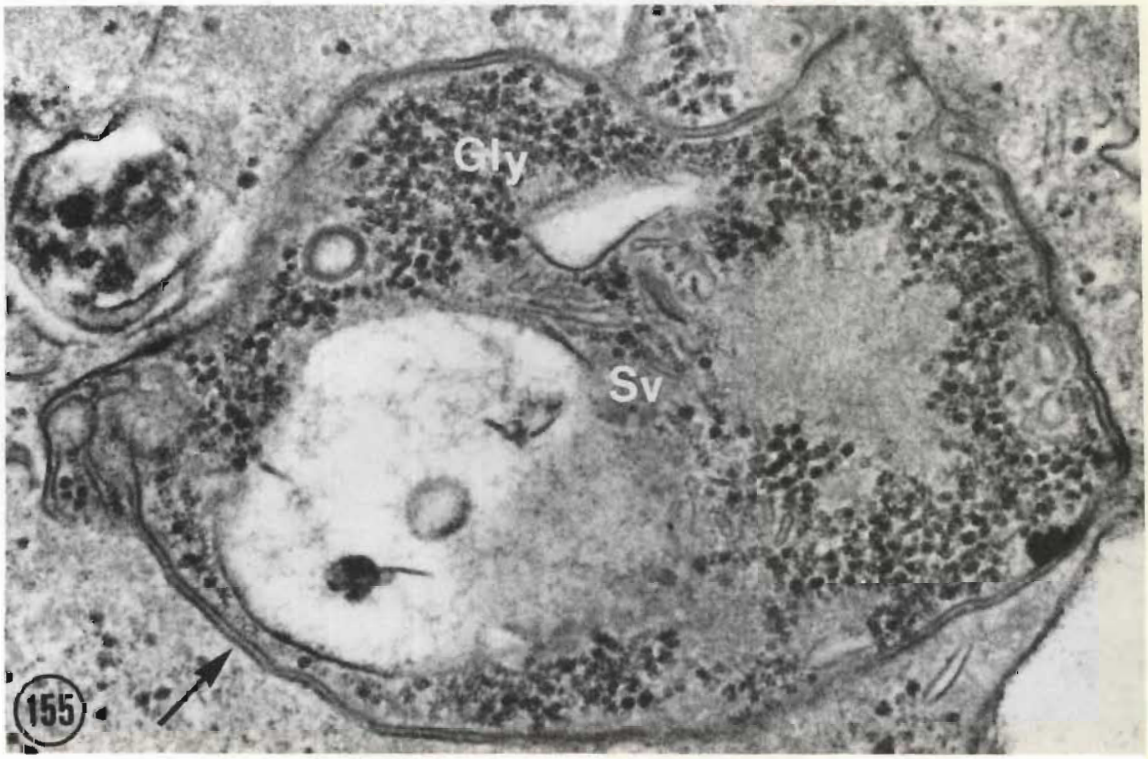
Fig. 153 Part of the cytoplasm showing numerous crystalloid aggregates (CB) and a vacuole containing a starch granule (St). x 20 160

Fig. 154 Part of a trophozoite from culture showing a lysosomal vacuole (L) with a trigger mechanism (T). x 39 900

Fig. 155 Shows a region of glycogen (Gly) and small vesicles (Sv) bound by two unit membranes (arrowed). x 53 360


CB
St
153


L
T
154


Gly
Sv
155

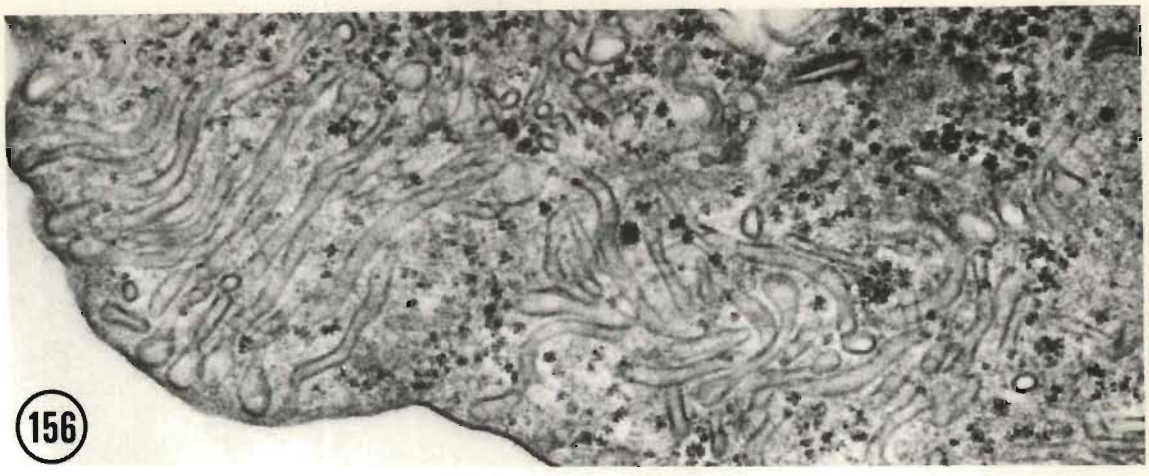
Trophozoites of *E. coli*

Fig. 156 Shows the concentrations of hollow membranous structures bearing a close resemblance to smooth endoplasmic reticulum. x 53 360

Fig. 157 Transverse section through the membranous structures. x 125 860

Fig. 158 Shows the elongated profiles of smooth walled vesicles. x 125 860

Fig. 159 Shows a stack of smooth walled vesicles which appear to be slightly swollen at their distal ends. Note the electron-dense body in close association with the vesicles. x 64 380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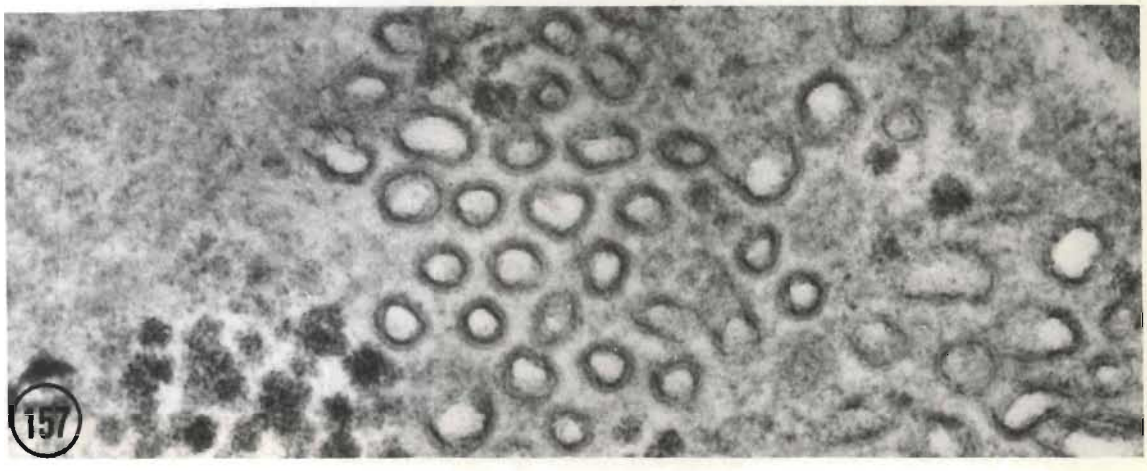
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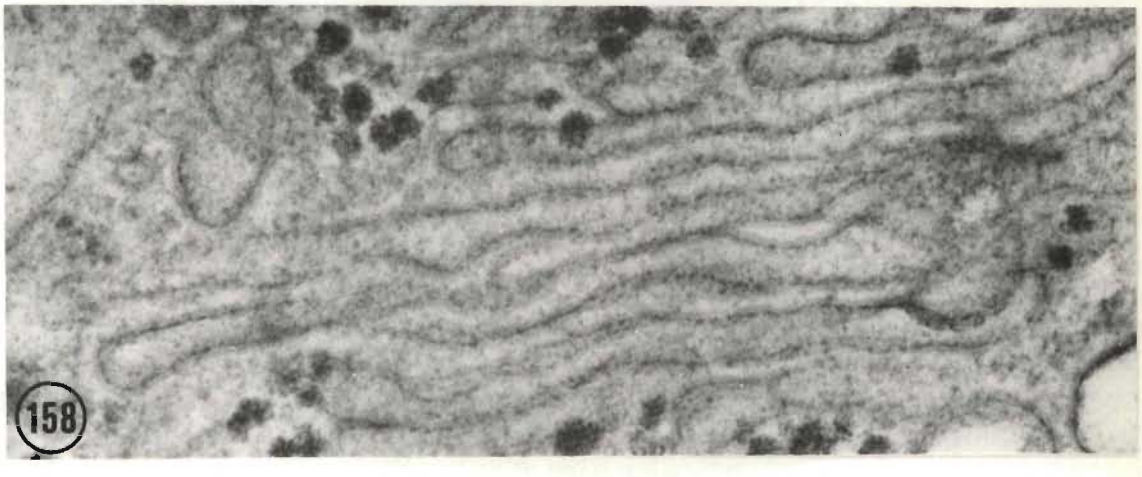
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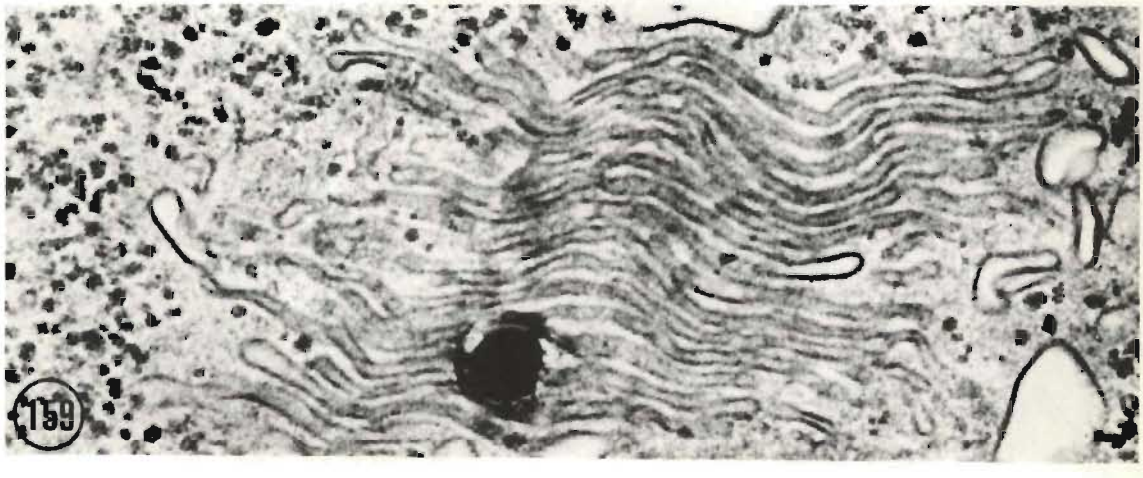

