

## Interstrain variation of murine arylsulfatase C<sup>1</sup>

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**Summary.** A method has been developed for the assay of arylsulfatase C in tissue extracts containing arylsulfatases A and B. Significant variation of enzyme activity was observed among 26 inbred murine strains. Activity differences were apparent at all stages evaluated between 1 and 70 days postnatal age. Arylsulfatase C from representative high- and low-activity strains exhibited similar Michaelis constants, temperature optima, pH optima, thermostabilities and inhibitor profiles.

Arylsulfatases (arylsulfate sulfohydrolase, EC 3.1.6.1) cleave sulfate from a variety of substrates, and several isozymes have been described which differ with respect to their physical and chemical properties, substrate affinities and intracellular distributions<sup>2</sup>. Arylsulfatase C (aryl C) is largely microsomal and may function as a steroid sulfatase. Although pathological variation of this isozyme has been reported in man<sup>3,4</sup>, relatively little is known concerning genetic control of aryl C expression in mammalian tissues. The value of murine inbred lines for analysis of genetic control of quantitative enzyme variation in mammalian systems has been amply demonstrated<sup>5</sup>; however, genetic and biochemical analyses of murine aryl C have been precluded by lack of a sensitive, reliable assay. This report describes an assay which is suitable for measurement of murine aryl C activity in mixtures of arylsulfatase isozymes, the general properties of the murine enzyme, and inter-strain and developmental variation of aryl C activity in selected organs.

**Assay.** Aryl C has an alkaline pH optimum and is relatively insensitive to phosphate<sup>6</sup>. Arylsulfatases A and B are completely inhibited by 0.2 M phosphate and have acidic pH optima<sup>2</sup>. Differences in these properties were exploited in the design of the following assay. Mice were killed by cervical dislocation; and tissues were excised, rinsed, weighed and frozen at -20°C until analysis. Appreciable loss of activity was not observed to occur during storage for at least 6 months. Tissues were homogenized in 9 volumes of 0.2 M sodium phosphate buffer, pH 8.6, sonicated at 80%-low setting with a Biosonic IV sonicator for 30 sec and centrifuged at 3000×g for 15 min. This supernatant was diluted with buffer to a protein concentration of 0.2–0.7 mg (liver) or 0.6–2.4 mg (kidney and brain) per ml and used as the source of the enzyme. The reaction mixture contained 0.05 ml of diluted supernatant and 0.1 ml of 5 mM repurified<sup>7</sup>, 4-methylumbelliferyl sulfate in 0.2 M sodium phosphate buffer, pH 8.6. 3 replicates per sample were employed. The reaction was terminated after 30 min at

37°C by addition of 3 ml 0.085 M glycine-carbonate, pH 10. The fluorescence was measured with a Turner 111 fluorometer using 7–60 primary and 3–73 secondary filters. Zero time blanks and appropriate 4-methylumbelliferone standards were used throughout. The reaction was linear, for at least 45 min. Fluorescence should be determined within 1 h of termination of the assay.

