STUDIES ON NUCLEAR DIVISION OF A MALARIAL PARASITE UNDER PYRIMETHAMINE TREATMENT

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INTRODUCTION

Pyrimethamine is an antimalarial drug which possesses schizonticidal activity in both tissue and blood phases. Though pyrimethamine is known to inhibit folic acid metabolism of malarial parasites by binding with folic acid reductase (6), the cytological effect on the parasites is not completely understood.

To our knowledge, there has been no study until now on the morphological effects of pyrimethamine on malarial parasites. However, Thurston (11), using light microscopy, reported the effects of a similar antimalarial drug, proguanil, on the nuclear division of the erythrocytic stages of *Plasmodium berghei*. Since proguanil and pyrimethamine exert qualitatively similar effects on malarial parasites (8), pyrimethamine is also thought to act against nuclear division of malarial parasites.

In this investigation, we have studied with electron microscopy the morphological effects of pyrimethamine on the erythrocytic stages of a malarial parasite, *Plasmodium gallinaceum*. It is hoped that pyrimethamine may be used for the study of nuclear division in malarial parasites, which is still poorly understood.

MATERIALS AND METHODS

2–3-wk-old White Rock chicks weighing an average of 450 g were used for this experiment. Experimental birds were inoculated intravenously with the erythrocytic stages of the 8A strain of *P. gallinaceum*. When the parasitemia reached approximately 50%, a single dose (10 mg/kg) of pyrimethamine was given to the birds by stomach tube. Blood samples for electron microscopy and smears for light microscopy were obtained at 2, 3, and 5 hr. Smears were dried and fixed in methanol and stained in Giemsa solution. For electron microscopy, about 0.5 ml of blood was taken for each sample and was prepared in the manner previously described (1, 2). Sections were cut on a Porter-Blum MT-2 ultramicrotome and were examined with a Siemens Elmiskop 1A.

OBSERVATIONS

Light microscopic observations of the erythrocytic stages of *P. gallinaceum* obtained after exposure to pyrimethamine revealed that there are more schizonts and fewer merozoites when compared with the control group. These changes are noted at 2 and 3 hr after administration of the drug, but they are more prominent at 5 hr. Electron microscopic studies show that the most prominent early effects after exposure to the antimalarial drug are seen in the nucleus. However, these effects are not changes in the morphology of the nuclear structures but rather changes in their behavior, as evidenced by manyfold increase in frequency of dividing nuclei observed.

The nucleus in uninucleate trophozoites and schizonts shows less prominent peripheral chromatin clumping than that in the control group. Many of these nuclei demonstrate bundles of nuclear microtubules (Figs. 1 and 2). Such bundles of nuclear microtubules radiate in fan-shape fashion from a centriolar plaque located on the nuclear membrane (Fig. 1). Each nuclear microtubule is composed of a densely stained cortex and a lightly stained central core, and measures 110–130 A in the inner diameter, and 190–220 A in the outer diameter. Occasionally, these nuclear microtubules are arranged in the manner usually seen in the spindle fibers of the metaphase or early anaphase of mitosis of mammalian cells. Sometimes, two centriolar plaques are seen in the same plane of section and their bundles of microtubules

