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Ultrastructure of an Unusual Erythrocytic Form of *Plasmodium berghei**

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SYNOPSIS. Unusual dense forms were discovered in ultrathin sections of *Plasmodium berghei*-infected rat erythrocytes. These parasites frequently occurred with one or more typical trophozoites in a single blood cell. They appeared darker than both the neighboring trophozoites and the host erythrocyte. Ribosomes were visible in clusters in their compact cytoplasm. The endoplasmic reticulum, when present, had dilated cisternae often containing a material of low density. Large food vacuoles were commonly seen along with the small vesicles harboring pigment granules. The single large nucleus had dense nucleoplasm. Multilaminated membraned bodies and sausage-shaped vacuoles were seen in some of the parasites.

The exact identity of this form of *P. berghei* is not known. Its possible significance is discussed with particular reference to the differentiation of gametocytes.

RUDZINSKA and Trager (20) outlined the fine structure of intraerythrocytic *P. berghei* as early as 1959. Several electron microscopists have since described the erythrocytic stages of this species within (2, 8, 11, 14), free of (4, 12, 14) and entering or leaving (15, 16, 18) the host cells. Enzymatic activities (23) and structural alterations in relation to drug resistance (10, 19) and effects (13, 17, 24) have also been illustrated in thin sections of *P. berghei*-infected blood.

In spite of the number and variety of studies with *P. berghei*, fresh insights into its fine structure continue to be revealed (14). We have observed an unusual dense form of the parasite during electron microscopic examination of *P. berghei*-infected erythrocytes. In our discussion we consider the significance of this form and the possibility that it is a pre-gametocyte stage.

MATERIALS AND METHODS

The NK65 strain of *P. berghei* was generously supplied by Dr. Meir Yoeli, New York University School of Medicine, New York and was producing gametocytes at the time of this study. The blood from acutely infected rats was prepared for electron microscopy according to the methods outlined previously (12).*

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* The phosphate buffer used in (12) was 0.1 M. It appears incorrectly as 0.2 M.

RESULTS

An unusual form of *P. berghei*, varying in size but never occupying more than ⅔ of the host cell, was found in some sections of infected cells. This form was often accompanied by one or more typical trophozoites (Figs. 1, 3) but also occurred alone (Fig. 2). It appeared very dark compared with the neighboring parasites and even with the host cell.

Ribonucleoprotein particles or ribosomes were usually concentrated in clusters in its compact cytoplasm (Figs. 1, 2, 3). When endoplasmic reticulum was present, its membranes limited dilated channels which seemed to contain a material of very low density (Fig. 3). Large food vacuoles were frequently visible (Figs. 1, 2, 3) along with small vesicles containing hemozoin granules (Figs. 1, 2). Single large nuclei with a compact nucleoplasm (Figs. 1, 3) were characteristic of these forms.

The plasma membrane usually appeared as a double unit membrane closely applied to the parasite cytoplasm (Figs. 1, 2, 3). However, some membrane elaboration was evident, particularly where vacuolar (Figs. 1, 2) or nuclear membranes were in close proximity to the marginal membranes of the cytoplasm. In a few cases, a complex membrane array was also observed to transept or cleave the parasite cytoplasm (Fig. 1). Membranous organelles including multilaminated membraned bodies and sausage-shaped vacuoles (Fig. 3) were evident in some of the dense parasites.

For purposes of comparison, Figs. 4 and 5 are included to show the features of *P. berghei* gametocytes. Note, in particular, the complex surface membrane structure and the single large compact nucleus in both illustrations. The gametocyte in Fig. 4 with its high concentration of ribosomes is probably a macrogametocyte. Wide endoplasmic reticulum with cisternae containing a material of low density and dense bodies or toxonemes are additional features of this parasite. In Fig. 5, the paucity of ribosomes is striking and suggests that this parasite is a microgametocyte.

DISCUSSION

The dense forms of *P. berghei* are considered in the following comments as possibly (1) nonviable or degenerating forms, (2) merozoites, (3) trophozoites, (4) schizonts, or (5) pregametocytes.

