Table 1. Hepatic enzyme activities 6 hr after aminotriazole (1 g/kg)

	Control	Aminotriazole	Assay method (ref.)	% of Control
MEOS	9.0 ± 0.2*	7.2 ± 0.7*	4	80
[nmol·min^{-1}·(mg protein)$^{-1}$]	10.9 ± 0.4	9.1 ± 0.3	5	83
Aniline hydroxylation				
[nmol·min^{-1}·(mg protein)$^{-1}$]	1.6 ± 0.1	1.0 ± 0.2	4	63
Methanol peroxidation	96 ± 10	38 ± 3	4	40
[μmol·hr^{-1}·(g liver)$^{-1}$]	317 ± 46	47 ± 8	14†	15

* Values are means ± SE of five animals.
† The published assay was modified as detailed in Materials and Methods.

or that this peroxisomal pathway represents a significant contribution to ethanol metabolism as proposed by Handler and Thurman [7]. Even in the absence of ADH, the data presented here, as well as previous studies [1, 3, 6], provide *in vivo* evidence which argues against a major role for this pathway.

In conclusion, our study demonstrated effective inhibition of *in vivo* ethanol elimination by the MEOS inhibitor, 1-butanol, but failed to confirm the effects of aminotriazole reported by Handler *et al.* [4] in ADH negative deermice. Moreover, these results agree with other published experiments conducted in the rat, and concur with previous studies of the *in vivo* roles of MEOS and catalase carried out in deermice using completely different methods [3, 6]. Thus, our present as well as past results clearly indicate a significant role of MEOS, but not of catalase, for ethanol metabolism *in vivo* in deermice.

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REFERENCES

1. Y. Shigeta, F. Nomura, S. Iida, M. A. Leo, M. R. Felder and C. S. Lieber, *Biochem. Pharmac.* **33**, 807 (1984).
2. E. B. Glassman, G. A. McLaughlin, D. T. Forman, M. R. Felder and R. G. Thurman, *Biochem. Pharmac.* **34**, 3523 (1985).
3. T. Takagi, J. Alderman, J. Gellert and C. S. Lieber, *Biochem. Pharmac.* **35**, 3601 (1986).
4. J. A. Handler, B. U. Bradford, E. Glassman, J. K. Ladine and R. G. Thurman, *Biochem. Pharmac.* **35**, 4487 (1986).
5. S. Kato, J. Alderman and C. S. Lieber, *Archs Biochem. Biophys.* **254**, 586 (1987).
6. J. Alderman, T. Takagi and C. S. Lieber, *J. biol. Chem.* **262**, 7497 (1987).
7. J. A. Handler and R. G. Thurman, *Alcohol* **4**, 131 (1987).
8. W. G. Heim, D. Appleman and H. T. Pyfrom, *Science* **122**, 693 (1955).
9. V. E. Price, W. R. Sterling, V. A. Tarantola, R. W. Hartley, Jr. and M. Rechcigl, Jr., *J. biol. Chem.* **237**, 3468 (1962).
10. F. W. Kinard, G. H. Nelson and M. G. Hay, *Proc. Soc. exp. Biol. Med.* **92**, 772 (1956).
11. M. E. Smith, *J. Pharmac. exp. Ther.* **134**, 233 (1961).
12. T. R. Tephly, R. E. Parks, Jr. and G. J. Mannering, *J. Pharmac. exp. Ther.* **143**, 292 (1964).
13. M. K. Roach, M. Khan, M. Knapp and W. N. Reese, Jr., *Q. Jl Stud. Alcohol* **33**, 751 (1972).
14. E. Feytmans and F. Leighton, *Biochem. Pharmac.* **22**, 349 (1973).
15. G. D. Wendell and R. G. Thurman, *Biochem. Pharmac.* **28**, 273 (1979).
16. B. Chance, *Acta chem. scand.* **1**, 236 (1947).
17. J. A. Handler and R. G. Thurman, *Fedn Proc.* **46**, 539 (1987).
18. R. Kato, *Jap. J. Pharmac.* **17**, 56 (1967).
19. A. Nakamura, T. Hara and S. Minakami, *J. Biochem., Tokyo* **73**, 47 (1973).
20. T. Nash, *Biochem. J.* **55**, 416 (1953).
21. N. Oshino, D. Jamieson, T. Sugano and B. Chance, *Biochem. J.* **146**, 67 (1975).
22. E. C. Foerster, T. Fährenkemper, U. Rabe, P. Graf and H. Sies, *Biochem. J.* **196**, 705 (1981).
23. J. R. Williamson, R. Scholz, E. T. Browning, R. G. Thurman and M. H. Fukami, *J. biol. Chem.* **25**, 5044 (1969).
24. M. N. Berry, D. G. Clark, A. R. Grivell and P. G. Wallace, *Eur. J. Biochem.* **131**, 205 (1983).

