0022-3565/88/2462-0758$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 246, No. 2
Printed in U.S.A.

Albendazole Sulfonation by Rat Liver Cytochrome P-450c

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Accepted for publication March 31, 1988

ABSTRACT

The metabolism of albendazole (ABZ) was studied in perfused livers from control and ABZ-treated rats (10.6 mg/kg, per os, each day for 10 days). In the perfusion fluid, the concentration of ABZ-sulfoxide (SO-ABZ) remained unchanged in treated, as compared to control animals, whereas ABZ-sulfone (SO_2-ABZ) was increased in treated animals. In bile, only SO-ABZ was present. The transformation kinetics of SO-ABZ to SO_2-ABZ in microsomes from rats treated with ABZ, 3-methylcholanthrene, Aroclor and isosafrole were biphasic. This suggests that enzyme activity was a consequence of two enzyme systems, one characterized by low affinity and high capacity, the other by high affinity and low capacity, the latter could be induced by 3-methylcholanthrene, ABZ, Aroclor and isosafrole. Cytochrome P-450c was induced potently *in vivo* by ABZ as proven by increased monooxygenase (7-ethoxyresorufin and 7-ethoxycoumarin-O-deethylase) activities and by Elisa test (a 5-fold increase in hemoprotein concentration was observed). Purified and reconstituted cytochrome P-450c from 3-methylcholanthrene or ABZ-treated rat liver were able to produce SO_2-ABZ (2.01 and 1.70 nmol/mg/15 min, respectively, whereas cytochrome P-450b produced 10 times less SO_2-ABZ). Immunological assays, as well as activity measurements showed a relationship between cytochrome P-450c-3-methylcholanthrene and cytochrome P-450c-ABZ. We conclude that induction of cytochrome P-450c by ABZ is the probable explanation for the enhanced formation of SO_2-ABZ *in vivo*.

ABZ (Zentel, Valbazen), is a broad spectrum anthelmintic, widely used in many countries in human and veterinary medicine (Saimot *et al.*, 1983; Theodorides *et al.*, 1976) and has shown evidence of good efficiency in the treatment of hydatid cyts in humans (Morris *et al.*, 1983). *S*-oxidation of the drug to SO-ABZ and SO_2-ABZ are the first steps of its bioconversion (Gyurik *et al.*, 1981). Previous studies have demonstrated that ABZ was converted to SO-ABZ by liver microsomes (Fargetton *et al.*, 1986; Souhaili-El Amri *et al.*, 1987). The anthelmintic activity and embryotoxicity of ABZ in rats were due principally to the SO metabolite. Conversely, the SO_2 metabolite is neither active nor toxic (Delatour *et al.*, 1981). A 10-day p.o. administration of ABZ (10.6 mg/kg/day) enhanced the rat liver microsomal P-450c monooxygenase activities, the UDP-glucuronosyltransferase type 1 activities and the microsomal epoxide hydrolase (Souhaili-El Amri *et al.*, 1988). After repeated ABZ administration to rats, the SO_2-ABZ plasma level was enhanced. However, *in vitro* microsomal ABZ sulfoxidation and sulfonation activities were unchanged (Souhaili-El Amri, 1988).

The aim of the present work was to explore further the relationship between the liver P-450c induction and the enhancement of *in vivo* ABZ-SO_2 production. We used perfused rat liver to demonstrate the role of this organ in sulfonation.

We then studied ABZ sulfonation in rat liver microsomes, after treatment of rats either by 3-methylcholanthrene, ABZ, Aroclor or isosafrole which induce P-450c (Reik *et al.*, 1982).

The role of induced P-450c in the increased sulfonation was demonstrated with purified and reconstituted P-450c from 3-methylcholanthrene- or ABZ-treated rat livers.

Received for publication August 12, 1987.

Materials and Methods

Chemicals. ABZ, SO-ABZ and SO_2-ABZ were supplied by Smith Kline and French Laboratories (West Chester, PA). 3-Methylcholanthrene was obtained from Sigma Chemical Co. (St. Louis, MO), Aroclor 1254 and isosafrole were purchased from Aldrich Chemical Co. (Milwaukee, WI) and phenobarbital bought from Fluka Chemical Co. (Bucks SG, Switzerland). Other chemicals and biochemicals were from commercial sources.

Animals. Male Sprague-Dawley rats (180–200 g) were purchased from Iffa Credo (L'Arbresle, France).

Treatment of animals and preparation of microsomes. Isosafrole (150 mg/kg/day in corn oil) was administered by i.p. injection on 4 consecutive days and the rats were sacrificed by decapitation on the 5th day. 3-Methylcholanthrene and Aroclor 1254 were administered once by i.p. injection in corn oil at a dose of 80 and 500 mg/kg,

ABBREVIATIONS: ABZ, albendazole; SO, sulfoxide; SO_2, sulfone; P-450, cytochrome P-450; DMSO, dimethyl sulfoxide; SDS, sodium dodecyl sulfate.

