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The Fine Structure of *Plasmodium falciparum* and its Host Erythrocytes in Natural Malarial Infections in Man*

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Electron micrographs of ultra-thin sections of erythrocytes taken from two Liberian children ill with Plasmodium falciparum *malaria show that the appliqué forms of this parasite are clearly within the host cell. The general fine structure of the parasites resembles that of other mammalian malaria parasites, as does the mode of ingestion of host cell material by pinocytosis. Granules of haemozoin were usually found in small vesicles pinched off from the large food vacuole. Scattered through the cytoplasm of infected red cells were narrow clear clefts bounded on each side by two unit membranes. These probably represent the Maurer's clefts seen in light microscopy. The surface of infected erythrocytes was notably distorted, a phenomenon which may have a bearing on the stickiness of the infected red cells in human falciparum malaria and the segregation of these cells in the capillaries. Many uninfected erythrocytes showed a multiple alveolar, blister-like abnormality of a portion of the cell membrane ; this was not seen in otherwise comparable blood from a case of* P. ovale *infection.*

Since the first electron microscopy studies of thin sections of malaria parasites by Fulton & Flewett (1956) with *Plasmodium knowlesi* and by Rudzinska & Trager (1957, 1959) with *P. lophurae* and *P. berghei*, the fine structure of the erythrocytic stages of a number of species has been described in some detail (see, for example, Aikawa et al., 1966). Reports of observations on human malaria parasites, however, have up to now been limited to material from splenectomized chimpanzees (Rudzinska, Trager & Bray, 1965) and from the low-grade infections available in human volunteers inoculated with *P. falciparum* (Ladda, Arnold & Martin, 1966).

It seemed desirable to examine severe infections as they occur naturally, especially in children. To this end one of us (W. Trager) spent a week at the Liberian Institute for Tropical Medicine, where children ill with falciparum malaria presented themselves at the clinic conducted by Dr Earl Reber, Acting Director of the Institute.

MATERIALS AND METHODS

With the co-operation of Dr Reber, small samples of blood were collected from a number of children heavily infected with *P. falciparum*, as well as from one child with *P. ovale*. These were fixed in several ways, none of which turned out to be entirely satisfactory. The best results were obtained with two samples.

The first (Case 1) was from a 14-month-old boy with about 150 000 *P. falciparum* parasites per mm^3. This blood was allowed to clot, the clot was cut into small fragments and fixed for 1 hour in 3% glutaraldehyde in 0.05 M phosphate buffer at pH 7.2. The material was washed 8 times with the same buffer containing 6% sucrose and left in this overnight in the cold. It was post-fixed in 1% osmium tetroxide, dehydrated and embedded in Epon.

The second (Case 2) was from a 12-year-old girl with about 60 000 *P. falciparum* parasites per mm^3 of blood. 0.75 ml of blood was mixed with 0.1 ml

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heparin solution (30 mg heparin, 0.85 g NaCl per 100 ml) and to this were immediately added 10 ml 5% glutaraldehyde in 0.05 M phosphate buffer at pH 7.2. The mixture was kept for 30 minutes in the cold and then centrifuged and washed 9 times in phosphate-buffered 6% sucrose solution. It was kept cold and brought back to New York, where it was post-fixed in 1% osmium tetroxide, dehydrated and embedded in Epon.

Sections were cut with a Porter-Blum microtome and double-stained with uranyl acetate and lead citrate. Electron micrographs at original magnifications up to 11 000× were taken with an RCA EMU 3F.

OBSERVATIONS AND DISCUSSION

Giemsa-stained blood films of Case 1 showed only young rings with no pigment visible by light microscopy. In Case 2 the parasites were thick rings with some fine pigment granules.

General fine structure

This does not differ conspicuously from that of other species infecting primates (see Rudzinska, Trager & Bray, 1965; Aikawa, Huff & Sprinz, 1966). As already shown by Ladda, Arnold & Martin (1966) the parasite, like all other erythrocytic malaria parasites, is separated from the host cell cytoplasm by two unit membranes, closely apposed and not surrounded by any vacuole (Fig. 1).

The cytoplasm contained numerous ribonucleoprotein particles. As seems to be typical for primate malarial parasites and for *P. berghei*, no nucleolus was seen. It is of interest that a nucleolus has been seen in older trophozoites of *P. falciparum* in a splenectomized chimpanzee (Rudzinska, Trager & Bray, 1965). Other differences between the chimpanzee and the human material are noted below.

