GENETIC ANALYSIS OF MURINE ARYLSULFATASE C AND STEROID SULFATASE

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ABSTRACT

SWR/J mice possess two- to threefold higher 4-methylumbelliferyl sulfate (4MUS), dehydroepiandrosterone sulfate (DHEAS) and estrone sulfate (E_1S) sulfatase activities in liver and kidney extracts than do A/J mice. These interstrain activity differences are maintained throughout the 6- to 45-day postnatal period. Characteristics of the hepatic activities of SWR/J mice suggest that all three activities reside in the same enzyme. Biochemical properties of the SWR/J and A/J enzyme were not significantly different. Expression of hepatic enzyme activity is subject to regulation by an autosomal locus possessing two alleles with additive effects. Postnuclear E_1S- and DHEAS-sulfatase activities are primarily microsomal. Although postnuclear hepatic 4MUS-sulfatase activity is predominantly microsomal, renal activity is primarily nonmicrosomal. Only that portion of 4MUS-sulfatase occurring in cell membranes appears capable of hydrolyzing E_1S and DHEAS. The hepatic- and renal-specific subcellular distributions of 4MUS-sulfatase activity may reflect tissue differences in enzyme processing. Renal 4MUS-sulfatase activity is also controlled by an autosomal gene with two alleles having additive effects. Positive correlation between hepatic and renal 4MUS-sulfatase activities indicates that both activities are most likely influenced by the same gene.

ARYLSULFATASE C (arylsulfate sulfohydrolase, EC 3.1.6.1) has been detected in a wide variety of organisms and tissues. The mammalian enzyme has an alkaline pH optimum, is resistant to inhibition by phosphate and displays activity toward a variety of synthetic substrates including *p*-acetylphenyl sulfate, *p*-nitrophenyl sulfate (pNPS) and 4-methylumbelliferyl sulfate (4MUS). The enzyme is largely associated with the microsomal fraction of rat liver, kidney and spleen (THOMAS and ROSE 1976); however, the major portion of the brain activity was recovered in the nuclear and mitochondrial/lysosomal fractions (PERUMAL and ROBINS 1973). Arylsulfatase C shares several properties with mammalian steroid sulfatase (EC 3.1.6.2); however, the question of whether arylsulfatase C activity and the respective steroid sulfatase activities reside in the same enzyme or two or more closely related enzymes remains unanswered (DOLLY, DODGSON and ROSE 1972; IWAMORI, MOSER and KISHIMOTO 1976). Steroid sulfatase may participate in the release of active steroids from their

stored sulfates in liver and placenta (HOLLER *et al.* 1977) and may play a role in the capacitation of sperm (LANGLAIS *et al.* 1981).

The genetics of mammalian arylsulfatase C and steroid sulfatase has not been fully elucidated. Arylsulfatase C and several steroid sulfatase activities are deficient in *X*-linked human placental estrogen deficiency (FRANCE and LIGGINS 1969) and in boys with *X*-linked icthyosis who are products of these pregnancies (SHAPIRO *et al.* 1978). The locus responsible for these sulfatase deficiencies has been mapped to the short arm of the *X* chromosome near the Xg^a blood group locus (MULLER *et al.* 1980). Arylsulfatase C and steroid sulfatase activities of women are approximately 50% higher than those of men, and recent studies have demonstrated partial expression of the allele situated on the inactivated *X* chromosome of women (MIGEON *et al.* 1982). Steroid sulfatase activities of wood lemmings (*Myopus schisticolor*) are also influenced by an *X*-linked locus, and female steroid sulfatase activities exceed those of males (ROPERS and WIBERG 1982). Although the *X*-linked locus affecting steroid sulfatase activity in man and wood lemming may be a structural locus (or cluster of closely linked structural loci), some evidence suggests that the *X*-linked locus may affect the membranous environment of steroid sulfatase (MCNAUGHT and FRANCE 1980), secondarily influencing its catalytic function.

Murine arylsulfatase C (4MUS-sulfatase) activity does not appear to differ significantly between sexes (NELSON 1979), and F_1 male progeny derived from reciprocal crosses of SWR/J (high activity) and A/J (low activity) mice possess similar hepatic and renal 4MUS-sulfatase activities, respectively (K. NELSON, B. M. KEINANEN and W. L. DANIEL, unpublished results). R. P. ERICKSON, K. HARPER and J. M. KRAMER (unpublished data) have recently described an autosomal gene that affects the expression of murine testicular and liver dehydroepiandrosterone sulfate (DHEAS) sulfatase activity. These observations suggest that one or more autosomal genes influence 4MUS-sulfatase and steroid sulfatase activities in murine tissues.

4MUS-sulfatase, estrone sulfate (E_1S) sulfatase and DHEAS-sulfatase activities are all two- to threefold higher in SWR/J liver compared with those of A/J mice. This report describes the developmental profiles of these three activities in murine tissues, compares the segregation of hepatic 4MUS-sulfatase, E_1S-sulfatase and DHEAS-sulfatase activities among backcross and F_2 progeny derived from SWR/J × A/J crosses and provides a preliminary biochemical characterization of the liver and kidney enzymes from these two inbred strains.

MATERIALS AND METHODS

Inbred mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Animals were maintained on a 12-hr light/12-hr dark cycle, commercial feed and water *ad libitum*. Animals were sacrificed by cervical dislocation at 45 ± 1 days unless otherwise specified. Tissues were excised, rinsed, weighed and frozen at −20° until use. Deterioration of enzyme activity was negligible for at least 1 yr at this temperature.

