オイゲノール投与ラット肝ミクロゾームにおけるベンツピレン水酸化活性の特異的低下

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Specific Reduction of Hepatic Microsomal Benzo[*a*]pyrene Hydroxylase Activity after Administration of Eugenol to the Rat

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ABSTRACT. Suppressed mutagenicity of benzo[*a*]pyrene (B[*a*]P) in the mutagenesis test (Ames test) using liver microsomes prepared from rats treated with eugenol (4-allyl-2-methoxyphenol) was previously reported [Yokota, H., Hoshino, J., and Yuasa, A., 1986, Mutation Res. 172: 231–236]. The mechanism of this suppression was examined. Specific reduction of B[*a*]P hydroxylase activity in rat liver microsomes was observed by administration with eugenol. However, eugenol treatment did not change either the contents or activities of other microsomal cytochrome P-450-dependent monooxygenase system. Cytochrome P-450s were solubilized with sodium cholate from eugenol-treated rat liver microsomes, and separated into three fractions by the DEAE-TOYOPEARL column chromatography at room temperature. Cytochrome P-450 content of the fraction having B[*a*]P hydroxylase activity decreased in contrast to the corresponding fraction from untreated rat liver microsomes. In the Ouchterlony double diffusion analysis, the antibody against P-450c (a major 3-methylcholanthrene inducible form of P-450 highly catalytic of B[*a*]P hydroxylation) did not show any reaction line with untreated microsomes. B[*a*]P hydroxylase activity in untreated microsomes was not inhibited by the antibody against P-450c. These results suggest that specific cytochrome P-450 isozyme(s) mediating the B[*a*]P hydroxylase activity was reduced in eugenol-treated rat liver microsomes, and that the isozyme(s) was a different isozyme from P-450c.—KEY WORDS: benzo[*a*]pyrene hydroxylase, cytochrome P-450, eugenol, microsome, rat liver.

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Some edible plant extracts have been reported to inhibit carcinogenesis or mutagenesis [2]. Recently, it was reported that ellagic acid and some plant phenols inhibited the mutagenicity of ultimate carcinogenic metabolites of benzo[*a*]pyrene (B[*a*]P) [4, 5, 25]. B[*a*]P is a well known potent carcinogen and mutagen. B[*a*]P is itself relatively inert compound and essentially acts as premutagen, and B[*a*]P requires metabolic activation before it exerts mutagenicity [2]. In this activation process, the first step is usually carried out by cytochrome P-450-dependent monooxygenase system of microsomes [12].

Eugenol, a principal constituent of clove oil, is a plant phenol widely used as flavor additive and chemical intermediate. We have been reported the enhancement of glucuronidation and the induction of liver microsomal UDP-glucuronyltransferase by oral administration with eugenol to rats [26, 28–30]. Recently, it was reported that eugenol is not carcinogenic in male or female rats [20], and that eugenol neither inhibit nor induce a mutagenic response observed in mutagenesis test using *Salmonella typhimurium* (Ames test) [20, 31]. In the previous paper [27], we reported that the mutagenicity of B[*a*]P in the Ames test by use of liver microsomes from eugenol-treated rats was suppressed to a lower level than that obtained using untreated rats. We also reported that the mutagenic activity of B[*a*]P and cytochrome P-450-dependent B[*a*]P hydroxylase activity were not inhibited by the direct addition of eugenol into assay mixtures [27].

In the present paper, we demonstrated that eugenol reduced B[*a*]P hydroxylase activity in rat liver microsomes, and that this reduction resulted from the decrease of specific isozyme(s) of cytochrome P-450.

MATERIALS AND METHODS

Materials: DEAE-TOYOPEARL was obtained from Toso Co., Tokyo, Japan. Phenobarbital (PB), 3-methylcholanthrene (3-MC), dilauroylphosphatidylcholine (DLPC) and 2', 5'-ADP-agarose were from Sigma Chemical Co., St. Louis, USA. Eugenol, B[*a*]P and cholic acid were purchased from Wako Pure Chemical Industries, Osaka, Japan. Cholic acid was further purified and converted to the sodium salt as described [10]. Benzphetamine was a generous gift from Dr. Y. Imai, Institute for Protein Research, Osaka University. Emulgen 911 was kindly supplied by Kao-Atlas Co., Tokyo, Japan.

