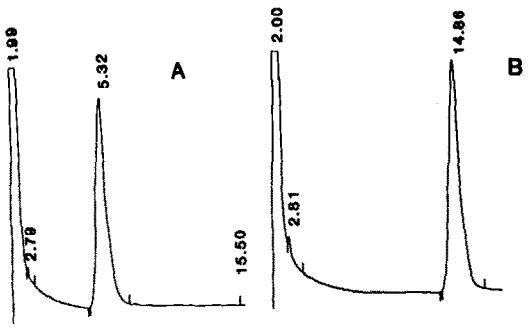

Fig. 1. HPLC separation of 4-hydroxybiphenyl and 4-hydroxybiphenyl-β-D-glucuronide. After extraction of the 4-HB GT reaction mixture with $CHCl_3$, aliquots (20 μl) of the aqueous phase were separated by HPLC as described in Materials and methods. The chromatogram shown in panel A received no additional treatment, whereas the chromatogram in panel B was obtained after incubation of the aqueous phase with β-glucuronidase.

ethanol does not appear similar to that produced by the group 1 inducer 3-MC or the group 2 inducer phenobarbital, since GT activities toward 4-MU (group 1) and 4-HB (group 2) were enhanced only slightly by ethanol feeding. Furthermore, chronic ethanol feeding was shown to have no effect on GT activity toward bilirubin (group 3) in Sprague–Dawley rats [10].

GT activities toward PP and APAP were increased markedly by ethanol administration, supporting the suggestion that these two substrates are glucuronidated by the same form of GT [19]. However, there is still much controversy as to which group of GT acceptors PP belongs, since PP has been classified as a group 1 [20–22] and a group 2 [23] substrate. Results of the present study indicate that PP is not a typical group 1 or group 2 substrate, thereby providing further evidence for functional heterogeneity within these enzymes forms. Moreover, the differential degree of induction within the group 1 substrates (3-HBP and 4-MU) also tends to support the contention of functional heterogeneity.

The inducing agents 3-MC [5] and β-napthoflavone [13] have been shown to produce a coordinated increase (2-fold) in microsomal monooxygenase and GT activities. Two weeks of ethanol feeding was shown previously to cause a 2-fold increase in cytochrome P-450 levels [24] and a 6-fold increase in the microsomal oxidation of *p*-nitrophenol to 4-NTC [4]. In the present study, GT activity toward 4-NTC was enhanced by only 52%, indicating that ethanol feeding caused a large increase in PNP monooxygenation without a coordinated increase in 4-NTC GT activity.

In conclusion, 2 weeks of ethanol feeding produced various degrees of induction of GT activity. However, with the exception of enzyme activity toward PP and APAP, GT activity was increased only moderately or slightly by ethanol.

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Impairment of the hepatic microsomal drug-metabolizing system in rats parasitized with *Nippostrongylus brasiliensis*

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Nippostrongylus brasiliensis, an intestinal nematode parasite of rats, causes a serious and even fatal disease called nippostrongylosis. The gross pathology of this disease is accompanied by marked physiological and biochemical derangements in the host [1–3]. The hepatic microsomal mixed-function oxidase (MFO) system plays an important role in the metabolism of a variety of xenobiotics and endogenous compounds, such as steroids, fatty acids and

prostaglandins. This system is of vital importance in chemotherapy since the efficacy of a drug is partly related to the mechanisms involved in its biotransformation and excretion. Environmental and physiological stresses have been shown to alter the drug-metabolizing capacity of the host [4]. Earlier investigations have indicated impairment of the hepatic microsomal MFO system during various parasitic infestations [5–11]. The present investigations were undertaken to study the changes in the hepatic microsomal MFO system in rats during experimental infection with *N. brasiliensis*.

