High-Affinity Accumulation of Chloroquine by Mouse Erythrocytes Infected with Plasmodium berghei

COY D. FITCH, NORMAN G. YUNIS, REKHA CHEVLI, and YOLANDA GONZALEZ

From the Departments of Internal Medicine and Biochemistry, Saint Louis University School of Medicine, Saint Louis, Missouri 63104

Abstract Washed erythrocytes infected with chloroquine-susceptible (CS) or with chloroquine-resistant (CR) P. berghei were used in model systems in vitro to study the accumulation of chloroquine with high affinity. The CS model could achieve distribution ratios (chloroquine in cells: chloroquine in medium) of 100 in the absence of substrate, 200-300 in the presence of 10 mM pyruvate or lactate, and over 600 in the presence of 1 mM glucose or glycerol. In comparable studies of the CR model, the distribution ratios were 100 in the absence of substrate and 300 or less in the presence of glucose or glycerol. The presence of lactate stimulated chloroquine accumulation in the CR model, whereas the presence of pyruvate did not. Lactate production from glucose and glycerol was diminished in the CR model, and ATP concentrations were higher than in the CS model. Cold, iodoacetate, 2,4-dinitrophenol, or decreasing pH inhibited chloroquine accumulation in both models. These findings demonstrate substrate involvement in the accumulation of chloroquine with high affinity.

In studies of the CS model, certain compounds competitively inhibited chloroquine accumulation, while others did not. This finding is attributable to a specific receptor that imposes structural constraints on the process of accumulation. For chloroquine analogues, the position and length of the side chain, the terminal nitrogen atom of the side chain, and the nitrogen atom in the quinoline ring are important determinants of binding to this receptor.

Introduction Erythrocytes infected with malaria parasites exhibit a remarkable capacity for accumulating chloroquine (1-5), amodiaquin (6), quinine (7), dihydroquinine (4), quinacrine (8) and probably other structurally related drugs. With glucose and a pharmacologically reasonable concentration of chloroquine in the bathing medium, 10 nM for example, there may be 6,000 or more nmol of chloroquine/kg mouse erythrocytes infected with chloroquine-susceptible (CS) Plasmodium berghei when steady-state conditions are reached (2). Under similar conditions, owl monkey erythrocytes infected with CS P. falciparum accumulate up to 750 nmol of chloroquine/kg (3). Presumably, this ability to accumulate chloroquine with high affinity is related to the susceptibility of CS parasites to the drug. Erythrocytes infected with chloroquine-resistant (CR) parasites have much less capacity to accumulate chloroquine with high affinity (1-3).

The process responsible for chloroquine accumulation produces a steady-state distribution of chloroquine within minutes and becomes saturated at low concentrations of chloroquine in the medium external to the erythrocytes (2). These findings have been interpreted as evidence of the existence of a drug receptor in infected erythrocytes; the dissociation constant for the interaction between chloroquine and this receptor is estimated to be 10⁻⁸ M (2). In accord with the putative role of drug accumulation in determining the susceptibility of malaria parasites to drugs, the accumulation of chloroquine by erythrocytes infected with CS P. berghei is competitively inhibited by the diverse drugs to which CR P. berghei is cross-resistant (9). Conversely, CR P. berghei is fully susceptible to effective antimalarial drugs that do not competitively inhibit chloroquine accumulation, such as primaquine and dapsone (9). Autoradiographic studies (10) and studies of pigment clumping in P. berghei exposed to chloroquine (11) raise the

Received for publication 1 November 1973 and in revised form 7 January 1974.
The possibility that high-affinity accumulation of chloroquine is a function of the food vacuole of the parasite. Nevertheless, the exact location of the process, as well as many other characteristics of the high-affinity accumulation of chloroquine, remains to be determined. The present work was undertaken to evaluate the effects of temperature, pH, substrates, and certain metabolic inhibitors on the process of chloroquine accumulation, with CS and CR P. berghei in model systems in vitro, and to identify those features of the chloroquine molecule that are important determinants of its participation in this process.

