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Parental influences on expression of glucose-6-phosphate dehydrogenase, *G6pd*, in the mouse; a case of imprinting

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Summary

Glucose-6-phosphate dehydrogenase (G6PD) activity was measured in blood from heterozygotes for the normal allele $G6pd^a$ and the low activity allele $G6pd^{a\text{-}m1Neu}$. In adult mice lower activity was found in $G6pd^a/G6pd^{a\text{-}m1Neu}$ than in the reciprocal heterozygote $G6pd^{a\text{-}m1Neu}/G6pd^a$ (the maternal allele being listed first). Thus, either the paternally derived allele was over-expressed or the maternally derived allele was under-expressed. By contrast, in younger mice the difference in G6PD activity in reciprocal crosses was less marked. The findings are interpreted in terms of differential imprinting of maternally and paternally inherited information. The explanation offered for age related differences is that, as a consequence of imprinting, either the paternal X-chromosome is preferentially reactivated, or cells in which the paternally derived allele is active are at a selective advantage, and proliferate better than those in which the maternally inherited allele is active.

1. Introduction

Both genetic factors and parental source can lead to non-random X-chromosome inactivation in mice. The proportion of cells with one or other X-chromosome active is determined by alleles at the *Xce* locus, the X-chromosome controlling element. Whereas X-chromosome inactivation is random in homozygotes, the three known *Xce* alleles act cis and in a hierarchical manner such that the probability of the X-chromosome on which they are located being active is in the order $Xce^c > Xce^b > Xce^a$ (Cattanach, 1975).

Two effects on the randomness of X-chromosome expression have been attributed to the parental source of the X-chromosome. The first is demonstrable in certain extra-embryonic tissues, for both cytogenetic and biochemical studies have shown that the paternally derived X-chromosome is preferentially inactivated in certain extra-embryonic membranes, although inactivation which is more nearly random occurs in the cells of the definitive embryo (Takagi & Sasaki, 1975; Takagi, 1978; West *et al.* 1977, 1978; Frels & Chapman, 1980; Frels *et al.* 1979; McMahon *et al.* 1981; Papaioannou & West, 1981). The second effect has been shown in somatic cells, and results in a slight preferential expression of either the paternally derived X-chromosome or the maternally derived X-chromosome. In several experiments, a slight increase in the probability of expression of the paternally derived X-chromosome has been found. These include investigations of expression of genes affecting the coat including tabby, *Ta* (Kindred, 1961), albino, *c*, when it is X-linked in Cattanach's insertion Is(In7;X)1Ct (Cattanach & Perez, 1970), brindled, Mo^{br} (Falconer & Isaacson, 1972; Falconer *et al.* 1982) as well as the gene determining the enzyme phosphoglycerate kinase, *Pgk-1* (Bücher & Krietsch, 1988; B. M. Cattanach, personal communication). However, other investigations of *Pgk-1* have shown a slightly increased probability of expression of the maternally derived X-chromosome (Forrester & Ansell, 1985), and yet other experiments have failed to demonstrate any parental source effect in the expression of *Pgk-1* in somatic tissues (Johnston & Cattanach, 1981; Bücher *et al.* 1985). One reason for these differing results may be that the effect is small and requires large numbers of mice to be demonstrated convincingly.

Both of these parental source effects could represent examples of chromosome imprinting. This term, first used by Crouse (1960) and later refined by Chandra & Brown (1975), relates to those processes which cause chromosomes to function differently according to their parental origin. Crouse (1960) described the elimination of paternal X-chromosomes in *Sciara* and Brown & Nur (1964) reported the inactivation and elimination of the entire paternal chromosome set in

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male coccids. In recent years evidence of the occurrence of chromosome imprinting of certain autosomes and autosomal regions has accumulated from nuclear transplantation experiments, and genetic experiments using chromosome rearrangements (reviewed by Cattanach & Beechey, 1990). It is envisaged that the imprinting results in modifications in the germ line that lead to subsequent inactivation or elimination of the paternal X-chromosome or to functional differences in expression of chromosomes or genes. Whether there is a single process or more than one for all forms of imprinting is unknown.

Here we report the results of investigations of glucose-6-phosphate dehydrogenase (G6PD) activity in blood from heterozygotes for the normal allele, *G6pd*^{a}, and a mutant allele, *G6pd*$^{a\text{-}m1Neu}$, that determines G6PD with only 15% normal activity (Pretsch *et al.* 1988). A parental source effect was found and, in common with most of the earlier results, the probability of expression of the paternally derived X-chromosome was slightly greater than that of the maternally derived X-chromosome.

