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EFFECT OF MALARIA INFECTION AND ENDOTOXIN-INDUCED FEVER ON PHENACETIN O-DEETHYLATION BY RAT LIVER MICROSOMES

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Abstract—We have investigated the effect of malaria infection with the rodent parasite *Plasmodium berghei* and fever induced by *Escherichia coli* endotoxin on the metabolism of phenacetin to paracetamol by rat liver microsomes from young (4 weeks old) male Wistar rats (N = 5 in control and fever groups; N = 10 in malaria-infected group). Following determination of % parasitaemia, the malaria-infected group was divided into a low parasitaemia subgroup (N = 5; mean % parasitaemia = 9.87 ± 2.6) and a high parasitaemia subgroup (N = 5; mean % parasitaemia = 36.6 ± 8.1). The control group received normal saline. Total microsomal protein was not significantly affected by fever or malaria infection while cytochrome P450 levels were reduced by approximately 50% in the high parasitaemia subgroup, 20% in the low parasitaemia subgroup and 20% in the endotoxin-treated group. Phenacetin-O-deethylation kinetics were biphasic in both control and malaria-infected rats, but monophasic in endotoxin-treated rats. Total apparent intrinsic clearance ($CL_{int.total}$; calculated as V_{max}/K_m; V_{max} is maximum velocity, K_m is Michaelis constant) of phenacetin was reduced approximately 6-fold in low parasitaemia, 30-fold in high parasitaemia and 35-fold in fever. There was a poor correlation between $CL_{int.total}$ and % parasitaemia ($r = -0.6$). However, log $CL_{int.total}$ correlated inversely with % parasitaemia ($r = -0.9$), suggesting that $CL_{int.total}$ decreased exponentially with an increase in % parasitaemia. Phenacetin O-deethylation is a marker for cytochrome P4501A2 activity and the results of the present study suggest that both malaria infection and fever might specifically reduce P4501A2 activity in the rat.

Malaria infection has been shown to be associated with the impairment of both phase I and phase II metabolism of several drugs and chemicals [1–9]. The clinical features of malaria due to infection with *Plasmodium falciparum* and *P. vivax* include episodes of high fever that recur every 48 hr. Experimentally induced fever *per se* also affects drug metabolism in animals [10–12], and it is possible that malaria infection and fever alter drug metabolism by a common mechanism.

Many clinically important phase I metabolic reactions are catalysed by isozymes of the cytochrome P450 superfamily. As a consequence it is essential to determine how these isozymes function in disease. We are interested in the effect of malaria in particular and febrile illness in general on the activity of well-characterized isozymes of cytochrome P450. The present study was aimed at investigating the effect of malaria infection and fever on the activity of cytochrome P4501A2, using the O-deethylation of phenacetin to paracetamol as an index. Additionally, we wanted to determine whether the effect, if any, of malaria infection on phenacetin O-deethylation can be attributed to fever. Cytochrome P4501A2 is expressed in human liver [13, 14] and is orthologous to form *d* in the rat [14, 15]. This isozyme is involved in the metabolism of caffeine [16], theophylline [17, 18] and carcinogenic arylamines [18], theophylline being clinically important due to its narrow therapeutic index.

The rat was chosen as a model to study the effects of malaria infection and fever on cytochrome P4501A2 activity because fever can easily be produced by injection of bacterial endotoxin, while malaria infection can be produced using the rodent parasite *P. berghei*. Moreover, it is possible to determine accurately the level of parasitaemia in this model. Both endotoxin-induced fever and malaria infection produced by *P. berghei* in the rat are well-established models and have been used in a number of studies [4–9, 11, 12].

MATERIALS AND METHODS

Chemicals and reagents. Phenacetin and 3-acetamidophenol were obtained from the Aldrich Chemical Co. (Gillingham, U.K.). Magnesium chloride and Tris base were obtained from BDH (Poole, U.K.). Anhydrous sodium acetate, acetonitrile (HPLC grade), dichloromethane (Analar), methanol (HPLC grade), ethyl acetate (Analar) and glacial acetic acid (Analar) were obtained from Fisons (Loughborough, U.K.). Bovine serum albumin (Factor V), NADP, *Escherichia coli* lipopolysaccharide (LPS§), glucose-6-phosphate,

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§ Abbreviations: LPS, lipopolysaccharide; TNF_α, tumour necrosis factor α.

glucose-6-phosphate dehydrogenase (type XV), paracetamol, Folin and Ciocalteu's Phenol Reagent and glycerol were obtained from the Sigma Chemical Co. (Poole, U.K.).

