# Taxol Arrests the Development of Blood-Stage *Plasmodium falciparum* In Vitro and *Plasmodium chabaudi adami* in Malaria-infected Mice

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## Abstract

**Taxol, a natural product used to treat a variety of human cancers, is shown here to be extremely effective against chloroquine- and pyrimethamine-resistant malaria parasites. Addition of Taxol (1.0 μM) for one cycle to cultures of human erythrocytes infected with *Plasmodium falciparum* prevents the establishment of new infections. Blood parasitemia is eliminated in mice infected with *Plasmodium chabaudi adami* when they are given a single intraperitoneal injection of Taxol at 150 mg/m². The majority of the animals treated immediately preceding parasite schizogony remain free of infection after eight replication cycles. The impressive antimalarial activity of Taxol, at a dosage that has been tolerated in humans, establishes its potential utility for treatment of severe, drug-resistant human malaria. (*J. Clin. Invest.* 1994. 94:413–417.) Key words: chemotherapy • cremophor • chloroquine • drug resistance • infusion**

## Introduction

Malaria continues to claim millions of lives annually. The spread of drug-resistant *Plasmodium falciparum* threatens to add to this total, intensifying the search for new antimalarial agents. Taxol is a novel diterpenoid isolated from the stem bark of the western yew *Taxus brevifolia.* It has a unique mode of action, being a mitotic spindle poison that stabilizes microtubules and inhibits their depolymerization to free tubulin (1–3). During the erythrocytic phase of malaria infection, *Plasmodium* parasites propagate by schizogony, wherein the nucleus divides three to five times to form 8–32 nuclei (4). Microtubular structures, containing α tubulin, have been shown recently to change their conformation during parasite replication (5). We hypothesized that Taxol might have antimalarial activity by interfering with nuclear division and/or production of nascent, infectious merozoites. We show here that in vitro or in vivo treatment of blood-stage malaria parasites with Taxol before schizogony prevents the establishment of new infections.

## Methods

### *Parasites*

The effects of Taxol on four parasite strains of different origin and drug sensitivity were examined. Strain FCR3-C5 (Gambia) is chloroquine and pyrimethamine sensitive, T9-94 (Thailand) is chloroquine resistant, HB3 (Honduras) is pyrimethamine resistant, and 7GB (Brazil) is both chloroquine and pyrimethamine resistant. Infected red blood cells (IRBC)[1] were cultured as described previously (6, 7). The infections were synchronized with sorbitol treatment (8) to ±2 h. One cycle lasts 44–46 h, during which time intracellular parasites undergo asexual development, and merozoites are released to produce new infections.

### *Taxol treatment in vitro*

Taxol, obtained from Molecular Probes, Inc. (Eugene, OR) (catalogue No. T-3456) was dissolved in DMSO. Cultures of infected erythrocytes were either untreated, treated with DMSO only, or treated with Taxol to a final concentration of 0.05, 0.1, or 1.0 μM. All treated cultures contained the same final concentration of DMSO (0.17%). Taxol was added to early ring-stage IRBC (4–6 h after invasion) infected with the *P. falciparum* clones FCR3-C5, T9-94, 7G8, or HB3, and maintained for two life cycles. The tissue culture medium was changed daily, and a fresh aliquot of DMSO or Taxol was added as required. The initial parasitemia of the cultures was 0.5–1.0%.

### *Effect of Taxol on parasite viability*

*Parasitemia.* The parasitemia (parasites/erythrocytes × 100) was measured by examining Giemsa-stained blood smears using a light microscopy. The efficacy of Taxol, expressed as parasitemia (percentage of control), was determined by counting the parasitemia in the treated cultures 10 h after invasion was complete in the untreated and DMSO-treated cultures and comparing that value with the parasitemia of the control cultures. A parasitemia of 0% indicates that Taxol totally inhibits new infections; a parasitemia of 100% indicates that Taxol has no effect. Parasitemia was calculated by pooling the results of two separate experiments, with 1,000 cells counted at each Taxol concentration.

