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The localization of *G6pd*, glucose-6-phosphate dehydrogenase, and *mdx*, muscular dystrophy in the mouse *X* chromosome

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Summary

A low activity mutant of glucose-6-phosphate dehydrogenase, *G6pd*$^{a\text{-}m1Neu}$ has been used to position *G6pd* in the mouse *X* chromosome. Linkage tests with *tabby*, *Ta* and *harlequin*, *Hq*, indicate a likely gene order of *Hq–G6pd–Ta*. Muscular dystrophy, *mdx*, has been located by two- and three-point crosses using *Hprt*, *Pgk-1* and *Mo*^{blo} and suggest a gene order of *Hprt–mdx–Pgk-1 –Mo*^{blo}. Together with existing linkage data a tentative order for the seven loci is *Hq–Hprt–G6pd–mdx–Ta–Pgk-1–Mo*^{blo}. The relative positions of *G6pd* and *mdx* have not been directly tested and *G6pd* is assigned provisionally proximal to *mdx*. In the three point test using *Hq*, *G6pd* and *Ta* the recombination frequency found between *Hq* and *Ta* was $9{\cdot}9 \pm 2{\cdot}6\,\%$, substantially less than the value of $20{\cdot}5 \pm 2{\cdot}1\,\%$ reported by Isaacson *et al.* (1974).

1. INTRODUCTION

Evidence for the *X*- chromosome assignment of glucose-6-phosphate dehydrogenase, *G6pd*, in the mouse has come from gene dosage (Epstein, 1969; Chapman & Shows, 1976) and somatic cell studies. Up until recently its position in the linkage map could not be established by linkage studies because no genetic variants were available but Martin-DeLeon *et al.* (1984) have located *G6pd* to the A region of the cytogenetic map by *in situ* hybridization. This result was supported by recombination data from two-point crosses (Peters & Ball, 1985) using a *G6pd* mutant with lowered activity, induced by ethylnitrosourea (Pretsch *et al.* 1988). The proximal position of *G6pd* was confirmed by pedigree and recombinational analysis using a molecular marker for *G6pd* (Avner *et al.* 1987; Brockdorff *et al.* 1987*a*,*b*; Chamberlain *et al.* 1987). In this paper we present further linkage data from two-point crosses and describe the results from three-point crosses.

Muscular dystrophy, *mdx*, is an *X* chromosome-linked myopathy in the mouse identified in a C57BL/10 substrain (Bulfield *et al.* 1984). We present evidence that *mdx* is closely linked to *G6pd* on the *X* chromosome. From the map position, as well as histological characteristics, it may be deduced that *mdx* may either be homologous to DMD, muscular dystrophy Duchenne type, or to the late onset Emery–Dreifuss muscular dystrophy in humans. The recent finding that dystrophin is absent in DMD-affected individuals and in *mdx* mice supports homology of *DMD* and *mdx* (Hoffman *et al.* 1987*a*,*b*).

2. Materials and methods

The original animal with a mutant form of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was a female derived from a mutagenesis experiment carried out in Neuherberg (Pretsch *et al.* 1988). In the experiment $(102/\mathrm{El} \times \mathrm{C3H/El})\mathrm{F}_1$ male mice were injected with 250 mg/kg ethylnitrosourea, mated to tester stock females and the activity levels of ten enzymes were measured in the offspring. The breeding regime was such that any induced mutations would have arisen in spermatogonial stem cells. The glucose-6-phosphate dehydrogenase activity in the blood of the original mutant was 60% of normal and subsequent breeding tests showed that the mutant was heterozygous for an allele determining normal activity (*G6pd*^{a}) and an allele determining low activity

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(*G6pd*$^{a\text{-}m1Neu}$), and that these alleles were at an *X*-linked locus (Pretsch *et al.* 1988). Homozygous and hemizygous descendants of the original mutant were sent to the MRC Radiobiology Unit for linkage testing with other *X* chromosome markers.

For the initial linkage tests females homozygous for the mutant allele, *G6pd*$^{a\text{-}m1Neu}$, were crossed to males carrying either *tabby*, *Ta*, or *harlequin*, *Hq* two loci in the proximal half of the *X*- chromosome (Lyon, 1987*a*). Hybrid female progeny were crossed to (C3H/HeH × 101/H)F_1 males and both male and female offspring classified for either *Ta* and *G6pd* or *Hq* and *G6pd*. For a subsequent three-point test, two female recombinants $+G6pd^{a\text{-}m1Neu}\ Ta/+$ $G6pd^{a}+$ were crossed to $Hq\,G6pd^{a}+/Y$ males. Then the $+G6pd^{a\text{-}m1Neu}\ Ta/Hq\,G6pd^{a}+$ female offspring were crossed to $+G6pd^{a}+/Y$ males of either the inbred strains C3H/HeH or 101/H or the hybrid (C3H/HeH × 101/H)F_1. Male offspring of this cross were scored for *Hq*, *G6pd* and *Ta*. G6PD was assayed and haemoglobin concentration measured using an automatic enzyme analyser (ACP 5040, Eppendorf, Hamburg, FRG) as described by Charles & Pretsch (1986).

