9 Hyvärinen, and Nikkilä, E.A., Clinica chim. Acta *7* (1962) 140.
10 Coulomba, J.J., and Favreau, L., Clin. Chem. *9* (1963) 102.
11 Neuweiler, O., Klin. Wschr. *12* (1953) 869.
12 Dole, W.P., Moutville, W.I., and Bishop, U.S., Am. J. Physiol. *240* (1981) 4709.
13 Dandona, P., James, I.M., and Becket, A.G., Lancet *1* (1981) 694.
14 Dandona, P., James, I.M., Newbury, P.A., Woollard, M.L., and Beckett, A.G., Br. med. J. *2* (1978) 325.
15 Ewald, U., Tuvemo, T., and Rooth, G., Lancet *1* (1981) 1287.
16 Kiss, Á., Grosz, Gy., Juhász-Nagy, S., Szinay, Gy. Wagner, M., and Pogátsa, G., Kísérl. Orvostud. *32* (1980) 490.
17 Feigl, E.O., Circulation Res. *37* (1975) 88.
18 Juhász-Nagy, S., and Szentiványi, M., Am. J. Physiol. *200* (1961) 125.
19 Mohrman, D.E., and Feigl, E.O., Circulation Res. *42* (1978) 79.
20 Kunos, G., and Szentiványi, M., Nature, Lond. *217* (1977) 1077.
21 Kunos, G., Br. J. Pharmac. *59* (1977) 177.
22 Stene-Larsen, G., and Hule, B.K., Life Sci. *23* (1978) 2681.
23 Savarese, J.J., and Berkowitz, B.A., Life Sci. *25* (1980) 2075.
24 Schuyler, M.R., Niewoehner, D.E., Inkeley, S.E., and Kohn, R., Am. Rev. resp. Dis. *113* (1976) 37.
25 Pogátsa, G., Bihari-Varga, M., and Szinay, Gy., Acta diabet. lat. *16* (1979) 129.
26 Sullivan, S., and Sparks, H.V., Am. J. Physiol. *236* (1979) H-301.

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Murine arylsulfatase C: Evidence for two isozymes[1]

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Summary. SWR/J mice possess high arylsulfatase C, estrone sulfatase, and dehydroepiandrosterone sulfatase activities in liver, spleen, and kidney compared to A/J mice. This interstrain activity variation appears to be determined by at least 1 autosomal gene. Murine arylsulfatase C activity occurs in both hydrophobic and hydrophilic forms which differ with respect to certain biochemical properties and exhibit different subcellular distributions. The hydrophilic isozyme is a major component in kidney and brain extracts and a minor isozyme in liver and spleen extracts. The hydrophobic arylsulfatase C isozyme appears to be identical to steroid sulfatase. The hydrophilic arylsulfatase C isozyme does not possess steroid sulfatase activity; however, hydrophilic and hydrophobic arylsulfatase C share certain properties, suggesting that they may be structurally related. The autosomal gene(s) affects both arylsulfatase isozymes.

Arylsulfatase C (arylsulfate sulfohydrolase, EC 3.1.6.1) hydrolyzes sulfate ester bonds of a number of synthetic substrates, including p-nitrophenyl sulfate and 4-methylumbelliferyl sulfate, and appears to function as a steroid sulfatase in vivo. Arylsulfatase C (steroid sulfatase) deficiency is associated with X-linked ichthyosis[3], and the presumptive steroid sulfatase locus has been mapped to the distal short arm of the X-chromosome[4]. Unlike most known X-linked loci, both steroid sulfatase alleles are functional in female cells[5]. Relatively little is known regarding genetic regulation of normal arylsulfatase C activity in mammalian cells. We have developed a reliable sensitive assay for arylsulfatase C in crude preparations and have described interstrain and developmental variation of the murine enzyme[6]. This report presents data supporting the occurrence of 2 arylsulfatase C isozymes in murine tissues, describes their properties, and provides preliminary support for autosomal control of arylsulfatase C activity.

