Homologue of the Golgi Apparatus in Herpetomonas Muscarum, Giardia Intestinalis, Lophomonas Blattarum and L. Striata

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Abstract

The cytoplasmic inclusions identified in Herpetomonas muscarum are the para­basal body, volutin granules and mitochondria, and in Giardia intestinalis (cysts), Lophomonas blattarum and L. striata (trophic forms) the para­basal bodies, lipid bodies and mitochondria.

The parabasal body in H. muscarum lies near the blepharoplast of the flagellum; but in G. intestinalis there may be as many as four such bodies, distributed at random in the cytoplasm. In L. blattarum and L. striata there is a pair of para­basals enclosing between them the calyx along with the nucleus. In H. muscarum the parabasal body appears to have a duplex structure, the externum showing the presence of lipids, proteins and DNA, while the composition of the internum is unknown. In G. intestinalis the parabasal bodies are in the form of large crescents composed of proteins and some lipids; and in L. blattarum and L. striata they are hyaline, thick and curved bodies, the cortices of which show positive reaction for lipids and proteins.

The lipid bodies (composed of lipids and proteins) identified as homogeneous or duplex spheres in G. intestinalis, L. blattarum and L. striata represent, in our view, the typical Golgi bodies as described in other protozan and metazoan cells. However, some investigators hold the view that in flagellates the para­basal is the homologue of the Golgi apparatus. This view seems to be true in H. muscarum despite the unusual presence of DNA in the para­basal, as separate lipid bodies independent of the para­basal are entirely absent in this form.

In G. intestinalis also the para­basals react positively to the Golgi techniques; but in L. blattarum and L. striata in spite of the presence of lipids in the cortex, the morphology of para­basals is widely different from that of the typical duplex para­basal of Herpetomonas.

Since the Golgi apparatus has a characteristic ultra-structure, the exact homologies of the para­basals in these flagellates can be settled only on the basis of their electron microscopical investigations.

Introduction

With the application of the Golgi techniques of osmium and silver impregnations, a variety of structures in flagellates, viz. (1) para­basal body, (2) osmiophil granules, rings, crescents and duplex spheres, (3) stigma, and (4) cortical part of the contractile vacuole, have been recognised by the earlier authors as the Golgi apparatus in spite of the fact that they are morphologi­cally and physiologically different (refer to the reviews on the Golgi apparatus...
of Protozoa by King 1927; Hill 1933; MacLennan 1941; and Smyth 1944).

The parabasal, in addition to its homology with the Golgi apparatus (Grasse 1926; and Duboscq and Grasse 1933), has also been regarded in trypanosomes and allied forms, as a secondary nucleus or kination nucleus by Fantham (1912) and Grasse (1926) in Herpetomonas, and by Sen Gupta et al. (1953) in Leishmania donovani. Lilie (1948) recognised the Feulgen positive nature of the blepharoplast (parabasal body) in Trypanosoma cruzi.

The duplex nature of the parabasal of Herpetomonas muscarum has been reported by Dutta (1939); the externum consists of neutral lipid (probably triglyceride), lipoprotein, and DNA, while the composition of the internum is unknown.

The ultra-structure of the parabasal of a large number of flagellates investigated by Anderson et al. (1956), Grassé (1956), Grassé and Carasso (1957), and Anderson and Beams (1959) is similar to the typical structure of the metazoan Golgi apparatus.

Kofoid and Christiansen (1915a, b and c) homologized the pair of deeply-staining curved or rounded bodies, which lie just posterior to the sucking disc in Giardia, to the parabasals of other flagellates; but Wenyon (1926) considered that there was no real evidence in support of this view.

Hirschler (1927) described the parabasal apparatus of Lophomonas as typical curved dictyosome-like bodies with chromophilic and chromophobic substance. He also observed sometimes the presence of a chain of two or three dictyosomes situated around the axostylar calyx in the neighbourhood of the nucleus. In addition to the parabasals, he identified some thick dictyosomes in the cytoplasm of L. blattarum which could be impregnated with osmium. Hirschler (1932) could not reach any definite conclusion as regards the homology of the parabasal of L. blattarum. Brown (1939) has also observed crescents, rings or spheres, which he considers as the Golgi bodies, structures distinct from the two parabasals in another hypermastigote flagellate, Microsporidia.

Material and Technique

The flagellates studied during the present investigation are Herpetomonas muscarum (Trypanosomidae, Protophomonadina), Giardia intestinalis (Hexamitidae, Polymastigina), Lophomonas blattarum and L. striata (Lophomonadidae, Hypermastigina). H. muscarum was obtained from the hind-gut of housefly; G. intestinalis parasite in the colon of man was obtained from the stools of infected patients; and L. blattarum and L. striata from the colon of cockroach.

