two days after nerves reach the distal limb tip. This result may indicate a G_1 block as the axolotl blastema cell cycle length is 40–53 h[21,22]. In the present study the MI_{lab} increased to control levels upon complete reinnervation (days 12–14). Days 12–14 in reinnervated limbs represented early blastema stages which occured on days 5–7 in innervated limbs. The MI_{lab} for these early blastema stages was virtually identical in reinnervated and innervated limbs (fig. 2). Thus, reinnervation on day 9 can be correlated with the resumption of mitosis in the blocked population by day 12, leading to formation of a blastema. The labeled population contributed significantly to the blastemal cell population as evidenced by the greater than 50% LI on day 14 (fig. 1). The concept of cell rescue using continuous labeling and the MI_{lab} parameter can now be utilized in a valid in vivo bioassay for NTF, one which involves a specific regeneration response – the contribution of previously blocked cells to the reinnervation blastema. The rescue bioassay may not be sensitive enough or quick enough for efficient use as an aid to purifying NTF from nerve or brain homogenates, yet it provides an excellent system for the definitive in vivo test of neurotrophic activity for any candidate molecule.

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23 Note that no MI_{lab} should be seen on day 4 after the 2-h pulse of ^{3}H-T because the length of G_2 is greater than 2 h for axolotl blastema cells (G_2 = 5 h[21,22]).
24 See also Petrosky et al.[17], Olsen and Tassava[18] and Olsen et al.[19].

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Effect of *Plasmodium berghei* infection on benzoic acid metabolism in mice

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Summary. The metabolism of benzoic acid was studied in *Plasmodium berghei* infected mice both in vitro and in vivo. Results of in vitro studies showed a considerable decrease in the ability of the infected liver to detoxify benzoic acid by hippuric acid formation. The in vivo study showed that hippuric acid formation decreases with increasing parasitemia and the emergence of benzoyl-glucoronide. This new pathway stops operating with further increase in parasitemia.
Key words. Mouse liver; malaria infection; benzoic acid detoxification; glucuronic acid conjugation; hippuric acid.

Pathological changes have been shown to occur in tissues of host animals after infection by the malarial parasites. Some of the organs affected are the spleen, kidney, liver and the adrenal glands. The liver shows a number of morphological and biochemical changes, the magnitude of which appear to be related to the severity of the infection. Studies of Mercado and Von Brand[8] and Singer[14] showed that livers of parasitized animal exhibited a decreased ability to synthesize glycogen from exogenous glucose and a reduction in coenzyme A content.

The studies of Rosen et al.[10] have shown changes in hepatic ultrastructure caused by malarial parasite. Electron microscopy has revealed alterations in mitochondrial appearance. Sharma et al.[13] have noticed some changes in the biochemical parameters while Patwari et al.[9] showed alteration in serum aspartate transaminase (Serum AST: SGOT) serum alanine transaminase (Serum ALT: SGPT) and alkaline phosphatase in children with *Plasmodium vivax* malaria.

McCarthy et al.[7] studied the effect of malaria infection on drug-metabolizing enzyme activity. This study was limited to phase I drug metabolism. There are possibilities that malaria infection may not affect the phase II drug-metabolizing enzymes. The metabolism of benzoic acid, a carboxylic acid, involves the conjugation of the acid with glycine to form hippuric acid. At low doses (below 100 mg/kg b.wt of mouse), the main metabolite is hippuric acid while at higher doses, a secondary pathway emerges; conjugation with glucuronic acid. When these two pathways are saturated by a further higher dose, benzoic acid is eliminated as the unchanged compound in addition to the glycine and glucuronic acid conjugates. Benzoic acid is here used as a test compound for the enzymes involved in these two pathways.

Materials and methods. The following compounds were purchased: [carboxyl-^{14}C] benzoic acid (Sp. act. 20 mCi/mmole) (Radiochemical Centre Amersham, UK), coenzyme A (CoA), adenosine triphosphate (ATP), glutathione and glycine, hippuric acid and benzoic acid, β-glucuronidase (Sigma Chemical Co. Surbiton, UK).

