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ELECTROPHORETIC VARIATION FOR X CHROMOSOME-LINKED HYPOXANTHINE PHOSPHORIBOSYL TRANSFERASE (HPRT) IN WILD-DERIVED MICE

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ABSTRACT

An electrophoretic variation for hypoxanthine phosphoribosyltransferase, HPRT, has been identified in samples of *Mus spretus*, a field mouse from southern Europe and in *M. m. castaneus*, a house mouse from southeast Asia. These mice will interbreed with laboratory mice to produce viable, fertile F_1 progeny. The variation for HPRT segregates as an X chromosome gene in F_1 and backcross progeny. Linkage analysis involving the markers *Pgk-1* and *Ags* indicated a gene order of centromere—*Hprt*—*Pgk-1*—*Ags* in crosses involving both stocks of wild mice.

THE X chromosome linkage of the structural gene for hypoxanthine phosphoribosyl transferase, HPRT (E.C.2.4.2.8) has been established for several mammalian species (FRANCKE *et al.* 1977; PEARSON *et al.* 1979; SEEGMILLER 1976). The X chromosome assignment of the *Hprt* locus in the laboratory mouse, *M. musculus*, is based upon gene dosage studies in oocytes of XX and XO females (EPSTEIN 1972) and confirmed by somatic genetic analysis (FRANCKE *et al.* 1977; CHAPMAN and SHOWS 1976; HASHMI and MILLER 1976). More recently, the regional localization of the *Hprt* locus on the X chromosome was established in a somatic genetic analysis using the *T(X;16)16H* X/autosome translocation (FRANCKE and TAGGART 1980). The assignment of a gene order of centromere—*Hprt*—*Pgk-1*—*Ags* for X chromosome loci was based upon the separation of the mouse genes for *Hprt* and *Ags* in mouse-hamster somatic cell hybrids. These results indicated concordance for the *Hprt* locus on the mouse X chromosome centromere and for the mouse *Ags* locus on the X chromosome segment that had been translocated to chromosome 16. A direct test for the X chromosome order of these genes has not been previously possible because genetic variation for *Hprt* has not been available in laboratory stocks of *M. musculus* (NESBIT *et al.* 1979).

Surveys of feral populations of mice has provided genetic variants of other X chromosome loci, *Pgk-1* (NIELSEN and CHAPMAN 1977) and *Ags* (LUSIS and WEST 1976). We have employed similar surveys of feral *M. musculus* populations and interbreeding Mus species and subspecies from Europe and Asia to look for variation for HPRT. In this report we describe electrophoretic variation for HPRT found in *Mus spretus* and *Mus musculus castaneus*, a field mouse from

southern Europe and a house mouse from Bangkok, Thailand, respectively. These mice will interbreed with laboratory mice and the HPRT variation segregates as an X chromosome gene. Linkage analysis in crosses involving *M. spretus* and *M. m. castaneus* indicates a gene order of centromere—*Hprt*—*Pgk-1*—*Ags*, which is consistent with the order indicated by somatic genetic analysis using the X autosome translocation (FRANCKE and TAGGART 1980).

MATERIALS AND METHODS

Feral mice: Feral trapped mice from several sources have been established as breeding colonies in our laboratory. *M. spretus* from Montpellier, France was kindly provided to us by FRANÇOIS BONHOMME, Laboratoire d'Evolution des Vertebres, Montpellier, France. Additional samples from Spain were sent to us by RICHARD SAGE, Museum of Vertebrate Zoology, University of California. These stocks were pooled and are maintained as a randomly breeding colony, avoiding sib-matings. *M. m. castaneus* was sent to our laboratory in 1973 from Thailand by JOE MARSHALL. They have been maintained since that time as a randomly breeding colony with a concomitant development of two separate inbred lines.

Laboratory mouse stocks: A congenic C3H/HeHa strain C3H/HeHa·*Pgk-1*^{a} ($N_{10}F_{14}$) (designated as C3H·*Pgk-1*^{a}) carries the *Pgk-1*^{a} allele originally found in feral mice from Denmark (NIELSEN and CHAPMAN 1977). Recent tests also indicate that this stock carries a thermolabile allele of *Ags* that has heat inactivation kinetics that are similar to *Ags*^{m} (V. M. CHAPMAN, unpublished results).

A congenic C3H/HeHa·*Ags*^{m} ($N_{10}F_{8}$) strain (designated as C3H·*Ags*^{m}) was also used. This strain was developed by transferring the *Ags*^{m} thermolabile allele from the *M. m. molossinus* background to C3H/HeHa.

Additional crosses involving C3H/StHa were also performed. This strain has been maintained as an inbred line at Roswell Park since 1956 by T. HAUSCHKA and transferred to our laboratory in 1975.

