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Age-Related Changes in Hepatic Microsomal Drug Metabolism are Substrate Selective[1]

LORA E. RIKANS and BRUCE A. NOTLEY
Department of Pharmacology, University of Oklahoma, Health Sciences Center, Oklahoma City, Oklahoma
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ABSTRACT

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Microsomes were isolated from livers of male Fischer 344 rats at 3 to 5, 14 to 15 and 24 to 25 months of age for the determination of monooxygenase components and drug metabolism activities. Microsomal cytochrome P-450, cytochrome b_5, NADPH-cytochrome *c* reductase activity and phospholipid were decreased in middle-aged and old rats compared with young-adult rats, but the enzymatic reduction of microsomal cytochrome P-450 was unchanged. Drug metabolism activities both decreased and increased as a consequence of aging, depending upon the substrate used. Differences were observed between young-adult and old rats in the CO maximum of reduced microsomal cytochrome P-450, in microsomal fatty acid composition and in the amounts of microsomal polypeptides having molecular weights of 52,500 and 53,000. The substrate selectivity of the age-related alterations in hepatic microsomal drug metabolism may be due to qualitative changes in the cytochrome P-450 and phospholipid components of the monooxygenase system.

A number of studies suggest that hepatic microsomal drug metabolism diminishes in old age, but the basis for this decline remains uncertain. Although the early work of Kato and Takanaka (1968a,b) indicated that cytochrome P-450 content and NADPH-cytochrome *c* reductase activity were diminished in liver microsomes from old rats, more recent studies of the effects of aging on concentrations of microsomal monooxygenase components have yielded contradictory results. For example, some investigators reported that hepatic microsomal cytochrome P-450 content diminished in old rats (Kao and Hudson, 1980; McMartin *et al.*, 1980; Schmucker and Wang, 1980, 1981), whereas others found that concentrations of the cytochrome were unaffected by aging (Baird *et al.*, 1975; Birnbaum and Baird, 1978a,b). Decreased levels of cytochrome *c* reductase activity were usually found in conjunction with diminished cytochrome P-450 content (Kao and Hudson, 1980; McMartin *et al.*, 1980; Schmucker and Wang, 1980, 1981); however, one study found that aging affected only the reductase activity (Baird *et al.*, 1975). Although age-related decreases in rat hepatic microsomal phospholipid content have also been reported (Grinna and Barber, 1972; Grinna, 1977; Birnbaum and Baird, 1978b), the decreases were not always associated with a decline in drug metabolism activity (Birnbaum and Baird, 1978b). In contrast to other monooxygenase components, microsomal cytochrome b_5 content in rat liver was reported to increase slightly or remain unaltered with aging (Birnbaum and Baird, 1978a; McMartin *et al.*, 1980). Finally, a study with senescent mice demonstrated that hepatic microsomal drug metabolism activities could be altered markedly (either increased or decreased) while cytochrome P-450 content, cytochrome *c* reductase activity and phospholipid content remained unchanged. Some of these conflicting findings may be due to differences between the various studies in animal species, strain and sex or in the ages used for comparison. Unfortunately, the results neither provide a clear picture of the effects of aging on microsomal monooxygenase components nor suggest a uniform mechanism for age-associated changes in drug metabolism activity.

A systematic study of the effects of aging on the microsomal monooxygenase system from rat liver was undertaken in this laboratory. Monooxygenase components and drug metabolism activities were measured in liver microsomes from male Fischer 344 rats maintained under identical conditions throughout adulthood. Young-adult, middle-aged and old rats were 3 to 5 months, 14 to 15 months and 24 to 25 months old, respectively. The effects of aging from young adulthood to middle age have been reported (Rikans and Notley, 1981).

Methods

Animals. Rats (Fischer 344 males) were purchased from Charles River Breeding Laboratories (Wilmington, MA) at 7 weeks of age and maintained in the Animal Facilities of the University of Oklahoma

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ABBREVIATION: SDS, sodium dodecyl sulfate.