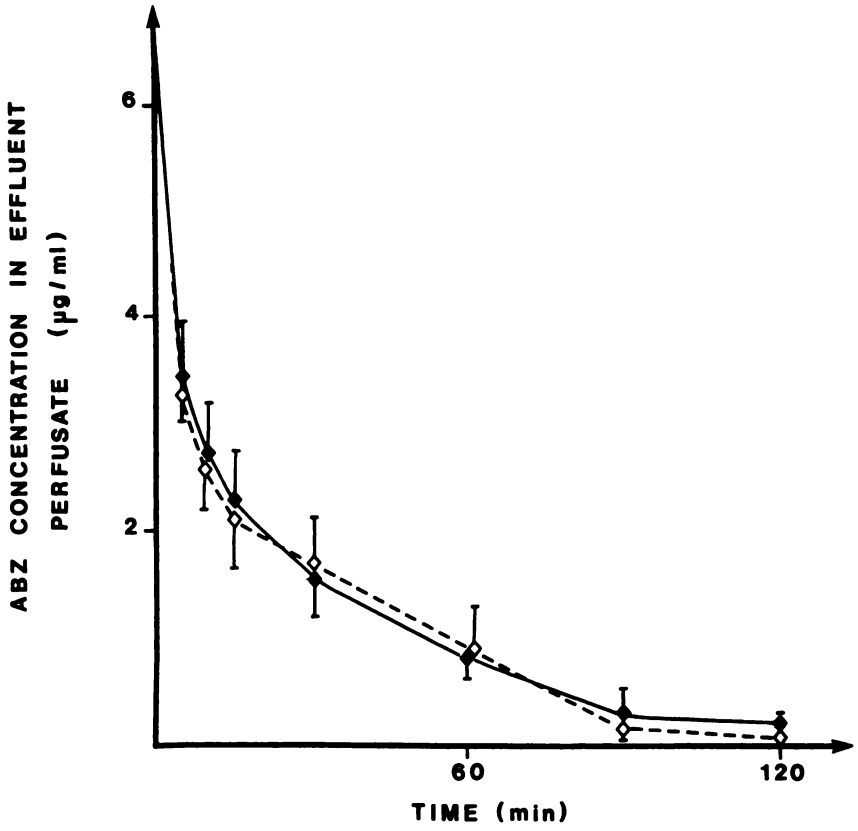

Fig. 1. Disappearance patterns of ABZ from perfusion medium of isolated liver. Results are the means ± S.D. from four different animals. △, rats treated with ABZ; ◆, untreated rats.

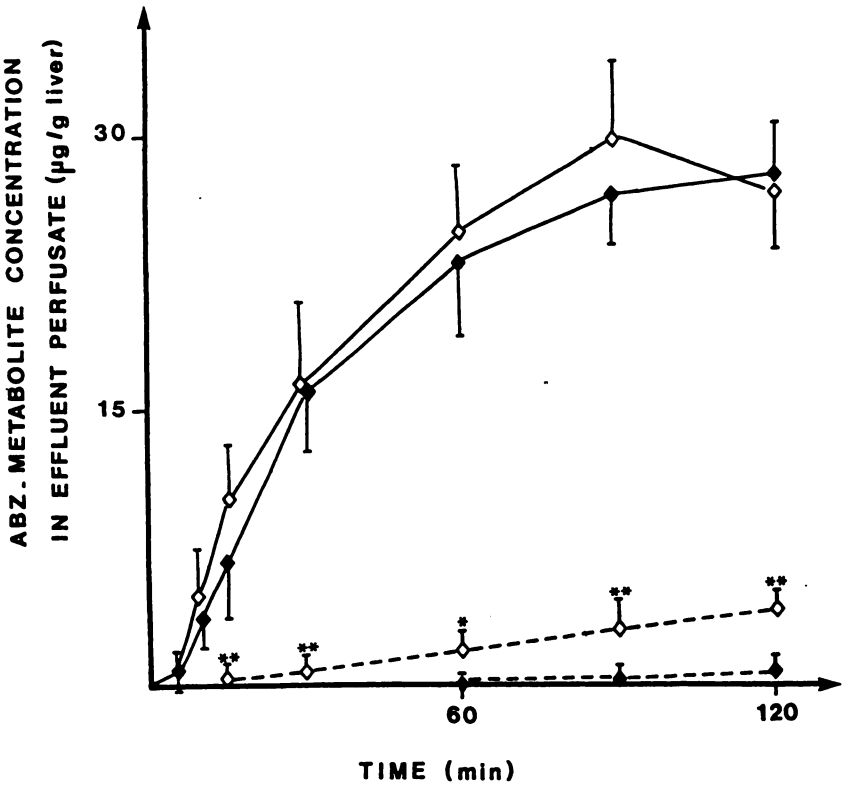

Fig. 2. Effect of ABZ pretreatment on ABZ metabolism by perfused livers. Results are the means ± S.D. from four different animals. ——, Production of SO-ABZ; – – –, production of SO_2-ABZ; ◆, untreated rats; ◇, ABZ-treated rats; * significantly different from control at $P < .05$ using Student's *t* test; ** significantly different from control at $P < .01$ using Student's *t* test.

respectively, and the rats were sacrificed on the 5th day. Albendazole was administered at a dose of 10.6 mg/kg by gastric tubage of aqueous suspension for 10 days and the rats were sacrificed on the 11th day. Phenobarbital was administered i.p. in saline for 4 days at a dose of 80 mg/kg.

Liver microsomes were prepared according to the previously published procedure (Souhaili-El Amri *et al.*, 1987).