2. Materials and methods

(i) *Mice*

Five stocks of mice were used that were homozygous or hemizygous at the X-linked loci *G6pd* and *Xce*. These were the inbred strains C3H/HeH (*G6pd*^{a} *Xce*^{a}), JU/FaCt (*G6pd*^{a} *Xce*^{b}), the outbred stocks STB (*G6pd*$^{a\text{-}m1Neu}$ *Xce*^{a}), PGK (*G6pd*^{a} *Xce*^{c}), and a stock carrying *G6pd*^{a} *Ta Xce*^{b} (Cattanach & Williams, 1972; Cattanach *et al.* 1989; Johnston & Cattanach, 1981). The *G6pd*^{a} allele will be referred to as *a*, and the *G6pd*$^{a\text{-}m1Neu}$ allele will be referred to as *a-m* for the rest of this paper, and the maternally derived allele will be listed first. Reciprocal crosses were set up to produce *a Xce*^{a}/*a-m Xce*^{a} and *a-m Xce*^{a}/*a Xce*^{a}, *a Xce*^{b}/*a-m Xce*^{a} and *a-m Xce*^{a}/*a Xce*^{b}, *a Xce*^{c}/*a-m Xce*^{a} and *a-m Xce*^{a}/*a Xce*^{c}, *a Ta Xce*^{b}/*a-m*+*Xce*^{a} and *a-m*+*Xce*^{a}/*a Ta Xce*^{b}.

The *a-m* allele arose in a mutation experiment in which (102/El × C3H/HeH)F_1 males were treated with ethylnitrosourea (Pretsch *et al.* 1988). Thus the mutation was probably induced in the X-chromosome derived from 102/El. The 102/El inbred stain is derived from 101 and C3H, both of which carry the *Xce*^{a} allele, and therefore the STB stock was expected to carry the *Xce*^{a} allele. This was later confirmed following experimentation by B. M. Cattanach (personal communication).

(ii) *Assay of glucose-6-phosphate dehydrogenase in blood*

Blood for quantitative assay of glucose-6-phosphate dehydrogenase, G6PD, was taken by retro-orbital bleeding while the mouse was subjected to ether anaesthesia. In general, mice were sampled at one and three months, but in one experiment the mice were sampled monthly between one and seven months of age. The blood samples were frozen at – 70 °C for up to three months. After thawing, 50 μl of blood was added to 500 μl lysis buffer (0·1 M-triethanolamine, 1 mM-EDTA, pH 7·5 containing 5 mM-dithiothreitol and 0·5 mg/ml saponin), and the mixture was allowed to stand for ten min at 4 °C. The mixture was centrifuged at 6500 r.p.m. in a Beckman microfuge 12, 250 μl of supernatant was diluted with 250 μl sample buffer (lysis buffer without saponin), G6PD was assayed and haemoglobin concentration measured using ar automatic enzyme analyser (ACP 5040, Eppendorf, Hamburg FRG) as described by Charles & Pretsch (1987, 1988).

3. Results

Table 1 and Fig. 1 show that G6PD activity was lower in *a/a-m* heterozygotes than in the reciprocal het-

Table 1. *G6PD activity in blood from reciprocal crosses between C3H/HeH* (a), *and STB* (a-m) *at different ages*

Age, months	*n*	*a/a-m*	*n*	*a-m/a*	Statistical significance of difference between reciprocal crosses: *t*	*P*
1·0	6	14·11 ± 1·71	7	15·15 ± 0·66	0·57	NS*
1·5	9	11·31 ± 1·01	7	13·75 ± 0·89	1·75	NS
2·5	9	9·89 ± 1·13	7	12·89 ± 0·76	2·07	NS
3·5	9	10·00 ± 1·15	7	13·41 ± 0·54	2·68	0·020
4·5	9	9·63 ± 1·28	7	13·59 ± 0·50	2·87	0·015
5·5	9	9·75 ± 1·11	7	14·39 ± 0·59	3·68	0·003
6·5	9	10·46 ± 1·18	7	15·11 ± 0·99	2·91	0·012

* NS, not significant.
The maternally derived allele is shown first; and enzyme activity ± S.E. was measured as μmol NADPH produced/min/g haemoglobin at 25 °C.