Health Sciences Center for the duration of the study. They were group-housed under filter caps on heat-sterilized, hard-wood bedding. Temperature, humidity and lighting cycles remained constant and exogenous chemicals (fumigants, disinfectants, pesticides and cigarette smoke) were avoided. The rats were fed a semipurified diet (AIN 76 Semipurified diet; ICN Nutritional Biochemicals, Cleveland, OH) to avoid seasonal variations in the composition of commercial rations. These conditions yielded values for enzyme activities and cytochrome P-450 concentrations that remained constant over a 3-month period when the animals were 3 to 5 months of age. Only clinically healthy animals were used in the study. Sections from each of the old rat livers were removed for histologic examination and data are included only for those livers with no significant lesions other than moderate cytoplasmic vacuolation (a common finding in old rats). The mean survival age for our colony was 24 months.

Some experiments required simultaneous analysis of microsomes from young and old rats (mixing experiments and gel electrophoresis). For those experiments, male Fischer 344 rats were purchased from the colony maintained for the National Institute on Aging by Charles River Breeding Laboratories. The rats were kept for 6 weeks in our facilities before their use and the conditions for their maintenance were exactly as described above.

Microsomal monooxygenase components. Rats were killed 3 hr after the onset of the 12-hr light period and liver microsomes were prepared by differential centrifugation (Rikans and Notley, 1981). Recovery of microsomes, based on NADPH-cytochrome *c* reductase activity, was similar in all three age groups (42 ± 2, 43 ± 2 and 45 ± 3%). Cytochrome P-450 was determined from the reduced CO difference spectrum in a Pye Unicam SP8-100 spectrophotometer and cytochrome b_5 was determined from the NADH-reduced difference spectrum (Omura and Sato, 1964). Cytochrome P-450 reduction in microsomes was measured at 15°C by following the formation of the reduced cytochrome P-450-CO complex after NADPH addition under anaerobic conditions (Zannoni *et al.*, 1972); cytochrome *c* reduction by NADPH was measured at 23°C in a high ionic strength buffer (Vermilion and Coon, 1978). Lipids were extracted from microsomes with chloroform-methanol (2:1, v/v) and the fatty acid methyl esters were analyzed by gas chromatography (May and McCay, 1968). Phospholipids were determined as inorganic phosphate in the total extracted lipid (Fiske and Subbarow, 1925). A modified Lowry procedure, which included 1% SDS in the alkaline copper reagent for solubilizing membrane samples, was used to assay microsomal protein (Markwell *et al.*, 1978).

Monooxygenase activities. Freshly prepared microsomes were used for the drug metabolism assays. The procedures used have been described elsewhere in detail (Rikans and Notley, 1981). In brief, benzphetamine N-demethylation was determined by measuring the production of formaldehyde according to Nash (1953); aniline hydroxylation was determined from the production of *p*-aminophenol by the phenol-indophenol reaction (Imai *et al.*, 1966); and *p*-nitroanisole O-demethylation was determined from the production of *p*-nitrophenol (Netter and Seidel, 1964). The conditions used for these assays resulted in activities that were linear with incubation time and proportional to the amount of microsomal protein.

SDS gel electrophoresis. Polyacrylamide slab gel electrophoresis in the presence of SDS was carried out as described by Haugen and Coon (1976), except that the samples contained 10 μg of microsomal protein. Reference proteins and their molecular weights were: bovine serum albumin, 68,000; catalase, 58,000; glutamate dehydrogenase, 53,000; ovalbumin, 45,000; and carbonic anhydrase, 30,000. A slight modification (Rikans *et al.*, 1978) of the heme staining procedure of Thomas *et al.* (1976) was used for the detection of the peroxidase activity of cytochrome P-450 in SDS gels.

Statistics. Groups of data were compared by Student's *t* test to determine whether a significant difference existed.

Results

Components of the monooxygenase system. Hepatic microsomes from middle-aged and old rats contained diminished concentrations of monooxygenase components compared with those from young-adult rats. Because microsomal recoveries and microsomal protein contents were similar, the results are the same whether expressed per milligram of microsomal protein or per gram of liver. Aging from young to middle adulthood was accompanied by significant decreases in microsomal cytochrome P-450 content (32%), NADPH-cytochrome *c* reductase activity (42%), phospholipid content (41%) and cytochrome b_5 content (22%) (table 1). Similar decreases were found in hepatic microsomes from old rats except that the decrease in cytochrome b_5 content was only 11%. Although reductase activity with cytochrome *c* as the substrate diminished markedly with increasing age, activity with cytochrome P-450 as the substrate was unchanged.

