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Antimalarial Activity of Selected Aromatic Chelators

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SUMMARY

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Disulfiram and its physiological reduction product diethyldithiocarbamate have been shown to inhibit the growth of the human malaria parasite *Plasmodium falciparum in vitro*. It has been suggested that activity of these agents against malaria depends on at least two factors: (1) inhibition of metalloprotein oxidases and (2) chelation by agents with favorable lipid/water partition coefficients (lipophilic) and favorable binding constants. The aromatic chelators 8-hydroxyquinoline and 2-mercaptopyridine-*N*-oxide possess these characteristics and also exhibit active antimalarial effects *in vitro* at concentrations to 6.89×10^{-10} and 7.86×10^{-10} M, respectively, the lowest level tested. On the other hand, either the introduction of a methyl group in the number 2 position of 8-hydroxyquinoline or the movement of the hydroxyl group to the 5 position impairs the chelating ability of this compound and, correspondingly, reduces toxicity to the parasite. Approximately 6.28×10^{-6} M 2-methyl-8-hydroxyquinoline and 6.89×10^{-5} M 5-hydroxyquinoline were required to achieve the same inhibition as 6.89×10^{-9} M 8-hydroxyquinoline. This suggests that formation of the ligand–metal complex by these chelating agents is associated with their antimalarial activity. In addition, 8-hydroxyquinoline at a level as low as 6.89×10^{-6} M inhibited parasite glycolysis with no statistically significant effect on the glycolysis of normal red cells. This is similar to effects of disulfiram reported previously.

INTRODUCTION

The malaria parasite satisfies its energy requirements by fermentative reactions. *Plasmodium knowlesi*, a primate malaria parasite infective to humans, degrades glucose to products other than CO_2 and H_2O and excretes organic end products (1, 2). There is no difference in glucose utilization when the parasite is incubated either aerobically or anaerobically, indicating the absence of a classical Pasteur effect. More recently, we reported that the human malaria parasite *Plasmodium falciparum* grown *in vitro* has a low oxygen requirement, growing best at 3% O_2 but tolerating O_2 levels as low as 0.5% without appreciable reduction in multiplication rates. There does appear to be a critical level of O_2 below which the parasite will not survive, in contrast to true anaerobic forms of life (3). These are major points of departure from the metabolism of mammalian cells and suggest oxygen may not play the same role in electron transport as it does in many host cells. Instead, oxygen may act mainly through other metalloprotein oxidase enzymes.

Therefore, we postulated that a potent chelating agent with proven activity against metalloproteins might provide a substance selectively toxic to the parasite. We also hypothesized that the compound must possess a favorable lipid/water partition coefficient to penetrate both the erythrocyte and the intracellular malaria parasite and a high enough binding constant to compete with naturally occurring chelators in the cell. Disulfiram (tetraethylthiuram disulfide, Antabuse), a safe drug presently used in aversion therapy of alcoholism, and its physiological reduction product, diethyldithiocarbamate, are compounds of this type. These compounds exhibited active antimalarial effects *in vitro* at concentrations to 6.46×10^{-7} and 4.4×10^{-7} M, respectively, the lowest level tested (4). On this basis, if chelation is responsible for the antimalarial activity of this drug, one would expect other lipophilic chelators with the same characteristics also to inhibit the growth of *P. falciparum in vitro* to a similar degree.

8-Hydroxyquinoline (oxine), an agent of this type, possesses an affinity for metallic cations due to the presence of an ionizable hydroxyl group peri to a tertiary heterocyclic nitrogen atom (5). It is highly lipophilic (6), has a high stability constant (5, 7), and is also inhibitory to metalloprotein oxidases (8). A second aromatic chelator used in this study is 2-mercaptopyridine-*N*-oxide (PNO). The metal is gripped in a five-membered ring similar to oxine but held between an oxygen and sulfur atom instead of between an oxygen and nitrogen (9). The mode of action appears similar to that of oxine (10, 11, 12).