Perfused liver. The animals were anesthetized with pentobarbital (50 mg·kg^{-1}). Then, the vena portae, the inferior vena cava and the bile duct were cannulated. The liver was removed from the animal and

TABLE 1

Values for the four Michaelis-Menten parameters of microsomal ABZ-sulfonase activities

Effect of ABZ, 3-methylcholanthrene, Aroclor and isosafrole pretreatment. The transformation of SOABZ to SO_2ABZ is determined as indicated under "Material and Methods." Values are the means of two determinations.

	Low Affinity Component		High Affinity Component	
	K_m apparent	V_{max}	K_m apparent	V_{max}
	mM	*nmol/mg/15 min*	*μM*	*nmol/mg/15 min*
Control	1.01	1.96		Not detected
ABZ	1.07	2.09	6.90	0.127
3-Methylcholanthrene	1.16	2.38	8.40	0.187
Aroclor	2.05	1.77	14.04	0.247
Isosafrole	1.26	2.18	12.50	0.101

connected to the perfusion apparatus. The liver was perfused at 37°C with 150 ml of Krebs-Henseleit medium at pH 7.4 with albumin (25 g·l^{-1}). The system was oxygenated by O_2-CO_2 (95/5 v/v) and the perfusion flow rate was adjusted to about 4 ml·min^{-1} per g of liver; pH values before and after the passage of the liver in the outflowing medium were measured continuously. After the preperfusion period of 30-min aliquots of perfusate were analyzed and ABZ (5 mg/kg b.wt.) was added to the perfusion reservoir. No ABZ or metabolites were found in the preperfusate.

The samples of perfusate were withdrawn after 5, 10, 15, 30, 60, 90 and 120 min. Bile was collected over the following 120 min of perfusion. The parent drug and the metabolites were extracted and analyzed, quantitatively according to the previously published procedure (Delatour *et al.*, 1984).

Extraction of ABZ and its metabolites from liver. The rats which had been starved overnight received a single dose of 10.6 mg ABZ per kg p.o. The animals were sacrificed by decapitation (three animals at each time) at 0, 0.25, 0.5, 0.75, 1, 1.5, 3, 6, 9, 12 and 18 hr postadministration. The liver was removed, weighed, placed in a few milliliters of phosphate buffer (pH 7.4) and crushed with an Ultrathurax apparatus (tissue grinder). The volume was adjusted to 25 ml. One milliliter of the homogenate was added to 10 ml of diethyl ether and the extraction carried out lasted 15 min on a rotor type agitator. The extraction was performed twice. The organic phase was evaporated and 500 μl of DMSO were added to the residue and the metabolites were analyzed, as described previously (Delatour *et al.*, 1984).

Purification of P-450. P-450 was purified from liver microsomes from 3-methylcholanthrene- or ABZ-treated rats according to the method described by Wolf and Oesch (1983). The liver microsomes from phenobarbital-treated rats were used for the purification of P-450b (Guengerich *et al.*, 1982).

Immunological assays. The preparation of antibodies against P-450c has been described previously (Souhaili-El Amri *et al.*, 1988). Immunodiffusion was carried out using Ouchterlony's method (1949). Enzyme-linked immunosorbent assays (Elisa) were carried out according to Engvall's method (1980) using a peroxidase-conjugated antibody.

Assays of Drug Metabolism Enzymes

Generalities. All assays were performed in 100 mM K-phosphate buffer (pH 7.5) containing 1 mM $MgCl_2$. Assays of catalytic activity using purified P-450 were carried out in the presence of dilaurylphosphatidylcholine (30 μg/ml) and sodium deoxycholate (100 μg/ml). Blanks contained all components except NADPH. Samples were preincubated for 2 min at 37°C before the addition of NADPH (0.2 mM) to initiate the reaction. Individual assays were carried out as follows.

Benzphetamine. The N-demethylation of benzphetamine were assayed by Yang and Strickhart's method (1974). Incubations were carried out for 5 min at 37°C using 0.2 nmol of P-450, 1 U of reductase and 7 mM of substrate in a final volume of 1 ml.

7-Ethoxycoumarin. The O-deethylation of 7-ethoxycoumarin was assayed as described by Ullrich and Weber (1972). Incubations were carried out for 5 min at 37°C using 0.05 nmol of P-450, 0.5 U of reductase and 0.3 mM of substrate in a final volume of 2 ml.

7-Ethoxyresorufin. The O-deethylation of 7-ethoxyresorufin was assayed by the method of Burke and Mayer (1974). The incubations were carried out directly in the fluorometric cuvette at 37°C using 0.05 nmol of P-450, 0.5 U of reductase and 4 μM substrate in a final volume of 1 ml.

p-Nitroanisole. The O-demethylation of *p*-nitroanisole was assayed by Netter and Seidel's method (1964) in the spectral cuvette *in situ* at 37°C using 0.2 nmol of cyt P-450, 1 U of reductase and 0.5 mM substrate in the final volume of 2.5 ml.