Analysis of the reduced cytochrome P-450-CO absorption maxima in microsomes from young-adult and old rats revealed a small but significant difference ($P < .01$) between the two age groups. The CO maximum in microsomes from old rats was 448.6 ± 0.1 nm compared with 449.4 ± 0.1 nm in microsomes from young-adult animals.

Monooxygenase activities. Liver microsomal monooxygenase activities were not uniformly decreased as a consequence of the decline in microsomal components (fig. 1). Benzphetamine N-demethylase activity was unchanged by aging to middle adulthood but declined markedly in old age. Aniline hydroxylase activity was decreased in middle-aged compared with young-adult rats and was decreased further in old rats. On the other hand, nitroanisole O-demethylase activity was greater in liver microsomes from middle-aged and old rats than in those from young-adult rats. The substrate selectivity of the age-related changes in drug metabolism can be visualized best by relating the amount of drug metabolized to the concentration of cytochrome P-450 available (*i.e.*, nanomoles of product formed per minute per nanomole of cytochrome P-450). When

TABLE 1

Effect of age on hepatic microsomal monooxygenase components

Male Fischer 344 rats were housed under strictly controlled environmental conditions and maintained on a semipurified diet and water *ad libitum*. Young-adult rats were 3 to 5 months of age and averaged 270 to 350 g b.wt., middle-aged rats were 14 months and 530 g; and old rats were 24 months and 480 g. Values are means ± S.E.; *N* ≥ 6.

	Young Adult	Middle Aged	Old
Microsomal protein (mg/g liver)	29.0 ± 0.9	30.2 ± 0.7	31.7 ± 1.2
Cytochrome P-450 (nmol/mg protein)	0.79 ± 0.01	0.54 ± 0.03[a]	0.55 ± 0.03[a]
Cytochrome *c* reductase[b] (nmol/min/mg protein)	240 ± 10	140 ± 10[a]	120 ± 10[a]
Phospholipids (nmol lipid P/mg protein)	750 ± 20	440 ± 10[a]	410 ± 20[a]
Cytochrome b_5 (nmol/mg protein)	0.46 ± 0.01	0.36 ± 0.01[a]	0.41 ± 0.01[a, c]
Cytochrome P-450 reduction[d] (nmol/min/mg protein)	0.67 ± 0.04	0.76 ± 0.08	0.66 ± 0.03

[a] Significantly different from young-adult value, $P < .01$.

[b] Nanomoles of cytochrome *c* reduced per minute per milligram of microsomal protein at 23°C.

[c] Significantly different from middle-aged value, $P < .01$.

[d] Nanomoles of cytochrome P-450 reduced per minute per milligram of microsomal protein at 15°C.

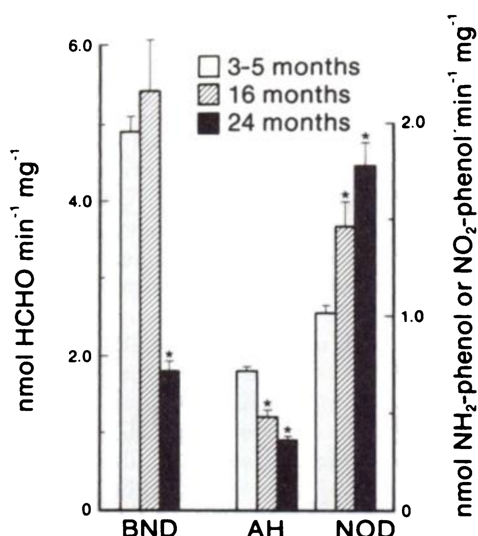

Fig. 1. Effect of aging on hepatic microsomal monooxygenase activities. Liver microsomes from rats at various ages were incubated at 37°C with the appropriate substrates and an NADPH-generating system. The activities are benzphetamine N-demethylase (BND), in nanomoles of formaldehyde per minute per milligram of microsomal protein; aniline hydroxylase (AH), in nanomoles of *p*-aminophenol per minute per milligram of protein; and nitroanisole O-demethylase (NOD), in nanomoles of *p*-nitrophenol per minute per milligram of protein. The values are means ± S.E. for six or more rats. Values which differed significantly (P < .01) from those for 3- to 5-month-old animals are indicated with an asterisk.