Treatment of animals: Male Wistar rats (250 g) were used. Eugenol (200 mg) diluted to 1 m*l* with olive oil was orally administered to the rats 4 times at 12 hr intervals, and the rats were sacrificed 2 days after final treatment. PB was dissolved in saline and intraperitoneally injected daily for 3 days at a dose of 100 mg/kg of body weight, and the rats were killed 2 days after the final treatment. 3-MC were dissolved in olive oil and intraperitoneally injected at a single dose of 80 mg/kg, and the rats were killed at the 4th day.

Preparation of microsomes: Rat liver, after being perfused with cold 0.15 M KCl, was minced and homogenized with 4 volumes of the same solution. The homogenate was centrifuged at 9,000×g for 20 min, then the supernatant was centrifuged at 105,000×g for 60 min to prepare the microsomal fraction.

Analytical procedures: The content of cytochrome P-450 was determined from the difference spectrum between the reduce-CO complex and reduced samples as described by Omura and Sato [21] and the Soret absorption maximum of reduced-CO complex was measured at the same time. The content of cytochrome b_5 was determined as described by Omura and Sato [22]. The activity of NADPH-cytochrome P-450 reductase [22] and NADH-cytochrome b_5 reductase [8] were assayed as described previously. B[*a*]P hydroxylase activity in microsomes was assayed by the method of Nebert and Gelboin [19]. In the assay for partially purified preparation, NADPH-cytochrome P-450 reductase and DLPC were added to the reaction mixture at each optimal concentration. The activities for *N*-demethylation of aminopyrine and benzphetamine, and for *O*-demethylation of *p*-nitroanisole were assayed by determining the product of formaldehyde, as described by Nash [18]. *O*-deethylase activity of 7-ethoxycoumarin was assayed as described by Ullrich and Weber [24]. Protein concentration was determined by the method of Lowry *et al.* [16] using bovine serum albumin as a standard protein.

Solubilization and separation of B[a]P hydroxylase activity from liver microsomes: For solubilization of B[*a*]P hydroxylase activity, liver microsomes were suspended at a protein concentration of about 3 mg/m*l* in 0.1 M potassium phosphate buffer (pH 7.4) containing 0.6% sodium cholate, 1 mM EDTA and 1 mM dithiothreitol (DTT). After stirring for 30 min, the resultant fluid was centrifuged at 105,000×g for 90 min, and the supernatant was dialyzed overnight against 5 mM potassium phosphate buffer (pH 7.4) containing 20% (v/v) glycerol, 0.2% (w/v) Emulgen 911, 0.5% sodium cholate, 0.1 mM EDTA and 1 mM DTT. The dialyzed sample was applied on a column packed with the same volume of DEAE-TOYOPEARL (1.4×7 cm), which was previously equilibrated with the di-

alyzed buffer and maintained at room temperature. After washing the column with one column volume of the dialyzed buffer, B[a]P hydroxylase activity was eluted with 4 column volumes of the buffer containing a liner gradient of 0 to 0.25 M NaCl.

Purification of P-450c and reductase: Cytochrome P-450c was purified from 3-MC-treated rat liver microsomes as described by Ryan *et al.* [23]. NADPH-cytochrome P-450 reductase was purified from liver microsomes of PB-treated rats as described by Imai [9].

Preparation of antibodies against P-450c and its effect on B[a]P hydroxylase activity: The antibody against P-450c was prepared as described by Kuwahara *et al.* [14]. The immunoglobulin fraction was prepared from the pooled antisera by ammonium sulfate fractionation (33–40%). The Ouchterlony diffusion plates containing 1.0% agar, 1.0% sodium cholate, 0.9% NaCl and 0.02% sodium azide in 10 mM potassium phosphate buffer (pH 7.4) were used, and the immunodiffusion was carried out at 4°C. B[a]P hydroxylase activity in the presence of the antibody against cytochrome P-450c were assayed after preincubation of microsomes with various amounts of the antibody at 37°C for 10 min. All the reaction mixture were made up with control immunoglobulin to the same final immunoglobulin concentration.