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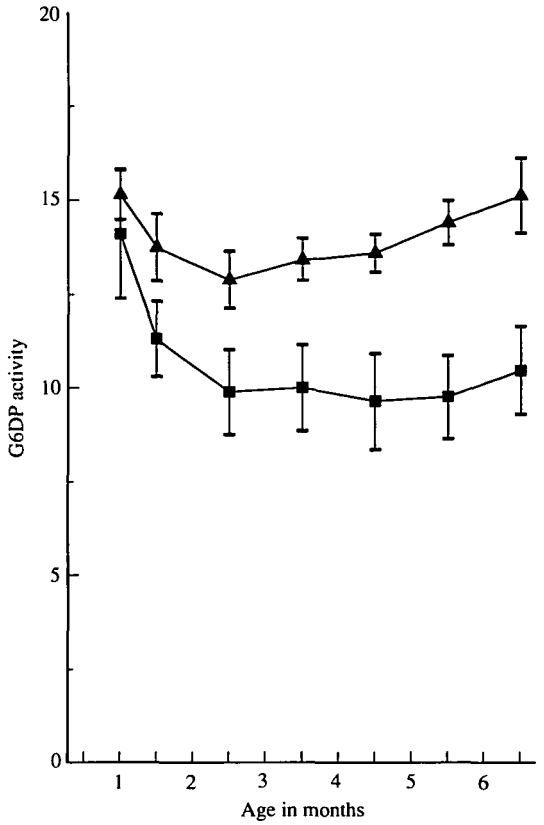

Fig. 1. G6PD activity in blood from *a-m/a* (▲) and *a/a-m* (■) mice at different ages. Means ± S.E. are shown, and the data used are given in Table 1.

erozygote *a-m/a*, and this dissimilarity was statistically significant in mice aged three and a half months or older. All animals of one age group were tested at the same time to eliminate disparities due to variability in assay conditions on different days. Thus it appeared that the maternally and paternally derived alleles were expressed to differing extents in reciprocal crosses, and this effect was particularly noticeable in adult mice. Furthermore, a drop in G6PD activity occurred in mice between one and two and a half months of age. A diminution in glucose phosphate isomerase activity between one and three months has also been reported (West *et al.* 1987; West & Flockhart, 1989). It was suggested that one month old mice may have a younger population of erythrocytes than three month old animals, and that enzyme activity may be higher in younger erythrocytes (West *et al.* 1987). This is because the mean life span of mouse erythrocytes is 40–43 days (Russell & Bernstein, 1966), and erythrocytes lack a nucleus with which to replenish depleted mRNA. After two and a half months G6PD activity remained steady in *a/a-m* heterozygotes but rose slightly in *a-m/a* mice.

These results were confirmed in a second experiment (Table 2) for G6PD activity was again found to be lower in *a/a-m* heterozygotes than in the reciprocal *a-m/a*, and this effect was more marked in three month old animals than in one month old mice. The expected activity of G6PD in a heterozygote is the mean of the

Table 2. *G6PD activity in blood from homozygotes, and heterozygotes from reciprocal crosses*

No. of cross	Stock	*G6pd* genotype (X^m/X^p)*	1 month's activity				Statistical significance of difference between reciprocal crosses		3 months' activity			Statistical significance of difference between reciprocal crosses	
			n	± SE	Expected†		*t*	*P*	*n*	± SE	Expected†	*t*	*P*
	C3H	*a/a*	10	24·91 ± 0·31					10	20·19 ± 0·22			
	STB	*a-m/a-m*	11	3·50 ± 0·22					11	2·60 ± 0·07			
1	C3H × STB	*a/a-m*	16	13·22 ± 0·82	14·21		1·18	NS	11	9·55 ± 0·66	11·40	2·49	0·022
2	STB × C3H	*a-m/a*	12	14·91 ± 1·25	14·21				11	12·87 ± 1·16	11·40		

* X^m = maternally derived *X*-chromosome; X^p = paternally derived *X*-chromosome.
† Expected G6PD activity in a heterozygote was calculated as the mean of the values in the respective homozygotes.
Enzyme activity was measured as μmol NADPH produced/min/g haemoglobin at 25 °C.

