Phagotrophy and Two New Structures in the Malaria Parasite *Plasmodium berghei**

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ABSTRACT

Blood collected from rats infected with *Plasmodium berghei* was centrifuged and the pellet was fixed for 1 hour in 1 per cent buffered OsO_4 with 4.9 per cent sucrose. The material was embedded in *n*-butyl methacrylate and the resulting blocks sectioned for electron microscopy.

The parasites were found to contain, in almost all sections, oval bodies of the same density and structure as the host cytoplasm. Continuity between these bodies and the host cytoplasm was found in a number of electron micrographs, showing that the bodies are formed by invagination of the double plasma membrane of the parasite. In this way the host cell is incorporated by phagotrophy into food vacuoles within the parasite. Hematin, the residue of hemoglobin digestion, was never observed inside the food vacuole but in small vesicles lying around it and sometimes connected with it. The vesicles are pinched off from the food vacuole proper and are the site of hemoglobin digestion.

The active double limiting membrane is responsible not only for the formation of food vacuoles but also for the presence of two new structures. One is composed of two to six concentric double wavy membranes originating from the plasma membrane. Since *no* typical mitochondria were found in *P. berghei*, it is assumed that the concentric structure performs mitochondrial functions. The other structure appears as a sausage-shaped vacuole surrounded by two membranes of the same thickness, density, and spacing as the limiting membrane of the body. The cytoplasm of the parasite is rich in vesicles of endoplasmic reticulum and Palade's small particles. Its nucleus is of low density and encased in a double membrane.

The host cells (reticulocytes) have mitochondria with numerous cristae mitochondriales. In many infected and intact reticulocytes ferritin was found in vacuoles, mitochondria, canaliculi, or scattered in the cytoplasm.

Phagotrophy in an intracellular parasite was first observed and described in the malaria parasite *Plasmodium lophurae* (42). An electron microscope study disclosed that the parasite engulfs parts of host cytoplasm by invaginations of its plasma membrane with the subsequent formation of food vacuoles. Within the vacuoles digestion takes place and hematin, the residue of hemoglobin (9), accumulates. Although it seemed reasonable to assume that this way of feeding is not limited to *P. lophurae* but represents a common phenomenon among malaria parasites, it was important to check on this assumption with other species of *Plasmodium*. Thus a study of *Plasmodium berghei* (43–45) has been made which has confirmed the occurrence of phagotrophy and in addition has uncovered two new structures hitherto not found in other cells. Incidental observations have been made also on the fine structure of the host reticulocytes and on the occurrence of ferritin granules within them.

Materials and Methods

Plasmodium berghei was generously supplied by Dr. Ira Singer, who has been maintaining this strain in

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young rats by biweekly blood passage (51). For electron microscopy blood was collected without an anticoagulant from rats decapitated on the 9th day of infection. Short centrifugation in the cold separated the erythrocytes from the plasma, which was discarded. The pellet of cells was cut into small pieces (40) and fixed for 1 hour at 0°C. in 1 per cent OsO_4 buffered with veronal acetate (27) at a pH 7.4. The addition of 4.9 per cent sucrose to the fixative (6) improved greatly the preservation of the cells. After fixation, the material was rapidly dehydrated in ascending concentrations of alcohol (50 per cent, 70 per cent, 95 per cent) and then kept in three changes of 100 per cent alcohol for a period of 1½ hours. It was subsequently impregnated with *n*-butyl methacrylate for about 3 hours and finally embedded for polymerization in *n*-butyl methacrylate with 2 per cent luperco either at 47°C. or 60°C. for about 24 hours. Polymerization at 60°C. gave better results, and all the electron micrographs included in this paper are of sections from blocks embedded at this temperature. The blocks were sectioned with a Porter-Blum microtome (35). Sections stretched occasionally by the application of xylol (47) or chloroform (53) were mounted on carbon-coated grids (55) and examined in a RCA microscope. Electron micrographs were made with a model EMU-2b at original magnifications from 8,000 to 12,700 and enlarged further photographically.

Observations

Plasmodium berghei usually invades reticulocytes (1, 16, 51), young red blood cells of relatively low density. There is little contrast between the density of parasite and host cell after OsO_4 fixation (Fig. 3), and this makes it difficult to distinguish an infected erythrocyte from an intact one in thin sections at lower, and sometimes also at higher magnifications. As many as five parasites were found in one sectioned host cell, two (Fig. 2) or three (Fig. 1) being very frequent.

Plasma Membrane.—The body of the parasite is surrounded by two very thin membranes (Fig. 3), closely applied to the cytoplasm of the host (see: Discussion II). Both membranes are of the same density and thickness (about 60 A), and the spacing between them varies from place to place, ranging from 60 to 170 A. Usually the membranes appear as wavy lines, apparently touching each other in places or forming loops. In some instances, invaginations of both membranes into the cytoplasm of the parasite can be seen (Figs. 3, 3 *b*, and 3 *c*). Occasionally papilla-like evaginations of both (Figs. 3, 3 *c*) or only the external membrane (Figs. 3, 3a) can be found. These observations suggest that the plasma membrane of the plasmodium is an active and flexible structure capable of frequent changes.

Food Vacuoles.—In almost all sections of *P. berghei* oval bodies of the same density as the host cytoplasm are present (Figs. 1 and 2). Their shape, number, size, and location vary from section to section. Although the oval shape prevails, elongated (Fig. 19) and dumb-bell–shaped profiles are also encountered. One to several such bodies can be found in a cell section. Their size ranges from 250 mμ to almost 2 μ in the longer diameter, the larger ones being more common. They are either centrally located (usually in young organisms) as in Figs. 1 and 4 or near the periphery of the body (Fig. 2). Each is surrounded by a double membrane (Figs. 3, 7), and contains a dense, structureless, and homogeneous matrix with a few small dense particles scattered at random. Since the same structure is characteristic for the cytoplasm of the host cell (Figs. 1 and 2) it seemed likely that these bodies represent parts of host cytoplasm, the same as found in *Plasmodium lophurae* (42). Confirmation for this view came from electron micrographs showing connection between these bodies and the host cytoplasm (Figs. 4 to 8, 17). Fig. 4 shows a section through part of an erythrocyte invaded by three young parasites. One of the parasites is in the early stage, showing clearly that the cup-shaped parasite encircles a large portion of host cytoplasm. Eventually this will be completely cut off and engulfed by the parasite, resulting in the formation of an oval body which, as in *Plasmodium lophurae*, may be regarded as a food vacuole. The connection between the not yet completed oval body and the cytoplasm of the host clearly shows the origin of this first food vacuole. Other electron micrographs show that food vacuoles, which appear in older parasites, are formed in a similar way. In Fig. 8 the formation of several oval bodies can be seen in a large and apparently very active parasite. One of the food vacuoles has already been formed, two others are in the formation stage. They appear as invaginations of the limiting membrane of the parasite. More advanced stages of the invagination process may be seen in Figs. 5 to 7. In Fig. 6 a small food vacuole is almost completed, but its connection with the cytoplasm of the host still persists in the form of a short narrow neck. In Fig. 7 one of the two food vacuoles appears to be in the last stage of pinching off from the erythrocyte cytoplasm.

The electron micrographs described here show different stages in the formation of food vacuoles. The double limiting membrane of food vacuoles still connected with the cytoplasm of the host is continuous with the double membrane surrounding the parasite. Completed food vacuoles are surrounded by two membranes which show the same thickness, density, and spacing as the plasma membrane. There is no doubt that their contents represent parts of host cytoplasm incorporated by invaginations of the plasma membrane into the body of the parasite.

Pigment Granules.—Close to the food vacuoles small, very dense granules, which represent most probably the pigment hematin, may be found (Figs. 12 to 14). They are present also in other parts of the cytoplasm (Fig. 10). Their size ranges from about 30 to 85 mμ in width and to 190 mμ in length. They are always encased in a vesicle limited by a thin membrane. The size of the vesicles varies, and ranges from about 250 mμ to about 374 mμ in longer diameter. The ground substances of the vesicles is homogeneous and varies in its density (Figs. 10 and 15). Usually one pigment granule is present in each vesicle but occasionally two or three may be found (Fig. 3). In some electron micrographs connection between the vesicles and the food vacuole can be noted (Figs. 10, 13). In Fig. 10 several vesicles containing pigment granules lie in the vicinity of a food vacuole. One of the vesicles is connected with the food vacuole. Similarly connected vesicles may be seen clearly in Figs. 9, 11 to 14. These electron micrographs suggest that the vesicles are derived from food vacuoles by a "pinching off" process. A great number of vesicles with pigment granules may surround or be attached to one food vacuole (Figs. 12 to 14). Of particular interest are Figs. 13 and 14 which are electron micrographs of two serial but not adjacent sections through the same organism. The sections are far enough apart not to pass through the same vesicles. The presence of numerous vesicles in both sections indicates the abundance of vesicles containing hematin around this food vacuole. In all electron micrographs studied, pigment granules were found only in the small vesicles, none in the food vacuole proper. It might be assumed, therefore, that the small vesicles are the site of hemoglobin digestion. Occasionally a food vacuole breaks into small units in which digestion apparently takes place as indicated by the presence of hematin granules. This is seen in Fig. 16 where one of the small vacuoles, most probably part of a larger food vacuole, contains two pigment grains. The same process seems to be represented in Fig. 20. Vesicles with hematin are present not only around food vacuoles but also in other parts of the parasite body, suggesting that they are able to move freely in the cytoplasm. Some of the vesicles at the periphery of the body show continuity with the internal double limiting membrane. (Fig. 23 *a*). It would seem that such pigment granules are on the way out of the parasite body. However, so far no hematin granules have been found outside the parasite, in the host cell.

Endoplasmic Reticulum and Palade's Small Particles.—Vesicles and canaliculi ranging from 25 to 130 mμ in shorter diameter appear in groups or singly scattered throughout the cytoplasm (Figs. 2, 15). They represent profiles of the endoplasmic reticulum, an interconnected vacuolar system, found and described first in electron microscopy by Porter in avian cells cultured *in vitro* (36). The identification of the endoplasmic reticulum in thin sections was accomplished thereafter in a variety of cells by Porter (35, 37), Palade and Porter (31), and other investigators.

The content of vesicles and canaliculi of the endoplasmic reticulum is of low density and bound by a thin smooth- or rough-surfaced membrane (31). The latter has on its outer surface small dense particles about 100 to 130 A in diameter (Fig. 20). They are most probably Palade's particles containing ribonucleoprotein (29, 32). Particles of the same size are present in the ground substance of the cytoplasm. They contain also ribonucleoprotein, but seem to differ from the particles attached to the membranes in their RNA turnover and the RNA/protein ratio, according to the latest work of Siekevitz and Palade (49). These free particles appear in great abundance in the form of clusters or rosettes (Fig. 24).

Although most of the vesicles of the endoplasmic reticulum are rough surfaced and oval in shape, in some sections groups of smooth surfaced vesicles may be found also. They are usually more closely packed and composed of small vesicles or canaliculi arranged often in parallel lines and accompanied by several larger ones at the periphery (Fig. 19). They look very similar to the Golgi apparatus described in cells of higher organisms (8, 18, 38, 52).

Concentric Double Membraned Structure.—In many sections a structure composed of several pairs of wavy membranes arranged concentrically has been found. Occasionally the structure has more than one center (Fig. 19). The size of this organelle varies from 250 mμ to about 1 μ in length and from 180 to 280 mμ in width. In section it may appear oblong (Figs. 10, 22) or circular (Fig. 10). Its major component, the double membranes, are smooth surfaced and vary in number from two to six. They are about 50 to 60 A thick and are closely packed. The spacing between two membranes is about 60 to 100 A and between two pairs about 300 A. The membranes have a wavy appearance, and are usually assembled at the periphery of the organelle, leaving its center of low density structureless. One to three such structures may be encountered in a single section. They are most frequently found near the periphery of the cell. In several instances a continuity between the double membrane of the parasite body and the concentric double membraned structure was noticed. This is well represented in Figs. 4, 4 *a*, 18, 18 *a*, where the connection between both structures is clearly defined, suggesting that the concentric structure might originate from the two plasma membranes by infoldings. In Fig. 17 an early stage in the formation of the concentric structure could be traced. In some electron micrographs, concentric membranes are connected with membranes surrounding the food vacuole (Figs. 7 and 11). In Fig. 11 the concentric structure is continuous with the plasma membrane and with the membranes of the food vacuole.