159

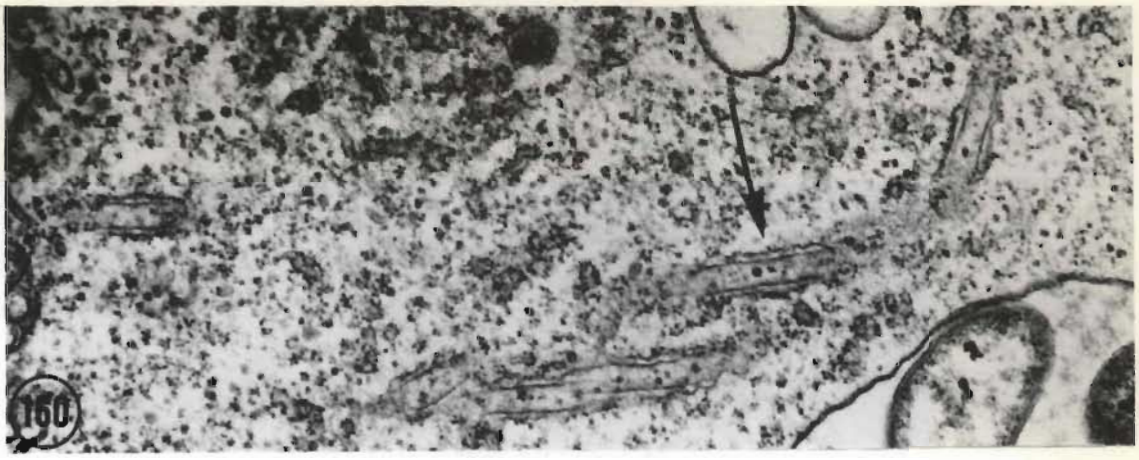
Trophozoites of *E. coli*

Fig. 160 Shows the double-walled tubules (arrowed) seen in the cytoplasm of strains C2 and C5. x 24 650

Fig. 161 Shows strands of unit membrane at right angles to the plasmalemma. x 98 600

Fig. 162 Shows the complex arrangement of unit membrane on the surface and in the cytoplasm. x 132 000

Fig. 163 High power showing the association between the unit membranes on the surface and within the cytoplasm. x 134 500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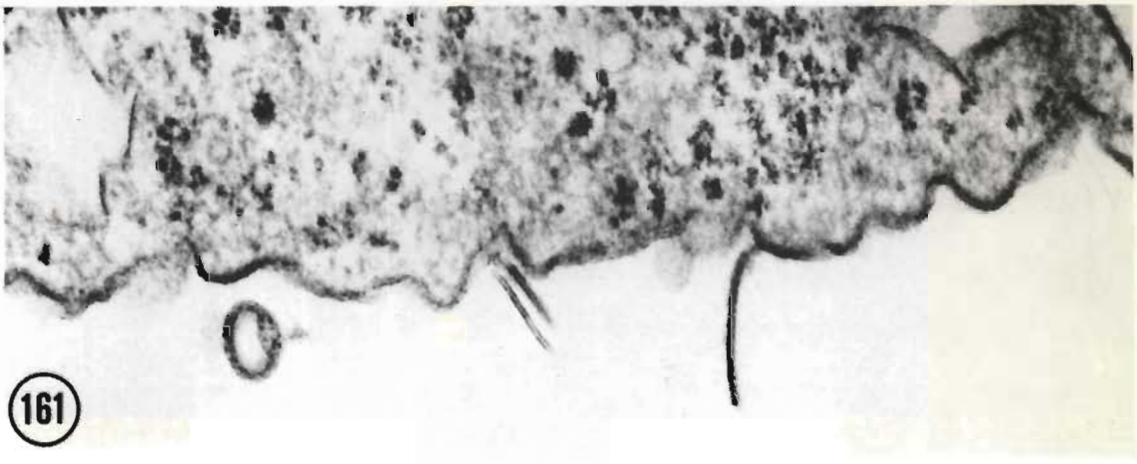
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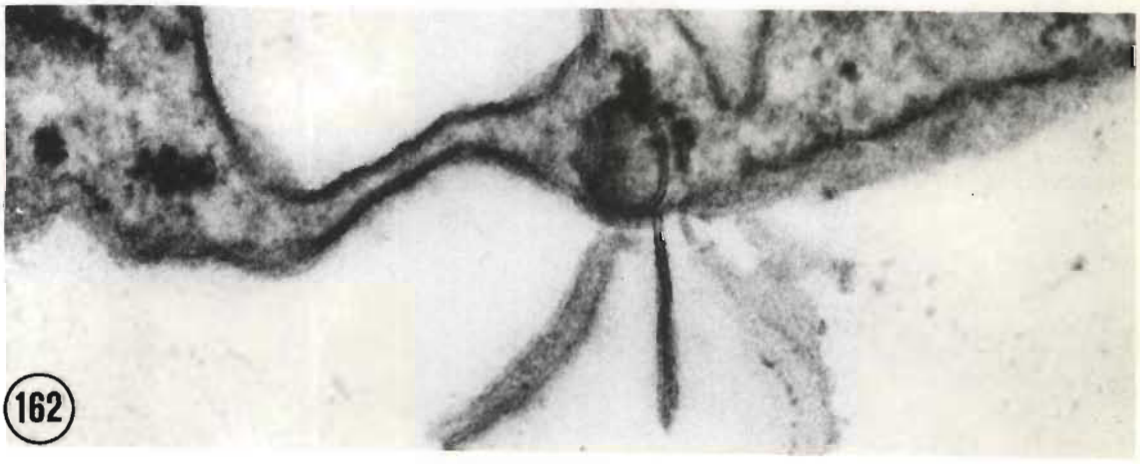
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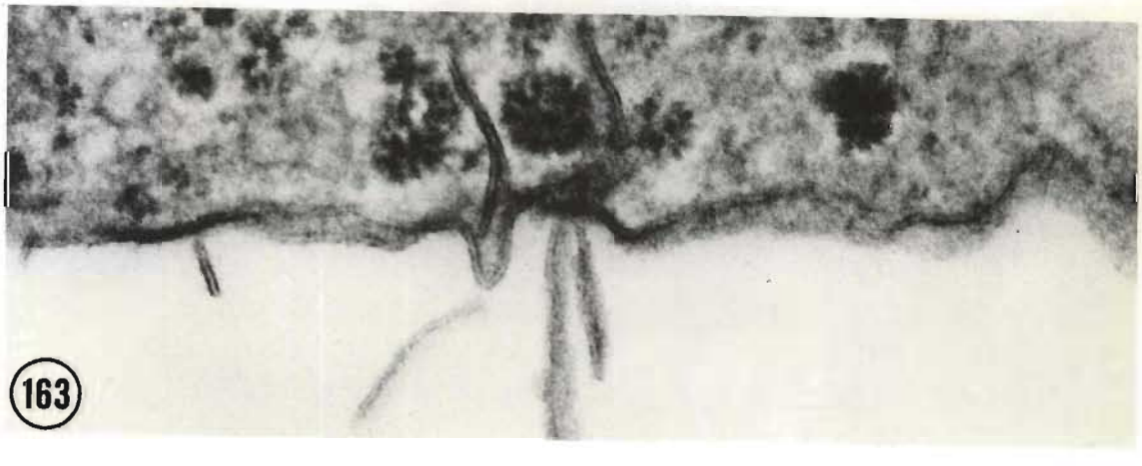
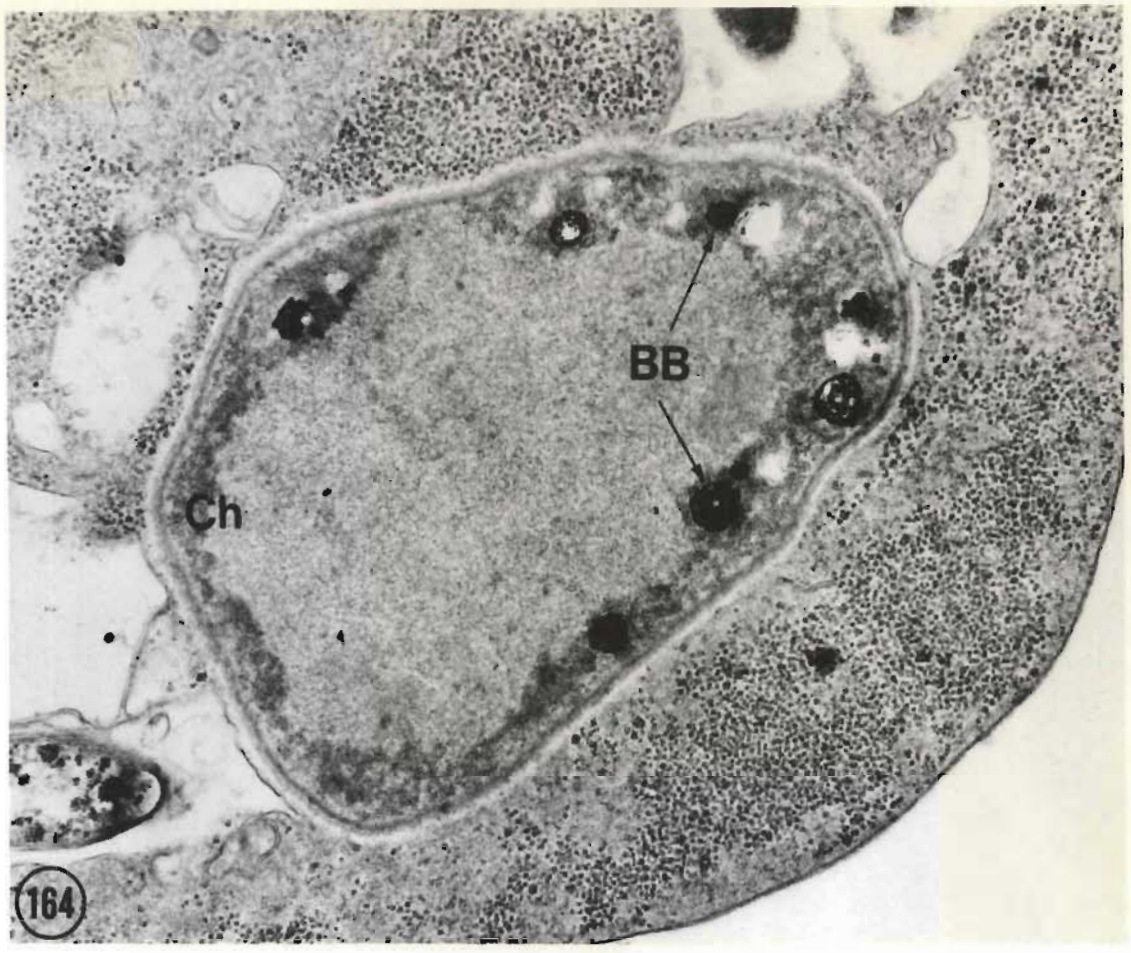
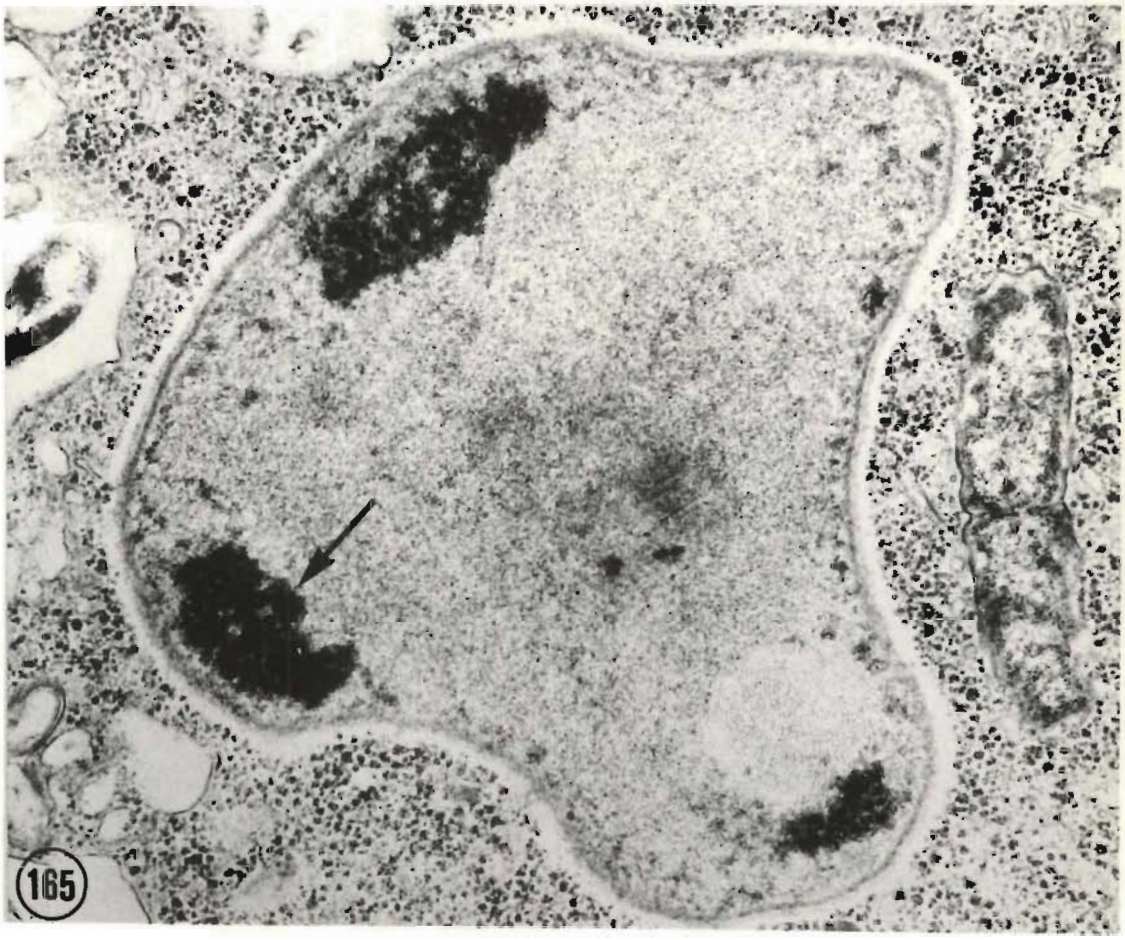

