Biochemical Pharmacology, Vol. 41, No. 11, pp. 1707–1711, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

THE EFFECT OF MALARIA INFECTION ON PARACETAMOL DISPOSITION IN THE RAT

SHARIF M. MANSOR,* GEOFFREY EDWARDS,*† PHILLIP J. ROBERTS* and STEPHEN A. WARD†‡

*Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX; and † Department of Parasitology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, U.K.

(*Received* 12 *November* 1990; *accepted* 14 *January* 1991)

Abstract—The effect of *Plasmodium berghei* infection, a rodent malarial model, on the dispostion of paracetamol (50 mg/kg, i.v.) was investigated in rats. Malaria infection (MI) resulted in a significant decrease in clearance (control: 21.6 ± 5.5 vs test: 11.8 ± 2.9 mL/min/kg, $P < 0.005$) with no change in volume of distribution and a significant prolongation of the elimination half-life (control: 30.7 ± 6.3 vs 53.3 ± 12.1 min, $P < 0.005$) of paracetamol in malaria infected rats. These changes were not related to the severity of MI. Malaria infection also decreased biliary clearance of paracetamol (64%) but not its glucuronide and sulphate conjugates in the bile compared with controls. In addition, glutathione conjugates were not detected in bile samples of malaria infected rats. These data suggest that important pathways of drug detoxification may be compromised by MI in a relatively selective fashion and the relevance of these findings to the clinical use of drugs eliminated by these pathways merits further study.

Disease states such as malaria infection (MI) may compromise hepatic function and affect drug metabolism and consequently pharmacological activity and toxicity [1, 2]. Most studies on the effect of MI on drug-metabolizing enzyme activity have been limited to Phase I reactions [3–5] and little is known of the effects of MI on Phase II conjugation. A limited study by Emudianughe *et al.* showed that MI could alter the conjugation of benzoic acid in mice [6]. The present study aims to investigate the effect of MI on Phase II metabolism using paracetamol as a probe drug for glucuronide, sulphate and glutathione conjugation reactions.

Paracetamol (4-hydroxyacetanilide) is a widely used non-prescription analgesic with few unwanted side effects. A hydroxyl group on the ring is the site of extensive conjugation through glucuronidation and sulphation to form non-toxic conjugates [7, 8]. However, oxidation by cytochrome P450 can also occur to form *N*-hydroxyl derivatives [9–11] which undergo spontaneous degradation to *N*-acety-*p*-benzoquinoneimine (NABQI), which is thought to be the toxic metabolite which reacts with tissue macromolecules causing damage [12–14] usually in situations following paracetamol overdosage [15]. Normally, NABQI is detoxified immediately by reaction with glutathione to form non-toxic conjugates. The multiple pathways of metabolism, availability of standard compounds and sensitive and selective analytical techniques make paracetamol a useful probe for the investigation of disease states such as malaria on phase II metabolism.

MATERIALS AND METHODS

Study design. Male T.H.W. mice (25–30 g) were infected with a chloroquine sensitive strain of *Plasmodium berghei* using an inoculation (i.p.) of 10^6 parasitized erythrocytes from previously infected animals. Blood samples were removed from the tail vein for daily monitoring of the degree of parasitaemia by evaluation of Giemsa (BDH, Poole, U.K.) stained thin blood films under a light microscope. When the degree of parasitaemia exceeded 25%, blood was collected from the brachial artery of each anaesthetized mouse and transferred into lithium heparin tubes (L.I.P. Ltd, Shipley, U.K.). Parasitized red cells (0.1 mL, 10^6) were then injected (i.p.) to young (5–8 weeks) male Sprague–Dawley rats (70–120 g). The development of MI was again determined by microscopic examination of Giemsa stained thin blood films obtained from daily tail bleeds. In addition, rectal temperature was monitored every 24 hr. When parasitaemia reached 25–40%, these rats (N = 6) were used to assess the disposition of paracetamol during malaria. Control male rats (N = 6) were non-infected and matched for age and weight. All animals were housed in a well ventilated room and allowed free access to food (Oxoid breeding diet, Oxoid Ltd.) and water until 12 hr prior to the study, when food was withdrawn.