MATERIALS AND METHODS

P. falciparum, strain FCR-4/6252 knobless (13), was grown *in vitro* in 6% red cell suspensions. The petri dish–

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candle jar method, described by Jensen and Trager (14), was used. Incubations in reduced oxygen tension were done in a modular incubator chamber (Billups Rothenberg, Del Mar, Calif.). Type A red cells and sera were used throughout the study. Four petri dishes of parasites were grown at each drug concentration unless otherwise specified. The media and parasitized erythrocytes were dispensed into petri dishes and parasites were allowed to multiply in the absence of inhibitor for 24 h. Smears were made of all cultures daily and stained with Giemsa's stain. A minimum of 1000 red blood cells was counted on each slide and growth was expressed each day in percentage red cells infected with *P. falciparum*. Initial parasitemia was determined by counting parasites in 40,000 red cells. Parasites were actively growing throughout the study. The chelator to be tested was dissolved in dimethyl sulfoxide (DMSO), certified spectranalyzed (Fisher Chemical Co.), to give a concentration of 80 mg/ml. Fifty microliters of this solution was added to 40 ml of medium. Controls contained equal quantities of DMSO only. Stock solutions were filter sterilized and appropriately diluted with sterile medium to achieve desired concentrations of drug. All chelators used in this study were recrystallized reagents, purchased from Aldrich Chemical Co.

For the determination of glucose, lactate, and protein, perchloric acid was added to the cultures to a final concentration of 3%. The protein was removed by centrifugation at 27,000*g* for 15 min and was determined in the pellet by the procedure of Lowry *et al.* (15). The supernatant was neutralized with KOH. Lactate was quantified enzymatically by a modification of the method of Lowry *et al.* (16). Lactate dehydrogenase (Worthington, Inc.) used in this assay was diluted with 0.9% NaCl rather than albumin and the buffer used was 2-amino-4-methyl-1-propanol, pH 9.7. Glucose was assayed spectrophotometrically at 340 nm by a modified method of Slein (17). The reaction mixture contained 40 μmol glycyl glycine buffer (pH 7.4), 0.8 μmol Mg Cl_2, 3.75 μmol adenosine triphosphate (ATP) and 0.3 μmol $NADP^+$, 0.56 unit of hexokinase, and 0.28 unit of glucose-6-phosphate dehydrogenase (Boehringer and Soehne) in 0.05 M glycyl glycine buffer (pH 7.4). The total volume was 1.0 ml.

RESULTS

Growth at different concentrations of oxine and PNO. Typical growth effects of *P. falciparum* in oxine (Fig. 1) or PNO (Fig. 2) at concentrations of 7.86×10^{-4} to 6.89×10^{-10} M are shown in Tables 1 and 2. Student's *t* test at the 0.05 level shows that there is no statistically significant multiplication of the parasites as early as 24 h after the addition of 6.89×10^{-4} and 6.89×10^{-5} M oxine and 7.86×10^{-4}, 7.86×10^{-5}, and 7.86×10^{-6} M PNO. This suggests a rapid onset of inhibition by these compounds. Within 48 h (third day of the experiment), 6.89×10^{-7} M concentrations of oxine inhibit growth by 47% (Table 1) and 6.89×10^{-8} M inhibit 67%. The difference in inhibition at these two concentrations is not significant from experiment to experiment. Concentrations of 7.86×10^{-9} M PNO result in approximately 53% inhibition in 48 h (Table 2). Incubations of *P. falciparum* for 72 h in

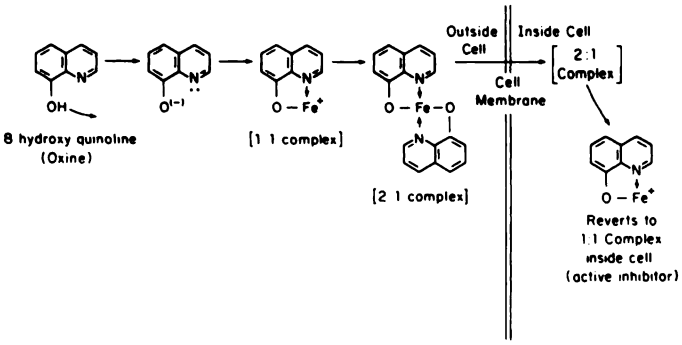

FIG. 1. *Proposed mechanism of entry of oxine into cell*

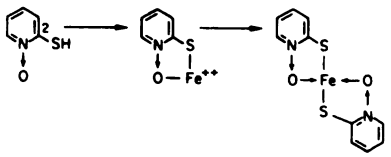

FIG. 2. *2-Mercaptopyridine-N-oxide*

6.89×10^{-9} M oxine result in 45% inhibition, and in 7.86×10^{-10} M PNO result in 55% inhibition.

