Primaquine-Induced Changes in Morphology of Exoerythrocytic Stages of Malaria

Abstract. *Exposure to primaquine for 48 hours caused lesions in the exoerythrocytic stages of* Plasmodium fallax *grown in cultivated cells derived from embryonic turkey brain. The lesions appeared in the form of cytoplasmic vacuoles when viewed under the light microscope. The electron microscope revealed these vacuoles as swollen mitochondria readily identifiable by their typical protozoan cristae. Mitochondria of the host cell were unaffected.*

The 8-amino quinolines, the first of the synthetic antimalarials to be discovered, have always been extensively used clinically; in fact primaquine and quinocide remain today the drugs of choice for attempts at radical cure of infections by *Plasmodium vivax* and *P. malariae*. Yet, in spite of their clinical importance very little is known about their action on the exoerythrocytic stages of the parasite against which they are effective. The principal reason for this has been lack of a satisfactory experimental system for study of the action of these compounds on their target parasites. However, recent advances in cultivation of the exoerythrocytic stages of an avian malarial parasite, *P. fallax*, provide the opportunity for direct observation of the action of curative drugs. In addition to facilitating separation of the exoerythrocytic forms from the blood stages, the techniques involved have developed sufficiently to allow routine production of large numbers of parasites (*1*), thus providing the needed experimental system as well as permitting studies with the electron microscope.

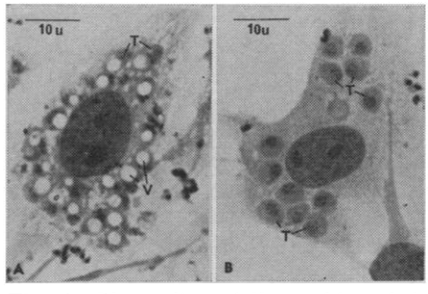

Fig. 1. *Plasmodium fallax* cultivated in turkey cells. Trophozoites (*T*) taken from (A) a culture that had been exposed to primaquine for 48 hours, and from (B) a normal culture. Note the large vacuole (*V*) within the cytoplasm of the parasite.

The activity of primaquine was studied in vitro with both light and electron microscopes. Minor modifications of reported techniques (*1*) yielded preparations for light-microscope studies. Briefly the method consists of inoculation of a suspension of parasitized turkey brain cells and media into Leighton tubes fitted with cover slips, allowance of 24 hours for the parasitized cells to overgrow the cover slips, and replacement of the old medium with fresh to which the drug, at a concentration of 10 mg/liter (twice the minimum dose effective on 50 percent of the parasites, as determined by previous light-microscopy), has been added. Controls receive no drug. After 48-hour exposure to the drug, the cover-slip preparation is removed, wet-fixed in Zenker solution, and stained in Giemsa. For study of the fine structure, a standard T-flask is substituted for the Leighton tubes; the inoculum is allowed to grow out, and the culture is exposed to the mixture of drug and medium for 48 hours; similarly, drugs are omitted from controls. Parasitized cells are removed by trypsinizing and concentrated by centrifugation. After decantation, parasites and cells are processed routinely for electron microscopy (*2*).

Observations with the light microscope reveal that treatment with primaquine results in formation of vacuoles within the cytoplasm of the parasites (Fig. 1A). These cytoplasmic vacuoles occur in all stages of the parasites, including young trophozoites, dividing schizonts, and merozoites. Furthermore, the vacuoles vary in size. In the extreme case, the vacuoles appear to displace the nucleus and other structures to the periphery. Since the vacuole was not observed in the control group (Fig. 1B) [nor ever seen in previous studies (*2*)], the lesion produced in the parasite treated with primaquine appeared to be a change produced by the drug.

