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COMPARISON OF MICROSOMAL DRUG-METABOLIZING ENZYMES IN 14 RAT INBRED STRAINS

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Abstract—Drug metabolic capacity in liver microsomes of 14 rat inbred strains was investigated. Cytochrome P-450 content as well as the following enzyme activities were measured: NADPH cyt. c(P-450) reductase (Red.), aminopyrine *N*-demethylase (APDM), ethoxycoumarin *O*-deethylase (ECOD), 1-naphthol: UDP-glucuronosyltransferase (NGT) and hydrolysis of acetylsalicylic acid (ASA; measured at pH 5.5 and pH 7.4). All enzymes measured were found to exhibit statistically significant inter-strain differences. In males the enzyme activities varied over a 7.3-fold (ECOD) to 1.4-fold (cytochrome P-450) range. Other inter-strain differences were generally larger than 2-fold: ASA-hydrolysis at pH 5.5 and 7.4 (3.9- and 3.3-fold variation, respectively), NGT and Red. (2.1-fold variation) and APDM (1.8-fold variation). In females similar, but somewhat smaller inter-strain differences were observed. Correlations between different enzyme activities were generally poor (correlation coefficients $r < 0.7$). An exception was the correlation between ASA-hydrolysis at pH 5.5 and pH 7.4 ($r = 0.79$). We conclude that ASA hydrolysis at pH 5.5 and 7.4 is mediated by the same enzyme or by coregulated enzymes and that all other activities are mediated by different or differentially regulated enzymes. Based on analysis of variance and subsequent inter-strain comparisons, all strains appear to express a unique profile of liver microsomal drug metabolism. No two strains are identical with respect to all activities measured. We suggest that differences between inbred rat strains and particularly the difference in balance between different enzymes in various strains can be used advantageously in pharmacological and toxicological experiments.

Drug metabolism in a given animal species is influenced by a large number of endogenous and exogenous factors [1]. Variability related to sex [2, 3], age [4, 5] and hormonal status [1, 6] ultimately relies on the genetic background of the animal. Inbred animal strains, in which over 98% of the genes are in the homozygous state, and other genetic models have proved to be invaluable in studying the heterogeneity of drug metabolizing enzymes [7, 8] and cell-membrane carriers [9], as well as in the study of genetic regulation of biotransformation processes [10, 11].

Inbred mouse strains are known to be highly variable in their rate of metabolism of various xenobiotics [7, 12–14] and these strain-related differences are thought to be involved in strainspecific metabolic profiles [13, 15], pharmacokinetics [16], toxicity [17] and longevity [14]. Although the rat is widely used in drug metabolism studies, inbred strains of this species are poorly characterized with respect to xenobiotic metabolism. Older literature indicates that 2–3-fold differences may exist in ethylmorphine *N*-demethylase and aniline-hydroxylase activity between individual inbred strains [18]. More recently, rat inbred strains have been introduced as a model for the 4-hydroxylation defect of debrisoquine in man [19]. The DA-strain was found to be a poor metabolizer of debrisoquine [19] and ochratoxin-A [20], but not of phenacetin [21]. Other strain-related differences in drug metabolism have been described in the literature [20, 22–24], but usually only a few rat strains were compared.

Recent evidence that rat strains are highly polymorphic in electrophoretic recognizable isoforms of cytochrome P-450 [25] and esterases [26, 27] prompted our study of microsomal drug metabolism in a larger number of rat inbred strains. It is shown in this paper that inter-strain differences can vary over a 1.3- to 7.3-fold range, depending on the enzyme activity being measured.

MATERIALS AND METHODS

Chemicals. Cytochrome c (type III, from horse heart), NADPH (sodium salt), 1-naphthol (grade III) and 1-naphthyl-β,D-glucuronide (sodium salt) were obtained from Sigma Chemical Co. (St. Louis, MO). Rotenone, 7-ethoxycoumarin, 7-hydroxycoumarin, aminopyrine and ASA were purchased from Aldrich (Beerse, Belgium), Boehringer (Mannheim, F.R.G.), Fluka (Buchs, Switzerland), Gist-Brocades (Delft, NL) and OPG (Utrecht, NL), respectively. All other chemicals were of analytical grade purity and used as supplied.