163

Trophozoites of *E. coli*

Fig. 164 Shows the even distribution of the chromatin (Ch) at the periphery of the nucleus. Note the button bodies (BB) and the clear region surrounding the entire nucleus. x 27 550

Fig. 165 Shows the chromatin confined to a few clumps which are entirely filled with intranuclear bodies (arrowed). x 27 550

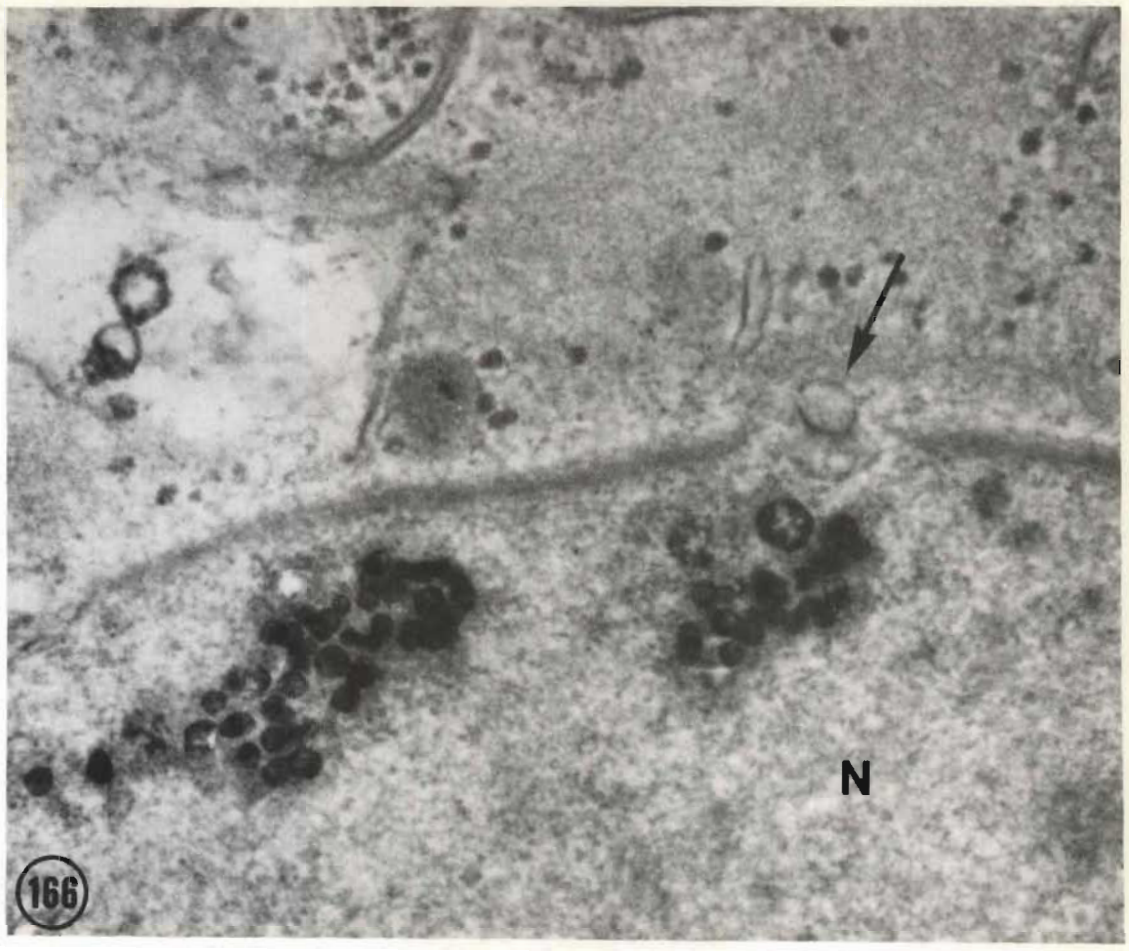

BB
Ch
164

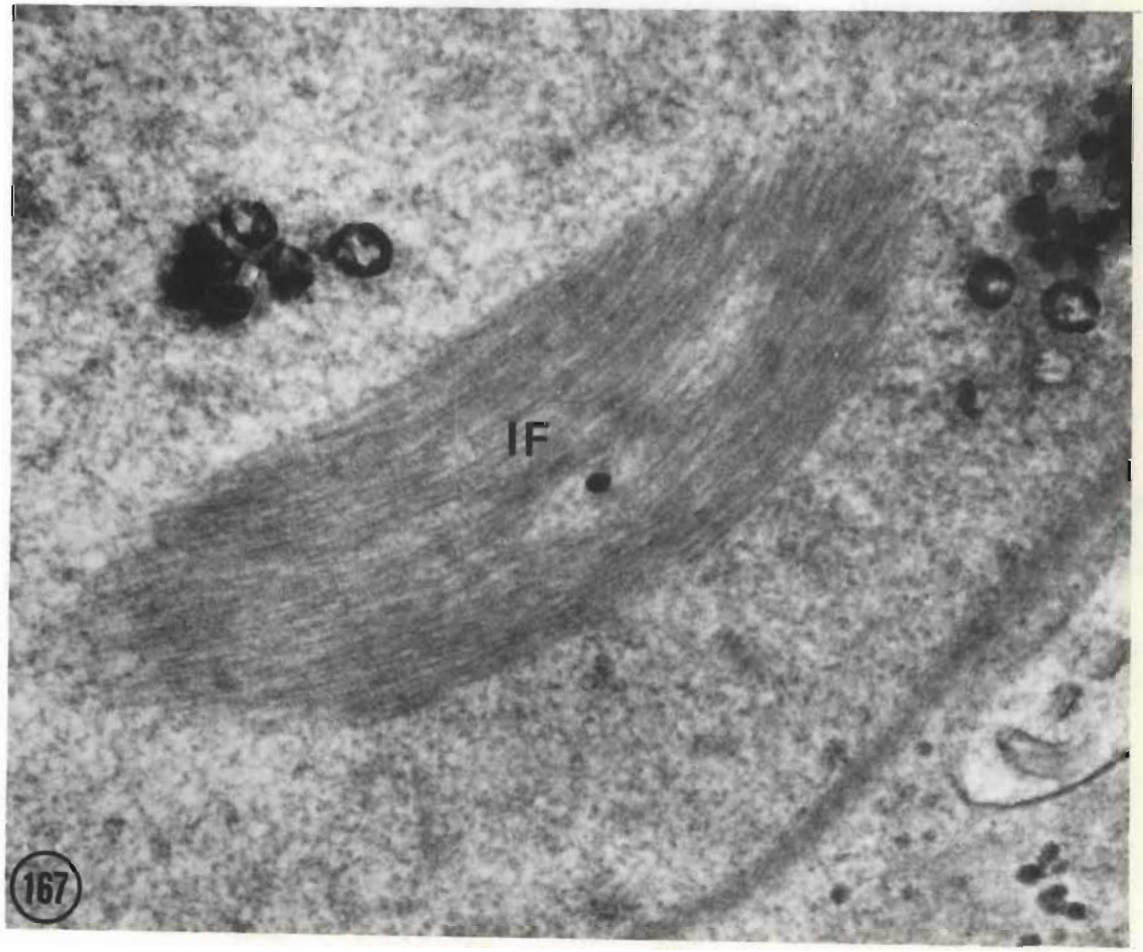

165

Trophozoites of *E. coli*

Fig. 166 Shows the nucleus (N) containing button bodies and an apparently empty body (arrowed) traversing the nuclear membrane possibly through a nuclear pore. x 69 000

Fig. 167 High power of the filamentous virus-like structure (IF) within the nucleus. x 69 000