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Differences in the induction of carboxylesterase isozymes in rat liver microsomes by xenobiotics*

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Carboxylesterase (EC 3.1.1.1) in liver microsomes catalyzes the hydrolysis of a large number of xenobiotics, including carboxylesters, thioesters, and aromatic amides [1–3], and plays an important role in drug metabolism. Recent studies have focused on the multiplicity of this enzyme, and more than twenty isozymes have been isolated and characterized [3, 4]. Only a few of these isozymes of hepatic microsomal carboxylesterases are induced by exogenous compounds such as phenobarbital [5] and DDT

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[6]. Kidney [7] and lung [8] carboxylesterases are not induced by phenobarbital. Satoh and Moroi [9] and Raftell *et al.* [8] reported that 3-methylcholanthrene has no inducing effect on hepatic microsomal carboxylesterase activities.

Although several xenobiotics do induce carboxylesterases, there is little understanding of the mechanism of such induction in liver microsomes because of the lack of specific assay methods. In a previous paper [10], we reported the purification of three forms of carboxylesterase and the preparation of specific antibody to each isozyme, and the quantitation of carboxylesterase isozymes in liver microsomes by using a radial immunodiffusion assay.

The purpose of the present study was to clarify whether the amounts of these three carboxylesterase isozymes in liver microsomes are affected by well-known inducing agents such as *trans*-stilbene oxide, aminopyrine, Aroclor 1254 (a complex mixture of polychlorinated biphenyls), and clofibrate.

Materials and methods

Male and female rats of the Sprague–Dawley strain were used. Animals were fed a laboratory chow and water *ad lib.* and were housed in wire-bottomed cages at constant temperature (22–24°) and humidity (50–60%) under a 12-hr light–12-hr dark cycle (7:00 a.m. to 7:00 p.m.). Male and female rats at 7 weeks of age were treated by intraperitoneal injection with *trans*-stilbene oxide (in corn oil, 150 mg/kg, daily, five times) or Aroclor 1254 (in corn oil, 40 mg/kg, daily, three times), or orally with aminopyrine (suspended in gum arabic, 600 mg/kg, daily, six times) and clofibrate (suspended in gum arabic, 300 mg/kg, daily, three times). The animals were killed approximately 24 hr after the last treatment. Preparation of the microsomal fraction was carried out as described previously [10].

Carboxylesterase activities towards four substrates, i.e. *p*-nitrophenylacetate, malathion, isocarboxazid, and butanilicaine, were determined essentially according to the methods described in previous papers [10, 11]. The hydrolysis of long-chain acyl-CoA* was assayed in 30 mM Hepes buffer (pH 7.4, 30°) by colorimetric determination (412 nm) of free thiol groups by reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) according to the method of Berge [12], as previously described [10].

Carboxylesterases RL1, RL2 and RH1 were purified from liver microsomes of untreated male rats as described previously [10]. Antibodies to these isozymes were prepared as described previously [10]. Immunochemical quantitation of the forms of carboxylesterase isozymes in liver microsomes was conducted by the method of Thomas *et al.* [13] with minor modifications [10]. Protein was determined by the method of Lowry *et al.* [14]. All data in this study were statistically analyzed by Student's *t*-test after Smirnoff's elimination test for outliers.