Animals. Liver samples of rat inbred strains (see Table 1) were obtained from the Department of Laboratory Animal Science, Veterinary Faculty, University Utrecht. The animals originated from four different breeding facilities: Central Institute of Laboratory Animal Breeding, Hannover, F.R.G. (/Han); University of Limburg, Maastricht, NL (/Mtr); Antoni van Leeuwenhoek Hospital, Amsterdam, HNL (/A) and REPGO/TNO-Institute of Radiobiology, Rijswijk, NL (/Rij). All strains used have recently been found to be homogeneous, using 28 different biochemical markers [26]. All animals were housed under identical conditions during at

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Table 1. Mixed function monooxygenase activities in liver microsomes of rat inbred strains

Strain code	Strain	Sex	Cytochrome P-450 (pmol/mg)	NADPH-cyt.c reductase (nmol/min·mg)	Aminopyrine-*N*-demethylase (nmol/min·mg)	7-ethoxycoumarin-*O*-deethylase (pmol/min·mg)
A	BDII/Han	male	430 ± 40	272 ± 6	3.15 ± 0.25	62 ± 5
B	BDV/Han	male	465 ± 32	352 ± 29	3.79 ± 0.33	191 ± 15
C	BDIX/Han	male	610 ± 12	465 ± 29	4.67 ± 0.20	345 ± 16
D	BN/Mtr	male	490 ± 12	190 ± 11	3.51 ± 0.06	74 ± 2
E	DA/Han	male	615 ± 32	269 ± 8	3.02 ± 0.09	68 ± 1
F	LE/Han	male	490 ± 12	187 ± 10	2.58 ± 0.10	47 ± 2
G	LEW/Mtr	male	510 ± 0	205 ± 4	3.30 ± 0.16	243 ± 10
H	R/A	male	560 ± 17	295 ± 8	4.51 ± 0.12	199 ± 4
I	U/A	male	470 ± 17	195 ± 7	3.14 ± 0.02	135 ± 17
J	WKY/Mtr	male	605 ± 14	408 ± 28	4.50 ± 0.05	100 ± 2
K	BDE/Han	female	380 ± 12	311 ± 32	2.45 ± 0.41	112 ± 20
L	OM/Han	female	375 ± 9	159 ± 11	2.81 ± 0.07	84 ± 2
M	R/A	female	580 ± 0	259 ± 9	2.66 ± 0.05	150 ± 3
N	SD/A	female	300 ± 17	186 ± 11	2.19 ± 0.04	81 ± 1
O	WAG/Rij	female	450 ± 35	307 ± 19	3.13 ± 0.21	249 ± 7
Mean over all strains (males)			525	291	3.61	146
Mean over all strains (females)			417	239	2.65	136

Microsomes were prepared and incubated as described in Materials and Methods. The values given represent the means ± SE of four determinations per strain (duplicate measurements with two microsomal preparations). ANOVA demonstrated that the between-animal within-strain and the within-animal duplicate mean square was not statistically significant ($P > 0.05$), while the inter-strain variation was statistically significant ($P < 0.001$) in all cases, except aminopyrine *N*-demethylase in females.

least 25 weeks before killing and had free access to tap water and a commercially available diet (RMH-B, Hope Farms, Woerden, NL). Strain nomenclature is according to Ref. 28; for ease of reference shorter strain codes are used throughout this paper (Table 1).

Preparation of microsomes. Rats, aged 35–78 weeks, were killed by cervical dislocation and livers were homogenized (Potter–Elvehjem homogenizer, three up-and-down strokes) in a three-fold volume of Na–K-phosphate buffer (9 mM KH_2PO_4, 34 mM $Na_2HPO_4 \cdot 2H_2O$, pH 7.4) containing 0.1 mM EDTA-Na_2, 1 mM dithiothreitol and 20% (v/v) glycerol. Microsomes were prepared by differential centrifugation (MSE-prepspin ultracentrifuge; 20 min at 12,000 g_{max}; 60 min at 105,000 g_{max}), resuspended (*ca.* 10 mg protein/ml) in homogenization buffer, quick-frozen and stored at −80° until measurement of enzyme activities. All procedures were carried out at 0–4°. Samples were stored in small aliquots and were thawed only once for determination of enzyme activity. A particular enzyme activity was measured in all samples simultaneously.

Incubations. Cytochrome P-450 was quantitated as the CO vs CO + dithionite difference-spectrum using an Aminco-DW2a UV-vis spectrometer in the split-beam mode [29]. Reductase† was quantitated at 37° by measuring the reduction of cytochrome-*c* (50 μM) by NADPH (50 μM) in the presence of 0.1 mM KCN and 2.5 μM rotenone (44 mM K-phosphate buffer, pH 7.6, containing 66 mM KCl). NADPH was omitted from the reference samples [30]. APDM was measured by incubation of microsomes at pH 7.4 (Na–K-phosphate buffer) with 1 mM aminopyrine and quantitation of released formaldehyde [31]. ECOD and NGT were measured by quantitation of 7-hydroxycoumarin [32] and 1-naphthyl-β,D-glucuronide [33] on a Perkin–Elmer LS-3 spectrofluorimeter after incubation with 7-ethoxycoumarin (0.2 mM) or 1-naphtol (0.5 mM), respectively. Glucuronidation was measured in microsomes maximally activated with Brij-58 (0.5 mg/mg protein). Hydrolysis of ASA by aspecific carboxylesterases (EC 3.1.1.1) was measured by fluorimetric quantitation of free salicylic acid (λ_{ex} = 298 nm, λ_{em} = 406 nm) and corrected for spontaneous hydrolysis (details will be published elsewhere). ASA-hydrolysis was measured at pH 5.5 (4 mM ASA, 200 mM Na-phosphate buffer) and pH 7.4 (8 mM ASA, 200 mM Na-phosphate buffer) because it has been suggested that hydrolysis at the lower pH (ASA-1 activity) and the higher pH (ASA-2 activity) is due to different esterases [34]. All enzyme activities were measured under conditions linear with time and protein concentration and expressed as specific activities. Protein was determined according to Lowry *et al.* [35] using crystalline bovine serum albumin as a standard.