Studies of thin sections with the electron microscope revealed that the vacuoles seen with the light microscope are apparently swollen mitochondria (Fig. 2A), readily recognizable by the

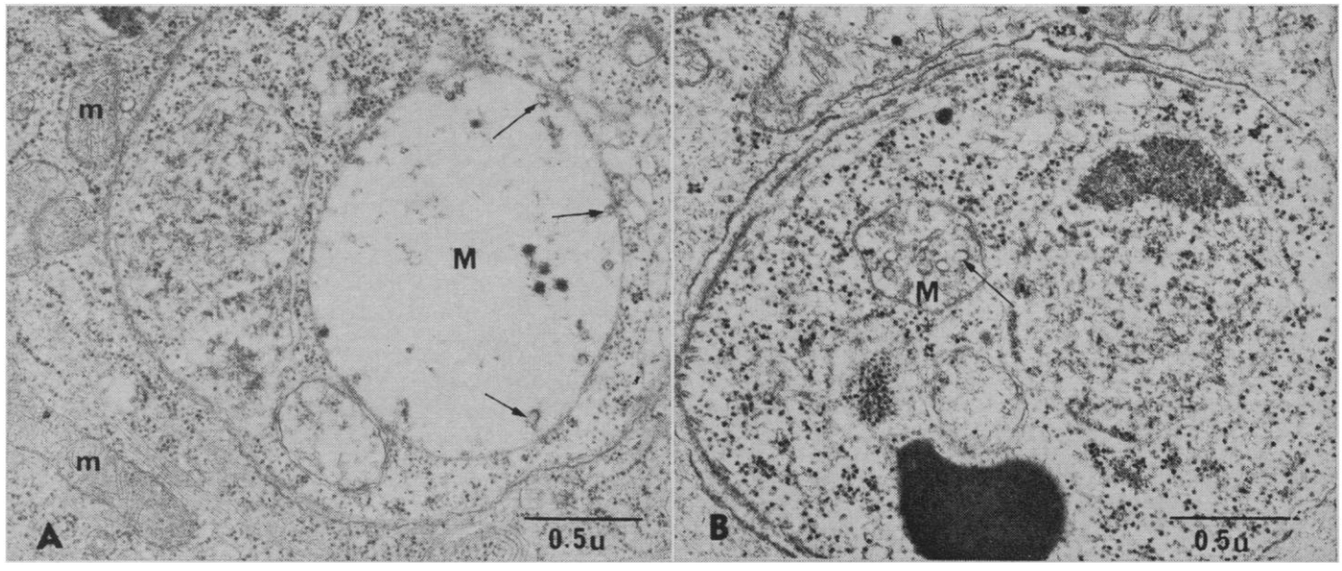

Fig. 2. (A) An exoerythrocytic trophozoite of *Plasmodium fallax* 48 hours after exposure to primaquine. A swollen mitochondrion (*M*) with its cristae (arrows) is noticeable; the mitochondria (*m*) of the host cell appear to be unaffected. (B) An exoerythrocytic trophozoite of *Plasmodium fallax*; note a mitochondrion (*M*) with the microtubular type of cristae (arrow).

presence within of microtubular cristae characteristic of protozoan mitochondria and similar to the cristae seen in the mitochondria of the control group (Fig. 2B). Furthermore the light microscope reveals only a single vacuole in uninucleate parasites, the merozoite, and young trophozoite, whereas larger dividing forms have several vacuoles; this finding corresponds well with electron-microscope observation of the normal parasite, showing the merozoite and young trophozoite with a single mitochondrion whereas schizonts have several. The drug had no detectable effect on the ultrastructure of the mitochondrion of the host cell (Fig. 2A). These observations support the view that the swelling of the mitochondria, seen in the parasites treated with primaquine, is not due to any special artifact of fixation but rather to the effects of the drug.

Certain agents are known to cause active swelling of mitochondria (*3*). Our observations indicate that primaquine probably acts similarly except that it is somewhat specific in its action, causing swelling only in the mitochondria of the parasite and not in those of the host cell.

The structure of the mitochondrion is closely linked to its metabolic functions (*3*), and any major disruption of this structure is undoubtedly accompanied by impairment of its associated metabolic processes. Although the mode of action of primaquine remains unknown, we feel that the morphological changes that we report are sufficiently severe to account for the strong action of this drug on the exoerythrocytic stages of malaria.

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4. From the Bureau of Medicine and Surgery, U.S. Navy; research tasks M4305.12-3007 and MR005.09.0003. Contribution 346 from the Army Research Program on Malaria. The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. We thank C. P. A. Strome, William Clutter, and Kittie Brown for technical assistance.