Enzyme extraction and assays: Homogenates, 10% (w/v), were prepared in 0.2 M sodium phosphate buffer, pH 8.6, containing 1% (v/v) Triton X-100. The homogenate was certrifuged at 20,000 × *g* for 30 min, and the supernatant was used as the source for arylsulfatase C. More than 85% of the homogenate activity was recovered in the supernatant, and no significant variation in enzyme recovery was observed among preparations from different inbred strains. Arylsulfatase C was assayed as previously described (NELSON and DANIEL 1979) using repurified 4MUS (RINDERKNECHT

et al. 1970) in the presence of 0.2 M phosphate and 1% Triton X-100. Brain, kidney and liver supernatants were diluted 1:8, 1:16 and 1:32, respectively, with assay buffer. Under these conditions, 4MUS-sulfatase activity is equivalent to arylsulfatase C activity. Extracts for assays of E_1S- and DHEAS-sulfatases were prepared as described earlier; however, a buffer containing 5 mM Tris, 2 mM EDTA, 7 mM mercaptoethanol, 0.5% (v/v) Triton X-100 and 0.1% (w/v) N_aN_3 (pH 8.0) was substituted for the phosphate/Triton buffer employed for extraction of 4MUS-sulfatase. Brain and kidney supernatants were assayed full strength; however, liver supernatants were diluted 1:10 or 1:2 prior to estimation of E_1S- and DHEAS-sulfatase activities, respectively. E_1S-sulfatase activity was determined using [6, 7-^{3}H(N)] E_1S ammonium salt (New England Nuclear, 59 Ci/mmol) as substrate (IWAMORI, MOSER and KISHIMOTO 1976). DHEAS-sulfatase activity was measured using ^{3}H-DHEAS ammonium salt (New England Nuclear; 22.1 Ci/mmol) as substrate (ROPERS *et al.* 1981). The labeled product was extracted directly into the scintillation cocktail. Zero time blanks were used for all assays, and an internal standard with known E_1S- and DHEAS-sulfatase activities was included with each assay series.

Developmental studies: SWR/J and A/J mice were sacrificed at 6-day intervals following birth. 4MUS-, E_1S- and DHEAS-sulfatase activities were estimated using enzyme extracted from duplicate pools containing brain, liver or kidney from three animals from each age group.

Genetic studies: F_1, backcross and F_2 progeny were obtained from an SWR/J × A/J cross using standard methods. Livers and kidneys were excised from 45-day-old animals, weighed and frozen. Liver from each animal was subdivided into 0.5-g pieces prior to freezing. Hepatic and renal 4MUS-sulfatase activities were determined as described previously. Joint segregation of 4MUS-, E_1S- and DHEAS-sulfatase activities among backcross and F_2 progeny was studied in a subset of animals previously tested for MUS-sulfatase activity by measuring E_1S- and DHEAS-sulfatase activities of duplicate liver slices. Possible influence of the *As-1* locus (putative structural locus for arylsulfatase B) upon 4MUS-, E_1S- and DHEAS-sulfatase activities was investigated using progeny derived from the F_1(SWR/J × A/J) × A/J backcross. As-1 phenotypes were determined by assaying renal arylsulfatase B (DANIEL, HARRISON and NELSON 1982). 4MUS-, E_1S- and DHEAS-sulfatase activities were then measured using liver from the same animals.

Biochemical characterization of arylsulfatase C and steroid sulfatases: Subcellular distribution of murine renal and hepatic sulfatases was investigated using the method of PERUMAL and ROBINS (1973). Hydrophobic chromatography of hepatic and renal sulfatases on phenyl-Sepharose CL-4B (Pharmacia) was conducted according to the method described by CARSON and KONIGSBERG (1981). Fresh tissue (0.5 g) was homogenized (10% w/v) in 0.05 M Tris-0.1 M NaCl-0.01% (w/v) NaN_3 buffer, pH 7.6, sonicated 15 sec and centrifuged at 3,000 × *g* for 20 min. The supernatant was directly applied to a 1 × 7 cm column of phenyl-Sepharose. Hepatic sulfatases were electrophoresed in 5% polyacrylamide slab gels containing 0.1% (v/v) Triton X-100 (DAVIS 1962). 4MUS-sulfatase was visualized under UV light following immersion in 5 mM 4MUS at 37° for 45 min and in 0.085 M glycine CO_3, pH 10, for 5 min. E_1S- and DHEAS-sulfatase activities were located following elution from parallel slices of the unstained gel using the assays previously described. Thermal denaturation of hepatic and renal sulfatases was performed in 0.2 M sodium phosphate/1% (v/v) Triton X-100 buffer, pH 8.6, at 55°. Inhibitors of sulfatase activity were incorporated in the respective substrate solutions at the specified concentrations. Kinetics of hepatic and renal sulfatases were examined using the following substrate ranges: 10 to 0.6 mM 4 MUS, 2 to 0.125 mM E_1S and 0.5 to 0.0312 mM DHEAS. Protein was measured by the method of LOWRY *et al.* (1951). Protein was precipitated from Triton-containing solutions with 30% (w/v) trichloroacetic acid prior to estimation.

RESULTS

4MUS-sulfatase activity measured at pH 8.6 in the presence of 0.2 M phosphate is contributed by arylsulfatase C. Arylsulfatases A and B are completely inhibited by these conditions (NELSON and DANIEL 1979). Therefore, the term 4MUS-sulfatase will be substituted for arysulfatase C throughout this report.

