Plasmodium falciparum in Owl Monkeys: Drug Resistance and Chloroquine Binding Capacity

Abstract. *Erythrocytes infected with chloroquine-sensitive* Plasmodium falciparum *bind chloroquine with an apparent intrinsic association constant of 1.5 × 10^7 liters per mole. Such high-affinity binding of chloroquine is absent or deficient in uninfected erythrocytes and in erythrocytes infected with chloroquine-resistant* Plasmodium falciparum.

With the discovery that the owl monkey, *Aotus trivirgatus*, is susceptible to infections with *Plasmodium falciparum* (*1*), it has become feasible to study accumulation of drugs by erythrocytes infected with strains of *P. falciparum* which exhibit varying degrees of susceptibility to chloroquine. The need for these studies has been apparent, since diminished accumulation of chloroquine is associated with resistance to chloroquine in a model system of animal malaria, *P. berghei* in the mouse (*2*). The diminished accumulation of chloroquine in the *P. berghei* model is due to a deficiency of drug-receptor sites that have high affinity for chloroquine (*3*). I now report diminished chloroquine accumulation by monkey erythrocytes infected with chloroquine-resistant *P. falciparum* and provide evidence that in this model, too, there is a deficiency of high-affinity drug-receptor sites.

Owl monkeys infected either with the Camp. or with the Monterey strains of *P. falciparum* were available for this study. Although the Camp. strain of *P. falciparum* was originally isolated from man as a chloroquine-resistant strain (*4*), it is relatively sensitive to chloroquine therapy in the owl monkey; it is eradicated by a 1-week course of chloroquine administered orally at a daily dose of 5 mg of the base per kilogram of body weight of the monkey, that is, a total dose of approximately 420 mg per square meter of body surface. For comparison, infections with the usual chloroquine-sensitive strains of *P. falciparum* in man are eradicated by a total dose of chloroquine of approximately 400 mg per square meter of body surface (*5*). The Monterey strain of *P. falciparum* in the owl monkey is resistant to treatment with a 1-week course of chloroquine at a daily dose of 40 mg per kilogram of body weight: this is a total dose of more than 3300 mg/m^2 of body surface and is in excess of the short-term dose that humans can tolerate without toxic reactions (*6*).

Except for the difference in susceptibility to chloroquine therapy, the Camp. and Monterey strains of *P. falciparum* are quite similar. No morphologic differences were detected by light microscopy, and parasites of both strains exhibited approximately the same amounts of malaria pigment. After an intravenous injection of whole blood containing 10^8 parasites, both strains had a prepatent period of 3 to 4 days, and both caused a fatal illness with severe anemia. After either of the infections became patent the percentage of infected erythrocytes increased rapidly, to as high as 60 percent within a week. The number of parasites per 10,000 erythrocytes ranged from 1000 to 8200 for the Camp. strain and from 2000 to 8600 for the Monterey strain. The development of the parasites was usually asynchronous, but in every study the majority of the parasites were trophozoites. Details of the maintenance of the monkeys were similar to those described by Hickman (*7*). One monkey infected with Monterey strain parasites was splenectomized.

The methods used to measure chloroquine accumulation with preparations of washed erythrocytes also have been described (*3*). The erythrocytes were suspended in media which contained various amounts of chloroquine, and the accumulation of chloroquine by the cells was allowed to proceed for 10 or 20 minutes. At the end of incubation, the cells and media were separated by centrifugation, and the amounts of chloroquine in each were measured radiochemically; chloroquine-3-[^{14}C] (specific activity, 1.71 mc/mmole;

