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Hepatic Drug Metabolism in Ten Strains of Norway Rat Before and After Pretreatment with Phenobarbital (33853)

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Differences among species and among strains of a given species in metabolism of certain drugs have been reported frequently (1–4) and it is established clearly that activities of drug-metabolizing enzymes differ not only among various species but also among certain strains of some species. However, strain differences in inducibility of hepatic microsomal drug-metabolizing enzymes have been reported in detail only once before (5). The results showed that in various strains of rabbits there existed large differences in the extent to which phenobarbital pretreatment elevated activities of drug-metabolizing enzymes (5). These results were so dramatic that the present study was undertaken to determine whether in a more commonly used laboratory species, *Rattus norvegicus*, there occurred similar large strain differences in the basal and induced levels of two mixed function oxygenases, aniline hydroxylase and ethylmorphine *N*-demethylase.

Materials and Methods. Ten strains of the species *R. norvegicus* were obtained mainly from the NIH animal production section; in addition, LE came from Blue Spruce Farms (Altmont, New York); LEW from Simonson Laboratories (Gilroy, California); W from Huntingdon Laboratories (Philadelphia, Pennsylvania); ACI, LEW, and F344 (Fischer) from Microbiological Associates (Walkersville, Maryland). Eight of the strains were inbred: ACI, Alb (Albany), Buf (Buffalo), F344 (Fischer), LE (Long Evans), LEW (Lewis), M520 (Marshall) and W (Wistar). OM (Osborne Mendel) and SD (Sprague-Dawley) were the two outbred strains employed. At the time that experiments were performed, animals weighed between 200 and 225 g, were approximately 2 months old and were maintained for at least 2 weeks in our animal quarters on water and Purina chow *ad libitum*. Wild Norwegian rats were trapped in a garbage dump in New Freedom, Pennsylvania and were used after only 1 day in the laboratory. Kangaroo rats are a wild strain of the species *Dipodomys merriami*. They are of a species distinct from *R. norvegicus* and were trapped in the deserts of southern California. They were kept in our laboratory under the conditions described above for only 4 days prior to use.

In initial experiments sodium phenobarbital was injected ip in a dose of 100 mg/kg every 24 hr for 5 days. Subsequent experiments on induction were performed after only 3 days because the results revealed that maximum elevations of aniline hydroxylase and ethylmorphine *N*-demethylase activities occurred after 3 days (Fig. 1). Kangaroo rats were much more sensitive to phenobarbital, and to prevent appreciable mortality the dose was reduced to 50 mg/kg every 24 hr for 3 days. Control animals received saline injections (0.8 ml., ip) on each of 3 days. Rats were sacrificed by decapitation. Livers were immediately removed, rinsed in saline, blotted dry, and weighed. A 1:4 homogenate was prepared with 1.15% KCl in 0.02 *M* Tris buffer, pH 7.4. The homogenate was centrifuged at 4° for 20 min at 9000*g*. Ethylmorphine *N*-demethylase (6) and aniline hy-