Results and discussion

In a previous paper, we investigated the induction of carboxylesterases RL2 and RH1 in liver microsomes in male and female rats by phenobarbital treatment; RL1 was not affected [10]. In the same paper, we also showed that the three carboxylesterase isozymes have different catalytic properties and are immunologically different from each other as determined by immunochemical blotting analysis and immunochemical inhibition of catalytic activity. Thus, in the present work, we used a rather specific substrate for each isozyme, i.e. *p*-nitrophenylacetate and malathion for RL1, butanilicaine for RH1 and isocarboxazid for RL2, to measure and discriminate the hepatic carboxylesterases.

Table 1 shows the carboxylesterase activities in liver

Table 1. Effects of aminopyrine on carboxylesterase activities and isozyme contents of hepatic microsomes in adult male rats

	Specific activity				Isozyme contents‡ (μg/mg protein)		
	p-Nitrophenylacetate hydrolase*	Malathion hydrolase†	Butanilicaine hydrolase†	Isocarboxazid hydrolase†	RL1	RH1	RL2
Male							
Control	1.52 ± 0.4	52.3 ± 10	116 ± 5.0	38.3 ± 8.9	7.5 ± 1.7	16.4 ± 1.6	44.9 ± 7.4
Aminopyrine	2.95 ± 0.1‖	89.5 ± 12¶	133 ± 4.0	135 ± 18**	21.1 ± 1.4‖	24.1 ± 3.3	86.7 ± 14¶
Female							
Control	0.87 ± 0.1	27.2 ± 8.3	32.5 ± 1.0	45.4 ± 3.8	1.1 ± 0.2	7.8 ± 0.5	99.8 ± 8.9
Aminopyrine	1.47 ± 0.1¶	54.1 ± 11¶	49.3 ± 1.0	112 ± 4.7‖	6.0 ± 0.4‖	11.7 ± 1.7	166 ± 23¶

Each value is the mean ± SE for four to six preparations. Male and female rats at 7 weeks of age were treated, by p.o. administration, with aminopyrine (suspended in gum arabic, 600 mg/kg, six consecutive days).

* Expressed in μmol/mg protein/min.

† Expressed in nmol/mg protein/min.

‡ Expressed in nmol/mg protein/30 min.

§ Immunochemical quantitation of the forms of carboxylesterase isozymes was conducted by the method of radial immunodiffusion analysis.

‖–** Significantly different versus control: Significantly different versus control: ‖$P < 0.01$, ¶$P < 0.05$, and **$P < 0.001$.

* Abbreviations: CoA, coenzyme A; IgG, immunoglobulin G; and Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

Table 2. Effects of *trans*-stilbene oxide (TSO) and Aroclor 1254 (PCB) on carboxylesterase activities and isozyme contents of hepatic microsomes in adult male rats

	Specific activity				Isozyme content§ (μg/mg protein)		
	p-Nitrophenylacetate hydrolase*	Malathion hydrolase†	Butanilicaine hydrolase†	Isocarboxazid hydrolase‡	RL1	RH1	RL2
Control	1.93 ± 0.1	74.6 ± 9.2	119 ± 25	60.0 ± 11	13.5 ± 4.8	24.5 ± 5.1	56.1 ± 8.9‖
TSO	2.38 ± 0.2‖	83.3 ± 3.9	144 ± 53	85.0 ± 11‖	12.6 ± 3.9	23.8 ± 5.3	72.1 ± 10‖
PCB	1.92 ± 0.1	76.1 ± 7.6	146 ± 20	71.5 ± 2.7	13.5 ± 4.6	25.7 ± 2.6	83.1 ± 13‖

Each value is the mean ± SE from three to five preparations. Male rats at 7 weeks of age were treated, by i.p. injection, with *trans*-stilbene oxide (dissolved in corn oil, 150 mg/kg, 5 consecutive days) or Aroclor 1254 (dissolved in corn oil, 40 mg/kg, 3 consecutive days).

