In the present experiment, 2-acetylaminofluorene was *N*-deacetylated primarily by mouse liver microsomes, whereas 2,4-diacetylaminotoluene was *N*-deacetylated primarily by mouse liver cytosol. These finding suggest that mouse liver has at least two *N*-deacetylase enzyme systems, a microsomal *N*-deacetylase enzyme which is more specific for the monoarylacetamide and a cytosol *N*-deacetylase enzyme which is more specific for the diarylacetamide. Furthermore, formation of the products was directly proportional to the incubation times up to 60 min only for 2,4-diacetylaminotoluene. The effect of pH on *N*-deacetylase activity was greater for 2-acetylaminofluorene. In addition, the K_m of *N*-deacetylase enzyme for 2-acetylaminofluorene (0.8×10^{-6}M) was lower than that for 2,4-diacetylaminotoluene. These results, therefore, support the possible conclusion that there are two separate *N*-deacetylase enzyme systems in mouse liver.

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Induction of the hepatic cytochrome P-450-dependent mono-oxygenase system in young and geriatric rats

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It has long been recognized that aging is associated with alterations in pharmacological variables which may be responsible for changes in the susceptibility to toxic effects of xenobiotics in senescent animals [1–3]. Investigations of drug metabolism *in vivo* have demonstrated that metabolic efficiency of rodents declines with advancing age [4–6]. Moreover, an age-related decline in metabolic activities has been observed in *in vitro* studies of the metabolism of a number of xenobiotics in hepatic microsomal preparations from rats of increasing age [7–9]. This decrease in activity has been shown to parallel the apparent decrease in the levels of components of the hepatic cytochrome P-450-dependent mixed function oxygenase (MFO) enzyme system [10]. Indeed, the decrease in drug-metabolizing activity in geriatric animals has been attributed specifically to an age-related loss of hepatic microsomal NADPH-cytochrome *c* reductase activity [6]. In contrast, Birnbaum and Baird [11] have reported no differences in NADPH-cytochrome *c* reductase activity in young and old rats.

The activities and components of the hepatic MFO enzyme system are sensitive to a wide variety of agents, being induced by a number of chemicals (typically the barbiturates and polycyclic hydrocarbons). However, controversy exists in the literature with regard to the inducibility of this enzyme system in senescent rats. Kato and Takanaka [10] showed that phenobarbital-induced elevation of MFO activities was markedly higher in young rats than in senescent rats. Recent reports, however, demonstrated no such age-related differences in the sensitivity of the functional components of the hepatic microsomal drug-metabolizing enzyme system following either phenobarbital or 3-methylcholanthrene treatment [6, 11].

The hepatic MFO enzyme system is a major determinant of toxicity of drugs and environmental agents. It is important, therefore, to define clearly any age-related differences in the responses of this enzyme system to xenobiotics, since such age-related differences may be a determining factor in the altered susceptibility to toxic effects of foreign chemicals in senescence.

In this paper, the effects of phenobarbital (PB) and β-naphthoflavone (BNF) on the induction of the hepatic MFO enzyme system were examined in both young adult and geriatric rats. BNF is a non-carcinogenic inducer which has inductive properties similar to 3-methylcholanthrene [12].

Male Fischer 344 rats (young adults, 10 weeks; geriatrics, 100 weeks) were housed in the same environmentally controlled animal room (20°, 50% humidity and 12-hr light cycles) and maintained *ad lib.* on Purina Laboratory Chow and tap water. The animals were treated for 4 consecutive days with daily i.p. doses of either PB (80 mg/kg) in 0.9% (w/v) saline (1 ml/kg) or BNF (80 mg/kg) in corn oil (10 ml/kg), and appropriate controls were treated in parallel. The animals were starved for 24 hr prior to being

Table 1. Effects of phenobarbital (PB) and β-naphthoflavone (BNF) on hepatic microsomal enzyme activities in young and old rats*