METHODS

Young male white mice of the NL/W strain, obtained from National Laboratory Animal Co, Creve Coeur, Mo., were fed Purina Laboratory Chow (Ralston Purina Co, St. Louis, Mo.) and water ad libitum. They were infected either with the CS, NYU-2 strain of P. berghei or with a newly isolated strain of CR P. berghei when they weighed approximately 20 g. The methods used to maintain these strains of parasites, the characteristics of the infections, and the methods of obtaining and preparing erythrocytes for study have been described previously (2).

The CR strain of P. berghei was isolated by a procedure involving selection of parasites infecting polychromatophilic erythrocytes and exposure of these parasites to chloroquine. The selection of parasites was undertaken because a previous strain of CR P. berghei, derived from the NYU-2 strain, infected polychromatophilic erythrocytes predominantly (1, 2). Hypotonic hemolysis was used to select for the younger, polychromatophilic erythrocytes. Blood from mice heavily infected with the NYU-2 strain of P. berghei was mixed 1:50 with a heparin-containing hypotonic saline solution to achieve a final osmolality of 114 mosmol/kg of water. After being mixed to permit hemolysis, 0.1 ml of the mixture was injected intraperitoneally into each of three mice. During the next 6 days, these three mice developed parasitemia with a relatively heavy infection of polychromatophilic erythrocytes. On the 6th day of infection, blood from one of these mice was mixed 1:12 with 0.9% NaCl solution containing heparin, and 0.1 ml of this mixture was injected intraperitoneally into each of 11 normal mice. Each of these mice was given 1 mg of chloroquine intraperitoneally immediately after being infected and daily thereafter for 3 days. Within 2 wk, 3 of the 11 mice developed heavy parasitemia with the infection limited to polychromatophilic erythrocytes. The blood from these mice was used to infect other chloroquine-treated mice; and after four additional passages through chloroquine-treated mice, this newly isolated strain of CR P. berghei regularly produced heavy parasitemia (approximately 800 parasites per 1,000 erythrocytes) despite treatment of the mice with 40 mg of chloroquine/kg of body weight each day for 14 days. By contrast, the parent strain is susceptible to treatment of infected mice with as little as 1.25 mg of chloroquine/kg of body weight daily for 4 days (12). The course and characteristics of infections with this new strain of CR P. berghei are indistinguishable from those of the CR strain developed by Macomber, O'Brien, and Halm (1) and used previously in studies of chloroquine accumulation (1, 2). The new strain of CR P. berghei had been maintained by regular passage through chloroquine-treated mice at 2-wk intervals for 2 yr before the present work. Chloroquine-treated mice were used only to maintain the CR strain of P. berghei for passage. None of the donors of blood for studies of chloroquine accumulation received chloroquine treatment.

The methods used to study chloroquine accumulation and to test for competitive inhibition of chloroquine accumulation have been described in detail previously (2, 9). Briefly, washed erythrocytes were incubated in a medium containing [3-14C]chloroquine (1.71 mCi/mmol; New England Nuclear, Boston, Mass.) for 1 h, after which the suspensions were centrifuged to obtain supernatant fluid and erythrocyte pellets. The hematocrits of these pellets were 90% or greater; other characteristics of pellets obtained in this way have been reported (2). The amounts of chloroquine in the supernatant fluid (external chloroquine) and in the pellets were determined radiochemically (2). All calculations of chloroquine content of pellets are based on wet weight. No attempt was made to determine directly the concentration of free chloroquine inside the erythrocyte. In the present work, the amount of chloroquine in the pellet is treated as a function of the external chloroquine concentration.

The appropriateness of using a 1-h incubation period to approximate steady-state conditions (2) was experimentally verified for the studies described in this report, except that an individual time-course study was not performed for each of the compounds evaluated for ability to inhibit chloroquine accumulation competitively. Under the experimental conditions used in these studies, the steady-state developed rapidly as previously reported (2), well before the end of 1 h of incubation.