Wet whole mount smears of these flagellates were fixed and examined as described below:—

1. Fixed in Zenker, Carnoy, Lewitsky saline (Baker 1956), or weak Bouin followed by pyridine extraction (Baker 1946), and stained with Heidenhain’s iron haematoxylin for the study of the morphology of these flagellates.
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(2) Fixed in Lewitsky saline or formaldehyde-calcium with or without post-chroming (Baker 1946), and coloured with Sudan black for the demonstration of lipids.

(3) Fixed in Lewitsky followed by post-osmication in 2 per cent osmium tetroxide according to the Kolatchev technique.

(4) Fresh material treated with 2 per cent osmium tetroxide and studied as such.

(5) Fixed in Zenker, Carnoy, formaldehyde-calcium, weak Bouin followed by pyridine extraction, and stained with mercuric-bromphenol blue (Mazia et al. 1953), or coupled tetrazonium (Pearse 1960) for proteins.

(6) Fixed in Zenker and stained in pyronin/methyl green (Jordan and Baker 1955) for the identification of nucleic acids.

(7) Fixed in Carnoy and stained with Feulgen for the demonstration of DNA (Feulgen and Rossenbeck 1924).

(8) Material fixed in Carnoy or Zenker was extracted with 4 per cent trichloroacetic acid as a control for the complete removal of DNA and RNA (Schneider 1945).

(9) Fixed in Lewitsky or Helly and stained with Cain's acid fuchsine (Cain 1948) for the identification of mitochondria.

(10) Stained with Janus green B supervitally for the identification of mitochondria.

(11) Stained with neutral red supervitally for the staining of lipid bodies.

OBSERVATIONS

The cytoplasmic inclusions identified in Herpetomonas muscarum are the parabasal body, volutin granules and mitochondria; and in Giardia intestinalis (cysts), Lophomonas blattarum and L. striata (trophic forms) the parabasal bodies, lipid bodies and mitochondria.

The mitochondria of these flagellates, which stain specifically with Cain's acid fuchsine, are in the form of short filaments in G. intestinalis, L. blattarum and L. striata; while in H. muscarum they are granular. They are evenly distributed in the cytoplasm and stain with Janus green B supervitally.

The parabasal body of H. muscarum associated with the blepharoplast and a flagellar vacuole, represents the kinetoplast. In G. intestinalis, there are one to four parabasal bodies which lie side by side in the cytoplasm; while in L. blattarum and L. striata there is a pair of parabasals enclosing between them the calyx along with the nucleus.

The parabasal body of H. muscarum is a duplex structure; the externum is osmiophil and sudanophil due to the presence of lipids; it also stains with mercuric-bromphenol blue, thus showing the presence of proteins. The parabasal gives positive reaction for DNA, as it stains with Feulgen reaction, and the methyl green component of pyronin/methyl green. The internum
remains unstained in all the techniques. The parabasal appears crescentic in optical section due to the uneven distribution of material in the externum.

In *G. intestinalis*, the parabasal bodies are in the form of large crescents. It is concluded that they are lipoidal in nature as they give a positive reaction to Kolatchev, and are coloured in Sudan black. Their staining with mercuric-bromphenol blue and coupled tetrazonium shows the presence of proteins in them.

In *L. blattarum* and *L. striata*, the parabasal bodies are hyaline, thick and curved bodies, the cortices of which show a slight positive reaction for lipids and proteins.

In addition to the parabasals, there are present in the cytoplasm of *G. intestinalis, L. blattarum* and *L. striata* a varying number of lipid bodies. The lipid bodies are in the form of homogeneous and duplex spheres. They stain with neutral red supervitally, the internum staining more lightly than the externum. The homogeneous lipid spheres as well as the externum of the duplex bodies are of lipoidal nature, as revealed by fresh osmium tetroxide and Kolatchev preparations, and on colouring with Sudan black; and their staining with mercuric-bromphenol blue and coupled tetrazonium shows the presence of proteins. The lipid bodies appear as spheres or crescents in the iron haematoxylin preparations. The internum of duplex bodies is not stained in these techniques; it is probably watery in nature.

