Malaria: The Effect of Iron and Chloroquine on the Erythrocytic Forms of *Plasmodium berghei*[1,2] (36240)

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The effect of iron on the growth and multiplication of the malarial parasite *in vivo* and the effect of chloroquine on the action of iron have not been previously reported. Extensive evidence, however, has been presented to suggest that chloroquine inhibits the replication process of the plasmodia (1, 2) or that it has a more direct action on the vesicles of the parasite (3, 4). It has also been reported that chloroquine inhibits enzymes to prevent the formation of intermediates for the plasmodia (5–7).

This paper presents evidence that iron protects against the growth and multiplication of the erythrocytic forms of the malarial parasite and that chloroquine stimulates the protective action of iron.

Materials and Methods. White mice,[3] Walter Reed strain, weighing approximately 25 g were employed. Radioactive iron-59 citrate was purchased from the New England Nuclear Corp.; Imferon-R (iron dextran injections) from the Lakeside Labs, Milwaukee; and the low iron diet from the Nutritional Biochemicals Corp.

The chloroquine-sensitive strain of *Plasmodium berghei* was cultured *in vivo* as previously described (8).

Iron and chloroquine base were administered separately as Imferon-R and chloroquine hydrochloride, respectively, by the intraperitoneal route. It should be noted that the effect of iron and chloroquine is lost when these two substances are mixed before injecting into the animals.[4] This phenomenon was previously observed with chloroquine and hematin against *Plasmodium berghei* (9, 10). For radioactive counting purposes, the blood was collected from the tail vein of the mouse into heparinized glass microcapillary tubes and centrifuged for 3 min. The plasma was removed by suction with a small Intramedic-R polyethylene tubing and syringe and the packed cells were washed once with isotonic saline solution in the same tubes.[4] The washed packed cells were placed into counting vials and the radioactivity was determined in a Nuclear Chicago Gamma Counter, Model 4233.

Thin blood smears were prepared at intervals from the tail vein of the mouse and stained with Giemsa staining solution after fixation with anhydrous methanol for 3 min.

Results and Discussion. The results for the *in vivo* effect of iron on the chloroquine-sensitive *Plasmodium berghei* under different conditions are presented in Tables I and II. The mice in Table I received relatively high-

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[2] The opinions and conclusions expressed herein are solely those of the author and do not necessarily reflect the view of the Department of Army or of any other agency of the Federal Government and should not imply Department of Army endorsement of or preference for the commercial products described.

[3] In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Science–National Research Council.

[4] Siu, P. M. L., and Forrester, L. J., unpublished data.

TABLE I. The *in Vivo* Effect of Iron on a Chloroquine-Sensitive *Plasmodium berghei*.[a]

	Average parasitemia (day)[b]					
	35		36		37	
Group	Mean	SD	Mean	SD	Mean	SD
A	2.0817	0.53577	2.6641	0.04393	3.1657	0.46135
B	1.8129	0.08514	2.4855	0.10618	2.9842	0.07684
C	1.6690	0.26174	2.1509	0.18629	2.6620	0.21747
D	2.1330	0.23033	2.6816	0.21686	3.1251	0.08799
E	0.9684	0.65183	1.2353	0.87360	1.7594	1.18619

[a] The experimental conditions are as follows: The experiment was started with day-1 when the mice were placed on a low iron diet and was terminated when one mouse expired in any one of the groups. Group A, mice kept on a low iron diet and distilled water; Group B, on normal chow and tapwater; Group C, on normal chow, tapwater, and iron; Group D, on normal chow, tapwater, and chloroquine; and Group E, on normal chow, tapwater, iron, and chloroquine. Each mouse (4 per group) was infected with 2×10^4 parasites by ip on the 29th day; the chloroquine base (40 mg/kg body wt) was administered on the 8th, 15th, and the 22nd day; and the iron (200 mg/kg body wt) on the 8th, 16th, 20th, 23rd, 27th, and the 30th day.