Parasitemia was determined by counting parasites and erythrocytes in Giemsa-stained blood films, and it is expressed throughout this report as the number of parasites per 1,000 erythrocytes. Multiparasitism of infected erythrocytes was common in both types of infection but occurred to a greater extent with CR P. berghei. No corrections for parasite counts were made in expressing the results of this study, but the parasite count is given for each of the experiments because the degree of parasitemia is known to have an effect on the amount of chloroquine accumulated (2). Likewise, no corrections were made for chloroquine accumulation by uninfected erythrocytes. The amount of chloroquine accumulated by normal erythrocytes is relatively small when the external concentration of chloroquine is small (2).

ATP concentrations were measured in a coupled spectrophotometric assay with phosphoglycerate kinase. Lactate production was also measured enzymatically (13).

RESULTS

The effect of temperature on chloroquine accumulation is shown in Fig. 1. Relative to the values obtained at 25°C, the process of chloroquine accumulation is inhibited both at 2°C and 37°C. The inhibition at 2°C is virtually complete. The degree of inhibition at 37°C is small and possibly is related to a fall in pH. The lower pH for erythrocytes infected with CR parasites (see legend to Fig. 1) probably is due to greater lactate produc-
A survey of compounds for ability to stimulate chloroquine accumulation is summarized in Fig. 3. For erythrocytes infected with CS parasites, glucose and glycerol are approximately equal in ability to stimulate chloroquine accumulation; pyruvate and lactate are less effective. In Fig. 3, inosine appears to be less effective than glucose or glycerol, and this was confirmed in other studies, which are not included in this report. Fructose was evaluated and was equivalent to glucose in its ability to stimulate chloroquine accumulation. A similar survey with erythrocytes infected with CR parasites yielded similar results, except that glycerol was not as effective as glucose and that pyruvate was completely ineffective in stimulating chloroquine accumulation. Subsequent experiments, some of which are shown in Figs. 4 and 5, confirmed the failure of pyruvate to stimulate chloroquine accumulation in erythrocytes infected with CR parasites. Whether or not pyruvate can enter these cells is not known, however (15).

Representative examples of studies to evaluate the effect of varying the concentrations of glucose, glycerol,
pyruvate, and lactate are shown in Fig. 4. These studies confirm the superiority of glucose and glycerol in stimulating chloroquine accumulation and reveal that 1-mM concentrations of these substrates are sufficient for maximal or nearly maximal stimulation of erythrocytes infected with CS parasites. Erythrocytes infected with CR parasites are less responsive to added substrate; but of the substrates studied, glucose is the best. Parasite counts are sufficiently close to permit quantitative comparisons between the CS and CR models only for two of the four substrates, glucose and glycerol; in agreement with earlier work (1, 2), erythrocytes infected with CS parasites accumulate more chloroquine than erythrocytes infected with CR parasites. When the difference due to responsiveness to substrate is minimized, as it is at low glucose and glycerol concentrations, the difference between CS and CR parasites with regard to chloroquine accumulation is also minimized. These observations are further substantiated by data recorded in Fig. 5.

Besides showing differences related to the type of parasite and the type of substrate, Fig. 5 demonstrates that effective substrates have a major effect at low concentrations of external chloroquine. Each of the effective substrates increases the maximal capacity to accumulate chloroquine, but there is no evidence that the affinity of the process for chloroquine changes. Because the process responsible for accumulating chloroquine is sensitive to change, and the magnitude of this change was presented in relationship to Fig. 1. Fig. 5 also demonstrates that 1 mM glucose and glycerol and 3 mM pyruvate have no effect on chloroquine accumulation by uninfected erythrocytes.

The effects of 2,4-dinitrophenol and iodoacetate on the accumulation of chloroquine are illustrated in Figs. 6, 7, and 8. Regardless of which substrate is used, both compounds are potent inhibitors of chloroquine accumulation by erythrocytes infected with CS parasites, causing maximal inhibition at 1 mM concentrations and half-maximal inhibition at approximately 0.1 mM concentrations. Less extensive studies were performed with erythrocytes infected with CR parasites; but in this model too, both compounds are potent inhibitors of chloroquine accumulation (Fig. 7). In related experiments, 1 mM ouabain did not inhibit chloroquine accumulation by erythrocytes infected with either strain of parasite.