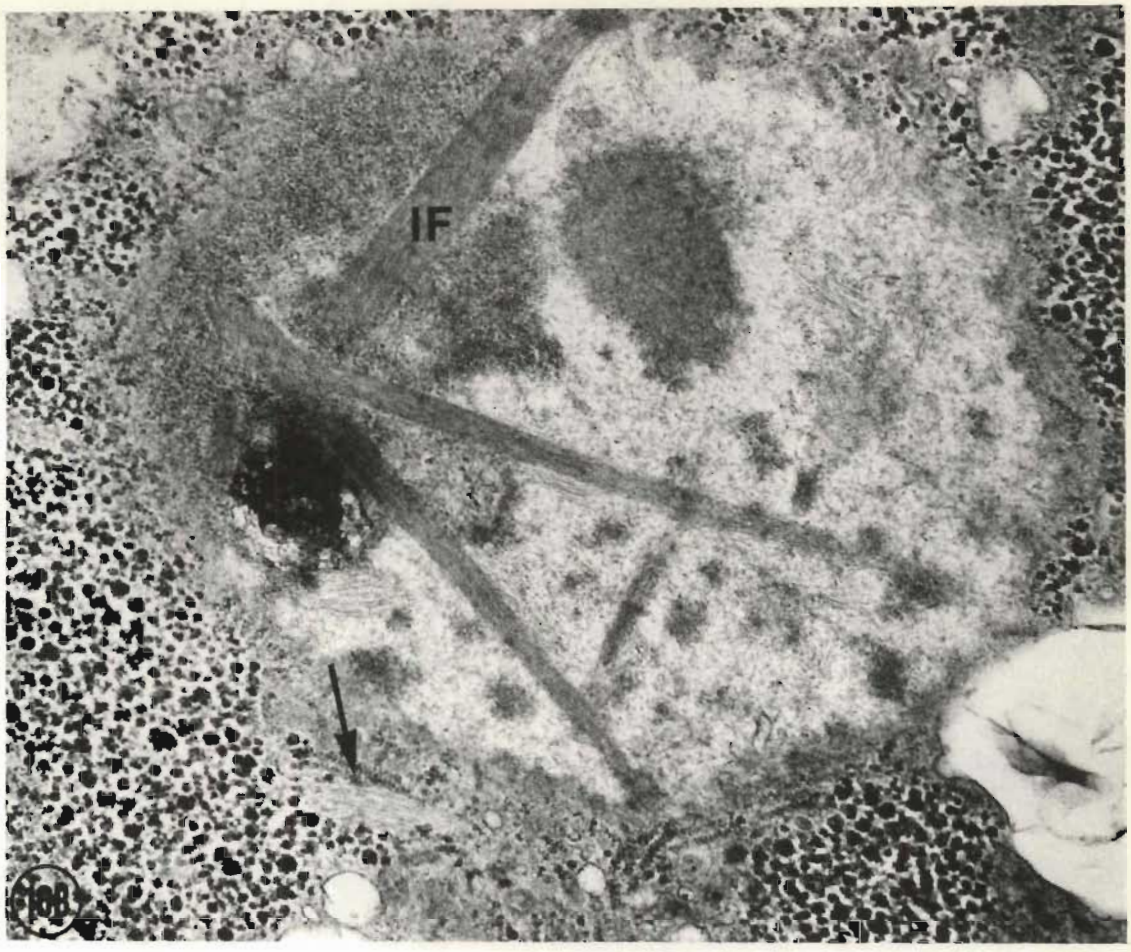

N
166

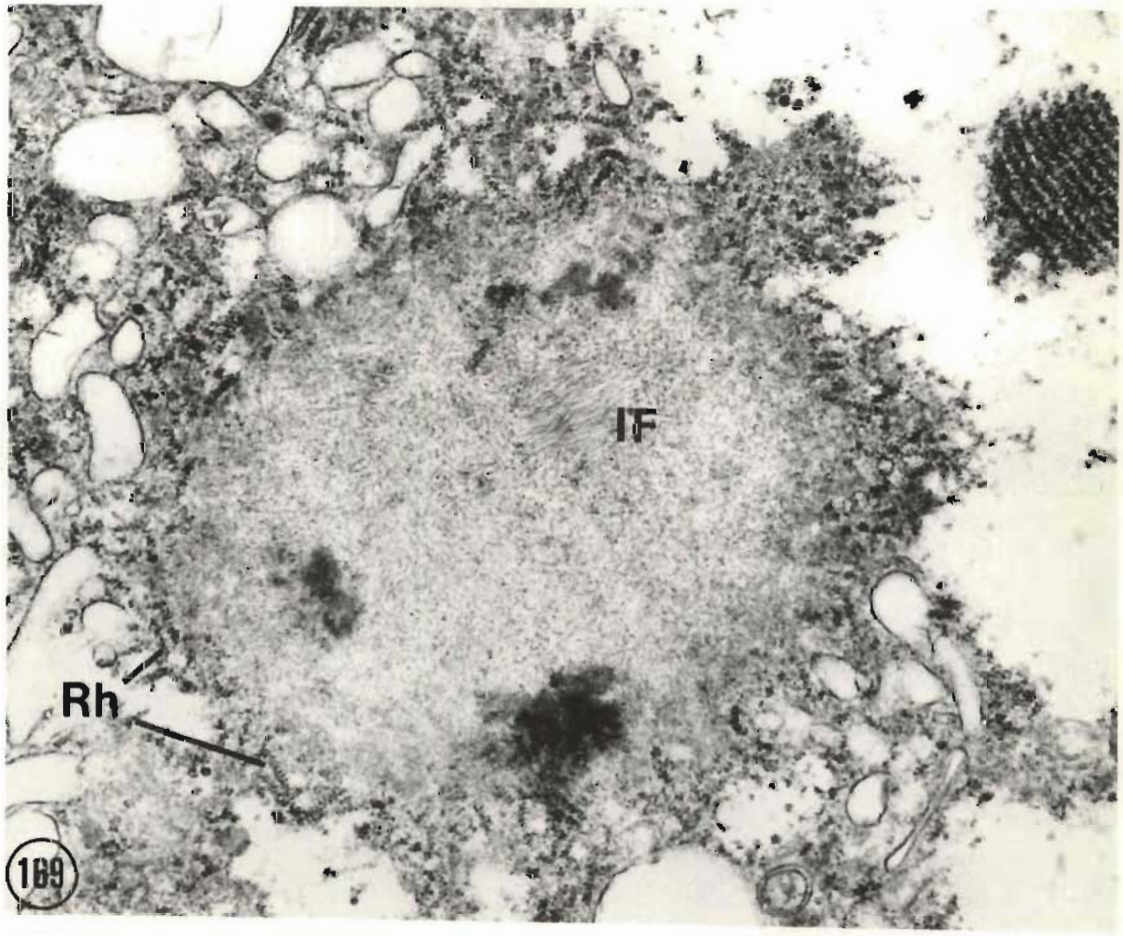

IF
167

Trophozoites of *E. coli*

Fig. 168 A nucleus from strain C 16 almost entirely filled with strands of filamentous material (IF). Note the electron-dense body within the nucleus and the filamentous material (arrowed) in the cytoplasm. x 38 640

Fig. 169 Section through a nucleus of a binucleate trophozoite in which the nuclear envelope was not apparent and the nucleoplasm was surrounded by ribosomal helices (Rh). Within the nucleoplasm is a bundle of filamentous material (IF). x 43 680


IF
168

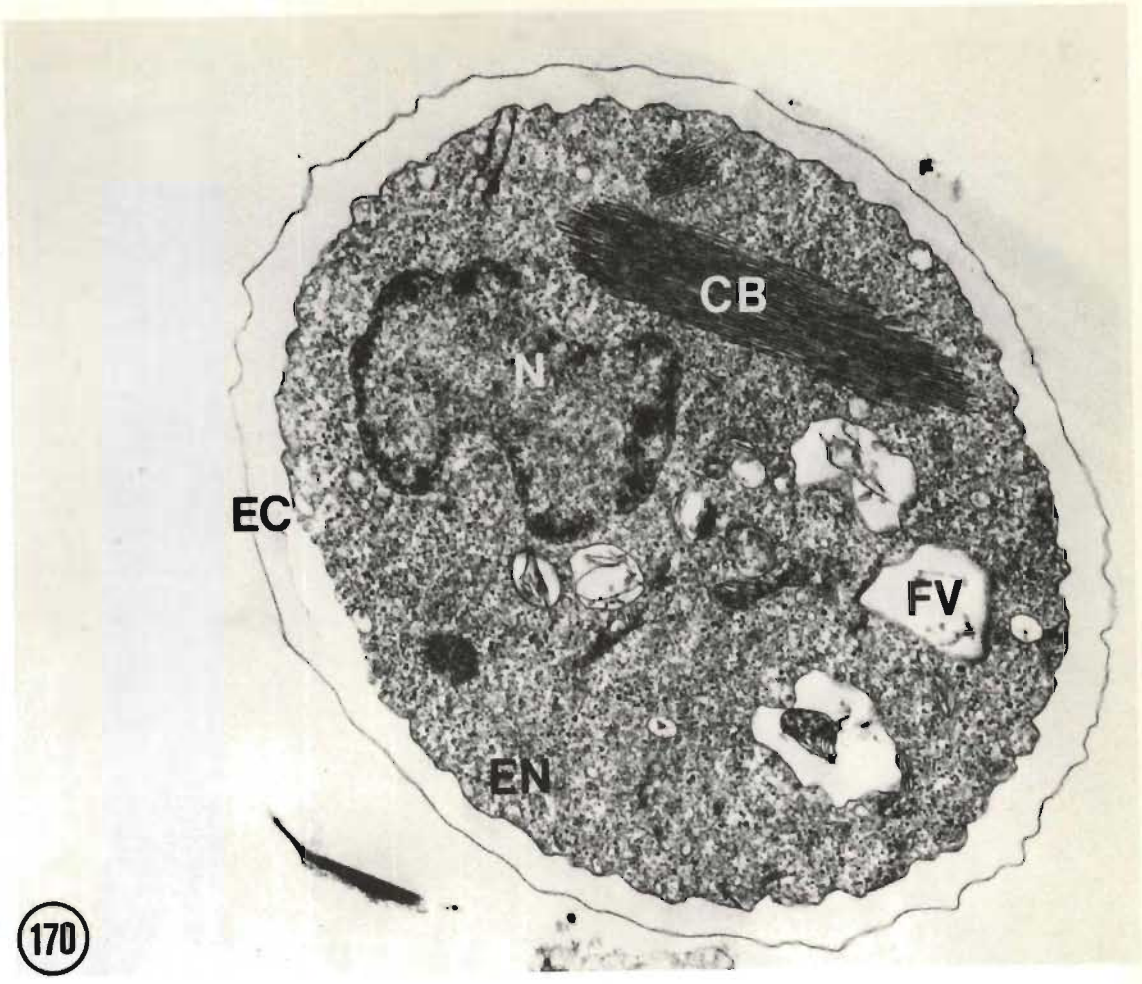

IF
Rh
169

Cysts of *E. coli*

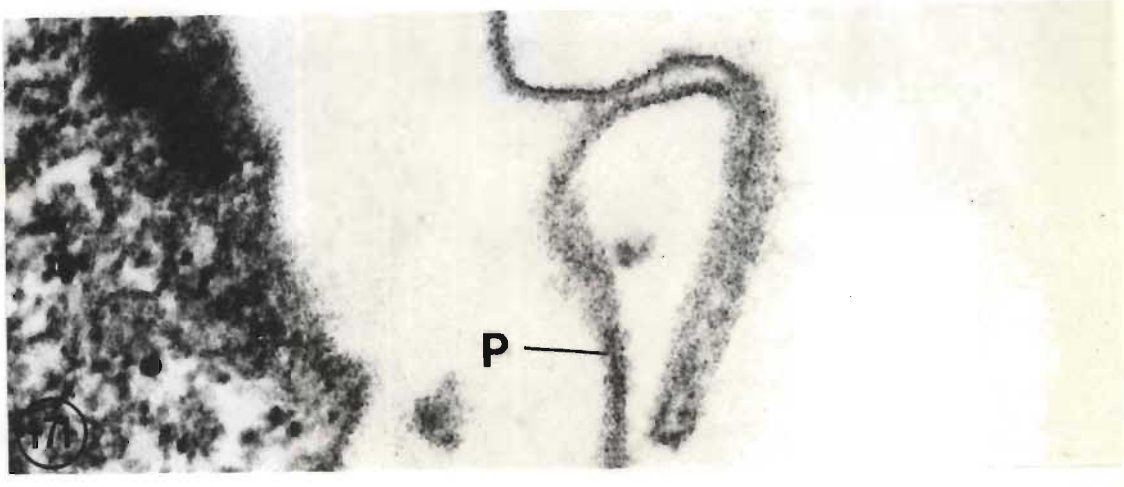
Fig. 170 An immature cyst showing the differentiation into ectoplasm (EC) and endoplasm (EN) and containing aggregates of ribosomal helices (CB) a nucleus (N) and numerous digestive vacuoles (FV). x 12 740