*DNA synthesis.* The effect of Taxol on parasite viability was assessed by measuring the incorporation of [$^3$H]hypoxanthine into untreated and treated cultures. Highly synchronous (±2 h) cultures of FCR3-C5–infected erythrocytes were grown in a 96-well plate. Each well contained 3 μl of IRBC at a parasitemia of 1–2%, 90 μl of hypoxanthine-free culture medium, and 10 μl of human serum. Eight wells were untreated, and eight wells were treated with 1.0 μM Taxol at 4 h after invasion. The culture medium in each well was replaced with Taxol-free, hypoxanthine-free medium after the first cycle. During the trophozoite stage

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1. *Abbreviation used in this paper:* IRBC, infected red blood cells.

(32 h after invasion) of the first cycle, 18 μl of culture medium containing [$^3$H]hypoxanthine (1–5 Ci/mmol; Amersham Corp., Arlington Heights, IL) at 20 μCi/ml and 2 μl of human serum was added to half of the wells for 4 h. The same additions were made at the trophozoite stage of the second cycle to the remaining wells. The radioisotopically labeled cultures were harvested onto glass fiber filters (Whatman Inc., Clifton, NJ) using distilled water and an automated cell harvester (Cambridge Technology, Inc., Watertown, MA). The filters were added to 10.0 ml of BioSafe II (Research Products International Corp., Mount Prospect, IL) and counted in a liquid scintillation counter. Values shown are the mean of four measurements.

### *Determination of parasite recrudescence*

To determine if the infections in Taxol-treated cultures were recrudescent, two cultures at 5% hematocrit containing 2.5 × $10^7$ IRBC were established. When the parasites were young rings, 1.0 μM Taxol (4.3 μl of Taxol in DMSO) was added to one culture, and 4.3 μl of DMSO was added to the other. After reinvasion, the medium was changed in both cultures to remove the Taxol and/or DMSO. The cultures were maintained for 8 d corresponding to four additional cycles. Parasitemia was counted after each cycle of reinvasion. The parasitemia of the control culture was kept below 5% by the addition of fresh blood.

### *Treatment of Plasmodium chabaudi adami–infected mice with Taxol*

30, 7-wk-old male BALB/c ByJ mice (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) were administered intraperitoneal injections of 2.0 × $10^5$ *Plasmodium chabaudi adami* parasites. The parasitemia, estimated from tail vein blood smears, was allowed to reach a mean of 1–2% before beginning the experiment. 7–10 d were required for the parasitemia to reach these levels. One life cycle is completed in 24 h. Taxol, obtained from the Thomas Jefferson University Hospital pharmacy, is supplied in an ampul at 6 mg/ml in 5.0 ml of 1:1, vol/vol, of polyethoxylated castor oil (cremophor EL; Sigma Chemical Co., St. Louis, MO) and dehydrated alcohol. On day 0, eight groups of mice (each group contained three animals) were given a single, 1.0-ml intraperitoneal injection of Taxol at 150 or 75 mg/m$^2$ at 2-h intervals beginning at the mid-trophozoite stage. The highest dosage was chosen by extrapolating the $LD_{10}$ for a single intraperitoneal Taxol injection in rats to mice (9). Conversion from milligrams per square meter to milligrams per kilogram is based on the empirical correlation that surface area (m$^2$) = $K \cdot W^{2/3}/100$, where K is a species-specific factor, and W is body weight in kilograms (10). K is 9 for rats and mice and 10.6 for a 70-kg human. A dose at 150 mg/m$^2$ is equivalent to 975 μg in a 20-g mouse and 260 mg in a 70-kg human. The injections consisted of the desired volume of the stock Taxol solution adjusted to 1.0 ml with saline. One group of control mice was treated identically as the treated groups, except that Taxol was omitted from the formulation. The other control group received saline. Daily blood smears were made over eight life cycles, and the parasitemia was measured to assess the effect of the treatment. Mean values were obtained for each group daily and divided by the mean value on day 0. This is defined as the relative parasitemia.

