GENETICS OF MURINE LIVER AND KIDNEY ARYLSULFATASE B[1]

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ABSTRACT

Mice from 12 inbred strains were surveyed for variation of kidney and liver arylsulfatase levels. Kidney variation was due to differences in the activity of arylsulfatase B. Twofold higher activities of arylsulfatase B in SWR/J kidney compared to A/HeJ kidney were determined by an autosomal gene which may be identical to the structural gene for arylsulfatase B since the SWR/J enzyme was more heat-stable than the A/HeJ enzyme. C57BL/6J mice possessed twofold higher liver arylsulfatase levels than did A/HeJ mice. The major portion of this variation could be attributed to differences in arylsulfatase B, and appeared to be inherited in autosomal fashion. Although some evidence supports the existence of a major locus influencing liver arylsulfatase activity, this must be substantiated by further studies. Whatever the nature of the genetic factors involved, they do not appear to involve structural genes since no differences were discernible between the enzymes of the two strains relevant to K_m, heat stability, electrophoretic mobility, pH optimum, activation energy, or response to several inhibitors. Furthermore, the rank ordering of strains on the basis of kidney arylsulfatase activity differed markedly from that which pertained to liver activity. Kidney arylsulfatase levels, but not brain or liver arylsulfatase activities, appear subject to androgenic influences.

Genetics of Murine Arylsulfatase B

MURINE inbred strains have been utilized by several investigators for the study of the genetic control of quantitative enzyme variation in mammalian systems. Catalase appears to be subject to one genetic factor determining its catalytic activity and to a second genetic factor controlling its rate of degradation (GANSCHOW and SCHIMKE 1969). The former locus was expressed in both liver and kidney cells, while the expression of the locus controlling the rate of catalase degradation was limited to hepatic cells. Murine β-glucuronidase is also under the control of two loci. The structural locus (*Gus*) has been located on chromosome 5 (PAIGEN and NOELL 1961; SIDMAN and GREEN 1965), and a second locus (*Eg*) which regulates the incorporation of β-glucuronidase into microsomes has been linked to the *Es-1* locus on chromosome *8* (KARL and CHAPMAN 1974). Androgens are believed responsible for higher β-glucuronidase activities in males (FISHMAN and FARMELANT 1953). Regulation of β-galactosidase may be effected by a gene closely linked to the β-galactosidase structural gene (*Bgs*) which is

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located on chromosome *9* (FELTON, MEISLER and PAIGEN 1974). No structural differences have been observed in the enzymes of high and low activity strains. Control of red cell glucose 6-phosphate dehydrogenase also appears to be complex. HUTTON (1971) has detected at least two autosomal loci which influence its activity. One of these loci may act by regulating NADP levels in erythrocytes (ERICKSON 1974). In an attempt to further delineate the nature of the genetic control of quantitative enzyme variation in mammalian systems, the arylsulfatases (arylsulfate sulfohydrolase, EC 3.1.6.1) of murine liver, brain and kidney were selected as a possible model system.

Arylsulfatases appear to be involved in the metabolism of sulfatides and mucopolysaccharides, and deficiencies of one or more species of enzyme appear to be associated with metachromatic leukodystrophy (AUSTIN, ARMSTRONG and SHEARER 1965) and its variant disease states in man (MURPHY *et al.* 1971). Arylsulfatases have been classified on the basis of their activity toward artificial substrates, intracellular location, sensitivity to inhibitors, and their optimal pH ranges (DODGSON and SPENCER 1957). Type I arylsulfatases (arylsulfatase C) are preferentially active toward simpler substrates such as p-nitrophenylsulfate, while Type II arylsulfatases (arylsulfatases A and B) exhibit highest affinity for more complex arylsulfates such as p-nitrocatecholsulfate. The Type I enzymes are located in the microsomal fraction, whereas the Type II enzymes are contained primarily in the lysosomal fraction. ROWDEN (1967) reported a heterogenous distribution of arylsulfatases A and B among murine liver and kidney lysosomes. Arylsulfatase A possesses a more acidic pH optimum than arylsulfatase B and tends to be less inhibited by chloride ion. Both arylsulfatases A and B are strongly inhibited by phosphate; however, arylsulfatase C is minimally affected by phosphate. The relative activities of arylsulfatase A and B differ among species, with birds having predominantly arylsulfatase A (FAROOQUI and BACHHAWAT 1971) and the rat, mouse and man possessing a considerable excess of arylsulfatase B (ROY 1958). The organ distribution of the two enzymes also varies. The ratios of arylsulfatase B to arylsulfatase A were 33:1, 20:1 and 12:1 for rat kidney, liver and brain, respectively (HOOK *et al.* 1973).

Relatively little is known regarding genetic determination of normal quantitative variation of arylsulfatase activities. Genetic and biochemical studies of arylsulfatase variation among inbred mouse lines are described in the present report.

MATERIALS AND METHODS

Mouse strains: BALB/c mice were purchased from Carworth, New City, Rockland County, New York. The remainder of the inbred lines selected for this study were obtained from Jackson Laboratories, Bar Harbor, Maine. The mice were fed commercial laboratory chow (23% protein and 4.5% fat for white strains) or mouse breeder blox (20% protein and 10% fat for the remaining strains, F_1, F_2, and backcross progeny). The animals were maintained for a minimum of three weeks on these diet regimens and a 12-hr light/12-hr dark cycle.