Fig. 8 shows the effects of 2,4-dinitrophenol and iodoacetate over a range of concentrations of external chloroquine, and demonstrates that these inhibitors are effective at low concentrations of chloroquine. Again the maximum capacity to accumulate chloroquine is

![Figure 3](http://www.jci.org) Effect of possible substrates on chloroquine accumulation by erythrocytes infected with CS *P. berghei*. 5% suspensions of washed, infected erythrocytes were incubated for 1 h at 25°C and pH 7.4 in the medium described for Fig. 1. The concentration of chloroquine at the beginning of incubation was always 215 nM. The various possible substrates were added to achieve 3-mM concentrations at the beginning of incubation, except glucose, which was added to achieve a 1-mM concentration. The distribution ratio was calculated by dividing the number of nanomoles per kilogram of erythrocyte pellet by the concentration of external chloroquine in nanomoles per liter. Each bar represents the average from duplicate determinations. The parasitemia was 1,170.

![Figure 4](http://www.jci.org) Effect of substrate concentration on chloroquine accumulation by erythrocytes infected either with CS or with CR *P. berghei*. 5% suspensions of washed, infected erythrocytes were incubated for 1 h at 25°C and pH 7.4 in the medium described for Fig. 1. The concentration of chloroquine at the beginning of incubation was 150 nM when pyruvate or lactate were substrates for erythrocytes infected with CR parasites and 215 nM for the other studies. The concentrations of different substrates at the beginning of incubation were varied as shown in the figure. Solid circles represent erythrocytes infected with CS parasites and the open circles represent erythrocytes infected with CR parasites. The parasitemias were as follows: pyruvate, CS 1,020 and CR 560; lactate, CS 1,300 and CR 678; glucose, CS 1,048 and CR 1,280; and glycerol, CS 840 and CR 786.

*Chloroquine Resistance and Drug Accumulation in Malaria* 27
affected without evidence that the affinity of the process for chloroquine changes. Both of these inhibitors cause significant inhibition in the presence or absence of added substrate. The inhibition by dinitrophenol is prevented partially by adding glucose but not by adding glycerol. Glycerol partially prevents the inhibition by iodoacetate.

The effects of substrates and inhibitors on ATP concentrations and lactate production are shown in Table I. As expected, glucose and glycerol increase ATP concentrations and lactate production in erythrocytes infected with either strain of P. berghei, although the increase in ATP concentration in response to glycerol is small in erythrocytes infected with CR parasites and may not be significant. Erythrocytes infected with CR parasites have high concentrations of ATP even in the absence of substrate. With either type of parasite, the presence of iodoacetate reduces ATP concentration and inhibits lactate production from glucose and glycerol. Dinitrophenol reduces ATP concentrations in every instance and inhibits lactate production from glycerol.

Figs. 9 and 10 and Table II summarize studies of the structural determinants of chloroquine accumulation. Double reciprocal graphs of the data (16) were used to assess for competitive inhibition of chloroquine accumulation by various analogues. Evidence of competition may be accepted as evidence that an analogue and chloroquine interact at the same site in the process of accumulation. An example of a study showing competitive inhibition is presented in Fig. 9. The apparent inhibitor constant (Ki) can be calculated from the intercept on the baseline of the graph of chloroquine accumulation in the presence of competitive inhibitor. Although subject to some error, the values for Ki permit a ranking of compounds according to the affinity with which they interact in the process of accumulation (9).

An example of a mixed type of inhibition is shown in Fig. 10. In this case, the graphs for inhibited and uninhibited accumulation intersect at a negative value for the reciprocal of external chloroquine rather than at zero. All of the compounds evaluated in the present work...
caused competitive or mixed types of inhibition or no inhibition.

Table II shows the structural formulas for chloroquine and most of the analogues tested. Some of the structural features of chloroquine are a quinoline ring system, a chlorine atom at position 7 of the quinoline ring and a 1-methyl-4-diethylaminobutylamino side chain attached to position 4 of the quinoline ring. One proton will associate with the diethylamino group with an apparent acid dissociation constant, $pK_a$, of 10.16 and another one will associate with the nitrogen atom in the quinoline ring with a $pK_a$ of 8.06 (17). At the physiologic pH of 7.4, therefore, chloroquine exists predominantly in the doubly protonated form.