## Results

The in vitro effects of Taxol on human erythrocytes infected with different strains of *P. falciparum* malaria parasites (IRBC) varying in origin and drug sensitivity are shown in Fig. 1. To ensure that DMSO, necessary to dissolve Taxol, did not adversely affect the parasites, the parasitemia of untreated cultures and of DMSO-treated cultures of IRBC were compared for up to six life cycles. DMSO had no significant effect on parasite viability or invasiveness. Taxol reduced the parasitemia of the cultures in a dose-dependent fashion (Fig. 1). In erythrocytes infected with the FCR3-C5 clone, 0.5 and 1.0 μM Taxol reduced the parasitemia by 95 and 100%, respectively, after treatment for one replication cycle (Fig. 1 *A*). Exposure of the IRBC to 0.5 μM Taxol for two replication cycles also totally eliminated parasitemia. Similar dose-dependence curves were obtained for erythrocytes infected with chloroquine- and/or pyrimethamine-resistant parasites (Fig. 1) as well as Gambian human isolates (data not shown). Thus, Taxol is equally effective in abolishing the erythrocytic phase of cloned, drug-resistant parasites and human isolates that had not been previously exposed to tissue culture. Furthermore, Taxol is as effective in vitro as classical antimalarials on a molar basis (e.g., chloroquine, 0.25 μM) (11). Taxol's effectiveness for killing parasites at low concentration may be due to its unique mode of action, since a 50 times greater concentration of colchicine (a microtubule antagonist that prevents polymerization of tubulin) is required to abolish all parasites in vitro (data not shown).

To ensure that the observed results were not because of an inability to detect low parasitemia from blood smears or a lengthening of the life cycle, IRBC were incubated for one cycle with 1.0 μM Taxol, the culture medium was changed, and the cells were cultured for an additional cycle in Taxol-free medium. Incorporation of [$^3$H]hypoxanthine into control or Taxol-treated cultures was similar during the first cycle. No incorporation of [$^3$H]hypoxanthine was detected in the treated cultures during the second cycle (Fig. 2). Thus, Taxol appears to act by preventing the establishment of new infections. When the cultures were grown for three additional cycles, the parasitemia increased nearly 300 times in the untreated culture while no IRBC were detected in the Taxol-treated culture (Fig. 3).

To determine if Taxol was effective at a particular time in the life cycle, 1.0 μM drug was added at different intervals up to 44 h after invasion and was maintained throughout the cycle. Taxol added up to 30 h after invasion completely blocked the formation of new infections (data not shown). Effectiveness decreased linearly between 30 and 40 h after invasion. Taxol added after 40 h had no effect on the production of new infections. Thus, it appears crucial that the parasites be exposed to micromolar Taxol just before and throughout parasite replication.

Examination of Giemsa-stained blood smears taken from the untreated and Taxol-treated cultures revealed the most striking differences between 42 and 44 h after invasion. The untreated cultures contained segmented schizonts, free merozoites, and new, ring-stage infections. By contrast, parasites within Taxol-treated IRBC often were condensed, the nuclei were extremely large, and only a few segmented schizonts were visible (data not shown). Examination of the segmented schizonts by electron microscopy suggested that nuclear division occurred in many of the IRBC in treated cultures. However, compared with untreated IRBC, merozoites were misshapen (they did not possess the classic pear shape), their radial distribution was disrupted, and the apical orientation of rhoptries and micronemes within the merozoites was often not apparent.

To assess whether this drug had similar efficacy in vivo, mice infected with the rodent malaria parasite *Plasmodium chabaudi adami* were treated with Taxol. This parasite was chosen since it produces a relatively synchronous infection (±2–3 h). This was an essential requirement for three reasons: (*a*) our in vitro results showed that Taxol was effective at a specific time in the life cycle; (*b*) Taxol is likely to be rapidly cleared from the bloodstream (12–14); and (*c*) the nature and volume of the Taxol formulation dictated that it be delivered in mice as a single intraperitoneal injection since administration by continu-