Tissue preparation: Tissue samples were homogenized in a 0.25 M sucrose, 0.02 M imidazole, pH 7.4 buffer (10% w/v) with a polytron homogenizer. Supernatant solutions of the homogenates were obtained by centrifuging the samples for 20 min at 20,000 × g.

Electrophoresis: HPRT electrophoretic forms initially were separated on starch gels, but more recently have been separated on horizontal isoelectric focusing gels, using the procedure described by CHASIN and URLAUB (1976). The 12% starch gels were run vertically for 16 hr at 150 volts, using a citrate phosphate, pH 6.8 buffer (0.006 M citric acid, 0.03 M phosphate (K_2HPO_4) in the gel and 0.006 M citric acid, 0.835 M phosphate in both electrodes). Identification of HPRT activity after electrophoresis was achieved isotopically by applying a reaction mixture over the gels as described by CHASIN and URLAUB (1976).

PGK-1 electrophoretic forms were separated on cellulose acetate gels (Titan III, Helena Laboratories) using a barbital-citrate, pH 8.8 buffer, containing 20 mM sodium barbital, 10 mM sodium citrate, 5 mM $MgSO_4$ and 2 mM EDTA with 0.1 mg/ml dithioerythritol and 0.36 mg/ml AMP added to the cathodal buffer. PGK-1 activity was identified using a modification of the staining procedure described by BUCHER *et al.* (1980). Visualization of the bands was achieved by adding 4.0 mg of MTT tetrazolium and 0.19 mg of phenazine methosulfate to the reaction mixture.

Enzyme assays: α-Galactosidase activities were measured fluorometrically using the substrate 4-methylumbelliferyl α-D-galactosidase. The procedures for heat inactivation and activity assay were those described by LUSIS and WEST (1976).

RESULTS

HPRT electrophoretic patterns

By starch gel electrophoresis, the HPRT band of C3H is slower migrating than the HPRT band of either *M. spretus* or *M. m. castaneus* (Figure 1). The two phenotypes are designated HPRT-B and HPRT-A, respectively. Electrophoretic mobility varies between tissues in the increasing order of kidney, brain, spleen

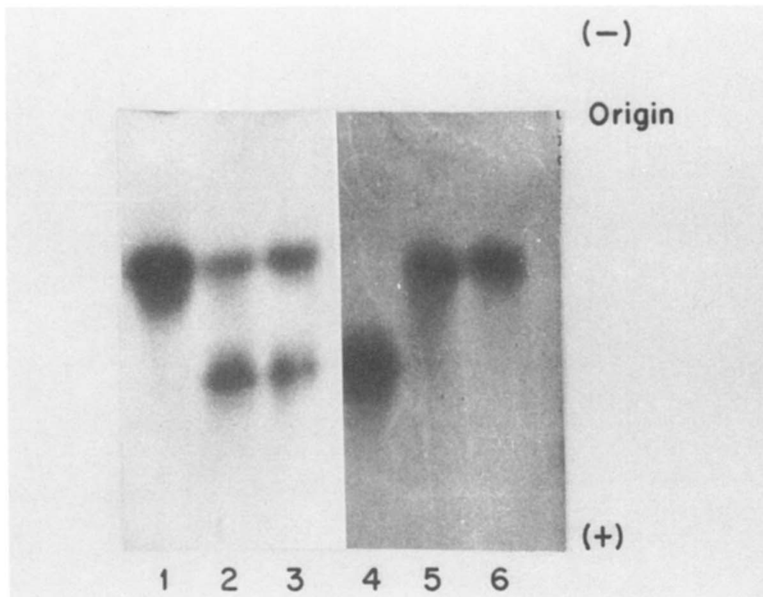

FIGURE 1.—Starch gel electrophoretic separation of HPRT in kidney homogenates. Lane 1: C3H/HeHa (HPRT-B); lanes 2, 3: (C3H/HeHa × *M. spretus*)F_1 females, (HPRT-AB); lane 4: *M. spretus* (HPRT-A); lanes 5 and 6: (C3H/HeHa × *M. spretus*)F_1 males (HPRT-B).

and liver. The mobility differences between tissues, however, are smaller than the differences between HPRT-A and -B.