RESULTS

Microsomal P-450-dependent monooxygenase activities from eugenol-, 3-MC- and PB-treated rat livers were shown in Table 1. 3-MC especially increased the activities of B[a]P hydroxylase and 7-ethoxycoumarin *O*-deethylase, and PB increased most microsomal drug monooxygenase activities except B[a]P hydroxylase activity. Similar findings have been reported elsewhere [1, 3]. In contrast, eugenol decreased microsomal monooxygenase activities, especially B[a]P hydroxylase activity (29% of untreated control). Effects of eugenol, 3-MC and PB on microsomal cytochrome P-450-dependent and cytochrome b_5-dependent electron transport system were shown in Table 2. PB and 3-MC increased the components of microsomal P-450-dependent monooxygenase system. Eugenol slightly decreased the content of cytochrome P-450 that directly mediates a monooxygenase,

Table 1. Effects on rat liver microsomal P-450-dependent monooxygenase activities by administration of eugenol, 3-methylcholanthrene and phenobarbital

Substrates	Specific activity (nmol of product/min/mg of protein)			
	Untreated	Eugenol	3-Methylcholanthrene	Phenobarbital
Benzo[a]pyrene	0.234±0.045[b] (100)[a]	0.067±0.012 (29)	0.951±0.184 (406)	0.142±0.022 (61)
7-Ethoxycoumarin	0.221±0.056 (100)	0.209±0.033 (95)	1.390±0.242 (628)	0.560±0.102 (253)
Aminopyrine	2.282±0.049 (100)	1.368±0.073 (60)	1.741±0.128 (76)	6.124±0.327 (268)
Benzphetamine	5.61 ±1.43 (100)	3.51 ±0.81 (63)	2.14 ±0.48 (38)	23.98±6.54 (427)
p-Nitroanisole	1.076±0.042 (100)	0.833±0.111 (77)	4.037±0.398 (375)	4.643±0.828 (431)

a) % of untreated control.
b) Means±S.E. for 6 animals.

while other monooxygenase system components, cytochrome b_5, NADPH-cytochrome P-450 reductase and NADH-cytochrome b_5 reductase were not influenced by the treatment with eugenol (Table 2.). As described above, eugenol was shown to be a negative inducer of B[*a*]P hydroxylase activity.

Microsomal cytochrome P-450s, which mediated the B[*a*]P hydroxylase and other monooxygenases were solubilized with 0.6% sodium cholate and separated into three fractions by DEAE-TOYOPEARL column chromatography at room temperature as described by Ryan *et al.*[23]. The elution profiles of B[*a*]P hydroxylase and cytochrome P-450s in untreated and eugenol-treated rat liver microsomes on DEAE-TOYOPEARL chromatography were shown in Fig. 1 (A) and (B), respectively. The chromatograms showed that the reduced-CO complex in the first and second fractions of cytochrome P-450 had a Soret absorption maximum at 450 nm, and that of the third fraction had that at 448 nm. B[*a*]P hydroxylase activity was almost detected in the second fraction. B[*a*]P hydroxylase activity and cytochrome P-450 content in the second fraction reduced to about 30% and 60%, respectively, by eugenol treatment. When we applied 3-MC-treated rat liver microsomes, B[*a*]P hydroxylase activity was not detected in the second fraction, but detected in the third fraction, of which the reduced-CO complex gave a Soret absorption maximum at 448 nm (data not shown).

Fig. 2 showed the Ouchterlony double diffusion analysis. The antibody prepared against P-450c reacted with P-450c and also solubilized microsomes from 3-MC-treated rat liver, and they formed a single fused precipitation line. On the other hand, the antibody did not show any detectable reaction lines with solubilized microsomes from untreated, PB-treated and eugenol-treated rat liver, respectively (Fig. 2). These observations indicated that the antibody was closely specific for the P-450c, and the contents of P-450c were very low in liver microsomes from untreated, eugenol-treated and PB-treated rats. Fig. 3 showed that the antibody against P-450c inhibited B[*a*]P hydroxylase activity in 3-MC-treated rat liver microsomes, but not in untreated rat liver microsomes.