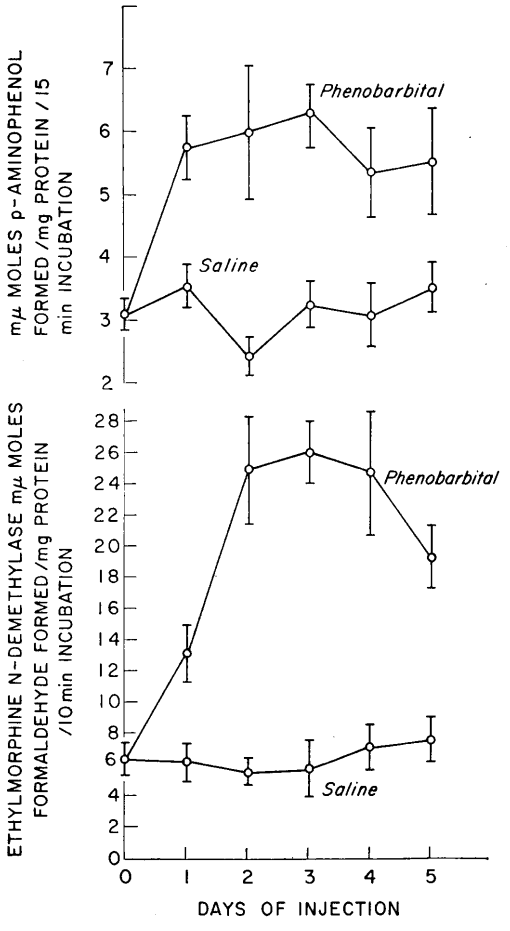

FIG. 1. Alterations in hepatic microsomal ethylmorphine *N*-demethylase and aniline hydroxylase activities when OM male rats received phenobarbital sodium, 100 mg/kg, ip, for 0 days (controls) or daily for 1–5 days prior to sacrifice: each (O) = the mean for 6 animals; SD are indicated by lines above and below the circles; 9000*g* supernatants were assayed for enzyme activities and protein concentrations.

droxylase (7) activities were assayed in the supernatant as previously described. The reaction mixture in a total volume of 3.0 ml contained substrate in a concentration of 10 μmoles; 0.15 *M* Tris buffer, pH 7.4; $MgCl_2$, 50 μmoles; glucose-6-phosphate, 25 μmoles; glucose-6-phosphate dehydrogenase, 2 units; and NADP, 1.25 μmoles; but no nicotinamide. Activity is expressed per milligram of protein in the 9000*g* supernatant of liver homogenate determined by the method of Lowry (8). Cytochrome P-450 activity was measured in the microsomal fraction prepared by centrifuging the 9000*g* supernatant for 1 hr at 78,000*g* (9). The apparent K_m and V_{max} for ethylmorphine *N*-demethylase and aniline hydroxylase were determined on this fraction by a computer program described previously (10).

Results. In 10 strains of rat, males exhibited a twofold range in ethylmorphine metabolism and females showed almost a fourfold range (Table I). Males metabolized ethylmorphine 50–300% more rapidly than females of comparable strain. Similar sex differences for aniline metabolism occurred only in Alb, F344 and LE. Aniline metabolism varied over a threefold range for males and females (Table II).

After phenobarbital pretreatment ethylmorphine metabolism increased 2.2- to 3.2-fold in male rats and 2.0- to 3.8-fold in females (Table I). Phenobarbital pretreatment elevated aniline metabolism 1.5- to 2.9-fold in male rats and 1.6- to 3.5-fold in females (Table II).

Male kangaroo rats exhibited lower metabolism of ethylmorphine and aniline than did rats from any of the 10 strains of *R. norvegicus*. The ethylmorphine metabolism of female kangaroo rats was comparable to that of female Norway rats, but aniline metabolism in female kangaroo rats was higher than in female Norway rats (Tables I and II). Phenobarbital pretreatment increased ethylmorphine metabolism 39.8-fold in male and 4.2-fold in female kangaroo rats (Table I). Phenobarbital pretreatment enhanced aniline metabolism 12.5-fold in male and 2.1-fold in female kangaroo rats (Table II).

Cytochrome P-450 values in the microsomal fraction were approximately 1 mμmole in all strains examined, except for the wild Norwegian rats where the value was only 0.5 mμmole (Table III).

The V_{max} for ethylmorphine *N*-demethylase was lower for the wild (47.9 ± 24.4[1]

[1] All values are the means ± SD for 6 rats. Measurements of apparent K_m and V_{max} for ethylmorphine *N*-demethylase and aniline hydroxylase in each of the 10 domestic strains will be presented elsewhere.

TABLE I. The Effect of Phenobarbital Pretreatment on the Hepatic Metabolism of Ethylmorphine in 10 Strains of *R. norvegicus*, in Wild Norwegian Rats, and in the Kangaroo Rat.[a,b]

	Male			Female		
	Treated	Control	Ratio (treated /control)	Treated	Control	Ratio (treated /control)
ACI	39.2 (6)	15.2 (6)	2.6	18.8 (12)	6.0 (12)	3.1
Alb	36.0 (6)	15.2 (6)	2.4	14.4 (5)	7.2 (5)	2.0
Buf	23.0[d] (6)	10.3[d] (6)	2.2	6.7[d] (13)	3.2[d] (13)	2.1
F344	46.1[c] (6)	16.4[c] (6)	2.8	20.5 (13)	7.1 (13)	2.8
LE	34.2 (6)	13.6 (6)	2.5	17.5 (8)	7.2 (8)	2.4
LEW	41.2[c] (6)	12.6 (6)	3.2	26.0[c] (8)	8.1 (8)	3.2
M520	41.0[c] (6)	18.4[c] (6)	2.2	31.2[c] (5)	11.3[c] (5)	2.8
OM	35.7 (6)	14.4 (6)	2.5	25.4[c] (9)	8.9[c] (9)	2.8
SD	30.8[d] (6)	9.6[d] (6)	3.2	20.7 (9)	5.9 (9)	3.5
W	29.3[d] (6)	9.4[d] (6)	3.1	14.3[d] (5)	3.7[d] (5)	3.8
Mean ± SD	35.6 (60) ±3.4	13.5 (60) ±2.2	2.7	18.9 (87) ±3.0	6.6 (87) ±1.9	2.8
K[e]	23.9 (5)	0.6 (5)	39.8	18.9 (6)	4.5 (6)	4.2
Wild rats		14.6 ±5.9			9.3 ±4.3	