Isoelectric focusing separates five HPRT bands for several C3H tissues, which we have designated HPRT-B_{1-5} (Figure 2). The entire pattern extends over approximately 0.4 pH units, ranging from pH 6.3 for HPRT-B_1 to pH 5.9 for HPRT-B_5. All five HPRT bands can be observed in brain, liver and spleen, with HPRT-B_1, B_3 or B_5 often being most intense. There is, however, much variation in the relative intensities of the bands between tissues from the same mouse, and for the same tissue between different mice of the same strain. By contrast, HPRT-B_1 is the major band in kidney homogenates, and this phenotype is stable with freezing and thawing of individual samples and between mice of the same strain. The HPRT-B form has also been observed in surveys of kidney in other laboratory stocks of mice, including the C3H congenic strains C3H·*Pgk-1*^{a} and C3H·*Ags*^{m}, the inbred strains C3H/St, C57BL/6J and BALB/cJ, and the random bred Ha/ICR.

Tissues from *M. spretus* and *M. m. castaneus* exhibit gel patterns that are uniformly more acidic than HPRT-B by approximately 0.4 pH units. We have designated the bands of the HPRT-A phenotype as HPRT-A_{0-5}, with HPRT-A_1 occurring at approximately the same pH as HPRT-B_5. As in the case of C3H, all six HPRT-A bands have been observed in brain, liver and spleen, with variable levels of activity in the different bands. The HPRT-A pattern of *M. spretus* and *M. m. castaneus* kidneys are likewise relatively stable between samples. A variable level of HPRT-A_0 may be present in all tissues. The electrophoretic

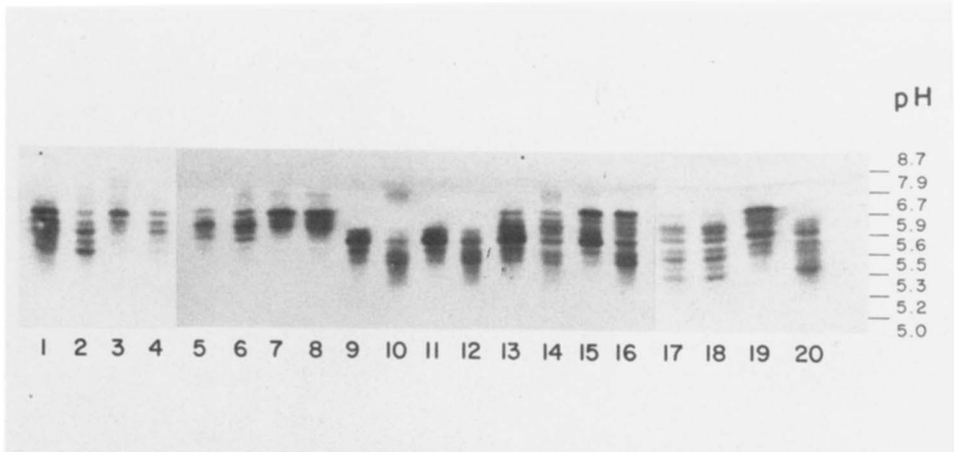

FIGURE 2.—Isoelectric focusing phenotypes of mouse tissue HPRT in a pH 5.0–8.0 gradient. *M. spretus* (HPRT-A) brain (lane 1); liver (2); kidney (3); spleen (4); *M. m. castaneus* (HPRT-A) brain (5); liver (6); kidney (7); spleen (8); C3H/HeHa (HPRT-B) brain (9); liver (10); kidney (11); spleen (12). 1:1 mixture of HPRT-A and HPRT-B brain (13); liver (14); kidney (15); spleen (16). (C3H/HeHa × *M. m. castaneus*)F_1 female (HPRT-A/B) brain (17); liver (18); kidney (19); and spleen (20).

pattern for *M. spretus* and *M. m. castaneus* tissues are similar, differing consistently only by more intense HPRT-A_3 and -A_5 bands in the liver of *M. m. castaneus*.

Segregation of HPRT electrophoretic forms

To determine whether the charge difference between HPRT-A and -B segregated as an X-chromosome gene variation, we examined the electrophoretic pattern of kidney HPRT in C3H × *M. spretus* or C3H × *M. m. castaneus* F_1 males and females (Table 1) and in backcrosses of F_1 females with C3H males (Table 1). F_1 males from C3H females crossed to either *M. spretus* or *M. m. castaneus* males were all HPRT-B like their mothers (Table 1), whereas F_1 females were all HPRT-AB heterozygotes. The HPRT phenotype of the F_1 females was similar to a 1:1 mixture of C3H and either *M. spretus* or *M. m. castaneus* (Figure 2). The relative intensities of the HPRT-A and -B bands were not grossly unequal (Figure 1). Some variation in the relative intensities of the HPRT-A and -B bands was present, which is consistent with an unequal proportion of cells expressing either the *M. musculus* or *M. spretus* (or *M. m. castaneus*) X chromosome, after random inactivation of one of the parental X chromosomes during development (LYON 1974).