The structure described above resembles a certain very special type of mitochondrion found in *Helix pomatia* in some stages of spermatogenesis (2, 17). Since no regular mitochondria are present in *Plasmodium berghei*, it might be suggested that these structures represent mitochondria.

Double Membraned Vacuole.—Another so far unidentified structure was found in many sections of *Plasmodium berghei*. It appears as an elongated sausage-shaped vacuole with bulb-like enlargements at its ends (Fig. 20). In some sections its central part is narrow and long and the end portions extended (Fig. 21). The size and shape of the structure vary, depending probably on the plane of sectioning. The longest so far found was 2¾ μ long with an inner diameter of 25 mμ in its narrowest part, and about 700 mμ at its broadest bulb-like ends. It is usually located parallel to the limiting membrane of the parasite body (Figs. 20 and 22), but it was also found in other parts of the body (Figs. 9 and 21). Its light and structureless matrix is surrounded by a thin, double, wavy membrane of the same thickness and density as the limiting double membrane of the parasite body. The significance and role of the double membraned vacuole are so far unknown.

The Nucleus.—One to four nuclei may be found in sections of *Plasmodium berghei*; however, in the majority of sections only one or two nuclei are present (Figs. 1 to 4, 6 to 8, 11 to 24). The nucleus is a large body over 2 μ long and over 1 μ wide. Its matrix is fairly homogeneous and of low density with denser small granules scattered at random. It is surrounded by two distinct membranes, each about 100 A thick and spaced about 170 A apart. To the external membrane Palade's small particles are attached at places. In sections where the nucleus lies at the periphery of the cell and it is closely adjacent to the plasma membrane occasionally three instead of four membranes (two belonging to the nucleus and two to the plasma membrane) are resolved. The middle membrane appears always in such instances to be thicker and denser than the two others (Figs. 15, 15 *a*). The middle membrane is most probably a result of coalescence of two membranes, the external nuclear and the internal plasma membrane.

The shape of the nucleus varies greatly. Oval (Fig. 23) and circular (Fig. 19) profiles are found along with a variety of irregular shapes. Of particular interest is the shape of nuclei which happen to be located close to food vacuoles, as seen in Figs. 1, 2, and 4. In all these electron micrographs the nucleus has a cup-like shape and lies around the food vacuole. The contact between both bodies is so intimate that their membranes are touching each other at places. An explanation of this very peculiar relationship between nucleus and food vacuole is provided in Fig. 8, which shows the beginning of the formation of a food vacuole in the vicinity of a nucleus. It is evident that a depression in the nucleus has the shape of the invaginating vacuole. No doubt as the process continues the nucleus is forced to give up more and more space for the developing ovoid food vacuole, thus finally acquiring a cup-like shape. A further result is a very close contact of both bodies as seen in Figs. 1, 2 and 4.

Fine Structure of the Host Cell.—As mentioned earlier, the parasite preferentially invades reticulocytes (1, 16, 51). These young red blood cells are bound by a thin plasma membrane (about 80 A)

which appears as a dense line (Fig. 25). In some cells many deep invaginations and folds of the plasma membrane can be noticed (Fig. 25). The cytoplasm is of comparatively low density. In its ground substance are suspended small dense particles about 170 A in diameter, probably Palade's ribonucleoprotein particles, which are sparsely scattered at random (Figs. 25 and 26). The endoplasmic reticulum is not too well developed and is represented by small vesicles and canaliculi (Figs. 25 and 26). Occasionally groups of canaliculi and vesicles closely assembled may be found, representing most probably the Golgi apparatus (Fig. 26). Mitochondria (Figs. 22 and 25) show the typical structure of mammalian cells as described by Palade (28). The double limiting membrane is clearly defined and also the cristae mitochondriales, which in some mitochondria appear in great abundance. This may be seen in Fig. 22 which shows a mitochondrion with a very rich internal structure in a reticulocyte containing two mature parasites. The numerous cristae mitochondriales disclose in many places continuity with the internal mitochondrial membrane from which they are formed by infoldings. Degenerating mitochondria with a few cristae or deprived completely of internal structure may be encountered also.

In a great number of reticulocytes ferritin granules were found (Fig. 27). The occurrence of these small, very dense iron particles in some cells has been reported by a number of investigators (3, 4, 26, 30, 39, 48). They appear in rat reticulocytes as very dense granules about 60 A in diameter densely concentrated either inside vacuoles or canaliculi or scattered in the ground substance of the cytoplasm (Fig. 27). They are present also often inside mitochondria. Some of the vacuoles containing ferritin approximate the size of mitochondria and are surrounded by a double membrane similar in spacing and thickness to the double mitochondrial membrane (Fig. 27). Inside such vacuoles double membranes resembling cristae mitochondriales may be found, leaving little doubt as to the origin of the vacuole itself. Canaliculi with ferritin are often located close to the periphery of the cell, suggesting that they are formed by pinocytosis as described by Bessis and Breton-Gorius (4). Deep pockets extending into some of the reticulocytes from the cell surface (Fig. 25) show that the plasma membrane of these cells is a flexible structure, able to form invaginations, thus supporting strongly Bessis' suggestion.

DISCUSSION

I

The electron microscope study of *Plasmodium berghei* supplies strong additional evidence that feeding in malaria parasites occurs by phagotrophy. In almost all sections of *P. berghei* food vacuoles of the same density and structure as the cytoplasm of the host are found. Continuity between these vacuoles and host cytoplasm in many electron micrographs makes it clear that these bodies represent parts of host cytoplasm engulfed by phagotrophy in the same way as in *Plasmodium lophurae* (42). Stages in the engulfing process from the beginning of the invagination of the plasma membrane till the pinching off of the complete food vacuole can be traced in a number of electron micrographs (Figs. 4 to 8). The double plasma membrane of the parasite is continuous with the double membrane surrounding the engulfed cytoplasm of the host. Each completed food vacuole is encased in a double membrane which is part of the original limiting membrane of the parasite body and, therefore, morphologically similar to the plasma membrane.

The process of formation of food vacuoles appears to be the same in both species. However, the mechanisms involved in the digestion of the content of food vacuoles differ greatly. It is well known from biochemical studies that hemoglobin is the main protein source for malaria parasites (25) and that the residue of its digestion is the pigment hematin (9). In almost all species of *Plasmodium* the pigment can be easily detected during the development of the parasite in light microscopy; however, in *P. berghei* the pigment hematin can be actually seen only just before segmentation. The puzzling phenomenon of lack of hematin in this species at earlier stages finds its explanation in the present study. The electron micrographs show that this is due to a peculiar way of digestion, entirely different than in *P. lophurae*, a species in which hematin can be seen in almost all stages during its life span. In the latter organism digestion takes place within the food vacuole where hematin accumulates forming aggregates large enough to be seen with light optics. In *P. berghei* digestion apparently does not take place inside the food vacuole proper. The latter never contains hematin. The pigment occurs as tiny grains, one to three in each of numerous small vesicles which often lie around the food vacuole. The membrane of some of the vesicles shows continuity with the outer

membrane of the food vacuole, suggesting that the vesicles are pinched off from the latter. There is reason to believe that digestion of hemoglobin takes place in the small vesicles because they are the only place in which hematin, the residue of hemoglobin, is to be found. Further support for this assumption comes from the varying degree of density of the matrix of the vesicles, probably representing stages in the digestion process. It could be also assumed that digestion takes place within the food vacuole proper and that the residue hematin is being removed from the food vacuole into the vesicles. If this were the case, one would expect to find hematin granules inside the food vacuole. It would also be expected that food vacuoles surrounded by many vesicles with pigment granules should have a matrix of lower density than those not surrounded by vesicles. Finally, all vesicles with hematin should be of the same density. The electron micrographs do not supply evidence for any of the enumerated possibilities. Only a few instances were found in which the food vacuole did not pinch off vesicles, but broke into smaller units (Fig. 16) in which digestion apparently took place as evidenced by the presence of hematin.

It seems that the digestive vesicles are able to change their position, since they are found not only in the vicinity of food vacuoles, but also scattered throughout the cytoplasm. The small size (on the average about 55 mμ in shorter diameter) and scattered appearance of hematin granules make them invisible by light microscopy. This explains why hematin could not be seen throughout almost the whole life span in the majority of organisms of *P. berghei* (16). Its sudden appearance at segmentation is probably the result of accumulation of all hematin vesicles in one area, just as in *P. lophurae* at the time of segmentation when all residual bodies and lipides are assembled in one large vacuole surrounded by a separate membrane (46).

In spite of the differences in the mechanism of digestion, the way of incorporating and utilizing the host cytoplasm is the same in *P. lophurae* and *P. berghei* and probably in all malarial parasites. The first food vacuole appears in both species to be large and centrally located, and there remains no doubt that its formation represents the ring stage of the parasite. Large food vacuoles may be divided into smaller units, and new food vacuoles are formed by engulfment of additional parts of host cytoplasm. In this way, the whole host cell in *P. berghei* and the whole cytoplasm in *P. lophura* is gradually incorporated into the parasite body where it is subsequently digested. This seems to be the simplest and at the same time the most efficient way of utilizing the host's body as food supply by an intracellular parasite.

As mentioned in a previous paper (42), this simple mode of ingesting globules of the surrounding medium appears to be a widespread phenomenon. Of special interest in this respect is pinocytosis, a form of phagotrophy, first observed by Edwards (12) in amoebae and thereafter by a number of investigators (7, 19, 22). Lewis (20), who introduced the term pinocytosis, which means drinking by cells, found it in macrophages, fibroblasts, sarcoma, and carcinoma cells. He stressed its significance, expressing the opinion that it might be a much more universal process than suspected. Confirmation for his supposition comes from recent electron microscope studies where pinocytosis was found in a great variety of cells of relatively fixed form (10, 30, 34, 38, 57). It could be concluded from these studies, that pinocytosis is not limited to organisms or cells known to have amoeboid movements. This seems to be justified by the fact that pinocytosis is not directly connected with pseudopodia formation but with the activity of the plasma membrane itself.

There is no doubt that pinocytosis performs a nutritional function. It is known that it may be induced by a number of substances (7, 12, 19, 22). Through pinocytosis a cell is able to incorporate great quantities of the substrate in which it is bathing, and this is exactly what is going on in *P. lophurae* and *P. berghei*. The parasite is suspended in the host which is being progressively incorporated.

II

The fine structure of *P. berghei*, although in general similar to other cells, shows a number of distinguishing features. The most characteristic are the richness of double membranes and the lack of typical mitochondria. The double membranes contribute to the formation of two new structures. Both originate most probably from the plasma membrane which is double. There seems to be little doubt that the two membranes belong to the parasite since merozoites in *P. lophurae* are covered by a double plasma membrane (46).

One of the new structures appears as an elongated body composed of two to six smooth double membranes arranged concentrically. Concentric

double membraned structures have been reported in a variety of cells (14, 15, 24, 33, 41, 54). Their membranes were found to be either smooth (33) or rough surfaced (14, 41). They are regarded by some investigators as a type of endoplasmic reticulum (14, 33, 54), by others as a secretory organelle (15, 24). None of the above structures seems to be homologous or analogous to the concentric, double membraned structure in *P. berghei*.

The concentric double membraned structure in *P. berghei* is usually located near the periphery of the cell. Its membranes are often continuous with the double limiting membrane of the body and occasionally with the double membrane of the food vacuole. The electron micrographs supplied good evidence that the concentric double membraned organelle derives from the double plasma membrane by its invaginations. This might explain the occasional connection of the concentric membraned organelle with food vacuoles, since the membranes of the latter are part of the original limiting membrane of the body. No information was found, so far, as to the way the invaginating double plasma membrane acquires the concentric pattern; it might be through multiple infoldings or by coiling.

The concentric double membraned organelle resembles a certain type of mitochondria found in some stages of spermatogenesis in *Helix pomatia* (2, 17). Since mitochondria of the usual type are lacking in *P. berghei*, the concentric structure may be assumed to represent mitochondria. Pertinent in this connection are recent findings in bacteria. It was established that in the latter organisms the total cytochrome system and the total succinic dehydrogenase activity reside in the plasma membrane (23, 56), which thus performs the function of mitochondria. The fact that the concentric structure derives from the plasma membrane is compatible with the hypothesis that the concentric double membraned organelle in *P. berghei* performs the function of mitochondria, as does the plasma membrane of bacteria.[1] Should further study confirm this hypothesis, a significant step in the evolution of a cell organelle might have been found.