SO-ABZ. The transformation of SO-ABZ to SO_2-ABZ was assayed with 5 mg of microsomal protein and varying quantities of SO-ABZ (3.5–700 μM) as substrate. SO-ABZ was dissolved in 10 μl of DMSO. Assays using the reconstituted system contained 0.5 nmol of P-450, 2 U of reductase and 50 μM of substrate in the final volume of 1 ml. SO-ABZ and SO_2-ABZ were extracted from incubation mixtures, identified (retention time, UV spectrum) by comparison with pure synthetic compounds and determined by high-performance liquid chromatography according to methods described previously (Delatour *et al.*, 1984). K_m and V_{max} values were obtained by graphical analysis of Eadie Hofstee plots and Michaelis-Menten kinetics.

The protein content was determined using the method of Lowry *et al.* (1951) with serum albumin as standard. Total P-450 was measured according to Omura and Sato (1964). SDS-polyacrylamide gel electrophoresis was carried out according to Laemmli's method (1970).

Statistics. All direct comparisons with a single control group were conducted with the unpaired Student's *t* test.

Results

Perfused liver. Figure 1 compares the rate of disappearance of ABZ in perfusate from control and ABZ-pretreated rat liver. ABZ (1 mg) disappeared totally within 120 min, this disappearance was unaffected by ABZ-pretreatment.

Figure 2 represents the kinetics of SO-ABZ and SO_2-ABZ appearance in the same livers as in Figure 1. ABZ was transformed into SO-ABZ and SO_2-ABZ with an average yield of 35 to 40% of the dose. ABZ treatment apparently does not affect the rate and yield of SO-ABZ production.

SO_2-ABZ appeared earlier (after 15 min instead of 60 min) and in a larger amount (8 times as much in 120 min) in perfused livers from ABZ-treated animals compared with those of the control.

In the corresponding bile, ABZ and SO_2-ABZ were absent. Only SO-ABZ was present. ABZ-pretreatment did not significantly change the amount of SO-ABZ (0.98 ± 0.29 μg/ml of bile in control rats, and 1.23 ± 0.36 μg/ml of bile in ABZ-pretreatment rats).

Microsomal fractions. The *in vitro* bioconversion of SO-ABZ into SO_2-ABZ was tested with liver microsomes from control rats or from rats treated with 3-methylcholanthrene, Aroclor, isosafrole and ABZ. SO-ABZ concentrations varied from 3.5 to 700 μM. Microsomal fractions from control rats led to a Michaelis-Menten kinetics with apparent K_m = 1.01 mM and V_{max} = 1.96 nmol/mg of protein per 15 min. The microsomal fractions which were enriched in P-450c *via* induction by 3-methylcholanthrene, Aroclor, isosafrole and ABZ, generated biphasic plots (table 1; fig. 3).

In the presence of *n*-octylamine (3 mM), the transformation of SO-ABZ into SO_2-ABZ in microsomes from control rats and pretreated rats (ABZ, 3-methylcholanthrene, Aroclor and isosafrole) was inhibited completely.