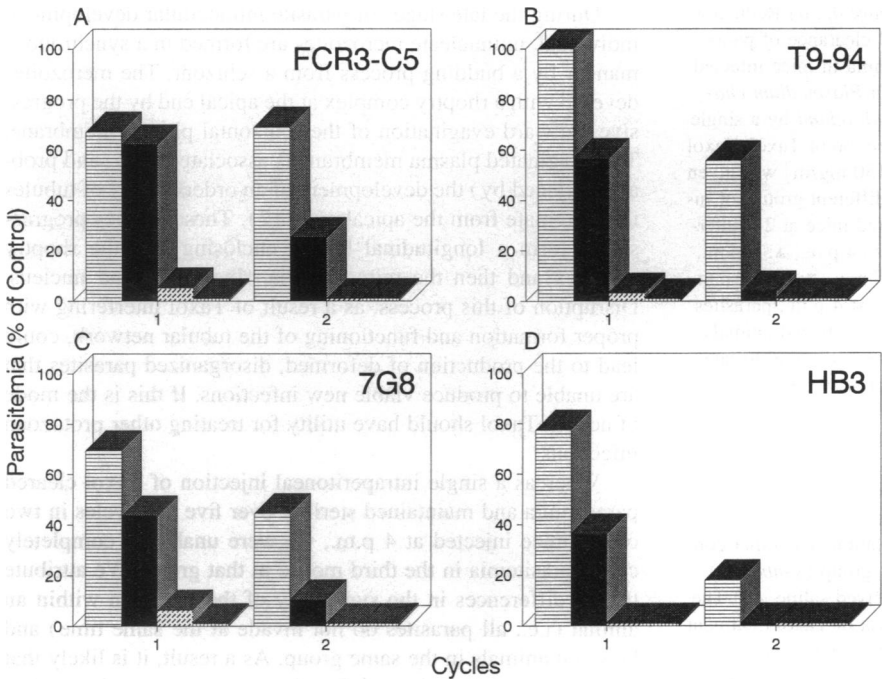


*Figure 1.* The effects of Taxol on four parasite strains, (*A*) FCR3-C5; (*B*) T9-94; (*C*) 7G8; and (*D*) HB3, of differing origin and drug sensitivity. Cultures of infected erythrocytes were either untreated, treated with DMSO only (data not shown), or treated with Taxol to a final concentration 0.05 μM (*horizontal striped bars*), 0.1 μM (*solid black bars*), 0.5 μM (*diagonal striped bars*), or 1.0 μM (*vertical hatched bars*). Taxol was added to early ring-stage IRBC (4–6 h after invasion) infected with the *P. falciparum* clones and maintained during two life cycles. Control parasitemia values used to calculate parasitemia (percentage of control) were from the DMSO-treated cultures. Similar results were obtained when the control parasitemia values from the untreated cultures were used.

ous intravenous infusion was not possible. The constraints of the animal model are considerable, but can be circumvented in humans, as an intravenous infusion would obviate the problems of asynchronous parasites, drug formulation, and drug clearance.

Eight groups of three male BALB/c mice infected with *Plasmodium chabaudi adami* were given a single, intraperitoneal injection of Taxol at 150 or 75 mg/m$^2$ at 2-h intervals beginning when the parasites were at the mid-trophozoite stage (4 p.m.). Daily blood smears were made over eight replication cycles, and the parasitemia was measured to assess the effect of the treatment. The parasitemia in the saline and cremophor control groups increased after each of the first four cycles of infection. However, the groups treated with 150 mg/m$^2$ of Taxol at 4 or 6 p.m. exhibited a dramatic decrease after the first cycle (Fig. 4). Giemsa-stained blood smears revealed that the remaining parasites had altered morphologies compared with IRBC from infected, untreated mice. Since the viability of these parasites could not be assessed directly, they were counted to calculate the parasitemia. It is possible that this leads to an overestimation of the parasitemia after the first cycle. Parasitemia was cleared completely in two of the mice in the 4 p.m. group and was drastically reduced in the third mouse after two cycles. Parasitemia was also reduced greatly in the group injected at 6 p.m., although no animals were cured completely. Taxol administered at the lower dose of 75 mg/m$^2$ only slightly reduced parasitemia (data not shown). The parasitemia de-