* Expressed in μmol/mg protein/min.

† Expressed in nmol/mg protein/min.

‡ Expressed in nmol/mg protein/30 min.

§ Immunochemical quantitation of the forms of carboxylesterase isozymes was conducted by the method of radial immunodiffusion analysis.

‖ Significantly different versus control, $P < 0.05$.

Table 3. Effects of clofibrate on carboxylesterase and palmitoyl-CoA hydrolase activities and isozyme contents of hepatic microsomes and cytosol in adult male rats

	Specific activity				Isozyme content§ (μg/mg protein)		
	p-Nitrophenylacetate hydrolase*	Butanilicaine hydrolase†	Isocarboxazid, hydrolase‡	Palmitoyl-hydrolase†	RL1	RH1	RL2
Microsomes							
Control	2.28 ± 0.1	65.8 ± 7.9	41.8 ± 3.6	41.8 ± 1.9	10.8 ± 1.4	16.4 ± 1.2	46.4 ± 3.6
Clofibrate	3.01 ± 0.2‖	275 ± 28¶	115 ± 16¶	51.2 ± 2.0**	17.0 ± 1.2**	35.2 ± 2.4‖	79.2 ± 3.6‖
Cytosol							
Control	0.25 ± 0.1	ND††	6.7 ± 0.8	24.8 ± 1.8	ND	ND	ND
Clofibrate	0.18 ± 0.01	ND	4.1 ± 0.4	89.2 ± 16‖	ND	ND	ND

Each value is the mean ± SE from four to five preparations. Male rats at 7 weeks of age were treated by p.o. administration, with clofibrate (suspended in gum arabic, 300 mg/kg, 3 consecutive days).

* Expressed in μmol/mg protein/min.

† Expressed in nmol/mg protein/min.

‡ Expressed in nmol/mg protein/30 min.

§ Immunochemical quantitation of the forms of carboxylesterase isozymes was conducted by the method of radial immunodiffusion analysis.

‖–** Significantly different versus control: ‖$P < 0.01$, ¶$P < 0.001$, and **$P < 0.05$.

†† Not detectable.

microsomes in aminopyrine-treated rats of both sexes. In control rats, the activities towards *p*-nitrophenylacetate, malathion and butanilicaine in male rats were significantly higher than in females; conversely, isocarboxazid hydrolase activity in males was lower than in females. The result of radial immunodiffusion assay for carboxylesterases reactive with anti-RL1-, -RL2- and -RH1-IgG are also shown in Table 1. It was confirmed that the amounts of RL1 and RH1 were greater in males than in females; conversely the amount of RL2 in females was greater than in males. This result was similar to that obtained in the case of each rather specific substrate. The amount of RL1 was increased more in females than in males by aminopyrine; this result is consistent with the change in malathion hydrolase activity. Conversley, the amount of RL2 was increased more in male rats than in female rats by aminopyrine; this result is similar to that in the case of isocarboxazid hydrolase activity. In both cases, butanilicaine hydrolase activity was not increased significantly, consistent with the finding for RH1. In a previous paper [10], We found that the amounts of RH1 and RL2, but not RL1, were increased by phenobarbital treatment in both sexes. We previously reported that aminopyrine is a specific inducer of gamma-glutamyl-transpeptidase in liver [15]. It appears that the mechanisms of enzyme induction by aminopyrine and phenobarbital are different. These results indicate that aminopyrine has both a sex- and an isozyme-dependent effect.

Table 2 shows the carboxylesterase activities and immunochemical quantitation of carboxylesterase isozymes by radial immunodiffusion assay in liver microsomes of adult male rats treated with *trans*-stilbene oxide and Aroclor 1254. These treatments caused an increase in the amount of RL2. This result was similar to that for isocarboxazid hydrolase activity. *Trans*-stilbene oxide is also an inducer of glutathione *S*-transferase in rat liver [16]. It appears that *trans*-stilbene oxide and Aroclor 1254 behave similarly in the induction of carboxylesterases in liver microsomes.