The sensitivity of *P. falciparum*, strain FCR-4/6252 (knobless) or FCR3/FMG (Gambian strain), to oxine or tetraethylthiuram disulfide is not changed by the addition of 100 mg/100 ml reduced glutathione to the medium or incubation in reduced oxygen tension (O_2 = 2.58%;

TABLE 1

Growth of P. falciparum in 8-hydroxyquinoline (oxine)

Parasites were grown for 24 h (1 day) in 1.5-ml petri dishes in a candle jar before exposing them to oxine.

	% Parasitemia[a] at		
	2 days	3 days	4 days
Oxine, 100 μg/ml (6.89×10^{-4} M)	0.5[b] (0.4–0.7)	0.3[b] (0.2–0.4)	0
DMSO control	2.0 (1.5–2.4)	3.6 (3.1–4.1)	4.9 (4.2–5.6)
Oxine, 10 μg/ml (6.89×10^{-5} M)	0.6[c] (0.5–0.9)	0.3[c] (0.1–0.5)	0
DMSO control	2.5 (1.8–2.8)	4.5 (3.5–5.1)	7.7 (7.4–8.1)
Oxine, 1 μg/ml (6.89×10^{-6} M)	0.9[d] (0.6–1.1)	0.5[c] (0.2–0.6)	0
DMSO control	2.4 (2.2–2.8)	3.9 (3.0–4.7)	5.7 (4.8–6.5)
Oxine, 0.1 μg/ml (6.89×10^{-7} M)	1.6[d] (1.2–1.9)	1.8[d] (1.3–2.4)	0.7[b] (0.4–1.2)
DMSO control	1.9 (1.0–2.6)	3.4 (3.2–3.5)	6.2 (3.5–8.9)
Oxine, 0.01 μg/ml (6.89×10^{-8} M)	1.4[d] (1.1–1.7)	1.5[d] (1.3–1.7)	0.7[c] (0.5–0.8)
DMSO control	2.6 (2.0–3.0)	4.6 (3.6–5.9)	5.1 (4.1–6.6)
Oxine, 0.0001 μg/ml (6.89×10^{-9} M)	2.4 (2.1–2.7)	3.0 (1.5–3.7)	3.2 (2.1–4.4)
DMSO control	2.2 (2.0–2.5)	4.1 (3.8–4.7)	5.8 (4.2–7.3)
Oxine, 0.0001 μg/ml (6.89×10^{-10} M)	2.1 (1.6–2.7)	3.8 (3.3–4.5)	4.6 (3.9–6.2)
DMSO control	2.3 (2.1–2.6)	3.8 (3.1–5.0)	7.0 (5.2–8.4)

[a] % Parasitemia is expressed as infected erythrocytes per 100 erythrocytes. The initial parasitemia was 0.40% (±0.03% SD). The average and range (in parentheses) are given from counts on four dishes. This is the result of one of four similar experiments.

[b] Essentially all dead.

[c] Many abnormal.

[d] Few abnormal.

TABLE 2

Growth of P. falciparum in 2-mercaptopyridine-N-oxide (PNO)

Parasites were grown for 24 h (1 day) in 1.5-ml petri dishes in a candle jar before exposing them to PNO.

	% Parasitemia[a] at		
	2 days	3 days	4 days
PNO, 100 μg/ml (7.86×10^{-4} M)	0.5[b] (0.4–0.5)	0	0
DMSO control	2.0 (1.5–2.4)	3.6 (3.1–4.1)	4.9 (4.2–5.6)
PNO, 10 μg/ml (7.86×10^{-5} M)	0.4[b] (0.2–0.7)	0.1[b] (0–0.1)	0
DMSO control	2.5 (1.8–2.8)	4.5 (3.5–5.1)	7.7 (7.4–8.1)
PNO, 1 μg/ml (7.86×10^{-6} M)	0.5[c] (0.4–0.6)	0.2[b] (0.1–0.3)	0
DMSO control	2.4 (2.2–2.8)	3.9 (3.0–4.7)	5.7 (4.8–6.5)
PNO, 0.1 μg/ml (7.86×10^{-7} M)	1.2[c] (0.9–1.8)	1.0[c] (0.8–1.3)	0
DMSO control	1.9 (1.0–2.6)	3.4 (3.2–3.5)	6.2 (3.5–8.9)
PNO, 0.01 μg/ml (7.86×10^{-8} M)	1.5[d] (1.2–1.8)	1.2[c] (0.5–1.8)	0.7[c] (0.4–0.9)
DMSO control	2.6 (2.0–3.0)	4.6 (3.6–5.9)	5.1 (4.1–6.6)
PNO, 0.001 μg/ml (7.86×10^{-9} M)	2.1 (1.8–2.7)	1.9[d] (1.4–2.3)	2.4[d] (1.4–3.4)
DMSO control	2.2 (2.0–2.5)	4.1 (3.8–4.7)	5.8 (4.2–7.3)
PNO, 0.0001 μg/ml (7.86×10^{-10} M)	2.1 (1.7–2.5)	3.0[d] (2.0–3.6)	3.1[d] (2.2–4.7)
DMSO control	2.3 (2.1–2.6)	3.8 (3.1–5.0)	7.0 (5.2–8.4)