Methods. Inbred mice were purchased from the Jackson Laboratory, Bar Harbor, Maine and raised to 45 days of age. Tissues were homogenized in 9 vols of 0.2 M sodium phosphate buffer, pH 8.6, containing 1.0% (v/v) Triton X-100. The homogenates were sonicated and centrifuged at 20,000 × g for 30 min. The supernatant was used as the source of the enzyme unless otherwise indicated. Arylsulfatase C activity was determined using 4-methylumbelliferyl sulfate (MUS) as previously described[6]. Steroid sulfatase activity was estimated using estrone sulfate (E_1S) and dehydroepiandrosterone sulfate (DHEAS) as substrates. E_1S-sulfatase activity was measured using [6,7-^{3}H(N)]E_1S ammonium salt (New England Nuclear; 59 Ci/mmole) as substrate[7]. DHEAS-sulfatase activity was estimated using ^{3}H-DHEAS ammonium salt (NEN; 22.1 Ci/mmole) as substrate[8]. The ^{3}H-DHEA product was extracted directly into the scintillation cocktail. Zero time blanks were used for all assays. Subcellular localization of liver and kidney arylsulfatase C was explored using the method of Perumal and Robins[9] using 1 g of tissue. Arylsulfatase C was chromatographed on phenyl-Sepharose CL-4B (Pharmacia) using a method modified from that of Carson and Konigsberg[10]. 10% (w/v) homogenates (0.5 g tissue) were prepared in 0.05 M Tris-0.1 M NaCl-0.01% NaN_3 (Tris-saline-azide) buffer, pH 7.6, sonicated 15 sec, and centrifuged at 3000 × g for 20 min. The supernatant was applied to a 1 × 7 cm column of phenyl-Sepharose preequilibrated with Tris-saline-azide buffer, and the column was washed with 50 ml of the same buffer. The column was developed with 100 ml of Tris-saline-azide buffer containing 0.5% (v/v) Triton X-100. 1.5 ml fractions were collected and

Table 1. Arylsulfatase C activities from selected tissues of 45-day male and female mice

Strain	Sex	Liver	Kidney	Spleen	Brain
SWR/J	M	1.80±0.08	0.78±0.07	1.63±0.14	0.33±0.02
	F	1.77±0.16	0.71±0.06	1.73±0.10	0.30±0.01
C57BL/6J	M	2.06±0.17	0.37±0.04	1.00±0.11	0.22±0.02
	F	1.78±0.16	0.36±0.03	1.00±0.09	0.20±0.01
SJL/J	M	1.90±0.12	0.85±0.06	1.57±0.13	0.34±0.01
	F	1.70±0.18	0.76±0.10	1.37±0.16	0.32±0.03
A/J	M	1.03±0.10	0.33±0.03	0.78±0.06	0.25±0.03
	F	1.04±0.07	0.37±0.03	0.74±0.05	0.25±0.02

Activities are expressed as μmoles MU formed/g wet tissue/h. The mean and range of 5 animals are presented in each category.

monitored for arylsulfatase C activity and protein. Appropriate fractions were pooled and used for enzyme characterization.

Characterization of arylsulfatase C. Arylsulfatase C was electrophoresed in 5% polyacrylamide slab gels at pH 8.3[11]. 0.1% (v/v) Triton X-100 was incorporated in both gel and buffer. The enzyme was stained by incubation in 5 mM MUS at 37 °C for 45 min and immersion in 0.08 M glycine-CO_3 buffer, pH 10. The stained gels were viewed under long wave UV-light. Parallel slices were removed from unstained portions of the gel, and their proteins were extracted overnight and assayed for E_1S- and DHEAS-sulfatase activities. Thermal denaturation of arylsulfatase C was conducted by heating the liver and kidney 20,000 × g supernatants (prepared in 0.2 M sodium phosphate buffer, pH 8.6, containing 1% Triton X-100) at 55 °C. Aliquots were removed at 10 min intervals, cooled in ice, clarified by centrifugation, and assayed for residual MUS-, E_1S-, and DHEAS-sulfatase activities. Inhibition studies were performed by including the inhibitor in the respective substrate solutions at the specified concentration. Michaelis constants for kidney and liver MUS-, E_1S-, and DHEAS-sulfatases were determined over the range 10–0.6 mM MUS, 2–0.125 mM E_1S, and 0.5–0.0312 mM DHEAS, respectively. Protein was estimated by the method of Lowry et al.[12]. Protein was precipitated from Triton-containing solutions with 30% TCA prior to measurement.