Surgical procedures. Rats (100–140 g) were anaesthetized with sodium pentobarbitone (60 mg/kg i.p.). The left femoral vein, and right carotid artery were cannulated with polyethylene tubing (PE 50, Portex Ltd., Hythe, U.K.) for administration of paracetamol and collection of blood samples respectively. The trachea was also exposed and cannulated to assist breathing before cannulation of the bile duct with polyethylene tubing (PE25). Heparin sodium (400 units/kg i.v.) was injected into the femoral vein to prevent clotting in the cannula.

Drug administration. Paracetamol (50 mg/kg) in warm physiological saline solution (15 mg/mL) was

‡ To whom correspondence should be addressed.

BP 41:11-A

administered intravenously over a 2 min period. Arterial blood samples (150 μL) were removed predose and at 15, 30, 60, 90, 120, 150, 180, 210 and 240 min and plasma separated following centrifugation (13,000 rev/min.). An equivalent volume of saline was infused to replace blood lost from sampling. Bile was collected for 10 min before paracetamol administration into pre-weighed vials and at 0–15, 15–30, 30–60, 60–90, 90–120, 120–150, 150–180, 180–210 and 210–240 min. The vials were then re-weighed to determine bile volume. Bile and plasma samples were stored at −20° until analysis by HPLC.

Drug analysis. Concentrations of paracetamol and its glucuronide (PG), sulphate (PS) and glutathione (PGSH) metabolites in plasma and bile were determined using the HPLC method of Tjia [16] adaped from Howie *et al.* [17]. All standards were donated by Sterling Winthrop, Alnwick, U.K.

Pharmacokinetic analysis. The elimination rate constant (β) of paracetamol was obtained by least square regression analysis of the plasma concentration versus time curve and the elimination half-life ($T_{\frac{1}{2}}$) was calculated from the ratio $0.693/\beta$. Other pharmacokinetic parameters were calculated using standard model-independent formulae [18]. Biliary clearance of paracetamol was calculated from the cumulative amount of unchanged paracetamol in the bile over 3 hr divided by the area under the plasma concentration–time curves from 0 to 3 hr. Similar calculations were performed for PG and PS to obtain the biliary clearance of each conjugate. Statistical comparisons were made using Student's *t*-test for unpaired data, accepting $P < 0.05$ as significant. Data in the text and tables are presented as mean ± SD and graphically as mean ± SEM.

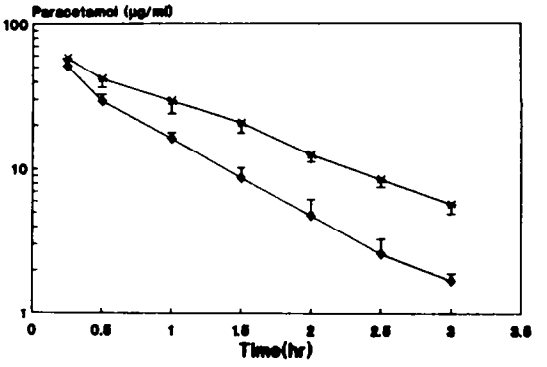

Fig. 1. The effects of malaria infection on the elimination of paracetamol in rat plasma. Mean values from six rats (◇) control, (*) malaria infected.

Table 1. The effect of malaria infection on the pharmacokinetic parameters of paracetamol in control and infected groups of rats

Parameter	Control	Infected
Cl (mL/min/kg)	21.6 ± 5.5	11.8 ± 2.9*
V_d (L/kg)	0.94 ± 0.26	0.90 ± 0.29
$T_{\frac{1}{2}}$ (min)	30.7 ± 6.3	53.3 ± 12.1*
AUC (μg min/mL)		
(a) PS	5941 ± 1055	5926 ± 904
(a) PG	2807 ± 617	1623 ± 324*

Values are mean (SD) for six rats.
* $P < 0.005$ significantly different compared to control.