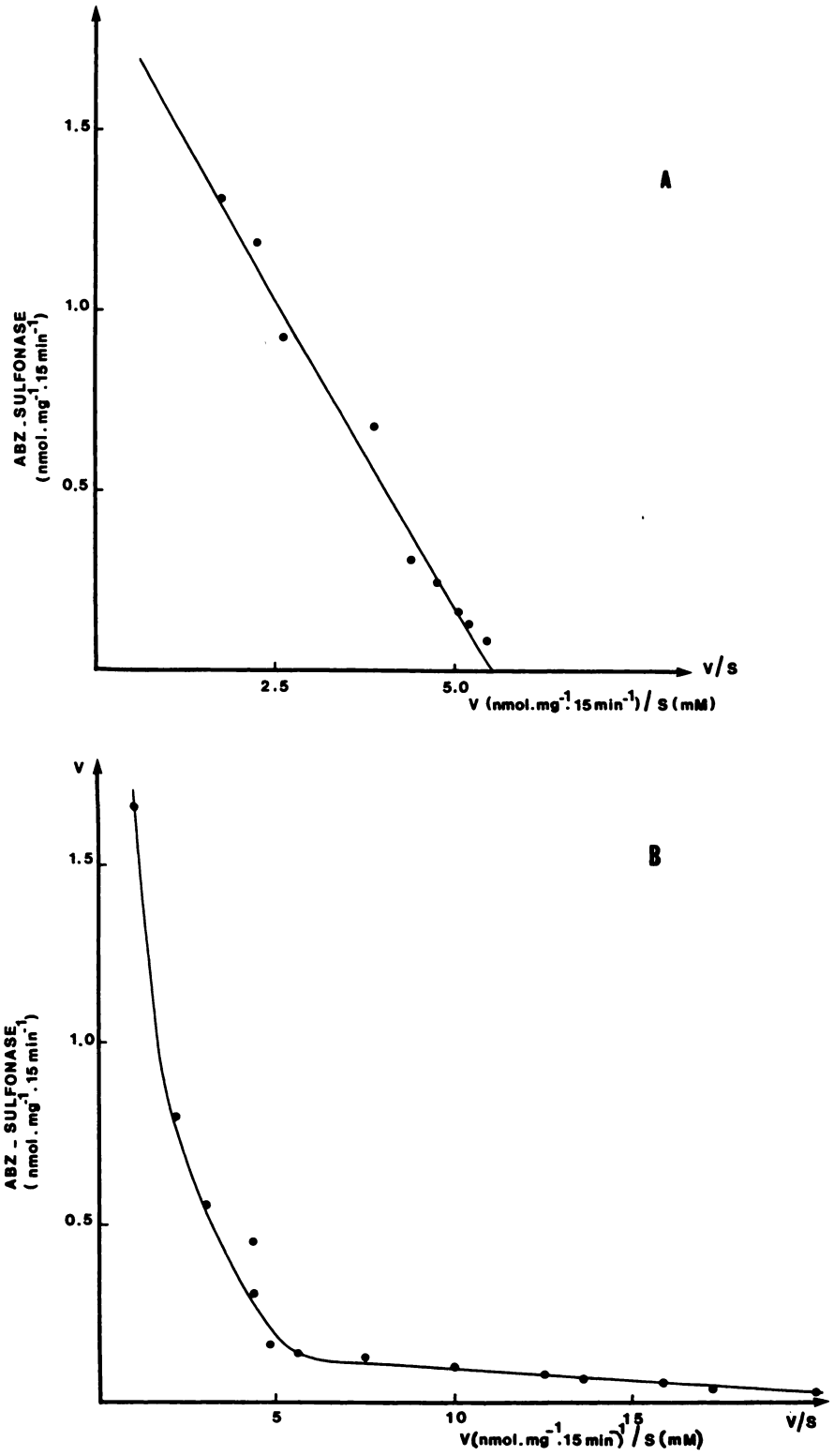

Fig. 3. Eadie Hofstee plot for ABZ sulfonation of rat hepatic microsomes from rat control (A) or rat treated with 3-methylcholanthrene (B).

Concentration of ABZ and its metabolites in the liver. When the rats were treated by a single ABZ dose, ABZ biotransformation to SO-ABZ was active (fig. 4). SO-ABZ concentration reached a maximum at about 12.44 ± 1.38 μg of SO-ABZ per g of liver, 6 hr post-p.o. dose. SO_2-ABZ also was present in the liver at a low concentration (0.57 ± 0.15 μg of SO_2-ABZ per g of liver at 9 hr).

Purification and characterization of liver microsomal P-450 after 3-methylcholanthrene and ABZ pretreatment. The specific contents of the P-450c and P-450_{ABZ} were 18 and 12 nmol/mg of protein, respectively. The monomeric molecular weight of the two proteins were similar (56,000). The P-450_{ABZ} was partially purified and two bands were revealed on SDS gels (fig. 5A). The absolute spectra of the two cytochrome

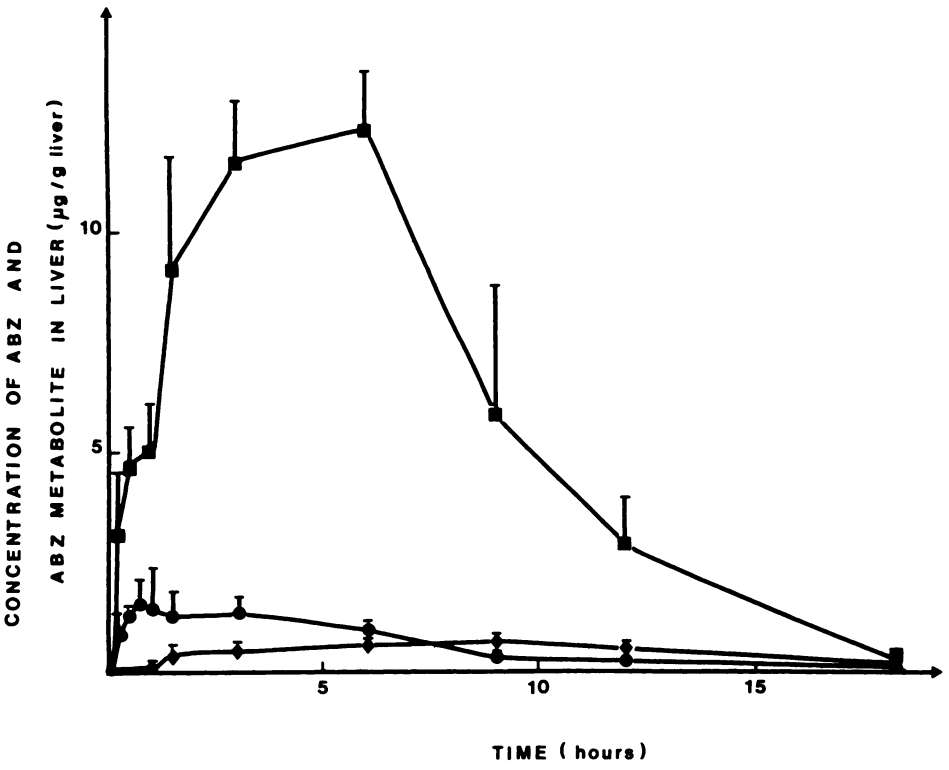

Fig. 4. Concentration of ABZ and ABZ-metabolites in liver after a single dose of ABZ (10.6 mg/kg). Results represents the means ± S.D. from three rat livers. ●, concentration of ABZ; ■, concentration of SO-ABZ; ◆, concentration of SO_2-ABZ.