Extraction methods: Adult mice, 60–90 days old, were killed by cervical dislocation, and 1/15 aqueous hemogenates (w/v) of kidney, brain, and liver tissues were prepared with a glass-teflon homogenizer. The homogenates were incubated for 20 min at 4° and centrifuged at 20,000 $\times$ g for 10 min. The supernatants were diluted 1 to 15 with distilled water prior to assay.

Enzyme assay: 0.5 ml of diluted supernatant was added to 1.5 ml of substrate (0.01 M p-nitrocatechol sulfate in 0.5 M sodium acetate-acetic acid buffer, pH 5.9) and incubated for 1 hr at 37°. The reaction was terminated with 1.5 ml of 1 N NaOH, and the optical density was measured at 515 nm against a zero time blank.

These procedures were followed for extraction and assay of the arylsulfatases from parental strains, F_1, F_2, and backcross animals.

Mixing experiments: 0.25 ml of diluted supernatant from liver or kidney of one strain was mixed with an equal volume of diluted supernatant from the corresponding tissue of the second strain and assayed as described above. Activities of the mixtures were compared with those of the unmixed strain preparations to determine the presence of inhibitors.

Centrifugation studies: 1/15 (w/v) homogenates were prepared as described above and centrifuged at 20,000 $\times$ g for 10 min. An aliquot of the supernatant was withdrawn for assay, and the remainder was centrifuged at 100,000 $\times$ g for 60 min. Activities of the 20,000 $\times$ g and 100,000 $\times$ g supernatants were compared to evaluate contribution of membrane-bound enzyme species to the total arylsulfatase activity.

Separation of arylsulfatases A, B and C: 20% (w/v) homogenates were prepared as described above and were centrifuged at 20,000 $\times$ g for 10 min. Five ml of supernatant were applied to a 60 $\times$ 2.5 cm column of Sephadex G-200 equilibrated with elution buffer and maintained at 4°. The proteins were eluted with a Tris-maleate buffer (0.01 M Tris, 0.01 M maleate, 0.001 M EDTA, 0.1 M KCl, 0.1% sodium azide; pH 7.4). Elution was downward at a flow rate of 9.6 ml/hr, and the fraction volume was 4 ml. Blue Dextran 2000, bovine serum albumin, bovine heart lactate dehydrogenase, and bovine chymotrypsinogen were used as markers for molecular weight estimates. The arylsulfatases were located by assaying at pH 5.9 using the assay described above. Under these conditions arylsulfatase C elutes with the void volume followed by arylsulfatase A and then arylsulfatase B.

Characterization of arylsulfatases A and B: Fractions from the portion of the arylsulfatase A peak proximal to the void peak were pooled, dialyzed for 18 hr against distilled water, and lyophilized. Fractions from the distal portion of the arylsulfatase B peak were treated similarly. Sufficient lyophilized material was suspended in the appropriate buffer to yield a protein concentration of approximately 0.15 mg/ml and processed as described below. Unless otherwise indicated, arylsulfatase B was assayed according to the procedure previously described. This procedure was modified for assay of arylsulfatase A by reducing the pH to 5.3 and shortening the incubation time to 30 min since the reaction was nonlinear for longer periods.

a) *Determination of pH optima*: The activities of arylsulfatases A and B were determined over the pH range 3.5 to 7.0 using 0.25 M citrate, 0.5 M acetate and 0.5 M imidazole-HCl buffers. The activities over the range of pH 5.0 to 7.0 were determined at closer intervals using 0.5 M acetate and 0.5 M imidazole-HCl buffers. Both enzyme and substrate were dissolved in buffer of appropriate constitutions and pH. The conditions of the assays were otherwise as described above.

b) *Effects of inorganic inhibitors*: Lyophilized enzyme extract was dissolved in either 0.5 M acetate buffer, pH 5.3 (arylsulfatase A), or 0.5 M acetate buffer, pH 5.9 (arylsulfatase B). Sulfate, sulfite, phosphate or chloride was incorporated in the substrate solution at concentrations of 0.0125 M, 0.001 M, 0.01 M and 0.2 M, respectively. The sodium salt of each inhibitor was used to minimize cationic effects.

c) *Heat inactivation studies*: Lyophilized material from the arylsulfatase A peak was suspended in 0.5 M acetate buffer, pH 5.3 as described above, and incubated at 65° for 0 to 120 min, cooled in ice, and assayed for residual activity. A similar procedure was used for arylsulfatase B with the exceptions that the pH of the acetate buffer was increased to 5.9, and the experiment was repeated with 0.5 M imidazole-HCl buffer, pH 5.9. In an additional experiment, sufficient lyophilized material from the arylsulfatase B peak was suspended in acetate buffer, pH 5.9, to yield a protein concentration of approximately 0.15 mg/ml. Equal volumes of suspension from each of two strains (BALB/c, A/HeJ, SWR/J or C57BL/6J) were then mixed and the above experiment was repeated. The heat inactivation profiles of the mixture were then compared with those of the parent fractions and with those of similar preparations derived from the respective F_1 hybrids.

d) *Temperature optima and apparent activation energies*: The temperature optima were determined for the two arysulfatase fractions by assaying over the temperature range: 27°–65°. The apparent activation energy was calculated over the range 30°–45°.

e) *Miscellaneous procedures*: The apparent Michaelis constants of the arylsulfatases were determined for the G-200 fractions prepared as described above. The substrate concentration was varied from 1 to 10 mM for arylsulfatase A and from 1 to 20 mM for arylsulfatase B.