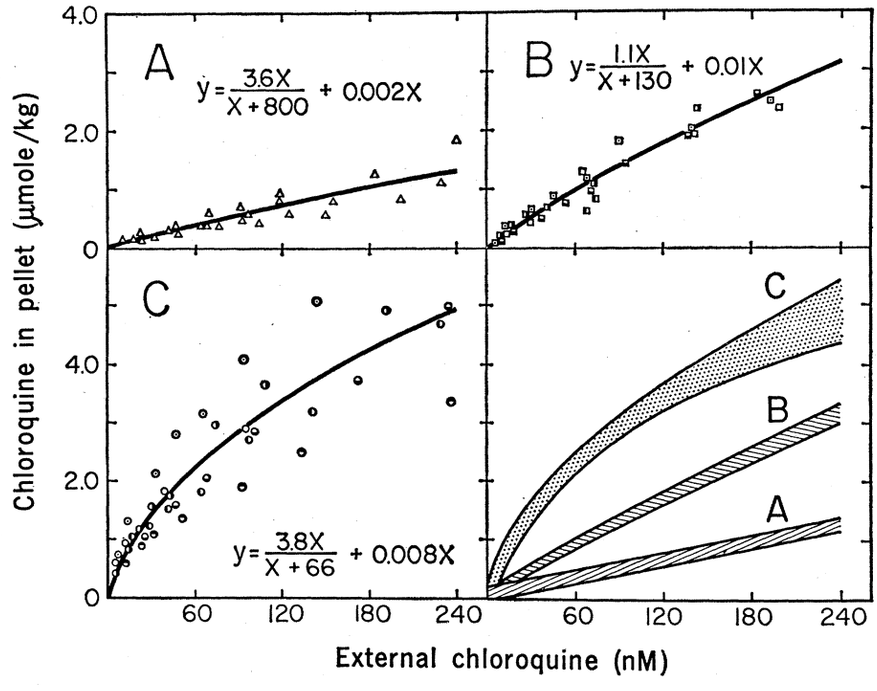

Fig. 1. Chloroquine accumulation by erythrocytes. Five percent suspensions of washed erythrocytes were incubated for 20 minutes under room air at 22°C and *p*H 7.4 in an aqueous medium of the following composition in millimoles per liter: NaCl, 25; KCl, 4.8; $MgSO_4$, 1.2; glucose, 86; and Na_2HPO_4, 50; the *p*H was adjusted with HCl and chloroquine-3-[^{14}C] was added to achieve the desired external concentration. The amount of chloroquine accumulated per kilogram of erythrocyte pellet (wet weight) is shown. Individual values, regression curves, and regression equations are shown in parts (A), (B), and (C); the 95 percent confidence limits of the regression curves (*10*) are shown in the bottom of the figure on the right; (A) 42 points from four uninfected monkeys; (B) 31 points from five monkeys infected with the Monterey strain of *P. falciparum;* (C) 45 points from six monkeys infected with the Camp. strain of *P. falciparum*. Some of the points fell outside the concentration range illustrated and are not shown. Since the degree of parasitemia varied, all of the data in (B) and (C) are corrected to represent 5000 parasites per 10,000 erythrocytes. The different symbols in (B) and (C) represent different monkeys.

New England Nuclear Corporation) was used for this purpose. The amount of chloroquine accumulated by infected erythrocytes after 10 minutes of incubation averaged 89 percent of the values after 20 minutes of incubation, which indicated that steady-state conditions were being approached rapidly; in uninfected erythrocytes the values at 10 minutes averaged 66 percent of the 20-minute values (Fig. 1).

There was considerable variation in the results from different monkeys within a group, particularly from the group infected with the Camp. strain. In infected erythrocytes of one monkey, not included in Fig. 1, the amounts of chloroquine accumulated were at least five times greater than corresponding values of any other monkey similarly infected with the Camp. strain. The variation between monkeys, however, does not hide the fact that erythrocytes infected with the Camp. strain accumulated more chloroquine than did those infected with the Monterey strain. Furthermore, the difference between Camp. and Monterey strains occurred with chloroquine concentrations of 250 nmole/liter or less; such concentrations are commonly found in plasma during chloroquine therapy in man (*8*).

Regression curves (Fig. 1) were fitted by the method of Tyson *et al.* (*9*). With each type of erythrocyte preparation a curvilinear regression equation fitted the data better than did the equation for a straight line, which indicated the presence of a saturable component in the process of accumulation. This saturability supports the hypothesis of chloroquine binding to receptor sites.

To evaluate the binding characteristics of the erythrocyte preparations, the curves in Fig. 1 may be treated as adsorption isotherms plus a linear component. Thus, in a preparation that contains one Camp. strain parasite for every two erythrocytes, the maximum binding capacity is estimated to be 3.8 μmole per kilogram of erythrocyte pellet for a class of receptor sites with an apparent intrinsic association constant of 1.5×10^7 liter/mole. These values are lower than those of the high-affinity drug-receptor sites of *P. berghei* (*3*), and they must be considered rough estimates since steady-state conditions may not have been reached. Nevertheless, the evidence for a class of receptor sites with high affinity for chloroquine is in agreement with results from work with chloroquine-sensitive *P. berghei* (*3*).

High-affinity chloroquine binding was not detected in uninfected erythrocytes from owl monkeys and was deficient, but possibly not absent, in erythrocytes infected with the Monterey strain. Since the Camp. strain of *P. falciparum* is easily eradicated from the owl monkey with chloroquine whereas the Monterey strain is highly resistant to chloroquine therapy, this deficiency of high-affinity drug-receptor sites may be the cause of resistance to chloroquine.

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Chromosome Pairing: Effect of Colchicine on an Isochromosome

Abstract. *Two separable stages in the process of chromosome pairing have been demonstrated. The first results in a close spatial relationship of homologs, and the second results in synapsis and formation of chiasmata. Colchicine reduces chiasma formation in conventional bivalents but not in an isochromosome. Thus, colchicine affects only the first stage of pairing.*

Orderly synapsis of chromosomes at meiosis depends upon an earlier phenomenon which prevents mutual interference of pairs of homologs. Cytological abnormalities arising from excess dosage of the long arm of chromosome 5B ($5B^L$) of hexaploid wheat *Triticum aestivum* were explained by Feldman (*1*) by interference to this earlier component of chromosome pairing. Colchicine, applied soon after the completion of the last premeiotic anaphase of hexaploid wheat, produces an effect similar to that of excess dosage of $5B^L$ (*2*). The conclusion that colchicine affects the earlier component of chromosome pairing and not synapsis and formation of chiasmata per se was based on the comparison of pairing behavior of chromosomes in different cells, namely, hexaploid and dodecaploid cells.

More direct evidence is now presented which differentiates these two stages of chromosome pairing and confirms that colchicine affects only the earlier component. This involved apply-

Table 1. Mean pairing behavior of monoisochromosome $5D^L$ with and without colchicine; 100 cells analyzed in each treatment.

Colchicine	Conventional chromosomes				Isochromosome	
	Ring bivalents (chiasma formed in both arms)	Rod bivalents (chiasma formed in one arm only)	Two univalents (chiasma formed in neither arm)	Chiasma formed per pair of chromosome arms	Chiasma formed	Chiasma not formed
Absent	18.65	1.25	0.10	0.96	0.97	0.03
Present	8.28	1.03	10.69	0.44	0.96	0.04