Experimental design and statistical analysis. All enzyme activities were measured in duplicate with two microsomal preparations, prepared from individual animals (two per strain). An initial ANOVA indicated that between-animal within-strain differences were not statistically significant (duplicate *plus* duplicate × treatment mean square used as

† Abbreviations used: ANOVA, analysis of variance; APDM, aminopyrine *N*-demethylase; ASA, acetyl salicylic acid; ECOD, ethoxycoumarin *O*-deethylase; NGT, 1-naphthol:UDP-glucuronosyltransferase (EC 2.4.1.17); reductase, NADPH cyt.c(P-450) reductase (EC 1.6.2.4).

Table 2. Glucuronidation and ester-hydrolysis in liver microsomes of rat inbred strains

Strain code	Strain	Sex	1-Naphthol -glucuronidation (nmol/min · mg)	Hydrolysis of acetyl-salicylic acid (nmol/min · mg) measured at pH 5.5 (ASA-1)	measured at pH 7.4 (ASA-2)
A	BDII/Han	male	9.6 ± 0.9	64.0 ± 14.5	37.0 ± 9.9
B	BDV/Han	male	20.8 ± 0.3	67.0 ± 5.8	36.5 ± 1.9
C	BDIX/Han	male	17.9 ± 0.3	119.0 ± 9.4	88.8 ± 10.0
D	BN/Mtr	male	9.8 ± 0.5	40.5 ± 2.2	27.0 ± 2.7
E	DA/Han	male	14.1 ± 0.4	113.0 ± 11.3	64.0 ± 7.4
F	LE/Han	male	14.0 ± 1.2	79.8 ± 6.8	27.3 ± 2.8
G	LEW/Mtr	male	15.3 ± 0.3	122.0 ± 6.5	74.0 ± 5.1
H	R/A	male	19.7 ± 0.4	80.5 ± 3.6	28.3 ± 2.3
I	U/A	male	12.6 ± 0.4	55.8 ± 1.3	29.5 ± 3.2
J	WKY/Mtr	male	11.0 ± 0.6	159.8 ± 10.3	78.0 ± 12.2
K	BDE/Han	female	13.8 ± 0.3	68.5 ± 6.0	43.3 ± 1.1
L	OM/Han	female	13.4 ± 0.7	30.8 ± 1.4	3.5 ± 2.0
M	R/A	female	16.0 ± 0.7	68.0 ± 7.6	23.0 ± 5.0
N	SD/A	female	16.2 ± 0.8	51.5 ± 2.7	34.0 ± 4.8
O	WAG/Rij	female	12.1 ± 0.2	59.3 ± 5.4	28.3 ± 8.1
Mean over all strains (males)			14.5	90.1	49.0
Mean over all strains (females)			13.9	55.6	26.4

Microsomes were prepared and incubated as described in Materials and Methods. The values given represent the means ± SE of four determinations per strain (duplicate measurements with two microsomal preparations). ANOVA demonstrated that the between-animal within-strain and the within-animal duplicate mean square was not statistically significant ($P > 0.05$), while the inter-strain variation was statistically significant ($P < 0.001$) in all cases.

error variance). Differences between strains were, therefore, assessed by an ANOVA, in which the between-animal within-strain, the duplicate within-animal and the second and third-order interactions' mean square was used as error variance. The values given in Tables 1 and 2 represent the mean ± SE of the four determinations. Inter-strain variability of enzyme activity is operationally defined as the *F*-value of the between-strain vs within-strain mean square comparison. Differences between individual strains were assessed by the sequential Newman–Keuls test, adopting a significance level of $P < 0.01$. Correlations between enzyme activities were assessed by correlation analysis and by ANOVA. The Pearson correlation coefficient *r*, the *F*-value of the regression vs error mean square comparison in the ANOVA and the probability level P are indicated. All statistical procedures were carried out according to Snedecor and Cochran [36] with the help of a commercially available statistical package [37], implemented on an Olivetti M24 personal computer.