Approximately 1 mg of protein from each fraction was applied to 7% (w/v) disc acrylamide gels 10 cm in length (DAVIS 1962). The proteins were electrophoresed approximately 90 min at 4° (5 mA/tube) using Tris-glycine buffer (0.005 M Tris, 0.038 M glycine; pH 8.3). The gels were sectioned at 5 mm intervals, and the enzyme from corresponding slices was extracted in 1 ml of either 0.5 M acetate buffer, pH 5.3 (arylsulfatase A), or 0.5 M acetate buffer, pH 5.9 (arylsulfate B), at 4° overnight. The respective enzyme activities were then determined by the procedures described above.

RESULTS

Biochemistry of murine arylsulfatases A and B: Sephadex G-200 gel filtration of C57BL/6J male liver extracts resolved arylsulfatase activity into three peaks. One eluted with the void volume and exhibited ninefold greater activity with p-nitrophenyl sulfate than with p-nitrocatechol sulfate. This peak was removed by centrifugation at 100,000 × g for 60 min prior to gel filtration. These properties suggest that this peak contained arylsulfatase C. The activity in the void peak accounted for approximately 3% of the total activity of the three peaks at pH 5.9.

The second peak of p-nitrocatechol sulfate arylsulfatase activity was associated with a protein with an apparent molecular weight of 143,000 ± 5,800 (Mean ± S.E.M.). This species of arylsulfatase exhibited maximal activity with p-nitrocatechol sulfate, had a temperature optimum of 45°, and possessed an apparent K_m of 6.2×10^{-4} M. The enzyme was inhibited by sulfate, sulfite, and phosphate, but not by 0.2 M chloride. Approximately 50% of its activity was lost after heating at 65° for 7 min. Its relative mobility with respect to bromphenol blue was 0.35. The optimal pH of this enzyme was 5.3, and it accounted for approximately 15% of the total activity at pH 5.9. These properties support the identification of the enzyme species in the second peak as arylsulfatase A.

The enzyme present in the third peak was relatively more heat-stable, exhibiting a $t_{1/2}$ at 65° of 30 min in 0.5 M acetate buffer, pH 5.9. The enzyme had an apparent molecular weight of 50,000 ± 2,500. Its relative electrophoretic mobility was 0.25 with respect to bromphenol blue. The apparent K_m was 1.06×10^{-3} M, and its pH otpimum was 5.9. The optimal temperature was observed to be 55°. This species of arylsulfatase was inhibited by sulfate, sulfite, and phosphate. In contrast to arylsulfatase A, this enzyme was moderately inhibited by 0.2 M chloride. These properties implicate the enzyme in peak three as arylsulfatase B. Approximately 82% of the total activity at pH 5.9 was attributable to arylsulfatase B.

Comparison of the G-200 elution profiles of C57BL/6J male liver and kidney extracts revealed much higher levels of arylsulfatase B activity in kidney. Male mice had 35% higher levels of arylsulfatase activity in kidney extracts compared to those of females; however, no significant sex differences were noted for the

TABLE 1

Kidney and liver arylsulfatase activities of selected male inbred mice

Kidney strain	Activity*	$t_{1/2}$	Liver strain	Activity	$t_{1/2}$
RF/J	595±8(5)	60	C57BL/6J	319±29(5)	30
BALB/c	588±17(5)	60	RF/J	233±19(5)	60
SWR/J	567±25(5)	60	SWR/J	180±12(5)	60
C3H/HeJ	461±15(5)	62	C57Br/cdJ	173±4(5)	30
DBA/2J	400±16(5)	55	C57L/J	165±15(5)	30
AKR/J	399±7(5)	54	BALB/c	137±8(5)	60
C57BL/6J	365±20(5)	30	DBA/2J	127±4(5)	50
C57Br/cdJ	352±16(5)	30	LG/J	120±6(5)	30
MA/MYJ	346±10(5)	60	AKR/J	120±7(5)	54
C57L/J	289±12(4)	30	A/HeJ	117±5(5)	30
A/HeJ	270±8(5)	30	MA/MYJ	106±5(5)	60
LG/J	206±3(5)	30	C3H/HeJ	101±8(5)	62
SWR/J mean *vs.* C57BL/6J mean***			SWR/J mean *vs.* A/HeJ mean†		
SWR/J mean *vs.* A/HeJ mean***			C57BL/6J mean *vs.* A/HeJ mean***		
C57BL/6J mean *vs.* A/HeJ mean***			C57BL/6J mean *vs.* SWR/J mean***		

* The activities are expressed as μmol/g wet wt/hr and have been corrected for strain differences in tissue protein (see text). $\bar{X} \pm$ SE(n).
† $0.005 > p > 0.001$. $t_{1/2}$ (mean of two experiments) refers to the time in minutes at 65° required to abolish one-half of the initial arylsulfatase B activity.
*** $p < 0.001$. Duncan's Multiple Range test of log-transformed data.

enzyme activities of brain or liver. The arylsulfatases of brain, liver and kidney appeared to have similar biochemical properties.