DISCUSSION

In the present paper, we found the administration of eugenol to rats caused the reduction of the activity of liver microsomal

Table 2. Effects of the components of microsomal cytochrome P-450-dependent and cytochrome b_5-dependent electron transport system by administration of eugenol, 3-methylcholanthrene and phenobarbital

Enzymes	Untreated	Eugenol	3-Methylcholanthrene	Phenobarbital
Cytochrome P-450 content (nmol/mg of protein)	0.90 ±0.01[b] (100)[a]	0.77 ±0.04 (85)	1.66 ±0.10 (185)	2.01 ±0.11 (222)
NADPH-cytochrome P-450 reductase activity (nmol/min/mg of protein)	0.105±0.007 (100)	0.122±0.008 (117)	0.122±0.005 (116)	0.223±0.015 (212)
Cytochrome b_5 content (nmol/mg of protein)	0.385±0:016 (100)	0.435±0.009 (113)	0.554±0.014 (144)	0.567±0.017 (150)
NADH-cytochrome b_5 reductase activity (nmol/min/mg of protein)	4.04 ±0.28 (100)	3.73 ±0.22 (92)	3.78 ±0.04 (93)	4.08 ±0.14 (101)

a) % of untreated control.
b) Means±S.E. for 6 animals.

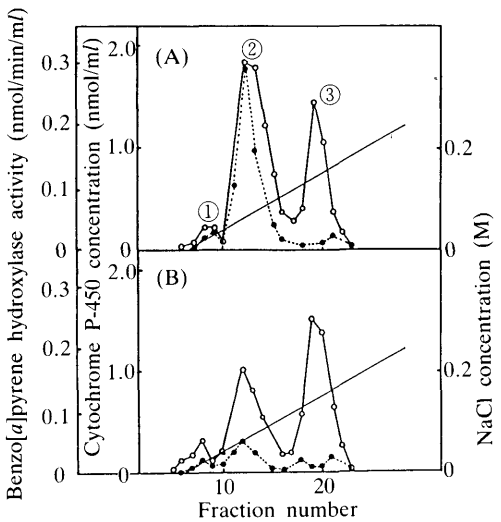

Fig. 1. Elution profiles of B[a]P hydroxylase by DEAE-TOTOPEARL column chromatography (at room temperature) of untreated rat liver microsomes (A) and eugenol-treated rat liver microsomes (B). Elution was performed with a linear gradient of 0–0.25 M NaCl. The experimental conditions are described in "MATERIALS AND METHODS". (○—○), Cytochrome P-450 concentration; (●—●), B[a]P hydroxylase activity; (——), NaCl concentration.

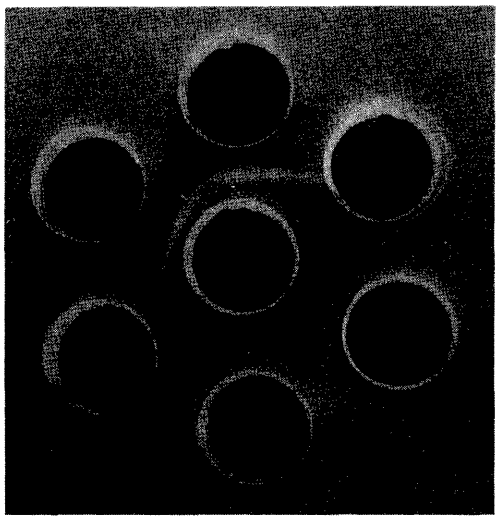

Fig. 2. Double immunodiffusion of the anti-P-450c immunoglobulin and homologous protein. The conditions of immunodiffusion are described in "MATERIALS AND METHODS". The center well (well 7) contained anti-P-450c immunoglobulin (0.5 mg). The samples in surrounding wells were as follows: well 1, purified P-450c (0.1 nmol); well 2, 3-MC-treated microsomes (120 μg); well 3, PB-treated microsomes (100 μg); well 4, untreated microsomes (220 μg); well 5, eugenol-treated microsomes (230 μg); well 6, saline. The microsomes were solubilized in 0.1 M potassium phosphate buffer (pH 7.4) containing 1.0% sodium cholate.

B[a]P hydroxylase, whereas contents or activities of enzymes of other cytochrome P-450-dependent monooxygenase system were not affected. Moreover, judging from the elution profiles of B[a]P hydroxylase activity and cytochrome P-450s by DEAE-TOYOPEARL column (Fig. 1A, B), the reduction of B[a]P hydroxylase activity by eugenol would be due to the decrease of the P-450 isozyme(s) catalyzing B[a]P hydroxylation.