Animals. Young male Wistar rats (4 weeks old, approximately 90 g weight) were bred in the Departmental Animal Unit. For malaria infection studies, animals were inoculated with saline-diluted blood from mice infected with the rodent parasite *P. berghei*, and infection was allowed to establish over 7 days. Blood was obtained from CD1 mice which had been previously infected with *P. berghei* and in which the level of parasitaemia was established to be between 40 and 50%. Mouse blood was diluted either 10-fold or 3-fold with sterile normal saline. Rats were injected with either the 10-fold diluted mouse blood (0.2 mL i.p.; N = 5) or the 3-fold diluted blood (0.2 mL i.p.; N = 5). After 7 days, parasitaemia was determined by microscopic examination of Giemsa-stained thin blood films. Rats with parasitaemia below 20% were placed in the low parasitaemia group while those with parasitaemia above 20% were placed in the high parasitaemia group. Young animals were used as it is difficult to establish infection in older rats. The rats were killed 7 days post inoculation and the livers removed as described below. For fever studies, rats were injected with *E. coli* LPS (4 mg/kg i.p.) as a solution in normal saline (total volume injected <0.5 mL). Rectal temperature was measured, using a temperature probe at zero time, and at 1, 4, 8, 22, 23 and 24 hr post LPS administration. Rats were killed 24 hr post LPS administration and livers removed as described below. Control rats (N = 5) were injected with sterile, pyrogen-free normal saline (0.4 mL i.p.) and livers removed 24 hr later. The 0 and 1 hr temperature readings were combined and the average taken as the baseline body temperature. The mean of the 22, 23 and 24 hr temperature readings was taken as the value for temperature change due to LPS (test) or saline (control) treatment 24 hr post treatment. During this period (22–24 hr post treatment) changes in temperature due to fever had stabilized. Animals were killed with a blow to the head and the livers were quickly removed, rinsed with cold Tris–HCl buffer (pH 7.4), blotted dry, weighed and used to prepare microsomes as described below.

Incubation of phenacetin with rat liver microsomes. Microsomes were prepared according to the classical procedure of differential centrifugation [19]. The washed microsomes were suspended in 1.15% KCl in 50 mM Tris–HCl buffer, pH 7.4 (2 mL) containing 20% glycerol [19] and stored at −80° until used. Microsomal protein content was determined using bovine serum albumin as standard [20] and cytochrome P450 content was determined according to a standard procedure [21]. Since the microsomal protein obtained from a single rat liver was insufficient both for incubation experiments and cytochrome P450 determination, separate groups of control, malaria-infected and endotoxin-treated rats (N = 5 in each group) were killed and the livers in each group pooled. The pooled livers were then used to prepare microsomes for cytochrome P450 determination.

Protein dependency of phenacetin-O-deethylation was assessed by incubating phenacetin (2.56 mM; 20 min) with microsomal protein (0.2–1 mg). Time dependency was determined by incubating phenacetin (2.56 mM) and protein (0.6 mg) for 5–60 min. Incubation was carried out at 37° in a shaking water bath. The cofactor solution (1 mL) contained an NADPH generating system comprising 2 U glucose-6-phosphate dehydrogenase, 0.5 mM glucose-6-phosphate, 1 mM $MgCl_2$ and 3 mM NADP. Following a 5 min equilibration period, the reaction was started by mixing 1 mL of cofactor solution with 1 mL of microsomal protein suspension in Tris–HCl buffer (pH 7.4). The reaction was stopped by addition of 0.5 mL of 0.025 N NaOH [22]. Rate of paracetamol production was linear for protein between 0.2 and 1 mg, and for incubation times between 5 and 40 min. Therefore, for subsequent experiments 0.6 mg microsomal protein (0.3 mg/mL in final incubation mixture) was used with an incubation period of 20 min.