Strain differences in kidney arylsulfatase activity: Considerable variation of mean kidney arylsulfatase activity levels was observed among 12 murine strains, with more than a twofold difference between the mean activities of the highest and lowest strains (Table 1). The data for male mice, only, are shown. Trends for female mice from the 12 strains were similar to those of males, although the kidney extracts from females uniformly contained less arylsulfatase activity than those of males. The means have been corrected for strain differences in protein content by using the formula of FELTON, MEISLER and PAIGEN (1974) in which the activity per gram wet weight is divided by:

$$\frac{\text{protein concentration of the specific strain}}{\text{average protein concentration of all strains}}$$

Two strains, SWR/J and A/HeJ, were selected as representative of the high and low activity groups, respectively, and were used for genetic analyses of the kidney arylsulfatase variation.

F_1 progeny derived from SWR/J × A/HeJ matings possessed kidney arylsulfatase activities that were intermediate between those of the two parental strains (Figure 1). No differences were observed between reciprocal crosses. Backcrosses of F_1 offspring to the SWR/J strain resulted in a bimodal distribution. The mean

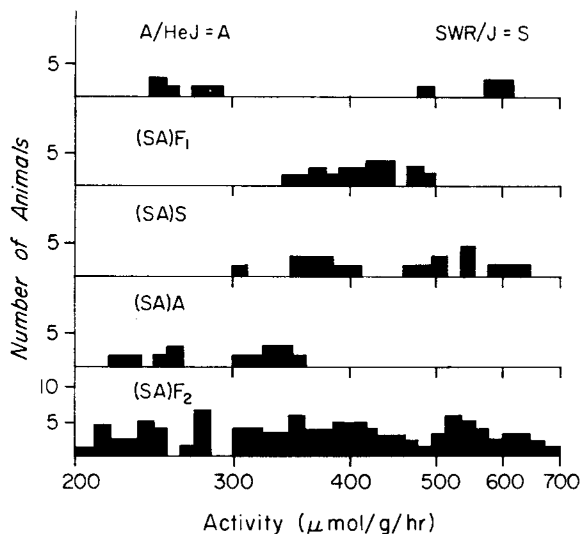

FIGURE 1.—Distribution of kidney arysulfatase activity among SWR/J, A/HeJ, F_1, backcross, and F_2 males. The designation "SA" refers to hybrids from both SWR/J × A/HeJ and from A/HeJ × SWR/J crosses. Although reciprocal crosses were studied in all cases, no significant differences were noticed between the means. The activities are entered on a logarithmic scale.

activity of the high group approximated that of the SWR/J parent strain, while the mean of the second group was similar to that of the hybrid. The backcross progeny of the F_1 × A/HeJ matings were also distributed among two groups—one approximating the F_1 hybrid mean and the other being similar to that of the A/HeJ parental strain. F_2 offspring fell into a trimodal distribution. Statistical analyses of the activity distributions among male progeny of the F_2 and backcrosses are presented in Table 2. The data are compatible with a single locus determining a major portion of the observed variability between these two strains.

Liver arylsulfatase activities also exhibited considerable interstrain variation (Table 1). The mean activities for the 12 strains have been corrected for tissue protein variation as described above. Arylsulfatase activities of female liver extracts closely approximated those of male liver extracts. High activity strains exhibited twofold greater arylsulfatase activities compared to those of low activity strains. C57BL/6J and A/HeJ strains were selected as representative of

TABLE 2

Distribution of male kidney arylsulfatase activities of SWR/J × A/HeJ crosses

Cross	Low <300	Intermediate	High >485	χ^2	P
F_2	24	52	29	0.48	$0.75<p<0.90$
F_1 × SWR/J		9	10	0.05	$0.75<p<0.90$
F_1 × A/HeJ	5	7		0.33	$0.50<p<0.75$

The χ^2 was calculated on the basis of a single locus controlling enzyme activity.

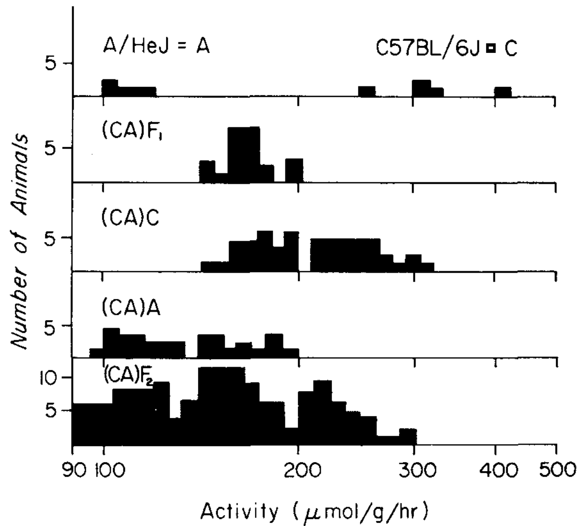

FIGURE 2.—Distribution of liver arylsulfatase activity among C57BL/6J, A/HeJ, F_1, backcross and F_2 males. The designation "CA" refers to hybrids from both C57BL/6J × A/HeJ and from A/HeJ × C57BL/6J crosses. Although reciprocal crosses were made in all cases, no significant differences were noticed between the means. The activities are entered on a logarithmic scale.

the high and low activity strains, respectively, and were used for genetic studies of liver arylsulfatase variation.