4MUS-, E_1S- and DHEAS-sulfatase activities of SWR/J liver extracts were two- to threefold higher than those of A/J mice (Table 1). Female activities

TABLE 1

Hepatic 4MUS-, E_1S- and DHEAS-sulfatase activities of 45-day SWR/J and A/J mice

Strain	Sulfatase	Males	Females
SWR/J	4MUS	1710 ± 58 (6)	1589 ± 36 (5)
	E_1S	46.8 ± 2.9 (5)	43.9 ± 1.9 (5)
	DHEAS	4.6 ± 0.2 (5)	4.2 ± 0.2 (5)
A/J	4MUS	761 ± 20 (6)	779 ± 17 (6)
	E_1S	19.9 ± 1.4 (5)	21.6 ± 1.4 (5)
	DHEAS	1.9 ± 0.1 (5)	1.7 ± 0.2 (5)

Activities [X SE(n)] are expressed as nanomoles of product formed per gram weight tissue per hour. Final substrate concentrations: 4MUS (3.3 mM); DHEAS (0.1 mM); E_1S (0.1 mM).

were comparable to those of males from the same inbred strain. Although the mean SWR/J male 4MUS-sulfatase activity appeared to exceed that of SWR/J females, the difference between means was not significant ($P(t = 1.51) > 0.05$).

Segregation of 4MUS-sulfatase activities among progeny derived from an SWR/J × A/J cross is illustrated in Figure 1. F_1 animals exhibited intermediate activities, and mean activities of males derived from reciprocal crosses were not significantly different (F_1(SWR/J × A/J): 1300 ± 34(11); F_1(A/J × SWR/J): 1270 ± 21(18); $0.10 > P(t = 0.81) > 0.05$). 4MUS-sulfatase activities of backcross progeny appeared to be continuously distributed throughout the F_1 and respective parental ranges. It is noteworthy that 29% of the F_1 × A/J animals possessed activities between 900 and 1000 nmol/g/hr, whereas only 11% of the A/J and 3% of the F_1 activities were in this range. A similar, although less obvious, trend was observed for F_1 × SWR/J backcross activites. F_2 4MUS-sulfatase activites appeared to be continuously distributed from the A/J through the SWR/J activity ranges and lacked clear demarcation into phenotypic classes.

Cosegregation of 4MUS-, E_1S- and DHEAS-sulfatase activites: F_1 × A/J and F_1 × SWR/J backcross progeny were scored for 4MUS-, E_1S- and DHEAS-sulfatase activities using enzymes from duplicate liver slices (Table 2). Correlation coefficients ranged from +0.79 to +0.83, suggesting that expression of all three activities is subject to coordinate genetic regulation.

Segregation of hepatic E_1S- and DHEAS-sulfatase activities: Segregation of hepatic E_1S-sulfatase activities is presented in Figure 2. F_1 activities were intermediate, and the distribution of progeny from the respective backcrosses were each bimodal. The observed upper and lower bounds of the F_1-like activities approximated 38 and 25 nmol/g/hr, respectively. If these values are used to define phenotypic classes, the activity distributions fit expectations of a two-allele, additive model for regulation of expression of hepatic E_1S-sulfatase activity (Table 3). Distributions of DHEAS-sulfatase activities were also compatible with a single locus-two allele additive model (data not shown).

Effect of As-1 upon hepatic 4MUS-sulfatase activity: As-1 is the putative structural locus for murine arylsulfatase B (DANIEL 1978). Elements within or near *As-1*

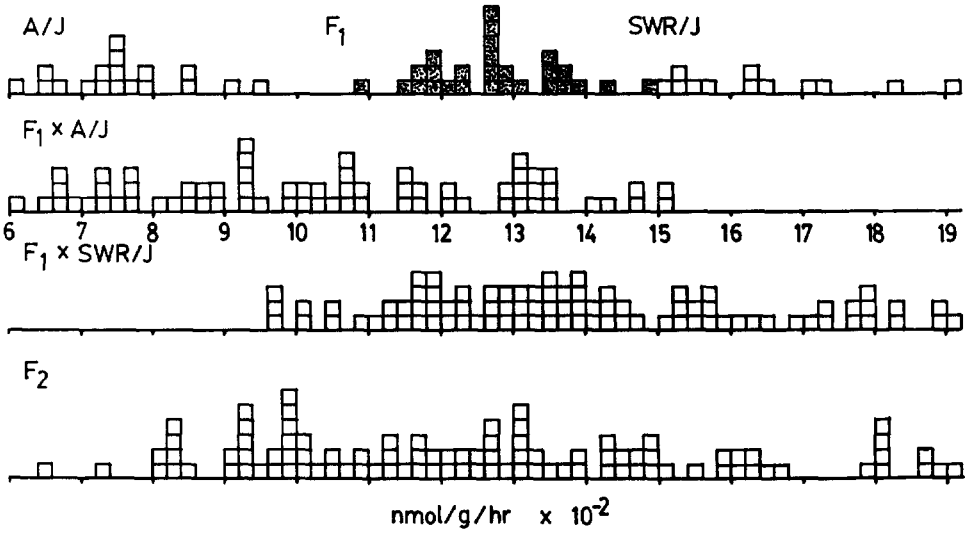

FIGURE 1.—Segregation of hepatic 4MUS-sulfatase activity among progeny derived from a SWR/J × A/J cross. Each square represents one animal. Stippled squares represent F_1 animals.

