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COMPARISON OF THE HEPATIC MIXED FUNCTION OXIDASE SYSTEM OF YOUNG, ADULT, AND OLD NON-HUMAN PRIMATES *(MACACA NEMESTRINA)*

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Abstract—The influence of age on the mixed function oxidase system from a non-human primate was studied. Microsomes were isolated from the livers of female *Macaca nemestrina* ranging from 2 to 21 years of age. No significant age-related change was observed in either the cytochrome P-450 content or the NADPH cytochrome *c* reductase activity. In addition, the ability of the microsomes to metabolize benzo[*a*]pyrene did not change significantly with age. These observations contradict studies with liver tissue from laboratory rodents in which an age-related decline in the mixed function oxidase system is generally observed. The lipid composition of the liver microsomes was studied also. Both the cholesterol and total phospholipid content of the liver microsomes increased significantly with age; however, the ratio of cholesterol to phospholipid remained constant. The percentage of individual phospholipids in the microsomes changed only slightly with age. These results provide new information on the effect of age on the mixed function oxidase system and indicate that one must be cautious in extrapolating from studies with liver tissue from laboratory rodents to primates.

In general, the elimination of drugs from the body appears to be altered with increasing age in humans. Several investigators have shown that the plasma half-life of drugs is higher for elderly subjects than young subjects [1–3]. The age-related decline in drug elimination in humans could be due to several factors, e.g. changes in plasma distribution, changes in renal clearance, and changes in drug metabolism. Currently, the exact mechanism responsible for the age-related changes in drug elimination in humans is unknown.

Using hepatic microsomes from laboratory rats, several investigators have shown that a decline in the *in vitro* metabolism of a variety of drugs occurs with increasing age [4–9]. This decline has been attributed to a decrease in the levels of the cytochrome P-450 and NADPH cytochrome P-450 reductase [5–21], the two major components of the mixed function oxidase (MFO) system. However, not all the data with rodents have shown an age-related decline in the hepatic MFO system [13]. Therefore, the decline in drug metabolism with increasing age may be species and strain specific.

Because of the research data with rodents, it is often suggested that hepatic drug metabolism declines with increasing age in humans. However, Kitani [13] has warned investigators against the over-interpretation of animal data to human geriatric pharmacology. Until recently there were no data directly measuring the MFO system in human liver as a function of age. Recently, James *et al.* [14] reported that the ethoxycoumarin-*O*-deethylase and aldrin epoxidase activities of liver microsomes of biopsies from humans did not change significantly between 24 and 75 years of age. However, because of the limited number of subjects studied and the large variation between subjects, it is possible that James *et al.* [14] might not have observed a decline in drug metabolism because of type 2 statistical error. To gain an insight into the effect of aging on the activity of the MFO system of human liver, we measured the cytochrome P-450 (P-450) content and NADPH cytochrome *c* reductase (reductase) activity of liver tissue from a relatively large number (twenty-four) of non-human primate *Macaca nemestrina*, which were maintained under uniform conditions. To our knowledge, this is the first information on the effect of aging on the MFO system for liver from a non-human primate.

MATERIALS AND METHODS

Liver samples from female *M. nemestrina* (pig-tailed macaque) were obtained from the Regional Primate Center at the University of Washington in Seattle, WA. The animals were housed in mixed-age groups consisting of eight to ten animals per cage and were fed a 25% protein monkey chow diet, which was supplemented with apples and oranges. These animals reach sexual maturity at 4 years of age, and the oldest *M. nemestrina* of known age in existence is presently 28 years of age (personal communication from Dr. Douglas Bowden, Associate Director of the Regional Primate Research Center at the University of Washington).

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The animals were anesthetised to deep surgical level with sodium pentobarbital and were exsanguinated. The liver was surgically removed, and sections (5–10 g) were frozen in liquid nitrogen at the University of Washington. The samples were shipped in dry ice to Illinois State University and were then stored at −80°. The frozen liver samples were ground to a powder in liquid nitrogen, and microsomes were prepared as described by Haugen and Coon [15].