RESULTS

Chromatography

The retention times of paracetamol, PG, PS, PGSH and metacetamol (internal standard) were 10, 4, 5.5, 8.5 and 13 min, respectively. The interassay coefficients of variation for paracetamol and the individual metabolites were as follows: paracetamol 5% at 20 μg/mL; PG 3.5% at 10 μg/mL; PS 4.5% at 10 μg/mL and PGSH 5.5% at 80 μg/mL. At these concentrations, the recoveries of paracetamol, PG, PS and PGSH were 90, 93, 95 and 89% respectively.

Malaria and paracetamol disposition

Percentage parasitaemias established at 3 and 5 days post-inoculation were 11.7 ± 2.0 and 31.5 ± 5.5% respectively. Rectal temperature measured at peak parasitaemia (4.0 ± 0.9 days post-inoculation) was 38.4 ± 0.7° in malaria infected rats compared with control rats (37.3 ± 0.5°, $P < 0.001$). Following the administration of paracetamol, plasma concentrations of the parent drug declined mono-exponentially (Fig. 1) in each groups. Significantly higher paracetamol concentrations were observed during malaria as indicated by the increase in $AUC_{0-\infty}$ (83%) in the infected rats. (control: 2452 ± 634 vs test: 4483 ± 1242 μgmin/mL, $P < 0.01$). Table 1 lists mean (SD) pharmacokinetic parameters for paracetamol in each group of rats. MI produced a significant decrease in clearance (Cl; 45%) without any change in volume of distribution (V_d). This resulted in a significant increase in elimination half-life ($T_{\frac{1}{2}}$; 74%) in malaria infected rats. The metabolite profile of paracetamol in plasma is presented in Fig. 2. These profiles suggest that the PS conjugate as the predominant metabolite in the plasma. In addition, MI resulted in a significant decrease in PG concentrations as indicated by the decrease in $AUC_{0-3\,hr}$ (42%) in infected rats (Table 1).

Biliary clearance of paracetamol and its metabolite

MI resulted in significant decrease in the biliary Cl of paracetamol (64%) compared with controls. No significant difference was observed in biliary Cl of PG and PS. Interestingly, PGSH was not detected in the bile of malaria infected rats (Table 2). Further, a significantly smaller volume of bile was produced in malaria infected rats compared with controls (control: 2.65 ± 0.67 vs test: 1.88 ± 0.27 mL, $P < 0.05$).

DISCUSSION

The disposition of paracetamol, comprising predominantly phase II processes differs markedly in malaria infected rats from control, animals.

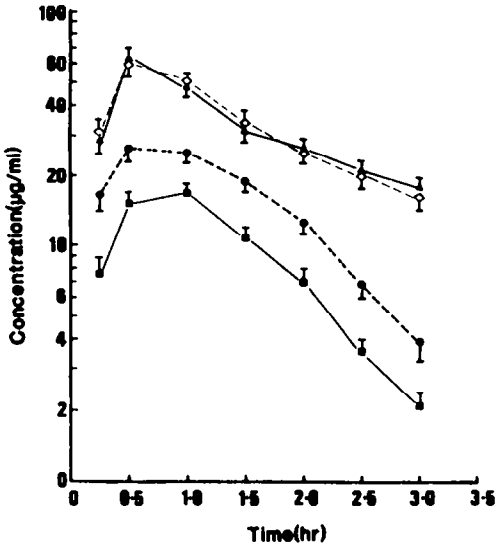

Fig. 2. The effect of malaria infection on the concentration of paracetamol metabolites (paracetamol glucuronide [PG] and paracetamol sulphate [PS]) in rat plasma. Mean values from six rats. (◇) PS control, (▲) PS malaria infected, (●) PG control, (■) PG malaria infected.