TABLE 2

Cosegregation of hepatic 4MUS-, E_1S and DHEAS-sulfatase activities among backcross progeny

Backcross	Correlation coefficients			
	N^a	4MUS *vs.* E_1S	N^a	E_1S *vs.* DHEAS
F_1 × A/J	50	+0.81	30	+0.79
F_1 × SWR/J	46	+0.79	30	+0.83

[a] N = number of pairs.

regulate expression of arylsulfatase B activity (DANIEL, ABEDIN and LANGELAN 1980; DANIEL, HARRISON and NELSON 1982). SWR/J mice possess the *As-1*^{a} allele (thermostable isozyme) and the *Asr-1*^{b} element (high systemic activity), whereas A/J mice have the *As-1*^{b} allele (thermolabile isozyme) and the *Asr-1*^{a} element (low systemic activity). If the *As-1* region influences 4MUS-sulfatase activity, then the mean 4MUS-sulfatase activity of *As-1*^{a}/*As-1*^{b} heterozygotes should be significantly higher than that of *As-1*^{b}/*As-1*^{b} homozygotes, since *Asr-1* is tightly linked to or within *As-1*. The mean hepatic 4MUS-sulfatase activities of 15 *As-1*^{a}/*As-1*^{b} and 15 *As-1*^{b}/*As-1*^{b} males derived from the F_1 × A/J backcross were not significantly different. The mean and standard error for the *As-1*^{a}/*As-1*^{b} and *As-1*^{b}/*As-1*^{b} males were 1163 ± 66 and 1116 ± 77 nmol/g/hr, respectively ($P(t = 0.46) > 0.2$). Therefore, the *As-1* region does not markedly influence 4MUS-sulfatase activity. Furthermore, hepatic 4MUS-sulfatase and arylsulfatase B activities did not cosegregate among 24 F_1 × A/J backcross progeny ($r = +0.10$), suggesting that these two sulfatases were not subject to the influence of the same gene.

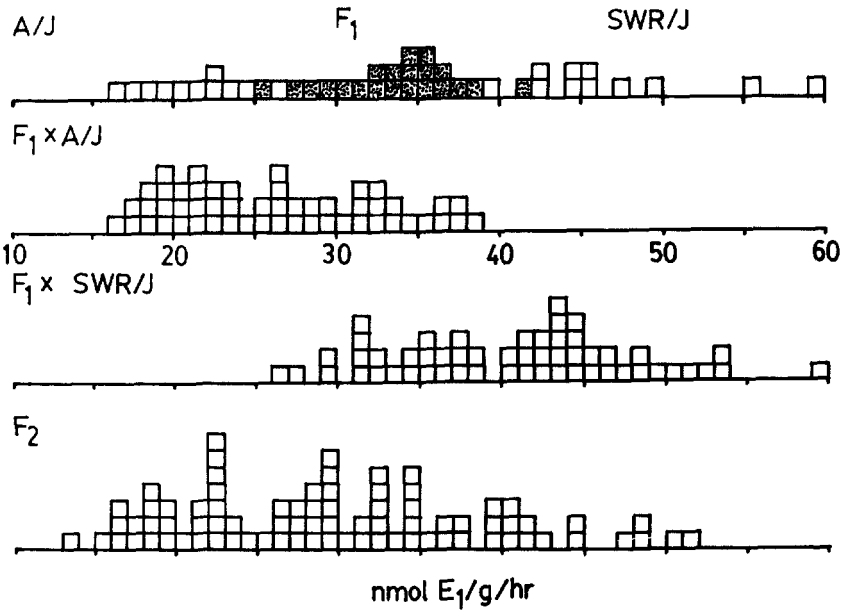

FIGURE 2.—Segregation of hepatic E_1S-sulfatase activity among progeny derived from the SWR/J × A/J cross. Each square represents one animal. Stippled squares represent F_1 animals.

Genetic regulation of kidney 4MUS-sulfatase activity: Renal 4MUS-sulfatase activity distributions for F_1, backcross and F_2 offspring from the SWR/J × A/J cross are displayed in Figure 3. The activities were apportioned according to expectations of a two-allele, additive model (Table 4). This segregation pattern was quite different from that for hepatic 4MUS-sulfatase activity, in which a substantial proportion of backcross animals possessed activities intermediate to those of the F_1 and respective parental strains. Therefore, hepatic and renal 4MUS-sulfatase activities of backcross animals were compared to determine whether activities in the two tissues were subject to similar genetic regulation. The correlation coefficient for hepatic and renal 4MUS-sulfatase activities of 42 F_1 × SWR/J animals was +0.39. This relatively low coefficient supports the hypothesis that hepatic 4MUS-sulfatase activity is subject to influences that are not operant in kidney cells.

Developmental variation of 4MUS-, E_1S- and DHEAS-sulfatase activities: Parallel variation of 4MUS- and E_1S-sulfatase activities was observed in brain and liver preparations throughout the 6- to 45-day interval examined (Figure 4). Both activities were 50–80% higher at 12 days postnatal age than at 45 days. Hepatic and brain E_1S- and 4MUS-sulfatase activities of SWR/J mice exceeded those of A/J mice throughout the 39-day interval. Both SWR/J and A/J renal 4MUS-sulfatase activities declined to approximately one-third of their 6-day activities by 24 days postnatal age and then remained relatively constant thereafter. The SWR/J renal E_1S-sulfatase activity profile paralleled that of 4MUS-sulfatase; however, A/J renal E_1S-sulfatase activity displayed little variation during the period monitored. Developmental variation of DHEAS-sulfatase

TABLE 3

Statistical analysis of E_1S-sulfatase activities of progeny derived from the SWR/J × A/J cross

	Phenotypic class[a]						
	SWR/J		F_1		A/J		
	N	$\bar{X} \pm SE$	N	$\bar{X} \pm SE$	N	$\bar{X} \pm SE$	χ^{2b}
SWR/J	12	46.0 ± 1.7					
A/J					11	20.6 ± 0.9	
F_1			20	32.8 ± 0.8			
F_1 × SWR/J	30	45.9 ± 0.8	23	31.9 ± 1.6			$0.50 > P(\chi^2 = 0.68) > 0.25$
F_1 × A/J			27	31.0 ± 0.7	24	20.1 ± 0.4	$0.90 > P(\chi^2 = 0.08) > 0.75$
F_2	16	43.3 ± 1.0	36	30.7 ± 0.6	28	19.5 ± 0.5	$0.25 > P(\chi^2 = 4.40) > 0.10$

[a] Activities are entered as nanomoles per gram per hour. Male and female data have been pooled. E_1S concentration = 0.1 mM.
[b] χ^2 calculated on goodness of fit to 1:1 or 1:2:1 ratio.