**Discussion**

With regard to the homologue of the Golgi apparatus in *Giardia intestinalis, Lophomonas blattarum* and *L. striata*, the lipid bodies, in our view, represent the Golgi apparatus, since they are identical with the Golgi bodies described in other Protozoa by Kudo (1934) and Dutta (1959b), and in Metazoa by Hirsch (1939), Nath (1944 and 1960), and Baker (1944, 1949 and 1959) and others. The lipid bodies are osmiophil in the Golgi techniques, and in addition they stain with neutral red supervitally. Hirsch (1939) also reported the neutral red-staining of the Golgi presubstance. The cytochemical investigations of these lipid bodies in Protozoa (Dutta 1959b) and Metazoa (Nath 1960) have also established their lipoidal nature. And finally, from the ultrastructure of lipid bodies, Chou and Meek (1958) have concluded that the lipid bodies represent the Golgi apparatus. Electron microscopy has demonstrated that the externum of the duplex lipid bodies is composed of several complete, concentric, dense double membranes, which are typical of the Golgi apparatus. These morphological, cytochemical and electron microscopical investigations, therefore, support our homology of the lipid bodies to the Golgi apparatus.

However, some investigators like Grassé (1926), and Duboscq and Grassé (1933), hold the view that in flagellates the parabasal because of its impregnation in the Golgi techniques, is the homologue of the Golgi apparatus. Evidence from electron microscopy also strongly supports this homology of the parabasal to the Golgi apparatus, as in some flagellates (viz. *Trypanosoma*
equiperdum, Anderson and others 1956; Joenia, Foania, Trimitus, Spirotrichonympha, Trichonympha, Grassé 1956; Joenia annectans, Foania dogieli, Trichonympha, Grassé and Carasso 1957; and Tritrichomonas type muris, Anderson and Beams 1959) the ultra-structure of the parabasal has been found to be identical to the typical Golgi apparatus.

The duplex parabasal body of *Herpetomonas muscarum* also morphologically resembles the typical lipid body or Golgi apparatus because of the osmiophil nature of its externum; moreover independent lipid bodies as such are absent in this flagellate. Cytochemical evidence also points to the lipid nature of the externum. Therefore, the homology of parabasal of *H. muscarum* to the Golgi apparatus seems to be true, despite the unusual presence of DNA. It must be emphasized, however, that DNA has never been described in association with the Golgi apparatus. DNA has also been identified in the parabasal of *Leishmania donovani* (Sen Gupta et al. 1953), and *Trypanosoma cruzi* (Lillic 1948). The nuclear nature of parabasal of *Herpetomonas* was also recognised by Fantham (1912) and Grassé (1926), who named it as secondary nucleus or kinetonucleus.

In *G. intestinalis* the parabasals react positively to the Golgi techniques; but in *L. blattarum* and *L. striata*, in spite of the presence of lipids in the cortex, the morphology of the parabasals is widely different from that of the typical duplex parabasal of *Herpetomonas*. Hirschler (1932) also could not establish any definite homology of the parabasals of *Lophomonas*. Moreover the presence of independent lipid bodies in these flagellates, which have been said to represent the Golgi apparatus, makes the homology of their parabasal to the Golgi apparatus still more doubtful. In *Microjienia*, which possesses both the crescents, rings or spheres (comparable to our lipid bodies), and the parabasals, Brown (1930) also favoured the homology of the crescents, rings or spheres to the Golgi apparatus.

Since the Golgi apparatus has a characteristic ultra-structure the exact homologies of the parabasals in these flagellates can be settled only on the basis of their electron microscopical investigations.

Furthermore, other structures of flagellates like the stigma and the contractile vacuole, which have been also homologized to the Golgi apparatus, are not present in these parasitic flagellates. It may be mentioned that in *Herpetomonas muscarum* a non-contractile flagellar vacuole is present, which, however, is non-osmiophil and non-sudanophil.

**References**

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CYTOCHEMISTRY OF THE CYTOPLASMIC INCLUSIONS
OF A FRESHWATER AMOEBA (AMOEBA SP.)

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CYTOCHEMISTRY OF THE CYTOPLASMIC INCLUSIONS
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Abstract

The cytoplasm of the Amoeba sp. contains mitochondria, lipid bodies, accessory and contractile vacuoles, and food vacuoles. The mitochondria, which are composed of triglycerides, phospholipids, lipoproteins and RNA, reveal a differentiated peripheral layer on staining with Janus green, acid haematein and Nile blue. It has been suggested that this differentiated peripheral layer corresponds to the double limiting membrane of the mitochondria seen with electron microscopy. The lipid bodies correspond to the Golgi bodies or Golgi apparatus described earlier by the light and electron microscopy. The lipid bodies usually show the presence of triglycerides and lipoproteins but in old cultures they contain in addition some phospholipids and fatty acids. The present investigation does not support the homology of the contractile vacuole to the Golgi apparatus. The alpha granules or crystals are absent in this amoeba.