It should be pointed out also, that the origin of the double membraned concentric structure from the limiting membrane of the body shows the remarkable potentialities of the plasma membrane. These potentialities are also revealed in the role of the plasma membrane in the feeding mechanisms of the parasite. As described earlier, invaginations of the limiting membrane engulf parts of host cytoplasm leading to the formation of food vacuoles.

The other new structure which appears in the form of a sausage-like vacuole surrounded by a double membrane seems to originate also from the plasma membrane, although no direct evidence was found so far. The density, thickness, and spacing of the membranes appear to be exactly the same as in the plasma membrane. The role and significance of this large vacuole are difficult to determine, since no similar organelle has been found in other cells. The low density and structureless appearance of its matrix suggest that it might be a reservoir of fluid and as such of some importance for an intracellular parasite embedded in a dense cytoplasm of the host cell. It is possible that large vacuoles in *P. lophurae* (42) play a similar role.

Other cellular structures usual for the majority of cells are richly represented in *P. berghei*. The endoplasmic reticulum is well developed in the form of numerous vesicles and canaliculi bound by rough surfaced membranes. In some cells vesicles of the Golgi apparatus were found also. The ground substance is abundant in Palade's small particles containing ribonucleoproteins. It might be assumed from these observations that *P. berghei* is a very active cell involved in extensive protein synthesis (21).

The nucleus shows very low density, often lower than the surrounding cytoplasm, and its double limiting membrane over limited areas is sometimes in close contact with the limiting membrane of the body. In such places, instead of four membranes only three may be seen, the middle being thicker and darker. It seems that the outer nuclear membrane and inner plasma membrane when very closely approximated fuse together, a phenomenon known in myelination (11).

III

The host cell is almost always represented by reticulocytes (1, 16, 51). It is remarkable that there seems to be no significant difference in the fine structure of an infected and intact cell. Mitochondria retain their structure and show numerous cristae mitochondriales even in cells containing more than one large parasite. The same applies to the endoplasmic reticulum. Vacuoles and canaliculi containing ferritin may be found in reticulocytes

[1] Of particular interest in this respect is the finding of a double membraned organelle formed by invaginations of the plasma membrane in the giant bacteria *Thiovulum majus* (13).

invaded by *P. berghei* as well as in cells free from parasites.

Ferritin was first found in cells of rat spleen and liver in electron microscopy by Palade (30), Schulz (48), and Novikoff *et al.* (26). Its occurrence in human erythroblasts was thereafter reported by Bessis and Breton-Gorius (4) and recently in avian erythrocytes by Benedetti and Leplus (3). Of particular interest is the work of Bessis and Breton-Gorius who followed the cycle of the ferritin molecule in reticular and red blood cells. According to their observations (4, 5), ferritin enters erythroblasts by ropheocytosis (a type of pinocytosis) and is present in all erythroblasts where it participates in hemoglobin synthesis. Red blood cells in which this process is completed are free of ferritin or may have only a few granules. However, large accumulations of ferritin may be found in the cytoplasm and inside mitochondria of erythroblasts and erythrocytes of certain hypochromic anemias (4, 5). In these diseases the amount of iron is normal, but the amount of hemoglobin low. Probably a blockage disturbs the synthesis of hemoglobin, and, as a result, extensive accumulations of iron particles are formed. Interesting in this connection is the occurrence of great amounts of ferritin granules assembled inside mitochondria or in vacuoles of reticulocytes in rats infected with malaria. It is known that a high degree of anemia accompanies this disease (1, 50). It is possible that this is due not only to the progressive destruction of reticulocytes invaded by the parasite, but also to a process similar to hypochromic anemia described above.

The most puzzling phenomenon is the presence of ferritin inside mitochondria. It was found only occasionally inside mitochondria of normal erythroblasts, but frequently and in great quantities in thallassemia and Cooley's anemia. In both these diseases iron appears not only in the form of ferritin but also as ferruginous micelles. The presence of these two types of iron inside mitochondria led Bessis (5) to a hypothesis that mitochondria might play an important role in hemoglobin synthesis changing ferritin into ferruginous micelles. These fine iron particles after liberation from mitochondria participate in the formation of the hemoglobin molecule. It is, however, difficult to understand how such a large molecule as ferritin enters mitochondria, organelles bounded by a continuous membrane.

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Explanation of Plates

Abbreviations

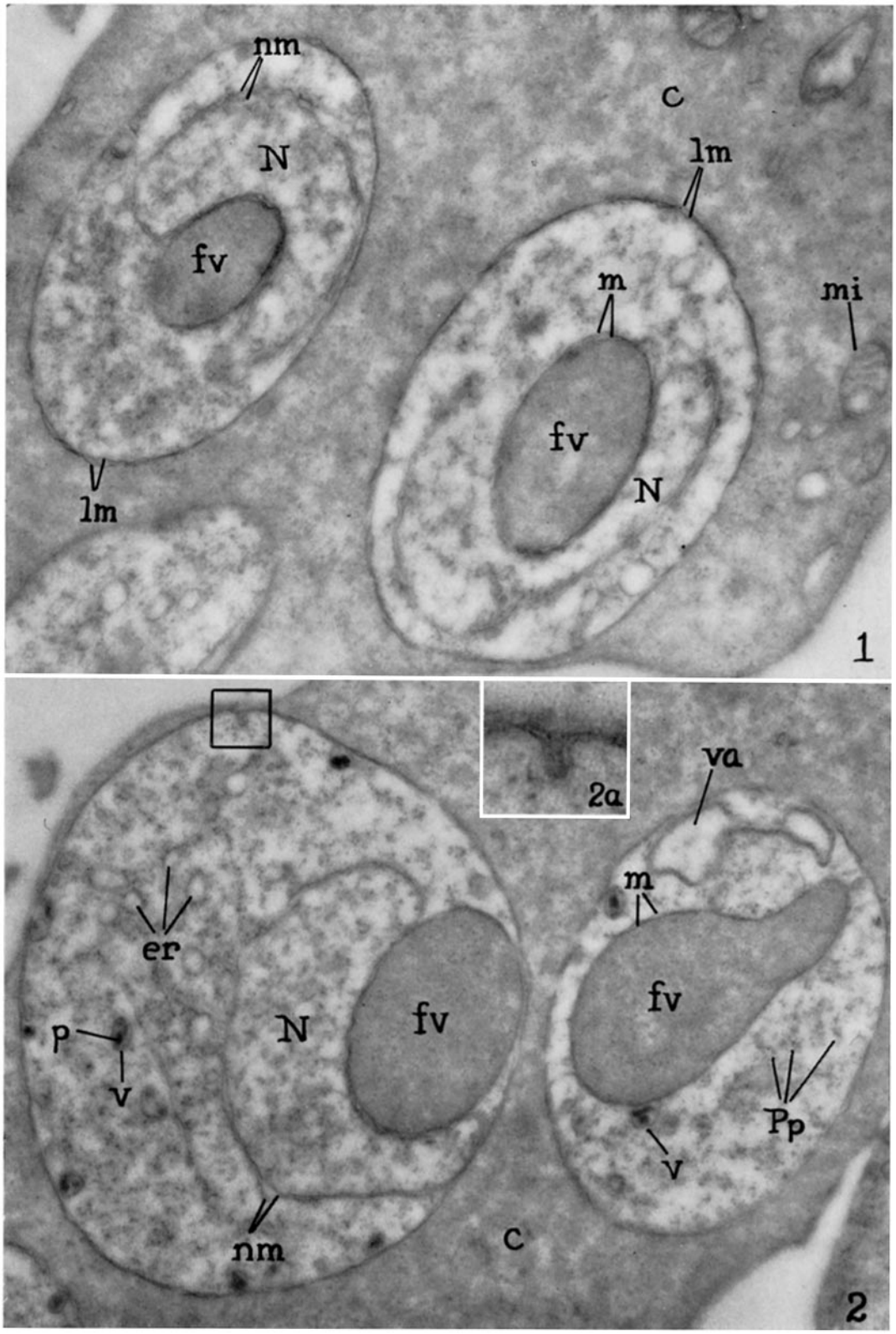
c—cytoplasm of host cell.
ca—canaliculus.
cm—structure composed of double concentric membranes.
em—evagination of external limiting membrane.
er—endoplasmic reticulum.
f—ferritin.
fv—food vacuole.
G—Golgi apparatus in parasite.
G_1—Golgi apparatus in reticulocyte.
lm—double limiting membrane.
m—double membrane surrounding food vacuole.
mi—mitochondria.
nm—double nuclear membrane.
N—nucleus.
p—pigment hematin.
pm—plasma membrane of host cell.
Pp—Palade's particles.
v—vesicle.
va—vacuolar structure surrounded by two membranes.
vac—vacuole.

Plate 63

Fig. 1. Electron micrograph of part of reticulocyte infected with three young parasites. Each of the parasites is surrounded by a double limiting membrane (*lm*). The large oval bodies seen in two parasites are food vacuoles (*fv*). They show the same structure and density as the cytoplasm of the host (*c*), and are surrounded by a double membrane (*m*). The nucleus (*N*) has a sausage-like shape and is in close contact with the food vacuole; it is encased by a double nuclear membrane (*nm*). In the cytoplasm of the reticulocyte (*c*) mitochondria (*mi*) may be seen. Magnification, 40,000.

Fig. 2. Two parasites containing large food vacuoles (*fv*) surrounded by two membranes (*m*). In one of the parasites the nucleus (*N*) encased in its double membrane (*nm*) lies around the food vacuole. Several small vesicles (*v*) contain the pigment hematin (*p*). In the ground substance of the cytoplasm numerous small dense particles represent Palade's particulate component (*Pp*). In the larger parasite rough surfaced vesicles of the endoplasmic reticulum (*er*) are present. A new so far unidentified vacuolar structure (*va*) may be seen. Outlined area is shown in higher magnification in Fig. 2 *a*. Magnification, 40,000.

Fig. 2 *a*. Higher magnification of area outlined in Fig. 2 showing an invagination of the double limiting membrane. Magnification, 100,000.