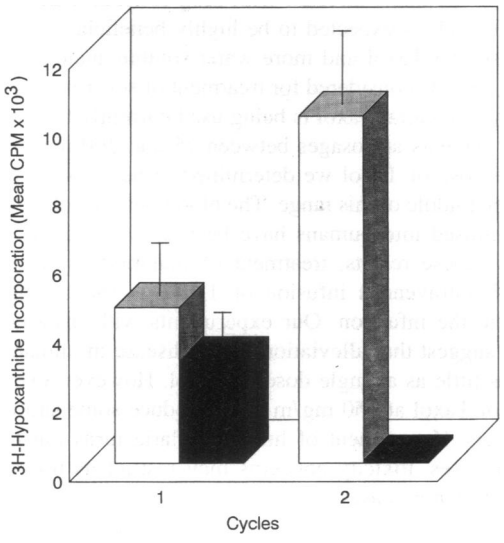


*Figure 2.* The effect of Taxol on parasite viability was assessed by measuring the incorporation of [$^3$H]hypoxanthine into untreated and treated cultures. Open bars represent untreated cultures, and filled bars represent Taxol-treated cultures. The Taxol concentration was 1.0 μM. See Methods for experimental details.

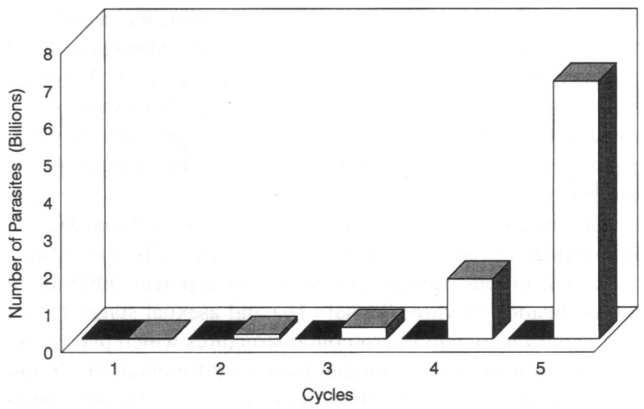


*Figure 3.* Determination of the effect of Taxol on parasite recrudescence. The infection disappeared in the treated culture (*black bars*) after the first cycle, while in the control culture (*white bars*) there was a 276-fold increase in the number of parasites after four additional cycles.

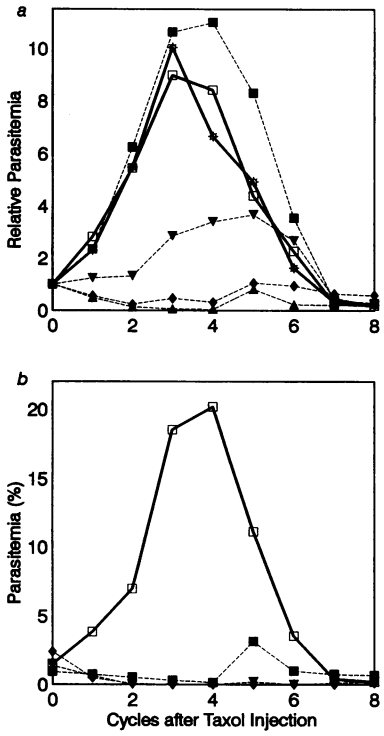


*Figure 4.* (*a*) Reduction and clearance of parasitemia in mice infected with *Plasmodium chabaudi adami* by a single injection of Taxol. Taxol at 150 mg/m$^2$ was given to different groups of infected mice at 2-h intervals (4 p.m., ▲; 6 p.m., ♦; 8 p.m., ▼; and 10 p.m., ■). At 4 p.m., parasites in the infected animals were at the mid to late trophozoite stage. One group of control mice (□) was treated identically as the treated groups, except that Taxol was omitted from the formulation. The other control group (*sunbursts*) received saline. (*b*) The effect of Taxol treatment on the individual mice in the 4 p.m. group (■, ♦, ▼). A cremophor-treated, control mouse (□) is shown for comparison. Data are presented as raw parasitemia.

creased in the untreated groups after three to four cycles as a result of a natural immune response in the mice.