Numerous studies investigating the erythrocytic phase of MI have demonstrated decreased hepatic cytochrome P450 and associated monoxygenase activity in rodents [2–5]. In the case of paracetamol, however, only a small proportion of the dose (10%) undergoes oxidation by cytochrome P450 to form a reactive metabolite which normally is detoxified immediately by conjugation with glutathione. Hence, the marked decrease in monoxygenase activities in livers of infected rats cannot account for the large changes in drug clearance. Since paracetamol is metabolized mainly via glucuronidation and sulphation, the search for an explanation must focus on the impairment of conjugation reactions. Interestingly, MI appears selectively to reduce the formation of PG but not PS. Sulphotransferase is a cytosolic enzyme while uridine diphosphate-glucuronosyl transferase is associated with the smooth-surfaced endoplasmic reticulum (SER). Ultrastructurally, the hepatocytes show changes during malaria [19, 20]. The studies of Rosan *et al.* [21] have reported alterations in SER and mitochondria but not the Golgi and the rough-surfaced endoplasmic reticulum suggesting that MI selectively can affect different organelles. Since the SER is importantly involved in the biotransformation of xenobiotics, impairment of its physiological function may contribute to reduced PG formation. Sulphation, the other conjugation pathway, may not be affected by MI since the enzyme involved has a different subcellular localisation. In addition, bile formation requires normally functioning of the hepatocytes [22] and a facility for glucuronidation which is unlikely to be preserved during malaria. Thus, specific alterations of hepatic ultrastructure, particularly the SER and mitochondria, may contribute to the significant decrease in bile volume observed in the malaria infected rat as compared to control. In addition, the carbohydrate metabolism of the host is thought to be disturbed during malaria [23, 24]. Livers of parasitized animals have shown decreased ability to synthesises glycogen from exogenous glucose [25]. Further, microscopic studies have revealed that cytoplasmic glycogen is depleted in mice infected with *P. berghei*. These factors may restrict the availability of uridine diphosphate-glucuronic acid for conjugation with paracetamol. Studies by Hjelle *et al.* [26] reported decreased hepatic concentration of adenosine 3′-phosphate 5′-phosphosulphate and uridine diphosphate-glucuronic acid after the acute administration of paracetamol (> 150 mg/kg). This phenomenon, depletion of co-substrate availability, is unlikely to be operating at the dosage of paracetamol (50 mg/kg) used in our study. Finally, it has been reported that in addition to causing a decrease in the hepatic monoxygenase activity of infected mice [28] the concentration of β-glucuronidase in the liver and spleen is increased in schistosomiasis [27]. This increase in β-glucuronidase may cause a breakdown of the already formed PG in the liver. Therefore the decrease in PG may be due to a reduction in its formation and (or) breakdown of the already formed PG in the liver. Reduction of glucuronic acid conjugation of the anthelmintic aryclonazide has also been reported in rats with *Fasciola hepatica* infections [30].

In contrast, to controls, glutathione conjugates were not detected in bile samples of malaria infected rats. As mentioned above, impairment of microsomal drug metabolizing activities of the host as the result of MI may inhibit or reduce the oxidation of

Table 2. Biliary excretion (% dose excreted in 180 min) and biliary clearance (mL/min) of paracetamol and its individual metabolites in control and infected groups of rats

	Biliary excretion		Biliary clearance	
	Control	Infected	Control	Infected
PG	7.15 ± 1.06	3.75 ± 1.07*	0.165 ± 0.064	0.138 ± 0.027
PS	5.00 ± 0.79	5.02 ± 0.82	0.055 ± 0.021	0.050 ± 0.011
P	0.77 ± 0.08	0.48 ± 0.15†	0.022 ± 0.009	0.008 ± 0.003†
PGSH	2.04 ± 0.72	ND		