Pinocytotic uptake and pigment formation

This again does not differ from that seen in other mammalian malaria parasites. A food vacuole in process of formation is shown in Fig. 2 and a formed large food vacuole in Fig. 1, 3 and 4. All of these figures also show small granules of haemozoin in separate vesicles, suggesting that digestion of haemoglobin does not occur in the large food vacuole (as it does in avian malaria) but rather in small vesicles pinched off from the food vacuole. Such a vesicle still connected to the food vacuole is shown in Fig. 4, a figure strikingly like Fig. 21 of Rudzinska, Trager & Bray (1965) for P. *berghei*. In *P. falciparum* in the chimpanzee most of the parasites showed pigment grains within the large food vacuole (Rudzinska, Trager & Bray, 1965). Present information is insufficient for an explanation of this important difference. It might be a result of the abnormal host (splenectomized chimpanzee) but it seems more likely that it will be accounted for on the basis of the stage of the parasite. In the chimpanzee material most of the parasites are advanced trophozoites, whereas they are rings in the human material.

Aikawa, Huff & Sprinz (1966) believe that all pinocytotic uptake by erythrocytic malaria parasites occurs through the cytostome (already figured for *P. falciparum* by Ladda, Arnold & Martin (1966) under the term " micropyle "). Their views, and our somewhat different view, are set out clearly in their paper and in our comments concerning it (Trager, 1966).

Appliqué forms

The early ring forms of *P. falciparum* as seen in Giemsa-stained films appear to lie on the erythrocyte rather than in it. It must, of course, be remembered that a dry film fixed in methyl alcohol is hardly a fine cytological preparation. Electron micrographs of thin sections show that these appliqué forms are clearly inside the cell, even though the peripheral layer of red cell cytoplasm may be less than 50 mμ thick (Fig. 2).

Maurer's clefts

Abnormal staining of host cell cytoplasm in primate malaria is a well-known phenomenon and is best exemplified by the Schüffner's dots seen in erythrocytes with *P. vivax*. In *P. falciparum* infections irregular darker-stained masses called Maurer's clefts appear in the infected erythrocytes under the light microscope. We believe that these are represented in electron micrographs by the clefts bounded by two unit membranes seen scattered through the cytoplasm of parasitized erythrocytes (Fig. 5, 9, 10, 11). In some sections they appear as a clear narrow circle bounded inside and out by two membranes. Such structures are also present in monkey erythrocytes infected with *P. coatneyi* (see Fig. 16 in Rudzinska, Trager & Bray, 1965). In the more abundant material on *P. coatneyi* (to be presented in detail in a later paper) there are indications that these structures represent extensions of the parasite membrane. What their biochemical function might be is a matter for interesting speculation.