The values for $K_i$ given in Table II indicate that compounds having bromine, iodine, methoxy, trifluoromethyl, or hydrogen at position 7 of the quinoline ring all cause strong competitive inhibition of chloroquine accumulation. In addition, two compounds with substitutions at position 6 of the quinoline ring cause strong competitive inhibition. Substitutions of other positions on the quinoline ring were not studied in as much detail, but it may be noted that two compounds having a side chain at position 8 rather than position 4, primaquine (9) and pamaquine, caused a mixed rather than a competitive type of inhibition. Similarly, a compound without nitrogen in the ring WR 94797, caused a mixed type of inhibition (Fig. 10).

**Figure 7** Effect of dinitrophenol and iodoacetate on chloroquine accumulation by erythrocytes infected with CR P. berghei. This experiment was identical to the one described in Fig. 6 except that 10-mM concentrations of glucose and glycerol were used and that 2,4-dinitrophenol and iodoacetate were used only at concentrations of 1 mM. The concentration of chloroquine at the beginning of incubation was 150 nM. The open bars indicate that neither substrate nor inhibitor was added to the incubation medium and the striped bars indicate that substrate was present. The type of substrate and the presence and type of inhibitor are given in the figure. Average values from duplicate experiments are shown. The parasitemia was 550.

**Figure 8** Effects of dinitrophenol and iodoacetate on high-affinity accumulation of chloroquine by erythrocytes infected with CS P. berghei. 5% suspensions of washed, infected erythrocytes were incubated for 1 h at 25°C and pH 7.4 in the medium described for Fig. 1. When used, glucose, glycerol, 2,4-dinitrophenol, and iodoacetate were each added to the incubation media to achieve concentrations of 1 mM at the beginning of incubation. Substrate alone, ○; inhibitor alone, ●; substrate plus inhibitor, ◆; neither substrate nor inhibitor, □. The types of substrates and inhibitors are given in the figure. The parasitemias were as follows: glucose and dinitrophenol, 1,120; glucose and iodoacetate, 1,080; glycerol and dinitrophenol, 1,360; and glycerol and iodoacetate, 1,140.
5% suspensions of washed erythrocytes, either uninfected or infected with one of the strains of *P. berghei*, were incubated for 1 h at 25°C and pH 7.4 in the medium described for Fig. 1. The concentrations of iodoacetate and 2,4-dinitrophenol were 1 mM. The concentration of glucose and glycerol were 1 mM for uninfected erythrocytes and CS parasites and 10 mM for CR parasites. At the end of the incubation period the erythrocytes were collected by centrifugation at 2°C for measurement of ATP. The lactate content of the total incubation mixture and the ATP content of the pellet are expressed as micromoles per milliliter of packed erythrocytes; *n* is 4 in each case and means±SE as shown (25). The parasitemias ranged from 640 to 1,300 with a mean value of 942 for CS parasites, and from 600 to 900 with a mean value of 758 for CR parasites.

Strong competitive inhibition was observed despite considerable variation in the side chain. Thus the length of the aliphatic portion between the two nitrogen atoms in the side chain can vary from two to five carbons, and the ethyl groups on the terminal nitrogen atom and the methyl group can be deleted without substantially interfering with the ability of the compound to compete with chloroquine. However, a compound without a terminal nitrogen atom in the side chain, WR 93156, is a relatively ineffective competitor with chloroquine. The apparent *Ki* of this compound calculated from Fig. 9 is 4×10^−8 M. Finally, a compound with six carbons between the two nitrogen atoms in the side chain, WR 7626, does not inhibit chloroquine binding.