Values are mean (SD) for six rats.
* $P < 0.001$, † $P < 0.005$ significantly different compared to control. ND, not detected.

paracetamol by cytochrome P450 to NABQI and thus restricting conjugation with glutathione. Drugs such as captopril, an angiotensin-converting enzyme (ACE) inhibitor which undergoes direct glutathione conjugation [31] could be used to determine if the impairment seen in the present study is a result of reduced monoxygenase acitivity or a direct action on the glutathione pathway. An alternative explanation for the absence of glutathione conjugates might relate to tissue damage and organ dysfunction resulting from anoxia due to circulatory disturbances [32]. Studies of oxygen carriage and delivery by blood in simian and rodent malarias suggests anoxia may not be the main cause of tissue damage despite the heavy oxygen demands of a rapidly growing parasite. However, at the late stage of the disease, impairment of oxygen transport as a result of haemolysis is likely to occur [33]. In addition, electron microscopy has revealed structural alterations and a marked diminuation of hepatocyte mitochondria [21, 34]. Such structural changes in liver mitochondria have been correlated with biochemical changes (i.e. abnormal respiration and oxidative phosphorylation) in mice infected with *P. berghei* [35, 36]. Evidence for local anoxia or stagnant anoxia has been demonstrated in monkeys with *P. knowlesi* malaria. There was marked constriction of the hepatic portal venous tree, the renal afferent vessels and the microcirculation of the small intestine. These were probable contributing mechanisms to tissue damage and organ dysfunction [37, 38]. Therefore tissue anoxia produced either by the cells' inability to use oxygen or by an inadequate oxygen supply is the principal cause of shock and eventual death in animals and humans with malaria. A recent study with rat hepatocytes suggests that glutathione synthesis is oxygen dependent [39, 40]. This, coupled with the above observations may offer some explanation as to the lack of glutathione conjugation derivatives as a result of disturbance of glutathione synthesis due to limited ATP production under anoxic conditions during MI.

In summary, MI alters the disposition of paracetamol in the rat and impairs selectively PG and PGSH formation but not PS. In order to have a better understanding of the selective impairment of these conjugation pathways, compounds should be selected for experimental study that are not metabolized by oxidation or sulphate conjugation. Probenecid, a uricosuric agent which undergoes only glucuronide conjugation [41] could be ideal for this purpose. The disposition during malaria of paracetamol and other drugs which undergo extensive conjugation now merits investigation.

Acknowledgements—The authors wish to thank Maureen Harwood for typing this manuscript. SMM is supported by the Academic Staff Training Scheme of the National Drug Research Centre, Universiti Sains Malaysia.