Materials and methods

Parasite and experimental hosts. The strain of *N. brasiliensis* used in the present study was originally obtained from Wellcome Laboratory, Beckenham, U.K. Healthy male albino rats (Duckrey strain; average weight 40–60 g), maintained in an air-conditioned room (temperature 24 ± 1°), were employed. Two thousand motile L_3 larvae of *N. brasiliensis* contained in 0.2 ml inoculum were administered subcutaneously to each albino rat. Peak changes were observed in the host 9 days after inoculation.*

Animals were killed at the peak of infection. Livers were excised, washed with cold normal saline, and homogenized (20%, w/v) separately in chilled KCl (150 mM) buffered with phosphate buffer (100 mM, pH 7.4). The homogenate was centrifuged at 11,000 *g* (20 min) at 0–5°, and aliquots of the supernatant fraction were used for the assay of aniline hydroxylase [12] and aminopyrine *N*-demethylase [13], while the remaining portion was used for the preparation of microsomes [14]. Cytochrome b_5 reductase [15] NADPH–cytochrome *c* reductase [16], cytochrome P-450, cytochrome b_5 and heme [17] were estimated in the isolated microsomal preparations. Protein was measured according to the method of Lowry *et al.* [18] using bovine serum albumin (BSA) as standard. Results were analyzed by Student's *t*-test, and P values less than 5% (0.05) were considered significant.

Results

Infection of rats with *N. brasiliensis* led to considerable reduction (23%) in body weight, whereas there was no significant reduction in fresh or dry weight of liver. Total and microsomal protein contents of liver remained unaltered during this infection. However, significant changes were observed in some components of the hepatic MFO system. The activities of aniline hydroxylase and aminopyrine *N*-demethylase in hepatic microsomes from infected rats were approximately half of those in the microsomes from the controls. Cytochrome P-450 and heme contents also decreased to the same extent. NADPH-cytochrome *c* reductase and cytochrome b_5 contents did not change significantly. However cytochrome b_5 reductase exhibited a marginal decrease (Table 1).

Marked differences were observed in the difference spectra of CO bound reduced microsomes from control and infected rat livers. The intensity of the peak at 450 nm declined with the concomitant appearance of a peak at 420 nm, which is ascribed to the denatured form of cytochrome P-450 (Fig. 1) [19]. In spite of the marked reduction

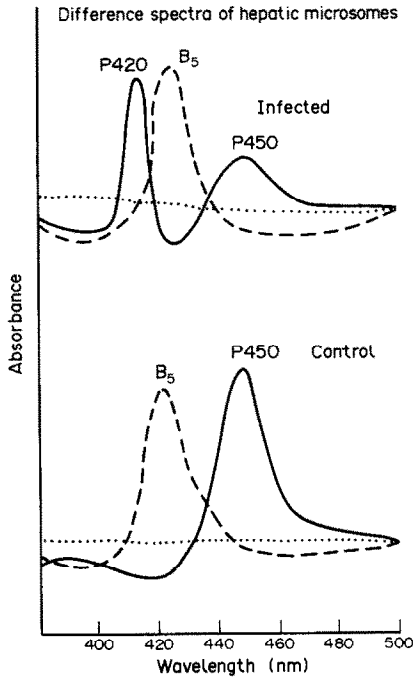

Fig. 1. Difference spectra of hepatic microsomes isolated from control and *N. brasiliensis* infected animals. Key: (. . . .) base, (---) dithionite reduced, and (——) CO bound.

* M. M. Khan. Ph.D. Thesis submitted to Kanpur University, Kanpur, India, 1985.

Table 1. Status of the hepatic microsomal mixed-function oxidase system in control and *Nippostrongylus brasiliensis* infected rats

Parameter	Control	Infected	Percent change over control (P value)
Aniline hydroxylase†	0.165 ± 0.017	0.085 ± 0.021	−48.2 (<0.001)
Aminopyrine *N*-demethylase†	1.369 ± 0.231	0.477 ± 0.121	−63.2 (<0.001)
Cytochrome b_5 reductase‡	1.793 ± 0.170	1.488 ± 0.273	−17.1 (<0.05)
NADPH–cytochrome *c* reductase‡	29.660 ± 4.720	25.660 ± 1.520	−13.4 (NS§)
Cytochrome P-450‖	1.105 ± 0.279	0.536 ± 0.191	−51.5 (<0.001)
Cytochrome b_5‖	0.556 ± 0.120	0.501 ± 0.231	−9.9 (NS)
Heme‖	2.460 ± 0.279	1.269 ± 0.235	−48.6 (<0.001)

Parameters are expressed as mean ± SD of six observations from separate animals.
† Expressed in nmol product formed·min^{-1}·$(mg\ protein)^{-1}$.
‡ Expressed in μmol product formed·min^{-1}·$(mg\ protein)^{-1}$.
§ Non-significant change over control.
‖ Expressed in nmole/mg microsomal protein.