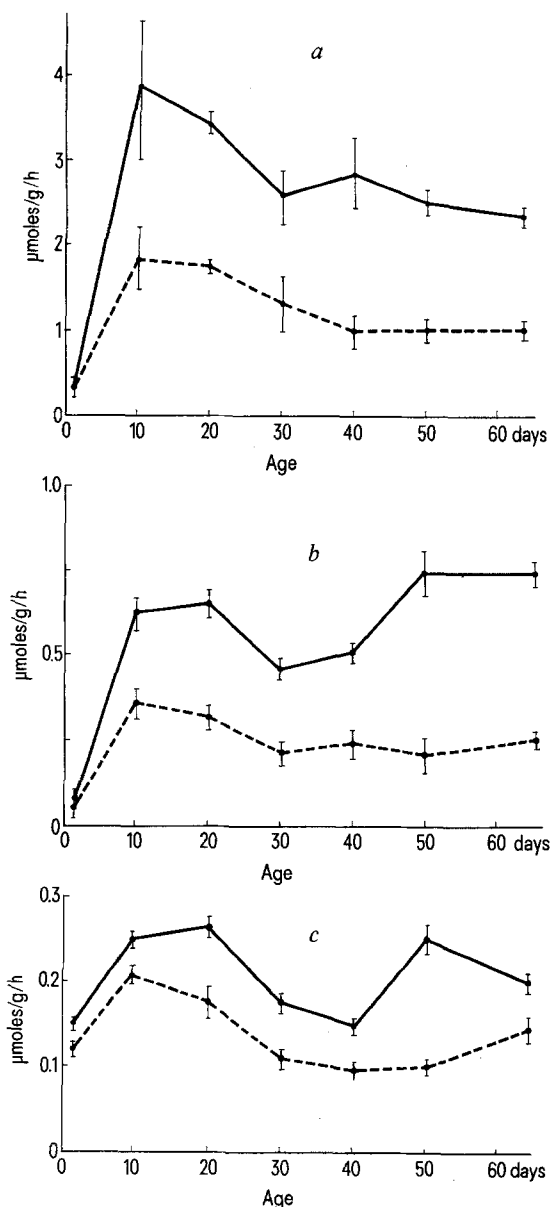


Table 1. Interstrain variation of adult male liver arylsulfatase C

Strain	Activity $\pm$ SEM	Strain	Activity $\pm$ SEM
CE/J	5.4 $\pm$ 0.53	MA/MYJ	2.4**
PL/J*	3.5 $\pm$ 0.27	RF/J	2.4 $\pm$ 0.25
SJL/J*	3.4 $\pm$ 0.73	RH/2J	2.3 $\pm$ 0.08
NZB/BINJ*	3.4 $\pm$ 0.12	SWR/J	2.2 $\pm$ 0.51
LP/J	3.3 $\pm$ 0.12	BDP/J*	2.2 $\pm$ 0.06
BALB/cJ*	3.2 $\pm$ 0.92	LG/J	2.1 $\pm$ 0.06
C57BR/cdJ	3.1 $\pm$ 0.10	MMM 272	1.9 $\pm$ 0.30
SEC/ReJ	3.0 $\pm$ 0.33	AU/SeJ	1.9 $\pm$ 0.10
SM/J	3.0 $\pm$ 0.15	C3H/HeJ	1.7 $\pm$ 0.42
MMM 287	2.9**	AKR/J	1.6 $\pm$ 0.38
DBA/2J	2.8 $\pm$ 0.22	BUB/BNJ	1.4 $\pm$ 0.60
C57BL/6J	2.8 $\pm$ 0.08	A/HeJ	1.1 $\pm$ 0.16
C57L/J	2.8 $\pm$ 0.06	A/J	1.0 $\pm$ 0.15

MMM=M. M. molossinus. Activities are expressed as  $\mu\text{moles}$  4-MU formed/g h and represent the means of 2 or 3 animals per strain. \*40 days of age. \*\*1 animal tested.

Developmental profiles of murine arylsulfatase C. a Liver, b kidney, c brain. Mean  $\pm$  SEM (3 animals/point). SJL/J (—), A/J (---).

Table 2. Tissue variation of arylsulfatase C

Tissue	SJL/J	A/J
Liver	2.51 ± 0.17	1.02 ± 0.15
Kidney	0.71 ± 0.07	0.17 ± 0.06
Brain	0.25 ± 0.02	0.10 ± 0.01
Testis	1.20 ± 0.10	0.55 ± 0.10
Spleen	0.59 ± 0.04	0.48 ± 0.08
Lung	0.40 ± 0.12	0.31 ± 0.04
Heart	0.14 ± 0.03	0.08 ± 0.01

Activities are expressed as  $\mu\text{moles/g/h}$ ; mean of 3 animals  $\pm$  SEM.

**Strain survey.** Liver aryl C activities for 26 murine strains are presented in table 1. SJL/J and A/J mice were selected as representatives of high- and low-activity strains, respectively. 2-fold or greater differences of aryl C activity were observed in liver, kidney, brain and testis. Variation of spleen, lung and heart aryl C activity appeared more conservative (table 2).