Introduction

Among the free-living amoebae, the cytoplasmic inclusions have been differently described by the earlier authors. Schaeffer (1916) described in Amoeba proteus a large number of clear bluish spheres, which he named excretion spheres. Taylor (1921) described these spheres in Amoeba sp. as nutritive spheres. In A. proteus, Hayes (1925) identified them as pale blue nutritive spheres composed of glycogen and described certain other starch granules also.

The Golgi bodies have been reported from time to time in the freshwater amoebae. Brown (1930) demonstrated two types of Golgi bodies in A. proteus, which appeared as rings or crescents, and granules. Mast and Doyle (1935a and 1935b) described the cytoplasmic bodies of A. proteus 'Y' and A. proteus 'X' (A. dubia) as alpha granules, beta granules (mitochondria), refractive bodies, crystals (lying inside crystal vacuoles), fat and contractile vacuole. They considered that the refractive bodies composed of carbohydrate-like shell, covered by a layer 'composed of a protein struma impregnated with a lipid containing fatty acid', represent the Golgi bodies. Pappas (1954) has worked out the detailed cytochemistry of five different amoebae, namely Amoeba proteus, Chaos chaos, Thecamoeba striata, Magorella bigemina and Amoeba...
and the cytoplasmic bodies reported in these species are the alpha and beta granules (mitochondria), the spherical refractive bodies, the contractile vacuoles, the crystals and crystal vacuoles, the food vacuoles, the fat globules and the permanent vacuoles. He also described the absence of some inclusions like the spherical refractive bodies in *H. bipennis* and *A. guttula*, and of crystals and crystal vacuoles in *T. striata* and *A. guttula*. He considered that the spherical refractive bodies of *A. proteus*, *C. chaos* and *T. striata* represented the Golgi bodies. He also observed that these bodies were stainable with neutral red. He recognized the homogeneous or duplex nature of these bodies, the latter consisting of an outer cortical layer composed of lipid and protein material and an inner medullary portion containing a fluid of unknown composition.

Das and Tewari (1955) identified three types of Golgi bodies in *Amoeba verrucosa*, namely (1) dark solid granules, (2) small vacuoles with a circular or crescent-shaped dark rim and (3) a few (three or four) large vacuoles. They reported that by the coalescence of large vacuoles a fully formed contractile vacuole was formed, which did not show any osmic impregnation. Singh (1938) demonstrated in the endoplasm of *Amoeba proteus* a single oval nucleus, contractile vacuole, food vacuoles, bipyramidal crystals, fat bodies, nutritive spheres containing glycogen, and mitochondria.

With electron microscopy, Cohen (1957) described in *Amoeba proteus* the presence of mitochondria, alpha particles, crystals, vacuoles and Golgi material. In the electron micrographs, the Golgi material reveals accumulation of flattened vesicles or membranes which, according to Cohen, represent the Golgi in higher forms. Cigada Leonard! (1958) has investigated the ultrastructure of the flagellated gamete of *Amoeba spumosa* and reported the presence of mitochondria, Golgi apparatus and chloroplast. The Golgi dictyosome in the electron micrograph shows the structure of the typical Golgi apparatus. Pappas (1959) has studied the ultrastructure of three amoebae, namely *Amoeba proteus*, *Pelomyxa carolinensis* and *Hartmannella rysodes*, and reported in the cytoplasm the presence of mitochondria, Golgi complex, crystals and crystal vacuoles, food vacuoles and contractile vacuole. He identifies the ‘arrays of flattened vesicles packed very closely together’, seen in the electron micrographs, as the Golgi complex. He has strongly emphasized that in these amoebae the contractile vacuole and its components bear no morphologic resemblance to the metazoan Golgi complex. He also reports that the spherical refractive bodies and alpha granules, described by the author earlier (Pappas 1954), were not identified with the electron microscopy. Mercer (1959) has investigated the ultrastructure of *Amoeba proteus* and described the presence of mitochondria, food vacuoles, crystals, contractile vacuole, ‘irregular vacuoles’ and ‘clusters of vesicles’ in the cytoplasm. Mercer (1959) states that these clusters of vesicles, which are randomly distributed throughout the cytoplasm, resemble the metazoan Golgi apparatus.
Ray and Sen Gupta (1954) have described the presence of mitochondria and Golgi complex in *Entamoeba histolytica*. They have identified the Golgi complex as spheroids which stain with neutral red supervitally. In *E. invadens* Deutsch and Zaman (1959), however, failed to identify the mitochondria with the electron microscopy. In *Hartmannella astronyxis* also, no trace of the Golgi apparatus was identified with the electron microscopy by Deutsch and Swann (1959), although the mitochondria, showing typical internal structure, were identified. The mitochondria of *H. astronyxis* were identified with the light microscopy by Ray and Hayes (1954).