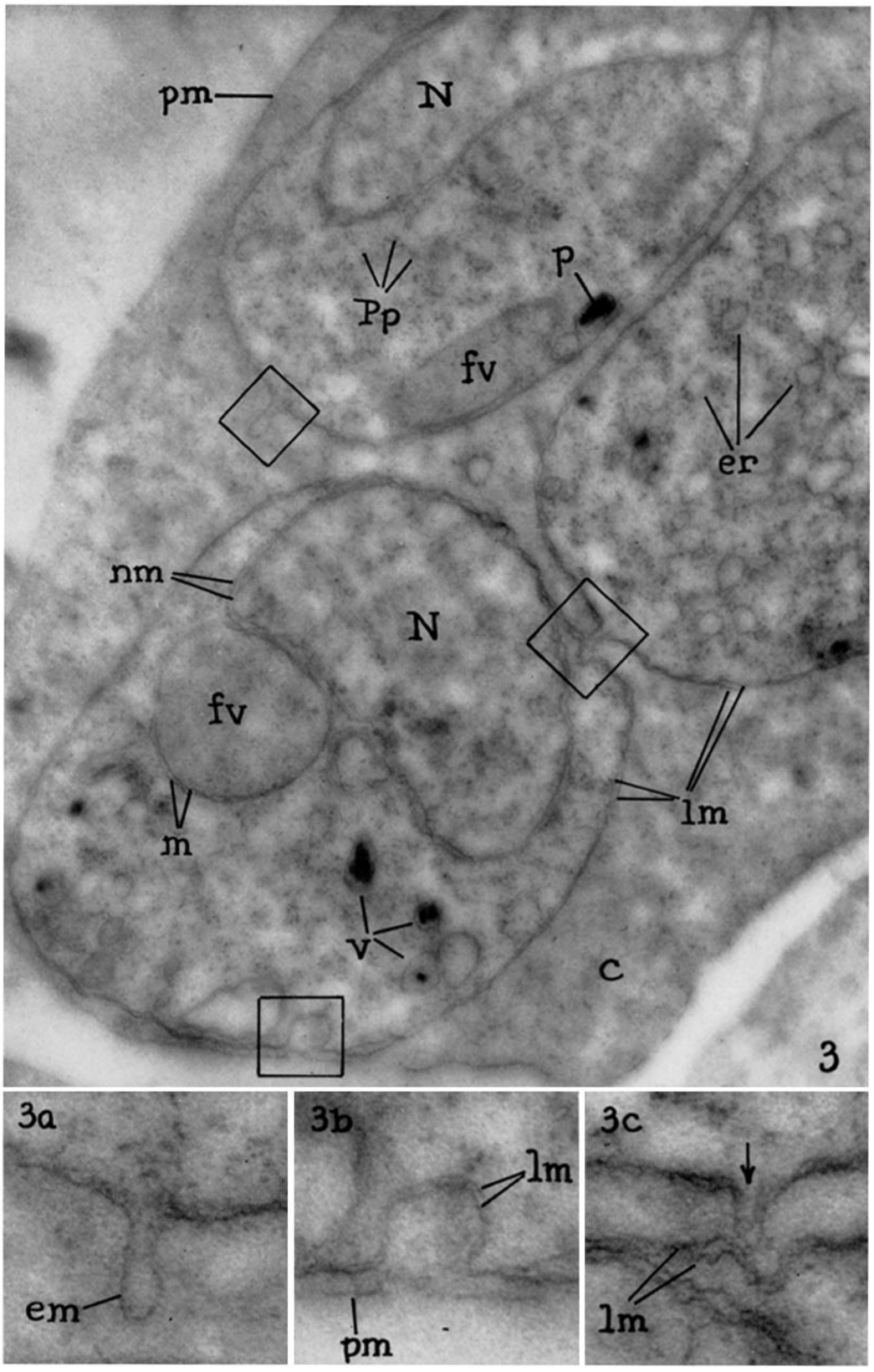


(Rudzinska and Trager: Phagotrophy in *Plasmodium berghei*)

PLATE 64

FIG. 3. Thin section through part of reticulocyte infected with three parasites. Note that the density of the host (*c*) and parasite are the same. Each of the parasites is surrounded by a double limiting membrane (*lm*). In the ground substance of the cytoplasm small dense particles represent Palade's particulate component (*Pp*). The endoplasmic reticulum (*er*) appears as rough surfaced vesicles. The bodies which show homogeneous structure similar to the cytoplasm of the host are food vacuoles (*fv*). They are surrounded by a double membrane (*m*). The pigment hematin (*p*) is within small vesicles (*v*). The large nucleus (*N*) is encased in a double membrane (*nm*). The cytoplasm of the host (*c*) is surrounded by the plasma membrane (*pm*). Magnification, 40,000.

FIGS. 3 *a*, *b*, and *c*. Higher magnification of areas outlined in Fig. 3 to show evagination of external limiting membrane (*em*) (Fig. 3 *a*), evagination of both membranes at arrow (Fig. 3 *c*), invagination of both membranes (*lm*) (Figs. 3 *b* and *c*). Magnification, 100,000.



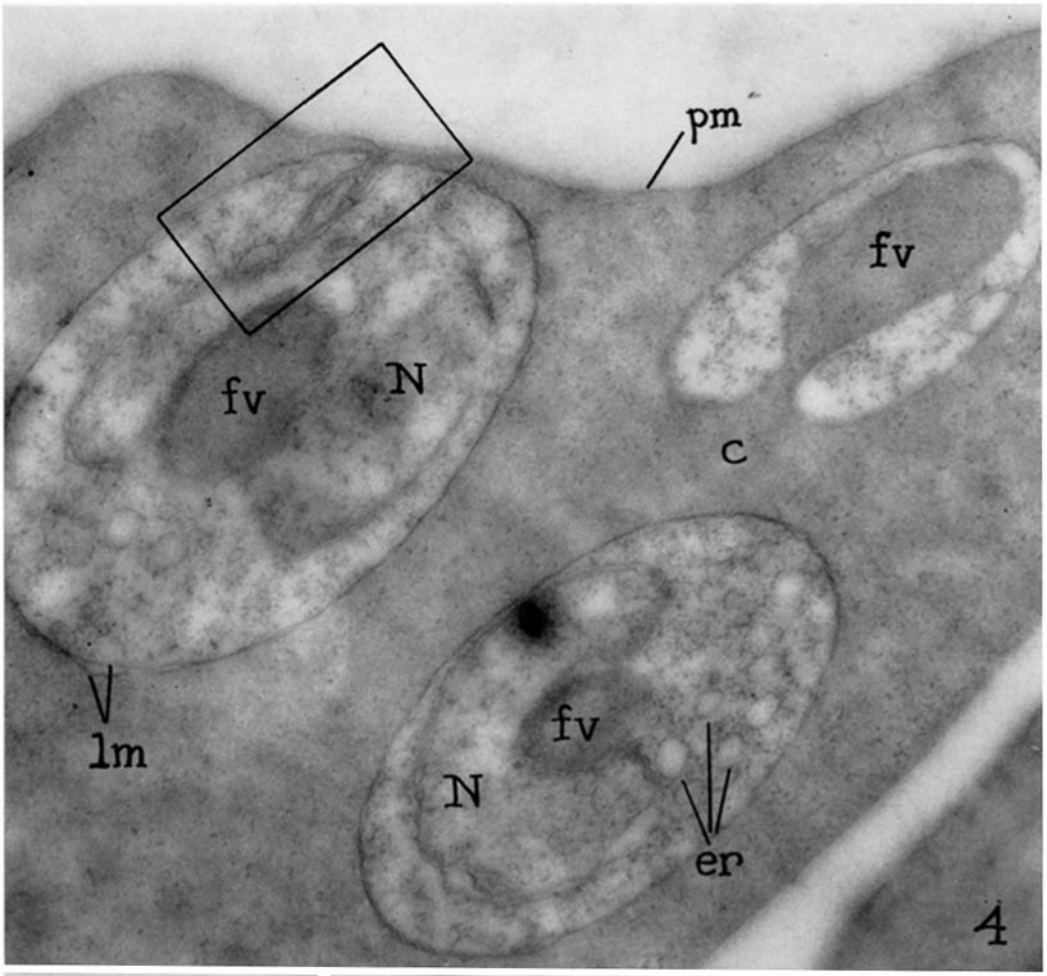
(Rudzinska and Trager: Phagotrophy in *Plasmodium berghei*)

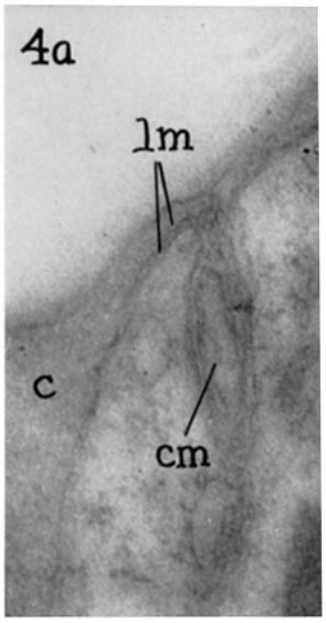
PLATE 65

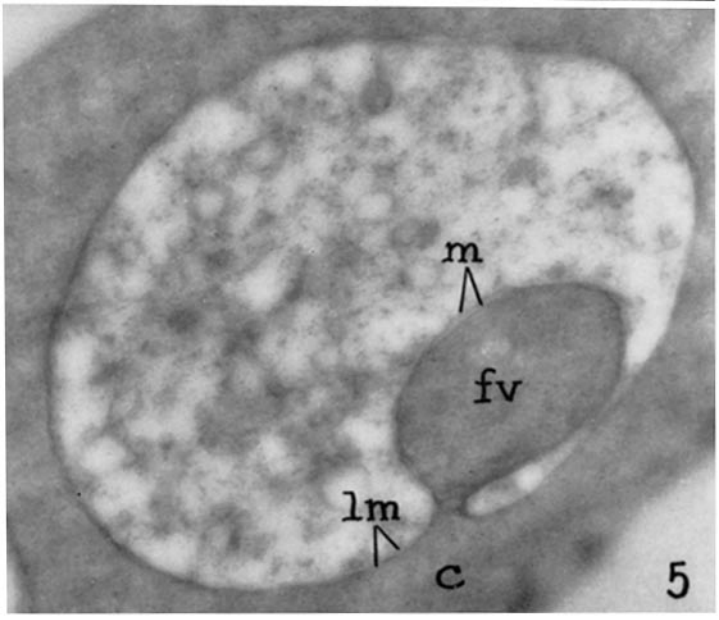
FIG. 4. Electron micrograph of three young *Plasmodia berghei*. One of the parasites on the right side is in the ring stage and shows clearly continuity between the content of the food vacuole (*fv*) and cytoplasm of host cell (*c*). Area enclosed in rectangle is shown at higher magnification in Fig. 4 *a*. Magnification 40,000.

FIG. 4 *a*. Higher magnification of area outlined in Fig. 4. It represents a new structure composed of double concentric membranes (*cm*) continuous with the limiting membrane of the body (*lm*). Magnification, 80,000.

FIG. 5. Section through parasite containing a food vacuole (*fv*) still connected with the cytoplasm of host (*c*). The double membrane of the food vacuole (*m*) is continuous with the plasma membrane (*lm*). Magnification, 40,000.







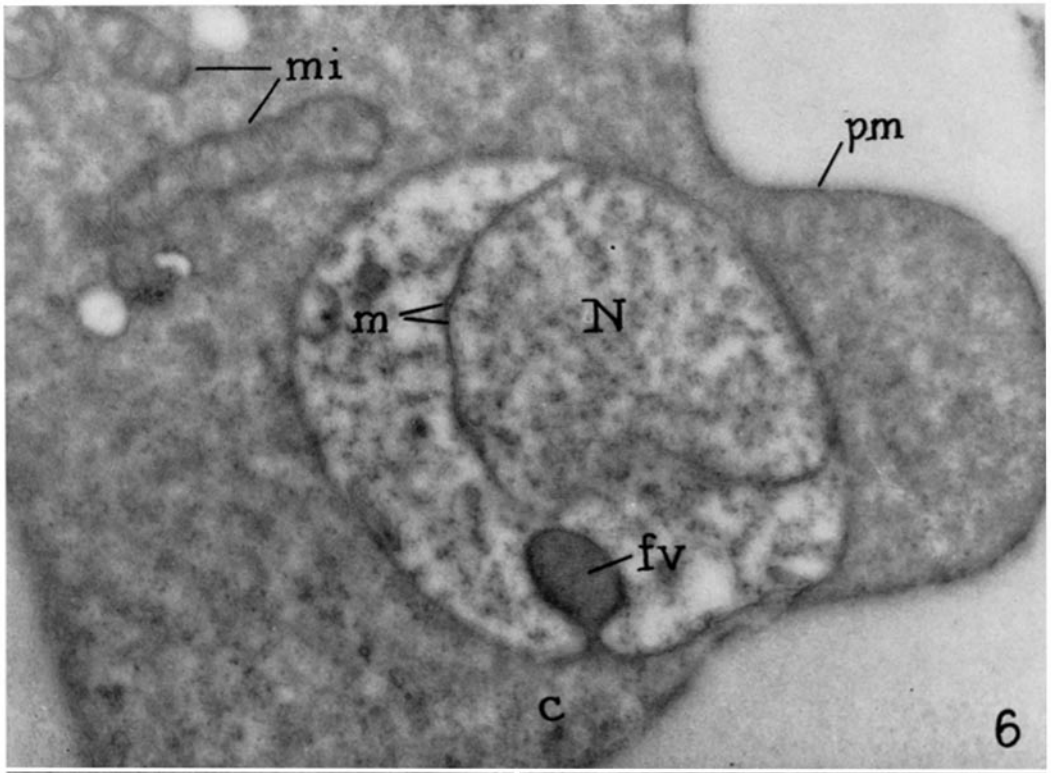
(Rudzinska and Trager: Phagotrophy in *Plasmodium berghei*)