## Discussion

Malaria remains one of the world's major primary health problems, inflicting illness and death on millions annually. Efforts to control this affliction have been hampered by lack of an efficacious vaccine and by rapid development of drug resistance to the limited spectrum of antimalarials. Current blood schizontocides, such as chloroquine, quinine, pyrimethamine, etc., are effective by disrupting selected parasite enzymatic or synthetic pathways (15). Similarities in chemical structure and mode of action of these agents have contributed to the development of drug resistance. In this investigation, we report that Taxol eliminates parasitemia in cultures of chloroquine- and pyrimethamine-resistant parasites with equal efficacy. Taxol appears to be more potent than other antimalarials in vitro, since, unlike chloroquine or protease inhibitors, it prevents the reappearance of parasitemia (recrudescence) in the cultures after a number of cycles.

The mechanism by which Taxol exerts its antimalarial effects remains to be elucidated. In mammalian cells, Taxol functions as a mitotic spindle poison and is a potent inhibitor of cell replication in vitro. Sexual (16) and asexual stages (5) of *P. falciparum* contain microtubular structures which play a crucial role in nuclear division and parasite differentiation. In our model, it does not appear that Taxol acts solely by preventing nuclear division or by directly killing parasites. A significant percentage of Taxol-treated IRBC was observed by electron microscopy to contain nucleated merozoites. In addition, Taxol had little if any effect on DNA synthesis in mature parasites.

During the late stages of parasite intracellular development, individual, uninucleate merozoites are formed in a synchronous manner by a budding process from a schizont. The merozoites develop with a rhoptry complex at the apical end by the progressive, outward evagination of the schizontal plasma membrane. The evaginated plasma membrane is associated with (and probably induced by) the development of an orderly array of tubules that originate from the apical end (17). These tubules progressively form a longitudinal basket enclosing first the rhoptry complex and then the mitochondria, ribosomes, and nucleus. Disruption of this process, as a result of Taxol interfering with proper formation and functioning of the tubular network, could lead to the production of deformed, disorganized parasites that are unable to produce viable new infections. If this is the mode of action, Taxol should have utility for treating other protozoan infections.

Whereas a single intraperitoneal injection of Taxol cleared parasitemia and maintained sterility over five life cycles in two of the mice injected at 4 p.m., we were unable to completely clear parasitemia in the third mouse in that group. We attribute this to differences in the synchrony of the infection within an animal (i.e., all parasites do not invade at the same time) and between animals in the same group. As a result, it is likely that the blood concentration of Taxol was too low at the critical time in the life cycle to completely disrupt merozoite formation. The inability of a lower dose of Taxol (75 mg/m$^2$) to clear the blood parasitemia is consistent with this explanation, as are our in vitro results. Treatment of *P. falciparum* infections in humans, which are asynchronous (e.g., different stages of the life cycle are present at any given time), would not be problematic since the drug can be administered and maintained at the desired concentration by continuous, intravenous infusion over long periods of time. Thus, while Taxol does not appear to directly kill parasites, its administration should quickly reduce parasite burden. Complete elimination of parasitemia may not be achieved until 48 h after the treatment was initiated. While reduction of parasite burden may be somewhat slower than achieved with chloroquine, a drastic reduction or elimination of parasitemia in individuals infected with drug-resistant malaria during one life cycle is expected to be highly beneficial.

We propose that Taxol and more water-soluble analogues, e.g., taxotere (18), be considered for treatment of severe (drug-resistant) human malaria. Taxol is being used currently to treat human cancer patients at dosages between 75 and 300 mg/m$^2$ (12–14). The dose of Taxol we determined to be curative in mice falls in the middle of this range. The blood pharmacokinetics of Taxol infused into humans have been established (12–14). Based on these results, treatment of malarious patients with a 6–24-h intravenous infusion of Taxol at 150 mg/m$^2$ should alleviate the infection. Our experiments with malaria-infected mice suggest that alleviation of the disease in humans may require as little as a single dose of Taxol. However, even a single dose of Taxol at 150 mg/m$^2$ will produce some short-term side effects. If treatment of human malaria turns out to require higher doses, toxicity concerns may restrict its use to treating life-threatening cases.