The author (Dutta 1959) has published the abstract of the cytochemical nature of the cell inclusions of *Amoeba* sp. The endoplasm reveals the presence of mitochondria, lipid bodies, contractile vacuole, accessory vacuoles and food vacuoles. The mitochondria are composed of neutral fats (triglycerides), phospholipids, lipoproteins and RNA, whereas the lipid bodies contain neutral fats (triglycerides) and complex lipoproteins. The author has also emphasized that there is apparently no direct relation between the formation of the contractile vacuole and the lipid bodies or the so-called Golgi bodies.

The ultrastructure of mitochondria of *Pelomyxa carolinensis* has been extensively investigated by Pappas and Brandt (1959), and Brandt and Pappas (1959), and these authors have described a complex mitochondrial pattern, consisting of double limiting membrane, the inner membrane being thrown into minute villi (cristae mitochondriales) which are branching and fusing in a complex manner. Pappas and Brandt (1959) have also observed some densely packed fibrillar material in the stroma of the mitochondria and these authors consider that these fibrillar arrays resemble the fibrillar component of the nuclear envelop described by Pappas (1959). Brandt and Pappas (1959) have observed a continuity of the limiting membranes of the mitochondria and postdivision nuclei and, therefore, they have suggested the possibility that the nucleus may be capable of forming mitochondria. The mitochondria have also been identified with electron microscopy in *Amoeba proteus* by Sedar and Rudzinska (1956) and in *Pelomyxa carolinensis* by Dalton and Felix (1957).

**Material and Technique**

The freshwater amoeba, *Amoeba* sp., was obtained from the culture of dry grass and other leaves made with tap-water. The amoebae appeared in large numbers in the surface scum in 3-4 days. The living amoebae were studied in the culture solution, under the phase-contrast microscope, using vital dyes, Janus green B and neutral red, in very dilute concentrations.

The cytochemical tests (Appendix I) were employed on the wet whole mount smears of the amoebae.
The amoebae fixed in Lewitsky or Helly were stained with acid fuchsine, according to the special technique of Cain (1948), for the study of mitochondria. For the routine cytological study, the amoebae fixed in Lewitsky or Helly were stained subsequently with Heidenhain's iron haematoxylin.

**Observations**

The living amoeba (Fig. 1) shows, under the phase-contrast microscope, the cytoplasm differentiated into clear hyaline ectoplasm produced into one or more broad pseudopodia and granular endoplasm. The endoplasm contains lipid bodies, mitochondria, contractile and accessory vacuoles, food vacuoles and nucleus. In this amoeba no alpha particles or crystals are present. The cell inclusions show a continuous steaming movement in the living amoebae.

**Figure 1.** Trophic form of *Amoeba* sp., fresh specimen treated with 2 per cent osmium tetroxide solution. Camera lucida sketch.

AV, accessory vacuole; CV, contractile vacuole; ECT, ectoplasm; END, endoplasm; FV, food vacuole; L, lipid bodies; M, mitochondria; N, nucleus; N1, nucleolus; U, uroid.
It has been calculated that the amoebae move at a speed of 1.8 mm per hour. The cell membrane or plasmalemma covers the cytoplasm externally and permits the amoeba to throw pseudopodia in all directions. The amoeba also develops a group of ectoplasmic filaments at the posterior end, which represent the 'uroid'.

Lipid bodies.—The lipid bodies in the younger amoebae are in the form of granules, whereas in the older amoebae they are in the form of homogeneous spheres. They appear in the living amoebae, examined under the phase-contrast microscope, as dark bodies distributed at random in the endoplasm. They are stainable with neutral red supervitally. In a few amoebae, which were probably dead, small vacuoles with dark rims were observed under the phase-contrast microscope. The dark rims give a crescentic appearance. It is believed that the disorganizing lipid bodies, in the dead amoebae, give rise to these 'vacuoles with dark rims'.

The osmiophile and sudanophil nature of the lipid bodies shows the presence of lipids in them. The violet staining of the lipid bodies in Nile blue and their colouring with Sudan III and IV mixture, and Fettrot, suggest the presence of some triglycerides, in addition to acidic lipids. The moderate staining of these bodies in acid haematein (in the old cultures of amoebae) and their weak positive staining in acid haematein, after the pyridine extraction control, shows the presence of some phospholipids which have been extracted in the control. The weak positive reaction in the control sections is probably due to the presence of some proteins, which are demonstrable on staining with mercuric bromphenol blue. These bodies in old cultures also contain some fatty acids (positive Fischler's test). The lipids, which are not extracted with cold ether or ethanol, are probably forming some lipoprotein complex. The blackening of the lipid bodies in fresh osmium tetroxide preparation and their positive reaction in performic acid-Schiff shows the unsaturated nature of the lipids. It may be concluded that the lipid bodies are composed of triglycerides, phospholipids, complex lipoproteins and fatty acids.