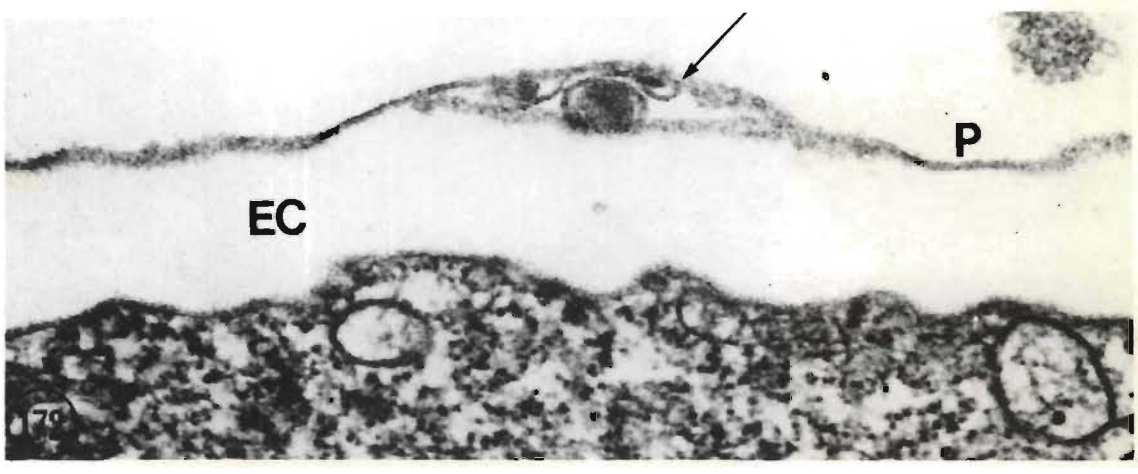
Fig. 171 Shows the convolution of the outer plasmalemma (P) in the early cystic stage. x 99 840

Fig. 172 Shows a dense mass in the ectoplasm (EC) surrounded by convolutions (arrowed) of the outer plasmalemma (P). x 71 920



170

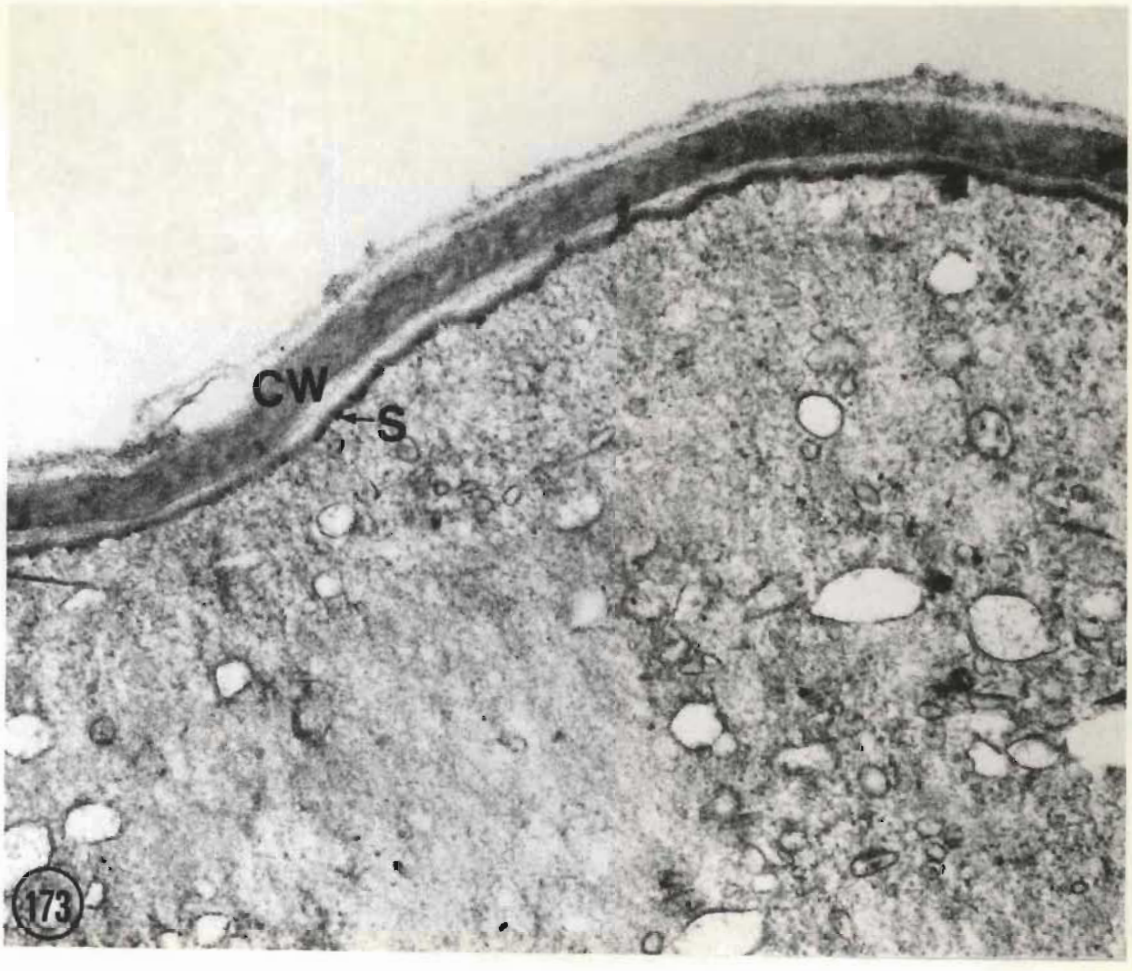


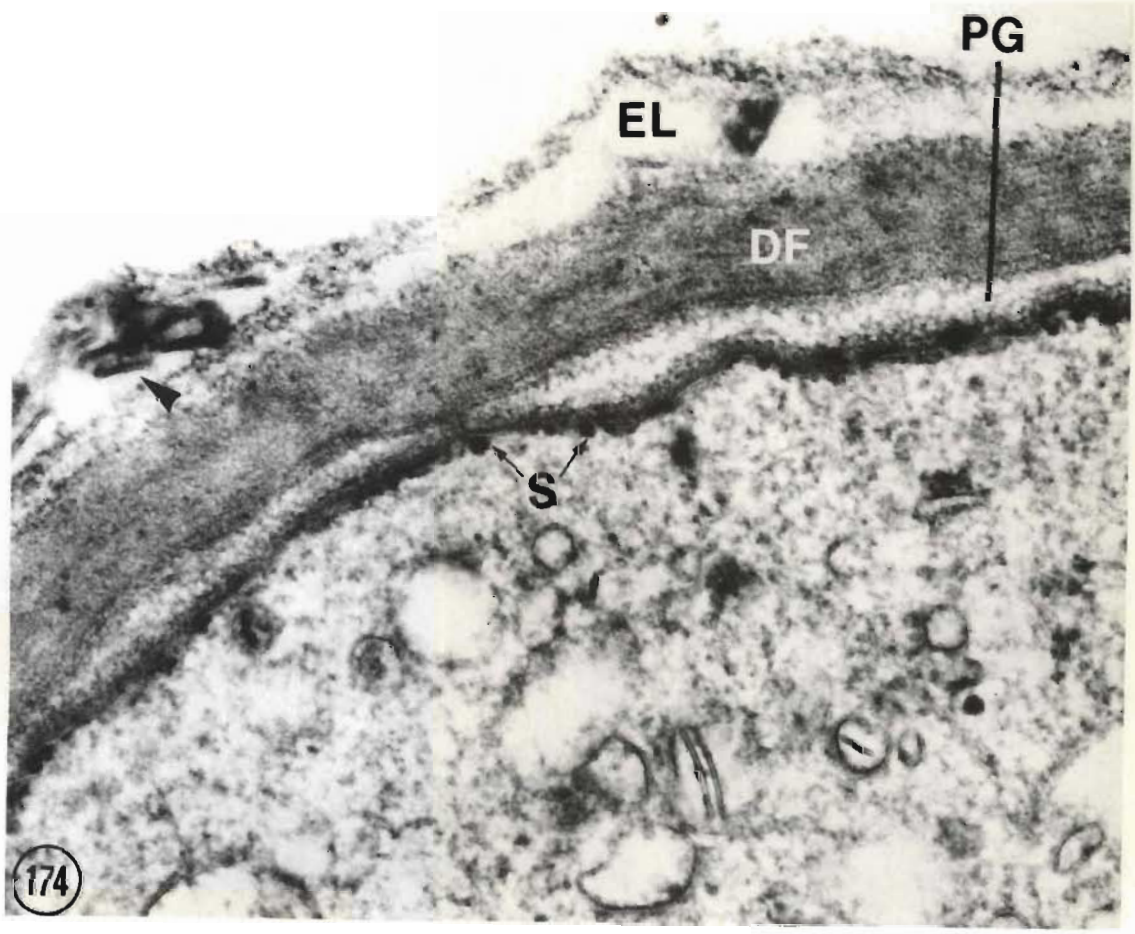


Cysts of *E. coli*

Fig. 173 Portion of the cyst wall (CW) of the intermediate stage showing the differentiation into the three layers and the osmiophilic bodies (S) on the inner surface of the plasmalemma. x 37 700

Fig. 174 High power showing the pale granular region above the plasmalemma (PG) the dense fibrous layer (DF) and the pale external region (EL). Note the osmiophilic bodies (S) on the inner leaflet of the plasmalemma and the parallel lamellae (arrowhead). x 89 900