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Table 3. *G6PD activity in blood from homozygotes, and heterozygotes from reciprocal crosses*

No. of cross	Stock	*G6pd* genotype (X^m/X^p)*	*n*	1 month's activity ±SE	Expected†	Statistical significance of difference between reciprocal crosses: *t*	*P*	*n*	3 months' activity ±SE	Expected†	Statistical significance of difference between reciprocal crosses: *t*	*P*
	C3H	*a Xce*^{a}/*a Xce*^{a}	20	22·32±0·31	—	—	—	12	18·51±0·20	—	—	—
	JU	*a Xce*^{b}/*a Xce*^{b}	23	21·69±0·52	—	—	—	8	16·89±0·49	—	—	—
	PGK	*a Xce*^{c}/*a Xce*^{c}	22	20·09±0·67	—	—	—	20	17·49±0·24	—	—	—
	STB	*a-m Xce*^{a}/*a-m Xce*^{a}	36	3·41±0·57	—	—	—	6	2·34±0·16	—	—	—
1	C3H × STB	*a Xce*^{a}/*a-m Xce*^{a}	33	12·88±0·52	12·87	3·01	0·0035	14	7·81±0·66	10·43	2·98	0·0049
2	STB × C3H	*a-m Xce*^{a}/*a Xce*^{a}	47	15·17±0·52	12·87			27	10·43±0·53	10·43		
3	JU × STB	*a Xce*^{b}/*a-m Xce*^{a}	47	15·91±0·42	12·55	1·82	NS	34	10·03±0·29	9·62	0·51	NS
4	STB × JU	*a-m Xce*^{a}/*a Xce*^{b}	43	14·62±0·57	12·55			21	10·27±0·39	9·62		
5	PGK × STB	*a Xce*^{c}/*a-m Xce*^{a}	45	16·24±0·51	11·75	1·00	NS	25	10·05±0·35	9·92	4·31	0·00017
6	STB × PGK	*a-m Xce*^{a}/*a Xce*^{c}	40	16·92±0·43	11·75			22	13·70±0·77	9·92		
7	Ta × STB	*a Ta Xce*^{b}/*a-m*+ *Xce*^{a}	34	13·63±0·56	12·38	0·41	NS	30	7·77±0·51	9·99	4·04	0·00022
8	STB × Ta	*a-m*+ *Xce*^{a}/*a Ta Xce*^{b}	23	13·99±0·64	12·38			15	11·06±0·52	9·99		

* X^m = maternally derived *X*-chromosome; X^p = paternally derived *X*-chromosome.

† Expected value for a heterozygote is calculated as the mean of the homozygote values; the homozygous value for *a Ta Xce*^{b} was not measured but was estimated as the mean of all values for *a*/*a*.

Enzyme activity measured as μmol NADPH produced/min/g haemoglobin at 25 °C.

values found in the respective homozygotes, and G6PD activity was found to be lower in *a/a-m* and higher in *a-m/a* than expected. Thus it appeared that either the maternally derived allele was under-expressed, or the paternally derived allele was over-expressed. As before, significantly lower activity was found in adult mice when compared with mice of one month of age.

In the first two experiments all the mice were homozygous Xce^a and G6PD activities were then investigated in reciprocal crosses heterozygous for alleles at both *G6pd* and *Xce*, (Table 3, crosses 3–8), as well as in a further larger series of Xce^a homozygotes (Table 3, crosses 1 and 2). In this experiment the data for the homozygotes were obtained at different times and at a later date than those for the heterozygotes, and although statistically significant differences in activity were found between JU/FaCt and PGK at one and three months of age, and between C3H/HeH and PGK at three months, the importance of these findings is unclear. These dissimilarities in G6PD activities may be real or due merely to variability in assay conditions on different days. In view of these uncertainties, the expected values for G6PD activities in heterozygotes which were calculated from the values found in homozygotes, must be treated with some caution.

The data shown in Table 3, crosses 1 and 2, confirm the earlier results shown in Tables 1 and 2 for Xce^a homozygotes, and furthermore G6PD activity was found to be significantly lower in *a/a-m* than in *a-m/a* heterozygotes in one month old as well as three month old animals, probably because greater numbers of animals were tested than in the earlier experiments. In heterozygotes for *Xce* no statistically significant effect attributable to parental source was found in one month old animals, but the trend in all but one (Table 3, crosses 3 and 4) was towards lower activity in *a/a-m* than in *a-m/a* heterozygotes. There was broad agreement with the earlier results when three month old animals were tested (Table 3, crosses 3–8) for in three out of four pairs of reciprocal crosses G6PD activities were significantly lower in *a/a-m* than in *a-m/a* heterozygotes (Table 3, crosses 1 and 2, 5 and 6, 7 and 8), but no difference was found between crosses 3 and 4.