Mitochondria.—The mitochondria which appear greyish in the living amoebae, examined under the phase-contrast microscope, are spherical or oval in shape. Janus green B stains mitochondria supervitally. But it is interesting to note that in the initial stages the outer layer of the mitochondria is stained, but with prolonged treatment they are stained homogeneously. This staining behaviour of the mitochondria clearly shows that they possess a differentiated limiting membrane which probably corresponds to the double limiting mitochondrial membranes revealed by the electron microscopy. No such differentiation is noticed in the unstained mitochondria of the living amoeba.

The mitochondria are slightly osmiophile in the fresh osmium tetroxide preparations, whereas they are intensely and homogeneously coloured with Sudan black, showing thereby that the lipids present in them are mostly
saturated, and they are, therefore, not easily blackened in 2 per cent osmium tetroxide solution.

In pyronin/methyl green preparations, their limiting membrane is stained with pyronin, while after salivary ribonuclease or perchloric acid extractions they are not stained, thus confirming the presence of RNA in them.

With mercuric bromphenol blue also, the limiting membrane is mainly stained, thus showing the concentration of protein in the peripheral portion of the mitochondria.

As in the Sudan black preparations, in Sudan III and IV, and Fettrot also, they colour homogeneously. The limiting mitochondrial membrane contains phospholipids (positive in ordinary acid haematein and negative in pyridine control) and complex lipoproteins (which resist extraction with cold acetone, ether or ethanol). In Nile blue also, only the limiting mitochondrial membrane is stained violet. These staining reactions clearly show that the mitochondria possess a differentiated peripheral layer composed of triglycerides, phospholipids, lipoproteins and RNA. The stroma of the mitochondria is largely lipoidal.

The mitochondria stain with acid fuchsine according to the special technique of Cain (1948).

Nucleus.—The nucleus appears rounded or oval in outline, with a dense body, the nucleolus, in its centre. In the living amoeba, the nucleolus appears of dark contrast with phase-contrast microscopy and constantly changes its form.

Cytoplasm.—The cytoplasm stains weakly with pyronin and mercuric bromphenol blue, thus demonstrating its basophil nature due to the presence of RNA. It also shows diffuse positive staining with periodic acid-Schiff and Best's carmine while, after extraction with salivary amylase or malt diastase, these reactions are completely negative, thus showing the presence of glycogen. The glycogen is dispersed in dilute colloidal state throughout the cytoplasm.

Contractile vacuole.—The contractile vacuole, in the living amoeba examined under the phase-contrast microscope, is seen to arise by the coalescence of small accessory vacuoles, which slowly aggregate into three or four vacuoles, the latter finally uniting to form a large contractile vacuole. Prolonged treatment of amoeba with 2 per cent osmium tetroxide has shown that the walls of the contractile vacuole or accessory vacuoles are not osmiophile.

The study of the living amoeba has clearly shown that there is apparently no direct relation between the formation of the contractile vacuole and the lipid bodies. The lipid bodies are not in any way transformed into the contractile vacuoles. The mitochondria and lipid bodies, however, aggregate round the growing vacuoles.

Uroid.—The amoebae develop at times a group of 5–15 finger-like ectoplasmic projections at the posterior end. Schaeffer (1918) described this group
of ectoplasmic filaments as ‘uroid’. The uroid acts as hold-fast and also helps the amoebae in locomotion and change of direction.

**Discussion**

*Lipid bodies.*—The lipid bodies are comparable to the ‘Golgi bodies’ described by Brown (1930) in *Amoeba proteus*, by Das and Tewari (1955) in *A. verrucosa* and by Ray and Sen Gupta (1954) in *Entamoeba histolytica*. These bodies correspond to the spherical refractive bodies described by Mast and Doyle (1935a and 1935b) in *A. proteus* 'Y' and *A. proteus* 'X' (*A. dubia*), and by Pappas (1954) in *A. proteus*, *Chaos chaos* and *Thecamoeba striata*, which according to them represent the Golgi bodies.

Hirsch (1939) reported that the presubstance of the Golgi material stains with neutral red. The present investigation also shows that the lipid bodies of amoeba stain homogeneously with neutral red, within a few seconds after treatment. These lipid bodies do not differentiate into duplex bodies recognized in other species of amoebae by Brown (1930), Mast and Doyle (1935a and 1935b), Pappas (1954) and Das and Tewari (1955). The staining of the Golgi bodies with neutral red has been reported earlier also by Ray and Sen Gupta (1954) and Pappas (1954).