F_1 hybrids derived from C57BL/6J × A/HeJ crosses exhibited intermediate levels of liver arylsulfatase activity compared to those of the parental strains (Figure 2). No differences in mean arylsulfatase activity were apparent between progeny of reciprocal crosses. Backcrosses of F_1 offspring to either parental strain resulted in a bimodal distribution of arylsulfatase activity among the progeny in each case. The mean activity of one group resembled that of the hybrids, while that of the other approximated the respective parental strain mean. The means of the high activity groups of progeny from C57BL/6J × F_1 and F_2 crosses were significantly lower than that of the C57BL/6J parental strain. The reason for this is not readily apparent. The distribution of the F_2 progeny appeared trimodal. The data are grouped into high, intermediate and low activity classes in Table 3. Although the distribution of backcross animals among the respective

TABLE 3

Distribution of male liver arylsulfatase activities of C57BL/6J × A/HeJ crosses

Cross	Low <140	Intermediate	High >200	χ^2	P
F_2	60	69	29	14.69	<0.001
F_1 × C57BL/6J		28	31	0.15	$0.50<p<0.75$
F_1 × A/HeJ	20	14		1.06	$0.25<p<0.50$

The χ^2 was calculated on the basis of a single locus controlling enzyme activity.

activity classes is compatible with a single-locus model, the F_2 ratios do not readily fit such a model due to an excess of animals in the low activity class.

Brain arylsulfatase activity levels varied less extensively among the 12 strains than either kidney or liver activities (Table 4). No sex differences were observed for brain arylsulfatase activity. Since the highest and lowest activity strains differed by less than a factor of two, genetic studies of brain arylsulfatase were not conducted.

Biochemical basis for liver and kidney arylsulfatase variation: The majority of kidney arylsulfatase activity in each of the three strains (SWR/J, A/HeJ, and C57BL/6J) at pH 5.9 was contributed by arylsulfatase B. The ratios of arylsulfatase B to arylsulfatase A activity were approximately 8:1, 14:1 and 20:1 for A/HeJ, C57BL/6J and SWR/J males, respectively. Preliminary studies indicate the ratio of arylsulfatase B activity to arylsulfatase A activity in BALB/c male kidney extracts is also approximately 20:1.

Heat denaturation at 65° separated the three strains into two groups. Kidneys from A/HeJ and C57BL/6J mice appear to contain a more heat-labile arylsulfatase B (Figure 3) than kidneys from SWR/J mice. The $t_{1/2}$ (incubation time at which one-half of the enzyme activity is lost) at 65° for A/HeJ and C57BL/6J mice approximated 30 min, while the $t_{1/2}$ for SWR/J mice was about 60 min. BALB/c arsylsulfatase B resembled that of SWR/J with respect to its heat denaturation properties. Heat denaturation profiles of mixtures of arylsulfatase B isolated from the pure strains are presented in Figure 4a. The profiles confirm the existence of two forms of arylsulfatase B. The denaturation profiles of the F_1 hybrids resembled those of the artificial mixtures of the enzymes derived from the parental strains (Figure 4b). Comparisons of the biochemical properties of kidney arylsulfatase B derived from all four strains are summarized in Table 5.

TABLE 4

Brain arysulfatase activities of selected male inbred mice

Strain	Activity*
MA/MYJ	229±5(5)
RF/J	219±5(5)
SWR/J	194±6(5)
BALB/c	187±6(5)
C57BL/6J	176±7(5)
AKR/J	175±5(5)
DBA/2J	169±5(5)
C3H/HeJ	168±1(5)
C57Br/cdJ	164±4(5)
C57L/J	164±4(5)
LG/J	157±2(5)
A/HeJ	149±5(5)

* The activities are expressed as μmol/g wet wt./hr. ($\overline{X}$ ± SE(n)). The activities have been corrected for differences in tissue protein (see text).