Results and discussion. Two patterns of arylsulfatase C (MUS-sulfatase) variation were observed: 1. increases in all 4 tissues tested (SWR/J and SJL/J vs A/J); and 2. increases in liver and spleen only (C57BL/6J vs A/J). E_1S- and DHEAS-sulfatase activity variation between SWR/J and A/J tissues paralleled that for MUS-sulfatase, suggesting that all 3 activities may be subject to similar genetic influences.

MUS-sulfatase activities were very similar in males and females from the same strain (table 1). Hepatic MUS-sulfatase activities (mean ± SE (n)) for 45-day F_1 (A x SWR) and F_1 (SWR x A) males were 1.28 ± 0.04 (15) and 1.35 ± 0.03 (15) μmoles/g/h, respectively. These means are not significantly different ($p(t=-1.70) > 0.10$). The corresponding renal MUS-sulfatase activities were 0.53 ± 0.05 (15) and 0.53 ± 0.02 μmoles/g/h, respectively. These data support autosomal determination of the interstrain MUS-sulfatase activity differences in these tissues, since activities of the hybrid males would have approximated those of the parental strains from which they received their X-chromosome if major X-linked genes were involved. Evidence for autosomal inheritance of a murine testicular DHEAS-sulfatase activity variant has been reported[13]. Moreover, X-linked genes appear to influence DHEAS-sulfatase activity in murine oocytes[14], suggesting that both autosomal and X-linked genes may contribute to steroid sulfatase activity in murine cells. Shapiro et al.[15] have published data supporting involvement of an X-linked gene affecting both MUS-sulfatase and steroid sulfatase activities in human placenta and skin fibroblasts. Studies of the genetic component responsible for the interstrain MUS-, E_1S-, and DHEAS-sulfatase activity variations are in progress.

There is disagreement whether MUS, E_1S, and DHEAS are hydrolyzed by the same active site[16] or by 2 or more distinct enzymes[7]. Our data generally support hydrolysis of these 3 substrates by the same hepatic enzyme (table 3) and by the same active site. E_1S-, DHEAS-, and MUS-sulfatase activities were resistant to inhibition by phosphate and sulfate and were competitively inhibited by pNPS. Both E_1S and DHEAS competitively inhibited MUS-sulfatase activity, and E_1S-sulfatase activity was competitively inhibited by DHEAS. All 3 sulfatase activities comigrated in polyacrylamide/Triton gels. Approximately 63, 73, and 72% of the hepatic postnuclear MUS-, E_1S- and DHEAS-sulfatase activities, respectively, were localized in the microsomal fraction. The somewhat lower pH optimum and greater thermostability of DHEAS-sulfatase activity may not be inconsistent with a single active site-multiple substrate model, if it is assumed that a somewhat different conformation is required for binding DHEAS. Further data are required for resolution of this problem.

Properties of renal E_1S- and DHEAS-sulfatase activities resembled those of the respective hepatic activities (table 3), and 74 and 83% of renal postnuclear E_1S- and DHEAS-sulfatase activities were located in the microsomal

Table 2. Comparison of MUS-, E_1S-, and DHEAS-sulfatase activities of SWR/J and A/J mice

	SWR/J			A/J		
	MUS*	E_1S**	DHEAS**	MUS*	E_1S**	DHEAS**
Liver	12.0 ± 0.7	399 ± 17	48 ± 3	6.1 ± 0.5	141 ± 12	21 ± 3
Kidney	5.0 ± 0.2	27 ± 6	4.2 ± 0.2	2.6 ± 0.1	10 ± 1	2.1 ± 0.1
Brain	1.6 ± 0.1	23 ± 2	2.6 ± 0.3	1.3 ± 0.1	20 ± 2	2.6 ± 0.2
Spleen	18.7 ± 1.9	76 ± 12	13.3 ± 1.6	8.4 ± 0.3	40 ± 4	5.8 ± 0.8

*nmoles/mg protein/h; MUS-sulfatase = arylsulfatase C. **pmoles/mg protein/h. Entries represent the mean and range of 2 45-day males.