### Table 1

**Effects of Substrates and Inhibitors on ATP Concentrations and Lactate Production**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Inhibitor</th>
<th>ATP Uninfected</th>
<th>CS <em>P. berghei</em></th>
<th>CR <em>P. berghei</em></th>
<th>Lactate Uninfected</th>
<th>CS <em>P. berghei</em></th>
<th>CR <em>P. berghei</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>1.35±0.08</td>
<td>0.79±0.04</td>
<td>2.04±0.07</td>
<td>4.9±0.3</td>
<td>3.7±0.4</td>
<td>3.6±0.4</td>
</tr>
<tr>
<td>None</td>
<td>Iodoacetate</td>
<td>1.01±0.11</td>
<td>0.71±0.05</td>
<td>1.82±0.11</td>
<td>4.1±0.2</td>
<td>3.8±0.6</td>
<td>2.8±0.4</td>
</tr>
<tr>
<td>None</td>
<td>2,4-dinitrophenol</td>
<td>0.60±0.03</td>
<td>0.30±0.06</td>
<td>0.53±0.02</td>
<td>4.8±0.9</td>
<td>4.2±0.8</td>
<td>3.2±0.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>None</td>
<td>1.48±0.12</td>
<td>1.34±0.07</td>
<td>2.57±0.14</td>
<td>7.8±0.4</td>
<td>24.9±1.6</td>
<td>36.7±3.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>Iodoacetate</td>
<td>0.17±0.01</td>
<td>0.18±0.03</td>
<td>0.79±0.09</td>
<td>3.9±0.3</td>
<td>8.0±0.7</td>
<td>10.7±1.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>2,4-dinitrophenol</td>
<td>1.06±0.08</td>
<td>0.82±0.04</td>
<td>2.23±0.04</td>
<td>14.7±2.5</td>
<td>21.0±2.7</td>
<td>66.9±5.6</td>
</tr>
<tr>
<td>Glycerol</td>
<td>None</td>
<td>1.28±0.08</td>
<td>1.10±0.06</td>
<td>2.25±0.11</td>
<td>4.9±0.2</td>
<td>9.2±0.9</td>
<td>11.0±1.6</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Iodoacetate</td>
<td>0.96±0.07</td>
<td>0.70±0.06</td>
<td>1.72±0.08</td>
<td>3.9±0.3</td>
<td>4.3±0.4</td>
<td>4.9±0.4</td>
</tr>
<tr>
<td>Glycerol</td>
<td>2,4-dinitrophenol</td>
<td>0.56±0.07</td>
<td>0.37±0.02</td>
<td>0.50±0.02</td>
<td>4.1±0.2</td>
<td>4.0±0.5</td>
<td>5.2±0.3</td>
</tr>
</tbody>
</table>

### Figures

**Figure 9** Competitive inhibition of chloroquine accumulation by erythrocytes infected with CS *P. berghei*, 5% suspensions of washed, infected erythrocytes were incubated at 25°C for 1 h in a medium with the following composition in millimoles per liter: NaCl, 25; KCl, 4.8; MgSO₄, 1.2; glucose 86; and Na₂HPO₄, 50; the pH was adjusted with HCl and [¹⁴C]chloroquine was added to achieve the desired external concentrations. Tween 80 (Atlas Chemical Industries, Inc., Wilmington, Del.) was added to the medium to achieve a concentration of 0.0125% (vol/vol) to help keep insoluble compounds suspended. The initial pH of the incubation mixture was 7.4 and the final pH 7.2. The inhibitor, WR 93156, was added as the hydrochloride salt. Open circles indicate the presence of inhibitor; closed circles represent uninhibited accumulation. The parasitemia was 881.

**Figure 10** Mixed inhibition of chloroquine accumulation by erythrocytes infected with CS *P. berghei*. The details of this study and the meaning of the symbols are the same as those stated for Fig. 9. The inhibitor, WR 94797, was added as the free base. The parasitemia was 928.
### DISCUSSION

**Effects of substrates and inhibitors.** Substrate involvement in the process of chloroquine accumulation with high affinity is demonstrated by the stimulating effects of glucose, glycerol, pyruvate, and lactate and by the inhibiting effects of cold, iodoacetate, and dinitrophenol. Substrate may be necessary either to support active transport of chloroquine or to make available a drug receptor or some other component of the process of chloroquine accumulation. In rhesus monkey erythrocytes infected with *P. knowlesi*, chloroquine accumulation is substrate-dependent (5); but the reason for the dependence is unknown; and it is not certain that the process of accumulation is otherwise similar to that of...
the *P. berghei* model of malaria. Substrate requirements for chloroquine accumulation have not been reported for *P. falciparum* malaria in the owl monkey or in other types of malaria.