Treatment	Microsomal protein (mg/g liver)	Cytochrome P-450 (nmoles/mg protein)	NADPH-cytochrome *c* reduction (nmoles reduced/ mg protein/min)	Benzphetamine *N*-demethylation (nmoles product/ mg protein/min)	7-Ethoxycoumarin *O*-deethylation (nmoles product/ mg protein/min)
Control†					
Young	16.4 ± 1.2	1.22 ± 0.07	265.7 ± 19.6	4.48 ± 0.32	2.42 ± 0.32
Old	11.4 ± 1.1	0.89 ± 0.04	178.3 ± 14.0	1.45 ± 0.20	1.87 ± 0.21
Phenobarbital					
Young	21.0 ± 1.8 (1.3)	2.64 ± 0.28 (2.2)	360.4 ± 36.4 (1.4)	6.71 ± 0.54 (1.5)	4.12 ± 0.07 (1.7)
Old	19.2 ± 2.3 (1.7)	2.03 ± 0.22 (2.3)	279.3 ± 28.7 (1.6)	6.60 ± 0.55 (4.6)	3.17 ± 0.23 (1.7)
β-Naphthoflavone					
Young	17.4 ± 2.2 (1.1)	2.21 ± 0.27 (1.8)	119.1 ± 16.1 (0.8)	1.59 ± 0.20 (0.4)	8.96 ± 0.79 (3.7)
Old	13.6 ± 2.0 (1.2)	2.11 ± 0.21 (2.4)	159.3 ± 9.7 (0.9)	0.77 ± 0.07 (0.5)	8.13 ± 0.42 (4.4)

* Results are given as the means ± S.E. from five experiments. Each experiment was performed with one animal from each age and treatment group. Figures given in parentheses are the ratios of enzyme activity in treated rats to the basal activity in control rats. Analysis of variance procedures were employed to assess the significance of age effect, treatment effect and their interactions [20], and pairwise comparisons were made using Fischer's LSD test [21].

Comparisons
(i) young vs old (controls) $P < 0.01$ [7-ethoxycoumarin *O*-deethylation $P < 0.05$].
(ii) PB vs controls (young) $P < 0.01$.
(iii) PB vs controls (old) $P < 0.01$.
(iv) BNF vs controls (young) $P < 0.01$ [microsomal protein NS].
(v) BNF vs controls (old) $P < 0.01$ [NADPH-cytochrome *c* reduction NS, microsomal protein NS].
(vi) old vs young (PB) NS [7-ethoxycoumarin *O*-deethylation $P < 0.01$].
(vii) old vs young (BNF) NS [benzphetamine *N*-demethylation $P < 0.01$].

Statistical significance applied to all five microsomal parameters assayed and exceptions are given in brackets.

† Combined values for saline and corn oil controls.

killed by cervical dislocation 24 hr after the last injection; the livers were excised and microsomes prepared [13]. The washed microsomal fractions were suspended in 0.25 M sucrose, 1 mM EDTA and 100 mM Tris–HCl buffer, pH 7.4 (microsomal protein 1–2 mg/ml). Microsomal *N*-demethylation of benzphetamine was determined in an assay system containing 250 μmoles Tris–HCl buffer (pH 7.4), 30 μmoles semicarbazide, 7.5 μmoles glucose-6-phosphate; 12.5 μmoles $MgCl_2$, 1.9 units glucose-6-phosphate dehydrogenase, 12.5 μmoles benzphetamine, 1.5–2.0 mg microsomal protein and 1.15 nmoles NADPH in a final volume of 2.5 ml. The mixture was incubated at 37° for 15 min. The amount of formaldehyde formed was determined by the method of Nash [14] as modified by Cochin and Axelrod [15]. 7-Ethoxycoumarin *O*-deethylation was measured in a reaction mixture containing cofactors as described above, omitting the semicarbazide and replacing the Tris with 625 μmoles of Hepes* buffer, pH 7.4. The reaction mixture also contained 0.5 μmole of 7-ethoxycoumarin and 20–30 μg of microsomal protein in a final volume of 2.5 ml. The mixture was incubated at 37° for 5 min, and the products were measured by the method of Aitio [16]. Cytochrome P-450 was determined by the method of Omura and Sato [17] and NADPH-cytochrome *c* reductase was measured by the method of Masters *et al.* [18]. Protein was measured by a modification of the Coomassie blue binding method [19] using bovine serum albumin as standard, and all assays were performed on freshly prepared microsomes.