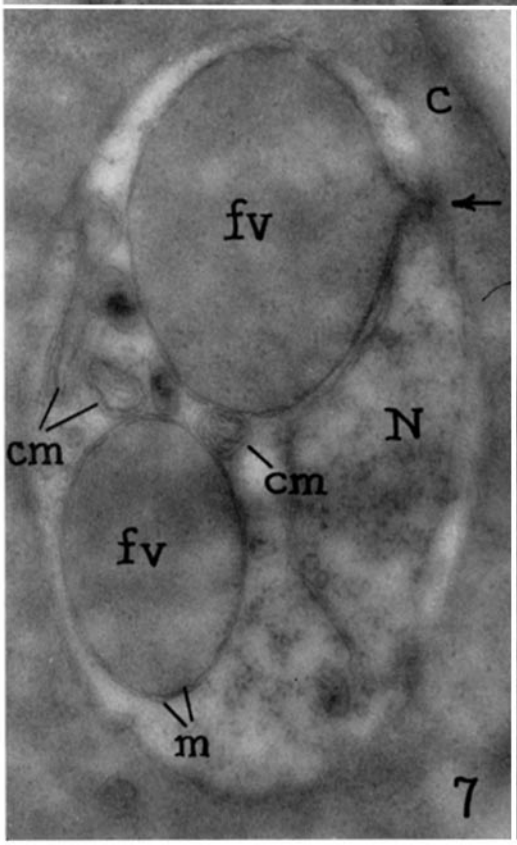
PLATE 66

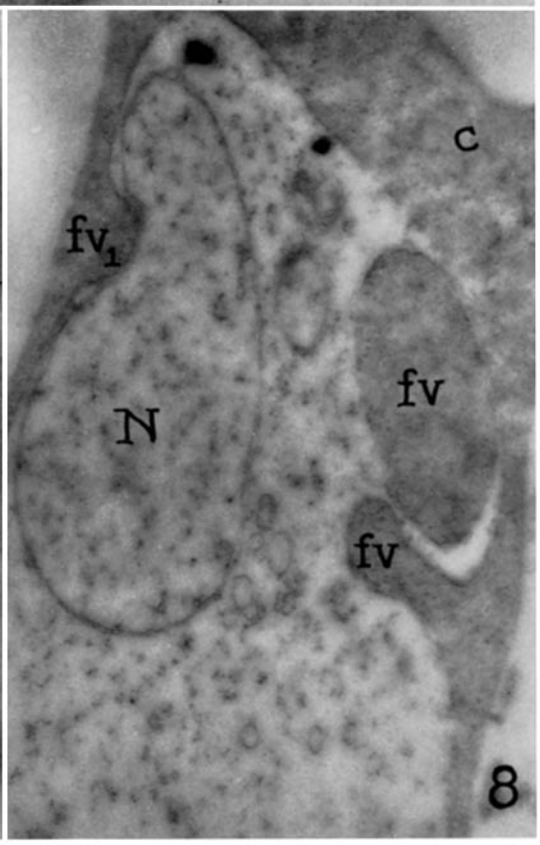
FIG. 6. Electron micrograph of part of reticulocyte with one parasite showing a small food vacuole (*fv*) connected by a narrow short neck with the cytoplasm of the host (*c*). The nucleus (*N*) surrounded by a double membrane (*m*) occupies a large part of the parasite's body. Magnification, 40,000.

FIG. 7. *Plasmodium berghei* with two large food vacuoles (*fv*) each encased in a double membrane (*m*). The large one is in the last stage of pinching off (arrow) from the cytoplasm of the host (*c*). Notice two vesicles containing the pigment hematin and three concentric double membraned structures (*cm*). Magnification, 51,000.

FIG. 8. Section through a large, very active parasite with three food vacuoles (*fv*) two of which are in early stages of formation. Of special interest is the food vacuole (fv_1), invaginating near the nucleus (*N*). In the latter a depression may be seen which has the shape of the invaginating food vacuole. Magnification, 26,000.





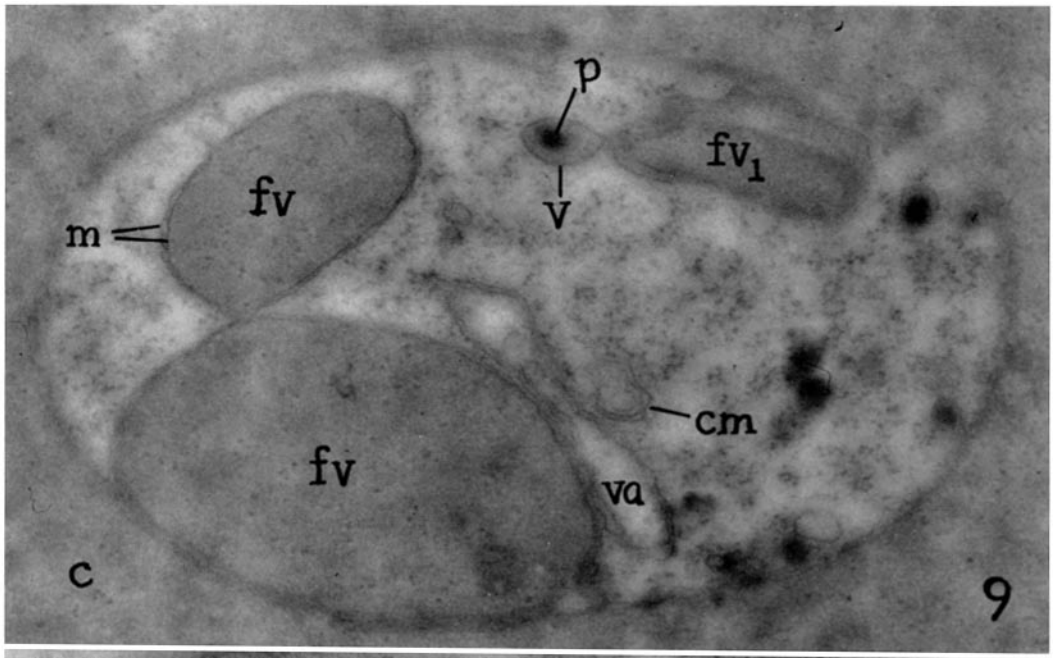


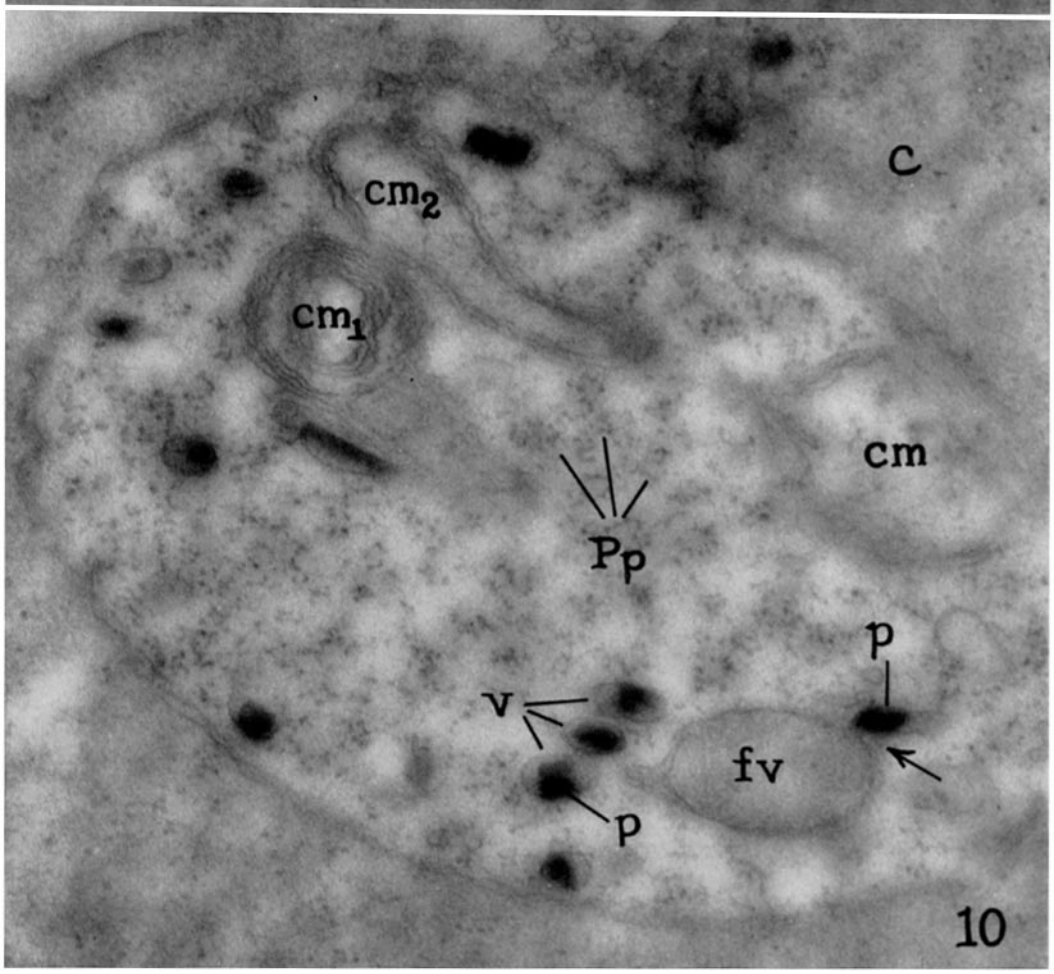
(Rudzinska and Trager: Phagotrophy in *Plasmodium berghei*)

PLATE 67

FIG. 9. Section through a parasite with three food vacuoles. The smallest food vacuole (fv_1) is irregular in shape and is connected with a vesicle (v), containing the pigment hematin (p). Magnification, 40,000.

FIG. 10. Electron micrograph of *Plasmodium berghei* showing several vesicles (v) containing pigment (p). One of the vesicles is connected (at arrow) with the food vacuole (fv). Notice three concentric membraned structures (cm, cm_1, cm_2) in cross- (cm_1) and longitudinal sections (cm_2). Magnification, 60,000.





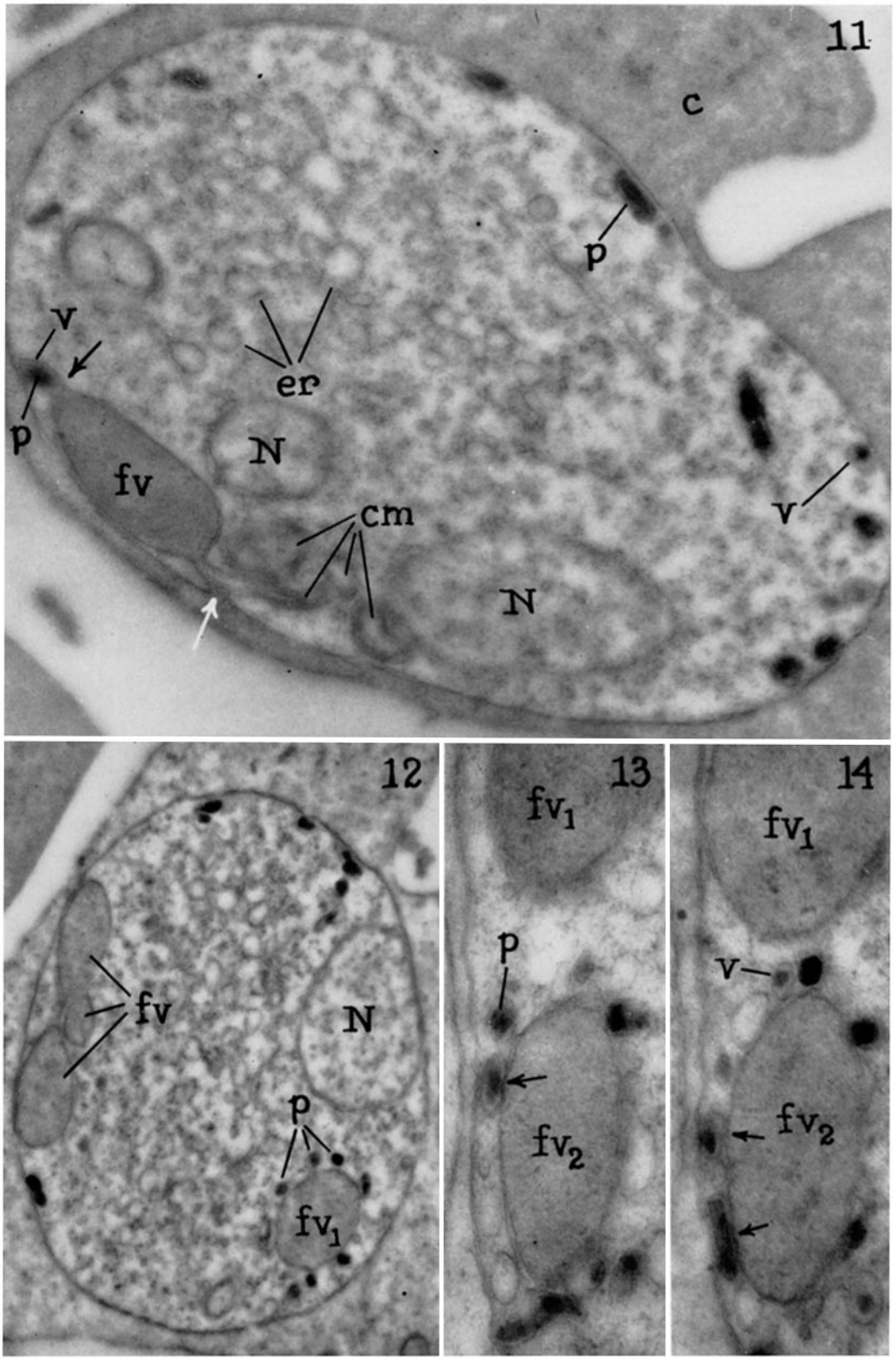
(Rudzinska and Trager: Phagotrophy in *Plasmodium berghei*)