**Developmental profiles.** Developmental variation of aryl C activity is illustrated in the figure. Liver aryl C activity peaked at approximately 10 days, declined and increased to adult levels at 50 days of age. SJL/J aryl C activities were significantly higher than those of A/J mice at all stages tested after day 1. Developmental profiles for brain and kidney aryl C activities were similar to those observed in liver.

**Biochemical characterization.** The general biochemical properties of SJL/J and A/J kidney and liver aryl C are summarized in table 3. No significant interstrain variation of these properties was apparent. However, the kidney

Table 3. Biochemical properties of arylsulfatase C

	Liver	Kidney
pH optimum	8.7	8.2
Temperature optimum	46 °C	43 °C
$t_{1/2}$ 55 °C (min)	37	7
$K_m$ (mM)	0.65 ± 0.15	1.58 ± 0.15
Inhibition (%)		
5 mM <i>p</i> -nitrophenyl-SO <sub>4</sub>	53.5	
5 mM Na <sub>2</sub> SO <sub>3</sub>	45.9	
5 mM Na <sub>2</sub> SO <sub>4</sub>	10.8	
200 mM NaH <sub>2</sub> PO <sub>4</sub> -Na <sub>2</sub> HPO <sub>4</sub>	28.0	

enzyme possessed a higher  $K_m$  and was more thermolabile than the liver enzyme in all strains evaluated. These differences suggest that distinct enzymes occur in these 2 tissues or that post-translational processing of aryl C may differ in liver and kidney.

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## Genetic validation of a *Drosophila* learning task

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**Summary.** 4 inbred strains of *Drosophila melanogaster* and crosses between them were tested in 2 types of multiple-choice T-maze. It is suggested that genetic analysis can distinguish learning from other behaviours implicated in maze performance. Directional dominance for high performance, which is characteristic of learning in many species, was found only for those aspects of behaviour previously hypothesised as involving learning.

The ability of *Drosophila* to learn has been demonstrated with several different paradigms<sup>1-3</sup>, opening the way to detailed genetic analyses of learning. However, if *Drosophila* are to be used as a general model of learning and memory, it is first necessary to show parallels between their behaviour and that of higher organisms. It is already known that cooling<sup>4</sup> and cycloheximide<sup>5</sup> disrupt memory in *Drosophila* just as in vertebrates. The present paper describes a genetic similarity, in that there is directional dominance on a *Drosophila* maze task, similar to that found for rodent learning<sup>6</sup>. Secondly, because this dominance is found only for learning and not for alternative behaviours which critics<sup>7,8</sup> have suggested might explain performance in the mazes, this task is further validated as a measure of learning.

This experiment involved four strains of *Drosophila melanogaster*, their F<sub>1</sub>s and the intercross between these F<sub>1</sub>s. From 10 Leslie Manor (LM) strains, described and tested previously<sup>3</sup>, LM 20, 26, 27 and 28 were chosen as covering the complete range of variation from learning to no-learning. For the F<sub>1</sub>s, reciprocal crosses were made of LM 20 with 26 and of 27 with 28. Because of the number of flies available, the intercross generation involved only the

2 reciprocal crosses of (26 × 20) with (28 × 27). The subjects were 8 samples of 300 flies (150 male and 150 female) from each of the 4 strains and the 4 F<sub>1</sub>s and 16 samples from each of the 2 intercrosses, to give an identical number of flies (9600) tested from each generation. All testing was done at 25 °C on flies 3 days after eclosion, with testing beginning at the same time each day to avoid the problem of circadian rhythms of activity<sup>9</sup>.

4 of the 8 samples of each type were tested in the 'forced-choice' maze used previously<sup>3</sup> to study learning. This is a 10-choice multiple T-maze of the kind normally used to study taxes, but modified with passages blocked off, so that after initially going left or right (no differential light or gravity cues are provided) the flies are forced to make a sequence of 5 left or 5 right turns, depending on their initial choice. Then they are given a second choice, forced to make another 2 turns and given a final choice. The learning explanation of the performance in this maze is that within 1 run in the maze, the flies associate the sequence of turns they have to make with progress through the maze towards a battery of lights behind the end tubes. The only obvious reinforcement is the opportunity to make their normal positive phototactic response. The criterion of learning is