Whether some of the large host inclusions (food vacuoles) observed in mammalian plasmodia represent ingestion or an oblique section thru a highly contorted parasite remains controversial. For the sake of our discussion, we will assume the former viewpoint.

1. *Nonviable or degenerating forms.* The dilated endoplasmic reticulum and dense nuclear areas found in some dense forms may indicate deterioration. This interpretation, for example, has been applied to parasites which show wide endoplasmic reticulum (5, 12) and dense nuclei (4, 12) after isolation procedures.

The occurrence of food vacuoles and pigment granules and the general appearance of intactness support the alternate view that the dense forms are viable. Furthermore, the juxtaposition of a dense form and one or more typical trophozoites in one host cell suggests that this dense form is not an artifact of the preparatory technics.

2. *Merozoites.* Merozoites have a very compact cytoplasm, condensed chromatin in the nuclei, and little or no endoplasmic reticulum (12, 14, 22). The small dense forms shared these features. However, unlike merozoites, the dense forms lacked conoid elements and a pear-shaped outline and often contained food vacuoles and vesicles with pigment granules.

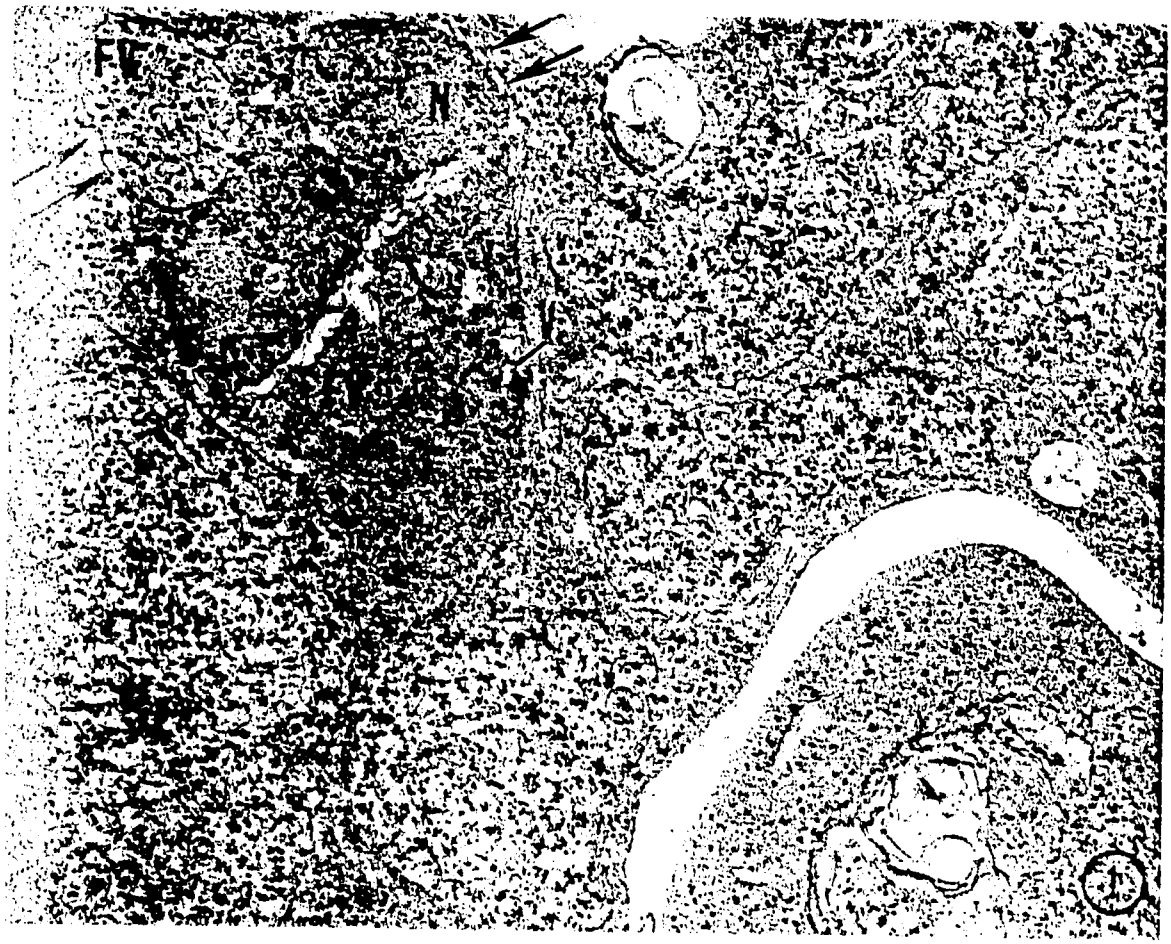
If certain of the dense forms are merozoites, they are either part of a segmenter the rest of which does not appear in the plane of section or they are parasites which have just invaded the host cell. In many cases, the large size of the dense forms and the frequent presence of typical trophozoites surrounding these forms tend to negate the former explanation. With regard to the 2nd explanation, Aikawa (1) and Ladda (16) described newly penetrated merozoites of *P. cathemerium* and *P. berghei*, respectively. These merozoites, in contrast with the dense forms, still had remnants of the conoid apparatus and the thickened inner membranes. In addition, the cytoplasm already appeared similar in density to the ring form of the trophozoites but lacked the prominent food vacuole, a structure seen in many of our dense forms.