REFERENCES

1. Mansor SM, Ward SA, Edwards G, Hoaksey PE and Breckenridge AM, The effect of malaria infection on the disposition of quinine and quinidine in the rat isolated perfused liver preparation. *J Pharm Pharmacol* **42**: 428–432, 1990.
2. McCarthy JS, Furner RL, Van Dyke K and Stitzel RE, Effect of malaria infection on host microsomal drug metabolising enzymes. *Biochem Pharmacol* **19**: 1341–1349, 1970.
3. Saxena N, Saxena A, Dutta GP, Ghatak S and Pandey VC, Effect of *Plasmodium yoelli nigeriensis* infection and chloroquine on the hepatic mixed function oxidase system of mice. *Mol Biochem Parasitol* **24**: 283–287, 1987.
4. Alvares AP, Ueng TH, Scheibel LW and Hollingdale MR, Impairment of hepatic cytochrome P450 dependent monoxygenases by malaria parasite. *Plasmodium berghei. Mol Biochem Parasitol* **13**: 277–282, 1984.
5. Sharma OP, Sukla RP, Singh C and Sen AB, Drug metabolising enzymes in mouse liver infected with *Plasmodium berghei. Ind J Parasitol* **2**: 29–30, 1978.
6. Emudianughe TS, Bickel QD, Taylor MG and Andrews B, Effect of *Plasmodium berghei* infection on benzoic acid metabolism in mice. *Experientia* **41**: 1407–1409, 1985.
7. Watari M, Iwai M and Kaneniwa N, Pharmacokinetic study of the fate of acetaminophen and its conjugate in rats. *J Pharmacokinet Biopharm* **11**: 245–272, 1983.
8. Cummings AJ, King ML and Martin BK, A kinetic study of drug eliminatin: The excretion of paracetamol and its metabolites in man. *Br J Pharmacol* **29**: 150–157, 1967.
9. Mitchell JR, Jollow DJ, Potter WZ, Davis DC, Gillette JR and Brodie BB, Acetaminophen-induced hepatic necrosis I. Role of drug metabolism. *J Pharmacol Exp Ther* **187**: 185–194, 1973.
10. Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR and Brodie BB, Acetaminophen-induced hepatic necrosis. II Role of covalent binding *in vivo J Pharmacol Exp Ther* **187**: 195–202, 1973.
11. Potter WZ, Davis DC, Mitchell JR, Jollow DJ, Gillette JR and Brodie BB, Acetaminophen-induced hepatic necrosis. III Cytochrome P450 mediated covalent binding *in vitro J Pharmacol Exp Ther* **187**: 203–210, 1973.
12. Corcoran GB, Mitchell JR, Vaishnav YN and Horning EC, Evidence that acetaminophen and *N*-hydroxyacetaminophen form a common arylating intermediate, *N*-acetyl-*p*-benzoquinone. *Mol Pharmacol* **18**: 536–542, 1980.
13. Rollins DE, Glaumann H, Moldeus P and Rane A, Acetaminophen: potentially toxic metabolite formed by human fetal and adult liver microsomes and isolated fetal liver cells. *Science* **205**: 1414–1415, 1979.
14. Davis DC, Potter WZ, Jollow DJ and Mitchell JR, Species differences in hepatic glutathione depletion, covalent binding and hepatic necrosis after acetaminophen. *Life Sci* **14**: 2099–2109, 1974.
15. Slattery JT and Levy G, Acetaminophen kinetics in acutely poisoned patients. *Clin Pharmacol Ther* **25**: 184–195, 1979.
16. Tjia JF, Inhibition of drug metabolism *in vitro* and *in vivo*. Ph.D. Thesis, University of Liverpool, U.K., 1987.
17. Howie D, Adriaenssens PI and Prescott LF, Paracetamol metabolism following overdosage: application of HPLC. *J Pharm Pharmacol* **29**: 235–237, 1977.
18. Rowland M and Tozer T, *Clinical Pharmacokinetics: Concepts and Application*. pp. 24–25. Lea and Febiger, Philadelphia, 1989.
19. Jervis HR, MacCullum DK and Sprinz H, Experimental *Plasmodium berghei* infection in the hamster. Its effect on the liver. *Arch Pathol* **86**: 328–337, 1968.
20. Jervis HR, Sprinz H, Johnson AJ and Wellde BT, Experimental infection with *Plasmodium falciparum* in *Aotus* monkeys. II Observation on host pathology. *Am J Trop Med Hyg* **21**: 272–281, 1972.