Additional evidence for X-linkage of *Hprt*, the gene coding for HPRT, is from backcross matings of HPRT-AB females to HPRT-B males (Table 1). Female progeny had either an HPRT-AB or HPRT-B phenotype and male progeny had either an HPRT-A or HPRT-B phenotype (Figure 3). For backcrosses involving *M. spretus*, an identical number of HPRT-AB and HPRT-B progeny were produced among 74 female progeny. The ratio of HPRT-A to HPRT-B progeny among 147 males tested, however, showed a deficiency of HPRT-A ($P < 0.02$). The deficiency of HPRT-A males was not a consequence of a defect carried by

TABLE 1

Segregation of HPRT electrophoretic phenotypes in F_1 and backcross matings involving M. spretus *and* M. m. castaneus

Parents		Progeny						
		Males			Females			
Female	Male	N	HPRT-A	HPRT-B	N	HPRT-A	HPRT-AB	HPRT-B
M. spretus								
C3H·*Pgk-1*^{a}	*M. spretus*	5	0	5	5	0	5	0
(C3H·*Pgk-1*^{a} × *M. spretus*)F_1	C3H·*Pgk-1*^{a}	15	9	6	19	0	10	9
(C3H × *M. spretus*)F_1	C3H·*Ags*^{m}	76	29	47	27	0	14	13
(C3H/St × *M. spretus*)F_1	C3H	23	8	15	13	0	6	7
BC_1 (HPRT-AB)	C3H·*Pgk-1*^{a}	33	12	21	15	0	7	8
Backcross total[1]		147	58	89	74	0	37	37
M. m. castaneus								
C3H·*Pgk-1*^{a}	*M. m. castaneus*	6	0	6	7	0	7	0
M. m. castaneus	C3H·*Pgk-1*^{a}	10	10	0	6	0	6	0
(C3H·*Pgk-1*^{a} × *M. m. castaneus*)F_1	C3H·*Pgk-1*^{a}	105	48	57	74	0	29	45
BC_1 (HPRT-AB)	C3H·*Pgk-1*^{a}	26	10	16	53	0	13	29
Backcross total[2]		131	58	73	116	0	42	74

[1] Segregation in backcross males differs significantly from 1:1, $\chi^2 = 6.54$, $P < 0.02$.
[2] Segregation in females differs significantly from 1:1, $\chi^2 = 8.83$, $P < 0.01$.

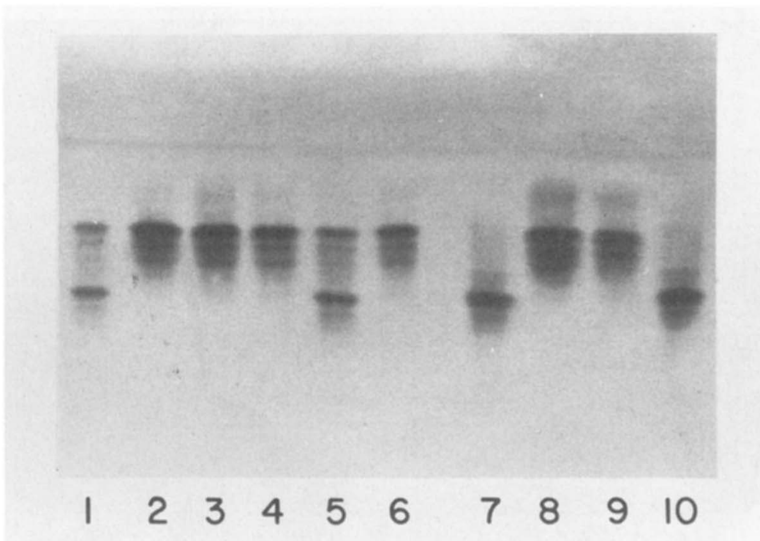

FIGURE 3.—Isoelectric focusing phenotypes of kidney HPRT in a pH 5.0–8.0 gradient. (C3H·*Pgk-1*^{a} × *M. m. castaneus*)F_1 × C3H·*Pgk-1*^{a} backcross females (lanes 1–5), HPRT-A/B (1); HPRT-B (2–4); HPRT-A/B (5); backcross males (6–10), HPRT-B (6, 8, 9); HPRT-A (7, 10).

a single *M. spretus* male. The situation was reversed for backcrosses involving *M. m. castaneus*, where the frequency of HPRT-A males was less than the frequency of HPRT-B males, but was not significantly different from 1:1 ($P > 0.10$). In females, the over-representation of HPRT-B progeny was greater than in males, and was significantly different from 1:1 ($P < 0.01$).