The protein content of the microsomes was determined as described by Peterson [16]. The cytochrome P-450 content was measured by the sodium dithionate-reduced carbon monoxide differential spectrum as described by Omura and Sato [17]. The NADPH cytochrome *c* reductase activity was measured as described by Williams and Kamin [18]. The data from cytochrome P-450 content and reductase activity were expressed per mg microsomal protein and per total liver. The aryl hydrocarbon hydroxylase activities of the liver microsomes were determined as described by Haugen [19] using benzo[*a*]pyrene as substrate and were expressed as nmoles of 3-hydroxy-benzo[*a*]pyrene formed per min per mg of microsomal protein.

The phospholipid and cholesterol composition of the liver microsomes was determined after extracting the microsomal fractions with 10 vol. of chloroform–methanol, 2:1 (v/v), according to the procedure of Folch *et al.* [20]. The extracted lipids were taken to dryness under nitrogen and resuspended in 3 ml of chloroform and stored at −20° until assayed. Aliquots of the lipid extract were assayed for lipid phosphorus and cholesterol content as described previously [21, 22]. Major phospholipids of the lipid extract were separated by two-dimensional thin-layer chromatography on silica gel G. The solvents used for the first and second dimensions were chloroform/methanol/ammonium hydroxide (135/55/10 ml) and chloroform/methanol/acetone/ammonium acetate/glacial acetic acid (140/60/55/10/3.5 ml) respectively. The quantification of individual phospholipids was based on the phospholipid phosphate content using the method described above for total lipid phosphorus [21].

RESULTS

The P-450 content and reductase activity of liver microsomes obtained from female *M. nemestrina*, which were divided into three groups, are shown in Table 1. A slight age-related increase in body and liver weight was observed; however, the microsomal protein isolated from the livers of these animals did not change significantly with age. Figure 1 shows the P-450 content and the reductase activity for each animal studied. The amount of variation in the data from *M. nemestrina* was less than that observed by James *et al.* [14] with liver biopsies from human subjects. It is apparent from Table 1 and Fig. 1 that no significant age-related change in the P-450 content or the reductase activity was observed in liver microsomes isolated from *M. nemestrina*. We also expressed our data per liver to determine if age-related changes in the total MFO activity of liver might occur with age. No significant change in either the P-450 content or the reductase activity was

Table 1. Levels of cytochrome P-450 and NADPH cytochrome *c* reductase in liver microsomes*

			Liver microsomes					
				P-450 content		Reductase activity		Aryl hydrocarbon hydroxylase activity
	Body wt (kg)	Liver wt (g)	Protein content (mg/g liver)	(nmoles/mg protein)	(μmoles/liver)	(nmoles/min/mg protein)	(μmoles/min/liver)	(nmoles/min/mg protein)
Young (2–5.5 years)	4.1 ± 0.8	105 ± 21	11.4 ± 0.6	2.14 ± 0.12	2.7 ± 0.6	196 ± 16	217 ± 37	0.457 ± 0.027
Adult (10–12 years)	5.1 ± 0.6	142 ± 19	10.7 ± 1.3	1.90 ± 0.21	2.3 ± 0.3	172 ± 19	216 ± 28	0.405 ± 0.010
Old (16–21 years)	6.5 ± 0.9	152 ± 22	11.2 ± 1.5	2.05 ± 0.14	2.9 ± 0.3	163 ± 12	253 ± 21	0.406 ± 0.025

* Values are the mean ± S.E. for data from eight animals for each age group.

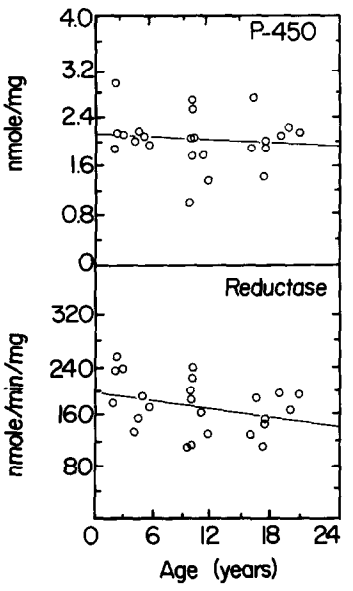

Fig. 1. Levels of cytochrome P-450 and NADPH cytochrome *c* reductase in liver microsomes of individual *M. nemestrina*. The lines drawn represent the linear least squares fit of the data.

observed when expressed per liver. The aryl hydrocarbon hydroxylase activity of the liver microsomes was also measured to determine if the ability of the liver to metabolize exogenous compounds, e.g. benzo[*a*]pyrene, changed with increasing age. The data in Table 1 show that the aryl hydrocarbon hydroxylase activity of liver from female *M. nemestrina* did not change significantly with age.