Mutagenic activity of B[a]P appeared after metabolic activation by cytochrome P-450-dependent B[a]P hydroxylase [12]. Recently, ellagic acid and some plant phenols which inhibited the mutagenicity of ultimate carcinogenic metabolites of B[a]P were reported [4, 5, 25]. These antimutagens inhibited the mutagenic activity of B[a]P in the Ames test during incubation with B[a]P and liver microsomes. On the other hand, as we reported previously [27], liver microsomes prepared from rats treated with eugenol suppressed the mutagenicity of B[a]P in Ames test. This effect was not direct inhibition by eugenol. Our present data suggested that the mutagenicity suppressed by eugenol was caused by the specific reduction of cytochrome P-450 isozyme(s) catalyzing B[a]P hydroxylation. This reduction of B[a]P hydroxylase activity should be a main factor on the suppression mechanism of B[a]P mutagenicity by oral administration with eugenol to rats.

Liver microsomal cytochrome P-450 isozyme catalyzing B[a]P hydroxylation had been purified from 3-MC-treated rats, and named P-450c [23] and so on [6, 11, 13, 15]. It is well known that P-450c mediates liver microsomal hydroxylation and mutagenicity

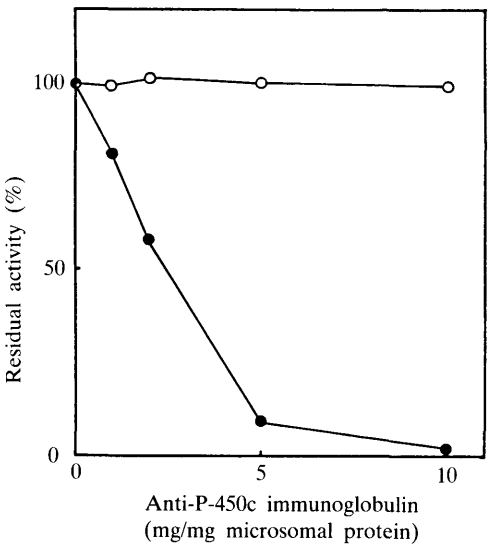

Fig. 3. Effects of the antibody against P-450c on B[*a*]P hydroxylase activity in rat liver microsomes. Liver microsomes from untreated rats (50 μg of protein, ○) and 3-MC-treated rats (40 μg, ●) were preincubated at 37°C for 10 min with various amounts of anti-P-450c immunoglobulin. Enzyme activities were assayed as described in "MATERIALS AND METHODS". 100% values of specific activities from untreated and 3-MC-treated rat liver microsomes corresponded to 0.206 and 0.982 nmol/min/mg of protein, respectively.

of B[*a*]P. So, it was conceived that the activity of B[*a*]P hydroxylase catalyzed by P-450c mediated B[*a*]P mutagenicity in untreated rat, even if the contents of P-450c was very low [12].

Our present data indicated that cytochrome P-450 catalyzing B[*a*]P hydroxylation in untreated and eugenol-treated rat liver microsomes was definitely different from that in 3-MC-treated microsomes. They had different charge and could be separated by an ion-exchange column chromatography, and Soret absorption maximum of reduced-CO complex was also different each other.

The B[*a*]P hydroxylase activity in liver microsomes of 3-MC-treated rats was almost completely inhibited by the specific antibody against P-450c (Fig. 3) as reported elsewhere [7, 17]; however, the activity in untreated rats was not affected by anti-P-450c immunoglobulin.

These data suggested that liver microsomal cytochrome P-450 isozyme(s), which mediated the B[*a*]P hydroxylase activity in untreated rat was different one from P-450c. Moreover, this P-450 isozyme(s) was reduced by the administration of eugenol.

