# Variation for $X$ chromosome expression in mice detected by electrophoresis of phosphoglycerate kinase

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## SUMMARY

The proportions of the two isozyme bands of the X-linked form of phosphoglycerate kinase (PGK-1) were compared in 16 tissues from four groups of adult heterozygous females. Little evidence was found for differences in expression of the two isozymes among tissues but there was a marked difference among the four groups of mice. The proportion of the PGK-1B enzyme was consistently lower in PGK-1AB heterozygous daughters of C3H/HeHa females than in corresponding heterozygotes with a C57BL/6Ha, DBA/2Ha or JBT/Jd mother. This difference was also observed in foetuses on the fourteenth day of gestation irrespective of whether the C3H/HeHa $X$ chromosome was derived from the mother or the father. Sequential sampling of blood from the same heterozygous females provided no evidence for genetically determined cell selection in the adult erythropoietic tissue. The observed differences probably reflect variation at an $X$-chromosome controlling element locus among inbred strains of mice, similar to that described by Cattanach & Williams (1972) using $X$-linked morphological markers, although this has yet to be tested.

## 1. INTRODUCTION

Female eutherian mammals are functional mosaics with respect to $X$-chromosome expression since it is now well established that only one of the two $X$ chromosomes is active in each cell (Lyon, 1961, 1974). Frequently the cell population expressing the maternally derived $X$ chromosome ($X^m$) and the population expressing the paternally derived $X$ chromosome ($X^p$) are present in almost equal proportions. However, unbalanced mosaicism, where one cell population predominates has been reported in a number of cases. Several lines of mouse $X$ inactivation mosaics have been established which are unbalanced for $X$-linked coat colour markers (Cattanach & Isaacson, 1965; Grahn, Lea & Hulesch, 1970; Krzanowska & Wabik, 1971; Falconer & Isaacson, 1972; Ohno *et al.* 1973). The studies of Cattanach and his colleagues (reviewed by Cattanach, 1975) revealed a locus, designated $X$ chromosome controlling element ($Xce$), located close to the tabby ($Ta$) locus. Variation for $Xce$ also appears to exist among different inbred strains of mice (Cattanach & Williams, 1972). This locus may be equivalent to the

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'inactivation centre' postulated by Russell (1964). Other loci that have similar effects and map in the same region of the $X$ chromosome as $Xce$ have been designated $Cg$ (Grahn *et al.* 1970) and $O^{hv}$ (Ohno *et al.* 1973; Drews *et al.* 1974) but these may be identical to $Xce$. Females, heterozygous at the $Xce$ locus, are normally unbalanced with respect to the expression of a number of $X$-linked marker genes with morphologically detectable effects. It is not yet known whether the imbalance is a result of non-random $X$ chromosome inactivation or a result of cell selection.

Variation among tissues for the balance in expression of the two $X$ chromosomes has been reported on several occasions. Hook & Brustman (1971) found preferential expression of the horse $X$ chromosome in three of six tissues and organs examined in the mule and there have been several cases of human $X$-inactivation mosaics that only had one of the two possible cell populations in the blood (Nance, 1964; Gandini *et al.* 1968; Nyhan *et al.* 1970). These examples may reflect tissue specific cell selection rather than non-random $X$ chromosome inactivation. Other studies of mouse and human $X$-inactivation mosaics (Linder & Gartler, 1965; Nesbitt, 1971; Fialkow, 1973) have revealed no striking differences in proportions of the two cell populations among different tissues.

This study represents a preliminary survey to look for variants of an $Xce$ type of locus among inbred strains of mice and to look for variation in $X$ chromosome expression among different tissues and organs, using the electrophoretic variant of phosphoglycerate kinase (PGK-1) discovered by Nielsen & Chapman (1977).

## 2. MATERIALS AND METHODS

### (i) *Genetics*

The electrophoretic variant recently described by Nielsen and Chapman (1977) for $X$-linked phosphoglycerate kinase (PGK-1) was used as a marker for $X$ chromosome expression. This variant form is designated PGK-1$A$ and is clearly separated from the more common PGK-1B enzyme using starch-gel electrophoresis. The structural genes coding for the PGK-1A and PGK-1B isozymes are designated $Pgk-1^a$ and $Pgk-1^b$ respectively. All males, with only one $X$ chromosome, are hemizygous and have either the PGK-1A or PGK-1B single-banded phenotype, whereas all the females from the crosses in our experiments were heterozygous $Pgk-1^a/Pgk-1^b$ and had the PGK-1AB double-banded phenotype.