For kinetic studies, phenacetin (0.005–2.56 mM) was added as a solution in methanol (20 μL) to the microsomal suspension and duplicate incubations were performed as described above. At the end of the incubation period the amount of paracetamol produced was determined as described below. It was assumed that further metabolism of paracetamol was negligible under these conditions.

Determination of paracetamol. Paracetamol was extracted from the incubation mixture according to previous procedures [23, 24], with some modifications. Briefly, the internal standard (3-acetamidophenol, 200 ng, dissolved in methanol) was added to the incubate which was then extracted by mechanical tumbling with dichloromethane (10 mL, 20 min) to remove excess phenacetin and interfering substances from microsomes. Following centrifugation (650 *g*, 10 min) the aqueous layer was separated and mixed with sodium acetate buffer (1 mL, pH 4.8). Paracetamol was extracted by shaking with ethyl acetate (10 mL, 30 min). Following centrifugation (650 *g*, 10 min), 9 mL of the ethyl acetate layer were removed and evaporated to dryness in a water bath (40°) under nitrogen. The residue was reconstituted in 60 μL of mobile phase (acetonitrile : 10 mM orthophosphoric acid; 3.75 : 96.25% v/v) and 50 μL were analysed using HPLC [25]. Calibration curves were constructed daily by spiking Tris–HCl buffer (1 mL, pH 7.4) containing microsomal protein (0.3 mg) with known amounts of paracetamol (20–600 ng) and the internal standard (200 ng; 20 μL of a solution in methanol) followed by extraction as described above. The HPLC system used comprised an LDC model III Constametric pump, a Rheodyne injector fitted with a 50 μL loop, a μ-Bondapak 10 μ C-18 column (25 cm × 4 mm), a Pye unicam LC871 UV-VIS detector (set at 245 nm and attenuation of 0.005–0.04 AUFS) and a Philips PM8251 single-pen recorder. Mobile phase flow rate was 2 mL/min at ambient temperature. Concentrations of paracetamol were determined from peak height ratios with reference to the standard curve.

Calculation of enzyme kinetic parameters. The rate of formation of paracetamol from phenacetin was

Table 1. Michaelis–Menten parameters in livers from control, malaria-infected and endotoxin-treated rats

Parameter	C	ML	MH	F
$K_m(1)$*	1.98 ± 1.5	336.5 ± 123[a]	492 ± 208[a,b]	658 + 338‡
$K_m(2)$	919.7 ± 318.5	482.5 ± 267.7[a]	481.5 ± 90.5[a,b]	—
$V_{max}(1)$†	0.23 ± 0.05	1.57 ± 0.89[a]	0.57 ± 0.51[a]	3.5 + 2‡
$V_{max}(2)$	2.98 ± 0.9	6.27 ± 1.73[a,d]	3.36 ± 0.94[c,d]	—

Values are means ± SD.

* Values in μM; † values in nmol/min/nmol cytochrome P450; ‡ values are calculated for a one-enzyme model.

[a] Significantly different ($P < 0.05$) from C; [b] not significantly different ($P > 0.05$) from ML; [c] significantly different ($P < 0.05$) from ML; [d] not significantly different ($P > 0.05$) from V_{max} in F.

Abbreviations: C, controls; ML, malaria infection, low parasitaemia; MH, malaria infection, high parasitaemia; F, fever.

calculated as nanomoles formed per milligram microsomal protein per minute. The untransformed rate data were fitted to the Michaelis–Menten equation for a one or a two enzyme model, using the iterative non-linear regression program GraFit [26] according to the following models:

Model 1

$$V = V_{max} \cdot S/K_m + S \quad (1)$$

Model 2

$$V = V_{max}(1) \cdot S/K_m(1) + S + V_{max}(2) \cdot S/K_m(2) + S \quad (2)$$

where $K_m(1)$ and $V_{max}(1)$ represent the high affinity, low capacity, and $K_m(2)$, $V_{max}(2)$ represent the low affinity, high capacity enzyme components, respectively. Under first order conditions Eqns 1 and 2 can be written as [27]:

$$V/S = V_{max}/K_m = CL_{int} \quad (3)$$

$$V/S = V_{max}(1)/K_m(1) + V_{max}(2)/K_m(2) = CL_{int}(1) + CL_{int}(2) \quad (4)$$

where V = formation rate of paracetamol; S = phenacetin concentration; CL_{int} = intrinsic clearance of phenacetin to paracetamol; $CL_{int}(1)$ = intrinsic clearance associated with the high affinity, low capacity enzyme component; $CL_{int}(2)$ = intrinsic clearance associated with the low affinity, high capacity enzyme component. Intrinsic clearance is a measure of the activity of drug metabolizing enzymes [27]. Initial parameter estimates for fitting data according to Eqns 1 and 2 were obtained from Eadie–Hofstee (V versus V/S) plots. The decision to use either model 1 or model 2 to fit data was based on visual inspection of the Eadie–Hofstee plots, significantly smaller standard errors associated with the estimated parameters and the distribution of residuals when V (observed) minus V (calculated) was plotted against V (observed).

The intrinsic clearance by the whole liver ($CL_{in,total}$) was calculated as the ratio of V_{max}, expressed per nmole cytochrome P450, and K_m, multiplied by the total amount of cytochrome P450 in the liver according to the following equation [28]:

$$CL_{int,total} = (V_{max}/K_m) \cdot \text{cytochrome P450}. \quad (5)$$

For the model described by Eqn 4, CL_{int} for each enzyme component was estimated using Eqn 5. $CL_{int,total}$ was then determined from the sum of CL_{int} for the two components. The K_m and V_{max} determined *in vitro* are apparent values [27]. Hence in subsequent discussion when referring to K_m and V_{max} the apparent values are implied.

Statistical analysis of estimated parameters. The non-parametric Mann–Whitney test was used for the comparison of the means of parameters from two groups. The Kruskal–Wallis test was used for the comparison of the means of parameters from more than two groups. $P < 0.05$ was taken as significant.

RESULTS

Drug analysis

The intra- and inter-assay coefficients of variation associated with analysis of paracetamol were 5.3 and 6.8%, respectively. The mean percentage recoveries of paracetamol from microsomal suspensions (0.3 mg protein/mL) spiked with paracetamol were 80.5 ± 10.5 (N = 5) at the 20 ng/mL level and 85.8 ± 7.6 (N = 5) at the 400 ng/mL level. The minimum detectable concentration of paracetamol was 5 ng/mL at a signal to noise ratio of 3. No paracetamol peaks were detectable from extracts of incubation mixtures which contained no phenacetin or those where cofactors were omitted.

Physiological and biochemical indices

Liver weight (g/100 g body weight) was not significantly different in malaria-infected rats or in rats with fever, compared with controls (controls: 5.5 ± 0.5; low parasitaemia malaria: 5.1 ± 0.4; high parasitaemia malaria: 5.9 ± 0.9; fever: 5.4 ± 0.7; $P > 0.05$). Microsomal protein content (mg/g liver) also was not significantly different in malaria infection and in fever compared with controls (controls: 6.6 ± 0.7; low parasitaemia malaria: 9.7 ± 2.7; high parasitaemia malaria: 7.7 ± 3.2; fever: 7.4 ± 1.3; $P > 0.05$). However, cytochrome P450 levels (nmol/mg microsomal protein) determined from pooled livers were reduced in malaria infection and in fever (controls: 0.34; low parasitaemia malaria: 0.28; high parasitaemia malaria: 0.16; fever: 0.27). Per cent