RESULTS

Enzyme activities

Cytochrome P-450 content and P-450-related enzyme activities in the strains A-O are given in Table 1. ANOVA indicated that for these activities statistically significant inter-strain differences are present both in males and females (Table 3). Two (P-450, reductase), to five (ECOD) groups of strains with mutual different activities can be discriminated (Fig. 1). Cytochrome P-450 content ranged over a 1.4-fold difference in males and 1.9-fold difference in females (Table 3). Reductase activity correlates with P-450 content ($r = 0.493$, $F = 12.2$ with 1 and 39 df, $P = 0.001$ for males; $r = 0.482$, $F = 5.5$ with 1 and 19 df, $P = 0.031$ for females). Correlation between APDM and total P-450 is better than between ECOD and P-450 ($r = 0.587$, $F = 30.5$, $P < 0.001$ for APDM and $r = 0.339$, $F = 7.5$, $P < 0.008$ for ECOD). This probably reflects the wide overlap in substrate-specificities of P-450 isoenzymes for APDM in comparison to ECOD [38]. When APDM and ECOD-activity are expressed per pmole P-450 (instead of per mg protein) similar inter-strain differences are found (data not shown).

Glucuronidation of 1-naphthol varied over a 2.1-fold range in males and a 1.3-fold range in females (Tables 2 and 3). On the basis of ANOVA and inter-strain comparisons four groups with mutually different NGT activities can be discerned (Fig. 1). Hydrolysis of ASA was measured at pH 5.5 (ASA-1) and at pH 7.4 (ASA-2) because it has been suggested [34] that these hydrolytic activities are mediated by different carboxylesterases. Our results indicate, however, that ASA-1 and ASA-2 are highly correlated (Fig. 2).

Sex differences

In the ANOVA male and female animals were treated separately, because sex-related differences are known to occur [2, 3]. Of one strain both males and females were available (H and M, respectively). Statistically significantly different activities between males and females were observed for APDM, ECOD, NGT and ASA-hydrolysis in this strain. For

Table 3. Variability of liver microsomal drug metabolism in rat inbred strains

Enzyme (units)	Sex	F-value of ANOVA	Lowest activity (strain)	Highest activity (strain)	Fold variation (highest/lowest)
Cytochrome P-450	male	9.5	430 (BDII/Han)	615 (DA/Han)	1.4
(pmol/mg)	female	32.5	300 (SD/A)	580 (R/A)	1.9
NADPH-cyt.c reductase	male	13.2	190 (BN/Mtr)	408 (WKY/Mtr)	2.1
(nmol NADPH ox./min·mg)	female	9.0	159 (OM/Han)	311 (BDE/Han)	2.0
Aminopyrine-*N*-demethylase	male	19.1	2.58 (LE/Han)	4.67 (BDIX/Han)	1.8
(nmol/min·mg)	female	2.8	2.19 (SD/A)	3.13 (WAG/Rij)	1.4
7-ethoxycoumarin-*O*-	male	98.5	47 (LE/Han)	345 (BDIX/Han)	7.3
deethylase (pmol/min·mg)	female	52.1	81 (SD/A)	249 (WAG/Rij)	3.1
1-naphthol-glucuronidation	male	43.1	9.6 (BDII/Han)	19.7 (R/A)	2.1
(nmol/min·mg)	female	13.0	12.1 (WAG/Rij)	16.2 (SD/A)	1.3
Hydrolysis of acetyl-salicylic	male	20.3	40.5 (BN/Mtr)	159.8 (WKY/Mtr)	3.9
acid (pH 5.5) (nmol/min·mg)	female	9.2	30.8 (OM/Han)	68.5 (BDE/Han)	2.2

Variability was operationally defined as the *F*-value of an ANOVA, in which the between-strains mean square was compared to the within-strain error mean square. Statistically significant differences ($P \leqq 0.001$) were found for all inter-strain comparisons except aminopyrine *N*-demethylase in females ($P = 0.061$).

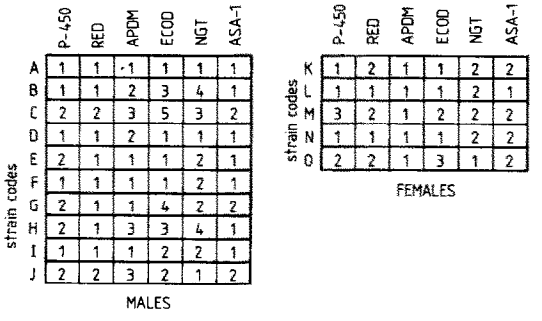

Fig. 1. Metabolic profile of rat inbred strains. Comparison of enzyme activities between strains were assessed by the sequential Newman–Keuls test for males and females separately adopting a significance level of $P < 0.01$. Strains that are not significantly different according to this criterion are indicated by the same number, 1 being given to the group with lowest activity.

all these activities, activity in females was the lower of the two. When the mean activities over all males (strains A–J) are compared to the mean activities over all females (strains K–O), the same conclusion is reached for APDM (3.62 ± 0.12 and 2.65 ± 0.11 nmol/min·mg in males and females, respectively) and ASA-1 (90.1 ± 6.1 and 55.6 ± 3.8 nmol/min·mg). ECOD and NGT-activities in males and females were not different when calculated for all strains together. P-450 (525 ± 12 and 417 ± 23 pmol/mg) and reductase (284 ± 15 and 244 ± 16 nmol/min·mg) were lower in females, compared to males.