[a] % Parasitemia is expressed as infected erythrocytes per 100 erythrocytes. The initial parasitemia was 0.40% (±0.03% SD). The average and range (in parentheses) are given from counts on four dishes. This is the result of one of four similar experiments.
[b] Essentially all dead.
[c] Many abnormal.
[d] Few abnormal.

CO_2 = 1.74%). Growth rates in DMSO controls are the same as those seen in the absence of DMSO.

Growth at different concentrations of 2 methyl-8-quinolinol (2-methyl oxine). 2-Methyl oxine (7) binds all cations less strongly than does oxine, but the difference is greatest for those divalent cations which have the smallest crystal radius (7). This is presumably due to steric hindrance (18), although other factors may also contribute (19). The results of growth of the parasite in 2-methyl oxine are given in Table 3. In the first 24 h of contact with the compound, inhibition of *P. falciparum* took place only at the 6.28×10^{-4} M dose (the decrease in multiplication at 6.28×10^{-5} M is not statistically significant). On the last day of the experiment there was a 67% inhibition at 6.28×10^{-6} M and no inhibition resulted at 6.28×10^{-7} M 2-methyl oxine.

Growth at different concentrations of 5-hydroxyquinoline. Of the seven isomeric monohydroxyquinolines, only oxine (8-hydroxyquinoline) is capable of forming nonionized complexes with divalent metallic ions through chelation (20) (Fig. 1). Multiplication of *P. falciparum in vitro* in the presence of 5-hydroxyquinoline is seen in Table 4. There is no statistically significant inhibition in growth of the parasite in the culture system within the first 24 h of addition of the compound. At the completion of the study, only the 6.89×10^{-4} M concentration inhibited growth completely and the 6.89×10^{-5} M dose inhibited growth 50%.

TABLE 3

Growth of P. falciparum in 2-methyl-8-quinolinol

Parasites were grown for 24 h (1 day) in 1.5-ml petri dishes in a candle jar before exposure to 2-methyl oxine.

	% Parasitemia[a] at		
	2 days	3 days	4 days
2-CH_3 "oxine," 100 μg/ml (6.28×10^{-4} M)	1.4[b] (1.1–1.9)	0.9[b] (0.5–1.1)	1.0[b] (0.6–1.2)
DMSO control	1.9 (1.5–2.2)	4.4 (4.2–4.6)	6.7 (6.6–6.8)
2-CH_3 "oxine," 10 μg/ml (6.28×10^{-5} M)	1.6[d] (1.3–1.9)	1.0[b] (0.7–1.2)	1.5[b] (1.3–1.9)
DMSO control	2.2 (1.7–3.0)	4.8 (4.1–5.4)	7.3 (6.3–8.3)
2-CH_3 "oxine," 1 μg/ml (6.28×10^{-6} M)	1.8 (1.4–2.1)	4.4 (3.0–5.7)	2.5[c] (1.4–3.8)
DMSO control	2.2 (1.8–2.5)	6.2 (4.0–7.9)	7.7 (7.0–8.5)
2-CH_3 "oxine," 0.1 μg/ml (6.28×10^{-7} M)	1.6 (1.1–2.3)	7.4 (6.2–8.9)	7.3 (6.6–7.7)
DMSO control	2.1 (1.8–2.8)	5.9 (4.8–7.2)	6.9 (6.5–7.3)

[a] % Parasitemia is expressed as infected erythrocytes per 100 erythrocytes. The initial parasitemia was 1.2% (±0.06% SD). The average and range (in parentheses) are given from counts on four dishes. This is the result of one of three similar experiments.
[b] Essentially all dead.
[c] Many abnormal.
[d] Few abnormal.