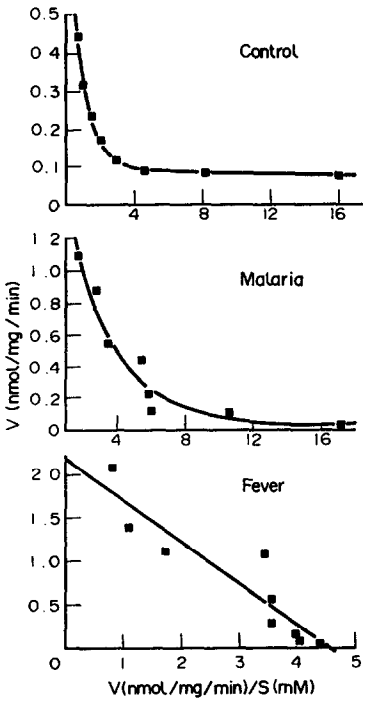

Fig. 1. Eadie–Hofstee plot for phenacetin O-deethylation by liver microsomes from a control, a malaria-infected (parasitaemia = 33%) and an endotoxin-treated rat.

Table 2. Intrinsic clearance (mL/min) of phenacetin to paracetamol in livers from control, malaria-infected and endotoxin-treated rats

Treatment	$CL_{int}(1)$	$CL_{int}(2)$	$CL_{int,total}$
C	2.0 ± 1.0	0.04 ± 0.02	2.1 ± 1.1
ML	0.09 ± 0.08	0.25 ± 0.19	0.34 ± 0.2*
MH	0.008 ± 0.005	0.05 ± 0.03	0.07 ± 0.03*†
F	NA	NA	0.06 ± 0.02*‡

Values are means ± SD.

NA, not applicable, as a one-enzyme model was suitable.

* Significantly different ($P < 0.05$) from C; † significantly different ($P < 0.05$) from ML; ‡ not significantly different ($P > 0.05$) from MH.

Abbreviations: C, controls; ML, malaria infection, low parasitaemia; MH, malaria infection, high parasitaemia; F, fever (endotoxin-induced); CL_{int}, intrinsic clearance.

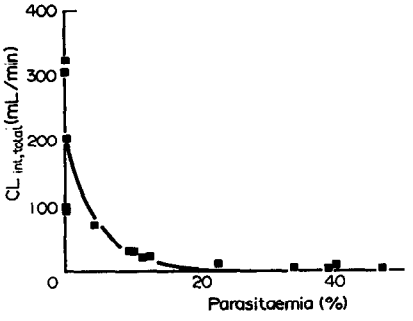

Fig. 2. Plot of total intrinsic clearance (×100) versus % parasitaemia, when clearance is assumed to decrease exponentially with increasing parasitaemia. Control values are indicated on the y-axis at the point corresponding to 0% parasitaemia.

parasitaemia was 9.9 ± 2.6 and 36.6 ± 8.1 in low and high parasitaemia groups, respectively.

Although fever caused a mean temperature rise of 0.5° at 24 hr compared to the baseline rectal temperature in control rats, this was not significant (controls: 37.4 ± 0.2; fever: 38 ± 0.6; $P > 0.05$). However, mean rectal temperatures at 4 and 8 hr were approximately 2° higher in rats with fever compared to mean rectal temperatures at corresponding times in control rats. This was statistically significant (controls: 36.8 ± 0.2 at 4 hr and 36.3 ± 0.4 at 8 hr; fever: 38.9 ± 0.1 at 4 hr and 39.1 ± 0.2 at 8 hr; $P < 0.05$).

Michaelis–Menten parameters and intrinsic clearance

Michaelis–Menten parameters for control and malaria-infected rats (two-enzyme model) and for rats with fever (one-enzyme model) are shown in Table 1.

Malaria and phenacetin O-deethylation

Phenacetin O-deethylation kinetic parameters in malaria infection are shown in Table 1. The Eadie–Hofstee plots for phenacetin O-deethylation were best described by a two-enzyme model in control and malaria-infected rats (Fig. 1). Total intrinsic clearance of phenacetin ($CL_{int,total}$; calculated using Eqn 5) was substantially reduced in malaria infection compared to control (Table 2). There was a significant difference in $CL_{int,total}$ between control and low parasitaemia malaria infection, between control and high parasitaemia malaria infection, and between low and high parasitaemia malaria infection (Table 2). $CL_{int}(1)$ accounted for approximately 97% of $CL_{int,total}$ in control, but this was reduced to 26% in low parasitaemia malaria and to 15% in high parasitaemia malaria. There was a relatively poor correlation ($r = -0.6$) between $CL_{int,total}$ and percentage parasitaemia. However, log $CL_{int,total}$ showed a better correlation ($r = -0.9$) when plotted against percentage parasitaemia. This implies that $CL_{int,total}$ decreases in an apparently exponential manner with increase in parasitaemia. Figure 2 shows a plot of $CL_{int,total}$ versus percentage parasitaemia assuming that $CL_{int,total}$ decreases exponentially with increasing parasitaemia.