The phospholipid content of the liver microsomes was also analyzed because it is known that phospholipids can affect the activity of the MFO system. The data in Table 2 show that both the cholesterol content and total lipid phosphorus increased significantly with age. Microsomes of the old age group had the greatest amount of both cholesterol and total lipid phosphorus. Differences between the adult and old groups were not significant, and the ratio of cholesterol to phospholipid did not differ significantly with age. The percentage of individual phospholipids differed only slightly with age (Table 3). The major phospholipid was phosphatidylcholine, which increased slightly with age; significant differences were observed between microsomes of the old as compared to the young age groups. A significant decrease was seen in microsomal cardiolipin of the old group as compared to the young and adult groups. The age differences among the other phospholipids were not significant.

DISCUSSION

In this study, the activity of the MFO system of liver microsomes from female *M. nemestrina* was measured as a function of age. The levels of P-450 (nmoles per mg microsomal protein) that we obtained from these animals (Table 1) were much

Table 2. Cholesterol and total phospholipid content of liver microsomes*

Age group	Cholesterol (μg/mg protein)	Phospholipid (μg/mg protein)	Cholesterol/phospholipid molar ratio
Young	49.65 ± 1.54	25.71 ± 1.73	0.1552 ± 0.0058
Adult	66.64 ± 3.67†	30.10 ± 2.57	0.1850 ± 0.0207
Old	72.35 ± 9.65‡	35.10 ± 4.26‡	0.1639 ± 0.0047

* Data are means ± S.E. for six animals per age group.
† $P < 0.01$ as compared to young.
‡ $P < 0.02$ as compared to young.

Table 3. Liver microsome phospholipids expressed as the percentage of total phospholipids*

Phospholipid	Phospholipids (% of total) Age group Young	Adult	Old
Phosphatidylcholine	51.19 ± 0.77	51.74 ± 0.55	53.28 ± 0.52†
Phosphatidylethanolamine	27.56 ± 1.23	26.88 ± 0.49	28.17 ± 0.78
Phosphatidylinositol	11.06 ± 0.10	10.76 ± 0.37	10.71 ± 0.18
Phosphatidylserine	3.03 ± 0.41	3.42 ± 0.40	2.71 ± 0.26
Sphingomyelin	3.51 ± 0.41	3.17 ± 0.46	3.42 ± 0.85
Cardiolipin	2.32 ± 0.35‡	2.67 ± 0.31‡	1.58 ± 0.21

* Data are the percentage of each phospholipid of the total phospholipids. Each value is the mean ± S.E. for six animals in each age group.
† $P < 0.02$ as compared to young.
‡ $P < 0.03$ as compared to old.

higher than those reported previously for liver microsomes from non-human primates [23, 24] and human subjects [25, 26]. It appears that this discrepancy is at least partially due to the protein content of the microsomal preparations. When one expresses the data per gram of liver tissue, the levels of P-450 that we obtained (approximately 22 nmoles/g liver) were essentially the same as reported by Rumack *et al.* [23] for *M. nemestrina* (18 nmoles/g liver) or by Litterst *et al.* [24] for *M. mulatta*, rhesus (23 nmoles/g liver). The reductase activity in the liver microsomes from *M. nemestrina* (Table 1) was similar to the levels reported for *M. mulatta* [24] and human subjects [25]. Although the aryl hydrocarbon hydroxylase activity of the liver microsomes found in this study was higher than that reported for *M. mulatta* [24] and human subjects [25], it was similar to the values Jakobsson *et al.* [26] obtained for liver microsomes from humans.

The P-450 content and reductase activity of liver microsomes from 2- to 21-year-old female *M. nemestrina* were compared. A major decline in most indices of aging, e.g. reproductive capacity and bone thinning, occurs by 20 years of age in *M. nemestrina* [27], and a 20-year-old *M. nemestrina* is approximately equivalent to a 60-year-old human (personal communication from Dr. Douglas Bowden, Regional Primate Research Centre, University of Washington, Seattle, WA). No significant change in either the P-450 content or the reductase activity of liver microsomes from *M. nemestrina* was observed between 2 and 21 years of age. Because P-450 and reductase are the major components of the drug-metabolizing system and because these two components do not change significantly with age, one might expect that the ability of the liver of *M. nemestrina* to metabolize drugs or xenobiotics would not change with increasing age. We found that the hydroxylation of benzo[*a*]pyrene by liver microsomes did not change significantly with age.