Table 3 shows the effects of clofibrate on hepatic carboxylesterase and palmitoyl-CoA hydrolase activities in liver microsomes and cytosol in adult male rats. Administration of clofibrate at a dose of 300 mg/kg daily to rats for 3 days caused a significant increase in the activities of *p*-nitrophenylacetate, isocarboxazid and butanilicaine and palmitoyl-CoA hydrolases in liver microsomes. *p*-Nitrophenylacetate and isocarboxazid hydrolase activities in liver cytosol were not induced by clofibrate, whereas palmitoyl-CoA hydrolase activity was induced significantly. These results were consistent with the finding on the amounts of three carboxylesterase isozymes in liver microsomes and cytosol by radial immunodiffusion assay (Table 3). It was confirmed that clofibrate induced all three isozymes of carboxylesterases in hepatic microsomes, but not in the cytosol. In a previous paper, we showed that the palmitoyl-CoA hydrolase activity in microsomes was inhibited strongly by anti-RL1 IgG, but not by anti-RH1 and -RL2-IgG [17]. On the other hand, palmitoyl-CoA hydrolase activity in liver cytosol was not inhibited by anti-RL1-, -RL2- or -RH1-IgG in clofibrate-treated rats.* Carboxylesterase RL1, which was induced by clofibrate, was found to possess palmitoyl-CoA hydrolase activity as well as long-chain monoglyceride lipase and cholesterol-oleate hydrolase activities.* Therefore, we consider that RL1 may play an important role in lipid metabolism in the endoplasmic reticulum.

In conclusion, we found that the three carboxylesterase isozymes in rat liver microsomes were induced by aminopyrine, *trans*-stilbene oxide, Aroclor 1254, and clofibrate to different extents.

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REFERENCES

1. K. Krisch, in *The Enzymes* (Ed. P. D. Boyer), Vol. 5, pp. 43–69. Academic Press, New York (1971).
2. R. Mentlein, S. Heiland and E. Heymann, *Archs Biochem. Biophys.* **200**, 549 (1980).
3. E. Heymann, in *Enzymatic Basis of Detoxication* (Ed. W. B. Jakoby), pp. 291–323, Academic Press, New York (1981).
4. T. Satoh, in *Reviews in Biochemical Toxicology* (Eds. E. Hodgson, J. R. Bend and R. M. Philpot), Vol. 8, pp. 155–81. Elsevier, New York (1987).
5. T. Satoh and K. Moroi, *Jap. J. Pharmac.* **27**, 233 (1977).
6. W. S. Schwark and D. J. Ecobichon, *J. Physiol. Pharmac.* **46**, 207 (1968).
7. B. R. Sonawane and C. O. Knoles, *Pestic. Biochem. Physiol.* **1**, 472 (1972).
8. M. Raftell, K. Bergins and F. Blomberg, *Archs Biochem. Biophys.* **181**, 534 (1977).
9. T. Satoh and K. Moroi, *Life Sci.* **12**, 169 (1973).
10. M. Hosokawa, T. Maki and T. Satoh, *Molec. Pharmac.* **31**, 579 (1987).
11. M. Hosokawa, T. Satoh, S. Ohkawara, S. Ohmori, T. Igarashi and H. Kitagawa, *Res. Commun. Chem. Path. Pharmac.* **46**, 245 (1984).
12. R. K. Berge, *Biochim. biophys. Acta* **574**, 321 (1979).
13. P. E. Thomas, D. Korzeniowski, D. Ryan and W. Levin, *Archs Biochem. Biophys.* **192**, 524 (1979).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
15. T. Satoh, T. Igarashi, T. Hirota and H. Kitagawa, *J. Pharmac. Exp. Ther.* **221**, 795 (1982).
16. H. Mukhtar, T. H. Elmamlook and J. R. Bend. *Chem. Biol. Interact.* **22**, 125 (1978).
17. M. Hosokawa, T. Maki, H. Kitagawa and T. Satoh, J. *Pharmacobio-Dynamics* **9**, s-22 (1986).

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