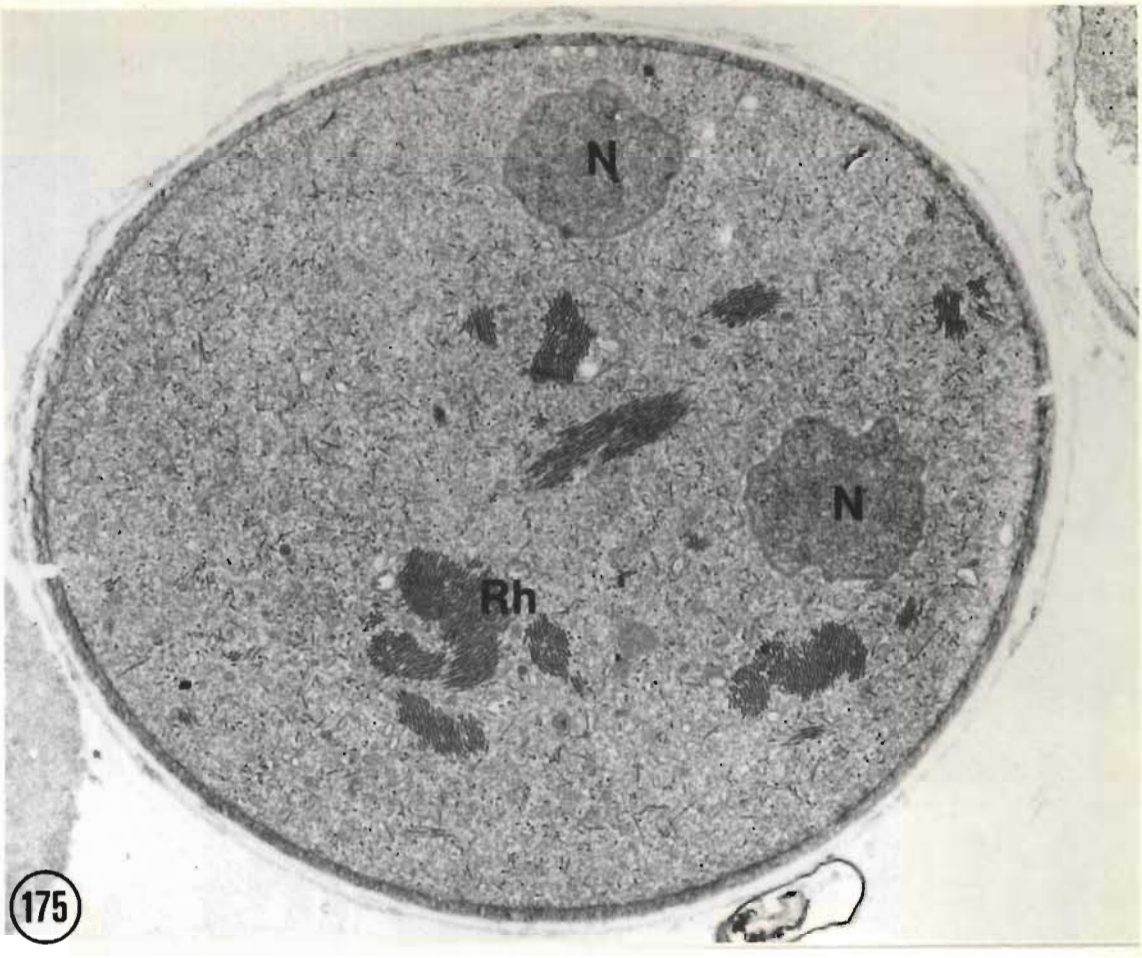

CW
S
173


PG
EL
DF
S
174

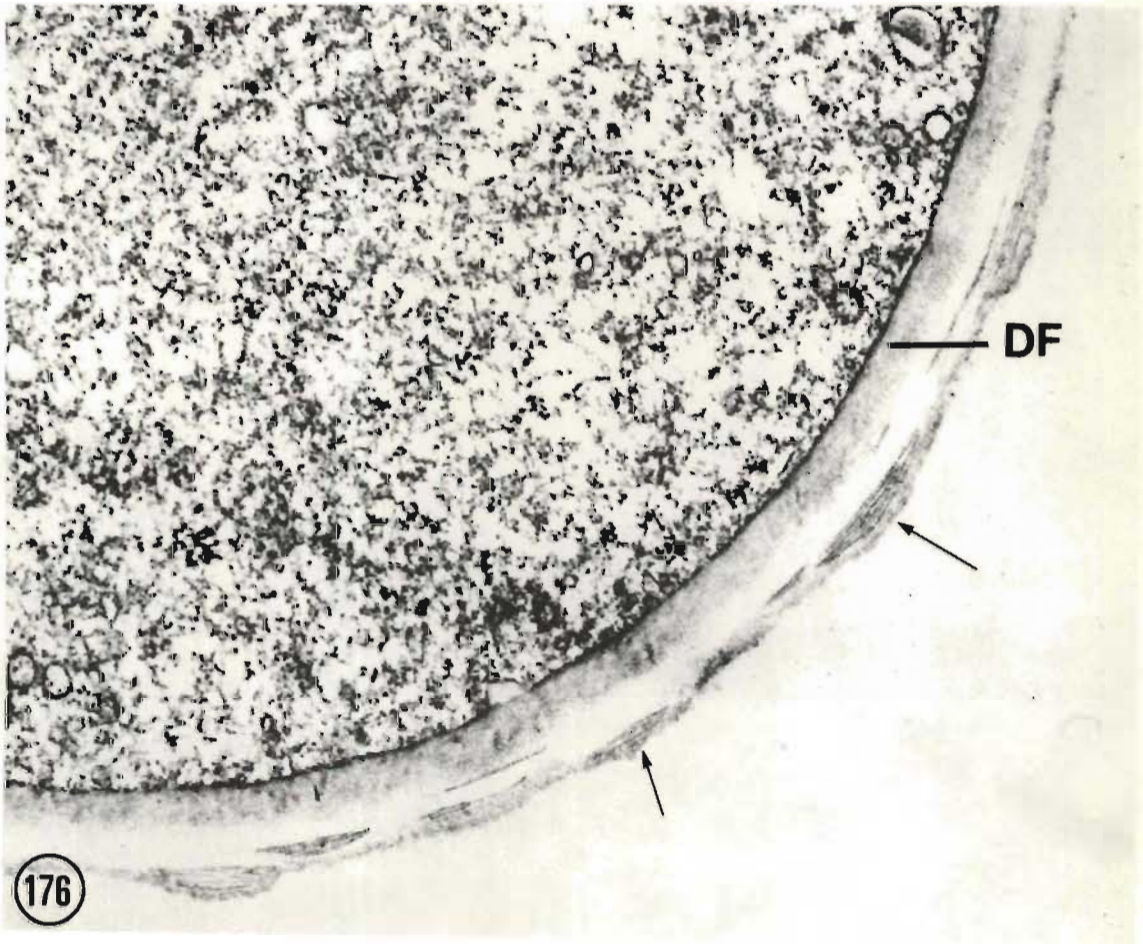
Cysts of *E. coli*

Fig. 175 A mature cyst containing two nuclei (N) and small bundles of ribosomal helices (Rh). Note the absence of digestive vacuoles. x 19 040

Fig. 176 High power showing the thickened wall of the mature cyst with the fibrous layer (DF) closely adherent to the plasmalemma and the groups of parallel lamellae (arrowed). x 29 680



175

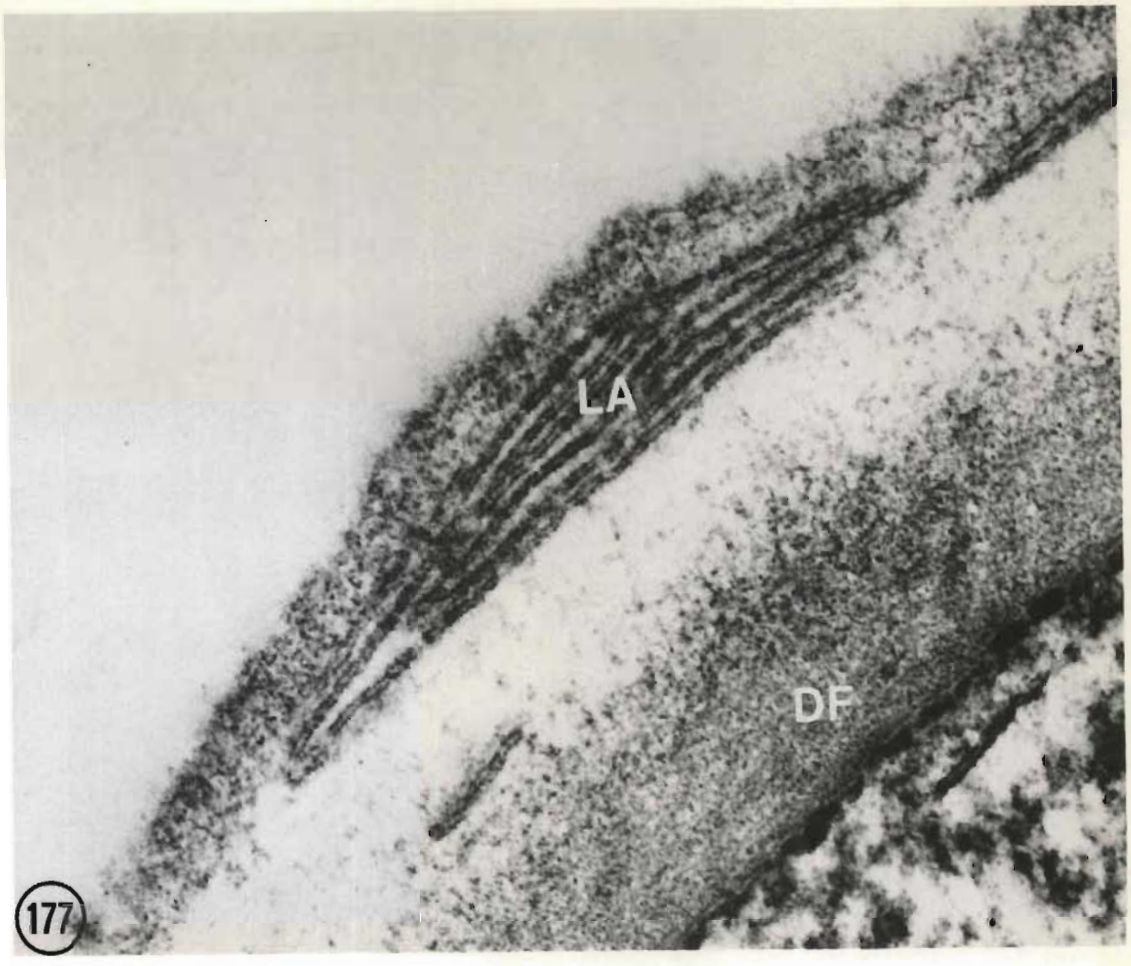


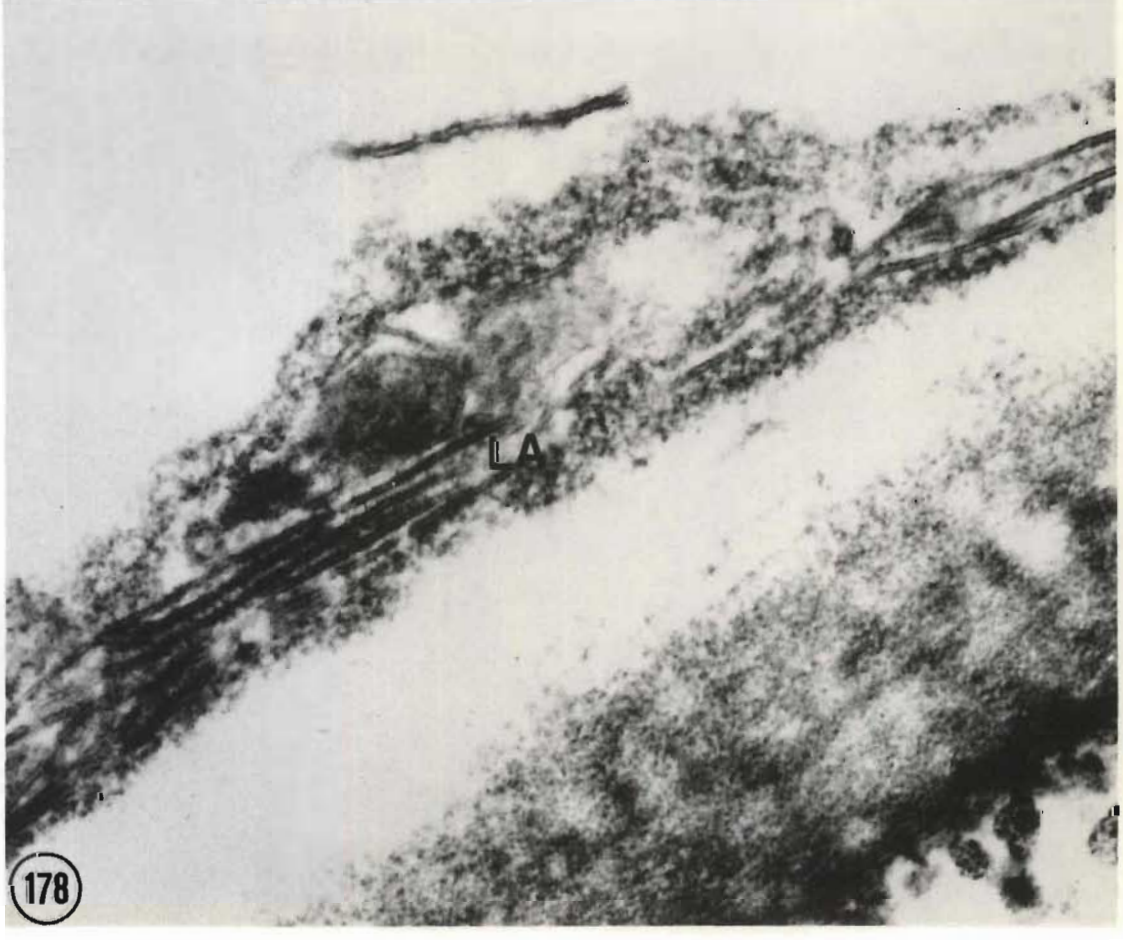
176

Cysts of *E. coli*

Fig. 177 High power of the cyst wall showing the parallel lamellae (LA) and the fibrous layer (DF). x 132 160

