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EFFECTS OF MALARIAL INFECTION ON HOST MICROSOMAL DRUG-METABOLIZING ENZYMES*

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Abstract—The present experiments are an attempt to correlate previously observed decreases in smooth endoplasmic reticulum during malaria infection with changes in drug-metabolizing enzyme activity. Rats were injected with parasitized (*P. berghei*) red blood cells from donor animals and then sacrificed 1–8 days later. The hepatic microsomal metabolism of ethylmorphine, aniline, *p*-nitroanisole and hexobarbital showed progressive decreases in rates over the 8-day period. The decreases in metabolism were accompanied by progressive increases in hexobarbital sleeping time. The per cent parasitemia also increased over this period. The presence of a rather severe malarial infection did not prevent the induction of microsomal enzymes by phenobarbital. The reduction in enzyme activity during malarial infection and the increases in activity seen after phenobarbital administration were accompanied by similar alterations in cytochrome P-450 content. Despite the decrease in enzyme activity, microsomal protein content remained essentially unchanged during the first 6 days of the infection. It remains to be determined whether the observed biochemical changes result from a direct action of the parasite or are secondary effects of the infection.

NUMEROUS pathological changes have been shown to occur in tissues of the host animal after infection by the malarial parasite. Among the organs affected are the spleen, kidney, liver and adrenal glands. The liver in particular shows a number of morphologic and biochemical changes, the magnitude of which appear to be related to the severity of the infection. Mercado and von Brand[1] demonstrated that livers of parasitized animals have a decreased ability to synthesize glycogen from exogenously supplied glucose and have a diminished endogenous glycogen content. The coenzyme A content of livers of infected animals is also reduced.[2]

Changes in hepatic ultrastructure caused by the malarial parasite also have been reported. Electron microscopy has revealed alterations in mitochondrial appearance and the presence within the mitochondria of large osmiophillic bodies.[3] Although the Golgi and the rough-surfaced endoplasmic reticulum (RER) were not obviously abnormal, the smooth-surfaced endoplasmic reticulum (SER) was very sparse and

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appeared vesicular.[3] Since the SER has been shown to be importantly involved in the metabolism of many foreign compounds,[4–6] impairment of its physiological function could have important consequences for the host. The present study was undertaken to determine if the above-noted changes in hepatic SER were accompanied by a decreased ability of the host's liver to metabolize drugs.

METHODS

Male Sprague–Dawley rats weighing 150–200 g were used. Control animals and animals receiving malarial parasites were given a single injection of phenylhydrazine (15 mg/kg, subcutaneously) in order to induce reticulocyte formation, since it has been shown that reticulocytes are much more vulnerable to malarial parasites than normal erythrocytes.[7] Malaria-infected blood obtained from donor animals was adjusted so that each rat received an intraperitoneal injection of approximately 1,000,000 parasitized red blood cells per gram of body weight. All studies used the KBG 173 strain of *Plasmodium berghei* obtained from Dr. William Trager of Rockefeller University. Animals were sacrificed 1–8 days after parasite and/or phenylhydrazine injection. Blood was taken for red blood cell and reticulocyte count. Parasitemia was quantified by examining 400 erythrocytes in thin blood smears stained with Wright's stain and expressed as a percentage of cells parasitized. The injection of equivalent volumes of blood obtained from normal animals into control animals resulted in no changes in microsomal enzyme activity.

Livers were rapidly excised, weighed and homogenized in ice-cold 1·15% KCl. Microsomes were prepared by differential centrifugation as described previously.[8] Reaction mixtures of 5-ml volume were incubated for 15 min in a Dubnoff metabolic shaker at 37° with air as the gaseous phase. Each mixture contained the following constituents: NADP, 2·0 μmoles; glucose 6-phosphate, 25 μmoles; glucose 6-phosphate dehydrogenase, 1·4 units; nicotinamide, 20 μmoles; magnesium chloride, 25 μmoles; and about 10 mg of microsomal protein. Forty-nine μmoles of semicarbazide hydrochloride was added to the reaction mixture when the *N*-demethylation of ethylmorphine was measured. The pH of the incubation mixture was adjusted to 7·4 with 0·1 M phosphate buffer.

The pathways studied, methods of assay and the amount of substrate added were: side chain oxidation of hexobarbital,[9] 3·0 μmoles; *N*-demethylation of ethylmorphine,[10] 5·0 μmoles; aromatic hydroxylation of aniline,[11] 10 μmoles; and *O*-demethylation of *p*-nitroanisole,[12] 10 μmoles. Aliquots of the microsomal suspension were assayed for protein content by the method of Lowry *et al.*[13] using a Technicon auto-analyzer. In some experiments the microsomal pellet was resuspended by homogenization in 0·1 M phosphate buffer, pH 7·4, and the P-450 content was determined as described by Omura and Sato.[14] The hypnotic effect (sleeping time) of hexobarbital was measured as the time interval from the loss till the restoration of the righting reflex.