PLATE 68

FIG. 11. *Plasmodium berghei* with one food vacuole (*fv*) connected (at black arrow) with a vesicle (*v*) containing pigment (*p*). At the other end of the same food vacuole its double membrane is continuous with a concentric membraned structure (*cm*) which shows connection with the plasma membrane (at white arrow). Magnification, 40,000.

FIG. 12. Parasite containing several food vacuoles, one of which (fv_1) is surrounded by vesicles containing pigment (*p*). Three food vacuoles (*fv*) look like parts of one larger food vacuole. Magnification, 26,000.

FIGS. 13 and 14. Two serial sections through parts of the same *Plasmodium berghei* possessing two large food vacuoles (fv_1, fv_2). Of special interest is food vacuole fv_2 surrounded by several vesicles with hematin (*p*). The limiting membrane of some of the vesicles (at arrow) seems to be continuous with the external membrane of the food vacuole. Magnification, 57,500.



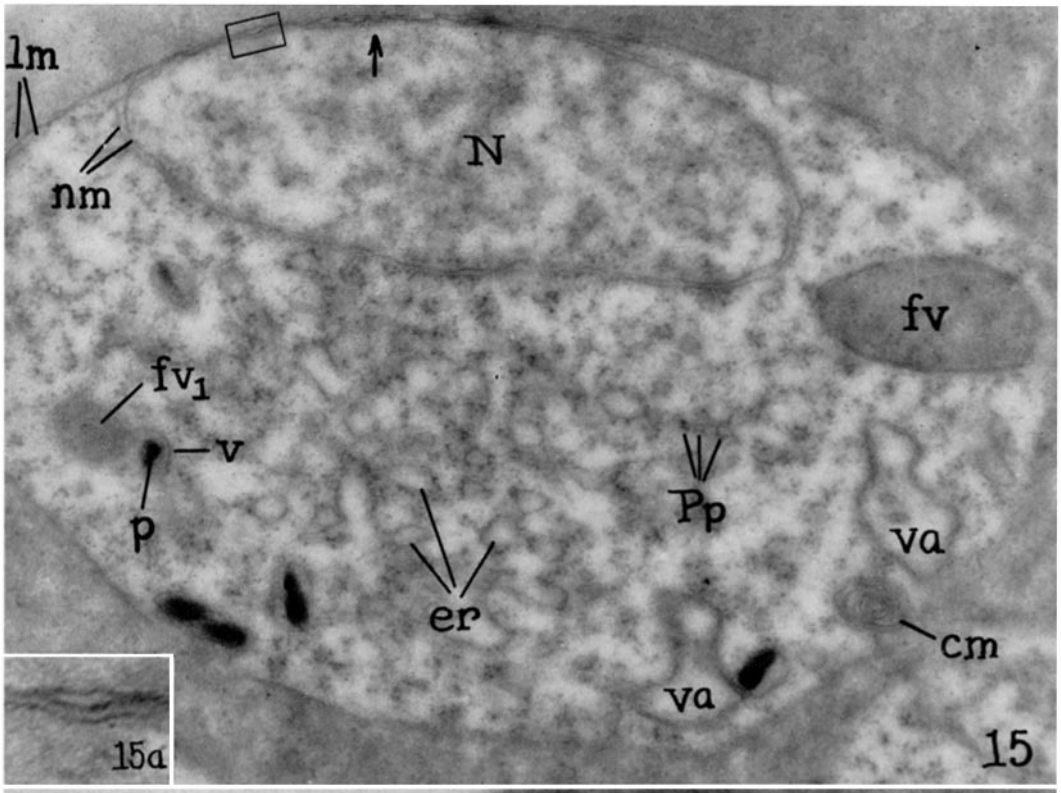
(Rudzinska and Trager: Phagotrophy in *Plasmodium berghei*)

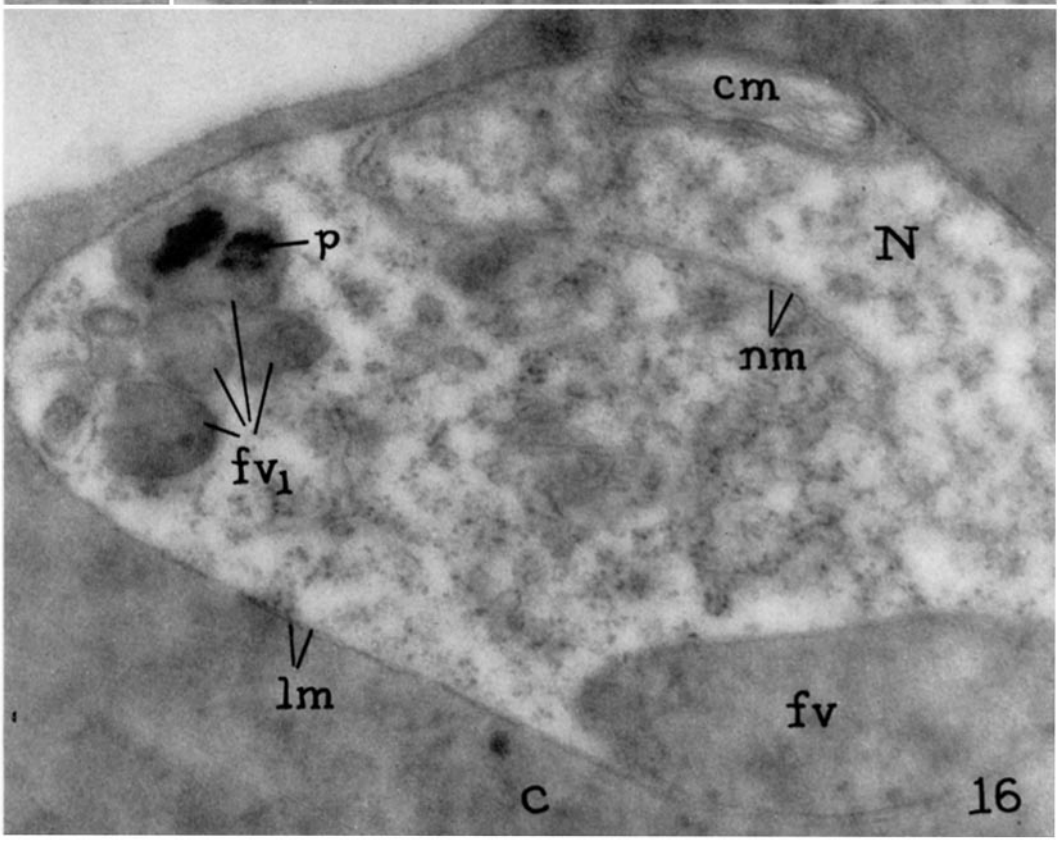
PLATE 69

FIG. 15. Section through a parasite with an elongated oval nucleus (*N*) encased in a double membrane (*nm*). In places where the latter comes in close contact with the double plasma membrane (*lm*) only three membranes may be seen (at arrow) instead of four. One of the smallest food vacuoles (fv_1) is connected with a vesicle (*v*) containing pigment (*p*). Magnification, 40,000.

FIG. 15 *a*. Higher magnification of area outlined in Fig. 15. This electron micrograph shows clearly that at places where the nuclear envelope comes in contact with the plasma membrane only three membranes are present instead of four, and that the middle membrane is much thicker than the two others, probably a result of fusion of the external nuclear and internal plasma membrane. Magnification, 125,000.

FIG. 16. *Plasmodium berghei* with one large (*fv*) and several small food vacuoles (fv_1) which look like parts of one large vacuole. One of the small food vacuoles contains two pigment granules (*p*). Note the elongated nucleus (*N*), surrounded by a double membrane (*nm*); and the concentric double membraned structure at *cm*. Magnification, 60,000.





(Rudzinska and Trager: Phagotrophy in *Plasmodium berghei*)

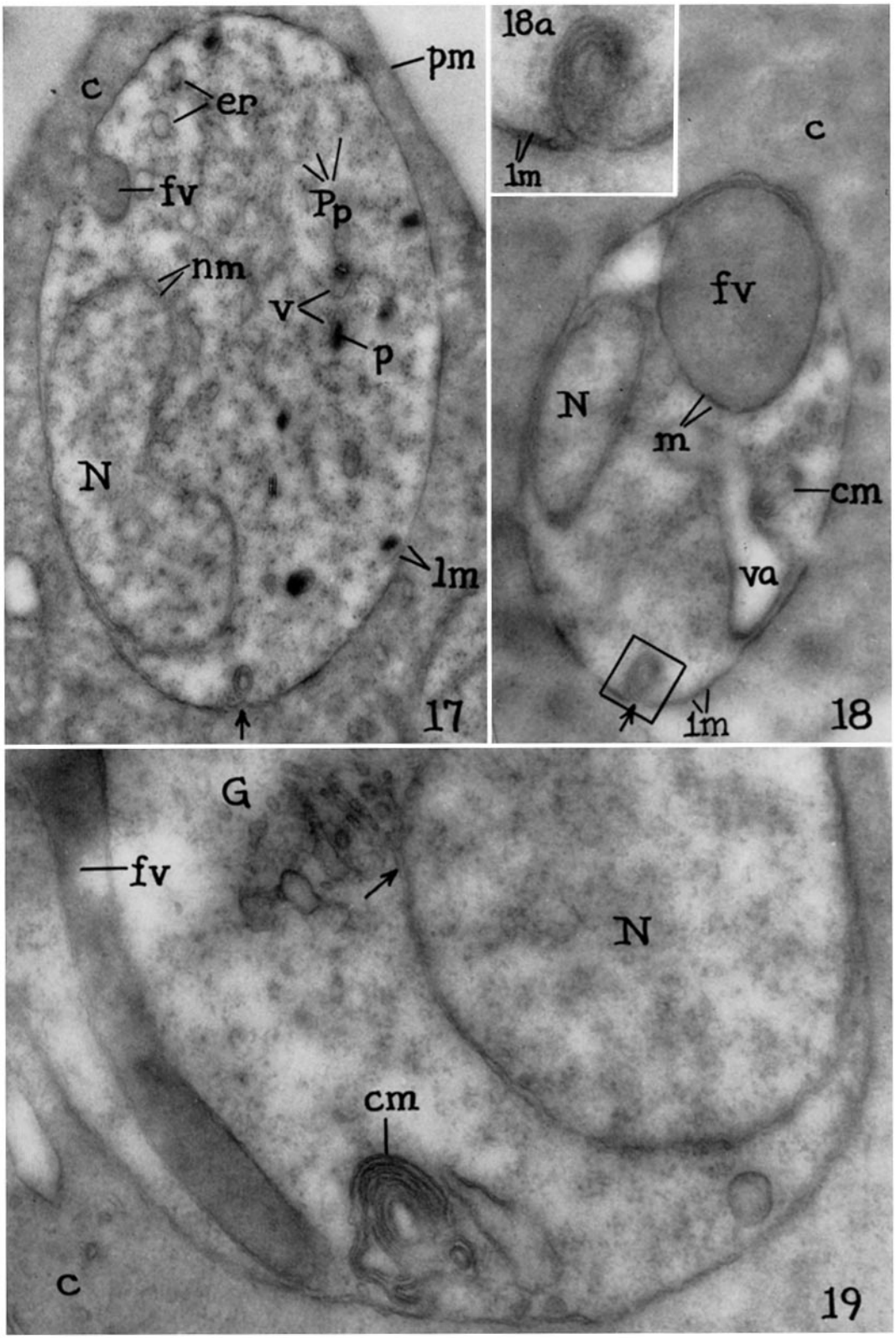
PLATE 70

FIG. 17. Parasite with large nucleus (*N*) encased in a double membrane (*nm*). Note the formation of a small food vacuole (*fv*) connected still with the cytoplasm of host cell (*c*). The arrow indicates the invagination of the double plasma membrane (*lm*) and probably the beginning of formation of the concentric double membraned structure. Several vesicles (*v*) containing hematin (*p*) are scattered in the cytoplasm. Magnification, 40,000.