Table 3. Comparison of the biochemical properties of SWR/J kidney and liver MUS-, E_1S-, and DHEAS-sulfatases*

	Liver			Kidney		
	MUS	E_1S	DHEAS	MUS	E_1S	DHEAS
pH Optimum	8.6	8.6	7.2	8.2	8.6	7.2
K_m (mM)	0.5 ± 0.1	0.21 ± 0.02	0.15 ± 0.02	1.8 ± 0.2	0.22 ± 0.02	0.15 ± 0.02
$t^1/_2$ 55 °C (min)	11 ± 1	10 ± 1	25 ± 2	11 ± 1	-	-
Electrophoretic mobility**	0.25 ± 0.01	0.24 ± 0.02	0.25 ± 0.02	-	-	-
Inhibition (%):						
10 mM pNPS	41 ± 2	73 ± 4	87 ± 3	26 ± 5	77 ± 4	87 ± 3
1 mM $Na_2B_4O_7$	74 ± 2	98 ± 2	82 ± 3	79 ± 3	95 ± 3	86 ± 2
10 mM Na_2SO_4	0	0	0	0	0	0
2.5 mM DHEAS	58 ± 3	-	-	12 ± 1	-	-
0.5 mM DHEAS	22 ± 2	53 ± 5	-	7 ± 1	35 ± 3	-
2.5 mM E_1S	53 ± 2	-	-	7 ± 1	-	-
0.5 mM E_1S	19 ± 2	-	-	5 ± 1	-	-

* Mean and range of 3 experiments. ** Measured in 5% separatory gel with bromphenol blue as a reference.

fraction. By contrast, less than 30% of renal postnuclear MUS-sulfatase activity occurred in the microsomal fraction, and about 50% was located in the mitochondrial-lysosomal fraction. Recoveries of all 3 renal sulfatase activities approximated 80±4% of initial postnuclear activity. The unusual distribution of renal MUS-sulfatase activity may be related to differences in the lipid and/or nonpolar amino acid content of the microsomal and non-microsomal enzymes. Only 16±5% of renal MUS-sulfatase activity bound to phenyl-Sepharose, a hydrophobic support, whereas 69±3% of hepatic MUS-sulfatase activity was retained by phenyl-Sepharose. More than 70% of E_1S- and DHEAS-sulfatase activities bound to phenyl-Sepharose, regardless of their tissue of origin. Renal MUS-sulfatase activity displayed a somewhat lower pH optimum, higher apparent K_m, and was less inhibited by E_1S and DHEAS (table 3). These differences are apparently due to enrichment of the hydrophilic MUS-sulfatase isozyme in renal tissue. The phenyl-Sepharose-bound MUS-sulfatase fraction possessed properties approximating those of hepatic MUS-sulfatase activity and was competitively inhibited by DHEAS and E_1S. The phenyl-Sepharose-void fraction of renal MUS-sulfatase had a higher K_m and was not inhibited by E_1S and DHEAS. These trends suggest that only the microsomal (phenyl-Sepharose-bound) MUS-sulfatase is capable of functioning as a steroid sulfatase. The hydrophilic and hydrophobic renal MUS-sulfatase isozymes may be structurally related. This conclusion is supported by their similar thermal denaturation properties, resistance to phosphate and sulfate inhibition, similar inhibition by pNPS (competitive), and the fact that both hydrophilic and hydrophobic MUS-sulfatase activities were increased in SWR/J kidney compared to A/J kidney.