RESULTS

Effect of phenylhydrazine and malarial infection on rat blood. Pretreatment of rats with a single subcutaneous injection of phenylhydrazine (15 mg/kg) resulted in a gradual appearance of reticulocytes in rat blood (Table 1). The number of red blood cells per ml of blood remained essentially constant at about 4×10^9 cells per ml. A similar temporal increase was observed in the percentage of reticulocytes in the blood

TABLE 1. EFFECT OF PHENYLHYDRAZINE AND MALARIAL INFECTION ON RAT BLOOD

Treatment	Day	RBC*	% Reticulocytes	% Parasitemia	% Mortality
Phenylhydrazine	0	4·98	< 1		0
	1	3·62	2		0
	2	4·47	9		0
	4	3·78	8		0
	6	4·17	16		0
	8	4·92	10		0
Phenylhydrazine + *P. berghei*	1	3·61	1	3	0
	2	2·64	6	6	0
	4	2·57	3	11	0
	6	2·75	7	20	20
	8	1·03	18	36	40

* Expressed as number red blood cells per ml × 10^9. Each value represents the mean of at least six determinations.

of animals injected with both phenylhydrazine and the malarial parasite, *Plasmodium berghei* (Table 1). The infected animals, however, showed a gradual decline in the total number of red blood cells per ml until a minimum value of 1·03 × 10^9 cells/ml was reached 8 days after the injection of the parasites. This was presumably because of the lysis of the cells by the rapidly dividing organisms.

There was a gradual increase in the number of parasitized red blood cells with time after the initial injection of *P. berghei*. The maximal parasitemia obtained was 36 per

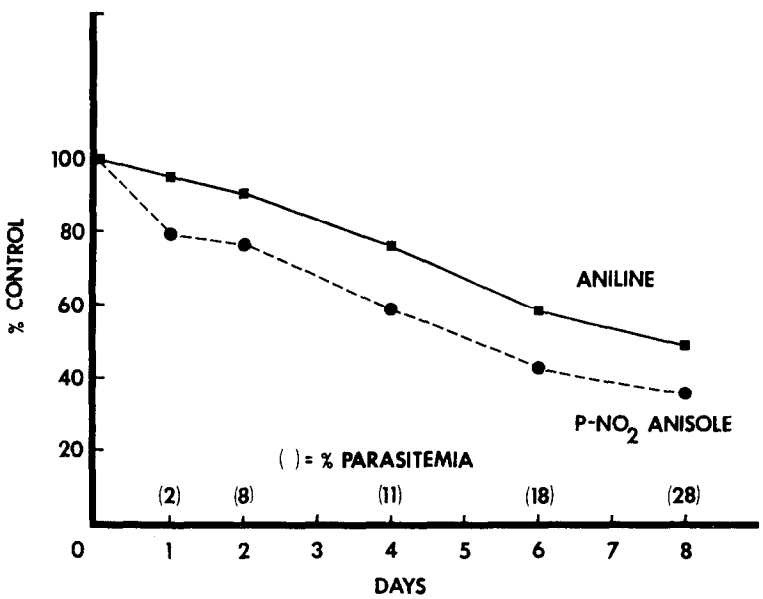

FIG. 1. Effect of malarial infection on the microsomal metabolism of aniline and *p*-nitroanisole. Liver microsomes were obtained from animals at various periods after injection of *P. berghei*. The degree of parasitemia was determined in each animal. The figure in parenthesis is the mean of at least 6 animals. Control rates of metabolism were determined concurrently with each experimental group.