Except for the report by James *et al.* [14] with liver biopsy samples from human subjects, these are the only data in which the MFO system has been studied as a function of age in primates. Although our data with liver microsomes from *M. nemestrina* agree with the study by James *et al.* [14], they do not agree with most of the studies with liver microsomes from rodents. In studies with rodents, a significant age-related decrease in P-450 content and reductase activity is generally observed [5–12], and the decrease in these components is paralleled by a decrease in the ability of the liver to metabolize a variety of drugs and xenobiotics [4–12].

The nature of the membrane lipid matrix can affect the function of membrane-bound proteins such as P-450 and reductase. It is well documented that P-450 requires phosphatidylcholine in a reconstituted system, and the fatty acid composition of the phospholipid affects the ability of P-450 to metabolize drugs [28, 29]. In addition, Becker *et al.* [30] showed that the ability of liver microsomes to demethylate *p*-nitroanisole or hydroxylate benzo[*a*]pyrene could be altered by changes in the lipid composition of the microsomes. Therefore, we also studied the lipid composition of liver microsomes from *M. nemestrina* to determine if age-related changes in the lipid matrix occurred that might affect the drug-metabolizing activity of the liver. The content of both cholesterol and total phospholipids in the microsomes increased significantly with age. These changes in the lipid composition of the microsomes would be consistent with the general concept that membranes become more rigid with increasing age [31, 32]. An age-related increase in cholesterol content has been reported for synaptic plasma membranes [33] and microsomes from brain, myelin, and liver [34, 35]. We also observed an increase with age in the total phospholipid content of the liver microsomes. An increase in the total phospholipid content of myelin and synaptosomes has been observed with increasing age [36, 37], while no change or an age-related decrease in the total phospholipid content of liver microsomes [31] or synaptic membranes [38] has been observed. Tissue and species differences may be important factors in the changes in phospholipid composition that occur with increasing age [38].

Although the cholesterol and total phospholipid content of the liver microsomes increased with age, the ratio of cholesterol to total phospholipid remained constant. In addition, the percentage of most of the types of phospholipids did not change significantly with age. An increase in the percentage of phosphatidylcholine and a decrease in cardiolipin was observed with increasing age. Szymanski and Kritchevsky [39] reported an increase in the phosphatidylcholine and phosphatidylethanolamine in sera of old rhesus monkeys. Because cardiolipin is localized in the mitochondria [40], the age-related change in this phospholipid might be due to changes in the contamination of the microsomes with mitochondria.

Our study has important ramifications to the area of geriatric pharmacology because it provides new information of the effect of age on the MFO system of primate liver. Although it is well documented that the elimination of many drugs by humans changes significantly with increasing age [1–3], it is not known if drug metabolism by the liver is important in these age-related changes in drug elimination. Essentially, the only data currently available in this area come from studies in which liver tissue from laboratory rodents has been used. The fact that we failed to observe the age-related decline in P-450 levels and reductase activity that has been reported for laboratory rodents points out the importance of using caution when extrapolating from rodents to primates, i.e. humans. Although our data agree with the data of James *et al.* [14] and suggest that the ability of primate liver to metabolize drugs does not change with age, additional studies should be conducted with primate liver. For example, the metabolism of a variety of drugs by liver tissue from primates should be studied as a function of age because the metabolism of drugs by the MFO system is dependent upon the drug *per se*. Changes in the P-450 species or subtle changes in the lipid component of the endoplasmic reticulum could also affect the metabolism of drugs differently. For example, Rikans and Notley [41] found that the demethylation of benzphetamine and the hydroxylation of aniline by rat liver microsomes decrease with age while the demethylation of nitro-

anisole increases. In addition, the activity of the MFO system should be studied in male primates of various ages because the species of P-450 in rat liver have been shown to be sex specific [42]. Although Kato and Takanada [9] observed a decrease in the P-450 content of liver microsomes of female rats, recent studies with female Fischer F344 rats indicate that the P-450 content of liver microsomes does not change with age [13, 43].

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