Non-inbred mice derived from feral *Mus musculus musculus* from Denmark (Nielsen & Chapman, 1977) were used as the source of the $Pgk-1^a$ allele. Four inbred strains, C3H/HeHa, C57BL/6Ha, DBA/2Ha and JBT/Jd, were used as the source of the $Pgk-1^b$ allele. The C3H/HeHa mice were obtained from the Roswell Park Production Colony at West Seneca, New York, and all other mice were bred and maintained in the Molecular Biology Department.

### (ii) *Dissections and sample preparation*

Conceptuses were removed from the uterus on the fourteenth day of gestation (13 days after vaginal plug). The foetuses were dissected free from the placentas

and membranes, under a dissecting microscope, and homogenized in 60 $\mu$l of water using a glass pestle. Blood samples were taken at 30-day intervals from the orbital sinus of adult heterozygous females under light ether anaesthesia. Red cells were pelleted by low-speed centrifugation, washed in isotonic saline and resuspended in distilled water. Adult females were partially exsanguinated under ether anaesthesia, killed in an atmosphere of carbon dioxide and intact organs were removed to ice. Before the organs were homogenized the contents of the stomach and intestine were washed out, the liver, lungs and heart were washed in ice-cold isotonic saline and the gall bladder and kidney capsules were removed. The organs used for electrophoretic analysis were homogenized in 0·2 ml (spleen, heart, thymus, tongue and stomach), 0·3 ml (lungs and uteri), 0·5 ml (brain, kidneys, intestines and pancreas) or 1·0 ml (liver and limb muscle) of distilled water, using a Polytron homogenizer. The bone marrow was flushed from both femurs with isotonic saline using a syringe and 26-gauge needle. The resulting suspension was centrifuged, and resuspended in distilled water. The foetal samples were coded and all samples were stored at $-20$ °C until used. Most samples were analysed within 2 weeks of collection. Immediately before electrophoresis the samples were centrifuged at a low speed (approximately 500$g$) for 20 min to remove cell debris from the supernatent. Although both male and female foetuses were analysed only the results from female foetuses were considered. The females were all heterozygous and were recognized by their double banded PGK-1AB phenotype.

Adult brains, livers and kidneys for spectrophotometric assays were homogenized in distilled water (10 %, w/v) in a Polytron homogenizer and centrifuged at 42000$g$ for 45 min. The supernatants were used for assays of PGK activity and protein content.

(iii) *Electrophoretic analysis*

Starch-gel electrophoresis was carried out with 12 % electrostarch using a pH 7·0 Tris-Citrate buffer system. Staining for PGK was done as described by Beutler (1969) using an agar overlay in place of the filter paper originally described. Bands of PGK activity were visualized under long-wavelength ultraviolet illumination as dark bands against a fluorescent background of NADH. The proportions of the two isozyme bands of PGK-1 were classified according to a five-point scale as previously described (West *et al.* 1977).

In order to test whether the PGK-1B enzyme from C3H/HeHa mice stained more intensely on the gels than the enzyme from C57BL/6Ha and DBA/2Ha, coded artificial mixtures of liver homogenates were analysed. Nine series of 11 artificial mixtures ranging from 0 to 100 % PGK-1A in steps of 10 % were prepared. Liver homogenates (33 %, w/v) from three *Pgk-1ᵃ*/Y males were each mixed in turn with one of three sets of male liver homogenates from each of the three strains of inbred mice. The 99 individual mixtures were coded and after electrophoresis they were scored for PGK according to the five point scale and then decoded.

### (iv) *Enzyme assays*

Phosphoglycerate kinase activity was determined spectrophotometrically as described by Yoshida & Watanabe (1972) and the protein content was determined by the method of Lowry *et al.* (1951).

### 3. RESULTS

The spectrophotometric assays of PGK, summarized in Table 1, show that there is no significant difference in PGK activity in liver, brain and kidney either among the four inbred strains used or between $Pgk-1^a$/Y and $Pgk-1^b$/Y mice.