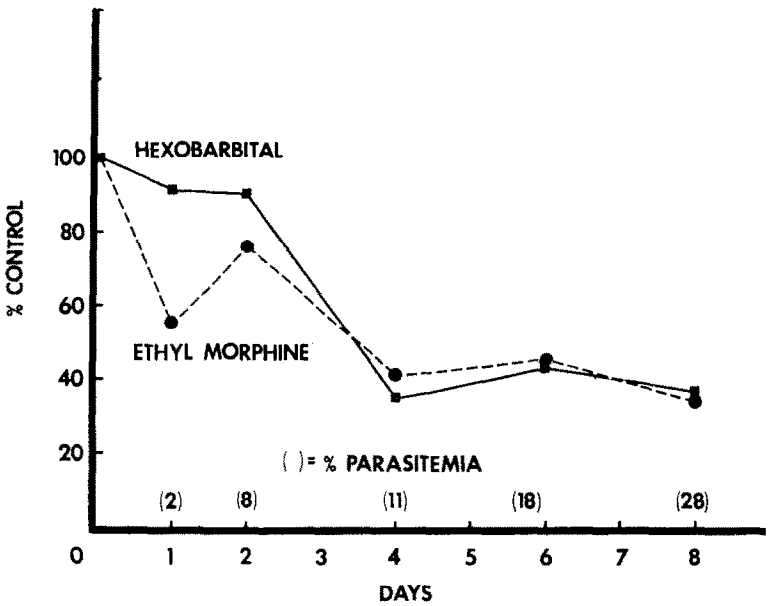

FIG. 2. Effect of malarial infection on the microsomal metabolism of hexobarbital and ethylmorphine. Liver microsomes were obtained from animals at various periods after injection of *P. berghei*. The degree of parasitemia was determined in each animal. The figure in parenthesis is the mean of at least 6 animals. Control rates of metabolism were determined concurrently with each experimental group.

cent and this occurred on the eighth day post injection (Table 1). An increased mortality was noted once a parasitemia of about 20 per cent was reached.

Effect of malarial infection on microsomal drug metabolism. In Figs. 1 and 2 are plotted the rates of microsomal metabolism (percent of control) against days after injection of *P. berghei*. The rates of metabolism of aniline, *p*-nitroanisole, hexobarbital and ethylmorphine all show a progressive decline until a maximal impairment is reached on the eighth day post injection. This correlates well with the gradually increasing parasitemia noted in these animals (Figs. 1 and 2); the higher the parasitemia, the more marked the impairment of drug metabolism. The maximum decreases in enzyme activity observed for the metabolism of hexobarbital, ethylmorphine and *p*-nitroanisole were about 65 per cent while the hydroxylation of aniline was reduced only about 50 per cent.

Changes in microsomal protein content in malaria-infected animals. Total protein was determined in liver microsomes taken from control and malaria-infected rats. The protein content remained relatively constant for the first 6 days after malarial parasite and/or phenylhydrazine administration (Table 2). Microsomes obtained on the eighth day post injection, however, showed a diminished protein concentration in both control and infected animals, although the protein content of the latter animals was somewhat lower.

Effect of malarial infection on hexobarbital sleeping time. Since the effectiveness and duration of action of hexobarbital as an anesthetic agent is greatly dependent upon normal hepatic function, it was of interest to examine the effects of malarial infection on hexobarbital's action. In these experiments control and malaria-infected animals

TABLE 2. INFLUENCE OF MALARIAL INFECTION ON MICROSOMAL PROTEIN LEVELS

Treatment	Days	Microsomal protein*
	0	34·0 ± 2·8
	1	33·3 ± 3·6
Phenylhydrazine	2	36·3 ± 1·5
	4	37·8 ± 1·0
	6	36·6 ± 1·4
	8	29·6 ± 1·9
	1	31·1 ± 2·9
Phenylhydrazine	2	35·9 ± 2·9
+	4	34·5 ± 0·6
P. berghei	6	35·1 ± 1·5
	8	25·3 ± 2·9

* Results are expressed as mg of protein per g of liver and are presented as mean values obtained from six animals.

were given hexobarbital (100 mg/kg, i.p.) and the duration of their sleeping time and degree of parasitemia were determined. Non-infected rats sleep about 26 min after this dose of hexobarbital (Fig. 3). There was no marked change in sleeping time until a parasitemia of 10 per cent was exceeded. Above this value there was a very close correspondence between increasing parasitemia and lengthening of the hexobarbital sleeping time (Fig. 3). Furthermore, the increase in sleeping time also corresponds well with the previously demonstrated impairment of hexobarbital metabolism (Fig. 2). Although sleeping time is plotted against per cent parasitemia in Fig. 3, a similar