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REFERENCES

1. Alveres, A. P., Schilling, G., Levin, W., and Kuntzmann, R. 1967. Studies on the induction of CO-binding pigments in liver microsomes by phenobarbital and 3-methylcholanthrene. *Biochem. Biophys. Res. Commun.* 29: 251–526.
2. Ames, B. N. 1982. Dietary carcinogens and anticarcinogens. *Science*, 221: 1256–1264.
3. Åström A., Maner, S., and DePierre, J. W. 1986. Induction of cytochrome P-450 and related drug-metabolizing activities in the livers of different rodent species by 2-acethylaminofluorene or by 3-methylcholanthrene. *Biochem. Pharmacol.* 35: 2703–2713.
4. Das, M., Mukhtar, H., Bik, D. P., and Bickers, D. R. 1987. Inhibition of epidermal xenobiotic metabolism in SENCAR mice by naturally occurring phenols. *Cancer Res.* 47: 760–766.
5. Dixit, R., Teel, R. W., Daniel, F. B., and Stoner, G. D. 1985. Inhibition of benzo[*a*]pyrene and benzo[*a*]pyrene-trans-7, 8-diol metabolism and DNA binding in mouse lung explants by ellagic acid. *Cancer Res.* 45: 2951–2956.
6. Elshourbagy, N. A., and Guzelian, P. S. 1980. Separation, purification, and characterization of a novel form of hepatic cytochrome P-450 from rats treated with pregnenolone-16α-carbonitrile. *J. Biol. Chem.* 255: 1279–1280.
7. Harada, N., and Omura, T. 1981. Selective induction of two different molecular species of cytochrome P-450 by phenobarbital and 3-methylcholanthrene. *J. Biochem.* 89: 237–248.
8. Hrycay, E. G., and Prough, R. A. 1974. Reduced nicotinamide adenine dinucleotide-cytochrome b_5 reductase and NADH-supported cytochrome P-450-dependent enzyme activities in liver microsomes. *Archs Biochem. Biophys.* 165: 331–339.
9. Imai, Y. 1979. Reconstituted *O*-dealkylase systems containing various form of liver microsomal

cytochrome P-450. *J. Biochem.* 86: 1697–1707.
10. Imai, Y., and Sato, R. 1974. An affinity column method for partial purification of cytochrome P-450 from phenobarbital-induced rabbit liver microsomes. *J. Biochem.* 75: 689–697.
11. Kamataki, T., Yamazoe, Y., Mita, S., and Kato, R. 1982. Mutagenic activation and covalent binding of 3-amino-1-methyl-5*h*-pyrido (4, 3-*b*) indole (Trip-P-2) by microsomal and reconstituted cytochrome P-450 system. pp. 463–470. *In*: Microsomes, Drug Oxidations and Drug Toxicity (Sato, R., and Kato, R. eds.), Japan Scientific Societies Press, Tokyo.
12. Kawajiri, K., Yonekawa, H., Gotho, O., Watanabe, J., Igarashi, S., and Tagashira, Y. 1983. Contributions of two inducible forms of cytochrome P-450 in rat liver microsomes to the metabolic activation of various chemical carcinogens. *Cancer Res.* 43: 819–823.
13. Kuwahara, S., Harada, N., Omura, T. 1982. Purification of five forms of cytochrome P-450 from rat liver microsomes and their contributions to microsomal drug oxidations. pp. 87–90. *In*: Microsomes, Drug Oxidations and Drug Toxicity (Sato, R., and Kato, R. eds.), Japan Scientific Societies Press, Tokyo.
14. Kuwahara, S., Harada, N., Yoshioka, H., Miyata, T., and Omura, T. 1984. Purification and characterization of four forms of cytochrome P-450 from liver microsomes of phenobarbital-treated and 3-methylcholanthrene-treated rats. *J. Biochem.* 95: 703–714.
15. Lau, P. P., ans Strobel, H. W. 1982. Multiple forms of cytochrome P-450 in liver microsomes from β-naphthoflavone-pretreated rats. Separation, purification, and characterization of five forms. *J. Biol. Chem.* 257: 5257–5262.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randal R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275.
17. Masuda-Mikawa, R., Fujii-Kuriyama, Y., Negishi, M., and Tashiro, Y. 1979. Purification and partial characterization of hepatic microsomal cytochrome P-450s from phenobarbital- and 3-methylcholanthrene-treated rats. *J. Biochem.* 86: 1383–1394.
18. Nash, T. 1953. The colorimetric estimation of formaldehyde by means of the Hanzsch reaction. *Biochem. J.* 55: 416–421.
19. Nebert, D. W., and Gelboin, H. V. 1968. Substrate-inducible microsomal aryl hydroxylase in mammalian cell culture. *J. Biol. Chem.* 243: 6242–6249.
20. NTP 1983. Carcinogenesis studies of eugenol (CAS No. 97–53–0) in F344/N rats and B6C3F1 mice (Food studies). TR No. 223, pp. 159. *In*: National Toxicology Program, Research Triangle Park, NC.
21. Omura, T., and Sato, R. 1964. The carbon monooxide-binding pigment of liver microsomes, II. Solubilization, purification and properties. *J. Biol. Chem.* 239: 2379–2385.
22. Omura, T., and Sato, R. 1970. A new method for simultaneous purification of cytochrome b_5 and NADPH cytochrome c reductase from rat liver microsomes. *J. Biochem.* 67: 249–257.
23. Ryan, D. E., Thomas, P. E., Korzeniowski, D., and Levin, W. 1979. Separation and characterization of highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital, 3-methylcholanthrene. *J. Biol. Chem.* 254: 1365–1374.
24. Ullrich, V., and Weber, P. 1972. The *O*-dealkylation of 7-ethxycoumarin by liver microsomes. A direct fluorometric test. *Hoppe-Seyler's Z. Physiol. Chem.* 353: 1171–1177.
25. Wood, A. W., Huang, M. -T., Chang, R. L., Newmark, H. L., Lher, R. E. Yagi, H., Sayer, J. M., Jerina, D. M., and Conney A. H. 1982. Inhibition of the mutagenicity of bay-reagion diol epoxides of polycyclic aromatic hydrocarbons by naturally occurring plant phenols: Exceptional activity of ellagic acid. *Proc. Natl. Acad. Sci. (U.S.A.)* 79: 5513–5517.
26. Yokota, H., Hashimoto, H., Motoya, M., and Yuasa, A. 1988. Enhancement of UDP-glucuronyltransferase, UDP-glucose dehydrogenase, and glutathione *S*-transferase activities in rat liver by dietary administration of eugenol. *Biochem. Pharmacol.* 37: 799–802.
27. Yokota, H., Hoshino, J., and Yuasa, A. 1986. Suppressed mutagenicity of benzo[*a*]pyrene by the liver S9 fraction and microsomes from eugenol-treated rats. *Mutation Res.* 172: 231–236.
28. Yuasa, A. 1974. Experimental studies of glucuronidation, III. UDP-glucuronyltransferase activity and glucuronide excretion enhanced by oral administration of eugenol. *Jap. J. Vet. Sci.* 36: 427–432.
29. Yuasa, A. 1982. Enhancement of liver UDP-glucuronyltransferase, UDP-glucose dehydrogenase and urinary glucuronide excretion in the rat, caused by oral administration of an effective glucosidurogenic compound, eugenol. pp. 419–420. *In*: Microsomes, Drug Oxidations, and Drug Toxicity (Sato, R., and Kato, R. Eds.), Japan Scientific Societies Press, Tokyo.
30. Yuasa, A., and Yokota, H. 1982. Induction of liver microsomal UDP-glucuronyltransferase by eugenol and inducers of microsomal monooxygenases in the rat. pp. 185–188. *In*: Cytochrome P-450, Biochemistry, Biophysics and Environmental Implications (Hietanen, E., Laitinen, M.,