Table 1. *Specific activity for PGK in* Pgk-1$^a$/Y *males and males from four inbred strains* (Pgk-1$^b$/Y)

| Males | Pgk-1 allele | PGK activity* | | | No. of mice |
|---|---|---|---|---|---|
| | | Liver | Brain | Kidney | |
| $Pgk-1^a$/Y† | a | $2.90 \pm 0.29$ | $3.25 \pm 0.02$ | $3.70 \pm 0.13$ | 2 |
| C3H/HeHa | b | $2.55 \pm 0.22$ | $3.17 \pm 0.19$ | $3.61 \pm 0.12$ | 5 |
| C57BL/6Ha | b | $2.40 \pm 0.06$ | $3.47 \pm 0.10$ | $4.08 \pm 0.01$ | 3 |
| DBA/2Ha | b | $2.19 \pm 0.07$ | $3.10 \pm 0.08$ | $3.22 \pm 0.11$ | 3 |
| JBT/Jd | b | $2.08 \pm 0.12$ | $3.12 \pm 0.16$ | $3.28 \pm 0.12$ | 3 |

\* PGK activity is expressed as m-mol of NADH oxidized/min/g protein. The mean specific activity and standard error is shown for each group.

† The $Pgk-1^a$/Y males assayed had been backcrossed to the C3H/HeHa strain for six generations and so were 98 % congenic with this strain.

The proportions of the two PGK-1 isozyme bands were classified for a large number of organs and tissues from adult heterozygous females and for homogenates of entire female foetuses dissected on the fourteenth day of gestation. The classification was according to the five-point scale described in Table 2. Previous experiments involving artificial mixtures indicated that both PGK-1A and PGK-1B stain with similar intensity on the gels and that a minor component representing 5–10 % of a homogenate mixture could be detected (West *et al.* 1977).

Table 2 shows the mean PGK scores for 16 organs and tissues from each of four groups of heterozygous females. Each of the 27 females analysed was sired by the same $Pgk-1^a$/Y father but the mothers were from four different inbred strains. No large consistent differences in mean PGK score were seen among the 16 organs and tissues although some small differences are apparent. For example, the pancreas tends to have a higher mean PGK score (more PGK-1A activity) than most other organs and tissues, in all four groups. A more sensitive quantitation technique will probably be necessary to determine whether these differences are significant.

A much more striking difference was observed for the overall mean PGK score between the four groups of heterozygotes. The mice with C3H/HeHa mothers had a markedly higher mean PGK score than any of the other three groups. This difference was consistent for all organs and tissues apart from limb muscle.

Table 2. *Mean PGK scores\* for 16 tissues and organs from four groups of 5- to 7-month-old heterozygous PGK-1AB females*

| Female parent | C3H/HeHa(9)† | C57BL/6Ha(10) | DBA/2Ha(4) | JBT/Jd(4) |
|---|---|---|---|---|
| Limb muscle | 2·44 ± 0·18 (9)‡ | 2·80 ± 0·20 (10) | 2·25 ± 0·25 (4) | 2·50 ± 0·29 (4) |
| Thymus | 3·00 ± 0·45 (5) | 2·56 ± 0·24 (9) | 2·25 ± 0·25 (4) | 2·25 ± 0·25 (4) |
| Red blood cells | 3·11 ± 0·31 (9) | 2·30 ± 0·15 (10) | 2·25 ± 0·25 (4) | 2·25 ± 0·25 (4) |
| Tongue | 3·11 ± 0·31 (9) | 2·30 ± 0·15 (10) | 2·00 ± 0 (4) | 2·25 ± 0·25 (4) |
| Intestine | 3·22 ± 0·22 (9) | 3·00 ± 0·19 (8) | 2·25 ± 0·25 (4) | 2·00 ± 0 (3) |
| Brain | 3·38 ± 0·18 (8) | 2·90 ± 0·10 (10) | 2·50 ± 0·29 (4) | 3·00 ± 0 (4) |
| Liver | 3·44 ± 0·18 (9) | 2·90 ± 0·10 (10) | 2·75 ± 0·48 (4) | 2·75 ± 0·25 (4) |
| Heart | 3·44 ± 0·24 (9) | 2·90 ± 0·18 (10) | 2·25 ± 0·25 (4) | 2·50 ± 0·29 (4) |
| Stomach | 3·56 ± 0·24 (9) | 2·70 ± 0·15 (10) | 2·50 ± 0·29 (4) | 2·50 ± 0·29 (4) |
| Uterus | 3·57 ± 0·20 (7) | 2·50 ± 0·19 (8) | 2·50 ± 0·29 (4) | 2·50 ± 0·29 (4) |
| Bone marrow | 3·63 ± 0·26 (8) | 2·33 ± 0·17 (9) | 2·00 ± 0 (2) | 2·25 ± 0·25 (4) |
| Left kidney | 3·67 ± 0·17 (9) | 2·80 ± 0·13 (10) | 3·00 ± 0·41 (4) | 2·50 ± 0·29 (4) |
| Spleen | 3·71 ± 0·18 (7) | 2·50 ± 0·22 (10) | 2·00 ± 0 (4) | 2·00 ± 0 (4) |
| Pancreas | 3·71 ± 0·18 (7) | 3·30 ± 0·21 (10) | 3·25 ± 0·48 (4) | 3·25 ± 0·48 (4) |
| Lungs | 3·86 ± 0·14 (7) | 3·20 ± 0·25 (10) | 2·50 ± 0·29 (4) | 2·75 ± 0·48 (4) |
| Right kidney | 3·89 ± 0·11 (9) | 3·00 ± 0·15 (10) | 2·50 ± 0·29 (4) | 2·75 ± 0·25 (4) |
| Mean | 3·42 ± 0·06 (130) | 2·75 ± 0·05 (154) | 2·44 ± 0·08 (62) | 2·51 ± 0·07 (63) |