FIG. 1-4

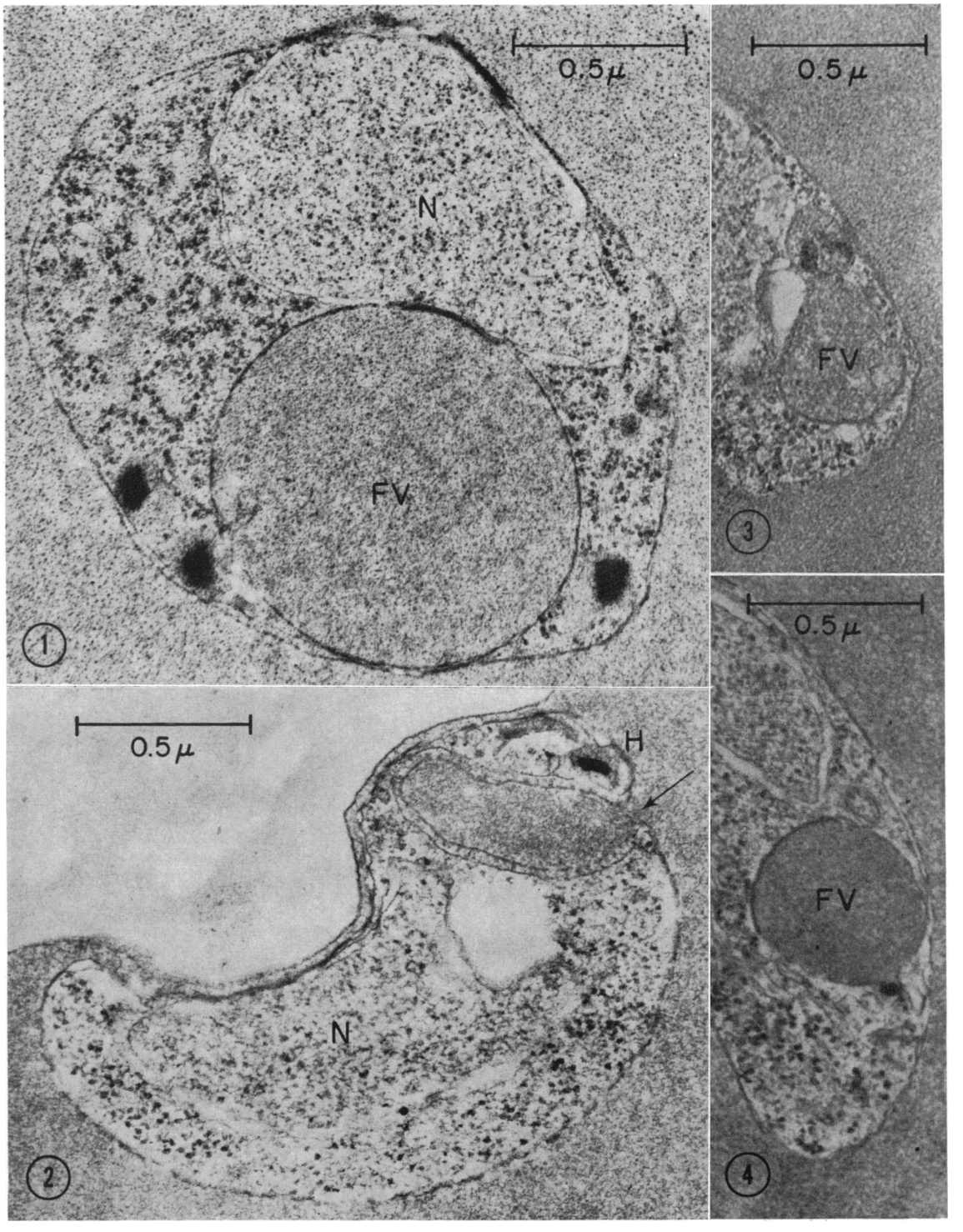

Fig. 1. Organism from Case 1 showing the nucleus (N) with its double membrane, a large food vacuole (FV) and three small haemozoin vesicles. Note the two membranes separating the parasite from its host cell.

Fig. 2. Appliqué form from Case 1. Arrow indicates forming food vacuole. Note vesicle with haemozoin (H).

Fig. 3 and 4. Organisms from Case 1, showing in each a vesicle with haemozoin pinching off from food vacuole (FV).