3. *Trophozoites.* That the dense forms are structurally different from younger trophozoites is obvious in Figs. 1, 3. The chromatin is condensed rather than dispersed in fine fibers, the cytoplasm is more compact, the endoplasmic reticulum, if present, is more dilated and the ribosomes are more clustered. That all of the dense forms are structurally different from Ladda's (14) middle-aged trophozoites is not so clear. Some, for example, have pigment granules, rough reticulum, abundant ribosomes and a regular profile typical of the parasites which Ladda (14) identified as middle-aged trophozoites. On the other hand, our dense forms still present an overall appearance much darker than the host cell. Ladda's trophozoites are lighter.

4. *Schizont.* Rudzinska and Vickerman (22) referred to the concentration of cytoplasm at the time of schizogony.

Fig. 1. A dense form of *P. berghei* surrounded by typical trophozoites in a single rat erythrocyte. Abundant ribosomes are visible in its cytoplasm which is transepted or cleaved by a membraned structure. Large food vacuoles (FV) and vesicles containing pigment granules (V) are evident. The dense form is limited by a double unit membrane which appears elaborate where the nuclear and food vacuole membranes are in close proximity (arrows). A single compact nucleus (N) with dense aggregations is also seen. × 29,000.

Fig. 2. A rat reticulocyte which appears to be singly infected with a dense form of *P. berghei*. Note the clusters of ribosomes, the food vacuoles (FV), the vesicles containing pigment granules (V) and the elaboration of the double unit surface membrane at the site of the large host inclusion (arrows). × 28,000.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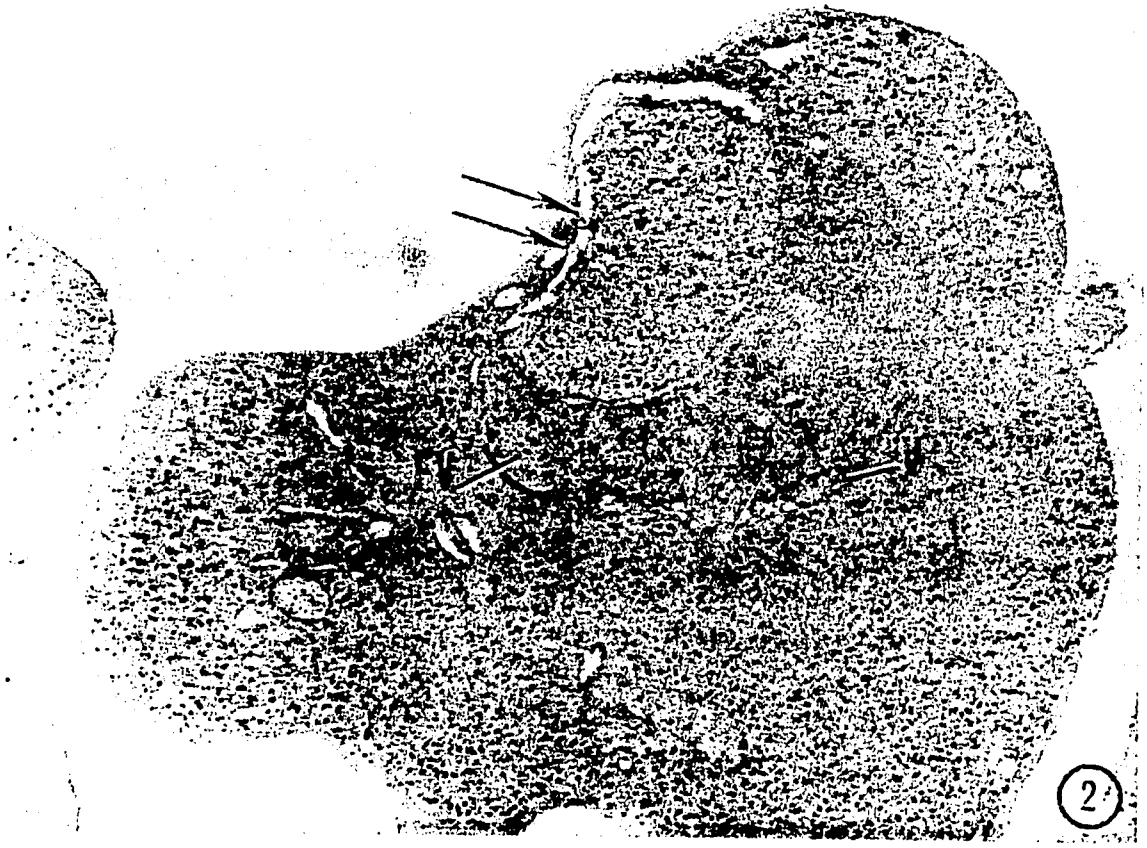
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Fig. 3. A dense form of *P. berghei* and a typical trophozoite infecting the same rat blood cell. Dilated endoplasmic reticulum (ER) with cisternae containing a material of low density is conspicuous in the dense parasite. A compact nucleus (N) and 2 large food vacuoles (FV) are also obvious. Note the sausa shaped vacuole (S) and multi-laminated membraned body (M along with the double unit plasma membrane. × 32,000.