\* The proportions of PGK-1A and PGK-1B were expressed on a five point scale: 1, only PGK-1B detected; 2, PGK-1B > PGK-1A; 3, PGK-1B > PGK-1A; 4, PGK-1A > PGK-1B; 5, only PGK-1B detected. All mice analysed were sired by the same *Pgk-1ᵃ*/Y male.

† Number of heterozygous females analysed.     ‡ Mean ± standard error (number in group).

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The observation that red blood cells had a lower mean PGK score than most other organs and tissues in the first group makes it unlikely that the overall difference was solely a result of blood contamination of all tissues and organs.
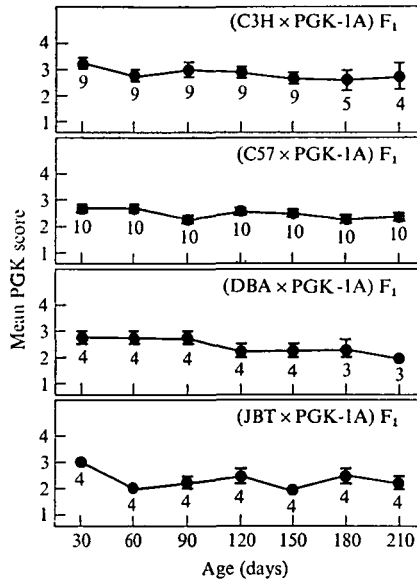


Fig. 1. Mean PGK scores for red blood cells from 30 to 210 days for four groups of heterozygous females. The 27 mice analysed were all sired by the same $Pgk\text{-}1^a/Y$ male and were sampled at 30-day intervals. The vertical bars represent the standard errors of the mean PGK scores and the numbers below each point represent the number of mice analysed. The numbers decrease with age for two of these groups because several mice were killed before 210 days. C3H = C3H/HeHa; C57 = C57BL/6Ha; DBA = DBA/2Ha; JBT = JBT/Jd.

The mean PGK scores for red blood cells for each of these four groups of heterozygous females remained approximately constant between 1 and 7 months after birth (Fig. 1). There was some fluctuation between consecutive sample times, particularly in the groups containing small numbers of mice, but no overall trend was apparent. The absence of a progressive widening of the gap, between the mean PGK scores for the first group and the mean scores for the other three groups, implies that no significant genetically determined cell selection occurred in erythropoietic tissue during this period.

Comparisons of the mean PGK scores, between heterozygous female foetuses deriving their $Pgk\text{-}1^b$ allele from C3H/HeHa parents and those deriving this allele from either C57BL/6Ha or DBA/2Ha parents, are shown in Table 3. The heterozygous foetuses shown in experiment 1 of Table 3 all shared the same $Pgk\text{-}1^a/Y$ father as the heterozygous females shown in Table 2 whereas numerous parents of both sexes were used to produce the foetuses shown in Exp. 2. The PGK scores for foetuses with C3H/HeHa parents were significantly higher than for foetuses with either C57BL/6Ha or DBA/2Ha parents in all three crosses shown in Table 3. Thus, the difference in mean PGK scores between hetero-