[b] The mean values with their standard deviations (SD) are expressed in logarithm to the base 10 and are derived from the sum of the logarithm-transformed values of the parasitemia of the individual mouse in each group divided by the number of mice in the group.

er, but noncurative, doses of chloroquine and the injections of chloroquine hydrochloride were discontinued after infection with the plasmodia. It is seen in Table I that the average parasitemia in the mice with iron alone (Group C) is approximately 3.2 times less than the mice kept on a low iron diet (Group A), *i.e.*, the antilogarithm of 0.5037 or 3.1657 minus 2.6620. Chloroquine alone (Group D) appears to have little or no effect on the average parasitemia probably due to the rapid decrease in the chloroquine concentration in the blood at the time of infection and thereafter. In this regard, Macomber *et al.* (11) had reported a rapid decrease in the concentration of chloroquine in the red blood cells of mice 4 hr after the injection of the radioactive chloroquine. When iron was administered to mice also given chloroquine (Group E), however, the average parasitemia on the 37th day is approximately 25 times less than the mice kept on a low iron diet (Group A), *i.e.*, the antilogarithm of 1.4063 or 3.1657 minus 1.7594. The stimulation of the protective effect of iron by chloroquine, therefore, is similarly calculated to be about 8 times greater than with iron alone (Group C).

The analysis of variance of the data in Table I gave $p < .001$ for comparisons of the combined group of A and B vs. the combined group of C, D, and E; the combined group of C and E vs. Group D; and Group C vs. Group E. The comparison for Group A versus Group B, however, gave $p > .05$ and, therefore, the difference between these two groups is considered not significant.

Table II presents similar results except that, under these conditions, chloroquine appears to have some protective effect on the animal. The iron and chloroquine injections were continued after the infection to maintain their levels in the blood. It is seen that on day 24 the average parasitemia in mice treated with iron alone (Group C) is approximately 6 times less than the mice kept on a low iron diet (Group A), *i.e.*, the antilogarithm of 0.7409 or 3.1785 minus 2.4376. The mice with iron and chloroquine injections (Group E), however, has an average parasitemia of approximately 19 times less than the mice kept on low iron diet (Group A), *i.e.*, the antilogarithm of 1.2802 or 3.1785 minus 1.8983. The increase in the protective effect of iron under these conditions by chloroquine is calculated to be approximately 3 times greater than with iron alone (Group C).

TABLE II. The *in Vivo* Effect of Iron on a Chloroquine-Sensitive *Plasmodium berghei.*[a]

	Average parasitemia (day)[b]					
	22		23		24	
Group	Mean	SD	Mean	SD	Mean	SD
A	2.0098	0.20888	2.6632	0.20218	3.1785	0.18805
B	1.8115	0.11759	2.4716	0.04283	3.0212	0.09030
C	1.3759	0.13748	2.0094	0.17035	2.4376	0.08463
D	1.0441	0.31864	1.7410	0.24586	2.4616	0.17005
E	0.7412	0.52703	1.4514	0.67904	1.8983	0.85436

[a] The experimental conditions are as follows: The experiment was started with day-1 when the mice were placed on a low iron diet and was terminated when one mouse expired in any one of the groups. Group A, mice kept on a low iron diet and distilled water; Group B, on normal chow and tapwater; Group C, on normal chow, tapwater, and iron; Group D, on normal chow, tapwater, and chloroquine; and Group E, on normal chow, tapwater, iron, and chloroquine. Each mouse (7 per group) was infected with 2×10^4 parasites by ip on the 17th day; the chloroquine base (1.6 mg/kg body wt) was administered on the 1st, 3rd, 5th, 7th, 9th, 11th, 13th, 15th, 17th, 19th, 21st, 23rd, and the 25th day; and the iron (60 mg/kg body wt) on the same days as the chloroquine base.

[b] The mean values with their standard deviations (SD) are expressed in logarithm to the base 10 and are derived from the sum of the logarithm-transformed values of the parasitemia of the individual mouse in each group divided by the number of mice in the group.