and Hänningen, O. Eds.), Elsevier Biomedical Press, Amsterdam.
31. Zeiger, E. 1987. Carcinogenicity of mutagens: Predictive capability of the *Salmonella* mutagenesis assay for rodent carcinogenicity. *Cancer Res.* 47: 1287–1296.

要　約

オイゲノール投与ラット肝ミクロゾームにおけるベンツピレン水酸化活性の特異的低下：扇谷信幸・横田博・湯浅　亮(酪農学園大学酪農学部獣医生化学教室)——植物精油成分オイゲノールを投与したラット肝ミクロゾームにおけるベンツピレン変異原性抑制現象の要因について検討し，以下の知見を得た．①オイゲノール投与ラット肝ミクロゾームでは，チトクローム P-450(P-450)により触媒される薬物酸化活性のうちベンツピレン水酸化活性のみが顕著に低下していた．他のミクロゾーム電子伝達系成分の量や活性値は殆ど変わらなかった．②肝ミクロゾームの P-450 を可溶化し，DEAE-TOYOPEARL を用いた陰イオン交換室温クロマトグラフィーで P-450 を分離した結果，オイゲノール投与肝ミクロゾームでは，ベンツピレン水酸化活性が存在する画分の P-450 含量が低下していた．③ゲル内沈降反応の結果，無処置ラット肝ミクロゾームは P-450c（3-メチルコラントレンで誘導され，高いベンツピレン水酸化活性を有している分子種)に対する抗体と反応しなかった．また，そのベンツピレン水酸化活性は P-450c 抗体で阻害されなかった．以上の結果から，オイゲノール投与ラット肝ミクロゾームでは，ベンツピレン水酸化活性を触媒する種類の P-450（P-450cとは免疫化学的に異なる)の量的低下が示唆された．