TABLE 2

Effect of age on cytochrome P-450-dependent drug metabolism

Liver microsomes from young-adult (3-5 months), middle-aged (14 months) and old (24 months) rats were incubated at 37°C with the appropriate substrates and an NADPH-generating system. Values are means ± S.E.; $N \geq 6$.

Age	Benzphetamine N-Demethylation	Nitroanisole O-Demethylation	Aniline Hydroxylation
	nmol product/min/nmol cytochrome P-450		
Young adult	6.08 ± 0.10[a]	1.28 ± 0.03[a]	0.90 ± 0.03[a]
Middle aged	10.06 ± 0.33[b]	2.68 ± 0.11[b]	0.89 ± 0.03[a]
Old	2.26 ± 0.16[c]	3.38 ± 0.36[b]	0.67 ± 0.04[b]

[a, b, c] Values within columns which differ statistically from each other (P < .01) are designated with different superscripts.

the activities were expressed in this fashion, the age-related changes in metabolism of nitroanisole and benzphetamine were accentuated (table 2). Nitroanisole metabolism in microsomes from middle-aged and old rats was 2.1 to 2.6 times greater than in those from young-adult rats. Benzphetamine metabolism in old age declined to less than one-fourth of the activity in middle-aged rats. On the other hand, the metabolism of aniline was decreased only slightly (25%) in microsomes from old compared with younger rats.

SDS gel electrophoresis. SDS polyacrylamide gel electrophoresis of liver microsomes from young-adult and old rats consistently showed that aging was associated with decreases in two polypeptide bands with apparent molecular weights of 52,500 and 53,000 (fig. 2). Although the two bands were not resolved under the electrophoretic conditions that are necessary for the heme-staining procedure, there were observable decreases in the amount of heme staining in the 50,000 to 55,000 MW region of the gels.

Lipid analysis. Gas chromatographic analysis of fatty acid methyl esters indicated that the composition of the microsomal fatty acids was significantly altered by the aging process (table 3). The changes were progressive as the animals aged from young to middle adulthood to old age and the percentages for individual fatty acids were completely different in microsomes from old rats compared with those in microsomes from young-adult rats. Importantly, the saturated to unsaturated fatty acids ratio increased significantly as the animals aged (0.68 ± 0.01, 0.75 ± 0.02 and 0.82 ± 0.01 in microsomes from young-adult, middle-aged and old rats, respectively).

Mixing experiments. Equal quantities of liver microsomes from young-adult and old rats were combined and the drug metabolism activities in the mixtures were compared with those in the original microsomes. In each case, the specific activity of the mixture equaled the sum of the specific activities contrib-

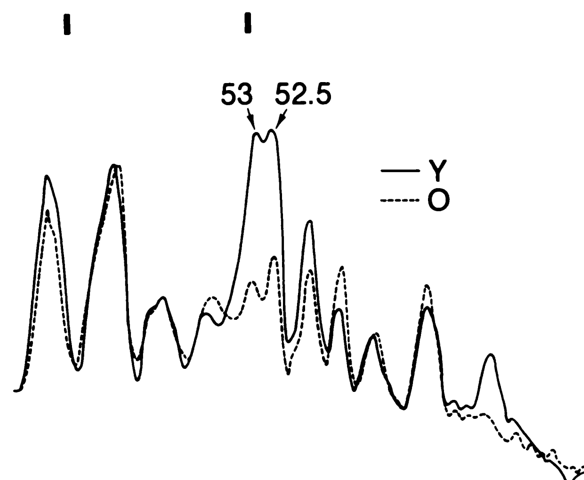

Fig. 2. SDS gel electrophoresis of hepatic microsomal proteins from young (Y) and old (O) rats. The gels were stained with Coomassie blue and scanned at 540 nm with a densitometer. Both samples contained 10 μg of protein and migration was from left to right. Cytochrome P-450 concentrations were 0.87 (Y) and 0.50 (O) nmol/mg of microsomal protein. The numbers are molecular weights × 10^{-3} and represent the average values obtained from eight experiments with bovine serum albumin, catalase, glutamate dehydrogenase, ovalbumin and carbonic anhydrase as reference proteins. The positions of catalase (MW 58,000) and glutamate dehydrogenase (MW 53,000) are indicated by bars at the top of the figure.

TABLE 3

Relative composition of liver microsomal fatty acids

Fatty acid composition was determined by using the Folch extraction procedure and gas chromatographic analysis of fatty acid methyl esters. Ages were 5, 16 and 25 months. Values are means for six rats ± S.E.