The electron microscopy has also established the presence of the Golgi apparatus in *Amoeba proteus* (Cohen 1957, Pappas 1959, Mercer 1959), which resembles the metazoan Golgi apparatus. Gatenby and Tahmisian (1959) consider that the structure of the Golgi apparatus of *A. proteus* (Cohen 1957) corresponds to that of the typical Golgi dictyosome. It is, therefore, reasonable to say that the Golgi apparatus described with electron microscopy represents the structures described as the Golgi bodies with the light microscopy. The electron microscopical study of the gamete of *Amoeba spumosa* (Cigada Leonardi 1958) strongly supports this view, as the Golgi apparatus undoubtedly appears as the section of a dictyosome. The Golgi dictyosomes with similar ultrastructure have been reported in *Chlamydomonas* by Sager and Palade (1957). In a recent review on the fine structure of Protozoa, Grimstone (1961) also concludes that the dictyosomes and the parabasal bodies represent the Golgi bodies.

*Mitochondria.*—The mitochondria of the amoeba possess a differentiated peripheral layer as revealed by the cytochemical tests, which probably corresponds to the double limiting membranes of the typical mitochondria, revealed with the electron microscopy. The supervital staining with Janus green B also shows the existence of such differentiated peripheral layer, which stains within a short time. This supervital staining of mitochondria with Janus green was established long ago by Michaelis (1900). Taking into account the mitochondria in general, it has been established that they are the exclusive sites
of the location of the cytochrome oxidase and of the entire succinic dehydrogenase enzymes of the cell (Palade 1956). Hogeboom, Claude and Hotchkiss (1946) and Hogeboom, Schneider and Palade (1948) have also established the close association of succinic dehydrogenase with the mitochondria. Scarpelli and Pearse (1958), Wigglesworth (1957), Hogeboom, Schneider and Palade (1948) and Palade (1952) have produced strong evidence in favour of the specificity of the supervital staining of mitochondria with Janus green, as they have shown that the sites of succinic dehydrogenase activity corresponded to the structures stained supervitally by Janus green B.

Siekevitz and Watson (1956) have gone a step further and they have demonstrated that the succinoxidase and cytochrome oxidase systems are tightly bound to the mitochondrial membranes, and the staining of a differentiated peripheral layer of the mitochondria of amoeba with Janus green, therefore, strongly suggests that these respiratory enzymes are abundantly present in the differentiated peripheral layer, and at the same time suggests that probably the \textit{cristae mitochondriales} in these mitochondria are poorly developed. The mitochondria of amoebae have been identified with the light microscopy by Mast and Doyle (1935a and 1935b), Singh (1938), Pappas (1954), Ray and Sen Gupta (1954) and Ray and Hayes (1954); and with the electron microscopy by Sedlar and Rudzinska (1956), Dalton and Felix (1957), Cohen (1957), Cigada Leonardi (1958), Pappas (1959), Mercer (1959), Deutsch and Swann (1959), Pappas and Brandt (1959) and Brandt and Pappas (1959). Among the amoebae, the mitochondria of \textit{Pelomyxa carolinensis} (Pappas and Brandt 1959) possess the most complex internal structure. Brandt and Pappas (1959) have further suggested that the nucleus may be capable of forming mitochondria in \textit{Pelomyxa carolinensis} since they have observed a continuity of the limiting membranes of mitochondria and postdivision nuclei.

\textit{Cytoplasm}.—The cytoplasm shows positive reactions for RNA, protein and glycogen. In this amoeba, no well-defined carbohydrate bodies are present, but the glycogen is dispersed in dilute colloidal state in the cytoplasm. Hayes (1925) and Singh (1938) reported the presence of nutritive spheres composed of glycogen in \textit{Amoeba proteus}. Schaeffer (1916) described them as 'excretion spheres', while Taylor (1924) described them as nutritive spheres. Pappas (1954) has not reported the presence of any such bodies in the five species of amoebae (including \textit{A. proteus}) he has investigated. The electron microscopic studies of Cohen (1957), Pappas (1959) and Mercer (1959) also have not reported the existence of any such bodies in \textit{A. proteus}. The author has also not observed any such body in the \textit{Amoeba sp.}, examined under the phase-contrast microscope.

Pappas (1959) and Deutsch and Swann (1959) described, in the cytoplasm of amoebae, elements comparable to endoplasmic reticulum of Palade (1955). Porter (1954a, 1954b and 1955) has shown that the number of small granules of
the endoplasmic reticulum is proportional to the basiphilia (i.e. the RNA content). The present investigation also shows the presence of RNA in the cytoplasm of amoeba. Mercer (1959), however, denied the presence of any membrane comparable to the α-cytomembrane (of the endoplasmic reticulum) in A. proteus.