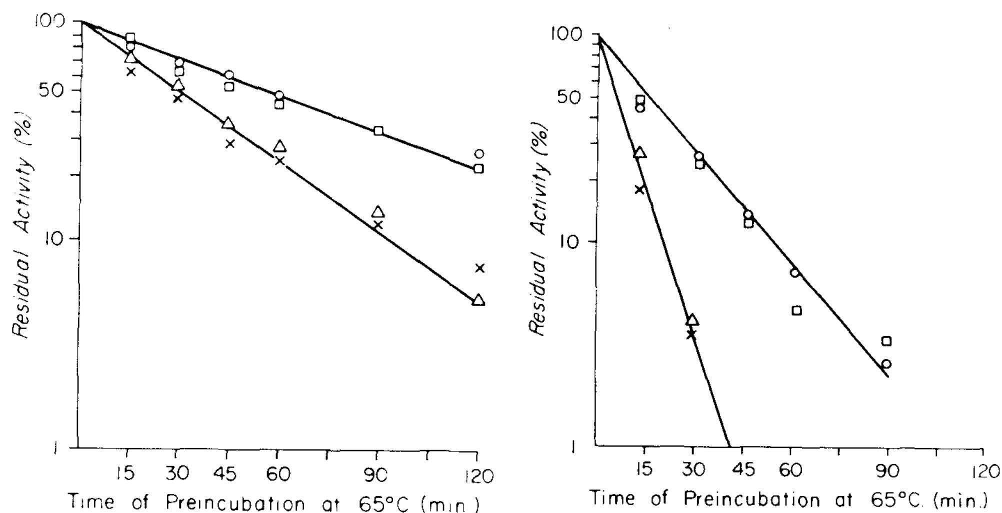

FIGURE 3.—Heat inactivation profiles of kidney arylsulfatase B (mean of two experiments per strain). (a) 0.5 M Acetate buffer, pH 5.9; (b) 0.5 M Imidazole-HCl buffer, pH 5.9. □ = SWR/J, ○ = BALB/c, △ = C57BL/6J, and × = A/HeJ.

With the exception of those properties related to the response of the enzymes to temperature, the enzymes appear to be quite similar.

Liver p-nitrocatechol-SO_4-arylsulfatases differ from those of kidney due to a greater proportion of arylsulfatase A. Table 6 presents the ratios of arylsulfatase B to arylsulfatase A for liver extracts from each of the four strains. Each species of arylsulfatase was assayed under optimal conditions (see MATERIALS AND

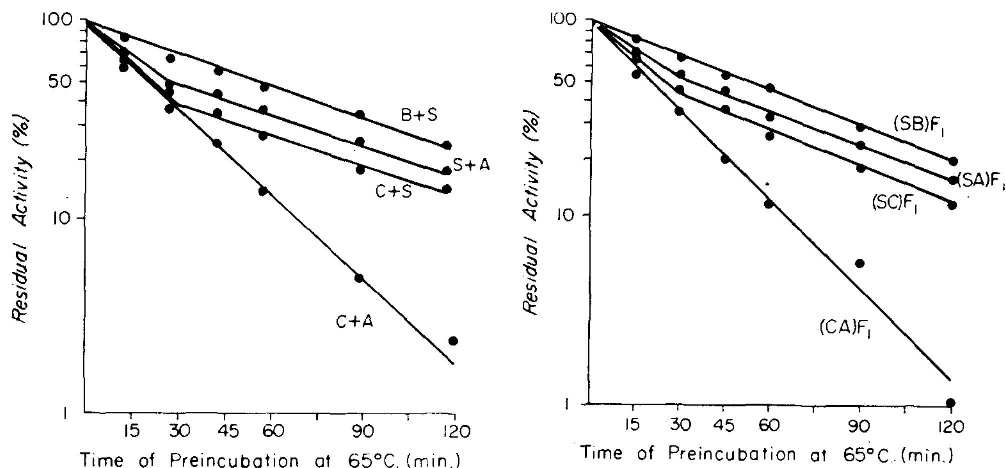

FIGURE 4.—Heat inactivation profiles for mixtures of parental arylsulfatase B preparations (a) and F_1 hybrids (b). Both incubation and assay of the enzyme were performed in 0.5 M Acetate buffer, pH 5.9. The points on each profile refer to the mean of two experiments. SB = SWR/J × BALB/c, SA = SWR/J × A/HeJ, SC = SWR/J × C57BL/6J, and CA = C57BL/6J × A/HeJ.

TABLE 5

Biochemical comparison of arylsulfatase B from kidney extracts of four inbred lines

	BALB/c SWR/J	A/HeJ C57BL/6J
Molecular weight	50,000	50,000
pH optimum	5.9	5.9
Temperature optimum	60°	55°
$t_{1/2}$ at 65°	60 min	30 min
Activation energy	12,840 cal mol^{-1}	10,670 cal mol^{-1}
Electrophoretic mobility	0.25	0.25
K_m	1.00×10^{-3} M	1.10×10^{-3} M
Inhibitor profile:		
(Percent inhibition)		
0.0125 M $SO_4^=$	21	22
0.001 M $SO_3^=$	97	92
0.001 M $PO_4^=$	61	56
0.2 M Cl^-	16	14

Each experiment was performed at least twice with two replicates per experiment.