They reviewed the features accompanying nuclear division which include the increase in tubular endoplasmic reticulum, the increase in multilaminated membraned bodies and the presence of abundant ribosomes. Some of our larger dense forms may be preschizonts. However, the single nuclei uniformly have condensed nucleoplasm cha acteristic of inactive nuclei rather than of nuclei prepari to divide. In addition, their endoplasmic reticulum, wh

Fig. 4. The presence of numerous ribosomes and dense bodies or toxonemes (T) suggest that this gametocyte of *P. berghei* is a macrogametocyte. The complex membrane system at the surface and the large compact nucleus (N) are conspicuous features of this parasite. A food vacuole (FV) is also evident. Note also the dilated endoplasmic reticulum and the occurrence of a lightly stained material in its wide channels. Coarse pigment granules (P) are also visible. × 28,000.

Fig. 5. A gametocyte, probably a microgametocyte, of *P. berghei* with a paucity of ribosomes. A prominent nucleus (N) is visible along with a complex surface membrane. × 23,000.

present, is dilated rather than tubular. In contrast to the more mature schizonts of *P. berghei*, our large dense forms lack periodic thickenings in their plasma membranes and the early appearance of paired organelles.

5. *Pregametocytes.* It is tempting to identify the unusual dense forms as pregametocyte stages. To date, the characteristics of parasites destined to become gametocytes rather than schizonts have not been well-defined. Rudzinska and Trager (21) reported on *P. coatneyi* gametocytes and suggested that the fine structure of the young gametocytes and trophozoites is indistinguishable. Aikawa et al. (3) in a later publication on avian and mammalian gametocytes suggested that the dense pellicle of the merozoites is retained in parasites which are fated to grow into gametocytes. In a recent study with *P. berghei yoeli*, Ladda (14) did not observe persistence of the pellicle. Instead, he defined the differentiating gametocyte as a cell having an enlarged nucleus without evidence of impending division, a highly organized endoplasmic reticulum and large numbers of dense bodies or toxonemes.