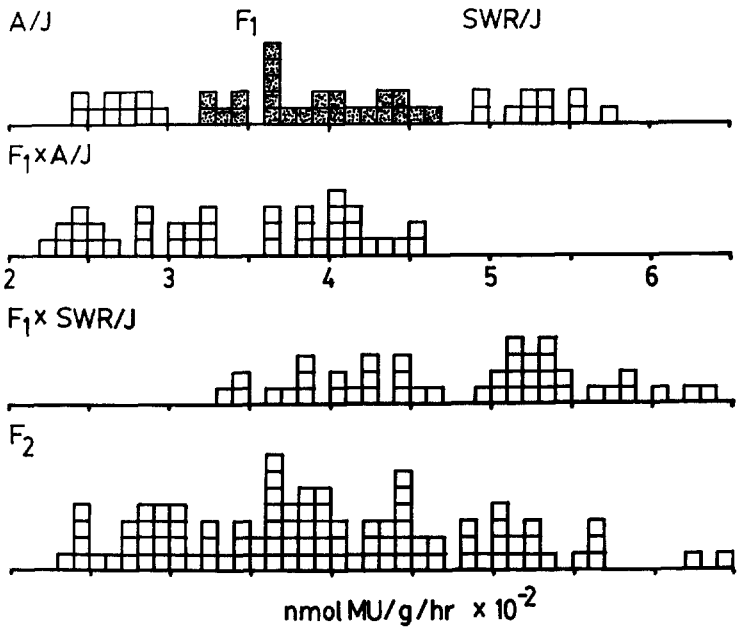

FIGURE 3.—Distribution of renal 4MUS-sulfatase activity among F_1, backcross and F_2 offspring from the SWR/J × A/J cross. Symbols are explained in the legend of Figure 2.

TABLE 4

Segregation analysis of renal 4MUS-sulfatase activities

	Phenotypic class[a]						
	SWR/J		F_1		A/J		
	N	$\bar{X} \pm$ SE	N	$\bar{X} \pm$ SE	N	$\bar{X} \pm$ SE	χ^{2b}
SWR/J	10	530 ± 8					
A/J					10	265 ± 5	
F_1			24	391 ± 8			
F_1 × SWR/J	24	539 ± 9	18	397 ± 8			$0.25 < P(\chi^2 = 0.60) < 0.50$
F_1 × A/J			19	411 ± 9	19	274 ± 8	$P(\chi^2 = 0) > 0.90$
F_2	20	531 ± 10	48	394 ± 6	26	279 ± 5	$0.50 < P(\chi^2 = 0.81) < 0.75$

[a] Activities are entered as nanomoles per gram per hour. Upper and lower bounds for F_1-like activities were 460 and 325 nmol/g/hr, respectively; 4MUS concentration = 3.3 mM.
[b] χ^2 calculated on goodness of fit to 1:1 or 1:2:1 ratio.

activity was similar to that of E_1S-sulfatase activity from the same tissue and strain.

Characterization of 4MUS-, E_1S- and DHEAS-sulfatases: Renal and hepatic 4MUS-sulfatase activities exhibited different subcellular distributions. Approximately 55% of the hepatic postnuclear SWR/J 4MUS-sulfatase activity occurred in the microsomal fraction, whereas 66% of the renal postnuclear activity appeared in nonmicrosomal fractions. A substantially larger proportion of renal 4MUS-sulfatase activity occurred in the cytosolic fraction. Similar

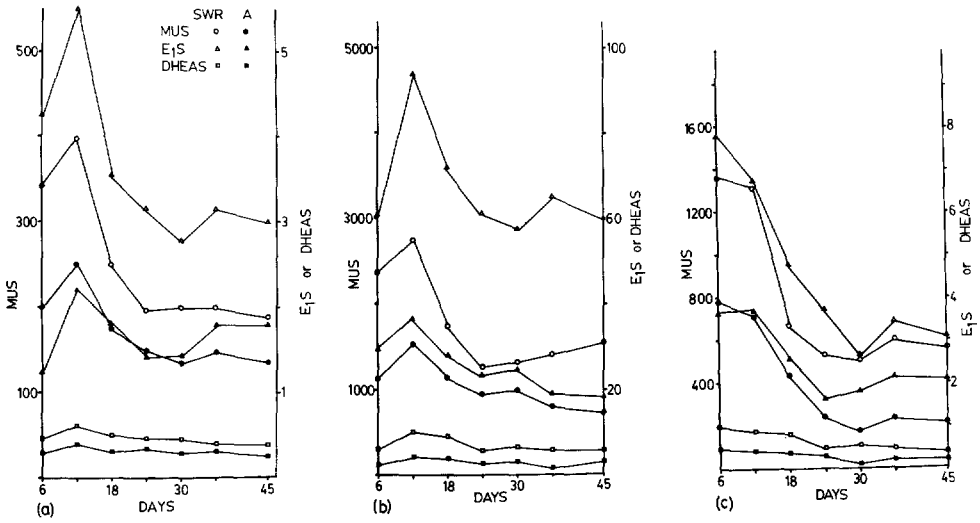

FIGURE 4.—Developmental variation of SWR/J and A/J 4MUS-, E_1S- and DHEAS-sulfatase activities in brain (a), liver (b) and kidney (c). Activities are expressed as nanomoles of product per gram per hour.