METHODS) for that species. The specific activities have been corrected for slight differences in protein in specific fractions of the four strains by dividing the specific activity by:

$$\frac{\text{mean mg protein for that strain in fraction}}{\text{mean mg protein for all four strains in that fraction}}.$$

As the total activity per gram liver declined (see Table 1), the ratio of arylsulfatase B/arylsulfatase A also declined, suggesting that differences in arylsulfatase B activities were primarily responsible for the strain variation in arylsulfatase activity. Furthermore, heat denaturation studies at 65° demonstrated the presence of a heat-labile enzyme in A/HeJ and C57BL/6J liver extract, and a heat-stable enzyme in SWR/J and BALB/c liver extracts. The heat denaturation profiles of F_1 hybrids derived from SWR/J and either A/HeJ or C57BL/6J mice were biphasic, with one phase of the curve corresponding to the profile of the SWR/J enzyme, and the other phase paralleling the A/HeJ or C57BL/6J

TABLE 6

Comparison of male liver arylsulfatases from four inbred strains

Strain	Arylsulfatase B/Arylsulfatase A*
C57BL/6J	1.86
SWR/J	1.28
BALB/c	1.24
A/HeJ	0.77

* The ratios were calculated from the specific activities of the arylsulfatase fractions obtained following G-200 gel filtration. The specific activities were corrected for slight differences in protein content (see text). Each experiment was performed at least twice.

denaturation curve. Similar results were also obtained for hybrids derived from BALB/c and A/HeJ or C57BL/6J mice.

Comparison of arylsulfatase activities of 20,000 × g supernatants with those of 100,000 × g supernatants failed to demonstrate appreciable differences between strains. The mean (two experiments) percent recovery of 20,000 × g supernatant activity in the 100,000 × g supernatants from SWR/J, C57BL/6J and A/HeJ male kidneys were 88.6, 77.6 and 87.4, respectively. The corresponding recoveries for liver from SWR/J, C57BL/6J and A/HeJ males were 92.8, 77.2 and 87.7, respectively. The percent recovereies for BALB/c kidney and liver were 83.4 and 94.6, respectively. The slight interstrain differences that were observed do not appear sufficient to account for the strain variation in arylsulfatase activity observed if binding to membranes is responsible for differential susceptibility to degradation or some other factor that would influence activity levels.

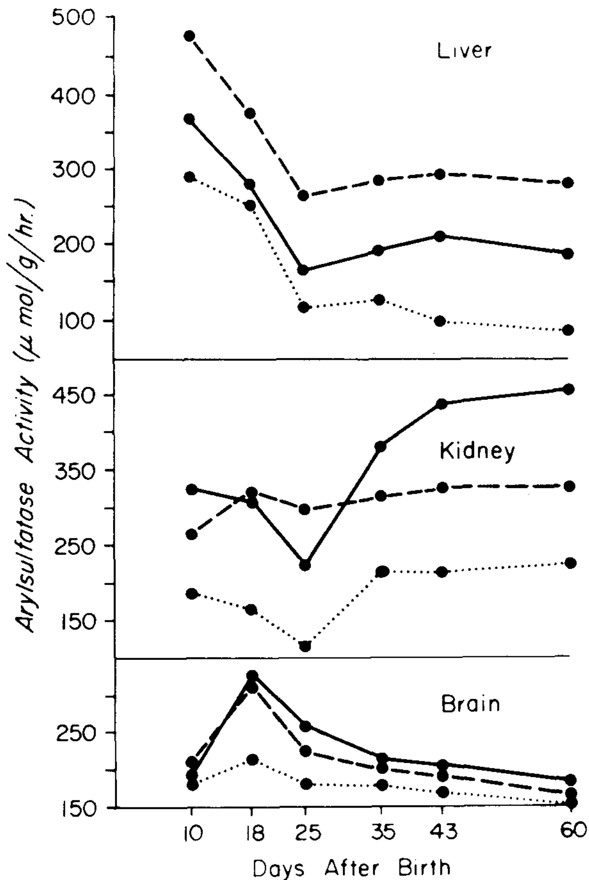

FIGURE 5.—Developmental profiles of arylsulfatase activities of liver, kidney, and brain. Activities have been corrected for stage-specific differences in protein content (see text). Activities at 10 and 18 days were determined on pooled samples consisting of three animals per pool. Points for older ages represent the means for 3–5 animals. Data for males only are presented. SWR/J (——), C57BL/6J (- - -), A/HeJ (. . . .).

The strain differences observed for kidney and liver arylsulfatase B were not affected by dialysis of the 20,000 × g supernatant against distilled water for 18 hr at 4°. Furthermore, mixtures of liver or kidney supernatants from high and low activity strains contained intermediate activities. These experiments tend to rule out the existence of dialyzable or nondialyzable inhibitors in low activity strains.

Developmental changes in arylsulfatase activity: Figure 5 illustrates the developmental profiles for arylsulfatase activities of brain, kidney and liver of SWR/J, C57BL/6J and A/HeJ males. The 10- and 18-day points represent the means of three pools consisting of three animals per pool. The points for older ages represent the mean of three animals. The activities have been corrected for age-related changes in organ protein content by dividing the activity per gram wet weight by:

$$\frac{\text{protein (mg/g) at a specific age}}{\text{average protein (mg/g) for all ages}}.$$

The data points for the 60–90-day interval differ from those listed in Table 1 by this factor. Brain arylsulfatase activities peaked at 18 days after birth and then steadily declined to adult levels which were reached at 60–90 days of age. Kidney activities started high, declined to a low at approximately 24–26 days, and then increased to adult levels which were attained about 44–46 days of age. Divergence of male and female kidney activities occurred after 26 days and before 34 days. Although male kidneys contained more protein per gram then female kidneys, correction for the protein difference did not equalize the mean arylsulfatase levels of the two sexes. The highest liver arylsulfatase activities were observed in the supernatants of 10-day-old mice. The activity levels declined to a low at 24–26 days and then increased to adult levels by 34–36 days. No significant differences were observed among the developmental profiles of SWR/J, C57BL/6J and A/HeJ mice.