Fatty acid	Young Adult	Middle Aged	Old
	%	%	%
16:0	18.8 ± 0.2	18.5 ± 0.2	14.3 ± 0.4[a, b]
16:1	3.7 ± 0.1	2.8 ± 0.1[a]	1.8 ± 0.1[a, b]
18:0	21.5 ± 0.3	24.1 ± 0.6[a]	30.8 ± 0.5[a, b]
18:1	12.2 ± 0.2	10.0 ± 0.2[a]	9.5 ± 0.3[a]
18:2	8.8 ± 0.5	6.4 ± 0.2[a]	5.9 ± 0.2[a]
20:4	27.4 ± 0.4	30.4 ± 0.3[a]	28.9 ± 0.4[b, c]
22:4	4.9 ± 0.1	5.2 ± 0.4	6.7 ± 0.2[a, b]
22:6	2.8 ± 0.2	2.5 ± 0.2	2.0 ± 0.2[a]
Saturated	40.4 ± 0.3	42.6 ± 0.5[a]	45.2 ± 0.2[a, b]
Unsaturated	59.6 ± 0.3	57.4 ± 0.5[a]	54.8 ± 0.2[a, b]

[a] Significantly different from young-adult values, P < .01.
[b] Significantly different from middle-aged values, P < .01
[c] Significantly different from young-adult values, P < .05.

TABLE 4

Effect of combining microsomes from young-adult and old rats

Aniline hydroxylase activity (nanomoles of *p*-aminophenol formed per minute per milligram of microsomal protein at 37°C) was determined in liver microsomes from young-adult (4 months) and old (25 months) rats and in mixtures containing equal quantities of the same microsomes. The analyses were performed in duplicate on three separate occasions using freshly prepared microsomes from one young and one old rat each time. Duplicate values differed by less than 10%.

Microsomes	Aniline Hydroxylase Activity
	nmol/min/mg protein
Experiment 1	
Young	0.637
Old	0.489
Young + old	0.570
Experiment 2	
Young	0.892
Old	0.467
Young + old	0.720
Experiment 3	
Young	0.984
Old	0.552
Young + old	0.683

uted by the microsomes from young and old rats. The results for aniline hydroxylase activity are given in table 4. Similar results were obtained for benzphetamine N-demethylase and nitroanisole O-demethylase activity. These experiments provided no evidence for the presence of age-related "factors" which stimulate or inhibit specific drug metabolism activities.

Discussion

Concentrations of hepatic microsomal monooxygenase components were decreased in male Fischer rats as a consequence of aging. On the other hand, aging produced increases as well as decreases in monooxygenase activity toward selected substrates. Apparently, the components that declined with age were not rate-limiting for the oxidation of some substrates. In fact, the putative rate-limiting step in microsomal monooxygenase reactions, the enzymatic reduction of cytochrome P-450 (Gillette and Gram, 1969; Taniguchi *et al.*, 1979; Yang *et al.*, 1978), was not diminished by aging. It should be noted that similar changes in the microsomal monooxygenase system were observed in middle-aged compared with young-adult rats (Rikans and Notley, 1981). The altered capacity for drug metabolism in old animals may be the result of gradual changes throughout adulthood rather than abrupt changes at the age of senescence.