Many of our dense forms showed a single large nucleus with no signs of division. A similar nuclear structure has been described by Ladda (14) and ourselves in *P. berghei* gametocytes. In some of the dense forms, the cisternae of the endoplasmic reticulum were dilated and contained a lightly stained material. Certain gametocytes also had this characteristic (Fig. 4). Food vacuoles and pigment granules in vesicles were found at random in the cytoplasm of both our gametocytes and dense forms; in the former, however, the pigment granules were more coarse. While dense bodies or toxonemes, common in gametocytes, were not visible in our dense forms, these structures would be difficult to distinguish in their compact cytoplasm.

The outer membranes of mammalian gametocytes have appeared as a 2-layered structure (8, 21), a 3-layered system in some areas (3), or, as we have seen in our sections, a complex of at least 3 membranes at many sites. Whether our dense forms are surrounded by 2 or more membranes, therefore, is not helpful in considering a possible relationship of these forms to young gametocytes. Indeed, the number of layers in the pellicle of mammalian gametocytes may vary with the condition of the parasites (3) or with different stages of maturity.

Altho Corradetti and Verolini (cited by Garnham, 7) using the light microscope, reported that only mature erythrocytes were invaded by the sexual forms of *P. berghei*, we found gametocytes in the reticulocytes as well. The reticulocytes were likewise a common host cell for the dense forms. We also observed multiple invasion of a single erythrocyte in the case of both the gametocytes and the dense forms. Whereas the mature gametocytes nearly filled the host cell, the dense forms always occupied less than ⅔ of the blood cell. In this regard, the dense parasites resembled the Type I stage of gametocyte maturation described by Hawking et al. (9) in *P. knowlesi* and *P. cynomolgi* preparations. Finally, the paucity of the dense forms compared with the typical trophozoites correlated with the relatively small number of gametocytes versus schizonts detected in our blood films.

It is possible that the dense forms are precursors of only the macrogametocytes. In their studies with *Leucocytozoon simondi*, for example, Desser et al. (6) observed that the female merozoites, like the mature macrogametocytes, have a cytoplasm considerably darker than that of the males while still in the megaloschizont. Possibly our dense forms also predict the formation of a female gametocyte. Unlike the microgametocyte, a high concentration of ribosomes and hence, a dense or darkly staining cytoplasm characterizes the macrogametocyte of plasmodia (7, 21).

CONCLUSION

In the dynamic series of events which lead to the development of a mature gametocyte or schizont from the invading merozoite, it is logical to have a condensed form at various times of cellular organization and growth. Whether the dense forms we have discovered in *P. berghei*-infected cells are artifactual and decomposing or precursors of an asexual or a sexual adult form remains open to challenge. At this time, we favor the interpretation that these dense forms are relevant to the process of gametocytogenesis.

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REFERENCES

1. Aikawa, M. 1966. The fine structure of the erythrocytic stages of three avian malarial parasites, *Plasmodium fallax, P. lophurae* and *P. cathemerium*. *Am. J. Trop. Med. Hyg.* **15**, 449-71.

2. Aikawa, M. & Antonovych, T. T. 1964. Electron microscopic observations of *Plasmodium berghei* and the Kupffer cell in the liver of rats. *J. Parasit.* **50**, 620-9.

3. Aikawa, M., Huff, C. G. & Sprinz, H. 1969. Comparative fine structure study of the gametocytes of avian, reptilian, and mammalian malarial parasites. *J. Ultrastruc. Res.* **26**, 316-31.

4. Bahr, G. F. 1966. Quantitative cytochemical study of erythrocytic stages of *Plasmodium lophurae* and *Plasmodium berghei*. *Mil. Med.* **131** (Suppl.), 1064-70.

5. Cook, R. T., Aikawa, M., Rock, R. C., Little, W. & Sprinz, H. 1969. The isolation and fractionation of *Plasmodium knowlesi*. *Mil. Med.* **134**, 866-83.