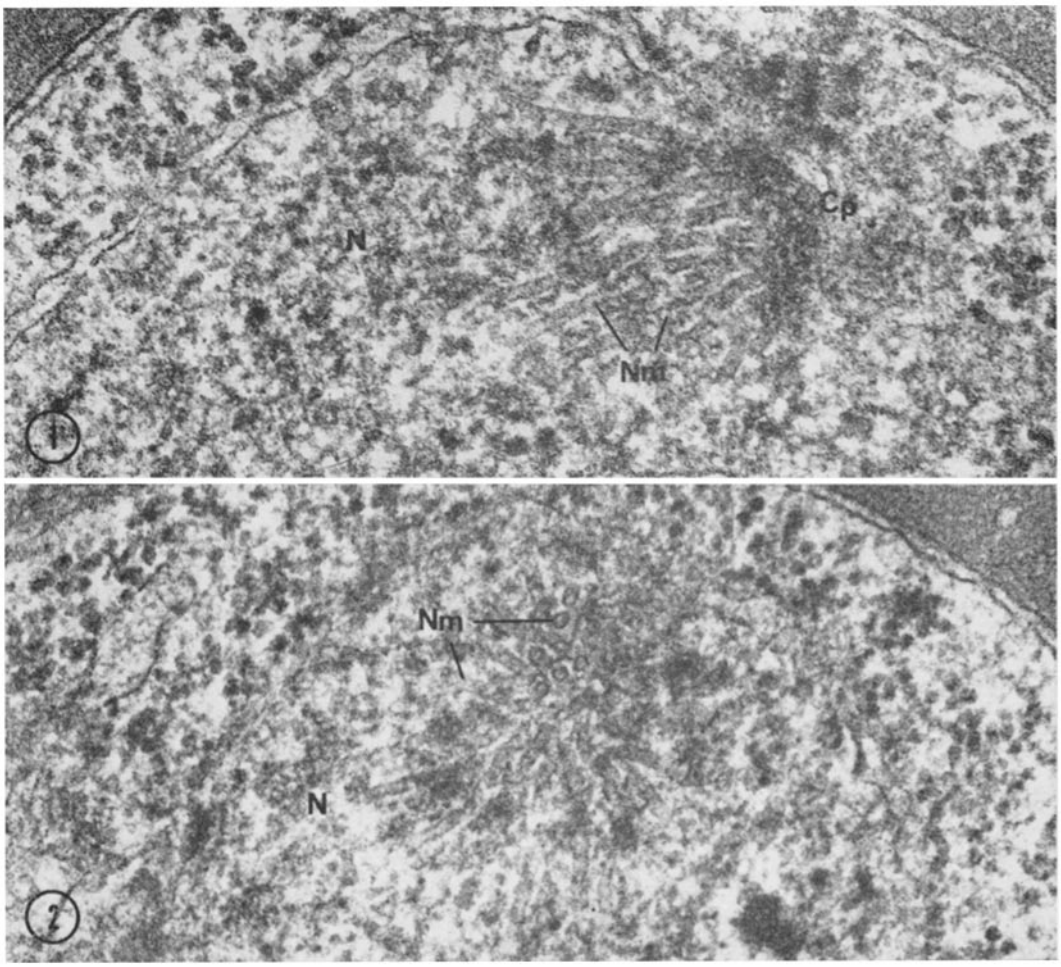

FIGURE 1 Trophozoite of *P. gallinaceum* after exposure to pyrimethamine. A nucleus (*N*) of the parasite shows a bundle of nuclear microtubules (*Nm*) radiating in fan-shape fashion from a centriolar plaque (*Cp*). × 84,000.

FIGURE 2 Cross-section of a bundle of nuclear microtubules (*Nm*) in a nucleus (*N*) of *P. gallinaceum* after treatment. × 97,000.

radiate toward each other and meet at a point roughly midway between the two centriolar plaques (Figs. 3 and 4). In general, the microtubules are not connected with those originating from an opposite centriolar plaque; sometimes, however, a single microtubule appears to connect two opposite centriolar plaques (Fig. 4).

At the midpoint at which bundles of microtubules from opposite plaques meet, we have observed paired, electron-opaque structures on and perpendicular to the microtubules (Figs. 3 and 4). Each of these paired dense structures is a short bar and measures approximately 750 A in length, and 740 A in width. Each bar is composed of three darkly stained subunits separated by lightly stained spaces (Figs. 3 and 4). The middle subunit of the bar is larger than the outer subunits and measures about 220 A in width, whereas the outer subunit measures 120–130 A. Though these structures are usually seen midway between two centriolar plaques, they also occur occasionally closer to each centriolar plaque (Fig. 4). In this instance, the