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Plantlets from Aspen Tissue Cultures

Abstract. *After 2 weeks in the dark, leafy shoots were initiated on subcultured callus tissue of triploid quaking aspen on Wolter's medium without auxin and with 6-benzylaminopurine at 0.05, 0.10, 0.15, or 0.20 milligram per liter substituted for kinetin. Shoots transferred to Wolter's medium under light (323 milliphots) grew large roots and were isolated as plantlets.*

Plants have been reproduced from single isolated cells of carrot, tobacco, endive, parsley, and asparagus (*1*), but plantlets of trees have not been reported from either cell or tissue cultures. Roots and shoots, not directly attached to each other, grew from the same pieces of subcultured callus of European aspen and birch (*2*) and of triploid quaking aspen (*3*). I now report the growth of large roots from the base of leafy shoots initiated on aspen callus, and the isolation of plantlets.

Firm white callus was isolated (*4*) from root sprouts of triploid quaking aspen (*Populus tremuloides* Michx.) and subcultured monthly for 1 year on Wolter's defined medium (*5*). Twenty seed pieces (33 ± 5 mg) from stock tissue grown 5 weeks from subculture were distributed among ten 125-ml erlenmeyer flasks for each medium made by adding 6-benzylaminopurine (BA) at 0.05, 0.10, 0.15, or 0.20 mg/liter to Wolter's medium without auxin and kinetin.

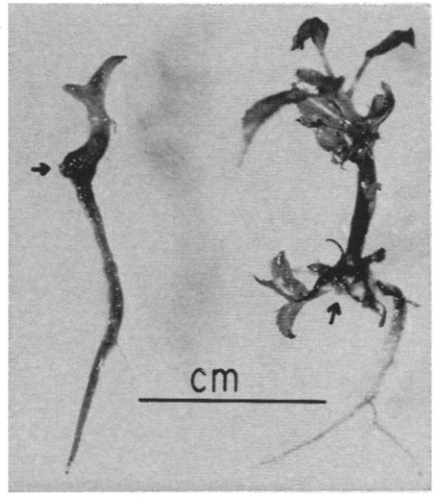

Fig. 1. Shoots, with attached roots, isolated from callus (arrows) as plantlets.

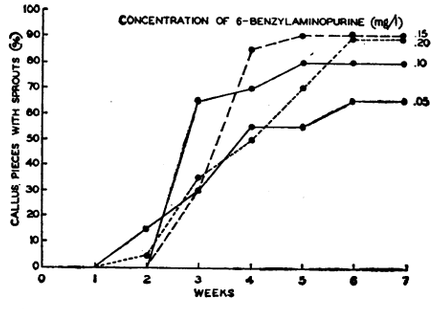

Fig. 2. Percentage of pieces of callus with shoots initiated on BA mediums without auxin.

Aerial shoots grew from cut surfaces of callus on 0.05-percent BA medium after 2 weeks in the dark at 27°C. Four shoots on separate pieces had large attached roots after 3 weeks when they were transferred to Wolter's medium (with 2,4-dichlorophenoxyacetic acid at 0.04 mg/liter and kinetin at 1.0 mg/liter) under 323-mphot light for 16 hours daily. Two plantlets (Fig. 1) dried during photography and two were placed in fixative for sectioning.

Vigorous shoots appeared on three pieces (15 percent) within 2 weeks and on 65 percent of the pieces within 6 weeks on medium containing BA at 0.05 mg/liter; smaller shoots grew from 30 to 65 percent within 3 weeks and from 80 to 95 percent of the callus pieces within 6 weeks on mediums containing more BA (Fig. 2). Shoots grew above and below the surface of mediums containing BA at 0.10, 0.15, or 0.20 mg/liter, but were not attached directly to roots. Shoots left on BA mediums in light or dark did not form roots.

Shoots grown from callus in the dark on medium containing BA at 0.05 mg/liter, in two subsequent experiments, are now on Wolter's medium in the light for initiation of roots. Roots but no shoots grew from callus in the dark on mediums made by substitution of 6-(γ,γ-dimethylallylamino) purine at 0.0001, 0.001, 0.01, 0.1, or 0 mg/liter for BA. The growth of aspen plantlets from callus tissue now opens the way for production of genetically identical trees from single cells.

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