G6PD activity was higher than expected for both heterozygotes for Xce^a and Xce^b, and Xce^a and Xce^c, particularly at one month of age, which suggests an effect of *Xce* genotype on *G6pd* expression. For example, in Xce^c/Xce^a heterozygotes the X-chromosome with the Xce^c allele (and therefore the normal activity *G6pd* allele) is more likely to be expressed. Furthermore, as would be predicted from previous work on *Pgk-1* (Johnston & Cattanach, 1981; Cattanach *et al.* 1982, 1983), the effect of Xce^c was more extreme than that of Xce^b.

In previous investigations of X-chromosome gene expression in heterozygotes from reciprocal crosses, the results have been expressed as the proportion of gene activity attributable to one of the alleles. In order to facilitate comparison between earlier results and those obtained in the present study, the proportion of activity due to the *a-m* allele has been calculated for each cross, at both ages tested. As reported by Krietsch *et al.* (1985) for phosphoglycerate kinase in man, this can be done from the known specific activity of the enzyme in homozygotes or hemizygotes for the normal and mutant alleles. Thus, in accordance with

Table 4. *Expression of the* a-m *allele in heterozygotes*

Xce and/or *Ta* genotype (X^m/X^p)*	Source of data	Percentage of G6PD activity attributable to the *a-m* allele at 1 month (X^m/X^p)		3 months (X^m/X^p)	
		a/a-m	*a-m/a*	*a/a-m*	*a-m/a*
Xce^a/Xce^a	Table 2, crosses 1 and 2	54·5	46·7	60·5	41·6
Xce^a/Xce^a	Table 3, crosses 1 and 2	49·9	37·8	66·2	50·0
Xce^b/Xce^a and Xce^a/Xce^b	Table 3, crosses 3 and 4	31·6	38·7	47·1	45·5
Xce^c/Xce^a and Xce^a/Xce^c	Table 3, crosses 5 and 6	23·1	19·0	49·1	25·0
Ta $Xce^b/+Xce^a$ and $+Xce^a/Ta\ Xce^b$	Table 3, crosses 7 and 8	44·6	42·6	66·5	44·5
Mean ± SE		43·5 ± 5·5	39·0 ± 4·4	59·2 ± 3·6	44·5 ± 4·6
		t	0·63	*t*	2·51
		P	0·54	*P*	0·031

* X^m = maternally derived *X*-chromosome; X^p = paternally derived *X*-chromosome.

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Krietsch *et al.* (1985), the percentage of activity due to *a-m* in heterozygotes = $(A_n - A_h) \times 100\% / (A_n - A_m)$ where A_n = G6PD activity in blood from *a/a* homozygotes; A_m = G6PD activity in blood from *a-m/a-m* homozygotes, and A_h = G6PD activity in blood from heterozygotes (the mean value for each cross was used). When the results are calculated in this way the parental source effect, favouring expression of the paternally derived X-chromosome, can be seen clearly (Table 4). For each pair of reciprocal crosses, except that involving Xce^b in the absence of tabby, *Ta*, (Table 3, crosses 3 and 4), when tested at one month of age, the proportion of activity attributable to the *a-m* allele is greater when paternally than when maternally inherited. Overall, the mean difference in one month old animals is not statistically significant, but in three month old animals the mean difference is about 15% and is statistically significant. A similar difference between reciprocal crosses was found for Mo^{br} (Falconer & Isaacson, 1972; Falconer *et al.* 1982), somewhat higher than the difference found for *c*-variegation in Is(In7;X)1Ct (Cattanach & Perez, 1970) or *Pgk-1* (Bücher & Krietsch, 1988; B. M. Cattanach, personal communication).

4. Discussion

With the inclusion of the work reported here on *G6pd*, there are now five examples of X-linked genes whose expression is modified by parental source. Three of these affect the coat (Kindred, 1961; Cattanach & Perez, 1970; Falconer & Isaacson, 1972; Falconer *et al.* 1982) and two determine enzymes (Bücher & Krietsch, 1988; B. M. Cattanach, personal communication; this paper). The broad findings are that there is a slightly increased probability that the paternally derived allele will be expressed in adult mice. Expression of three of the genes has been studied in younger mice and whereas the effect on expression of *Ta* is clearly seen in mice of five to ten days of age, (Kindred, 1961), over-expression of the paternally derived *Pgk-1* or *G6pd* has either not yet been found, or is much less marked in young mice. For *G6pd*, in spite of testing greater numbers of animals at one month of age than at three months, significant over-expression of the paternally derived allele was found in only one (Table 3, crosses 1 and 2) out of six pairs of reciprocal crosses (Tables 1, 2, and 3). It may be pertinent that the effect was seen in homozygotes for Xce^a, and that in two other crosses also homozygous for Xce^a (Tables 1 and 2) there was a trend towards over-expression of the paternally derived allele in one month old mice. In these other two crosses fewer mice were tested, and the number may have been insufficient to demonstrate a parental effect. When heterozygotes for Xce^a and one of the 'stronger' *Xce* alleles were examined (Table 3, crosses 3–8) there was no evidence for over-expression of the paternally derived *G6pd* allele in one month old mice. Thus, somewhat surprisingly, the effect of parental source appears weaker at one month of age than at three months and may be overridden by 'strong' alleles at *Xce*.