Characterization of α-galactosidase variation

Variation for α-galactosidase was further characterized in this study to serve as a marker along with *Pgk-1* in the mapping of *Hprt* on the X chromosome. Previously, a thermolabile variant for α-galactosidase was observed in the Japanese feral mouse *Mus musculus molossinus* (LUSIS and WEST 1976). The structural gene coding for α-galactosidase, *Ags*, has been transferred to the C3H congenic strain C3H·Ags^m. *Ags* has been mapped to a position 14.0 cM distal to *Pgk-1* on the X chromosome (LUSIS and WEST 1976).

In the course of linkage tests with *Ags*, we have discovered that α-galactosidase activity is increased in all the *M. spretus* tissues tested. The specific activity in the kidney is approximately twice as high in *M. spretus* as in C3H/HeHa and C3H·Ags^m (Table 2). In backcross males, the activity levels form a nonoverlapping bimodal distribution, with peak means similar to those in the parents, *M. spretus* and C3H·Ags^m. The increased α-galactosidase activity thus segregates as a single X-linked gene effect. The α-galactosidase activity distributions for backcross females are not as distinct as for the males, presumably because X inactivation reduces the upper peak to a broad average of the two parental levels.

Thermostability of *M. spretus* α-galactosidase is similar to that in inbred mice. In backcross males (Table 2), the high activity class had 40.2% activity remaining after 3 hr at 53°, whereas the low activity class had only 14.7% activity remaining. No recombinants for α-galactosidase activity and thermolability were observed in nearly 100 backcross progeny, suggesting that the increased α-galactosidase activity levels are allelic to the *Ags* locus established by thermostability. Thus, α-galactosidase activity levels, as well as thermolability, were used in the linkage analysis to more reliably indicate *Ags* segregation.

Mapping of HPRT electrophoretic variation

The position of *Hprt* on the *M. spretus* X chromosome relative to *Ags* and *Pgk-1* was determined by two-point and three-point crosses. Two-point linkage tests between *Hprt* and *Ags* were conducted in two crosses (Table 3). Cross 1 used the C3H·Ags^m congenic strain in the mating (C3H·Ags^m × *M. spretus*)F_1 × C3H·Ags^m and cross 2 combined matings of F_1 females derived from crosses of the inbred strains C57BL/6, C3H/St and BALB/c in the mating (inbred × *M. spretus*)F_1 × C3H·Ags^m. The combined recombination frequency between *Hprt* and *Ags* was 25.3 ± 4.6 and did not differ for the two crosses ($P > 0.10$). The recombination frequency was also similar among HPRT-A and -B progeny ($P > 0.10$), although there was an overall deficit of HPRT-A males.

The results of three-point crosses involving *M. spretus* are shown in Table 4. Cross 1 was the first backcross of the C3H·$Pgk\text{-}1^a$ congenic mated to *M. spretus*.

TABLE 2

Kidney α-galactosidase activity levels

Source of homogenate	Division of progeny	N	Specific activity[1] (mean ± S.E.)
M. spretus			6.2 ± 0.40
C3H/HeHa			3.7 ± 0.25
C3H·Ags^m			3.2 ± 0.18
(C3H·Ags^m × *M. spretus*)F_1 × C3H·Ags^m			
Males	Hi	28	7.7 ± 0.34
	Lo	30	3.2 ± 0.12
BC_1 (high α-gal) × C3H·Ags^m			
Males	Hi	46	8.4 ± 0.22
	Lo	44	4.0 ± 0.08
Females	Hi	38	6.3 ± 0.21
	Lo	29	4.0 ± 0.11

[1] Units μmol $hr^{-1}g^{-1}$ kidney.

TABLE 3

Recombination between Ags *and* Hprt *in* M. spretus *backcrosses*

Mating	Non-recombinant genotype			Recombinant genotype		
	Hprt	*Ags*	N	*Hprt*	*Ags*	N
(C3H·Ags^m × M. spretus)F_1 × C3H·Ags^m	*a*	s	16	*a*	*m*	2
$Hprt^b$ Ags^m/$Hprt^a$ Ags^s	b	*m*	28	b	s	12
Subtotal			44			14
(Inbred × M. spretus)F_1 × C3H·Ags^m	*a*	s	9	*a*	*h*	3
$Hprt^b$ Ags^h/$Hprt^a$ Ags^s	b	*h*	15	b	s	6
Subtotal			24			9
Total			68			23

Combined % recombination ± S.E. is 23/91 = 25.3 ± 4.6. In the second mating, the inbred strains were C57BL/6, C3H/St, and BALB/c.