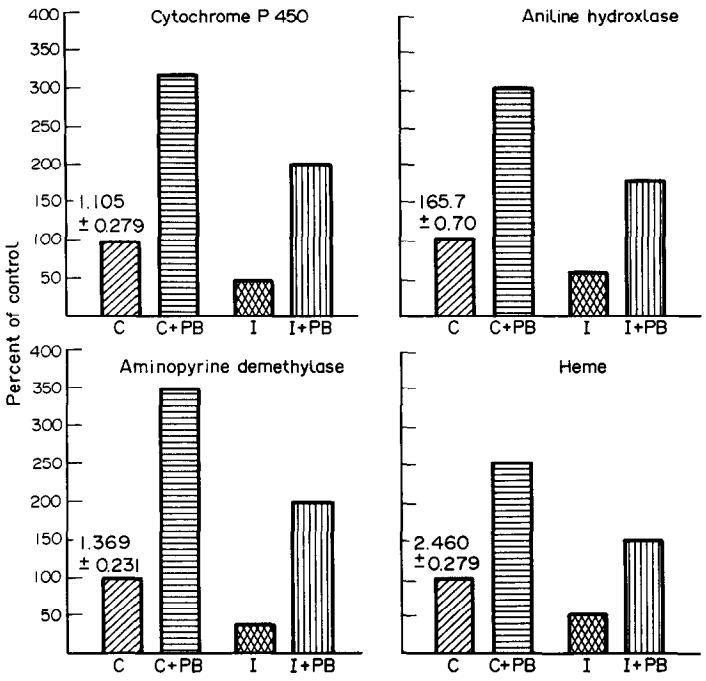

Fig. 2. Induction patterns of hepatic microsomal cytochrome P-450 and associated activities in control and *N. brasiliensis* infected rats. Phenobarbitone (80 mg/kg body weight) was administered i.p. from day 8 post-infection for 3 days, and the animals were killed on day 4 after drug treatment. Key: (C) control, (C + PB) phenobarbitone-treated controls, (I) infected, and (I + PB) phenobarbitone-treated infected rats.

in cytochrome P-450 and drug-metabolizing enzymes, the inducibility of the hepatic MFO system was not affected adversely. Phenobarbitone treatment (details are described in Fig. 2) caused a 3- to 4-fold increase in cytochrome P-450 and heme contents in both control and infected groups. Aniline hydroxylase and aminopyrine *N*-demethylase activities were also induced to the same degree in both groups of animals.

Discussion

Gross histopathological changes during *N. brasiliensis* infection in rats are generally associated with abnormal jejunal physiology [1]. Due to migration of larvae, during the course of infection, systemic effects also occur in the host [11]. Earlier reports have indicated that the oxidative metabolism of liver remains normal during this infection [20]. The results reported here indicate that, although liver tissue did not exhibit gross alterations, the activity of the hepatic MFO system was decreased dramatically as reflected by a marked reduction in the activities of aniline hydroxylase and aminopyrine *N*-demethylase and the contents of cytochrome P-450 and heme. The adverse effects of *N. brasiliensis* infections on the hepatic MFO system seem to be specific since other associated components, viz. NADPH–cytochrome *c* reductase, cytochrome b_5 reductase and cytochrome b_5 contents, were not altered significantly during this infection. Specific suppression of the hepatic MFO system during nippostrongylosis may be due to specific alterations in the synthesis, function or catalysis of cytochrome P-450. Similar induction patterns of cytochrome P-450 and associated activities by phenobarbitone treatment in both control and infected groups of animals indicated that the complete sequence of events involved in the synthesis of the components was preserved during this infection. Hence, the loss in cytochrome P-450 and associated activities may be attributed to an accelerated degradation of cytochrome P-450, rather than decreased synthesis of this hemoprotein. This is further supported by the appearance of a peak at 420 nm. The molecular mechanisms of this specific impairment of the MFO system during *N. brasiliensis* infection are not understood clearly and, hence, require further investigation.

Impairment of the MFO system common to hosts infected by *N. brasiliensis* as well as variety of other parasites may affect the efficacy and pharmacological properties of drugs as well as the metabolism of endogenous substances with possible physiological, pharmacological and toxicological consequences.

The results presented in this communication establish specific impairment of the hepatic microsomal MFO system during infection of rats with *N. brasiliensis*. Denaturation/degradation of cytochrome P-450 appears to be a possible cause of this specific impairment of the MFO system.