Malaria infection caused a significant increase in $K_m(1)$ while $K_m(2)$ was significantly reduced (Table 1). After correcting for estimated cytochrome P450 content, $V_{max}(1)$ and $V_{max}(2)$ were increased in low parasitaemia malaria. Corrected $V_{max}(1)$ was also increased in high parasitaemia malaria, but corrected $V_{max}(2)$ in high parasitaemia malaria and corrected $V_{max}(2)$ in control rats were not significantly different.

Fever and phenacetin O-deethylation

Phenacetin O-deethylation kinetic parameters in rats with fever are shown in Table 1. The Eadie–Hofstee plot for paracetamol production was best described by a one-enzyme model (Fig. 1). $CL_{int,total}$ was decreased significantly in fever. This change in the Eadie–Hofstee plot from the apparent biphasic profile in controls to a monophasic profile in fever appeared to be due mainly to an effect of fever on the high affinity, low capacity enzyme component since the difference between $CL_{int,total}$ in fever and $CL_{int}(2)$ in controls was less than 2-fold (Table 2). Compared to malaria infection, fever reduced $CL_{int,total}$ by a magnitude similar to that produced in the livers from rats with high parasitaemia malaria. After correcting for cytochrome P450 content, there was no significant difference ($P > 0.05$) between $V_{max}(2)$ in controls and high parasitaemia malaria and V_{max} (one-enzyme model) in fever (Table 1).

DISCUSSION

Both malaria infection and fever affected the metabolism of phenacetin by rat liver microsomes. The overall effect was a significantly decreased $CL_{int,total}$ of phenacetin in malaria infection and fever compared to controls (Table 2). Phenacetin O-deethylation kinetics in both control and malaria-infected livers were best described by a two-enzyme model but a one-enzyme model best described the data from rats with fever (Fig. 1). However, visual inspection of the fits for the two-enzyme model indicated that the biphasic characteristic appeared less pronounced in malaria infection compared to controls. Both malaria infection and fever appear to affect the high affinity, low capacity enzyme component with apparently little effect on the low affinity, high capacity component. This is apparent from the values of $CL_{int,total}$ in fever and $CL_{int,total}$ in high parasitaemia malaria (here the sum of CL_{int} associated with the two enzyme components) which were only approximately 2-fold higher than $CL_{int}(2)$ for controls (Table 2). In control livers, $CL_{int}(2)$ accounted for only 2.5% of $CL_{int,total}$ whereas in high parasitaemia livers $CL_{int}(2)$ accounted for 85% of $CL_{int,total}$.

The two-enzyme model for phenacetin O-deethylation is in accord with previous reports on phenacetin O-deethylation in Wistar [22], DA and Fischer rats [29] and in human liver microsomes [22, 24], and the range of substrate concentrations used in the present study was comparable to that used in a previous study with rat liver microsomes [29]. The apparent biphasic profile for phenacetin deethylation has been explained by postulating the participation of at least two forms or populations of cytochrome P450, which differ in their substrate affinity [29]. The relative contributions of $CL_{int}(1)$ and $CL_{int}(2)$ to $CL_{int,total}$ in control livers are also in accord with values which can be determined from a previous report [22]. Phenacetin O-deethylation by the high affinity, low capacity enzyme component is mediated by cytochrome P4501A2 [14, 16]. Thus, both malaria infection and fever appear to impair cytochrome P4501A2 activity in the rat selectively, with little effect on the isozyme associated with the low affinity, high capacity component. The identity of the latter isozyme is unknown.