We currently lack evidence for a structural mutation that could account for the SWR/J-A/J interstrain MUS-, E_1S-, and DHEAS-activity variation.

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3 Shapiro, L.J., Weiss, R., Webster, D., and France, J.T., Lancet *1* (1978) 70.
4 Tiepolo, L., Zuffardi, O., Fraccaro, M., diNatale, D., Gargantini, L., Muller, C.R., and Ropers, H.H., Hum. Genet. *54* (1980) 205.
5 Bedin, M., Weil, D., Fournier, T., Cedard, L., and Frezal, J., Hum. Genet. *59* (1981) 256.
6 Nelson, K., and Daniel, W.L., Experientia *35* (1979) 309.
7 Iwamori, M., Moser, H.W., and Kishimoto, Y., Archs Biochem. Biophys. *174* (1976) 199.
8 Ropers, H.H., Migl, B., Zimmer, J., Fraccaro, M., Maraschio, P.P., and Westerveld, A., Hum. Genet. *57* (1981) 354.
9 Perumal, A.S., and Robins, E., Brain Res. *59* (1973) 349.
10 Carson, S.D., and Konigsberg, W.H., Analyt. Biochem. *116* (1981) 398.
11 Davis, B.J., Ann. N.Y. Acad. Sci. *121* (1962) 404.
12 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.L., J. biol. Chem. *193* (1951) 267.
13 Erickson, R., Harper, K., and Kramer, J., Mouse News Lett. *66* (1982) 69.
14 Gartler, S.M., and Rivest, M., Genetics *103* (1983) 137.
15 Shapiro, L.J., Cousins, L., Fluharty, A.L., Stevens, R.L., and Kihara, H., Pediat. Res. *11* (1977) 894.
16 Dolly, J.O., Dodgson, K.S., and Rose, F.A., Biochem. J. *128* (1972) 337.

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The occurrence of 7-hydroxybiopterin in the scorpion fly, *Panorpa japonica*

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Summary. A purple fluorescent compound was isolated from the integument of scorpion flies. Based on paper chromatographic, UV-spectrophotometric, fluorometric and HPLC analysis, as well as a chemical color test and various degradation tests, the compound was identified as 7-hydroxybiopterin.

There are many reports of the occurrence of 7-hydroxybiopterin (fig. 1), which has also been named ichthyopterin, in the skin and scales of various fish[2–8]. The chemical structure of the compound was found to be 2-amino-4,7-dihydroxy-6-(1′,2′-dihydroxypropyl) pteridine[9]. Although numerous types of pteridines have been isolated in insects[10–12], 7-hydroxybipoterin has not been reported among them. Two papers discussed the occurrence of a purple fluorescent pteridine in silkworm eggs[13,14]. This compound was named fluorescyanine and it was thought to be identical to or related to 7-hydroxybiopterin. Further studies showed, however, that fluorescyanine in silkworm eggs was isoxanthopterin[15]. In studies on the scorpion fly, *Panorpa japonica*, we found that 7-hydroxybiopterin occurs naturally in this insect's integument. The present paper deals with the isolation and identification of this pteridine from the insect.

Materials and methods. Purified synthetic 7-hydroxybiopterin was kindly supplied by Dr Sugiura of Gakushuin University. Isoxanthopterin and isoxanthopterin-6-carboxylic acid were generous gifts of Prof. Matsuura of Nagoya University. Ethanolyzed cellulose was prepared by the method of Lee and Montgomery[16]. Phospho-Sephadex and Ecteola-cellulose were prepared by the method of Peterson and Sober[17]. All other chemicals were obtained from commercial sources. Scorpion flies were collected at Sagamihara City. The heads and wings were cut from their bodies and the integument was separated from other tissues by pressing between 2 sheets of filter paper.

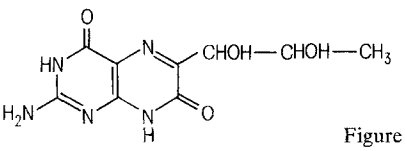

Figure 1. 7-Hydroxybiopterin.