TABLE 5

Subcellular distribution of SWR/J and A/J hepatic and renal sulfatases

	Liver				Kidney			
Fraction	Protein	4MUS	E_1S	DHEAS	Protein	4MUS	E_1S	DHEAS
SWR/J								
PN[a]	85	1513	30	3.6	43	287	1.2	0.2
M + L[b]	27	41	32	36	24	44	27	30
C[b]	56	4	1	1	56	22	7	0
M[b]	17	55	67	63	20	34	66	70
A/J								
PN	90	800	16	1.6	55	188		
M + L	23	37	32	39	30	46		
C	57	5	5	0	54	26		
M	20	58	63	61	16	28		

[a] PN (postnuclear) protein is expressed as milligrams, and PN enzyme activities are entered as nanomoles of product per gram per hour and represent the mean of two experiments. See Table 1 for substrate concentrations.

[b] M + L (mitochondrial + lysomal), C (cytosol) and M (microsomal) entries are expressed as percent of the PN entry and are normalized to 100% of the PN value. Actual recoveries were: protein (93 ± 4%), 4MUS-sulfatase (80 ± 2%), E_1S-sulfatase (79 ± 4%) and DHEAS-sulfatase (77 ± 3%).

patterns of distribution were observed in liver and kidney preparations from A/J mice. In contrast with 4MUS-sulfatase, the subcellular arrays of E_1S- and DHEAS-sulfatase activities were comparable for the two tissue types. A/J 4MUS-, E_1S- and DHEAS-sulfatase activities were uniformly reduced in all hepatic subcellular fractions compared with those from SWR/J mice.

The chromatographic behaviors of the hepatic and renal sulfatase activities reflected their respective subcellular distributions. Approximately 68% of the SWR/J hepatic 4MUS-sulfatase activity bound to phenyl-Sepharose, a hydrophobic support (Table 6); however, only 20% of the renal activity was retained by this column. Similar proportions of the respective A/J 4MUS-activities were bound to phenyl-Sepharose. Recoveries of hepatic and renal 4MUS-sulfatase activities averaged 85 ± 5% in these experiments. Both E_1S- and DHEAS-sulfatase activities displayed strong affinities for phenyl-Sepharose irrespective of their tissue of origin. Similar proportions of the A/J and SWR/J enzymes were bound by this support. Yields of E_1S- and DHEAS-sulfatase activities recovered from the columns ranged from 80 to 88%.

If phenyl-Sepharose-bound 4MUS-, E_1S- and DHEAS-sulfatase activities correspond to membrane-associated enzyme, then the microsomal fraction of these activities should be retained by phenyl-Sepharose, whereas cytosolic activities should pass freely through the column. This hypothesis was tested by applying the respective subcellular fractions to phenyl-Sepharose and monitoring the eluate fractions for 4MUS- and DHEAS-sulfatase activities. Approximately 89% of hepatic and 79% of renal microsomal 4MUS-sulfatase activity was retained by phenyl-Sepharose (hepatic, 89 ± 2%; renal, 79 ± 7%; mean and range of two experiments). By contrast, 20 ± 3% of cytosolic 4MUS-sulfatase activity from either tissue bound to phenyl-Sepharose. The affinity of microsomal DHEAS-sulfatase activity from liver and kidney for phenyl-Sepharose paralleled that of 4MUS-sulfatase (hepatic, 82 ± 2%; renal, 82 ± 4%; mean and range of two experiments). Cytosolic DHEAS-sulfatase activity was undetectable following phenyl-Sepharose chromatography. 4MUS-sulfatase activity occurring in the mitochondrial/lysosomal fraction was predominantly hydrophilic with respect to both hepatic (32 ± 2% bound to phenyl-Sepharose) and renal (20 ± 3% bound) preparations. More than 80% of mitochondrial/lysosomal DHEAS-sulfatase activity was retained by phenyl-Sepharose irrespective of the tissue of origin.

Biochemical properties of SWR/J hepatic and renal 4MUS-, E_1S- and DHEAS-sulfatase activities have been described (K. NELSON, B. M. KEINANEN and W. L. DANIEL, unpublished results). Hepatic 4MUS-sulfatase activity displayed a pH optimum of 8.6, a K_m of 0.5 mM and a relative electrophoretic mobility with respect to bromphenol blue of 0.25 in 5% acrylamide/0.1% Triton *X*-100 gels. SWR/J 4MUS-sulfatase activity denatured at 55° with a half-life of 11 ± 1 min. In contrast with the hepatic enzyme, SWR/J renal 4MUS-sulfatase activity exhibited a higher K_m (1.8 mM) and somewhat lower pH optimum (pH 8.2). Thermal denaturation kinetics of hepatic and renal 4MUS-sulfatase activities appeared identical. No significant differences were observed between A/J and SWR/J 4MUS-sulfatase activities from kidney and liver with respect to any of these properties.

Properties of 4MUS-, E_1S- and DHEAS-sulfatase activites were compared in an attempt to enumerate the number of active sites responsible for their hydrolysis. All three activities coelectrophoresed in acrylamide/Triton gels. E_1S-sulfatase activity exhibited a half-life at 55° of 10 ± 1 min and a pH optimum

TABLE 6

Affinity chromatography of hepatic and renal sulfatases on phenyl-Sepharose

Tissue	Activity	Percent bound[a]	
		SWR/J	A/J
Liver	4MUS	68 ± 3	70 ± 4
	E_1S	75 ± 1	77 ± 4
	DHEAS	79 ± 4	72 ± 3
Kidney	4MUS	20 ± 4	24 ± 2
	E_1S	73 ± 4	70 ± 3
	DHEAS	68 ± 5	71 ± 4

[a] Mean and range of two experiments. See Table 1 for substrate concentrations.

of 8.6, whereas DHEAS-sulfatase activity was more thermostable ($t_{1/2}$=25 ± 2 min) and had a lower pH optimum (pH 7.2). Each of the sulfatase activities was competitively inhibited by both of the other substrates. Properties of hepatic E_1S- and DHEAS-sulfatase activities were similar to those of the respective renal activities. Furthermore, no significant differences were observed between corresponding A/J and SWR/J activity with respect to any of these properties.