In malaria infection, $CL_{int,total}$ correlated poorly ($r = -0.6$) with percentage parasitaemia. However, correlation was improved ($r = -0.9$) when log $CL_{int,total}$ was plotted against percentage parasitaemia. This suggests that $CL_{int,total}$ decreases in an apparent exponential nature as percentage parasitaemia increases. A plot of $CL_{int,total}$ against percentage parasitaemia based on this assumption is shown in Fig. 2. $CL_{int,total}$ decreases rapidly as percentage parasitaemia increases up to approximately 20% at which point $CL_{int,total}$ decreases less rapidly with further increase in parasitaemia. Malaria infection resulted in an apparent increase in enzyme capacity. We have shown recently [30] that the glucuronidation of paracetamol in malaria infection is also associated with an apparent increase in the capacity of the enzyme involved. Currently, we have no explanation for this observation.

Both malaria infection and fever have been shown previously to depress phase I and phase II metabolism of drugs and several chemicals [1–12]. The exact mechanisms accounting for these observations have not been fully elucidated, but several explanations may be suggested based upon the known pathophysiological changes which occur during fever and malaria infection. For example, decreased cytochrome P450 levels and/or alterations in hepatic structure have been the most consistent changes observed during malaria infections [31]. Impairment of hepatic P450-dependent monoxygenase activity by *P. berghei* has also been reported [32]. In the present study using the rat model, pooled cytochrome P450 levels were reduced by approximately 50% and 20% in malaria infection and fever, respectively. Therefore, the reduced levels of cytochrome P450 may partly explain the reduction in intrinsic clearance of phenacetin observed in the present study. However, what causes this reduction in malaria infection and fever is unknown. A second factor which needs to be considered is the possible role of cytokines such as tumour necrosis factor α (TNF_α) and interleukin-6 in drug metabolism. Although in the present study fever was produced in a separate group of rats, it is well documented that cytokines such as TNF_α are released as a result of bacterial endotoxin administration to rats [33]. Elevated cytokine levels are a clinical feature of malaria infection [34] and cytokines could provide a link between the reported decreased drug metabolizing capacity in fever and in malaria infection. TNF and interleukin-6, for example, have been reported to depress microsomal drug metabolism [35–38]. The difference in phenacetin O-deethylation kinetics in malaria infection and fever may simply reflect a difference in circulating levels of cytokines. A third factor which might contribute to decreased drug metabolism in malaria infection is the presence of the malaria pigment haemozoin. The presence of haemozoin in hepatocytes has also been shown to be associated with decreased cytochrome P450 activity in experimental animals [39, 40]. However, it is unlikely that haemozoin accumulation alone could account for the results obtained with malaria-

infected livers in the present study. For example, the rapid decrease in $CL_{int,total}$ as percentage parasitaemia increases up to approximately 20%, followed by a less rapid decline with further increase in percentage parasitaemia, is difficult to explain solely on the basis of the intrahepatic accumulation of haemozoin. Such accumulation might be expected to produce a linear rather than exponential relationship between per cent parasitaemia and drug metabolizing activity. In addition, it has been shown previously [41] that malaria infection in rats results in a selective decrease in O-dealkylation of 7-ethoxycoumarin with no effect on N-dealkylation of aminopyrine. This selectivity is not easily explained if it is attributed to the effect of haemozoin accumulation only. The apparent specificity of inhibition of the metabolism of these compounds and phenacetin appears to be due to a selective inhibitor or inhibitors. Therefore, a combination of factors, including cytokines and haemozoin, might be responsible for the decreased clearance of phenacetin in malaria infection. We are currently investigating the relationship between systemic levels of TNF_α and other cytokines during malaria infection and following LPS administration to rats and the metabolism of phenacetin and other compounds which are substrates of well-characterized isozymes.

In conclusion, we have shown that both malaria infection and fever decrease phenacetin O-deethylation by rat liver microsomes. Decreased cytochrome P450 levels may account partly for this observation. There is need for further investigation of the factors involved since decreased phenacetin O-deethylase activity may be clinically important, especially in patients taking drugs such as theophylline, a widely used bronchodilator with a narrow therapeutic index.

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