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## References

1. Wani, M. C., H. L. Taylor, M. E. Wall, P. Coggon, and A. T. McPhail. 1971. Plant antitumor agents. VI. The isolation and structure of a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J. Am. Chem. Soc.* 93:2325–2327.

2. Schiff, P. B., J. Fant, and S. B. Horwitz. 1979. Promotion of microtubule assembly *in vitro* by taxol. *Nature (Lond.)*. 277:665–667.

3. Schiff, P. B., and S. B. Horwitz. 1980. Taxol stabilizes microtubules in mouse fibroblast cells. *Proc. Natl. Acad. Sci. USA.* 77:1561–1565.

4. Bray, R. S., and P. C. C. Garnham. 1982. The life cycle of primate malaria parasites. *Br. Med. Bull.* 38:117–126.

5. Read, M., T. Sherwin, S. P. Holloway, K. Gull, and J. E. Hyde. 1993. Microtubular organization visualized by immunofluorescence microscopy during erythrocytic schizogny in *Plasmodium falciparum* and investigation of post-translational modifications of parasite tubulin. *Parasitology.* 106:223–232.

6. Pouvelle, B., R. Spiegel, L. Hsiao, R. J. Howard, R. L. Morris, A. P. Thomas, and T. F. Taraschi. 1991. Direct access to serum macromolecules by intraerythrocytic malaria parasites. *Nature (Lond.)*. 353:73–75.

7. Gormley, J. A., R. J. Howard, and T. F. Taraschi. 1992. Trafficking of malarial proteins to the host cell cytoplasm and erythrocyte surface membrane involves multiple pathways. *J. Cell Biol.* 119:1481–1495.

8. Lambros, C., and J. P. Vanderberg. 1979. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. *J. Parasitol.* 65:418–420.

9. National Cancer Institute Clinical Brochure: Taxol (NSC 125973). Bethesda, MD: Division of Cancer Treatment, National Cancer Institute, July 1991. 1–36.

10. Freireich, E. J., E. A. Gehan, D. P. Rall, L. H. Schmidt, and H. E. Skipper. 1966. Quantitative expression of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. *Cancer Chemother. Rep.* 50:219–244.

11. Zhang, Y., K. S. O. Asante, and A. Jung. 1986. Stage-dependent inhibition of chloroquine on *Plasmodium falciparum in vitro*. *J. Parasit.* 72:830–836.

12. Wiernik, P. H., E. L. Schwartz, J. J. Strauman, J. P. Dutcher, R. B. Lipton, and E. Paietta. 1987. Phase I clinical and pharmacokinetic study of taxol. *Cancer Res.* 47:2486–2493.

13. Wiernik, P. H., E. L. Schwartz, A. Einzig, J. J. Strauman, R. B. Lipton, and J. P. Dutcher. 1987. Phase I trial of taxol given as a 24-hour infusion every 21 days: responses observed in metastatic melanoma. *J. Clin. Oncol.* 5:1232–1239.

14. Rowinsky, E. K., L. A. Cazenave, and R. C. Donehower. 1990. Taxol: a novel investigational antimicrotubule agent. *J. Natl. Cancer Inst.* 82:1247–1259.

15. Peters, W. 1980. Malaria: Epidemiology, Chemotherapy, Morphology and Metabolism. J. P. Krier, editor. Academic Press Inc., New York. 145–273.

16. Kumar, N., M. Aikawa, and C. Grotendorst. 1985. *Plasmodium gallinaceum:* critical role for microtubules in the transformation of zygotes into ookinetes. *Exp. Parasitol.* 59:239–247.

17. Shaw, M. K., and L. G. Tilney. 1992. How individual cells develop from a syncytium: merogony in *Theileria parva* (Apicomplexa). *J. Cell Sci.* 109:109–123.

18. Bissery, M.-C., D. Guenard, F. Gueritte-Voegelein, and F. Lavelle. 1991. Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue. *Cancer Res.* 51:4845–4852.