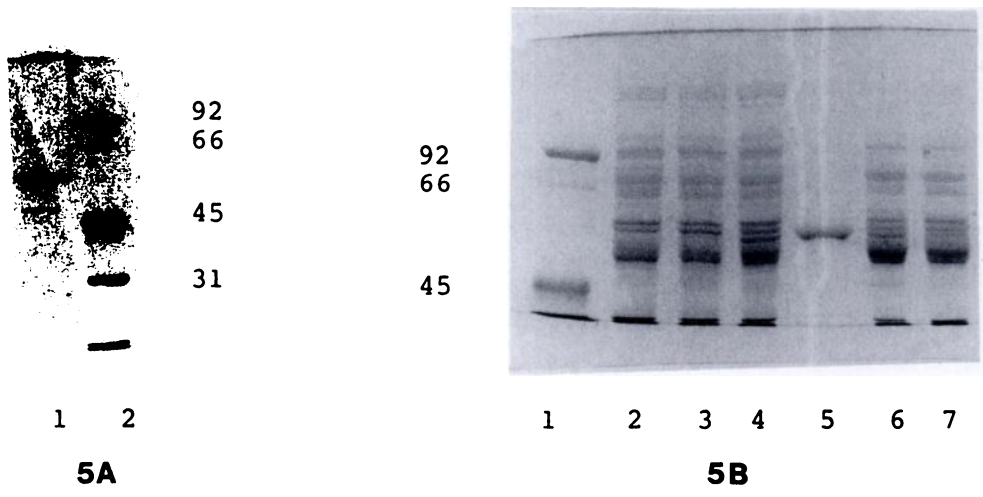

Fig. 5. Polyacrylamide gel electrophoresis. A, purified P-450c from ABZ-treated rats (Track 2). Track 1 contained standard proteins with the indicated molecular weights (K_D). Electrophoresis was carried out according to Laemmli's method (1970) using 12% acrylamide in the separating gel and a 5% stacking gel. B, profile of liver microsomes from rat treated with different xenobiotics. Electrophoresis was carried out according to Laemmli's method (1970) using 9% acrylamide in the separating gel and a 5% stacking gel. Track designations are as follows: Track 1 contained standard proteins with the indicated molecular weights (K_D); Track 2, 25 μg of control rat microsomes; Track 3, microsomes of ABZ-treated rats (25 μg); Track 4, microsomes of the 3-methylcholanthrene-treated rat (25 μg); Track 5, purified P-450c from 3-methylcholanthrene-treated rats (2 μg); Track 6, microsomes of Aroclor-treated rats (25 μg); Track 7, microsomes of isosafrole-treated rats (25 μg).

fractions were similar: both forms were low spin with a Soret peak at 419 nm. The ferrous cytochrome carbon monoxide complex of both cytochromes had a maximum at 447 nm. The Ouchterlony immunodiffusion plate (fig. 6) shows that anti-P-450c reacts with $P\text{-}450_{ABZ}$ to give one immunoprecipitation band. This immunoprecipitation band formed between the anti-P-450c antibody and $P\text{-}450_{ABZ}$ fused with the immunoprecipitation band obtained with anti-P-450c and P-450c. These data demonstrate an immunochemical identity between $P\text{-}450_{ABZ}$ and P-450c.

SDS gel electrophoretic profiles of rat liver microsomes. The Coomassie blue staining intensity of a microsomal protein that comigrates with purified P-450c was greater after treatment of rats with ABZ, 3-methylcholanthrene, Aroclor or isosafrole (fig. 5B). This protein was not detectable in the microsomes from untreated rats.

Immunoquantification of P-450c. Quantification of P-450c in different types of microsomes revealed that ABZ-treatment caused a 5-fold increase in this protein level (table 2). The rates of 7-ethoxyresorufin and 7-ethoxycoumarin O-deethylation were increased in parallel with the amount of the P-450c in the microsomes.

Metabolic activity of the P-450 fractions from 3-methylcholanthrene- and ABZ-pretreated rat liver. P-

450c fractions from both 3-methylcholanthrene- and ABZ-pretreated rat liver had high and similar turnover numbers toward 7-ethoxycoumarin, 7-ethoxyresorufin and SO-ABZ as substrates as can be seen in table 3. P-450b on the other hand showed a high activity toward benzphetamine and produced 10 times less SO_2-ABZ.

Discussion

SO_2-ABZ production by the liver. The perfused rat liver preparation metabolized ABZ into SO-ABZ and SO_2-ABZ. This clearly confirmed that the liver has a major role in the metabolism of ABZ including the second step of oxidation converting SO-ABZ into SO_2-ABZ. The kinetic data indicated that sulfonation was enhanced in livers of ABZ-pretreated rats as compared with control animals. This was in agreement with the pharmacokinetic data observed previously in rat plasma (Souhaili-El Amri *et al.*, 1988). In ABZ-pretreated animals the SO_2-ABZ appeared earlier and in a greater yield. This suggests that SO_2-ABZ was produced by two different enzymes in control and ABZ-pretreated animals, or by an unique enzyme system induced in pretreated animals.