Variability

All enzyme activities measured demonstrated strain-specific differences. Differences between strains range from 1.3 to 7.3-fold, depending on the enzyme under consideration (Table 3). No statistically significant correlations were found between age of the animals and various enzyme activities (data not shown). Furthermore, close examination of Fig. 1 reveals that all strains investigated are unique in their metabolic profile.

DISCUSSION

This is the first report describing inter-strain variability of a number of microsomal drug metabolizing enzymes in a large number of rat inbred strains. Earlier reports suggested that strain-related differences in liver microsomal drug metabolism do exist in the rat [18, 19, 22, 23], but only recently two rat inbred strains (DA and LEW) have been characterized for a larger number of enzymes, including phase-I and phase-II activities [19, 20, 24]. Rat inbred strains have been found to be highly polymorphic for cytochrome P-450 b, e, g and h [25] and various carboxylesterases [27] on the basis of acrylamide gel electrophoresis and isoelectric focusing, but the consequences of these genetically determined polymorphisms for microsomal drug metabolism are not known.

We quantitated total cytochrome P-450 as well as reductase, APDM and ECOD activities in 14 strains. APDM and ECOD were used as representative activities for phenobarbital and 3-methylcholanthrene inducible P-450s, respectively [38]. NGT was measured as a representative phase-II metabolic activity. Hydrolysis of ASA at two different pHs was measured because it has been suggested that ASA-1 and ASA-2 hydrolysis are mediated by different carboxylesterases [34] and because all rat strains used are genetically characterized for their esterase patterns [26, 27]. Statistically significant inter-strain differences were found for all enzyme activities measured. These differences may be as large as 7.3-fold (Table 3). Although we established that differences in enzyme activities between inbred rat strains exist, great care should be exercised in the use of the absolute enzyme activities, reported in this study, as reference values. Apart from strain-related differences, a large number of variables (interindividual variation, age-related differences, storage conditions and analytical

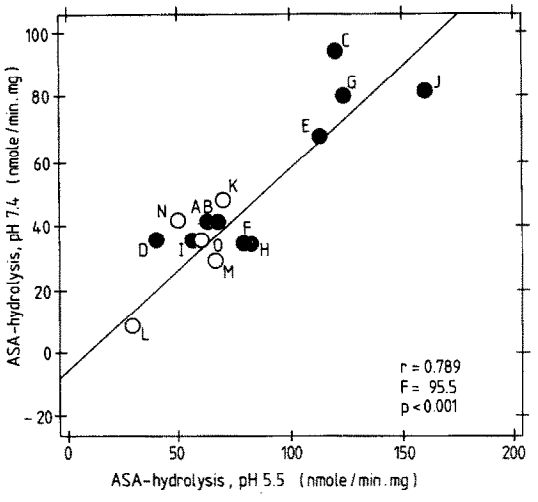

Fig. 2. Correlation between hydrolysis of ASA at pH 5.5 (ASA-1) and pH 7.4 (ASA-2). For reasons of clarity only the means per strain are indicated. Statistical evaluation was based on all individual measurements. Symbols (●: males; ○: females) and upper case characters refer to the strain-codes mentioned in Table 2.

variability) may have influenced the measurements. Special care was taken in this study to minimize experimental variation. Liver microsomes of all animals were prepared within one week, whereas activities of a particular enzyme were measured in all samples simultaneously using freshly thawed microsomes. The absolute values of the enzyme activities can, therefore, be influenced by storage conditions, but it is reasonable to assume that this affected all samples similarly. Because an initial ANOVA demonstrated that within-strain between-animal variances were not statistically significant, the measurements per strain were pooled for the final ANOVA. The values, given in Tables 1 and 2 represent, therefore, the means ± SE of four determinations. The SE is the result of analytical variation and includes variability due to homogenization, storage, thawing, incubation and quantitation of enzyme activity. Absence of statistically significant within-strain between-animal differences is not surprising, because the individuals of inbred strains (in contrast to the more commonly used random-bred strains) are identical with respect to at least 98.6% of their genes [17]. Moreover, the strains used in this study have recently been found to be homogeneous on the basis of 28 different biochemical markers [26]. All individual animals used in this study expressed their characteristic esterase-banding pattern when liver microsomes were analysed on starch gel electrophoresis [26].