Infection of mice. Male TFW mice purchased from a local supplier were infected by caudal vein injection of *plasmodium berghei* (N78-strain). The amount of parasite injected was adjusted in such a way that each animal received approximately 10^6 infected red blood cells.

In vitro studies. Animals were killed by a blow to the head and the livers rapidly excised, weighed and homogenized in 5 vol. of

Table 1. Hippuric acid formation in noninfected and *Plasmodium berghei* infected mouse liver

Infection time	Parasitemia (%)	No. of animals	Liver wt	Hippuric acid (%)	Hippuric acid formation (nmoles 20 mg/60 min)
Noninfected	0	5	1.6 (1.5–1.7)	37.5 (32.7–43.7)	74.9 (64.2–87.2)
Infected					
+ 48 h	8.7 (6.4–10.4)	5	1.8 (1.7–1.8)	21.13 (15.6–24.9)	42.27 (31.2–49.8)
+ 72 h	79.4 (77.2–81.5)	5	2.3 (2.1–2.5)	15.8 (14.2–17.9)	31.6 (28.4–35.8)
+ 96 h	100	5	2.17 (2.1–2.3)	9.4 (9–10.1)	18.8 (17.8–20.2)

ice-cold, 1.15% KCl. A blood film from each infected animals was made and percent parasitemia determined[7]. Hippuric acid formation was assayed as described by Caldwell et al.[1]. Typical incubation mixtures (1 ml) contained [^{14}c]-benzoic acid (200 nmoles; 0.2 μCi), CoA (100 nmoles) and glycine (60 μmoles) with tissue homogenate (equiv. to 20 mg tissue) in 0.2 M tris buffer, pH 8.0. Incubation was carried out at 37 °C for 60 min in a water bath shaker and the reaction stopped by addition of acetone (1 ml) containing carrier hippuric acid and benzoic acid (5 mg/ml of each). After centrifugation to remove denatured protein, 20–30 μl of the supernatant was chromatographed on a Silica Gel GF_{254} aluminium backed plate (16 × 2 cm) (Merck AG, Darmstadt, FRG) and developed as described by Caldwell et al.[1]. Benzoic and hippuric acids were employed as standards. The bands corresponding to benzoic and hippuric acids were located as dark quenching areas under UV light and these areas were cut out and placed in scintillation vials containing 5 ml of Luma scintillation cocktail. Radioactivity was determined using an LKB Scintillation Spectrometer.

In vivo studies. Control (noninfected) and infected animals were given benzoic acid as sodium salt (75 mg/kg b.wt) and housed in groups[2–5] in metabolic cages. Urine was collected for 24 h over mercuric chloride. Urinary ^{14}C was determined as described above. The above procedure was repeated 48 h, 72 h and 96 h after inoculation. A blood film was made at each time point to determine percent parasitemia.

Urine samples (0.1 m) were chromatographed on Silica Gel GF_{254} aluminium backed plates (16 × 2 cm) (Merck AG, Darmstadt, FRG) and developed as described by French[5]. The bands corresponding to benzoic acid, hippuric acid and benzoyl glucoronide were located as dark quenching areas under UV light and these areas were cut out, and their radioactivity determined as previously described.

Benzoyl glucuronide was further identified as described by Emudianughe et al.[4].

Results. Table 1 gives the results of in vitro studies of control and animals infected with *Plasmodium berghei.* The liver weight increases with increase in parasitemia; the range is from an average of 1.6 g in control liver to 2.17 g in an animal with 100% parasitemia. The ability to form hippuric acid decreases with increase in parasitemia (table 1).