Cross 2 was developed from backcross type matings from (inbred × *M. spretus*)F_1 females mated to C3H·*Pgk-1*^{a} congenics. The phenotypes for HPRT, PGK-1 and α-galactosidase were established in female progeny using tissue biopsy procedures. The gene order determined by the three-point cross is *Hprt*—*Pgk-1*—*Ags*. Recombination frequencies between *Hprt*—*Pgk-1* and *Pgk-1*—*Ags* were 17.1 ± 4.0% and 15.9 ± 4.0%, respectively. The overall *Hprt*—*Ags* recombination frequency of 32.9 ± 5.2% was not significantly different from that determined in the two-point cross ($P > 0.10$). No double recombinants were observed between these two regions, where the expected frequency was 2.7%.

The position of *Hprt* relative to *Pgk-1* and *Ags* on the *M. m. castaneus* X chromosome was determined by the three-point cross shown in Table 4. Only

TABLE 4

Three-point cross of Hprt, Pgk-1 *and* Ags *involving* M. spretus *and* M. m. castaneus

	Allelic combinations segregating			Cross 1		Cross 2			Cross 3			
								Combined spretus crosses	Tested for Ags	Not tested for Ags		Combined castaneus crosses
	Hprt	*Pgk-1*	*Ags*	♂	♀	♂	♀		♂	♂	♀	
Nonrecombinant	*a*	*b*	*s*	7	7	9	6	29	16	16	18	50
	b	*a*	*m*	3	6	15	2	26	37	9	36	82
Recombinant I	*a*	*a*	*m*	0	2	3	1	6	1	13	11	25
Hprt—Pgk-1	*b*	*b*	*s*	2	1	3	2	8	5	4	9	18
Recombinant II	*a*	*b*	*m*	2	1	0	0	3	2			2
Pgk-1—Ags	*b*	*a*	*s*	1	2	3	4	10	2			2
Double recombinant	*a*	*a*	*s*	0	0	0	0	0	0			0
	b	*b*	*m*	0	0	0	0	0	0	—	—	0
Total				15	19	33	15	82	63	42	74	179

Combined % recombination ± S.E. for crosses 1 and 2:
Cross 1: (C3H·*Pgk-1*^{a} × *M. spretus*)F_1 × C3H·*Pgk-1*^{a}
Cross 2: BC_1 (*Hprt*$^{a/b}$, *Pgk-1*$^{a/b}$, *Ags*$^{s/m}$) × C3H·*Pgk-1*^{a}
Hprt—Pgk-1 14/82 = 17.1 ± 4.2
Pgk-1—Ags 13/82 = 15.9 ± 4.0
Hprt—Ags 27/82 = 32.9 ± 5.2
% Recombination ± S.E. for cross 3:
(C3H·*Pgk-1*^{a} × *M. m. castaneus*)F_1 × C3H·*Pgk-1*^{a}
Hprt—Pgk-1 43/179 = 24.0 ± 3.2
Pgk-1—Ags 4/57 (136/179) = 5.3 ± 3.0
Hprt—Ags 29.3 ± 6.0

a selected group of mice were tested for α-galactosidase thermostability and activity, hence the percent recombination between *Pgk-1* and *Ags* is the product of the frequency of *Pgk-1—Ags* recombinants among nonrecombinants for the *Hprt—Pgk-1* region (4/57) and the frequency of nonrecombinants for *Hprt—Pgk-1* among total progeny (136/179). The percent recombination between *Pgk-1* and *Ags* is therefore 5.3 ± 3.0%, and the percent recombination between *Hprt* and *Pgk-1* is 24.0 ± 3.2%. The overall percent recombination between *Hprt* and *Ags* of 29.3 ± 6.0% and the frequencies for the intervals between the three genes in *M. m. castaneus* do not differ from their corresponding values in *M. spretus* ($P > 0.05$).

DISCUSSION

The HPRT electrophoretic pattern consists of combinations of six possible bands on isoelectric focusing gels. A consistent pattern from one mouse to the next is seen in the kidney, but other tissues have a variable pattern. The variation in band pattern from tissue to tissue suggests that post-translational modification is responsible for the multiple bands. No obvious correlations are

observed between the electrophoretic pattern in various tissues and the thermal stability reported for HPRT in these tissues (LO and PALMOUR 1979). The shift of the bands to more acid pHs in *M. spretus* and *M. m. castaneus* defines an HPRT-A phenotype that is similar to, but more acidic than the HPRT-B phenotype observed in C3H and other laboratory strains. The structural gene for HPRT is probably involved in the uniform acidic shift in the HPRT-A phenotype because of the additive expression of the HPRT-A and -B phenotypes in mixtures and in F_1 females.