Table 3. *Mean PGK scores for heterozygous female foetuses from different genetic crosses on the fourteenth day of gestation*

| Exp. no. | Cross Female × Male | Source of $Pgk\text{-}1^b$ allele | No. of foetuses | Mean PGK score for foetuses ($\pm$ s.e.m.) | Significance |
|---|---|---|---|---|---|
| 1* | $Pgk\text{-}1^b/Pgk\text{-}1^b \times Pgk\text{-}1^a/Y$ | C3H/HeHa | 26 | $3\text{·}38 \pm 0\text{·}10$ | $\chi^2_{(2)} = 18\text{·}097;\ P < 0\text{·}001$ |
| | | C57BL/6Ha | 20 | $2\text{·}60 \pm 0\text{·}11$ | |
| 2 | $Pgk\text{-}1^b/Pgk\text{-}1^b \times Pgk\text{-}1^a/Y$ | C3H/HeHa | 54 | $3\text{·}39 \pm 0\text{·}07$ | $\chi^2_{(2)} = 6\text{·}116;\ P < 0\text{·}05$ |
| | | DBA/2Ha | 52 | $3\text{·}15 \pm 0\text{·}06$ | |
| | $Pgk\text{-}1^a/Pgk\text{-}1^a \times Pgk\text{-}1^b/Y$ | C3H/HeHa | 34 | $3\text{·}50 \pm 0\text{·}09$ | $\chi^2_{(2)} = 9\text{·}901;\ P < 0\text{·}01$ |
| | | DBA/2Ha | 36 | $3\text{·}11 \pm 0\text{·}08$ | |

* In Exp. 1 the same $Pgk\text{-}1^a/Y$ male parent was used to produce all the foetuses analysed whereas in experiment 2 several mice of each genotype were used as parents.

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zygous females with a C3H/HeHa mother and the other groups of adults shown in Table 2 also occurs in foetuses on the fourteenth day of gestation. This difference was maintained irrespective of whether the C3H/HeHa $X$ chromosome was derived from the mother or from the father in experiment 2 shown in Table 3. The consistently higher mean PGK scores for heterozygous offspring of C3H/HeHa mice, compared to offspring from other strains, could either result from non-random $X$ chromosome expression or indicate a trivial difference between inbred strains in the staining of PGK-1B. The second possibility seems unlikely from the similar specific activities of PGK among the four inbred strains used, shown in Table 1. This possibility was rejected after electrophoretic analysis of nine series of coded artificial mixtures showed no consistent difference between the PGK scores for the mixtures containing C3H/HeHa liver homogenate and the equivalent mixtures involving DBA/2Ha or C57BL/6Ha tissue. Thus, the expression of the variant $X$ chromosome, carrying the $Pgk\text{-}1^a$ allele, occurs more frequently in heterozygous females with a C3H/HeHa parent than in heterozygotes with a C57BL/6Ha, DBA/2Ha or JBT/Jd parent.

## 4. DISCUSSION

Cattanach & Williams (1972) reported that a similar increase in the expression of the variant $X$ chromosome (carrying either the tabby ($Ta$) or viable brindled ($Mo^{vbr}$) gene) occurred in $F_1$ hybrids with a C3H/HeH parent compared with $F_1$ hybrids with a parent from either C57BL/H-$a^t$ or JU/FaCt strains. These observations on different $X$-linked loci, using two similar strains of mice to those used in the present study, imply that the expression of the whole $X$ chromosome is probably similarly affected. The results reported here provide evidence for a strain difference in the expression of the two $X$ chromosomes in heterozygous female offspring. This strain difference could be the result of autosomal or $X$-linked genes but, from previous work (reviewed by Cattanach, 1975), it seems likely that the relative expression of the two $X$ chromosomes is controlled by the differences, between the alleles or 'states' of a locus such as $Xce$, in the two $X$ chromosomes. Females homozygous at the $Xce$ locus are balanced $X$-inactivation mosaics with both $X$ chromosomes having an equal probability of being expressed. However, one $X$ chromosome is predominantly expressed in females heterozygous at the $Xce$ locus. The experiments of Cattanach & Williams (1972) and the present results are most readily interpreted as evidence that different inbred strains of mice carry different alleles of the $X$-linked $Xce$ locus. If so, the strains C57BL/6Ha, DBA/2Ha and JBT/Jd may carry the same $Xce$ allele. This allele is presumably different from the C3H/HeHa $Xce$ allele and both may be different from the allele carried by the $Pgk\text{-}1^a$ stock used in these experiments. Assuming that $Xce$ is involved, the predominance of one cell population in the foetus could arise either if the $Xce$ locus acted *cis* and influenced the probability of the inactivation of its chromosome or if the $Xce$ locus exerted a strong selection pressure in favour of one cell population after $X$ chromosome inactivation occurred.