6. Desser, S. S., Baker, J. R. & Lake, P. 1970. The fine structure of *Leucocytozoon simondi*. I. Gametocytogenesis. *Can. J. Zool.* **48**, 331-6.

7. Garnham, P. C. C. 1966. *Malaria parasites and other Haemosporidia*. Blackwell, Oxford, 465-71.

8. Garnham, P. C. C., Bird, R. G. & Baker, J. R. 1967. Electron microscope studies of motile stages of malaria parasites. V. Exflagellation in *Plasmodium, Hepatocystis*, and *Leucocytozoon*. *Trans. Roy. Soc. Trop. Med. Hyg.* **61**, 58-68.

9. Hawking, F., Worms, M. J. & Gammage, K. 1968. 24- and 48-hour cycles of malaria parasites in the blood; their purpose, production and control. *Trans. Roy. Soc. Trop. Med. Hyg.* **62**, 731-60.

10. Howells, R. E., Thomas, A. E. & Peters, W. 1968. Morphological changes associated with drug resistance in malarial parasites. *Parasitology* **58**, 5 p.

11. Jerusalem, C. & Heinen, U. 1965. Elektronenmikroskopische untersuchungen am malariaerreger (*Plasmodium berghei*) in polychromatischen and oxyphilen erythrozyten. *Z. Tropenmed. Parasit.* **16**, 377-94.

12. Killby, V. A. A. & Silverman, P. H. 1969. Isolated erythrocytic forms of *Plasmodium berghei*. An electron microscopical study. *Am. J. Trop. Med. Hyg.* **18**, 836-59.

13. Ladda, R. L. 1966. Morphologic observations on the effect of antimalarial agents on the erythrocytic forms of *Plasmodium berghei in vitro*. *Mil. Med.* **131** (Suppl.), 993-1008.

14. ———. 1969. New insights into the fine structure of rodent malarial parasites. *Mil. Med.* **134**, 825-65.

15. Ladda, R. L., Arnold, J., Martin, D. & Luehrs, F. 1965. Electron microscopy of *Plasmodium berghei*. I. On the migration of trophozoites from infected erythrocytes in the rat. *Trans. Roy. Soc. Trop. Med. Hyg.* **59**, 420-2.

16. Ladda, R. L., Aikawa, M. & Sprinz, H. 1969. Penetration of erythrocytes by merozoites of mammalian and avian malarial parasites. *J. Parasit.* **55**, 533-44.

17. Macomber, P. B. Sprinz, H. & Tousimis, A. J. 1967. Morphological effects of chloroquine on *Plasmodium berghei* in mice. *Nature* **214**, 937-9.

18. Peters, W. 1963. Penetration of a mouse erythrocyte by a merozoite of *P. berghei* as revealed by electron microscopy. *Trans. Roy. Soc. Trop. Med. Hyg.* **57**, 269-70.

19. Peters, W., Fletcher, K. A. & Stäubli, W. 1965. Phagotrophy and pigment formation in a chloroquine-resistant strain of *Plasmodium berghei*. Vincke and Lips, 1948. *Ann. Trop. Med. Parasit.* **59**, 126-34.

20. Rudzinska, M. A. & Trager, W. 1959. Phagotrophy and two new structures in the malaria parasite, *Plasmodium berghei*. *J. Biophys. Biochem. Cytol.* **6**, 103-12.

21. ———. 1968. The fine structure of trophozoites and gametocytes in *Plasmodium coatneyi*. *J. Protozool.* **15**, 73-88.

22. Rudzinska, M. A. & Vickerman, K. 1968. The fine structure. In Weinman, D. and Ristic, M., eds., *Infectious Blood Diseases of Man and Animals*. Academic Press, New York **1**, 217-306.

23. Theakston, R. D. G., Howells, R. E., Fletcher, K. A., Peters, W., Fullard, J., & Moore, G. A. 1969. The ultrastructural distribution of cytochrome oxidase activity in *Plasmodium berghei* and *P. gallinaceum*. *Life Sci.* **8**, 521-9.

24. Warhurst, D. C. & Hockley, D. J. 1967. Mode of action of chloroquine on *Plasmodium berghei* and *P. cynomolgi*. *Nature* **214**, 935-6.