Contractile vacuole.—The hypothesis of Nassonov (1924 and 1925), that the contractile vacuole, together with the associated osmiophile material in Protozoa, represented the Golgi apparatus, was strongly supported by Gatesby, Dalton and Felix (1955) who investigated the ultrastructure of the contractile vacuole of Protozoa and Parazoa, and described typical double membranes in the cortices of the contractile vacuoles, similar to the Golgi apparatus of metazoan cells. The recent studies on the ultrastructure of the contractile vacuole of Takophrya infusionum (Rudzinska 1958), Amoeba proteus (Pappas and Brandt 1958, Mercer 1959), Amoeba proteus, Pelomyxa carolinensis and Hartmannella rysodes (Pappas 1959) do not lend any support in favour of the homology of the contractile vacuole with the Golgi apparatus. Pappas (1959) strongly repudiates this homology and states that ‘the contractile vacuole and its components bear no morphologic resemblance to the metazoan Golgi complex’. The author (Dutta 1959) has also stressed the view that in Amoeba sp. there is apparently no direct relation between the formation of the contractile vacuole and the lipid bodies or the so-called Golgi bodies.

Uroid.—Schaeffer (1918) coined the term ‘uroid’ to describe the group of filaments in amoeba. In this species of Amoeba, the ‘uroid’ appears as a group of 5–15 ectoplasmic finger-like projections at the posterior end. The uroid acts as hold-fast and also helps the amoebae in locomotion and change of direction.

de Fromental (1874), Leidy (1879), Penard (1902) and Schaeffer (1926) used this posterior group of filaments as a taxonomic criterion for some amoeba. Taylor (1947) observed that the uroid of Amoeba kerrii is used as a pivot. Mercer (1959) also described the uroid or tail in A. proteus.

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References

Michelis, L. (1900). *Arch. mikr. Anat.*, 55, 558. (Quoted from Palade 1956.)


## APPENDIX I

### Showing the cytochemical reactions of *Amoeba sp.*

<table>
<thead>
<tr>
<th>Technique</th>
<th>Fixation</th>
<th>Reference</th>
<th>Lipid bodies</th>
<th>Mitochondria</th>
<th>Cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBB in 70% ethanol</td>
<td>FCa and FCa + PC</td>
<td>Baker 1944, 1946</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>SBB in 70% ethanol at 90°C</td>
<td>FCa and FCa + PC</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>SBB in propylene glycol</td>
<td>FCa and FCa + PC</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>SBB in cold acetone</td>
<td>Fresh or FCa</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Sudan III and IV in 70% etha-</td>
<td>FCa and FCa + PC</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>nol/acetone</td>
<td></td>
<td></td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Fastrot in 70% ethanol</td>
<td>FCa and FCa + PC</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Nile blue sulphate (NBS)</td>
<td>FCa and FCa + PC</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Acid haematin (AH)</td>
<td>F/G + PE</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>AH* pyridine (PE)</td>
<td>Baker 1948</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Fischler’s reaction</td>
<td>F/D + PE</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Perls’s iron blue</td>
<td>F/D + PE</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2% sodium tetroxide</td>
<td>F/D + PE</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Cholesterol reactions</td>
<td>F/D + PE</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Periodic acid-Schiff (PAS)</td>
<td>B, C, H</td>
<td>Hotchkiss 1948; Pearse 1960</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>PAS* oxidation</td>
<td>B, C, H</td>
<td>McManus and Cason 1950</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>PAS* in KOH restoration</td>
<td>B, C, H</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Mercuro-hemoporphin blue</td>
<td>F/G, F/G + PC</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>P/MG</td>
<td>Z</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>P/MG* salivary ribonuclease</td>
<td>C</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Best’s carmine</td>
<td>C</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>P/MG or Best’s carmine* sali-</td>
<td>C</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>vary amylase</td>
<td></td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Plasmin reaction</td>
<td>Fresh</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

### Key:
- **B** = Bouin; **C** = Carnoy; **FCa** = formaldehyde-calcium; **H** = Helly; **P** = paraffin; **PC** = with postchroming; **P/MG** = pyronin/methyl green; **SBB** = Sudan black B; **WB + PE** = weak Bouin’s followed by pyridine extraction; **Z** = Zenker; **+** = weak positive reaction; **++** = moderate positive reaction; **+++** = strong positive reaction; **—** = negative reaction; *** = after treatment with.