Studies of *Pgk-1* show that over-expression of the paternally derived allele is confined to adult blood, and has not been found in other tissues tested in adults, namely liver and kidney (Johnston & Cattanach, 1981; B. M. Cattanach, unpublished), or in younger mice. In common with the present results for *G6pd*, Forrester & Ansell (1985) obtained no evidence of a parental origin effect for *Pgk-1* expression in blood from four week old animals heterozygous for Xce^a and Xce^c. Furthermore they found over-expression of the maternally derived *Pgk-1* allele in heterozygotes for Xce^b and Xce^c; in one of the two reciprocal crosses involving Xce^b in the present study (Table 3, crosses 3 and 4) there was a trend towards over-expression of the maternally derived *G6pd* allele. Over-expression of the maternally derived *Pgk-1* allele has also been found at prenatal stages (Johnston & Cattanach, 1981; Bücher *et al.* 1985).

Age related differences in X-chromosome expression have been associated with reactivation of the inactive X-chromosome, or cell selection. Reactivation of the inactive X-chromosome in mouse somatic tissues has been found for genes affecting the coat (Cattanach, 1974; Brown & Rastan, 1988) and the enzyme ornithine carbamoyl transferase (Wareham *et al.* 1987). The action of cell selection has been invoked to explain differences in expression of alleles at the X-linked locus, hypoxanthine phosphoribosyl transferase (HPRT) with age in haematopoietic tissue in man (Albertini & De Mars, 1974; Migeon *et al.* 1988). In rapidly dividing haematopoietic tissue from heterozygotes for the $HPRT^+$ allele and the deficiency allele $HPRT^-$ which leads to Lesch–Nyhan syndrome, an extreme form of cell selection results in virtual elimination of $HPRT^-$ cells in heterozygotes for Lesch–Nyhan syndrome after adolescence (Albertini & De Mars, 1974). Even in skin fibroblasts, where the effect is much less extreme, an excess of $HPRT^+$ cells is found in heterozygotes over ten years of age (Migeon *et al.* 1988). The most likely explanation for the excess of $HPRT^+$ cells is that they proliferate better than $HPRT^-$ cells.

Either cell selection or reactivation could account for the increased difference in G6PD activity with age in reciprocal crosses. If cell selection occurs, then by analogy with studies of HPRT, blood cells in which the paternally derived X-chromosome is active may proliferate slightly better, or survive slightly longer, than those in which the maternally derived X-chromosome is active. Thus, G6PD activity would be expected to rise with age in *a-m/a* but fall in *a/a-m*. If reactivation takes place, then the paternally derived X-chromosome must be preferentially reactivated. G6PD activity in *a-m/a* would rise with age, but there would be little difference in *a/a-m* because the low activity *a-m* allele would be reactivated. No firm

conclusions can be drawn from the data, but it is intriguing that in the experiment where G6PD activities were measured between three and six months of age, (Table 1, Fig. 1), G6PD activity rose in *a-m/a* but remained steady in *a/a-m*, which would be predicted by the reactivation hypothesis.

Imprinting is thought to involve modifications to chromosomes as they pass through the germ line, and in general in the mouse the effects are seen early, in embryonic or neonatal development. These effects include preferential inactivation of the paternal X-chromosome in extra embryonic membranes, as well as differences in expression of certain autosomal chromosome segments resulting in lethality or abnormality. Thus, if imprinting phenomena share a common origin, the late effect found for differential expression of *G6pd* is unusual in mice. Other possible examples of late manifestations of imprinting are Huntington's chorea (Ridley *et al.* 1988) or spinocerebellar ataxia (Harding, 1981) in man, which show differences in age of onset according to parental transmission.

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