The results shown in Table 1 demonstrate that in control animals there was an age-related decline in the levels and activities of the hepatic microsomal MFO enzyme system. Thus, in control geriatric rats, all five variables which were assayed were significantly ($P < 0.01$) lower, by about 30 per cent, and this is in agreement with the observations of Kato and Takanaka [10]. Baird *et al.* [6], on the other hand, reported that there was no decrease in cytochrome P-450 levels associated with aging, but an age-related decrease in microsomal NADPH cytochrome *c* reductase activity was observed and these authors concluded that this was responsible for the decrease in the *in vitro* rates of drug-metabolizing activities in microsomal preparations from senescent rats. The same laboratory, however, reported that there was no such difference in NADPH-cytochrome *c* reductase activity between 13-week-old and 130-week-old rats [11], although age-related decreases in P-450 levels, benzphetamine *N*-demethylase, and other P-450 mediated activities were observed.

Using doses of enzyme inducers which gave maximal stimulation of MFO activity, no toxicity effects were observed in treated animals with the exception of senescent rats treated with PB. These animals were heavily sedated throughout the treatment, and a few failed to survive the treatment period. However, characteristic inductive effects of barbiturates (PB) and polycyclic hydrocarbons (BNP) were observed in both young and surviving old rats. The characteristic hypsochromic shift of the Soret peak of the reduced hemoprotein–CO complex to 448 nm [12] was observed in microsomes prepared from both old and young rats treated with BNF. By comparison, in microsomes from both young and old control and PB-treated animals, the Soret peak was at 450 nm. The distinctive differential effects of the two inducers [22–24] were further demonstrated, in that PB and BNF preferentially induced microsomal benzphetamine *N*-demethylation and *O*-deethylation of 7-ethoxycoumarin, respectively, in both young and geriatric rats. Moreover, benzphetamine *N*-demethylation was significantly ($P < 0.01$) lower in BNF-treated rats. The characteristic induction of cytochrome *c* reductase activity by PB treatment in young animals [22] was also observed in the old. On the other hand, a significant ($P < 0.01$) decrease with respect to this activity was observed in the young but not in the old animals following BNF treatment. In addition, both inducers significantly ($P < 0.01$) increased hepatic cytochrome P-450 levels [12, 22] of both old and young animals, although only PB treatment increased the microsomal protein concentration of the liver.

Following PB induction of young and old rats, there were no significant quantitative differences in the capabilities of their hepatic MFO enzyme system. Thus, similar microsomal protein and cytochrome P-450 contents and NADPH-cytochrome *c* reductase and benzphetamine *N*-demethylase activities were observed. However, the age-related differences in 7-ethoxycoumarin *O*-deethylation observed in control animals persisted following PB induction. This microsomal activity, although induced slightly by PB, responds preferentially to induction by polycyclic hydrocarbons [23, 24]. Similarly, benzphetamine *N*-demethylase activity, which is preferentially induced by PB [20], retained significant ($P < 0.01$) age-related differences following BNF treatment. No significant quantitative differences in other variables of the microsomal enzyme system assayed were observed between old and young BNF-induced rats.

These results demonstrate that the livers of old and young rats respond to exogenous inducers in a similar manner. Furthermore, the inducibility of hepatic enzyme is apparently unimpaired on aging. Indeed, in many incidences the degree of response to these two chemicals is apparently greater in senescent rats, since the basal activities in control animals were significantly ($P < 0.01$) higher in young rats than in old rats, yet no significant differences were observed in old and young induced animals. These results are contrary to previous observations which demonstrated that the inducibility of the hepatic MFO system to PB was impaired progressively with aging and no significant induction was observed in 86-week-old rats [10]. However, other investigators reported that there was no such impairment with age in response to PB [9], 3-methylcholanthrene or pregnenolene-16α-carbonitrile [11].

Our observations, illustrated by the five variables assayed, demonstrate that senescence is associated with a decline in the activities of the hepatic MFO enzyme system. This age-related difference, however, is eliminated following maximal induction by either PB or BNF and indicates that the optimal inducibility of the hepatic MFO enzyme system is unaltered by aging. This similar response capacity of the hepatic MFO enzyme system in young and sensecent rats suggests that aging is not associated with a loss in the capacity of the system to respond to inducers; MFO differences observed may reflect an age-dependent alteration in homeostatic factors responsible for regulating basal enzyme activities. Thus, the age-related decline in the activities of the hepatic MFO enzyme system may, in part, be responsible for the reported difference between young and old animals in their toxicological and pharmacological responses to xenobiotics. However, it is possible that age-dependent alterations in factors controlling the physiological disposition of xenobiotics may be more important in the altered susceptibility of senescent animals to toxic effects of foreign chemicals.

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* Hepes = 4-(2 hydroxyethyl)-1-piperazine-ethanesulphonic acid.

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