The excretion and recovery of metabolites from urine over a 24 h period for both control and infected animals are shown in table 2. Over this period most of the administered benzoic acid was recovered in both the control and experimental animals; 99.4% in the control and 92.6% in 100% parasitemia. At time point 48 h there was a reduction of hippuric acid 90.3% as compared to the control, which was 100% hippuric acid. There was also an emergence of glucuronic acid conjugate at a level of 9.7%. With increase in parasitemia there is a considerable decrease in hippuric acid formation and a corresponding increase in glucuronide formation. At 100% parasitemia, that is, at time point 95 h, hippuric acid formation decreases to 44.9% of the control value and glucuronic acid also decreases. There is a considerable quantity of benzoic acid being excreted at 100% parasitemia.

Discussion. Various studies have shown that the primary metabolic pathway of benzoic acid is by conjugation with glycine to form hippuric acid. At higher doses, a secondary pathway (i.e. conjugation with glucuronic acid) emerges. Further overdose causes benzoic acid to be eliminated as the unchanged compound in addition to glycine and glucuronic acid conjugates.

The present results of in vitro studies show a reduction of 46.6% in hippuric acid formation 48 h after infection and 74.9% 96 h after infection. These reductions parallel increased parasitemia. Other studies on drug metabolizing enzymes, especially the phase 1 reactions, show a decrease in enzymes involved in these reactions which parallels increase in parasitemia[7]. These results can be explained on the following hypothesis a) that there is a reduction in the synthesis of acyl CoA synthetase and glycine-N-acyl-transferase, the two enzymes necessary for glycine conjugation or b) that a mechanism is activated which partially inhibits one or both enzymes during hippuric acid formation. Singer[14] has shown that during malaria infection the CoA content of liver is reduced. This means that the amount of CoA available for activation of benzoic acid to benzoyl CoA is reduced and it thereby becomes a limiting factor.

In the present study, it has been shown that hippuric acid formation decreases with increase in parasitemia and a switch to glucuronic acid conjugation occurs which is inhibited as there is an increase in parasitemia. Studies on the effect of schistosomiasis on hepatic drug metabolism of mice showed that UDP-glucuronyl transferase was reduced considerably[2]. This reduction during schistosome infection may also occur in malaria infection, as in both cases hematin is deposited in the liver[6]. A reduction in UDP-glucuronyl transferase will decrease the ability of the animal to use this metabolic pathway. It has also been shown that schistosome infection causes an increase in the level of β-glucuronidase both in the liver and in the urinary bladder[11, 12]. This increase may cause a breakdown of the already-formed benzoyl glucuronide in either the liver or the bladder or in both organs. The decrease therefore in glucuronide formation may be due to a reduction in its formation and/or a breakdown in the liver or urinary bladder of already formed glucuronide.

The significance of conjugation has been reviewed by Caldwell[2]

Table 2. Urinary ^{14}C recovery in urine (%) and the various benzoic acid metabolites present in mouse dosed with ^{14}C benzoic acid 75 mg/kg

Infection time	Parasitemia (%)	No. of groups	^{14}C urinary recovery (%)	Hippuric acid (%)	Glucuronic acid conjugate	Benzoic acid
Noninfected	0	3	99.4 (98.3–100.9)	100	–	–
Infected challenged with benzoic acid						
+ 48 h	8.5 (6.4–10.1)	3	80.8 (73.3–90.2)	90.3 (90.2–90.4)	9.7 (9.6–9.8)	–
+ 72 h	79.0 (77.2–81.5)	3	88.6 (79.6–99.8)	66.4 (59.4–73.7)	33.9 (27.3–40.6)	–
+ 96 h	100	3	92.6 (70.0–105.8)	44.9 (36.77–49.4)	9.8 (7.3–12.5)	45.2 (41.6–50.8)

and this review shows that the major aim of this synthetic process is the deactivation of the drug. This means that the inhibition of any of the enzymes necessary for this process may cause the drug to reach a toxic blood level. Also, it has been shown that some drugs are more active than the parent compounds, for example, morphine-6-glucuronide is more active than the parent drug. Inhibition of glucuronide formation therefore reduces the potency of the drug. The probable increase of β-glucuronidase in the bladder may cause the breakdown of glucuronide to an active unchanged compound which may cause the drug to attain a toxic level in this organ.