DISCUSSION

The interstrain variation of kidney p-nitrocatechol arylsulfatase activity is largely due to differences in the activity of arylsulfatase B since the ratio of arylsulfatase B to arylsulfatase A increased in proportion to the increase in total activity. SWR/J males have twofold greater activities than A/HeJ males. The segregation of arylsulfatase activities in the F_1, backcross and F_2 progeny indicates that a single locus plays a major role in determining the level of arylsulfatase B in kidney supernatants derived from these two strains. Lack of significant differences in mean arylsulfatase activity between the respective F_1 hybrids derived from reciprocal parental crosses indicates that the locus is autosomal.

Arylsulfatase B from SWR/J mice has a higher temperature optimum, a somewhat larger activation energy, and is more heat-stable than the corresponding enzyme from A/HeJ mice. On the basis of these observations, the strain difference in arylsulfatase B activity could be attributed to a structural mutation, although none of the other properties of the SWR/J and A/HeJ enzyme tested

appear to differ. Inspection of the $t_{1/2}$ values for the kidney enzymes listed in Table 1 reveals that the strains possessing the higher arylsulfatase activities generally contain the more stable enzyme while the converse is generally true for lower activity strains. Such a trend is supportive for the participation of a structural locus in the determination of a major portion of the observed interstrain variability. Additional factors must also be involved since C57BL/6J males have 35% higher enzyme levels than A/HeJ mice despite the presence of apparently structurally similar enzymes. Experiments comparing the segregation of activity with that of heat stability among backcross and F_2 progeny are currently in progress. These data supplemented with linkage studies should provide definitive evidence for the presence or absence of the participation of a structural locus in the determination of kidney arylsulfatase levels.

The inheritance of liver arysulfatase activities appears to be different from that responsible for kidney arylsulfatase levels. There is a general lack of agreement between the rank orders of the 12 strains based upon activities in the two organs. Arysulfatase B appears to be primarily involved in the differences observed in arylsulfatase activities between C57BL/6J and A/HeJ males. The ratio of arylsulfatase B to arylsulfatase A in C57BL/6J male liver was approximately 2:1, while that for A/HeJ male liver was less than one. Although the segregation of arylsulfatase activities among progeny of (C57BL/6J $\times$ F_1) and (A/HeJ $\times$ F_1) backcrosses appears bimodal, the separation of the modes is not as great as observed for the kidney enzymes. Furthermore, there was a lack of agreement of the F_2 data with the 1:2:1 distribution expected on the basis of a single-locus model. A portion of the difficulty stems from the presence of much higher levels of arylsulfatase A in liver tissue. Subtle variation of this enzyme may be responsible for the poor segregation of activity levels observed since the arylsulfatase A present in the unfractionated supernatants employed for the genetic studies retains about 40% of its activity at pH 5.9. Preliminary studies in this laboratory indicate that it may be possible to inactivate arylsulfatase A by heating at a temperature that does not denature arylsulfatase B. Such pretreatment will permit a more accurate measurement of arylsulfatase B levels in relatively crude preparations and should provide more definitive data pertinent to the segregation of arylsulfatase B activities among progeny of informative genetic crosses.

C57BL/6J mice have higher liver arylsulatase activities than A/HeJ mice, although no apparent evidence for structural differences between liver arylsulfatase B from the two strains was discernible. Heat sensitivity has proven to be one of the most sensitive means for detecting structurally altered enzymes (BERNSTEIN, THROCKMORTON and HUBBY 1973). Comparison of the stabilities of liver arylsulfatase B derived from the 12 inbred strains does not reveal any apparent relationship between activity level and heat stability of the enzyme. This lack of agreement of activity with enzyme stability coupled with the segregation of activity levels among progeny of genetic crosses of two strains (C57BL/6J and A/HeJ) which have structurally similar enzymes makes it unlikely that the observed variation of liver arylsulfatase activity is due to a

structural gene mutation. Experiments are in progress which include differential heating to remove arylsulfatase A followed by a protocol outlined above with reference to the kidney enzyme that should provide data that will resolve this problem.

The strain differences observed for kidney and liver arylsulfatase activities were apparent 10 days after birth, with liver activities exceeding those of both brain and kidney at this stage. The brain enzyme peaked at 18 days after birth, a time of intensive myelination in the mouse. Kidney arylsulfatase levels markedly increase in males but not in females between 26 and 34 days after birth. The increased kidney activity most likely reflects the effects of androgens and is similar to the response of other enzymes such as kidney alcohol dehydrogenase and β-glucuronidase to androgen stimulation. The response does not appear to be due to a generalized increase in protein synthesis at this time since correction for protein differences between male and female kidney protein did not reduce the male activity to female levels. Experiments are now in progress to determine the species of enzyme responsible for the developmental fluctuations observed in the three tissues.

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