The substrate selectivity observed in this study with Fischer rats is consistent with results reported for other senescent rodents (Birnbaum and Baird, 1978a; Birnbaum, 1980). It implies that age-related changes in the microsomal enzyme system are more complicated than would be predicted from an overall decline in concentrations of monooxygenase components. Microsomes contain multiple forms of cytochrome P-450, the terminal oxidase and specificity conferring component of the monooxygenase system. Age-related changes in the relative proportions of functionally-different forms could selectively increase or decrease the metabolism of different substrates. This hypothesis is attractive, especially as the substrate specificity of age-related changes in some monooxygenase activities became more pronounced when results were expressed as product formed per nanomole of cytochrome P-450 rather than per milligram of microsomal protein (table 2). It appeared as though the cytochrome P-450 fraction in liver microsomes from old rats was much less efficient in metabolizing benzphetamine but much more efficient in metabolizing nitroanisole than the corresponding fraction from young-adult rats. Large differences in aniline metabolism (per nanomole of cytochrome P-450) were not observed, possibly because several different forms of cytochrome P-450 hydroxylate aniline at rates that are quite similar (Ryan *et al.*, 1979; Haugen *et al.*, 1975). The idea that aging affects the individual forms of cytochrome P-450 in different ways is supported by the work of McMartin *et al.* (1980). By using regioselective metabolism of R-warfarin to identify the presence of different forms of the cytochrome, they showed that liver microsomes from old rats (Wistar males) had reduced amounts of one form of cytochrome P-450 and approximately equal amounts of two other forms, compared with microsomes from younger rats. Our experiments with SDS gel electrophoresis consistently demonstrated substantive differences between young and old rats in the quantity of two microsomal polypeptide bands migrating in the 50,000 MW region. Unfortunately, the heme-staining procedure did not provide sufficient resolution to identify them as heme-containing bands. Furthermore, it is very likely that these bands also contain other proteins (Lu and West, 1980). Therefore, the results must be interpreted with caution. Nevertheless, when taken together with the drug metabolism data and the change in absorption maximum of the reduced cytochrome P-450-CO complex, the results with SDS gel electrophoresis are at least consistent with differences in specific cytochromes P-450.

The substrate-dependent changes that were observed in the present study may have resulted also from age-related changes in the lipid composition of the microsomal membrane. Various lines of evidence suggest that phospholipid plays an important role in determining the substrate specificity of the monooxygenase system. Reconstitution experiments with purified components of the system have demonstrated that different forms of cytochrome P-450 require different types and amounts of phospholipid for maximal activity (Strobel *et al.*, 1970; Warner and Neims, 1979; Ingelman-Sundberg *et al.*, 1980). Moreover, compositional changes in the fatty acids of microsomal phospholipids have been shown to occur as the result of dietary or pharmacological manipulations which change the activity and substrate selectivity of the microsomal enzyme system (Becker *et al.*, 1978; Ilyas *et al.*, 1978; Hammer and Willis, 1979). Our studies with aging Fischer rats demonstrated age-related changes in weight percentages of microsomal fatty acids. Furthermore, liver microsomes from old rats had a greater percentage of longer fatty acids (18 carbons or more) and a higher ratio of saturated to unsaturated fatty acids than microsomes from young-adult rats. These differences, which suggest a change in the fluidity of the microsomal membrane, may have important effects on the monooxygenase system.

Differences in the hepatolobular distribution of specific cytochromes P-450 and monooxygenase activities have been reported (James *et al.*, 1981; Baron *et al.*, 1978; Gooding *et al.*, 1978). Therefore, the possibility was considered that the substrate selectivity of the age-related modification in drug metabolism reflected a selective loss of functioning hepatocytes from a certain region of the liver lobule. Experiments are under way in this laboratory to examine this possibility.

It is concluded that aging alters the hepatic microsomal monooxygenase system in a substrate-specific fashion. The most probable cause for this substrate dependency is that individual forms of cytochrome P-450 are differentially affected by aging. Because different cytochromes P-450 are concentrated

in hepatocytes from different regions of the liver lobule, it may be that the hepatocytes in some regions "age" at a faster rate than those in other regions. Another possibility is that changes in drug metabolism occur because of age-related changes in the fatty acid composition of the microsomal membrane. Clinical studies have demonstrated also that the effects of senescence on liver microsomal drug metabolism in humans vary with different drug substrates (Stevenson *et al.*, 1979). Further studies to elucidate the basis for the marked substrate dependency of these age-related alterations may provide a rational basis for dosage modification in geriatric patients.

References

BAIRD, M. B., NICOLOSI, R. J., MASSIE, H. R. AND SAMIS, H. V.: Microsomal mixed-function oxidase activity and senescence. I. Hexobarbital sleeptime and induction of components of the hepatic microsomal enzyme system in rats of different ages. Exp. Gerontol. **10:** 89-99, 1975.

BARON, J., REDICK, J. A. AND GUENGERICH, F. P.: Immunohistochemical localization of cytochrome P-450 in rat liver. Life Sci. **23:** 2627-2632, 1978.

BECKER, J. F., MEEHAN, T. AND BARTHOLOMEW, J. C.: Fatty acid requirements and temperature dependence of monooxygenase activity in rat liver microsomes. Biochim. Biophys. Acta **512:** 136-146, 1978.