With knowledge of the substrate involvement in chloroquine accumulation, it is necessary to ask whether a change in substrate utilization causes drug resistance. CS *P. berghei* converts glucose to lactate by anaerobic glycolysis (18-21), consumes oxygen (18, 19), and presumably conserves energy liberated by electron transport (22), although it does not have a well-defined mitochondrion (23). Apparently it does not have a functional citric acid cycle (24). As far as is known, the metabolism of CR *P. berghei* is similar to that of CS *P. berghei* (24). Nevertheless, the present studies show that erythrocytes infected with CR *P. berghei* have high ATP concentrations. It is possible that this ATP is produced by the immature erythrocytes that host CR parasites and is inaccessible to the parasite. It is also possible that ATP is not the limiting factor for chloroquine accumulation with high affinity in the CR model. Further work is needed to determine whether or not a change in substrate utilization causes chloroquine resistance.

The drug receptor. The process of chloroquine accumulation has a relatively low degree of specificity in comparison to that commonly exhibited by enzymes. It is saturable (2), however, and it is competitively inhibited only by certain analogues of chloroquine. These findings are best explained by the interaction of chloroquine with a structurally defined receptor. They cannot be explained by a nonspecific interaction, such as an electrostatic attraction without structural constraints.

While the nature of the receptor involved in chloroquine accumulation remains obscure, an examination of the structures with which it interacts suggests the following topography: (a) A flat surface large enough to accommodate planar ring systems of 30-40 Å². The existence of this surface would explain the ability of the receptor to interact with a heterogeneous group of compounds, including derivatives of 4-aminoquinoline, of quinoline-4-methanol, of pyridine, of pyrimidine, and of phenanthrene (9). (b) A chemical grouping in the flat surface that favors interaction with compounds having a nitrogen in their ring system, such as the quinoline derivatives. This would account for increased accumulation of chloroquine with increasing pH (Fig. 2). Also, interactions with this grouping would help explain the differing affinities of the receptor for the various compounds with which it interacts (9). Furthermore, the proximity of the side chain to the nitrogen atom in the ring of 8-aminoquinoline derivatives might hinder interaction with this grouping and account for the failure of primaquine and pamaquine to inhibit chloroquine accumulation competitively. And (c) an anionic site located in the proper geometric relationship to the flat surface to attract the protonated terminal nitrogen atom of the side chain. The existence of this site would explain the higher affinity of the receptor for compounds with a terminal nitrogen atom in the side chain, and it might explain the apparent restriction on length of the side chain.

The fact that this receptor accommodates drugs with diverse chemical structures can be exploited in the design of new antimalarial drugs. Unfortunately, however, strong interaction with the receptor by itself does not assure a desirable chemotherapeutic result. On the contrary, when CS *P. berghei* becomes resistant to one of the drugs served by this receptor, it develops resistance to other drugs served by the receptor (9). For this reason, strong interaction with the receptor predicts resistance of CR *P. berghei* to the drug rather than susceptibility. In addition, the ultimate effectiveness of an antimalarial drug is determined not only by the interaction between the drug and the parasite but also by the handling of the drug by the host and by the response of the host to the drug.

**ACKNOWLEDGMENTS**

We are indebted to Dr. L. W. Laughlin for assistance in isolating the CR *P. berghei* and to Dr. L. H. Schmidt of the Southern Research Institute, Dr. A. R. Surrey of the Sterling-Winthrop Institute, and Drs. C. J. Canfield, T. R. Sweeney, B. T. Poon, and B. J. Boone of the Walter Reed Army Institute of Research, Washington, D. C., for supplying the structural analogues of chloroquine used in this work.

This work was supported in part by grant number AI-09973 from the National Institutes of Health and in part by contract number DADA 17-72-C-2008 from the United States Army Medical Research and Development Command. This is contribution number 1164 from the Army Research Program on malaria.

**REFERENCES**


32 C. D. Fitch, N. G. Yunis, R. Checchi, and Y. Gonzalez