FIG. 18. Part of reticulocyte with small parasite. Note the large oval food vacuole (*fv*) with its double membrane (*m*); the elongated nucleus (*N*) encased in two membranes; the vacuolated body (*va*); the concentric double membraned structure (*cm*), and the double plasma membrane (*lm*) invaginating at arrow to form a concentric double membraned structure. Magnification, 40,000.

FIG. 18 *a*. Higher magnification of area outlined in Fig. 18 to show formation of concentric double membraned structure from the double limiting membrane (*lm*) of the parasite's body. Magnification, 100,000.

FIG. 19. Part of parasite, with well developed concentric double membraned organelle (*cm*), which has more than one center. The food vacuole (*fv*) covered by two membranes has an unusually elongated form. Near the nucleus (*N*) is an aggregation of vesicles which represent most probably the Golgi apparatus (*G*). At arrow one of the vesicles is continuous with the double nuclear membrane. Magnification, 60,000.

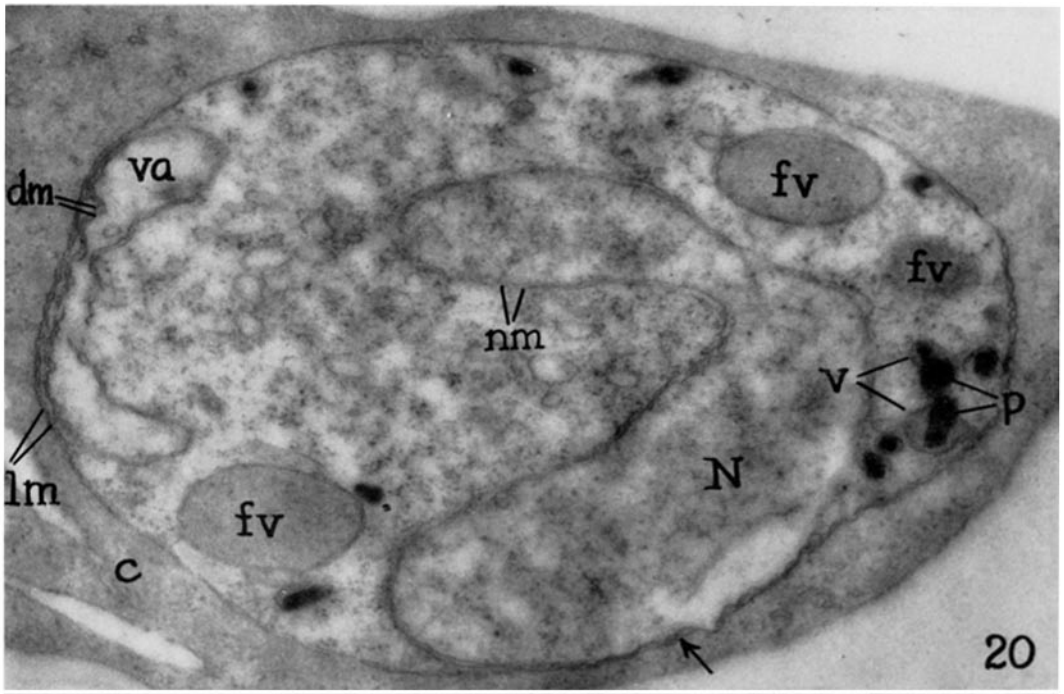


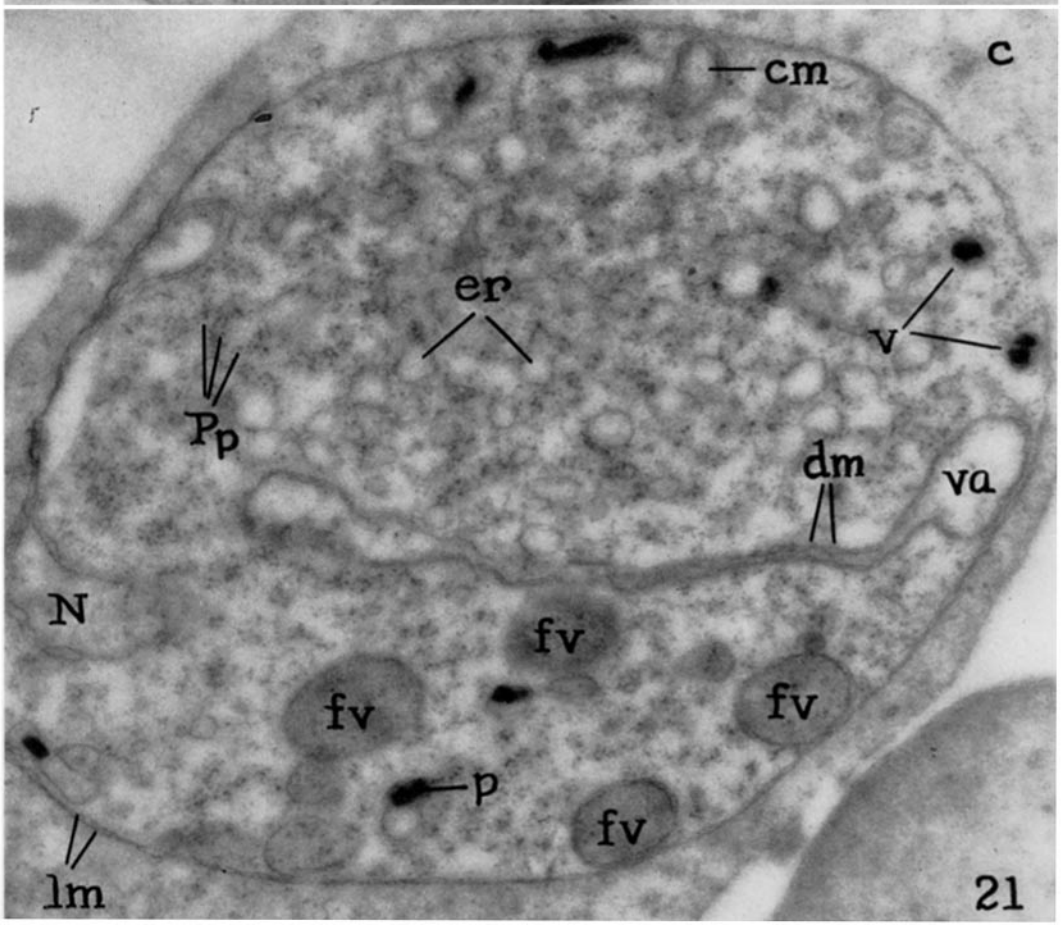
(Rudzinska and Trager: Phagotrophy in *Plasmodium berghei*)

PLATE 71

FIG. 20. Reticulocyte with one parasite. Near the limiting membrane (*lm*) of the body and in parts parallel to it is a double membraned (*dm*) vacuole (*va*). The nucleus (*N*) is large and irregular in shape. In places where the double nuclear membrane (*nm*) comes in contact with the double limiting membrane (*lm*) of the body (at arrow) only three membranes instead of four may be seen. Several small food vacuoles (*fv*) and a number of vesicles (*v*) with hematin (*p*) appear in groups or loosely scattered. Magnification, 40,000.

FIG. 21. Section through large parasite with several small food vacuoles (*fv*) and vesicles (*v*) containing pigment granules (*p*). A long double membraned (*dm*) vacuole (*va*) is located in the center of the body. Magnification, 40,000.

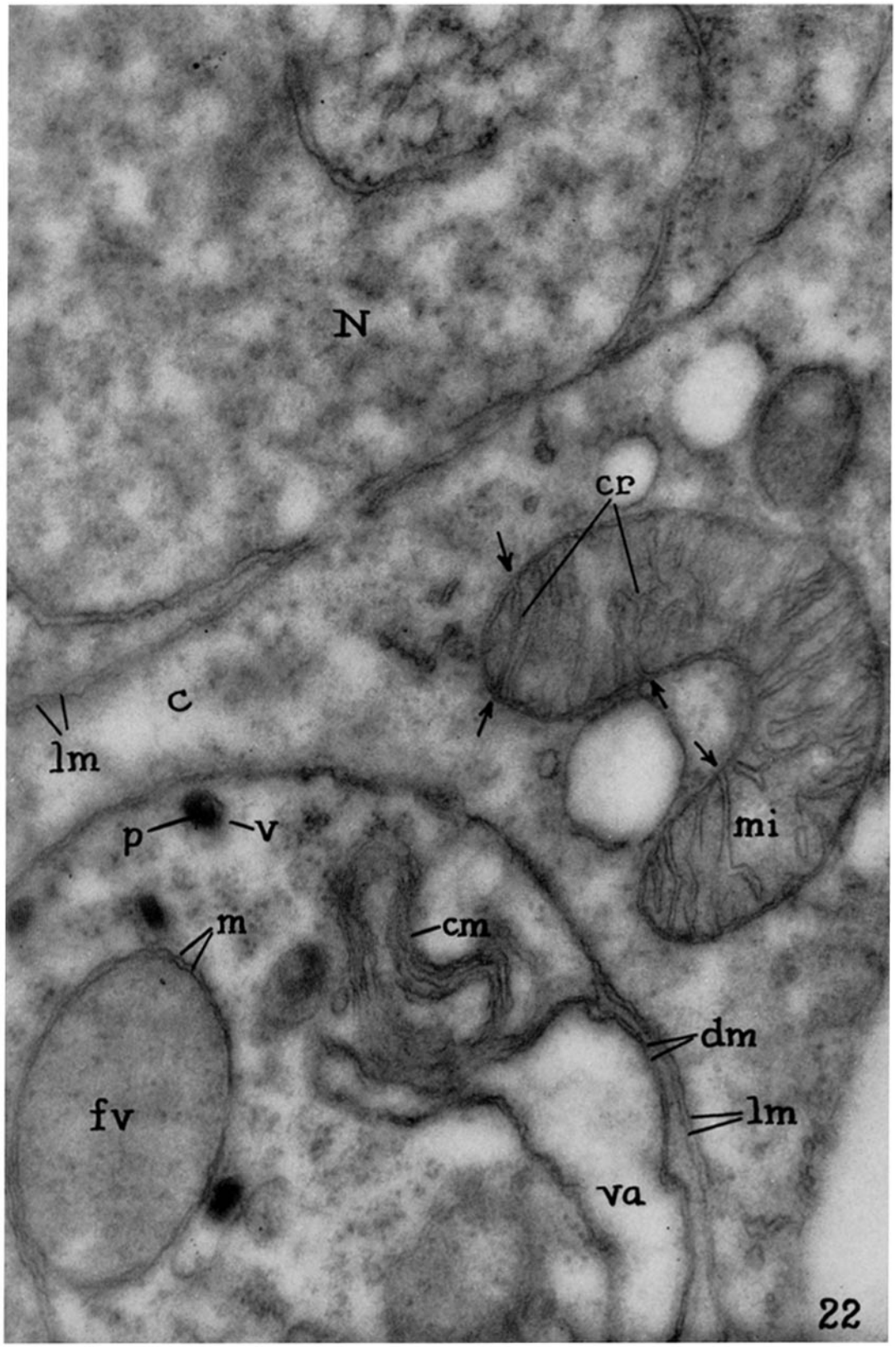




(Rudzinska and Trager: Phagotrophy in *Plasmodium berghei*)

PLATE 72

FIG. 22. Section through two plasmodia parasitizing in the same reticulocyte. In one of the organisms a large food vacuole (*fv*), encased in a double membrane (*m*), may be seen, four vesicles (*v*) with pigment granules (*p*), and also the two new structures: the double membraned (*dm*) vacuole (*va*), and the concentric double membraned organelle (*cm*). In the cytoplasm of the host cell a large mitochondrion (*mi*) shows clearly (at arrow) that the cristae mitochondriales (*cr*) are continuous with the internal mitochondrial membrane. Magnification, 76,000.