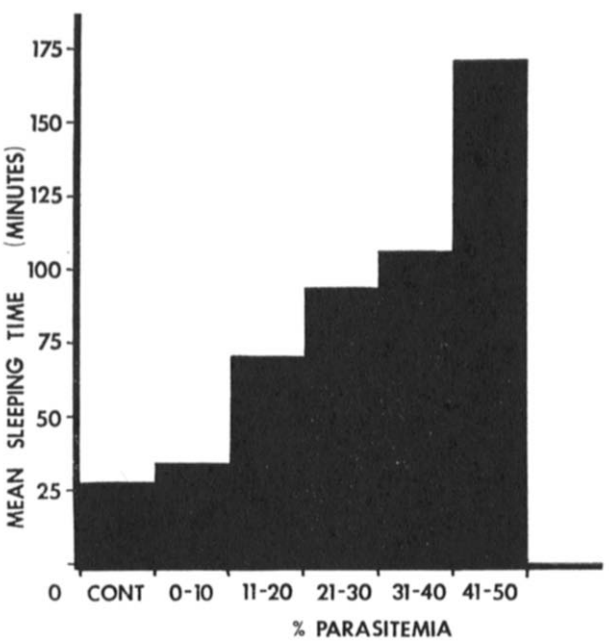

FIG. 3. Effect of increasing parasitemia on hexobarbital sleeping time. Animals received hexobarbital (100 mg/kg, i.p.) 1–8 days after the injection of *P. berghei* and sleeping time and per cent parasitemia were determined in each animal.

correlation is obtained if one graphs sleeping time against days after injection of *P. berghei*. In the latter case, however, there is somewhat more variability in the data.

Effect of phenobarbital on microsomal drug metabolism in control and malaria-infected rats. Animals were given phenobarbital (80 mg/kg, i.p.) daily for 4 days beginning on the fourth day after the injection of the malarial parasites and/or phenylhydrazine. Animals were killed 24 hr after the last injection of phenobarbital. Parasitized animals treated with phenobarbital showed a marked increase in the metabolism of ethylmorphine, *p*-nitroanisole and aniline (Table 3) when compared to

TABLE 3. EFFECT OF PHENOBARBITAL ON MICROSOMAL DRUG METABOLISM IN CONTROL AND MALARIA-INFECTED RATS*

Treatment	Aniline*	*p*-Nitroanisole*	Ethylmorphine*
Untreated	0·8 ± 0·1	0·7 ± 0·1	4·3 ± 1·0
Phenobarbital	2·7 ± 0·3	4·2 ± 0·2	10·4 ± 1·1
Phenylhydrazine	1·0 ± 0·1	1·1 ± 0·1	4·0 ± 0·5
Phenylhydrazine + phenobarbital	2·2 ± 0·4	3·7 ± 0·5	8·2 ± 1·8
Malaria (8 day)	0·5 ± 0·1	0·4 ± 0·1	1·2 ± 0·8
Malaria (8 day) + phenobarbital	1·6 ± 0·1	2·5 ± 0·2	7·0 ± 0·9

* μmole per g per hr. The data presented are mean ± standard error and are based on values obtained from at least six animals.

infected animals not receiving barbiturate. The per cent parasitemia of the two groups of animals was quite similar. The rates of metabolism were increased to values above those found in the untreated or phenylhydrazine-treated rats (Table 3). Although the total increases in metabolism produced in parasitized animals were less than those obtained in uninfected rats, the percentage increases were quite similar.

Effect of phenobarbital on hexobarbital sleeping time and metabolism. Treatment with

TABLE 4. EFFECT OF PHENOBARBITAL ON HEXOBARBITAL SLEEPING TIME AND METABOLISM IN CONTROL AND MALARIA-INFECTED RATS*

Treatment	Sleeping time (min)	Hexobarbital (μmoles/g/hr)	% Parasitemia
Untreated	25·8 ± 1·4	7·0 ± 1·8	0
Phenobarbital	9·1 ± 2·0	12·4 ± 0·9	0
Phenylhydrazine	26·2 ± 1·3	6·0 ± 0·4	0
Phenylhydrazine + phenobarbital	10·3 ± 0·5	11·3 ± 0·6	0
Malaria (8 day)	117·3 ± 10·5	2·2 ± 0·4	36 ± 5
Malaria (8 day) + phenobarbital	17·5 ± 0·9	9·6 ± 1·1	31 ± 3

* The data presented are means ± standard error and are based on values obtained from at least six animals.

phenobarbital resulted in increased rates of hexobarbital metabolism and decreased sleeping times in untreated, phenylhydrazine-treated and parasitized animals (Table 4). The values obtained for sleeping time and hexobarbital metabolism in infected, induced animals approached those found in control, induced animals.

Alterations in cytochrome P-450 content in rat-liver microsomes. Cytochrome P-450 has been implicated in the oxidation of foreign compounds by the microsomal

system.[15, 16] It was of interest, therefore, to see if changes in P-450 content paralleled the alterations in microsomal enzyme activity. The results are summarized in Table 5. There was a 40 per cent decrease in cytochrome P-450 content 8 days after animals were injected with *P. berghei*. This effect could, however, be reversed by phenobarbital treatment. Thus, the decrease in enzyme activity seen in malaria and its reversal by phenobarbital (Table 3) were accompanied by similar changes in P-450 content (Table 5).