BIRNBAUM, L. S.: Altered hepatic drug metabolism in senescent mice. Exp. Gerontol. **15:** 259-268, 1980.

BIRNBAUM, L. S. AND BAIRD, M. B.: Induction of hepatic mixed function oxidases in senescent rodents. Exp. Gerontol. **13:** 299-303, 1978a.

BIRNBAUM, L. S. AND BAIRD, M. B.: Induction of hepatic mixed function oxidases in senescent rodents. II. Effect of polychlorinated biphenyls. Exp. Gerontol. **13:** 469-477, 1978b.

FISKE, C. H. AND SUBBAROW, Y.: The colorimetric determination of phosphorus. J. Biol. Chem. **66:** 375-400, 1925.

GILLETTE, J. R. AND GRAM, T. E.: Cytochrome P-450 reduction in liver microsomes and its relationship to drug metabolism. *In* Microsomes and Drug Oxidations, ed. by J. R. Gillette, A. H. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts and G. J. Mannering, pp. 133-149, Academic Press, New York, 1969.

GOODING, P. E., CHAYEN, J., SAWYER, B. AND SLATER, T. F.: Cytochrome P-450 distribution in rat liver and the effect of sodium phenobarbitone administration. Chem.-Biol. Interact. **20:** 299-310, 1978.

GRINNA, L. S.: Age-related changes in the lipids of the microsomal and the mitochondrial membranes of rat liver and kidney. Mech. Ageing Dev. **6:** 197-205, 1977.

GRINNA, L. S. AND BARBER, A. A.: Age-related changes in membrane lipid content and enzyme activities. Biochim. Biophys. Acta **288:** 347-353, 1972.

HAMMER, C. T. AND WILLIS, E. D.: The effect of dietary fats on the composition of the liver endoplasmic reticulum and oxidative drug metabolism. Br. J. Nutr. **41:** 465-475, 1979.

HAUGEN, D. A. AND COON, M. J.: Properties of electrophoretically homogeneous phenobarbital-inducible and β-naphthoflavone-inducible forms of liver microsomal cytochrome P-450. J. Biol. Chem. **251:** 7929-7939, 1976.

HAUGEN, D. A., VAN DER HOEVEN, T. A. AND COON, M. J.: Purified liver microsomal cytochrome P-450. Separation and characterization of multiple forms. J. Biol. Chem. **250:** 3567-3570, 1975.

ILYAS, M. S., DE LA IGLESIA, F. A. AND FEUER, G.: The effect of phenobarbital and carbon tetrachloride on fatty acid content and composition of phospholipids from endoplasmic reticulum of liver. Toxicol. Appl. Pharmacol. **44:** 491-504, 1978.

IMAI, Y., ITO, A. AND SATO, R.: Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. J. Biochem. **60:** 417-428, 1966.

INGELMAN-SUNDBERG, M., GLAUMANN, H. AND JOHANSSON, I.: Properties of purified forms of rabbit liver microsomal cytochrome P-450 and NADPH-cytochrome P-450 reductase in reconstituted phospholipid vesicles. *In* Microsomes, Drug Oxidations, and Chemical Carcinogenesis, ed. by M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette and P. J. O'Brien, pp. 513-516, Academic Press, New York, 1980.

JAMES, R., DESMOND, P., KUPFER, A., SCHENKER, S. AND BRANCH, R. A.: The differential localization of various drug metabolizing systems within the rat liver lobule as determined by the hepatotoxins allyl alcohol, carbon tetrachloride and bromobenzene. J. Pharmacol. Exp. Ther. **217:** 127-132, 1981.

KAO, J. AND HUDSON, P.: Induction of the hepatic cytochrome P-450 dependent monooxygenase system in young and geriatric rats. Biochem. Pharmacol. **29:** 1191-1193, 1980.

KATO, R. AND TAKANAKA A.: Effect of phenobarbital on electron transport system, oxidation and reduction of drugs in liver microsomes of rats of different age. J. Biochem. **63:** 406-408, 1968a.

KATO, R. AND TAKANAKA, A.: Metabolism of drugs in old rats. I. Activities of NADPH-linked electron transport components and drug-metabolizing enzyme systems in liver microsomes of old rats. Jpn. J. Pharmacol. **18:** 381-388, 1968b.