M. spretus does not mate with *M. musculus* in its natural habitat, but will interbreed in the laboratory environment and produce viable F_1 progeny. Segregation of the HPRT phenotype, as well as the possibility of heterozygous females but not males, is consistent with X-linkage of *Hprt*, as has been demonstrated for *Hprt* in dosage (EPSTEIN 1972) and somatic genetic analysis (FRANCKE *et al.* 1977; CHAPMAN and SHOWS 1976; HASHMI and MILLER 1976). The gene order for *Hprt*, *Pgk-1* and *Ags* has been determined by three-point crosses and agrees with the gene order of centromere—*Hprt*—*Pgk-1*—*Ags* determined by somatic genetic analysis of the *T(X;16)16H* X/autosome translocation (FRANCKE and TAGGART 1980). Map distances averaged over *M. spretus* and *M. m. castaneus* for the regions *Hprt*—*Pgk-1* and *Pgk-1*—*Ags* are 20.6 and 10.6 cM, respectively. The relative map order of these genes on the long arm of the human X chromosome is centromere PGK—GALA—HPRT (FRANCKE and TAGGART 1980). Recombinational estimates for the relative distances of these genes is not available, but these findings demonstrate that the relative order of these genes has not been evolutionarily conserved between mouse and human.

The HPRT-A variant is similar in both *M. spretus* and *M. m. castaneus* in electrophoretic phenotype and in map distance from *Pgk-1* and *Ags* on the X chromosome. The recovery of $Hprt^a$ in progeny differs, however, between *M. spretus* and *M. m. castaneus*. In *M. spretus*, recovery of $Hprt^a$ from $Hprt^{a/b}$ mothers is deficient in sons but not daughters, whereas in *M. m. castaneus*, recovery of $Hprt^a$ in daughters is deficient, but only marginally so in sons.

In *M. spretus*, the 35% deficit of HPRT-A males is not caused by unequal segregation of the *M. spretus* X chromosome because of the equal recovery of the $Hprt^a$ and $Hprt^b$ alleles in females. The unequal frequency of HPRT-A and -B males could be accounted for by a 14% loss of males. HPRT phenotypes were not determined in all progeny. Of 650 total backcross progeny weaned, 349 were females and 301 were males. The deficit of males with the *M. spretus Hprt* is not accompanied by a deficit from the more distally located *Ags*. These results can be explained by any of several situations. 1) The $Hprt^a$ allele could produce reduced viability compared to the $Hprt^b$ allele, similar to the more severe human HPRT deficiency disease in the Lesch-Nyhan syndrome (SEEGMILLER 1976). Overtly, there are no pathological characteristics associated with males that are $Hprt^a$ or $Hprt^b$. The specific activity of HPRT is at least similar and possibly higher in $Hprt^a$ males than in $Hprt^b$ males in all tissues, with the exception of erythrocytes. In erythrocytes, the HPRT activity of $Hprt^a$ males is approximately 50- to 100-fold higher than that observed for $Hprt^b$ males (G. JOHNSON and V. M. CHAPMAN, unpublished data). A reduced viability associated specifi-

cally with *Hprt*^{a} seems unlikely, however, the *Hprt*^{a} of *M. spretus* may be coadapted generally with some autosomal loci. Coadaptation with autosomal loci would be consistent with a deficit of males in F_1 progeny, but not in *M. spretus*. 2) A DNA sequence that is located near *Hprt* could be lethal if interrupted by a crossover and combined with a non-*M. spretus* Y chromosome. According to this explanation, suppression that normally maintains the lethal in a benign state would be interrupted by a crossover with a frequency of about 30%. 3) The reciprocal situation to (2) is also possible, *i.e.*, that a chromosomal segment is lethal in combination with a non-*M. spretus* Y chromosome, if the segment is not interrupted by a crossover. Such a chromosomal segment could contain a series of genes that are coadapted to function with the *M. spretus* Y chromosome, and when in combination with a Y chromosome from other sources functions poorly. In both (2) and (3), the chromosomal segment would function normally in combination with X chromosomes from any source. These latter two possibilities could be differentiated by examining progeny from the reciprocal mating of *M. spretus* × C3H. Unfortunately, this mating so far has been infertile.

The unequal recovery of progeny receiving *Hprt*^{a} and *Hprt*^{b} in crosses with *M. m. castaneus* appears to result from a different cause than in the case of *M. spretus*. The sex ratio in the progeny from backcross matings is nearly equal (138 females:145 males) and the reduced recovery of the *M. m. castaneus Hprt*^{a} allele (147 *Hprt*^{b}:100 *Hprt*^{a}) is accompanied by a parallel reduction in the recovery of the distal *Pgk-1*^{b} allele (109 *Pgk-1*^{a}:70 *Pgk-1*^{b}). It appears either that the segregation of the *M. m. castaneus* X chromosome is at a disadvantage, or that the viability of embryos receiving the *M. m. castaneus* X chromosome is reduced. Reciprocal backcross matings to *M. m. castaneus* males are in progress to distinguish between these possible explanations.