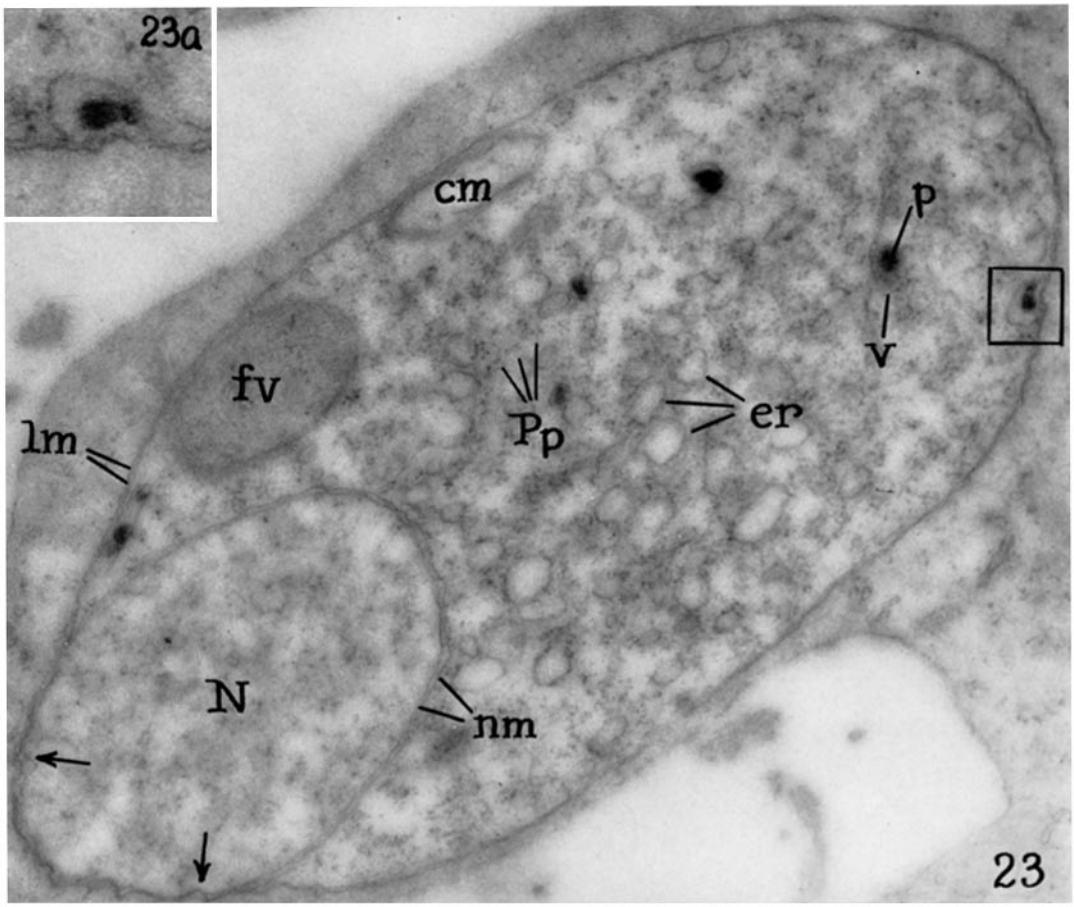
(Rudzinska and Trager: Phagotrophy in *Plasmodium berghei*)

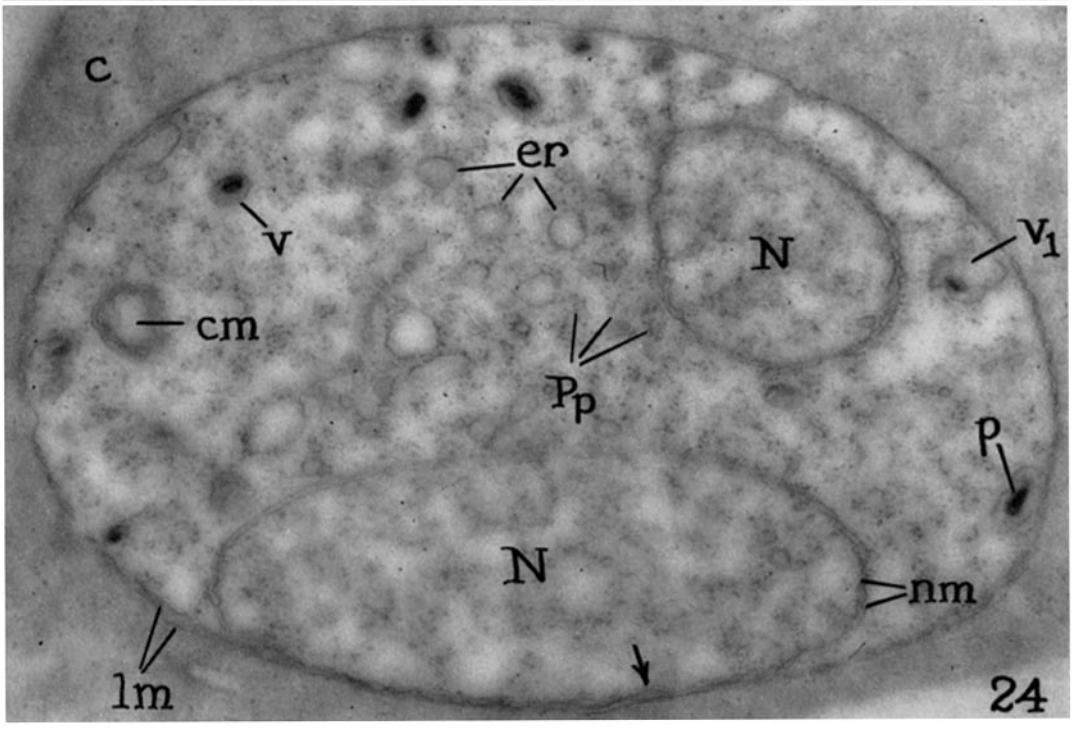
PLATE 73

FIG. 23. *Plasmodium berghei* with large oval nucleus (*N*) encased in a double nuclear membrane (*nm*). In places where it comes in close contact with the double limiting membrane (*lm*) only three membranes may be seen (at arrows) instead of four. Magnification, 40,000.

FIG. 23 *a*. Higher magnification of area outlined in Fig. 23, to show continuity between vesicle containing a pigment granule and the internal double plasma membrane. Magnification, 80,000.

FIG. 24. Section through *Plasmodium berghei* with two nuclei (*N*). The nuclear membrane (*nm*) of the larger nucleus shows in places (at arrow) fusion with the limiting membrane (*lm*) as described in Fig. 23. The matrix of the vesicles with pigment granules (*p*) varies as to the density. It is much denser in vesicle *v* than in vesicle v_1. Magnification, 40,000.





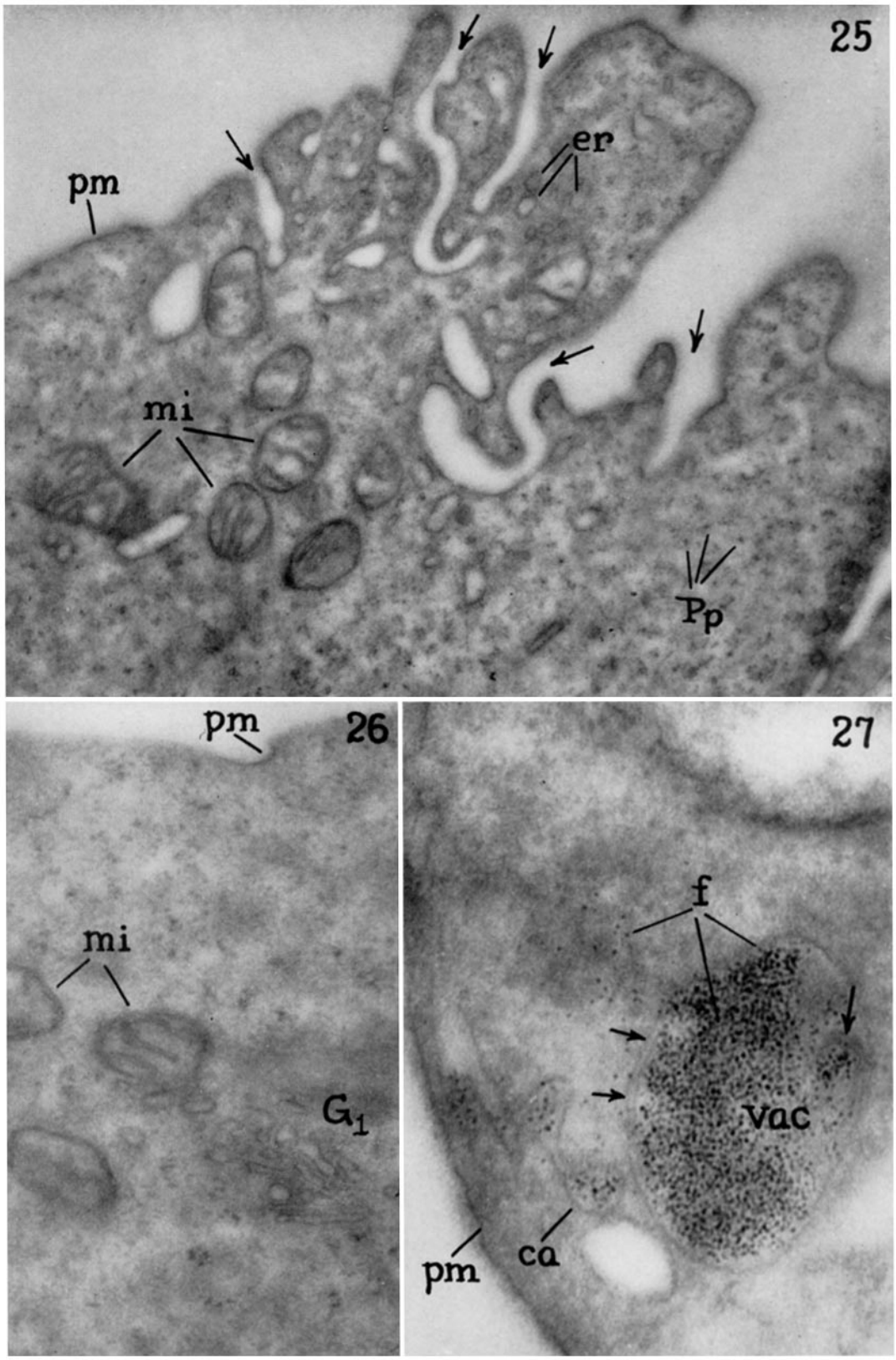
(Rudzinska and Trager: Phagotrophy in *Plasmodium berghei*)

PLATE 74

FIG. 25. Part of uninfected reticulocyte showing many deep invaginations (at arrows) of the plasma membrane (*pm*) and several mitochondria (*mi*). Magnification, 40,000.

FIG. 26. Section through part of uninfected reticulocyte with Golgi apparatus (G_1), composed of elongated vesicles. Magnification, 60,000.

FIG. 27. Higher magnification of part of infected reticulocyte containing a vacuole (*vac*) and a winding canaliculus (*ca*), both containing ferritin granules (*f*). The canaliculus leads most probably to the outside of the cell. Note that the membrane surrounding the vacuole is double at places (two arrows) and that inside the vacuole (at arrow) a short double membrane may be seen. These details suggest that the vacuole represents a mitochondrion filled with ferritin granules. Ferritin can be seen also loosely scattered in the cytoplasm. Magnification, 120,000.



(Rudzinska and Trager: Phagotrophy in *Plasmodium berghei*)