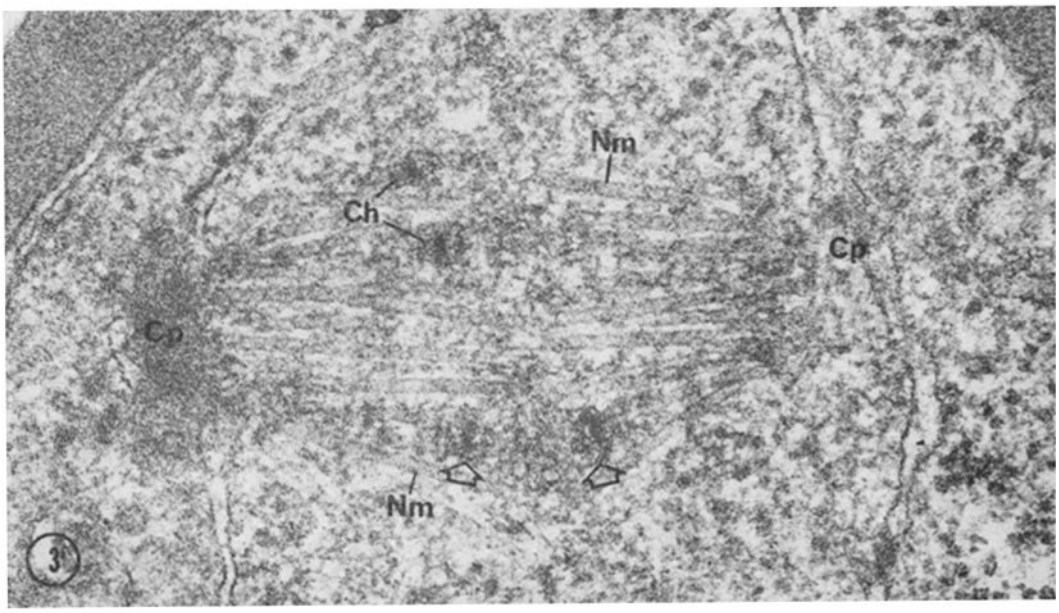

FIGURE 3 A schizont of *P. gallinaceum* after exposure to pyrimethamine. Two centriolar plaques (*Cp*) are seen in this plane of section; the centriolar plaque to the right is slightly off from this plane and only its periphery can be observed. Bundles of microtubules (*Nm*) radiate toward each other and meet at approximately the midpoint between these two plaques (*Cp*). Electron-opaque structures (*Ch*) are seen on the microtubules as well as perpendicular to them. Two electron-opaque structures are seen as a pair in the lower portion of this micrograph (double-shafted arrows). Each of these dense structures is composed of three darkly stained subunits. × 81,000.