Liver specific variation in α-galactosidase activity has been characterized among inbred strains of mice as an autosomally inherited locus (LUSIS and WEST 1978). The variation observed in *M. spretus* differs from the liver specific phenotype by altering activity levels in all tissues similar to the systemic variation observed for mouse β-galactosidase activity levels (FELTON, MEISLER and PAIGEN 1974). Furthermore, genetic crosses of *M. spretus* with mice which carry the thermolabile *Ags*^{m} allele indicate that the *M. spretus* α-galactosidase phenotype segregates as an allele of the X-linked *Ags* locus.

The occurrence of variation for *Hprt* in *M. spretus* and *M. m. castaneus* demonstrates the importance and utility of surveying the *Mus* gene pool for variation not present in inbred strains. The *M. m. castaneus* used in these studies were derived from a single population in Thailand. The *M. spretus* were derived from mice trapped in France and Spain. No polymorphism for *Hprt* has been detected among our samples suggesting that the *Hprt*^{a} allele may be monomorphic in *M. spretus*. However, our sampling of *M. spretus* is relatively limited and we cannot adequately estimate the extent of the *M. spretus* gene pool surveyed.

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LITERATURE CITED

BUCHER, T., W. BENDER, R. FUNDELE, H. HOFNER and I. LINKE, 1980 Quantitative evaluation of electrophoretic allo- and isozyme patterns. FEBS Lett. **115:** 319–324.

CHAPMAN, V. M. and T. B. SHOWS, 1976 Somatic cell genetic evidence for X chromosome linkage of three enzymes in the mouse. Nature **259:** 665–667.

CHASIN, L. A. and G. URLAUB, 1976 Mutant alleles for hypoxanthine phosphorylribosyl transferase: Codominant expression, complementation and segregation in hybrid Chinese hamster cells. Somatic Cell Genet. **2:** 453–467.

EPSTEIN, C. J., 1972 Expression of the mammalian X chromosome before and after fertilization. Science **175:** 1467–1468.

FELTON, J., M. MEISLER and K. PAIGEN, 1974 A locus determining β-galactosidase activity in the mouse. J. Biol. Chem. **249:** 3267–3272.

FRANCKE, U., P. A. LALLEY, W. MOSS, J. IVY and J. D. MINNA, 1977 Gene mapping in *Mus musculus* by interspecific cell hybridization: Assignment of the genes for tripeptidase-1 to chromosome *10*, dipeptidase-2 to chromosome *18*, acid phosphatase-1 to chromosome *12*, and adenylate kinase-1 to chromosome *2*. Cytogenet. Cell Genet. **19:** 57–84.

FRANCKE, U. and R. TAGGART, 1980 Comparative gene mapping: order of loci on the X chromosome is different in mice and humans. Proc. Natl. Acad. Sci. USA **77:** 3595–3599.

HASHMI, S. and O. J. MILLER, 1976 Further evidence of X-linkage of hypoxanthine phosphoribosyltransferase in the mouse. Cytogenet. Cell Genet. **17:** 35–41.

LO, Y-F. V. and R. M. PALMOUR, 1979 Developmental expression of murine HPRT. I. Activities, heat stabilities, and electrophoretic mobilities in adult tissues. Biochem. Genet. **17:** 737–746.

LUSIS, A. J. and J. D. WEST, 1976 X-linked inheritance of a structural gene for α-galactosidase in *Mus musculus*. Biochem. Genet. **14:** 849–855.

LUSIS, A. J. and J. D. WEST, 1978 X-linked and autosomal genes controlling mouse α-galactosidase expression. Genetics **88:** 327–342.

LYON, M. F., 1974 Mechanisms and evolutionary origins of variable X chromosome activity in mammals. Proc. Royal Soc. Lond. B **187:** 243–268.

NESBITT, M. N., B. BAKAY, M. B. GARDNER and C. DAY, 1979 Isoenzyme pattern of HPRT in murine erythrocytes: control by an autosomal locus. Biochem. Genet. **17:** 957–964.

NIELSEN, J. T. and V. M. CHAPMAN, 1977 Electrophoretic variation for X chromosome phosphoglycerate kinase (PGK-1) in the mouse. Genetics **87:** 319–325.

PEARSON, P. L., T. H. RODERICK, M. T. DAVISSON, J. J. GARVER, D. WARBURTON, P. A. LALLEY and S. J. O'BRIEN, 1979 Report of the committee on comparative mapping. Cytogenet. Cell Genet. **25:** 82–95.

SEEGMILLER, J. E., 1976 Inherited deficiency of hypoxanthine guanine phosphoribosyltransferase in X-linked uric aciduria (the Lesch-Nyhan syndrome and its variants). Adv. Human Genet. **6:** 75–163.

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