DISCUSSION

The term, activities, has been deliberately employed throughout this report to avoid unwarranted assumptions regarding their physical interrelationships, since the number of enzymes contributing to these activites remains controversial. 4MUS is a general substrate for arylsulfatases A, B and C; however, the natural substrates of these enzymes are markedly different. Arylsulfatases A and B are completely inhibited by 0.2 M phosphate at pH 8.6. The phosphate-resistant 4MUS-sulfatase activity is contributed by arylsulfatase C. This conclusion is supported by its subcellular distribution, pH optimum, resistance to inhibition by sulfate and competitive inhibition by pNPS (K. NELSON, B. M. KEINANEN and W. L. DANIEL, unpublished results).

Genetic regulation of murine 4MUS-, E_1S- and DHEAS-sulfatase activities appears to be primarily autosomal. Male and female mice possessed similar 4MUS-, E_1S- and DHEAS-sulfatase activities, and F_1 males derived from reciprocal crosses of the SWR/J (high activity) and A/J (low activity) strains exhibited similar hepatic and renal sulfatase activities. ERICKSON, HARPER and KRAMER (unpublished data) have reported that murine testicular and hepatic DHEAS-sulfatase activity is also subject to autosomal regulation.

Genetic analyses of hepatic 4MUS-, E_1S- and DHEAS-sulfatase activity variation among F_1 × SWR/J, F_1 × A/J and F_2 progeny indicated: (1) Factors regulating expression of hepatic 4MUS-, E_1S- and DHEAS-sulfatase activities cosegregate; (2) Hepatic E_1S- and DHEAS-sulfatase activities are each subject to regulation by a single autosomal locus possessing two alleles with additive effects; (3) Hepatic 4MUS-sulfatase activity is influenced by at least two independent factors and (4) Major genes influencing 4MUS-sulfatase activity are

not linked to *As-1*, the putative structural locus for arylsulfatase B. We would like to propose that a major autosomal locus, distinct from *As-1*, is accountable for a substantial proportion of the hepatic 4MUS-sulfatase activity variation and the majority of the E_1S- and DHEAS-sulfatase activity variation between the SWR/J and A/J inbred strains. The simplest way in which this might occur is for the gene to influence expression of a structural gene coding for an enzyme that hydrolyzes all three substrates.

The strongest evidence in favor of hydrolysis of 4MUS, E_1S and DHEAS by the same enzyme is provided by the inhibition experiments. Each sulfatase activity was competitively inhibited by the other two substrates. The facts that the three hepatic sulfatase activities have similar subcellular distributions, co-segregate among backcross progeny, exhibit covariation during development and comigrate in electrophoretic gels are compatible with the hypothesis that the three activities reside in the same enzyme. The lower pH optimum for DHEAS-sulfatase activity compared with that of E_1S- and 4MUS-sulfatase activity is not necessarily incompatible with a single enzyme-multiple specificity model. For example, ox liver arylsulfatase A displays a pH optimum of 5.6 for hydrolysis of *p*-nitrocatechol sulfate and a pH optimum of 4.5 for hydrolysis of cerebroside sulfate (ROY 1979). The greater thermostability of DHEAS-sulfatase activity compared with those of both E_1S- and 4MUS-sulfatase activities is more difficult to reconcile with the single enzyme model.

IWAMORI, MOSER and KISHIMOTO (1976) have described a partially purified (33-fold) preparation of rat liver steroid sulfatase that preferentially hydrolyzed pNPS and E_1S compared with other steroid sulfatases including DHEAS. E_1S-sulfatase activity was competitively inhibited by pNPS, a substrate for arylsulfatase C, indicating that the two substrates shared the same active site. In contrast, inhibition of E_1S-sulfatase activity by DHEAS was noncompetitive. The reason for observation of noncompetitive inhibition of rat liver E_1S-sulfatase activity and competitive inhibition of murine liver E_1S-sulfatase activity by DHEAS is unclear. However, DHEAS does not reach appreciable levels in murine tissues, and mice may not possess a distinct enzyme for its hydrolysis. IWAMORI, MOSER and KISHIMOTO (1976) stated that DHEAS-sulfatase activity was partially denatured by the detergent Miranol H2M and sonication. It is unlikely that use of Triton X-100 and sonication during extraction of the murine sulfatase activities selectively denatured DHEAS-sulfatase activity, since neither of these treatments was employed during the cell fractionation experiments. The ratio of 4MUS- to E_1S- to DHEAS-sulfatase activity was comparable to that observed in sonicated preparations.

The complex segregation patterns of murine hepatic 4MUS-sulfatase activity among backcross and F_2 progeny derived from the SWR/J × A/J cross are not necessarily inconsistent with participation of a major gene in the regulation of 4MUS-sulfatase expression in this tissue. Similar segregation patterns were observed for hepatic arylsulfatase B (DANIEL 1978), and it was subsequently demonstrated that approximately 75% of the variation could be attributed to allelic variation in the *As-1* region and 25% to genetic background (DANIEL, HARRISON and NELSON 1982). It is unclear why the E_1S- and DHEAS-sulfatase

activity patterns did not show similar complexity. Measurement error does not appear to be responsible, since the 4MUS-sulfatase activity of the internal standard exhibited minimal daily variation.