A more serious problem in interpreting the strain-related differences results from the variation in age of the animals used. Using regression analysis no statistically significant correlations were found between age and any of the enzyme activities measured. This demonstrates that the differences found could not be explained simply on the basis of age. Nevertheless, age-related factors may have influenced the results. Available literature indicates that a decrease in metabolic capacity occurs during aging [39], but this decrease is not very extensive (up to 30%) before 80 weeks of age [40, 41]. In particular with cytochrome P-450, NADPH-cytochrome c-reductase and various conjugating enzymes no decrease is observed up to 2 years of age [40–43]. We consider it therefore likely that the differences observed in this study are—at least in part—strain-related. Obviously, more detailed studies should be carried out to evaluate and/or to corroborate our findings.

All enzyme activities measured are in the same ranges as reported in the literature, generally [20, 34]. Our results confirms the observation of Hietanen *et al.* [20], that APDM and ECOD activities were lower in the DA-rat compared to the LEW-rat. Direct comparison with other literature data [18, 19, 21–24] is impossible because different enzyme activities were measured or because different rat strains were used. Unfortunately, the genetic composition of all strains used in this study is only known for the esterases [26, 27, 43]. However, this genetic characterization is only based on the electrophoretic mobility of allelic variants and cannot predict enzyme activity in microsomal preparations. No correlation could be detected between high or low ASA-1 activity (as determined in microsomes) and various allelic forms of the main liver microsomal esterases, Es-3, Es-4, Es-8/10, Es-14 and Es-15 [27, 43, 44]. It has been suggested that ASA is hydrolysed with a certain degree of specificity by Es-4 [44, 45] but out results demonstrate that this cannot be the only factor involved. All rat strains investigated are identical in expressing the Es-4B allele [26].

Correlations between different enzyme activities were generally poor, although statistical significance was reached in a number of cases. Correlation coefficients were not fundamentally altered when enzyme activities were expressed per pmole P-450 (instead of per mg protein) and inter-strain differences remained statistically significant. Poor correlations between various enzyme activities have been observed before in the rat [20] and in the mouse [7], and it was concluded that the enzyme activities measured are mediated by different or differentially regulated enzymes. High correlation coefficients are usually indicative for metabolism by the same isoenzyme or for metabolism by coregulated enzymes [7, 20]. This has been observed, for example for the correlation between ECOD, ethyoxyresorufin *O*-deethylase, aryl hydrocarbon hydroxylase and ochratoxin-A 4-hydroxylase in the rat [20]. The high correlation between ASA-1 and ASA-2 activity, observed in our study, suggests that both activities are regulated in an identical way in all strains investigated. Because all strains are widely differing in their allelic composition of the main liver esterases [26, 27, 43] and because ASA is hydrolysed by more than one isoenzyme [45], we consider it highly unlikely that ASA-1 and ASA-2 activities are mediated by different esterases [34]. ASA-2 activity was therefore not further used for inter-strain comparisons.

Cytochrome P-450 content, reductase and APDM,

ECOD, NGT, ASA-1 activities can be used to characterize the various strains. On the basis of ANOVA and subsequent inter-strain comparisons, all strains investigated must be considered unique. No two strains are identical with respect to all enzyme activities investigated (Fig. 1). Literature data are too scanty to relate differences in enzyme activities or differences in balance between enzyme activities to inter-strain variation in, for example, spontaneous tumorigenesis [28]. It has been shown that differences in metabolic capacity of the DA- and the LEW-strain affect the urinary excretion of metabolites of debrisoquine [19]. Moreover, spectral interaction with cytochrome P-450 [24] as well as metabolic activation of aflatoxin B1 [20] is different in microsomes from DA- and LEW-rats. Similar phenomena have been observed in inbred mouse strains [13–17] and we suggest that inbred rat strains can be used advantageously in pharmacological and toxicological studies as well. In particular when balances between various metabolic routes are of interest, the use of inbred strains offers distinct advantages. Experimental manipulation of one enzyme activity at a time by dietary factors, inducers or hormones is virtually impossible [6, 11, 46]. Inbred strains can be used directly to answer relevant questions and may even be used as models for human polymorphisms [19–21]. The results presented in this paper may suggest further applications of inbred rat strains in this area.