21. Rosen S, Roycroft DW, Hano JE and Barry KG, The liver in malaria. *Arch Pathol* **83**: 271–277, 1967.
22. Javitt NB, Hepatic bile formation. *N Eng J Med* **295**: 1464–1469, 1976.
23. Fulton JD, Experiments on the utilization of sugars by malaria parasites. (*Plasmodium knowlesi*). *Ann Trop Med Parasitol* **33**: 217–227, 1939.
24. Sinton JA and Hughes TA, The functional capacity of the liver in malaria, with special reference to the levulose tolerance test. *Indian J Med Res* **12**: 409–422, 1924.
25. Mercado TI and von Brand T, Glycogen studies on white rats infected with *Plasmodium berghei*. *Exp Parasitol* **3**: 259–266, 1954.
26. Hjelle JJ, Hazelton GA and Klaassen CD, Acetaminophen decreases adenosine 3′-phosphate 5′-phosphosulphate and uridine diphosphoglucuronic acid in rat liver. *Drug Metab Dispos* **13**: 35–41, 1985.
27. Saad AA, Farag HF, El-Zoghby SM, Mustafa MH and Girgis LH, beta-Glucuronidase activity in liver and spleen homogenates of mice experimentally infected with *Schistosoma mansoni*. *Acta Vitaminol Enzymol (Milano)* **31**: 179–182, 1977.
28. Cha Y-N and Edwards R, Effect of *Schistosoma mansoni* infection on the hepatic drug-metabolizing capacity of mice. *J Pharmacol Exp Ther* **199**: 432–440, 1976.
29. Homewood CA, Jewsbury JM and Chance ML, The pigment formed during haemoglobin digestion by malaria and schistosomal parasites. *Comp Biochem Physiol* **43**: 517–523, 1972.
30. Maffei Facino R, Carini M and Genchi C, Decrease in hepatic microsomal UDP-glucuronosyl-transferase activity in rats and cattle with fascioliasis: impaired *in vitro* glucuronidation of oxyclozanide. *Toxicol Lett* **26**: 65–71, 1985.
31. Migdalof BH, Antonaccio MJ, McKinstry DN, Singhvi SM, Lan SJ, Egli P and Kripalani KJ, Captopril: pharmacology, metabolism and disposition. *Drug Metab Rev* **15**: 841–869, 1984.
32. Maegraith BG, *Pathological Processes in Malaria and Blackwater Fever*. pp. 366–367. Blackwell, Oxford, 1948.
33. Maegraith BG, Jones ES and Andrews WHH, Pathological processes in malaria: progress report. *Trans R Soc Trop Med Hyg* **45**: 15–42, 1951.
34. Gutierrez Y, Aikawa M, Fremount HN and Sterling CR, Experimental infection of *Aotus* monkeys with *Plasmodium falciparum*: Light and electron microscopic changes. *Ann Trop Med Parasitol* **70**: 25–44, 1976.
35. Riley MV and Deegan T, The effect of *Plasmodium berghei* malaria on mouse-liver mitochondria. *Biochem J* **76**: 41–46, 1960.
36. Riley MV and Maegraith BG, Changes in the metabolism of liver mitochondria in mice infected with rapid acute *Plasmodium berghei* malaria. *Ann Trop Med Parasitol* **56**: 473–482, 1962.
37. Migasena P and Maegraith BG, Intestinal absorption in malaria I. The absorption of amino acid (AIB-I-14C) across the gut membrane in normal and in *Plasmodium knowlesi*-infected monkeys. *Ann Trop Med Parasitol* **63**: 439–448, 1969.
38. Skirrow MB, Chongsuphajaisiddhi T and Maegraith BG, The circulation in malaria II. Portal angiography in monkeys (*Macaca mulatta*) infected with *Plasmodium knowlesi* and in shock following manipulation of the gut. *Ann Trop Med Parasitol* **58**: 502–510, 1964.
39. Shan X, Aw TY, Shapira R and Jones DP, Oxygen dependence of glutathione synthesis in hepatocytes. *Toxicol Appl Pharmacol* **101**: 261–270, 1989.
40. Aw TY and Jones DP, Control of glucuronidation during hypoxia. Limitation by UDP-glucose pyrophosphorylase. *Biochem J* **219**: 707–712, 1984.
41. Conway WD and Melethil S, Excretion of probenecid and its metabolites in bile and urine of rats. *J Pharm Sci* **63**: 1551–1554, 1974.