P-450c involvement in SO_2-ABZ production. When the Michaelis Menten constants were determined in microsomes, the biotransformation of SO-ABZ into SO_2-ABZ was biphasic in ABZ, 3-methylcholanthrene, Aroclor or isosafrole pretreated rat liver microsomes, again suggesting the involvement of two different enzymes in the conversion of SO-ABZ into SO_2-ABZ by liver microsomes of induced animals. The biotransformation of SO-ABZ was totally inhibited by *n*-octylamine which suggests that both P-450s are involved in this biotransformation and that flavin containing monooxygenase was not responsible for this step of the transformation as distinct from the conversion of ABZ to SO-ABZ (Souhaili-El Amri *et al.*, 1987). Analogous examples are already known concerning ethoxycoumarin and phenacetin metabolism *via* P-450 (Boobis *et al.*, 1981). ABZ induced an isoenzyme of P-450 similar to P-450c (MW, substrate specificity and immunoreactivity) which was able to transform SO-ABZ into SO_2-ABZ. ABZ is thus able to induce its own metabolism at least at the bioconversion step of SO-ABZ to SO_2-ABZ, this SO_2-ABZ being therapeutically and toxicologically inactive (Delatour *et al.*, 1981).

The relative contribution of each enzyme system involved in ABZ sulfonation *in vivo* depends on the SO-ABZ concentration in the medium. The low affinity system requires at least 286 μg/ml (K_m = 1 mM) in the presence of 5 mg of microsomal protein in the medium to reach half its metabolic capacity. The high affinity component only requires 1.97 μg/ml of SO-ABZ (K_m = 6.90 μM) in the presence of 5 mg of protein from ABZ-treated rat liver microsomes. *In vivo*, the determination of residues of ABZ and its metabolites in the rat liver showed that SO-ABZ reached a maximum average at 14 μg/g of liver. In the plasma the corresponding concentration was less than 3 μg/ml (Souhaili-El Amri *et al.*, 1988). The same phenomenon was

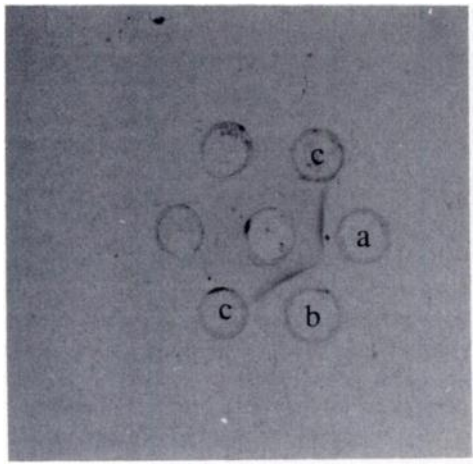

Fig. 6. Immunochemical comparison of P-450 purified from 3-methylcholanthrene- and ABZ-treated rats. The central well was filled with anti-P-450c. The well (labeled a) contained P-450c from 3-methylcholanthrene-treated rats, the well (labeled b) contained P-450c from ABZ-treated rats and the well (labeled c) contained P-450b from phenobarbital-treated rats.

TABLE 3

Metabolic activity of the P-450c fractions from 3-methylcholanthrene- and ABZ-treated and the P-450b from phenobarbital-treated rats

Assays were performed as described under "Materials and Methods." The data represent the means of two separate experiments.

Substrate	Turnover No.		
	3-Methylcholanthrene	ABZ	Phenobarbital
Benzphetamine (nmol/mg/min)	0.78	1.00	29.03
7-ethoxycoumarin (nmol/mg/min)	9.2	10.41	4.22
7-ethoxyresorufin (nmol/mg/min)	2.9	2.73	0.37
p-Nitroanisole (nmol/mg/min)	6.2	4.94	10.87
SO-ABZ (nmol/mg/15 min)	2.01	1.70	0.18

TABLE 2

7-Ethoxycoumarin and 7-ethoxyresorufin activities and levels of P-450c in liver microsomes after treatment with several xenobiotics

Animals were treated with the above compounds. Microsomes were prepared, P-450 was determined spectroscopically and the P-450c was determined immunologically (results are the means ± S.D. for five different animals). 7-Ethoxycoumarin and 7-ethoxyresorufin O-deethylase activities were determined as described under "Materials and Methods."

Treatment	Specific Content of P-450		7-Ethoxycoumarin O-Deethylase	7-Ethoxyresorufin O-Deethylase
	Spectral assays	Immunochemical assays		
	nmol/mg		*nmol/min/mg*	
Control	0.79 ± 0.12	0.07 ± 0.04	0.12 ± 0.01	0.023 ± 0.004
3-Methylcholanthrene	1.10 ± 0.36*	0.69 ± 0.26***	2.02 ± 0.16***	0.910 ± 0.230***
Aroclor	3.28 ± 0.65**	1.52 ± 0.25***	10.51 ± 2.35***	1.960 ± 0.530***
Albendazole	0.82 ± 0.10	0.34 ± 0.18**	1.27 ± 0.20***	0.345 ± 0.060***

* Significantly different from control at $P < .05$ using Student's *t* test; ** significantly different from control at $P < .01$ using Student's *t* test; *** significantly different from control at $P < .001$ using Student's *t* test.

observed in humans (Meulemans *et al.*, 1984) where 3 to 4 times more SO-ABZ was formed in the target tissue (liver) than in plasma. These SO-ABZ tissue concentrations and the kinetic data suggest a major participation of the high affinity system in the conversion of SO-ABZ to SO_2-ABZ, namely after ABZ pretreatment.

In conclusion, SO_2 was produced by the liver; the bioconversion of SO to SO_2 depends on a P-450c; it was induced by ABZ.