The analysis of variance of the data in Table II gave $p < .001$ for the comparisons of the combined group of A and B vs. the combined group of C, D, and E and of Group D vs. Group E. Again, the comparison of Group A vs. Group B gave $p > .05$ and the difference is considered statistically not significant. It should be noted here that the group of mice kept on a low iron diet (Group A) was included in our studies to accentuate the differences between the groups.

Figure 1 shows the relation between the incorporation of radioactive iron-59 citrate into the blood cells of uninfected mice and time. All the mice looked healthy with progressive gains in body weight when they were kept on a low iron diet for 12 days prior to the administration of the radioactive iron. It is seen in the figure that maximal incorporation of radioactivity was reached at approximately the 40th hr after the administration of the radioactive iron. The rate and the amount of incorporation of radioactivity were increased when the mice were given chloroquine (Curve B). The maximum level of radioactive iron incorporated into the blood cells of chloroquine-treated mice (Curve B) was approximately 33% higher than the saline-treated mice (Curve A). The difference in the incorporation between the two groups of mice is shown in the insert of the figure and was found to be linear up to approximately the 28th hr after the administration of the radioactive iron.

The present data suggest that iron may play an important role in the control of the growth and multiplication of the erythrocytic forms of the malarial parasite and that chloroquine can act to stimulate the protective action of iron, conceivably by increasing the concentration of iron inside the cell. The data in Table I suggest a stimulatory role of chloroquine in that the protective effect of iron is increased at least 8 times under the conditions when chloroquine has been lost from the red blood cells, as reported by Macomber *et al.* (11). Further studies to elucidate the mechanism of action of iron and the role of chloroquine on the effect of iron on the plasmodia are in progress in our laboratory.

Summary. A novel finding is presented for the *in vivo* regulation of the growth and multiplication of the erythrocytic forms of the malarial parasite by iron and chloroquine.

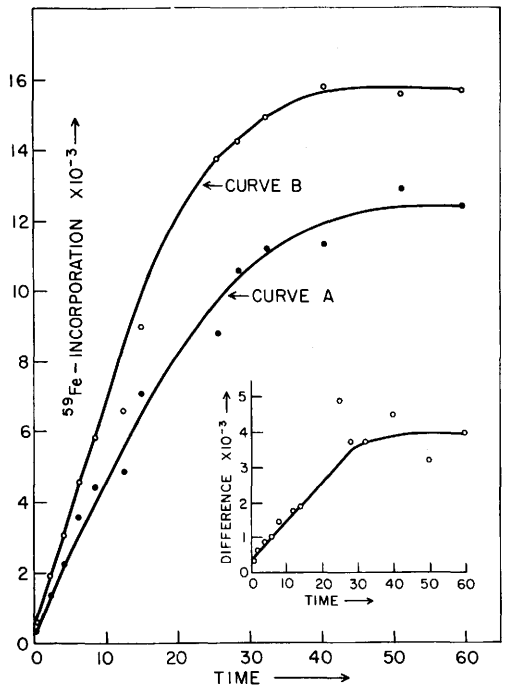

FIG. 1. The effect of chloroquine on the uptake of iron by the blood cells of uninfected mice.

The experimental conditions are as follows: the mice (10 per group) were placed on a low iron diet for 12 days before the administration of the radioactive iron-59 citrate (10 μCi/2.6 μg iron-59 per mouse) by ip injections. The mice in Curve A were injected with normal saline solution on the 12th, 13th, and the 14th day after they were placed on a low iron diet; and Curve B mice were injected with chloroquine base (40 mg/kg body wt) on the same days as the saline.

The water fed to both groups of mice had a specific conductivity of at least 15 Mohms.

The ordinate is the radioactive iron-59 incorporated into the blood cells and is expressed as the counts per minute of iron incorporated into 1.0 cm of packed cells of the mice. The abscissa is the time expressed in hours.

The insert represents the difference in the rates of radioactive iron incorporated between Curves A and B.

Mice treated with iron showed average parasitemias approximately 3–6 times less than in mice kept on a low iron diet. The protective effect of iron is increased approximately 3–8 times by chloroquine injections. Chloroquine is shown to increase the uptake of iron into the blood cells of uninfected mice to suggest another action of chloroquine.

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