[a] All rats were sacrificed 24 hr after the third injection of saline or phenobarbital and 9000*g* supernatants prepared from the liver in Tris–KCl buffer. Results are expressed as mμmoles of formaldehyde formed/mg of protein/10 min incubation at 37°.

[b] Values are the means for the number of animals given in parentheses.

Mean values for the strains were significantly higher, [c], ($p < 0.05$) or significantly lower, [d], ($p < 0.05$) than the general mean for the 10 strains, as determined by an analysis of variance.

[e] Kangaroo rats are a wild strain of the species *D. merriami*. Data for this species were not included in the intraspecies statistical comparisons for *R. norvegicus*.

mμmoles of formaldehyde formed/mg of microsomal protein during 10-min incubation at 37°) than for 10 domestic strains, which ranged from 116.2 ± 23.1 to 217.4 ± 35.8 mμmoles. The V_{max} for aniline hydroxylase was similar for wild (15.9 ± 4.0 mμmoles of *p*-aminophenol formed/mg of microsomal protein during 15-min incubation) and for 10 domestic strains which ranged from 12.5 ± 2.1 to 25.9 ± 3.5 mμmoles. The apparent K_m for ethylmorphine *N*-demethylase was 0.529 ± 0.139 m*M* in wild rats, whereas the apparent K_m was lower in 10 strains of domestic rats and ranged from 0.204 ± 0.035 to 0.323 ± 0.031 m*M*. The apparent K_m for aniline hydroxylase was 0.107 ± 0.043 m*M* in wild rats, which resembled in this respect 10 strains of domestic rats. These ranged in their apparent K_m from 0.035 ± 0.012 to 0.102 ± 0.021 m*M*.

Discussion. Previously, 2-fold variations in the activity of hepatic microsomal hexobarbital oxidase of 11 strains of mice were reported (4). The results of the present investigation show 2- to 3-fold variations in ethylmorphine and aniline metabolism in the 9000*g* liver supernatant from 10 domestic rat strains and wild rats of the same species, but differences between this species and another species, the kangaroo rat, are much greater. Equality of P-450 in all strains of domestic Norway rat in the uninduced state and similarity of these values to those of kangaroo rat indicate that variations in drug metabolism in the uninduced state cannot be related to alterations in P-450 levels. The metabolism of aniline and ethylmorphine was enhanced by phenobarbital pretreatment in all strains of the Norway rat; approximately 2-fold differences in the extent of induction among

TABLE II. The Effect of Phenobarbital Pretreatment on the Hepatic Metabolism of Aniline in 10 Strains of *R. norvegicus*, in Wild Norwegian Rats, and in the Kangaroo Rat.[a,b]

	Male			Female		
	Treated	Control	Ratio (treated /control)	Treated	Control	Ratio (treated /control)
ACI	24.8 (8)	9.1[d] (8)	2.7	23.3[c] (8)	14.8[c] (8)	1.6
Alb	20.9 (5)	11.0[d] (5)	1.9	20.5 (5)	5.9[d] (5)	3.5
Buf	29.0[c] (5)	16.8[c] (5)	1.7	26.4[c] (5)	15.7[c] (5)	1.7
F344	28.8[c] (9)	19.2[c] (9)	1.5	21.5 (9)	8.4[d] (9)	2.5
LE	31.1[c] (6)	20.6[c] (6)	1.5	21.6 (8)	11.5[c] (8)	1.9
LEW	18.3[d] (4)	9.7[d] (4)	1.9	13.9[d] (4)	7.2[d] (4)	1.9
M520	20.3 (5)	7.5[d] (5)	2.7	19.5 (5)	7.1[d] (5)	2.7
OM	20.2[d] (9)	11.5[d] (9)	1.7	17.6[d] (9)	7.0[d] (9)	2.5
SD	23.8 (9)	13.0 (9)	1.9	20.8 (9)	10.6 (9)	1.9
W	20.2[d] (5)	6.9[d] (5)	2.9	18.1 (5)	6.6[d] (5)	2.7
Mean ± SD	23.7 (65) ±2.3	13.2 (65) ±1.8	2.0	20.5 (67) ±2.6	9.5 (67) ±2.0	2.3
K[e]	52.6 (5)	4.2 (5)	12.5	40.4 (6)	19.0 (6)	2.1
Wild rats		13.6 ±2.4			9.4 ±3.7	