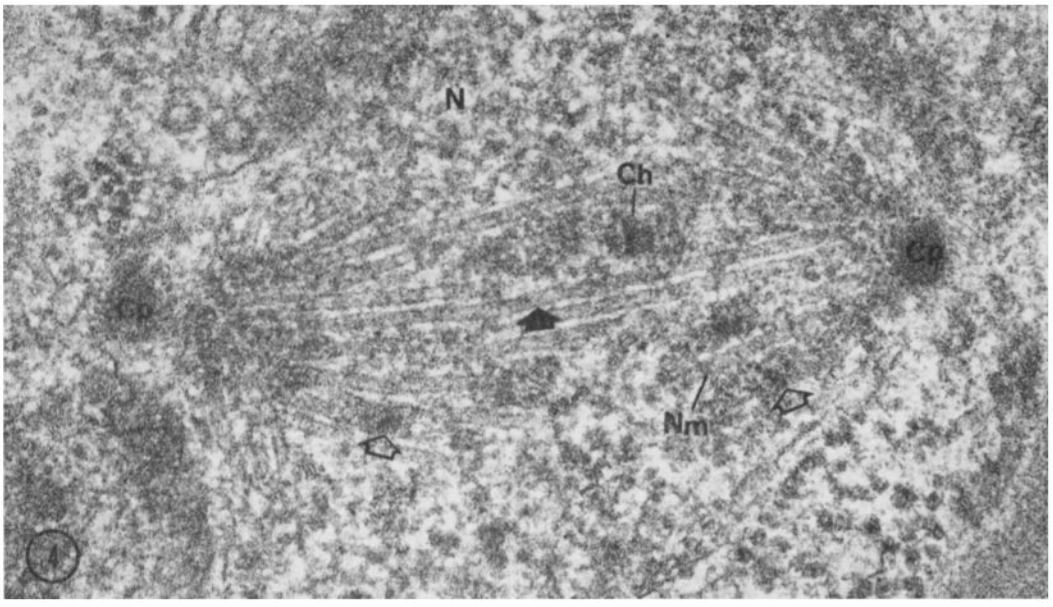

FIGURE 4 Two centriolar plaques (*Cp*) in the same plane of a section in the nucleus (*N*) of a schizont treated with the drug. Occasionally, a single microtubule appears to connect these plaques (arrow). Paired electron-opaque structures (*Ch*) are observed along these microtubules (*Nm*). One set of the paired dense structures (double-shafted arrows) is seen closer to each plaque. × 84,000.

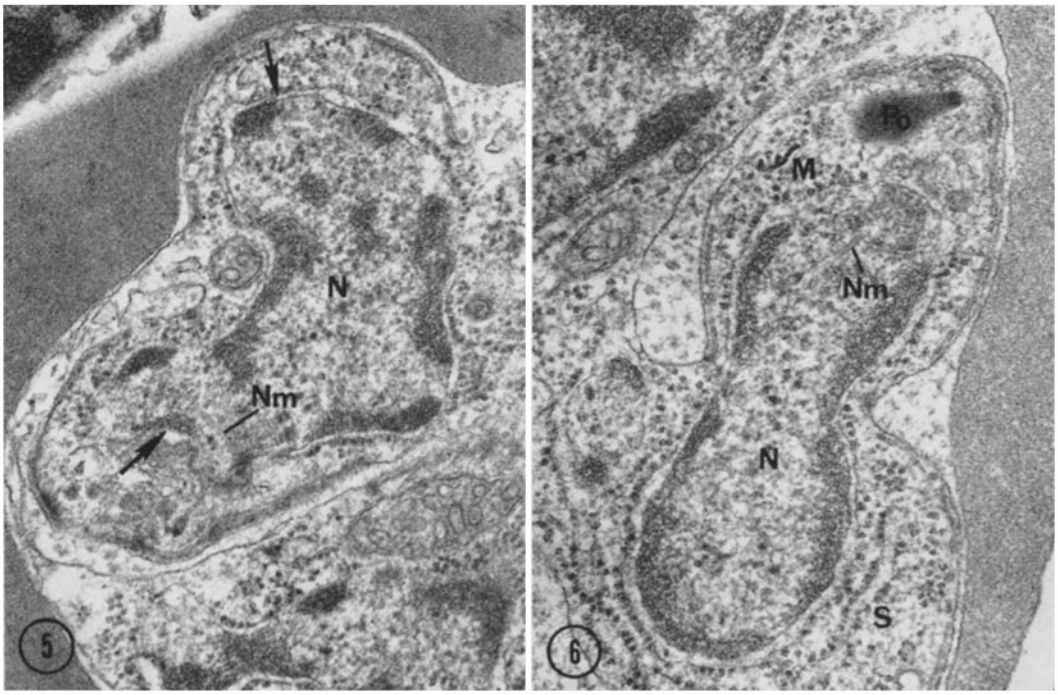

FIGURE 5 A single nucleus (*N*) of a pyrimethamine-treated schizont is seen extending to two adjacent newly forming merozoites (arrows). A bundle of microtubules (*Nm*) is seen in this nucleus. × 34,000.

FIGURE 6 After exposure to pyrimethamine, a large nucleus (*N*) of a schizont is observed halfway between a newly forming merozoite (*M*) and a mother schizont (*S*). A bundle of microtubules (*Nm*) is also seen in this nucleus. Paired organelles (*Po*). × 39,000.

distance from one centriolar plaque to its bar is approximately the same as the distance from the opposite plaque to its bar. Similar paired, electron-opaque structures have been observed in the nuclei of the control group, though they are rarely encountered, perhaps because of the rapidity of the process of nuclear division. In both treated and control nuclei, the nuclear membrane remains intact during division except at the location of the centriolar plaques (Figs. 1 and 3).