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REFERENCES

1. Gibson GG and Skett P, *Introduction to Drug Metabolism*. Chapman and Hall, London, 1986.
2. Kato R, Sex-related differences in drug metabolism. *Drug Metab Rev* **3**: 1–32, 1974.
3. Mulder GJ, Sex differences in drug conjugation and their consequences for drug toxicity. Sulfation, glucuronidation and glutathione conjugation. *Chem Biol Interact* **57**: 1–15, 1986.
4. Klinger W, Biotransformation of drugs and other xenobiotics during postnatal development. *Pharmacol Ther* **16**: 377–429, 1982.
5. Kiechel JR, Biotransformation of drugs during aging. *Gerontology* **28**(Suppl 1), 101–112, 1982.
6. Skett P, Nutritional, hormonal and physiological regulation of drug metabolism. In: *Drug Metabolism. Molecular Approaches and Pharmacological Implications* (Ed. Siest G), pp. 111–119. Pergamon Press, Oxford, 1985.
7. Lang MA, Gielen JE and Nebert DW, Genetic evidence for many unique liver microsomal P-450-mediated monooxygenase activities in heterogeneic stock mice. *J Biol Chem* **256**: 12068–12075, 1981.
8. Matsui M and Nagai F, Genetic deficiency of androsterone UDP-glucuronosyltransferase activity in Wistar rats is due to the loss of enzyme protein. *Biochem J* **234**: 139–144, 1986.
9. Jansen PLM, Groothuis GMM, Peters WHM and Meijer DKF, Selective hepatobiliary transport defect for organic anions and neutral steroids in mutant rats with hereditary-conjugated hyperbilirubinaemia. *Hepatology* **7**: 71–76, 1987.
10. Malik N and Owens IS, Genetic regulation of bilirubin-UDP-glucuronosyltransferase induction by polycyclic aromatic compounds and phenobarbital in mice. Association with aryl hydrocarbon (benzo(*a*)pyrene) hydroxylase induction. *J Biol Chem* **256**: 9599–9604, 1981.
11. Whitlock JP, The regulation of cytochrome P-450 gene expression. *Ann Rev Pharmacol Toxicol* **26**: 333–369, 1986.
12. Wood AW and Conney AH, Genetic variation in coumarin hydroxylase activity in the mouse (*Mus musculus*). *Science (Wash)* **185**: 612–614, 1974.
13. Gurtoo HL, Genetic expression of aflatoxin-B1 metabolism: Effects of 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the metabolism of aflatoxins B1 and B2 by various inbred strains of mice. *Mol Pharmacol* **18**: 296–303, 1980.
14. Koizumi A, Hasegawa L, Walford RL and Imamura T, H-2, Ah, and aging: the immune response and the inducibility of P-450 mediated monooxygenase activities, xanthine oxidase, and lipid peroxidation in H-2 congenic mice on C57Bl/10, C3H, and A strain backgrounds. *Mech Ageing Devl* **37**: 119–136, 1986.
15. Zaleski J, Bansal SK and Gessner T, Formation of glucuronide, sulphate and glutathione conjugates of benzo(*a*)pyrene metabolites in hepatocytes isolated from inbred strains of mice. *Carcinogenesis* **4**: 1359–1366, 1983.
16. Atlas SA, Zweier JL and Nebert DW, Genetic differences in phenytoin pharmacokinetics. *In vivo* clearance and *in vitro* metabolism among inbred strains of mice. *Dev Pharmacol Ther* **1**: 281–304, 1980.
17. Festing MFW, Inbred strains and the factorial experimental design in toxicological screening. In: *Animal Quality and Models in Biomedical Research* (Eds. Spiegel A, Erichsen S and Solleveld HA), pp. 59–66. Gustav Fischer Verlag, Stuttgart, 1980.
18. Page JG and Vesell ES, Hepatic drug metabolism in ten strains of Norway rat before and after treatment with phenobarbital. *Proc Soc Exp Biol Med* **131**: 256–261, 1969.
19. Al-Dabbagh SG, Idle JR and Smith RL, Animal modelling of human polymorphic drug oxidation—the metabolism of debrisoquine and phenacetin in rat inbred strains. *J Pharm Pharmacol* **33**: 161–164, 1981.
20. Hietanen E, Malaveille C, Camus A-M, Bérézíat J-C, Brun G, Castegnaro M, Michelon J, Idle JR and Bartsch H, Interstrain comparison of hepatic and renal microsomal carcinogen metabolism and liver S9-mediated mutagenicity in DA and Lewis rats phenotyped as poor and extensive metabolizers of debrisoquine. *Drug Metab Dispos* **14**: 118–126, 1986.
21. Kahn GC, Rubenfield M, Davies DS and Boobis AR, Phenacetin *O*-deethylase activity of the rat: strain differences and the effects of enzyme inducing compounds. *Xenobiotica* **17**: 179–187, 1987.
22. Gold G and Widnell CC, Response of NADPH cytochrome *c* reductase and cytochrome P-450 in hepatic microsomes to treatment with phenobarbital—differences in rat strains. *Biochem Pharmacol* **24**: 2105–2106, 1975.
23. Greenspan P and Baron J, Hepatic microsomal oxidative drug metabolism in the spontaneously hypertensive rat. *Biochem Pharmacol* **30**: 678–682, 1981.
24. Küpfer A, Al-Dabbagh SG, Ritchie JC, Idle JR and Smith RL, Spectral binding studies of the polymorphically metabolized drugs debrisoquine, sparteine and phenformin by cytochrome P-450 of normal and hydroxylation deficient rat strains. *Biochem Pharmacol* **31**: 3193–3199, 1981.