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Inhibition of retinoic acid metabolism by imidazole antimycotics in F9 embryonal carcinoma cells*

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Retinoic acid is a metabolite of retinol [1–3] that promotes growth, maintains epithelia *in vivo* [4], and is more active than retinol in the induction of differentiation of most cultured cells and tissues [5]. Inhibitors of retinoic metabolism would be useful to help assess the structure/activity relationships of retinoids, since extensive metabolism occurs simultaneously with retinoid-induced differentiation. The F9 embryonal carcinoma cell line, for example, is induced to differentiate into parietal endoderm by retinoic acid [6, 7], as it converts retinoic acid into polar metabolites [8], and is, therefore, one model to relate retinoid metabolism with function.

This report compares inhibition of retinoic acid metabolism in F9 cells by four inhibitors of cytochromes P-450. The imidazole antimycotics, ketoconazole, clotrimazole, and miconazole, inhibit: the metabolism of xenobiotics in microsomes [9] and vitamin D_3 in LLC-PK_1 cells [10, 11]; sterol biosynthesis [12, 13]; and oxidative metabolism of steroids [14, 15]. Metyrapone is a relatively nonspecific inhibitor of cytochromes P-450 [16].

Methods

Materials. [11, 12-^{3}H]Retinoic acid (23–32 Ci/mmol) was a gift of Hoffmann-La Roche, Inc. Ketoconazole was obtained from Janssen Pharm., Inc. Clotrimazole, miconazole, and metyrapone were purchased from Sigma.

Cell culture. F9 cells were grown in Dulbecco's modified Eagle medium with 10% heat-inactivated fetal bovine serum. The cells were seeded in 35 mm dishes (8×10^5 cells/plate). After 12 hr, the medium was replaced, and an inhibitor in ethanol (2 μl) or ethanol alone was added. Thirty minutes after the additions, 100 pmol of all-*trans*-[^{3}H]retinoic acid, purified to 98% by HPLC, was added in ethanol.

Quantification of retinoic acid in F9 cultures treated with inhibitors (Table 1). Each incubation (cells plus medium) was quenched with 0.2 N HCl in methanol (2 ml). Radioinert retinoic acid (5 μg) was added, and each sample was extracted with hexane [2]. The hexane was evaporated and the residues were dissolved in methanol (100 μl) and analyzed by HPLC (see below). The radioinert retinoic acid recovered (80%) was determined from a standard curve relating peak height to mass. [^{3}H]Retinoic acid was measured by liquid scintillation counting.

Analysis of retinoic acid metabolites (Figures). After the incubations, medium was removed from cells. Cells were harvested with 0.02% EDTA in phosphate-buffered saline (PBS) and were washed twice with PBS. The washes were combined with the medium. The medium and cells were dehydrated separately by azeotropic distillation with ethanol. Internal standards were added, and the residues were extracted with three portions of methanol (1–3 ml) containing butylated hydroxytoluene (BHT) (5 μg/ml). Solvents were concentrated and samples were analyzed with a reverse-phase column (radially-compressed C-18, 0.8 × 10 cm) eluted at a flow rate of 2 ml/min with a linear gradient of 10 mM ammonium acetate in methanol/water (45/55) to 10 mM ammonium acetate in methanol/water (75/25) over 30 min. Retinoids were detected at 340 nm. Liquiscint (5 ml) was added to each fraction (1 ml), and the [^{3}H]retinoids were measured with an LKB scintillation counter. Detection limits were 10 fmol in cell and 500 fmol in medium extracts.

Results and discussion

The four inhibitors of cytochromes P-450 were tested at three concentrations (Table 1). Ketoconazole and clotrimazole were about equipotent. Miconazole had no more than 10% of the activity of clotrimazole, i.e. 100 μM miconazole inhibited retinoic and metabolism by 56%, compared to 10 μM clotrimazole inhibiting 62%. Metyrapone had about 1% of the activity of clotrimazole—100 μM metyrapone inhibited 26% compared to 1 μM clotrimazole inhibiting 22%.

Incubation for more than 8 hr with 100 μM ketoconazole or clotrimazole resulted in cell death. The cytotoxic effects were not observed at inhibitor concentrations of 10 μM during 24-hr incubations. The presence of 10 μM ketoconazole does not prevent F9 cell proliferation or retinoic acid-induced differentiation [8]. Consequently, careful use

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