A coordination between the process of nuclear division and cytokinesis is observed during merozoite formation of the control group. In the parasites exposed to pyrimethamine, this coordination appears to be disrupted. Because of interference of nuclear division, a single curving nucleus is seen extending into two adjacent budding merozoites (Fig. 5), and sometimes a large nucleus is seen halfway between a newly forming merozoite and a part of the mother schizont (Fig. 6). These features are not encountered in the control group. Bundles of nuclear microtubules are also seen in these nuclei (Figs. 5 and 6). Though the migration of the nucleus to the budding merozoite is disturbed, the protrusion of the cytoplasm is seen as in the control group.

DISCUSSION

Thurston (11) in 1951 reported morphological changes in the erythrocytic stages of *P. berghei* after exposure to an antimalarial drug, proguanil. She observed with the light microscope that the drug acts chiefly on the early development of the schizont, causes the chromatin to divide into numerous small particles, and interferes with the production of the merozoites. Effects of pyrimethamine on the erythrocytic stages of *P. gallinaceum* studied in this investigation are roughly similar to those of proguanil which interferes at a different point in the same pathway; thus pyrimethamine also appears to impede nuclear division of the malarial parasite. A higher percentage of

schizonts with their nuclei showing metaphase and fewer resultant merozoites, as well as the interference with nuclear migration into newly forming merozoites, suggested that the ultimate morphological result of pyrimethamine is the arrest or retardation of nuclear division of malarial parasites. However, we must stress that the effects of pyrimethamine appear not to be disruption of the mitotic apparatus. In this respect, they differ from those of chemical compounds, such as colchicine (4), which interfere with nuclear division by disrupting the mitotic apparatus of cells. While expected, in view of the mode of action of the drug which blocks a biosynthetic pathway to nucleic acids (6), the observation of a nuclear effect with pyrimethamine was interesting in that it appeared to interfere at metaphase rather than at prophase, when chromosome replication is believed to take place.

Despite numerous studies on the nuclear division of asexual stages of malarial parasites, information concerning this process is still inadequate. Aikawa (1), by studying the fine structure of the erythrocytic stages of *P. fallax*, *P. lophurae*, and *P. cathemerium*, reported occasional nuclear microtubules and minute, paired dense structures along these microtubules in a dividing nucleus. Since then, nuclear microtubules in the nucleus of the erythrocytic (2) and exoerythrocytic stages (5) and sporozoites (10, 12) of malarial parasites have been reported. Similar nuclear microtubules have been described in the nucleus of yeast, *Saccharomyces cerevisiae*, by Robinow and Marak (9) and separately by Moor (7). Robinow and Marak suggested the role of nuclear microtubules as one of "form-giving rigidity" for maintenance of nuclear shape rather than as having any function in nuclear division. It seems more likely, however, that the nuclear microtubules observed in malarial parasites are related to nuclear division, because of their similarity to the spindle fibers seen in metaphase or early anaphase of mitosis and because of the paired nature of the electron-opaque bars which may correspond to chromosomes.

Wolcott has repeatedly reported structures which he interpreted as chromosomes in the erythrocytic stages of various malarial parasites (13). He described the chromosomes as two small, elongated structures of equal size measuring about 1 μ in length, which were usually observed in late asexual stages and in merozoites. There is, however, strong doubt about the true nature of the structures he described as chromosomes. These structures may, in fact, be the paired organelles which appear at the anterior end of merozoites and the periphery of schizonts of malarial parasites (1).

Though the nature of the paired, dense structures described by us is unknown, we suggest that they may represent the chromosomes of malarial parasites, because they are repeatedly seen paired as in the metaphase or early anaphase of mitosis of mammalian cells and are located along the nuclear microtubules at points roughly midway between the centriolar plaques. These structures are also found closer to the centriolar plaques, a fact which may indicate that these structures are in the act of migrating to each portion of a future daughter nucleus. The presence of a weak Feulgen reaction in *P. gallinaceum* has been reported by Chen (3). This observation suggests a small amount of DNA in the nucleus of malarial parasites which corresponds well with the formation of small chromosomes. However, it should be remembered that morphological observations alone cannot prove the identity of the paired dense structures as the chromosomes of malarial parasites, even though it seems likely that this is what they are.

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