25. Rampersaud A and Walz FG, Polymorphisms of four hepatic cytochromes P-450 in twenty-eight inbred strains of rat. *Biochem Gen* **25**: 527–534, 1987.
26. Bender K, Adams M, Baverstock PR, Den Bieman M, Bissbort S, Brdicka R, Butcher GW, Cramer DV, Von Deimling O, Festing MFW, Günther E, Guttman RD, Hedrich HJ, Kendall PB, Kluge R, Moutier R, Simon B, Womack JE, Yamada J and Van Zutphen B, Biochemical markers in inbred strains of the rat (*Rattus norvegicus*). *Immunogenetics* **19**: 257–266, 1984.
27. Simon B, De Looze S, Ronai A and Von Deimling O, Identification of rat liver carboxylesterase isozymes (EC 3.1.1.1) using polyacrylamide gel electrophoresis and isoelectric focusing. *Electrophoresis* **6**: 575–582, 1985.
28. Altman PL and Katz DD (Eds.) *Inbred and Genetically Defined Strains of Laboratory Animals. Pt 1: Mouse and Rat.* FASEB, 1979.
29. Estabrook RW, Peterson JA, Baron J and Hildebrandt A. In: *Methods in Pharmacology*, Vol. 2: *Physical Methods* (Ed. Chignell CF), pp. 303–350. Appleton-Century-Crofts, New York, 1972.
30. Ragnotti G, Lawford GR and Campbell PN, Biosynthesis of microsomal nicotinamide-adenine dinucleotide phosphate-cytochrome *c* reductase by membrane-bound and free polysomes from rat liver. *Biochem J* **112**: 139–147, 1969.
31. Parkinson A and Safe S, The detection of enzyme induction by rat liver microsomes prepared by isoelectric precipitation. *J Pharm Pharmacol* **31**: 444–447, 1979.
32. Greenlee WF and Poland A, An improved assay of 7-ethoxycoumarin *O*-deethylase activity: induction of hepatic enzyme activity in C57Bl/6J and DBA/2J mice by phenobarbital, 3-methyl-cholanthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J Pharmacol Exp Ther* **205**: 596–605, 1978.
33. Koster ASj and Noordhoek J, Glucuronidation in the rat intestinal wall. Comparison of isolated mucosal cells, latent microsomes and activated microsomes. *Biochem Pharmacol* **32**: 895–900, 1983.
34. Ali B and Kaur S, Mammalian tissue acetylsalicylic acid esterase(s): Identification, distribution and discrimination from other esterases. *J Pharmacol Exp Ther* **226**: 589–594, 1983.
35. Lowry AH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
36. Snedecor GW and Cochran WG, *Statistical Methods*, 6th Edn. Iowa State University Press, Ames, 1967.
37. *Systat, the System for Statistics.* Systat Inc., Evanston, IL, 1985.
38. Burke MD and Wolf CR, Substrates and inhibitors as probes of individual forms of drug metabolising systems. In: *Drug Metabolism from Molecules to Man* (Eds. Benford DJ, Bridges JW and Gibson GG), pp. 219–243. Taylor & Francis, London, 1987.
39. Van Bezooijen CFA, Influence of age-related changes in rodent liver morphology and physiology on drug metabolism—a review. *Mech Ageing Devl* **25**: 1–22, 1984.
40. Kitigawa H, Fujita S, Suzuki T and Kitani K, Disappearance of sex difference in rat liver drug metabolism in old age. *Biochem Pharmacol* **34**: 579–581, 1985.
41. Kamataki T, Maeda K, Shimada M, Kitani K, Nagai T and Kato R, Age-related alteration in the activities of drug-metabolizing enzymes and contents of sex-specific forms of cytochrome P-450 in liver microsomes from male and female rats. *J Pharmacol Exp Ther* **233**: 222–227, 1985.
42. Borghoff SJ and Birnbaum LS, Age-related changes in glucuronidation and deglucuronidation in liver, small intestine, lung, and kidney of male Fischer rats. *Drug Metab Dispos* **13**: 62–67, 1985.
43. Hedrich HJ, Von Deimling O and Kluge R, Biochemical genetics of *ES-14* (formerly *ES-Si*) and a new esterase variation, *Es-15*, of the laboratory rat (*Rattus norvegicus*): biochemistry, tissue expression and linkage to *Es-1* in linkage group V. *Biochem Gen* **25**: 79–93, 1987.
44. Mentlein R, Ronai A, Robbi M, Heymann E and Von Deimling O, Genetic identification of rat liver carboxylesterases isolated in different laboratories. *Biochem Biophys Acta* **913**: 27–38, 1987.
45. Mentlein R and Heymann E, Hydrolysis of ester- and amide-type drugs by the purified isoenzymes of non-specific carboxylesterase from rat liver. *Biochem Pharmacol* **33**: 1243–1248, 1984.
46. Guengerich FP, Effects of nutritive factors on metabolic processes involving bioactivation and detoxication of chemicals. *Ann Rev Nutr* **4**: 207–231, 1984.