In conclusion, the results obtained suggest that during malarial infection the ability of the liver to deactivate xenobiotics is reduced, tending towards overdosing of the animal. Further investigation is needed to clear up some unanswered questions.

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Benzamide potentiation of behavioral apomorphine-induced effects; mechanism involved

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Summary. A new N-pyridinyl benzamide was found to potentiate strongly the effects of apomorphine on the motility of reserpinized mice and on circling behavior. Since dopaminergic agonist activity could not account for this potentiation, involvement of α_2-adrenergic agonist activity provided the only consistent explanation.
Key words. Apomorphine-induced behavior; N-pyridinyl benzamide; α_2-adrenergic agonists.

Because of its direct dopaminergic agonist effect, apomorphine elicits characteristic behavioral modifications in rodents[2,3]. Administration of reserpine or 6-OH dopamine leads to development of hypersensitivity to dopaminergic agonists and allows more specific investigations about related central phenomena. For instance, the reversal of reserpine-induced akinesia indicates activation of postsynaptic dopamine receptors[4], and a distinction between a direct or indirect mechanism can be determined by observing the circling behavior elicited in unilaterally striatal 6-OH dopamine-lesioned mice[5]. In addition, the circling behavior and/or hypermotility induced by apomorphine can be increased by drugs such as caffeine, central anticholinergics, parachlorophenylalanine and clonidine[6-9].

The N-(4,6-dimethyl 2-pyridinyl) 3-chloro benzamide (N-PCB; fig. 1) like other pyridinyl benzamide derivatives[10] exhibits some antiinflammatory properties. However, its structural features led us to investigate its possible effects on dopaminergic transmission. In our work, potentiation of the above-mentioned apomorphine effects was observed with N-PCB, and the mechanism involved was studied.

Materials and methods. Male Swiss C.F. mice weighing 25 ± 5 g, kept in a quiet room at 21 ± 1 °C with artificial lighting (lights on between 07.00 and 19.00 h), were used for motility and circling behavior experiments. Food and water were given ad libitum. All drugs were injected i.p., except N-PCB which was administered orally in a 10% acacia gum aqueous suspension as it is insoluble in water. All controls also received this vehicle (10 ml/kg). Results were compared using Student's t-test.

Motility experiments on reserpinized mice were performed between 09.00 and 13.00 h in a Boissier photoactimeter (Apelex, France). Each individual activity cage (L = 25.5 cm, W = 20 cm, H = 9 cm) was fitted with two photoelectric units with IR lights located 1 cm above the floor of the cage in the middle of each side. These cages were placed in a closed compartment and connected to a counter. Motility was expressed as the number of interruptions of photocell beams. Mice received apomorphine hydrochloride (2 mg/kg) 20 h after reserpine (10 mg/kg) and were placed one per cage. The motility recording started immediately after apomorphine injection and lasted 60 min. N-PCB

Table 1. Effects of N-PCB, yohimbine and clonidine on apomorphine-induced motility and circling behavior

	Motility of reserpinized animals (counts per 60 min)	Circling behavior (turns per 2 min)
Apomorphine	435 ± 44 (40)	14.5 ± 1.1 (23)
Apomorphine and N-PCB[d]	721 ± 139[c] (27)	19.0 ± 1.9[a] (16)
Apomorphine and yohimbine[d]	424 ± 53 (19)	16.0 ± 2.7 (8)
Apomorphine and clonidine[d]	704 ± 106[b] (12)	26.3 ± 2.1[c] (7)
Apomorphine, clonidine and yohimbine	436 ± 87[e] (13)	14.6 ± 4.4[f] (5)

Values are means ± SEM, with number of animals in parentheses. [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.005$, compared to apomorphine-treated mice, [d]N-PCB, clonidine or yohimbine given alone, without apomorphine, had no significant effect either on motility or on circling behavior at the doses used; these results are in agreement with previous works[5,6,9,16]. [e]$p < 0.10$, [f]$p < 0.01$, compared to mice treated with both apomorphine and clonidine.