Fig. 178 High power showing the expansion of the lamellar region (LA). x 114 240

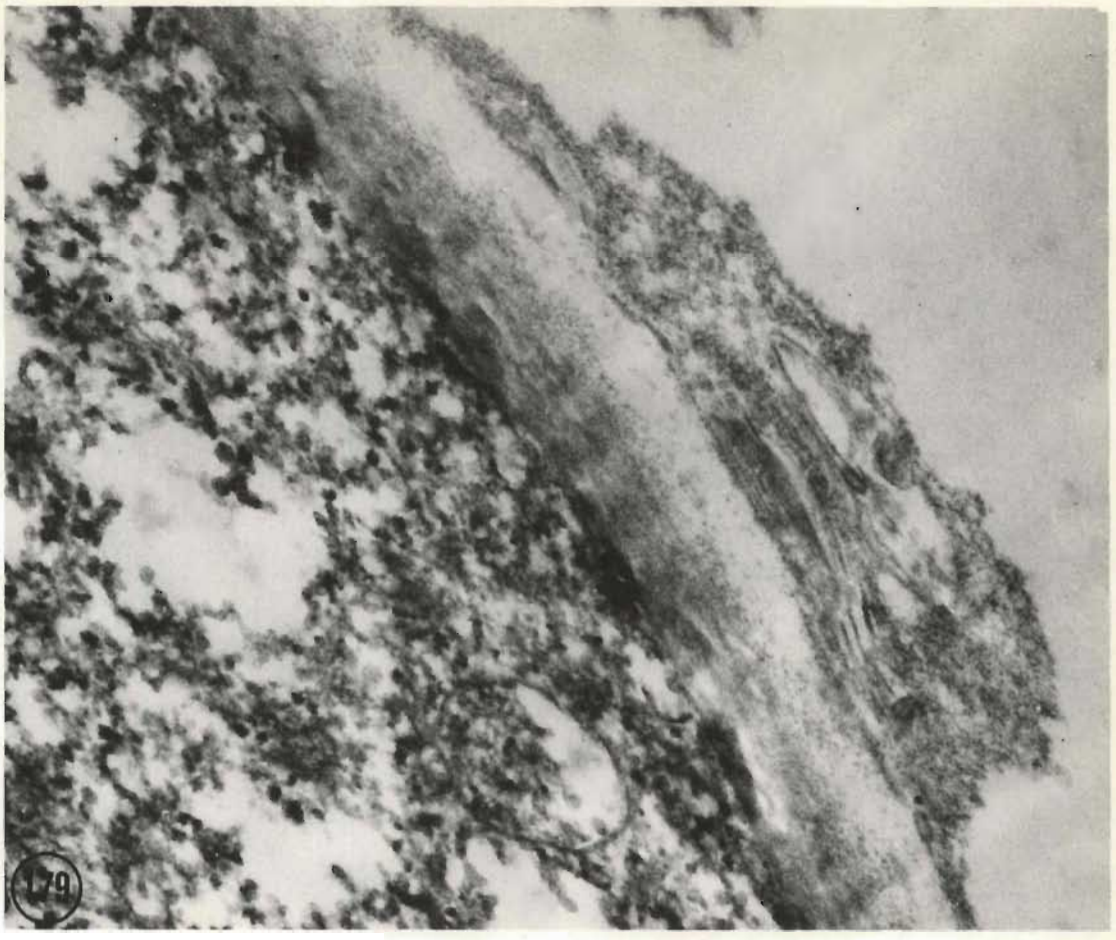

LA
DF
177

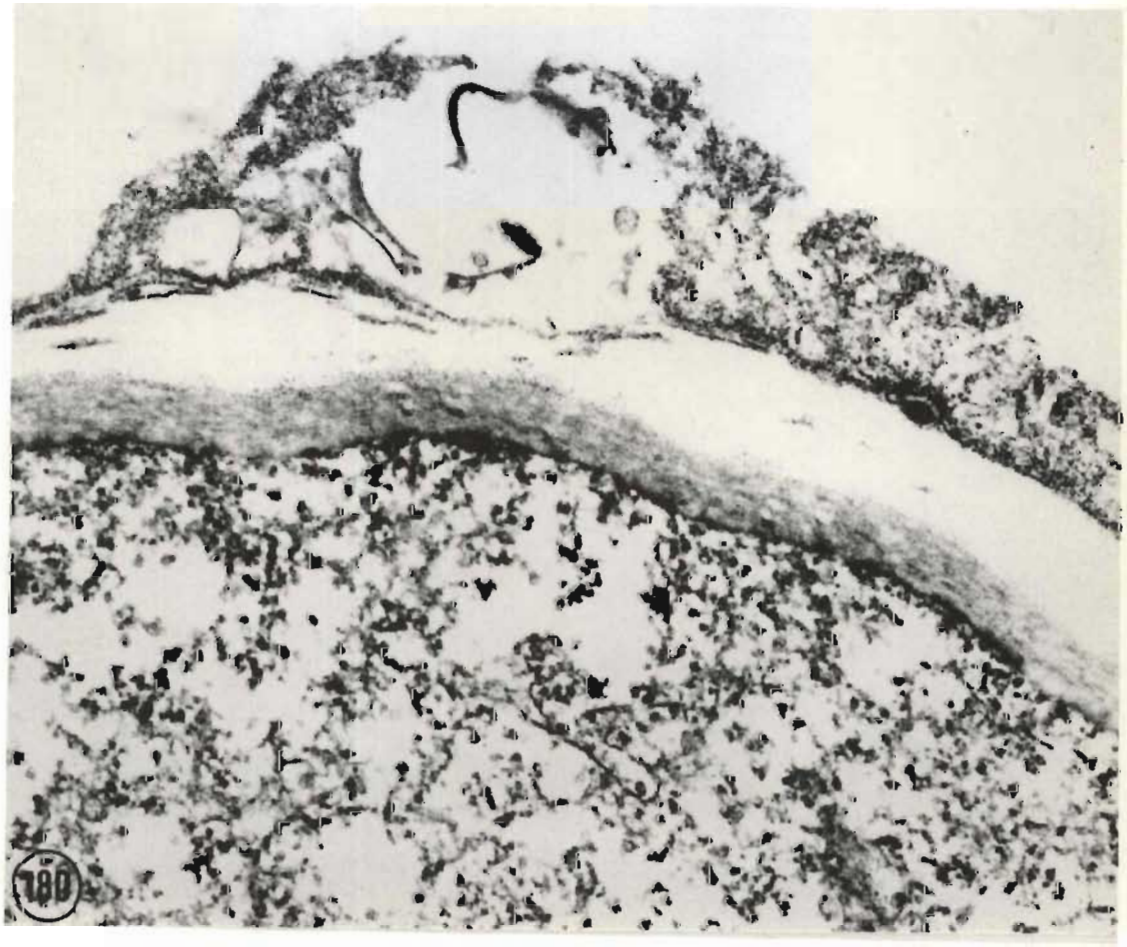

LA
178

Cysts of *E. coli*

Fig. 179 Shows the progressive increase in volume in the region of the parallel lamellae prior to explosion. x 41 440

Fig. 180 Shows the lamellar region after explosion and expulsion of the lamellae to the exterior. x 31 920