The correlation coefficient for hepatic and renal 4MUS-sulfatase activity approximated 0.4, indicating that some of the factors influencing hepatic activity may not be operating in kidney cells. Renal 4MUS-sulfatase activity appeared to be subject to regulation by a single autosomal locus possessing two alleles with additive effects. A substantial proportion of enzyme contributing to renal 4MUS-sulfatase activity appeared incapable of hydrolyzing E_1S or DHEAS. The ratios of 4MUS- to E_1S- to DHEAS-sulfatase activity for liver and kidney approximated 400:10:1 and 1200:5:1, respectively. These ratios did not differ significantly between strains. Although pNPS inhibited hepatic and renal 4MUS-sulfatase activity to a similar extent, inhibition of the renal 4MUS-sulfatase activity by E_1S averaged 15% of that observed for hepatic 4MUS-sulfatase activity at comparable E_1S:4MUS ratios. This percent inhibition reflects the smaller proportion of hydrophobic 4MUS-sulfatase activity in kidney extracts (20 *vs.* 68%) and the smaller proportion of 4MUS-sulfatase activity present in the renal microsomal fraction (31 *vs.* 56%) compared with corresponding hepatic 4MUS-sulfatase preparations. Renal 4MUS-sulfatase activity also exhibited a small but reproducible decrease in pH optimum and a higher apparent K_m. In contrast, the subcellular distribution and biochemical properties of E_1S- and DHEAS-sulfatase activity, respectively, present in liver and kidney extracts were indistinguishable. These data suggest that only that portion of 4MUS-sulfatase occurring within membranes of kidney cells is capable of hydrolyzing E_1S and DHEAS. This hypothesis is supported by the strongly hydrophobic character of hepatic and renal E_1S- and DHEAS-sulfatase activities, by the lack of appreciable DHEAS-sulfatase activity in hydrophilic fractions of 4MUS-sulfatase activity and by the similar proportions of hydrophobic 4MUS- and DHEAS-sulfatase activities occurring in microsomes. Processing of hepatic and renal 4MUS-sulfatase may differ. This possibility is supported by the similar thermal denaturation kinetics of the renal and hepatic 4MUS-sulfatase activities and by their similar response to inhibition by pNPS. The positive correlation between hepatic and renal 4MUS-sulfatase activities indicates that both activities may be controlled by the same autosomal gene; however, differential processing of the enzyme in the two tissues and its functional consequences may be responsible for the unexpectedly low correlation between hepatic and renal 4MUS-sulfatase activities.

All properties examined of A/J and SWR/J 4MUS-, E_1S- and DHEAS-sulfatase activities appeared identical. Therefore, it is unknown whether the activity variations occurring between these two strains have a structural or regulatory basis. Because of this uncertainty, we recommend that assignment of gene symbols be delayed until more is known regarding the mechanism of action of the major gene hypothesized.

ERICKSON, HARPER and KRAMER (unpublished results) employed recombinant inbred strains derived from F_2 (C57BL/6J × A/J) animals to explore the genetics of the DHEAS-sulfatase activity difference occurring between the

C57BL/6J and A/J inbred strains. The recombinant inbred strains segregated into high activity and low activity classes approximating DHEAS-sulfatase activities occurring in the C57BL/6J and A/J parental strains, respectively. This result coupled with data derived from studies of progeny obtained from a C57BL/6J × A/J cross indicated that murine testicular and hepatic DHEAS-sulfatase activity is controlled by an autosomal locus. Since the recombinant inbred strains were concordant for both hepatic and testicular DHEAS-sulfatase activity, this gene most likely influences expression of enzyme in both tissues. Although these researchers did not assay their preparations for E_1S- and 4MUS-sulfatase activities, their autosomal gene system and the one described in this report may be the same. We have found that both SWR/J and C57BL/6J testicular 4MUS, E_1S- and DHEAS-sulfatase activities have similar subcellular distributions. The subcellular distributions of the three testicular activities and their ability to bind to phenyl-Sepharose were comparable to those observed for the respective hepatic sulfatase activities. Responses of the testicular sulfatase activities to inhibitors also resembled those of the corresponding hepatic sulfatase activities. R. ERICKSON, K. HARPER and J. KRAMER (unpublished results) reported that C57BL/6J DHEAS-sulfatase possessed a higher apparent K_m than the A/J enzyme. We presently lack evidence for a significantly higher K_m for SWR/J DHEAS-sulfatase.

Human arylsulfatase C, DHEAS-sulfatase, E_1S-sulfatase and cholesterol sulfate sulfatase all appear to be influenced by an *X*-linked gene (SHAPIRO *et al.* 1977; SHAPIRO *et al.* 1978). DHEAS-sulfatase activity in the wood lemming is also subject to the effects of an *X*-linked gene (ROPERS and WIBERG 1982). The human *X*-linked gene appears to alter the membrane environment of steroid sulfatase (MCKEE, ABEYSEKERA and FRANCE 1981). Conservation of *X*-linked genes among mammals implicates presence of a similar gene in mice; however, allelic variation at this locus has not been described. GARTLER and RIVEST (1983) have reported twofold higher levels of steroid sulfatase activity in oocytes from *XX* mice compared with those of *XO* mice; however, kidney activities of *XX* and *XO* mice were comparable. A number of human sulfatases, including arysulfatases A, B and C and steroid sulfatase, are also affected by an autosomal gene (MURPHY *et al.* 1971). It is unlikely that our murine autosomal gene resembles the human autosomal gene, since covariation of hepatic murine 4MUS-sulfatase and arylsulfatase B activities among F_1 × A/J backcross progeny was not observed. Furthermore, murine 4MUS-sulfatase activity segregated independently from *As-1* and elements adjacent to or within *As-1* that regulate arylsulfatase B activity. C3H/An mice have been reported to have deficinet hypoxanthine phosphoribosyl transferase, steroid sulfatase and arylsulfatase C activities (BALAZS *et al.* 1982). These deficiencies were inherited as an autosomal recessive. The relationship between this gene and the one described in our report is unclear.

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