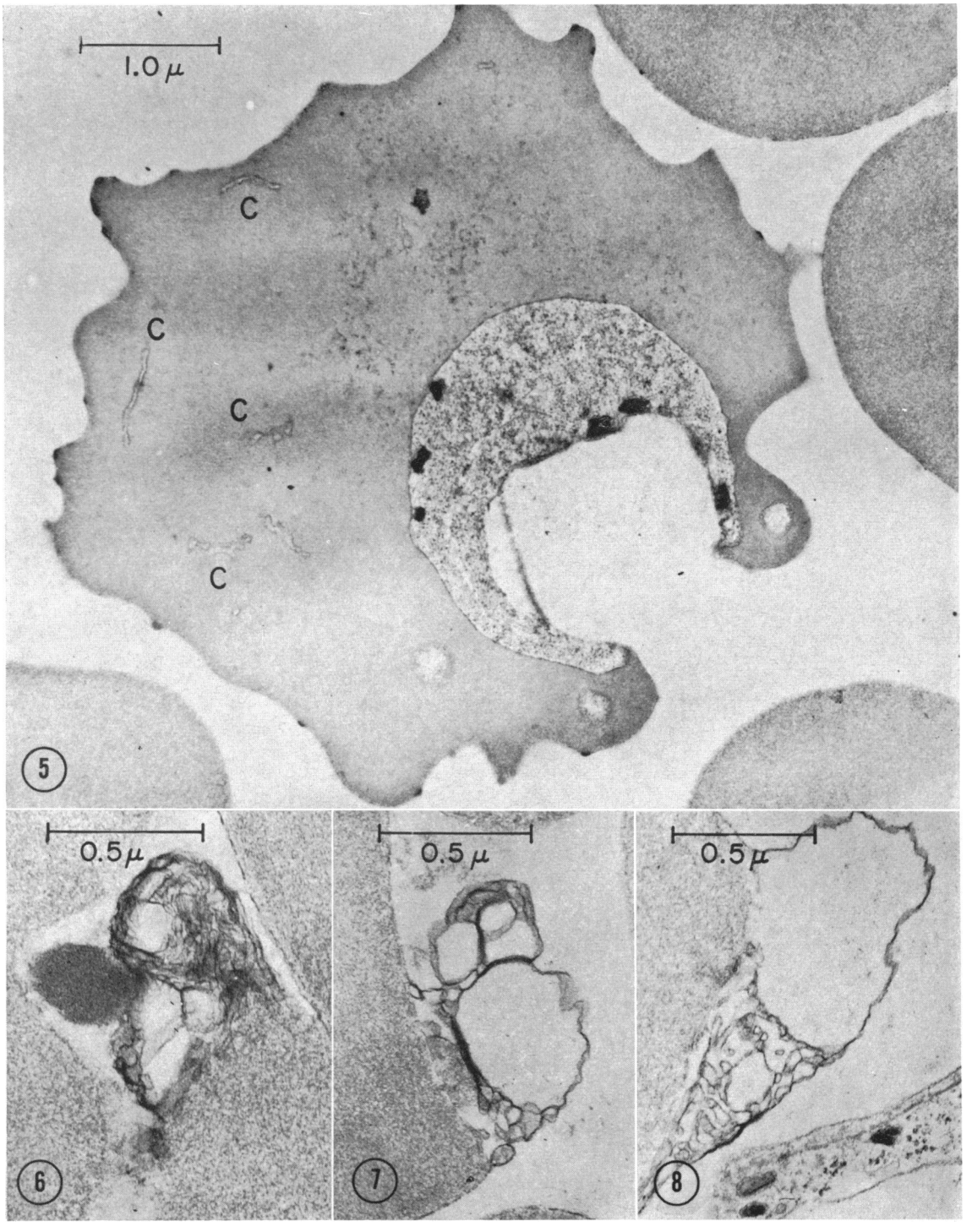

Fig. 5. Infected cell from Case 2, illustrating the distortion of the host cell. Note the membrane-bounded clefts (C).

Fig. 6, 7 and 8. Alveoli on uninfected red cells (Case 1). The dark mass in Fig. 6 is a fibrin fibre. In Fig. 8 a portion of a parasite is seen in an adjacent cell.