References

BOOBIS, A. R., KAHN, C. G., WHYTE, C., BRODIE, M. J. AND DAVIES, D. S.: Biphasic O-deethylation of phenacetin and 7-ethoxycoumarin by human and rat liver microsomal fractions. Biochem. Pharmacol. **30:** 2451–2456, 1981.

BURKE, D. AND MAYER, R. T.: Ethoxyresorufin: Direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metab. Dispos. **2:** 583–588, 1974.

DELATOUR, P., GARNIER, F., BENOIT, E. AND LONGIN, C.: A correlation of toxicity of albendazole and of oxfendazole with their free metabolites and bound residues. J. Vet. Pharmacol. Ther. **7:** 139–145, 1984.

DELATOUR, P., PARISH, R. C. AND GYURIK, R. J.: Albendazole: A comparison of relay embryotoxicity with embryotoxicity of individual metabolites. Ann. Rech. Vet. **12:** 159–167, 1981.

ENGVALL, E.: Enzyme immunoassay ELISA and EMIT. Methods Enzymol. **70A:** 419–439, 1980.

FARGETTON, X., GALTIER, P. AND DELATOUR, P.: Sulfoxidation of albendazole by a cytochrome P-450 independent monooxygenase from rat liver microsomes. Vet. Res. Commun. **10:** 314–324, 1986.

GUENGERICH, F. P., DANNAN, G. A., WRIGHT, S. T., MARTIN, H. V. AND KAMINSKY, L. S.: Purification and characterization of liver microsomal cytochromes P-450: Electrophoretic, spectral, catalytic and immunochemical properties and inducibility of eight isoenzymes isolated from rats treated with phenobarbital or β-naphtoflavone. Biochemistry **21:** 6019–6030, 1982.

GYURIK, R. J., CHOW, A. W., ZABER, B., BRUNER, E. L., MILLER, J. A., VILLANI, A. J., PETKA, L. A. AND PARISH, R. C.: Metabolism of albendazole in cattle, sheep, rats and mice. Drug Metab. Dispos. **19:** 503–508, 1981.

LAEMMLI, U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.) **272:** 680–685, 1970.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. AND RANDELL, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275, 1951.

MEULEMANS, A., GIOVANANGELI, M. D., MOHLER, J., VULPILLAT, M., HAY, J. M. AND SAIMOT, A. G.: High performance liquid chromatography of albendazole and its sulfoxide metabolite in human organs and fluids during hydatidosis. J. Liquid Chromatog. **7:** 569–580, 1984.

MORRIS, D. L., DYKES, P. W., DICKSON, B., MARRINER, S. E., BOGAN, J. A. AND BUNNOWS, F. G. O.: Albendazole in hydatid disease. Br. Med. J. **286:** 103–104, 1983.

NETTER, K. J. AND SEIDEL, B.: An adaptively stimulated O-demethylating system in rat liver microsomes and kinetics properties. J. Pharmacol. Exp. Ther. **146:** 61–65, 1964.

OMURA, T. AND SATO, R.: The carbon monoxide-binding pigment of liver microsomes. 1. Evidence for its hemoprotein nature. J. Biol. Chem. **239:** 2370–2378, 1964.

OUCHTERLONY, O.: Antigen-antibody reaction in gels. Acta Pathol. Microbiol. Scan. **26:** 507–515, 1949.

REIK, L. M., LEVIN, W., RYAN, D. A. AND THOMAS, P. E.: Immunochemical relatedness of rat hepatic microsomal cytochromes P-450c and P-450d. J. Biol. Chem. **257:** 3950–3957, 1982.

SAIMOT, A. G., CREMIEUX, A. C., HAY, J. M., MEULEMANS, A., GIOVANANGELI, M. D., DELAITRE, B. AND COULAUD, F. P.: Albendazole as a potential treatment of human hydatidosis. Lancet **17:** 652–656, 1983.

SOUHAILI-EL AMRI, H., FARGETTON, X., BENOIT, E., TOTIS, M. AND BATT, A. M.: Inducing effect of albendazole on rat liver drug metabolizing enzymes, and metabolite pharmacokinetics. Toxicol. Appl. Pharmacol. **92:** 141–149, 1988.

SOUHAILI-EL AMRI, H., FARGETTON, X., DELATOUR, P. AND BATT, A. M.: Sulphoxidation of albendazole by the FAD-containing and cytochrome P-450 dependent monooxygenases from pig liver microsomes. Xenobiotica, **17:** 1159–1168, 1987.

THEODORIDES, V. J., GYURIK, R. J. AND KINGSBURY, W. D.: Anthelmintic activity of albendazole against liver flukes, tapeworms, lung and gastrointestinal round worms. Experientia (Basel) **32:** 702–703, 1976.

ULLRICH, V. AND WEBER, P.: The O-dealkylation of 7-ethoxycoumarin by liver microsomes: A direct fluorimetric assays. Hoppe Seyler's Z. Physiol. Chem. **353:** 1171–1177, 1972.

WOLF, C. R. AND OESCH, F.: Isolation of a high spin form of cytochrome P-450 induced in rat liver by 3-Methylcholanthrene. Biochem. Biophys. Res. Commun. **111:** 504–511, 1983.

YANG, C. S. AND STRICKHART, F. S.: Inhibition of hepatic mixed function oxidase activity by propylgallate. Biomed. Pharmacol. **23:** 3129–3138, 1974.

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