[a] All rats were sacrificed 24 hr after the third injection of saline or phenobarbital and 9000*g* supernatants prepared from the livers in Tris–KCl buffer. Results are expressed as mμmoles of *p*-aminophenol formed/mg of protein/15 min incubation at 37°.

[b] Values are the means for the number of animals given in parentheses for each strain.

Mean values for the strains were significantly higher, [c], ($p < 0.05$) or significantly lower, [d], ($p < 0.05$) than the general mean for the 10 strains, as determined by an analysis of variance.

[e] Kangaroo rats are a wild strain of the species *D. merriami*. Data for this species were not included in the intraspecies statistical comparisons for *R. norvegicus*.

strains were observed.

A previous report described much larger differences in drug-metabolizing activity in the 9000*g* liver supernatant from various strains of rabbit (5). However, most of the variation was contributed by cottontail and jackrabbits, the latter representing a different species from that of the 5 other rabbit strains. The cottontails and jackrabbits were wild, whereas the other four strains were domestic; wild animals had less drug -metabolizing activity with several substrates than did domestic rabbits. Certain drug-metabolizing enzymes of wild hares and rabbits exhibited greater response to phenobarbital pretreatment than did corresponding enzymes of domestic rabbits.

Wild Norwegian rats had lower P-450 values than 10 domestic strains but resembled them in aniline hydroxylase and ethylmorphine *N*-demethylase activities, assayed in the 9000*g* supernatant. However, kinetic studies performed on the microsomal rather than the 9000*g* fraction revealed that wild rats had an appreciably lower V_{max} and higher apparent K_m for ethylmorphine *N*-demethylase than did 10 domestic strains. No differences between the wild and domestic strains occurred in aniline hydroxylase activity either with respect to apparent K_m or V_{max}. It should be noted that the standard deviations of the values both for apparent K_m for ethylmorphine *N*-demethylase and for P-450 were much larger for the wild Norwegian rats than for the domestic strains. The greater variability of the wild rats may be due to their wider range in nature and hence to the exposure of each wild rat to different amounts of those substances capable of inducing drug-metabolizing enzymes.

TABLE III. P-450 Content of Livers from Male Rats.[a,b]

Strain		P-450 (mμmoles ± SD)
ACI	(5)	0.99 ± 0.03
Alb	(6)	0.98 ± 0.03
Buf	(5)	0.98 ± 0.01
F344	(5)	0.97 ± 0.04
LE	(6)	0.96 ± 0.06
LEW	(6)	0.98 ± 0.05
M520	(6)	1.08 ± 0.13
OM	(6)	0.92 ± 0.11
SD	(5)	0.98 ± 0.02
W	(6)	0.99 ± 0.03
Wild Norwegian	(6)	0.49 ± 0.19
Kangaroo	(6)	1.01 ± 0.43

[a] The P-450 content was determined on a Shimadzu recording spectrophotometer from the optical density difference at 450 mμ and 490 mμ, with a microsomal suspension of 2 mg protein/ml (9).

[b] The number of animals studied is given in parentheses.

As in wild rabbits (5), wild Norwegian rats exhibited lower drug-metabolizing activity than did domestic strains. It might have been anticipated that greater exposure to potent inducing agents in the form of plant alkaloids would occur in nature than in captivity and that therefore wild animals would metabolize drugs more rapidly than domestic strains. Perhaps the laboratory environment contains more inducing agents than that in nature. Differences in inbreeding apparently do not account for these observations, since the outbred strains, OM and SD, were similar to eight inbred strains, both in basal metabolism of aniline and ethylmorphine and in response to phenobarbital pretreatment.