TABLE 5. EFFECT OF MALARIA AND DRUG TREATMENT ON THE CONTENT OF CYTOCHROME P-450 IN RAT-LIVER MICROSOMES

Treatment	% Parasitemia	P-450*
Control	0	1·14 ± 0·08
Control + phenobarbital	0	3·01 ± 0·29
Phenylhydrazine	0	1·19 ± 0·08
Malaria	20 ± 2	0·70 ± 0·09
Malaria + phenobarbital	20 ± 3	2·20 ± 0·31

* Amount expressed as ΔE (450 minus 500 mμ) per g of liver.

DISCUSSION

Stress,[17, 18] starvation,[11] obstructive jaundice,[19] hepatic tumors,[20] alloxan diabetes[21] and bacterial infections[22] are all capable of altering the rates of microsomal drug metabolism. Many conditions that lead to changes in the activity of drug-metabolizing enzymes in liver microsomes are accompanied by changes in the amount or structure of SER. Recent observations by Rosen *et al.*[3] demonstrated changes in the endoplasmic reticulum taken from a patient with malaria, i.e. there was a marked decrease in the quantity and organization of the SER but not the RER. It was of interest, therefore, to examine the drug-metabolizing capabilities of microsomes obtained from malaria-infected animals.

Injection of the malarial parasite, *P. berghei*, to rats resulted in a progressive impairment of microsomal drug metabolism. This inhibition appeared to be correlated with an increasing severity of the malarial infection, at least as judged by an increasing per cent parasitemia. In general, the most marked impairment of metabolism occurred once a parasitemia of at least 10 per cent was established. Although this was the case for the metabolism of hexobarbital, *p*-nitroanisole and aniline, the *N*-dealkylation of ethylmorphine appeared to be more sensitive to the effects of the malarial parasite. The metabolism of ethylmorphine was reduced to about 55 per cent of control values within 24 hr after the injection of *P. berghei*, i.e. at a time when there was only a 2 per cent parasitemia. Differential sensitivities of microsomal enzymes have been reported previously.[23–25]

Alterations in sleeping time in infected animals are probably more closely related to changes in hexobarbital metabolism than to changes in drug distribution. Noordhoek[26] has pointed out that in the rat diffusion of hexobarbital from plasma and brain to fat is quantitatively of minor importance in terminating biologic activity compared with metabolism. In our studies changes in hexobarbital sleeping time in parasitized rats were associated with both an increasing parasitemia and a decreasing rate of

microsomal hexobarbital oxidation. A generalized debilitation may also influence hexobarbital sleeping time since parasitized animals, particularly in the later stages of the infection, weigh less than their corresponding controls.

Although malarial infection reduces the activity of microsomal enzymes, it does not prevent the hepatic cells from responding to phenobarbital administration. Phenobarbital increased the rates of metabolism of all 4 substrates examined. The magnitude of the increases was similar to that seen in non-infected animals, although the absolute rates achieved were lower in the parasitized animals.

The progressive decrease in microsomal metabolism in parasitized animals cannot be attributed to a loss of microsomal protein since control and infected animals had similar concentrations of protein per g of liver. During the first 6 days after the injection of *P. berghei*, the rates of drug metabolism gradually diminished while microsomal protein content remained essentially unchanged. There was, however, a decrease in microsomal cytochrome P-450 content in the parasitized animals. Since it has been shown that cytochrome P-450 is importantly involved in the binding and metabolism of drugs by the microsomal hydroxylating system,[27, 28] a loss of this component might account for a decreased metabolism. This is supported by the observation that changes in enzyme activity paralleled changes in the amount of cytochrome P-450 present in liver microsomes. Decreased enzyme activity in parasitized animals was accompanied by a decrease in P-450 content, while induction of enzyme activity by phenobarbital paralleled increases in microsomal P-450. Thus, the impairment of drug metabolism seen in malaria-infected rats may be due to an effect of the parasite on cytochrome P-450.

Further studies are needed to determine whether the observed biochemical changes are produced by the direct effect of the malarial parasite or by secondary effects of the infection, such as hypoxia and hemolysis. Our results do, however, provide biochemical support for the morphologic observation[3] that malarial infection results in a loss of the structural and functional integrity of the smooth endoplasmic reticulum.

Impaired drug-metabolizing ability in patients with malarial infections may have important consequences for drug therapy. Compounds that depend upon metabolic conversion for their activity may have their effectiveness diminished, while those compounds that are active *per se* might reach toxic blood levels after normal therapeutic doses because of diminished rates of biotransformation.

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