Effect of oxine on glycolysis of P. falciparum infected and normal erythrocytes. Evidence has been reported that oxine and the thiocarbamates are toxic to fungi by inhibiting the paired mercapto groups of the lipoic acid/

TABLE 4

Growth of P. falciparum in 5-hydroxyquinoline

Parasites were grown for 24 h (1 day) in 1.5-ml petri dishes in a candle jar before exposure to 5-OH-quinoline.

	% Parasitemia[a] at		
	2 days	3 days	4 days
5-OH-quinoline, 100 μg/ml (6.89×10^{-4} M)	1.8[c] (1.5–2.1)	1.0[b] (0.6–1.3)	0.1[b] (0–0.4)
DMSO control	1.9 (1.5–2.2)	4.4 (4.2–4.6)	6.7 (6.6–6.8)
5-OH-quinoline, 10 μg/ml (6.89×10^{-5} M)	2.2 (2.0–2.4)	4.0 (2.5–5.4)	3.6 (2.1–5.2)
DMSO control	2.2 (1.7–3.0)	4.8 (4.1–5.4)	7.3 (6.3–8.3)
5-OH-quinoline, 1 μg/ml (6.89×10^{-6} M)	2.4 (2.3–2.4)	6.7 (6.1–7.3)	8.7 (8.2–9.2)
DMSO control	2.2 (1.8–2.5)	6.2 (4.0–7.9)	7.7 (7.0–8.5)
5-OH-quinoline, 0.1 μg/ml (6.89×10^{-7} M)	2.2 (1.9–2.5)	7.9 (7.7–8.0)	8.9 (8.5–9.2)
DMSO control	2.1 (1.8–2.8)	5.9 (4.8–7.2)	6.9 (6.5–7.3)

[a] % Parasitemia is expressed as infected erythrocytes per 100 erythrocytes. The initial parasitemia was 1.2% (±0.06% SD). The average and range (in parentheses) are given from counts on four dishes. This is the result of one of three similar experiments.
[b] Essentially all dead.
[c] Few abnormal.

TABLE 5

Effects of oxine on glucose utilization and the formation of lactate in infected red cells by P. falciparum

Parasites were grown in 4-ml petri dishes to a 10% parasitemia. Slides were made to assess parasitemia. Oxine was added at specified concentrations, each accompanied by a dimethyl sulfoxide (DMSO) control. Zero-time dishes were immediately harvested. Growth in the remaining dishes was allowed to continue for 6 h, and the supernatant removed for analysis. Fresh media containing oxine or DMSO were replaced and parasites allowed to grow an additional 16 h. Slides were made on all petri dishes to assess growth and morphology. All reactions were stopped by the addition of perchloric acid to a final concentration of 3%. This is the result of one of two similar experiments.

Incubation	Concentration oxine	Glucose utilized		Lactate produced	
		Infected RBCs		Infected RBCs	
		μmol/mg protein	% Inhibition	μmol/mg protein	% Inhibition
6 h	100 μg/ml (6.89 × 10^{-4} M)	0.243	1.5	0.151	34.2
	DMSO control	0.246		0.229	
	10 μg/ml (6.89 × 10^{-5} M)	0.234	6.4	0.122	39.3
	DMSO control	0.250		0.202	
	1 μg/ml (6.89 × 10^{-6} M)	0.244	1.9	0.192	2.4
	DMSO control	0.248		0.197	
Add'l. 16 h	100 μg/ml (6.89 × 10^{-4} M)	0.028	93.9	0.211	77.7
	DMSO control	0.458		0.949	
	10 μg/ml (6.89 × 10^{-5} M)	0.061	86.4	0.173	81.1
	DMSO control	0.450		0.916	
	1 μg/ml (6.89 × 10^{-6} M)	0.237	51.9	0.412	57.0
	DMSO control	0.492		0.958	