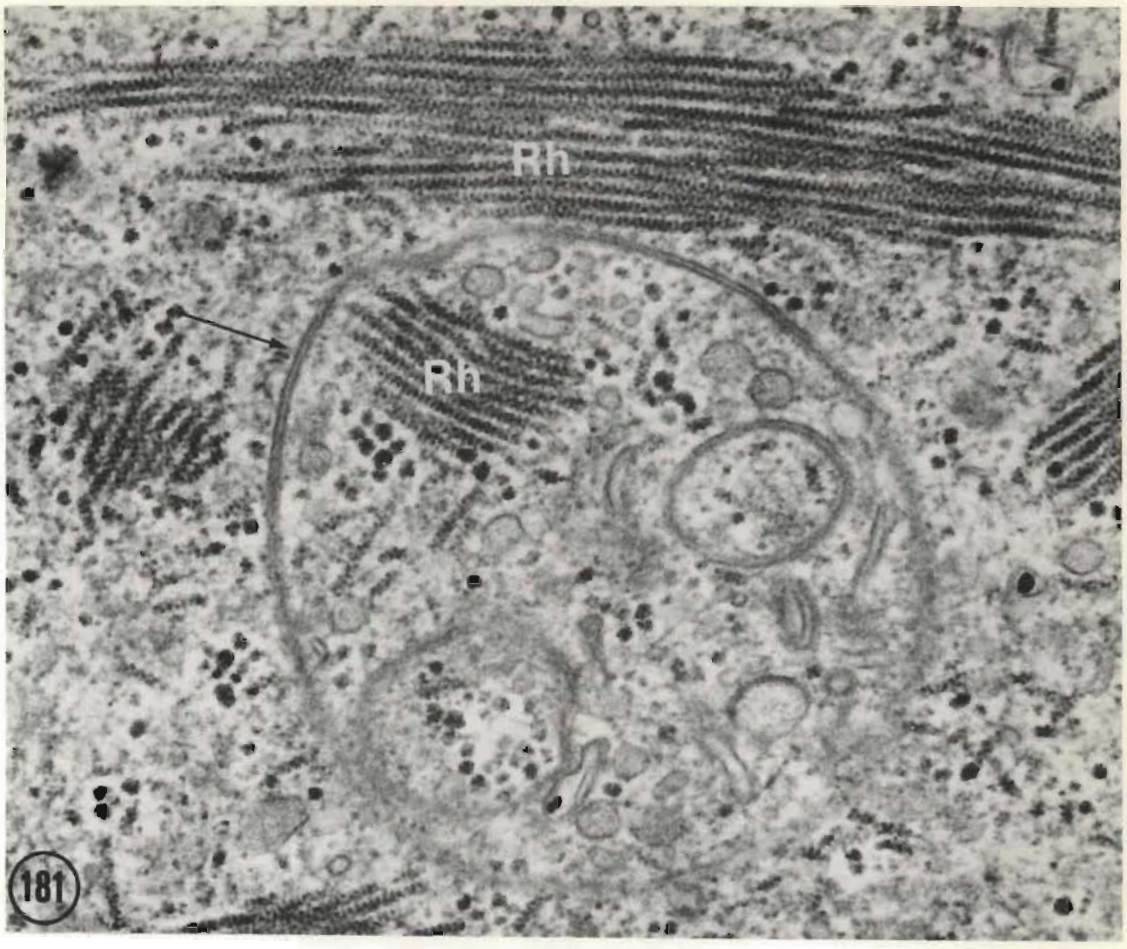


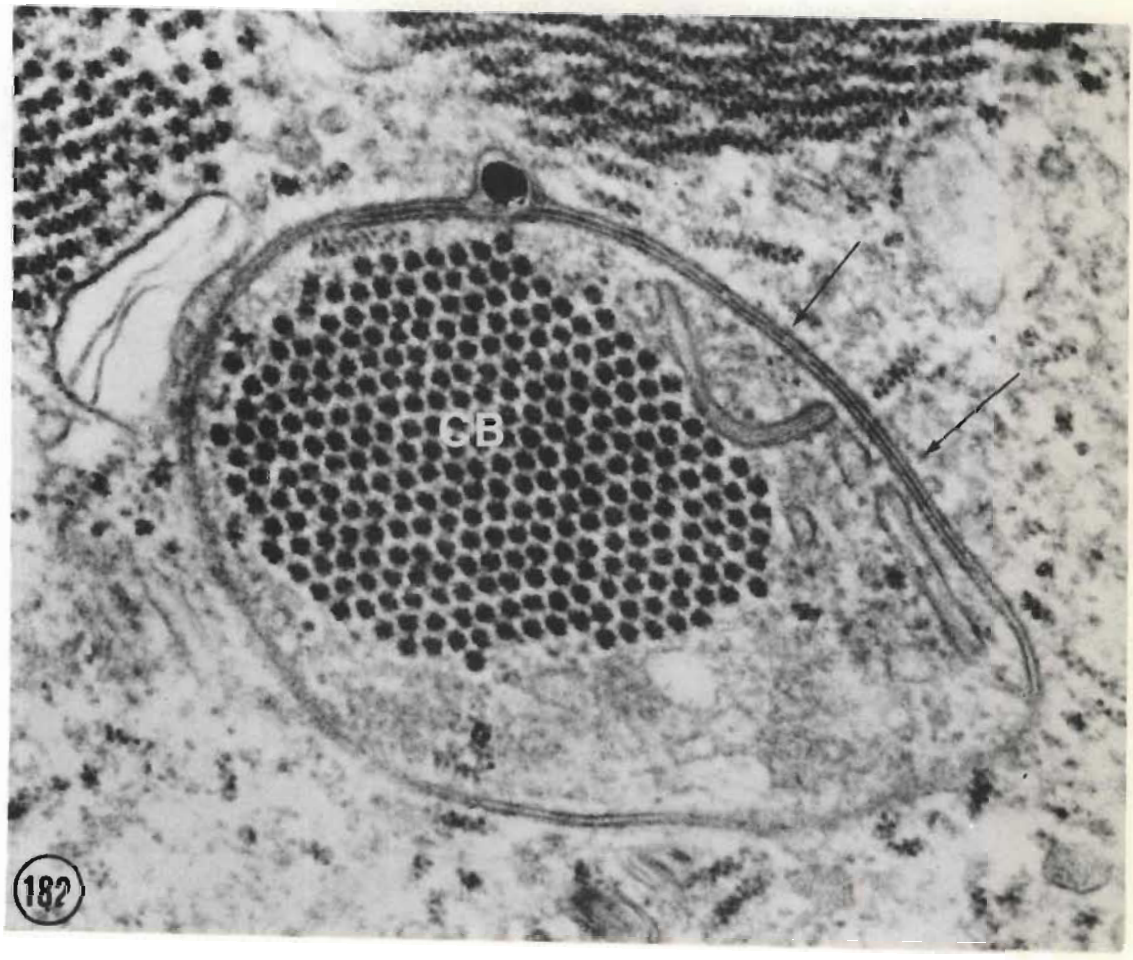


Cysts of *E. coli*

Fig. 181 Shows an area of cytoplasm separated from the rest of the cell by two unit membranes (arrowed). Note the bundle of helices (Rh) within and exterior to the membranes. x 47 000

Fig. 182 Three unit membranes (arrowed) surrounding a chromatoid body (CB) in transverse section. Note the outer membrane around the electron-dense body. x 66 640

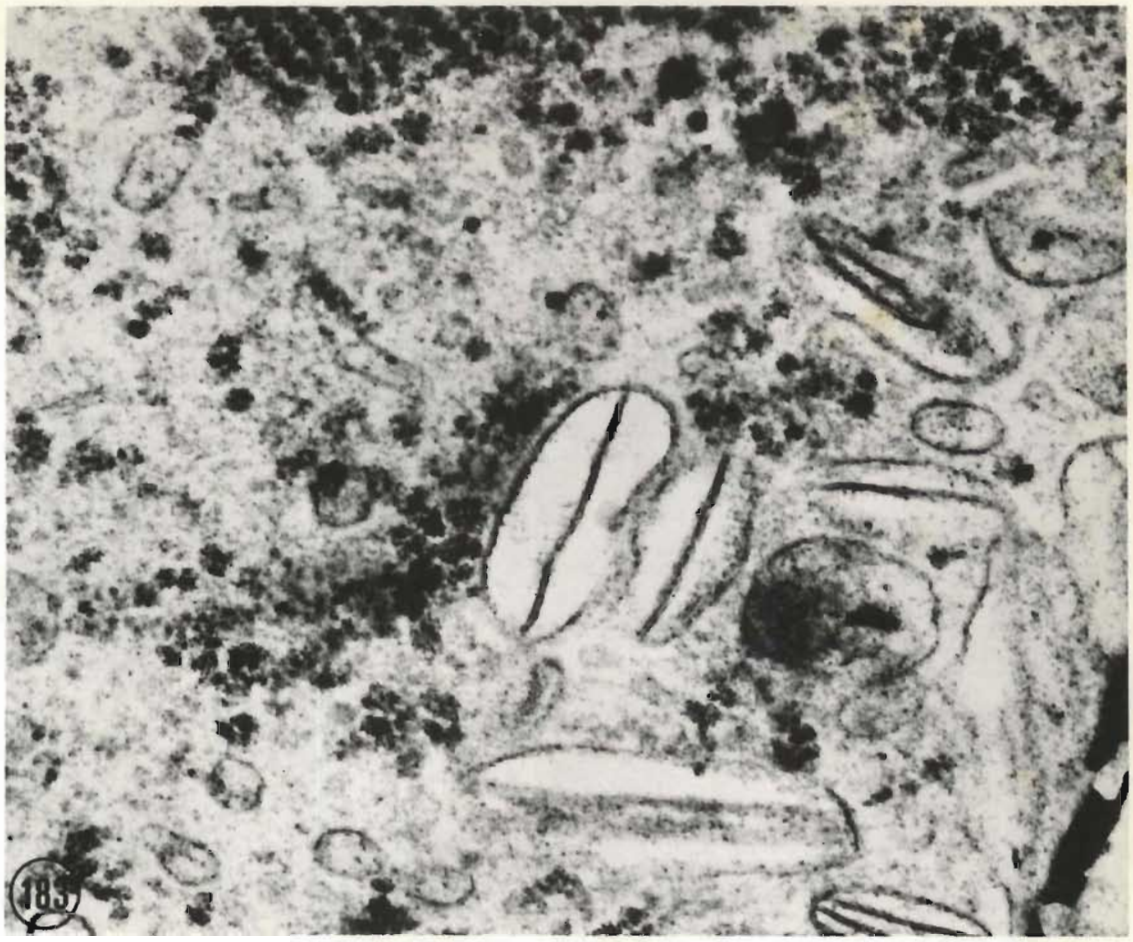

Rh
Rh
181

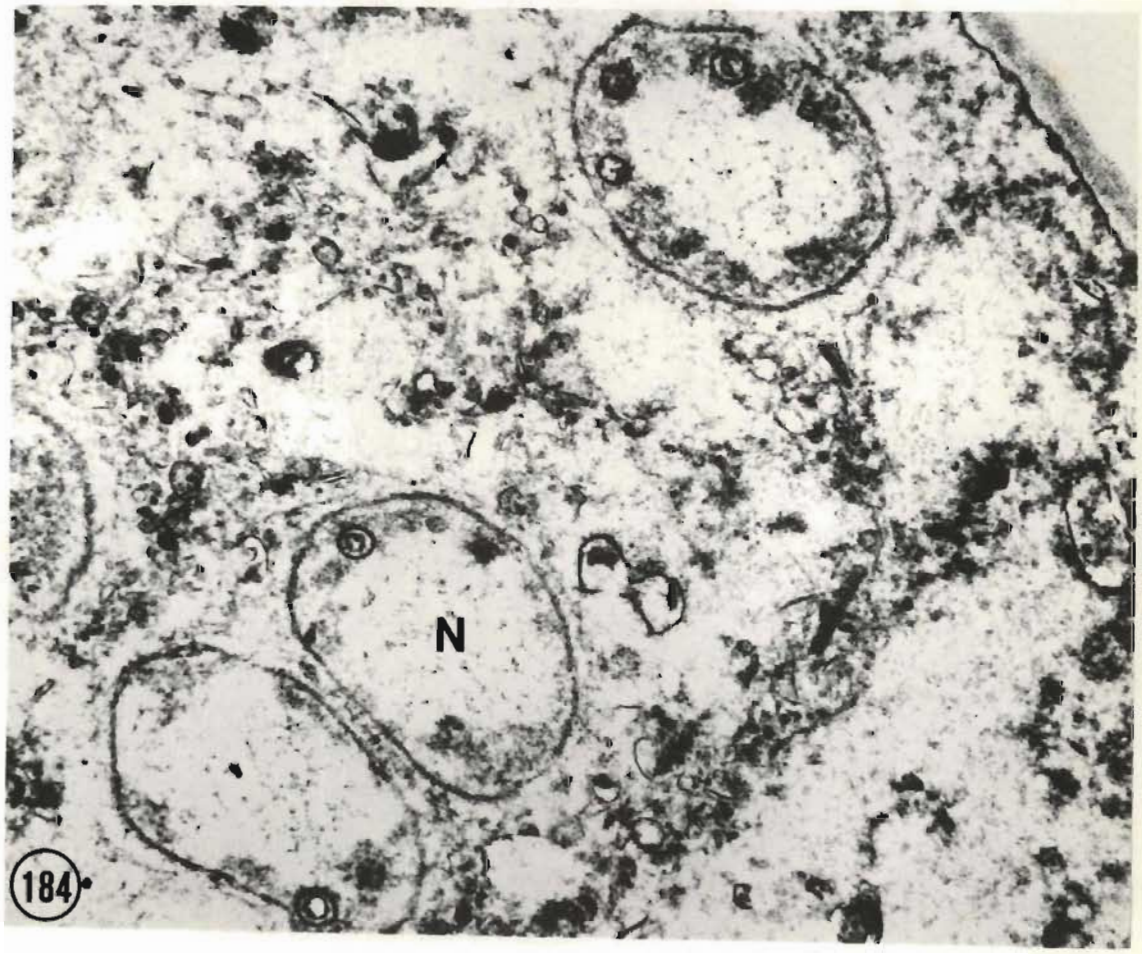

CB
182

Cysts of *E. coli*

Fig. 183 Shows numerous vacuoles containing strands of unit membrane. x 87 360

Fig. 184 Part of a mature cyst showing the nuclei (N) containing button bodies. The membranes of the intranuclear bodies are clearly visible. x 20 720


183


N
184