In spite of its name, the kangaroo rat is from a different species than the Norway rat; sex differences in drug-metabolizing enzymes of kangaroo rats differ from those in 10 strains of domestic Norway rats (Tables I and II) and resemble those observed in mice (4). Although in all strains of Norway rats ethylmorphine metabolism was lower in females than in males, for aniline metabolism this sex difference occurred only in females of strain Alb, F344 and LE; in other strains males and females were comparable in their metabolism of aniline. A sex difference in response of mice to hexobarbital has been demonstrated to exist in certain strains, but not in others (4). In OM rats kinetic analysis of ethylmorphine *N*-demethylase revealed that apparent K_m and V_{max} values in males and females differ markedly (2). We reported kinetic studies of ethylmorphine *N*-demethylase activity in various strains of the Norway rat (11). Strain differences in both apparent K_m and V_{max} were observed. Recently we obtained similar results for aniline hydroxylase (8). Therefore, strain variations in metabolism of aniline and ethylmorphine may arise not only because of quantitative variations in the amount of a given protein, but also because of the presence of a qualitatively altered protein. Different genetic mechanisms may account for these two causes of strain variations; metrical traits are frequently determined through the operation of multiple genes, whereas qualitative differences in proteins performing identical functions result usually from single point mutations, transmitted according to Mendelian laws. A lower V_{max} may result from decreased amounts of normal enzyme, in which case the apparent K_m would be unaltered, or it may result from the presence of a different or atypical enzyme possessing lowered substrate affinity and higher apparent K_m. The present experiments reveal that for ethylmorphine *N*-demethylase wild rats exhibit a lower V_{max} and higher apparent K_m than 10 domestic strains. Numerous environmental factors could produce in certain rat strains different concentrations of allosteric effectors, activators or inhibitors in hepatic microsomes, thereby altering their drug-metabolizing activities. Particular care was devoted to keeping as many environmental variables as uniform as possible. Animals were kept on hard wood bedding (4) with Purina food and tap water available *ad libitum* for 2 weeks in our animal room prior to use. Rats were used at two months of age because by this time differences in rate of growth that exist among strains have disappeared and rats of all strains may be considered mature. Finally, because we obtained several strains from different sup-

pliers, we compared the kinetics of the 2 enzymes from a given strain derived from 3 separate sources. No significant differences occurred in any of the 3 strains, each purchased from 2 or 3 different suppliers.

Current interest among pharmacologists in strain differences is based in part on the conviction that, since strains of a species are genetically more similar than animals of different species, investigations of strain differences may reveal fine gradations in the process of evolution of drug-metabolizing enzymes. Generally strains of a species diverged during a shorter time and are therefore separated by fewer mutational events than members of different species. Studies of strain and species differences have provided and may continue to provide insights into the development of drug-metabolizing enzymes during evolution (12). Large strain and species differences in drug metabolism offer serious obstacles both to comparing results of differenent laboratories using different strains and to extrapolating drug testing results from rodents to man.

Summary. Two- to three-fold differences in the metabolism of ethylmorphine and aniline have been described in the 9000*g* liver supernatant from 10 strains of Norway rat. In the 9000*g* supernatant wild Norway rats resembled domestic Norway rats in ethylmorphine and aniline metabolism, but in the microsomal fraction wild rats had lower V_{max} for ethylmorphone *N*-demethylase and higher apparent K_m than 10 domestic strains. In accordance with this observation, the P-450 values of the hepatic microsomal fraction were approximately 1.0 mμmoles for 10 domestic strains of the Norway rat and 0.5 mμmoles for the wild Norway rat. Greater variability of wild rats both for P-450 and apparent K_m for ethylmorphine *N*-demethylase suggests exposure of individual animals to different amounts of inducing agents. Male Norway rats metabolized ethylmorphine 50 to 300% more rapidly than did females, but in only three strains were similar sex differences observed for aniline. Conversely, in the distinct species *Dipodomys merriam* (kangaroo rats), females metabolized both drugs more rapidly than did males. In 10 strains of domestic rat, phenobarbital pretreatment elevated ethylmorphine metabolism 2.0- to 3.8-fold; aniline metabolism increased 1.5- to 3.5-fold. Implications of decreased hepatic microsomal drug-metabolizing activity in wild compared to domestic animals of the same species are discussed.

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