Non-random $X$ chromosome expression has been reported in a number of different situations. In some cases one $X$ chromosome is either not expressed or expressed at a very low level. This occurs in marsupials (reviewed by Cooper *et al.* 1975), certain extraembryonic membranes of mice and rats (Takagi & Sasaki, 1975; Wake, Takagi & Sasaki, 1976; West *et al.* 1977) and where one $X$ chromosome carries a deletion (Grumback, Morishima & Taylor, 1963) or a reciprocal $X$ chromosome/autosome translocation, like Searle's translocation in the mouse, (Eicher, 1970). Exclusive expression of one $X$ chromosome in the blood of certain human $X$-inactivation mosaics (Nance, 1964; Gandini *et al.* 1968; Nyhan *et al.* 1970) also occasionally occurs. Less extreme cases of non-random $X$ chromosome expression have been reported for humans (Nance, 1964; Ropers *et al.* 1977) mules (Hook & Brustman, 1971) and the various stocks of mice already discussed. There is some evidence for cell selection in mosaic cell populations *in vitro* (Gartler & Linder, 1964; Hamerton *et al.* 1971; Rattazzi & Cohen, 1972) and it seems likely that cell selection also produces some of the cases of unbalanced mosaicism seen *in vivo* (Gartler, 1976). Despite attempts to rule out cell selection as the cause of non-random $X$ chromosome expression (Drews *et al.* 1974; Takagi, 1976; West *et al.* 1977) there are still no examples where cell selection has been unequivocally disproved.

A temporal shift towards the predominance of one of two cell populations has been reported for red blood cells of some spontaneous chimaeric cattle twins (Stone, Friedman & Fregin, 1964), human blood chimaeras (Race & Sanger, 1968), sheep chimaeras (Tucker, Moor & Rowson, 1974) and mouse aggregation chimaeras (Mintz & Palm, 1969; West, 1977). The absence of such a temporal shift in peripheral red blood cells from our four groups of mouse $X$-inactivation mosaics indicates that no marked genetically determined cell selection is acting on the erythropoietic tissue of the adult although cell selection could occur before the first blood sample was taken. The similarities between the results from adult and foetal heterozygotes, however, suggests that if selection is the sole cause of the unbalanced mosaicism it must act before the fourteenth day of gestation.

Although no consistant temporal shift in the proportions of the two red blood cell populations occurred, quite marked fluctuations were occasionally seen in blood samples from individual mice. The most extreme example was seen in a mouse that was bled on the day of dissection and 6 days previously. During these 6 days the PGK score changed from 2 to 4. This probably represents a minimum increase from 40 % to 60 % PGK-1A. Rapid changes such as this may reflect the mitotic activity of only a proportion of the erythropoietic stem cells during the 6 days after the depletion of the blood at the first of these two sampling times. This observation underlines the importance of sampling from several animals in each group over a long period of time.

The general similarities in PGK score among tissues within a group of mice is in keeping with other investigations in both humans (Linder & Gartler, 1965; Fialkow, 1973) and mice (Nesbitt, 1971). Two of the major difficulties that arise when comparing the proportion of two isozymes of enzymes, such as PGK-1 or

G6PD, in different tissues are contamination from blood and the difficulty of cleanly dissecting the different tissues from organs. No attempt was made in this preliminary survey to overcome either problem since we were initially looking for large differences among tissues. Although the adults were partially exsanguinated before death no attempt was made to perfuse the organs. Whole organs or tissues were analysed wherever possible to avoid sampling unrepresentative areas but in organs comprising several tissue types any variation between the tissues will have been missed.

In conclusion, we have demonstrated an influence of the genome of different inbred strains of mice on the relative expression of the two alleles of $Pgk$-$1$ in a large number of tissues from heterozygous females. These results are similar to those described by Cattanach & Williams (1972) for $Ta$ and $Mo^{vbr}$ and we assume that our results are also produced by variation for an $Xce$ type of locus among inbred strains of mice. This difference can be detected on the fourteenth day of gestation but analysis of younger embryos will be necessary to determine whether the unbalanced $X$-chromosome mosaicism is a result of cell selection or non-random $X$-chromosome inactivation.

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