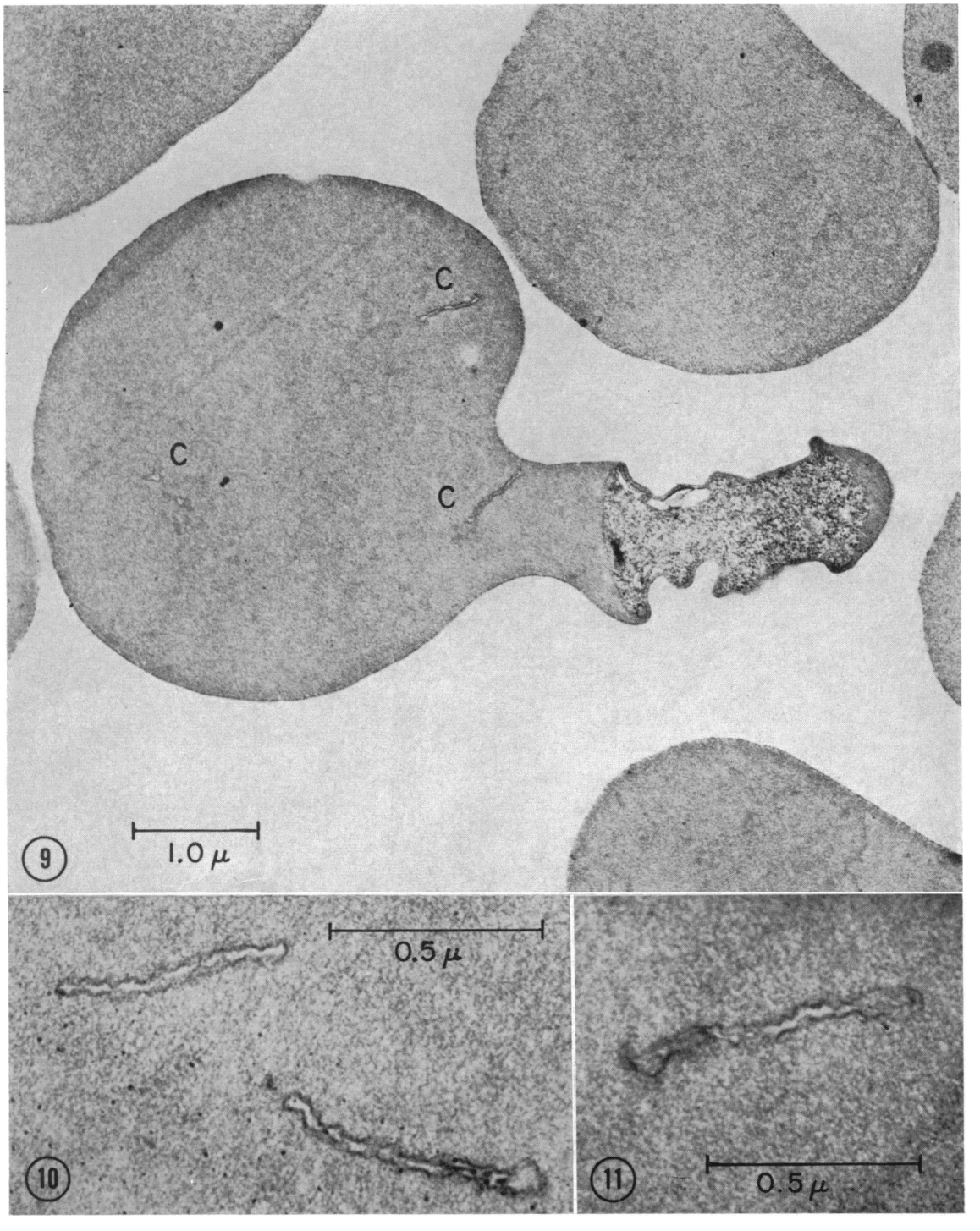

Fig. 9. A distorted infected cell from Case 2. Note the regular contours of the adjacent uninfected red cells. Note membrane-bounded clefts (C).

Fig. 10 and 11. Membrane-bounded clefts at higher magnification, from another infected erythrocyte (Case 2).

Changes in erythrocytes

Human red cells containing *P. falciparum* invariably appeared distorted, often grossly so, especially in the vicinity of the parasite (Fig. 5, 9). While this may be in part an artefact, it is noteworthy that adjacent uninfected cells show a very regular form (Fig. 9). Whether the host cells are actually distorted in the living condition or whether their membrane is affected so that they become distorted on fixation, this phenomenon might well have a bearing on the stickiness of human red cells infected with *P. falciparum* and their segregation in capillaries of the brain and other organs.

Finally, an abnormality was observed in unparasitized erythrocytes (Fig. 6, 7, 8). A portion of the membrane of an otherwise well-fixed cell would be raised in multiple alveoli. These peculiar blisters were very common in both of the falciparum infections studied, but were not seen at all in the material from a patient with *P. ovale* (a 10-month-old girl with about 5000 parasites per mm^3 of blood). It seems possible that these defects could be related to the haemolysis which occurs in some *P. falciparum* infections in man. The so-called " spotted red cells " seen by light microscopy during the acute phase of falciparum malaria (Gramiccia, 1947) might correspond to cells with these membrane defects. It is of interest that preparations of membranes of erythrocytes from cases of paroxysmal nocturnal haemoglobinuria showed unusual amounts of electron-dense material (Lewis, Danon & Marikovsky, 1965), but nothing closely resembling the abnormalities illustrated here.

RÉSUMÉ

L'examen par microscopie électronique de coupes ultra-minces a permis de préciser certains détails de la structure de *Plasmodium falciparum* et des hématies qui les hébergent au cours d'une étude menée au Libéria chez des enfants atteints de paludisme.

La morphologie générale du parasite ne diffère guère de celle d'autres espèces infectant les primates. Deux membranes distinctes étroitement accolées l'isolent du cytoplasme de la cellule hôte. La grande vacuole nutritive ne semble pas jouer un rôle dans la désintégration de l'hémoglobine, l'hémozoïne étant répartie en fines granulations dans des vésicules filles. Les formes annulaires jeunes de *P. falciparum* qui, après coloration classique au Giemsa, semblent appliquées sur l'érythrocyte, apparaissent au microscope électronique à l'intérieur de la cellule parasitée. Les taches de Maurer se présentent sous la forme d'amas allongés, limités par deux membranes, et répandus dans le cytoplasme des érythrocytes. Ces derniers apparaissent toujours déformés, surtout au voisinage du parasite. Enfin l'examen d'hématies non parasitées montre des altérations de la membrane cellulaire avec aspect bulleux caractéristique qui, selon les auteurs, pourrait avoir un rapport avec les phénomènes d'hémolyse observés dans certaines infections à *P. falciparum*.

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