LU, A. Y. H. AND WEST, S. B.: Multiplicity of mammalian microsomal cytochromes P-450. Pharmacol. Rev. **31:** 277-295, 1980.

MARKWELL, M. A. K., HAAS, S. M., BIEBER, L. L. AND TOLBERT, N. E.: A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. **87:** 206-210, 1978.

MAY, H. E. AND MCCAY, P. B.: Reduced triphosphopyridine nucleotide oxidase-catalyzed alterations of membrane phospholipids. I. Nature of the lipid alterations. J. Biol. Chem. **243:** 2288-2295, 1968.

MCMARTIN, D. N., O'CONNOR, J. A., JR., FASCO, J. J. AND KAMINSKY, L. S.: Influence of aging and induction on rat liver and kidney microsomal mixed function oxidase systems. Toxicol. Appl. Pharmacol. **54:** 411-419, 1980.

NASH, T.: The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. J. Biol. Chem. **55:** 416-422, 1953.

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

OMURA, T. AND SATO, R.: The carbon monoxide-binding pigment of liver microsomes. J. Biol. Chem. **239:** 2370-2378, 1964.

RIKANS, L. E. AND NOTLEY, B. A.: Decline in hepatic microsomal monooxygenase components in middle-aged Fischer 344 rats. Exp. Gerontol. **16:** 253-259, 1981.

RIKANS, L. E., SMITH, C. R. AND ZANNONI, V. G.: Ascorbic acid and cytochrome P-450. J. Pharmacol. Exp. Ther. **204:** 702-713, 1978.

RYAN, D. E., THOMAS, P. E., KORZENIOWSKI, D. AND LEVIN, W.: Separation and characterization of highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital and 3-methylcholanthrene. J. Biol. Chem. **254:** 1365-1374, 1979.

SCHMUCKER, D. L. AND WANG, R. K.: Age-related changes in liver drug metabolizing enzymes. Exp. Gerontol. **15:** 321-329, 1980.

SCHMUCKER, D. L. AND WANG, R. K.: Effects of aging and phenobarbital on the rat liver microsomal drug-metabolizing system. Mech. Ageing Dev. **15:** 189-202, 1981.

STEVENSON, I. H., SALEM, S. A. M. AND SHEPHERD, A. M. M.: Studies on drug absorption and metabolism in the elderly. *In* Drugs and the Elderly, ed. by J. Crooks and I. H. Stevenson, pp. 51-63, University Park Press, Baltimore, 1979.

STROBEL, H. W., LU, A. Y. H., HEIDEMA, J. AND COON, M. J.: Phosphatidylcholine requirement in the enzymatic reduction of hemoprotein P-450 and in fatty acid, hydrocarbon and drug hydroxylation. J. Biol. Chem. **245:** 4851-4854, 1970.

TANIGUCHI, H., IMAI, Y. IYANAGI, T. AND SATO, R.: Interaction between NADPH-cytochrome P-450 reductase and cytochrome P-450 in the membrane of phosphatidylcholine vesicles. Biochim. Biophys. Acta **550:** 341-356, 1979.

THOMAS, P. E., RYAN, D. AND LEVIN, W.: An improved staining procedure for the detection of the peroxidase activity of cytochrome P-450 on sodium dodecyl sulfate polyacrylamide gels. Anal. Biochem. **75:** 168-176, 1976.

VERMILION, J. L. AND COON, M. J.: Purified liver microsomal NADPH-cytochrome P-450 reductase. Spectral characterization of oxidation-reduction states. J. Biol. Chem. **253:** 2694-2704, 1978.

WARNER, M. AND NEIMS, A. H.: Multiple forms of ethoxyresorufin O-deethylase and benzphetamine N-demethylase in solubilized and partially resolved rat liver cytochromes P-450. Drug Metab. Dispos. **7:** 188-193, 1979.

YANG, C. S., STRICKHART, F. S. AND KICHA, L. P.: Interaction between NADPH-cytochrome P-450 reductase and hepatic microsomes. Biochim. Biophys. Acta **509:** 326-337, 1978.

ZANNONI, V. G., FLYNN, E. J. AND LYNCH, M.: Ascorbic acid and drug metabolism. Biochem. Pharmacol. **21:** 1377-1392, 1972.

Send reprint requests to: Dr. Lora E. Rikans, Department of Pharmacology, University of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73190.