dehydrogenase system (7, 12, 21). Lipoic acid is the essential coenzyme for the oxidative decarboxylation of pyruvic acid. Therefore, assessment of glycolysis by measuring glucose utilization and lactate production in parasitized and normal erythrocytes was done in oxine and DMSO controls (Table 5). Examination of slides made at the start of incubation with the drug and 22 h later, at termination, showed no change in parasite numbers. There was an increase in morphologically abnormal parasites in the presence of oxine, most marked at the 6.89 × 10^{-4} M dose (90% abnormal), with 82% abnormal at 6.89 × 10^{-5} M and 69% abnormal at 6.89 × 10^{-6} M. In confirmity with this finding, there was a 94% inhibition of glucose utilization and a 78% inhibition of lactate production over the last 16 h at 6.89 × 10^{-4} M. At 6.89 × 10^{-5} M there was an 86% inhibition of glucose utilization and an 81% inhibition of lactate production. The 6.89 × 10^{-6} M dose of oxine produced correspondingly less inhibition over the last 16 h of incubation in this experiment (i.e., 52% inhibition of glucose uptake and 57% inhibition of lactate production). In a second experiment there was 96% inhibition of glucose utilization and 92% inhibition of lactate production at a concentration of 6.89 × 10^{-6} M oxine during the final 16 h of incubation. In general, the inhibition is proportional to the amount of chelator added to the system. In contrast to the results seen in the last 16 h, the inhibition of glucose utilization during the first 6 h was low. This was similar to the results seen in the disulfiram study (4). On the other hand, there was a 34% inhibition in lactate production at 6.89 × 10^{-4} M, 39% inhibition at 6.89 × 10^{-5} M, and insignificant inhibition at 6.89 × 10^{-6} M in the first 6 h. This inhibition of glycolysis appeared to be directly proportional to the concentration of compound used.

It is interesting to note, however, that oxine at 6.89 × 10^{-4}, 6.89 × 10^{-5}, or 6.89 × 10^{-6} M has no statistically significant effect on the glycolysis of uninfected erythrocytes. The possible exception to this is at the 6.89 × 10^{-4} M oxine concentration in the last 16 h. Under these conditions there was a 25% reduction in lactate production in two trials out of three. Uninfected erythrocytes, over the first 6 h, used glucose at an average rate of approximately 0.093 μmol/mg protein and produced lactate at 0.042 μmol/mg protein. In the final 16 h, they utilized 0.081 μmol glucose/mg protein and produced about 0.114 μmol/mg protein of lactate. This was found in three experiments. The results agree in general with those obtained in the disulfiram study (4). The ratio of glucose utilized to lactate produced in the DMSO controls decreased considerably over the last 16 h of incubation, approaching 1:2. These findings were obtained in similar controls of a study on the effects of disulfiram on glycolysis in *P. falciparum* (4) and may be due to decreasing levels of glucose in the longer incubation times. Our studies showed that free *P. knowlesi* approached a 1:2 ratio when incubated in low levels of glucose (1). As substrate concentrations were increased, the ratio of glucose:lactate approached 1:0.3, suggesting that increasing the concentration of glucose may facilitate the use of ancillary pathways not involving lactate.

DISCUSSION

The effect of chelators is related to a direct interaction with the enzyme and to the rate of entrance into the cell (7). These agents (dialkyl dithiocarbamate, 8-hydroxyquinoline, and PNO) (12) are capable of forming a polar bidentate ligand at a 1:1 ratio with a divalent cation (Fig. 1). This polar 1:1 complex enters the cell poorly but once it is inside, it has the greatest potential to destroy vital cell components. As the concentration of chelator is raised, a lipophilic 2:1 complex of chelator to metal ion is formed, which is less polar and can enter the cell more readily. In the case of oxine, as the concentration of chelator is raised further, an insoluble 3:1 complex is formed which is inactive. According to Albert the 2:1 complex enters the cell and in turn breaks down to a 1:1 complex (chelator:metal) and free chelator which inhibits lipoic acid-mediated metabolic decarboxylation of pyruvate (7). "Concentration quenching," i.e., the decrease in biological activity (in terms of growth and end product formation) as the concentration of chelator is increased, is cited in support of this hypothesis (7).

In this study it was found that, as disulfiram, oxine at low concentrations rapidly inhibits the growth and multiplication of *P. falciparum in vitro*. A similar compound, 2-mercaptopyridine-*N*-oxide, appears even more potent

against the malaria parasite. This is in agreement with studies by other investigators on bacterial systems (9).

The sterically hindered 2-methyl oxine (24) produced complete growth inhibition only at 6.28×10^{-4} and 6.28×10^{-5} M in 72 h. At 6.28×10^{-6} M, inhibition amounted to 67%. This compound is significantly less potent than the unsubstituted 8-hydroxyquinoline, which inhibited growth of the parasite to an extent of 45% at 6.89×10^{-9} M and 86% at 6.89×10^{-8} M, in 72 h. These results suggest the operation of factors which reduce chelation and potency. Oxine chelates divalent cations by virtue of the location of the hydroxyl group in relation to the basic ring nitrogen. The hydroxyl dissociates as an anion. The nitrogen in turn supplies two electrons, forming a coordinate bond between metal and oxine. In this way the five-membered ring is closed (23, 25). Presumably any factors that interfere with availability of electrons from the nitrogen atom or with ionization of the hydroxyl group will interfere with chelation. Moving the hydroxyl from position 8 to position 5 effectively eliminates the ability of oxine to chelate. Such a change greatly reduced antimalarial effects, supporting the hypothesis that antimalarial activity correlates with chelation. The inhibition produced by high concentrations of the 5-hydroxyquinoline is probably a result of other mechanisms.

The 8-hydroxyquinoline, like disulfiram, rapidly inhibits glycolysis at low concentrations. In contrast to the results obtained in the disulfiram study, however, the inhibition of glycolysis by oxine is directly proportional to the concentration of inhibitor. There is no evidence of concentration quenching. In addition, the inhibition of growth by all compounds tested is, in general, directly proportional to the concentration. It was concluded, therefore, that the "biphasic response" on glycolysis by disulfiram in cultures of *P. falciparum* may result from other biochemical mechanisms not related to antimalarial activity.

It remains to be seen whether oxine is exhibiting its pharmacologic effect through the oxidation of sulfhydryl groups of glycolytic enzymes or is affecting some vital biosynthetic metalloprotein oxidase such as the activity of phenol oxidase in *Schistosoma mansoni* (26). In this homolactate fermentor, small amounts of oxygen are required for tanning eggshells by oxidation of phenolic compounds. It was also shown that oxine inhibits a wide variety of reactions, in addition to oxidase enzymes. Recently it was found to inhibit ribonucleic acid synthesis in yeast (27). In any event, oxine inhibits glycolysis and this inhibition appears to be selective for infected erythrocytes. This specificity is also shared by disulfaram, even though both the malaria parasite and the erythrocyte use the Embden-Myerhof scheme to degrade carbohydrate. Pyruvate oxidase itself is a metalloenzyme (7) of glycolysis and products of pyruvate oxidation in the malaria parasite are known to differ from those produced by mammalian tissue (1, 2). Inhibition at this site may partly explain the selective mechanism of action of these compounds, but this does not exclude other sites of action.

The quinoline nucleus is present in several effective antimalarials (i.e., 4-aminoquinoline and 8-aminoquinoline), amoebicides, and topical antibacterial/antifungal agents (i.e., Vioform). However, unsubstituted 8-hydroxyquinoline has been shown to be diabetogenic at doses of 50 mg/kg in rabbits (28), presumably because it is reacting with zinc in islet cells (29). This study does provide a rationale for the development of new antimalarials, since antimalarial effects appear to correlate with the ability of these compounds to chelate metals. In this group of heterocyclic compounds the metal binding effect is related to a hydroxyl group *ana* to a ring nitrogen (23, 30). The metal forms a new ring by linking simultaneously with a phenolic oxygen (through a primary valency) and with nitrogen (by coordination) (23). With these properties in mind, agents of this type can now be reinvestigated for selective toxicity to plasmodia. 2-Mercaptopyridine-*N*-oxide (9) and the 5-methyl derivative of oxine (23) are reported to have low toxicity for mammals. These compounds, in addition to other 5-substituted oxines, chelate metal ions well and are known to be markedly bactericidal (31). In addition, other promising possibilities are 5:6 benzooxine, 6-hydroxy-*m*-phenanthroline, and, to a lesser extent, 4-hydroxyacridines and 1-hydroxyphenazine (23, 30). Attention to these properties of molecular architecture may prove fruitful in future chemotherapeutic approaches to malaria, still the world's most severe health problem.

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