For linkage tests of *X-linked muscular dystrophy*, *mdx*, with the visible marker genes *harlequin*, *Hq*, and *blotchy*, *Mo*^{blo}, males carrying *mdx* were crossed to females carrying *Hq* or *Mo*^{blo}. Hybrid female progeny were backcrossed to males carrying *mdx*. In these linkage tests *mdx* was classified by measurements of pyruvate kinase levels in blood taken from the retro-orbital sinus. The classification was unequivocal; *mdx*/*Y* and *mdx*/*mdx* have 5–20× higher levels of pyruvate kinase in plasma than +/*Y* and +/+ (Bulfield *et al.* 1984).

For linkage tests of *mdx* with *hypoxanthine phosphoribosyl transferase*, *Hprt*, and *phosphoglycerate kinase-1*, *Pgk-1*, males of the coisogenic strain C57BL/10-*mdx* homozygous for *Hprt*^{b}, *mdx* and *Pgk-1*^{b} were crossed to females of the congenic strain C3H-*Hprt*^{a} *Pgk-1*^{a}; *homozygous for Hprt*^{a} and *Pgk-1*^{a}. Hybrid female progeny *Hprt*^{a} + *Pgk-1*^{a}/*Hprt*^{b} *mdx* *Pgk-1*^{b} were backcrossed to C57BL/10-*mdx* males, *Hprt*^{b} *mdx* *Pgk-1*^{b}/*Y*. HPRT was analysed after separation by isoelectric focusing and PGK-1 by electrophoresis as described by Chapman *et al.* (1983).

The *mdx* phenotype in the *Hprt–mdx–Pgk-1* linkage test was determined by analysis of circulating levels of creatine phosphokinase (CK) in blood plasma. Blood plasma CK levels were assayed fluorometrically using a coupled assay system which detects the formation of ATP by the reaction of hexokinase-G6PD. Hemizygous *mdx*/*Y* and homozygous *mdx*/*mdx* mice have a 10-fold increase in plasma CK compared with *mdx*/+ heterozygotes or +/+, +/*Y* wild-type mice (Chapman *et al.* in preparation).

3. Results

(i) *Assays of G6PD activity*

Hemizygotes and mutant homozygotes have very low G6PD activities; 12 and 9% of normal respectively (Table 1). The mean activity in heterozygotes was intermediate; 56% of normal (Table 1). These results show reasonable agreement with those of Charles & Pretsch (1984*a*,*b*) and Pretsch *et al.* (1988) who reported that hemizygous, heterozygous and homozygous mutants had 20, 60 and 15% normal G6PD activity in blood respectively. Charles & Pretsch (1984*a*,*b*) and Pretsch *et al.* (1988) also reported that haematological parameters did not show significant differences between mutant and wild type and this was confirmed in the present study.

In backcross males from the linkage tests the activity levels formed a non-overlapping bimodal distribution with peak means of a similar order to those found in parental stocks (Table 1, Fig. 1*a*). The low activity mutant of glucose-6-phosphate dehydrogenase thus segregates as an allele of a single *X*-linked gene as demonstrated by Pretsch *et al.* (1988). Among backcross males a greater number were found

Table 1. *Glucose-6-phosphate dehydrogenase activity levels in blood*

Source of blood	Division of progeny	*n*	Specific activity[a] (mean ± S.E.)
G6pd$^{a\text{-}m1Neu}$/*Y*		11	2·58 ± 0·12
G6pd^{a}/*Y*		12	21·36 ± 0·60
G6pd$^{a\text{-}m1Neu}$/*G6pd*$^{a\text{-}m1Neu}$		12	1·97 ± 0·11
G6pd^{a}/*G6pd*^{a}		11	22·49 ± 0·50
G6pd^{a}/*G6pd*$^{a\text{-}m1Neu}$		13	12·56 ± 0·73
Backcross *G6pd*$^{a\text{-}m1Neu}$/*G6pd*^{a} × *G6pd*^{a}/*Y*			
Males	Low	139	3·71 ± 0·11
	High	189	24·18 ± 0·25
Females	Intermediate	55	11·93 ± 0·30
	High	51	21·20 ± 0·28

[a] Enzyme activity measured as μmol/min/g haemoglobin.

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Table 2. *Recombination between* Hq *and* G6pd

Offspring (parental mating + $G6pd^{a\text{-}m1Neu}/Hq\ G6pd^{a} \times +G6pd^{a}/Y$)			
Non-recombinant	*n*	Recombinant	*n*
Experiment 1			
$+G6pd^{a\text{-}m1Neu}/Y$	23	$+G6pd^{a}/Y$	1
$Hq\ G6pd^{a}/Y$	26	$Hq\ G6pd^{a\text{-}m1Neu}/Y$	1
$+G6pd^{a\text{-}m1Neu}/+G6pd^{a}$	17	$+G6pd^{a}/+G6pd^{a}$	2
$Hq\ G6pd^{a}/+G6pd^{a}$	17	$Hq\ G6pd^{a\text{-}m1Neu}/+G6pd^{a}$	0
Total	83		4
Experiment 2			
$Hq\ G6pd^{a}Y$	70	$Hq\ G6pd^{a\text{-}m1Neu}/Y$	2
$+G6pd^{a\text{-}m1Neu}/Y$	7	$+G6pd^{a}/Y$	0

From Exp 1, recombination frequency ± S.E. = 4/87 = 4·6 ± 2·3 %.
From Expt 2, recombination frequency ± S.E. = 2/79 = 2·5 ± 1·8 %.
From Expts 1 and 2 combined, recombination frequency ± S.E. = 6/166 = 3·6 ± 1·5 %.

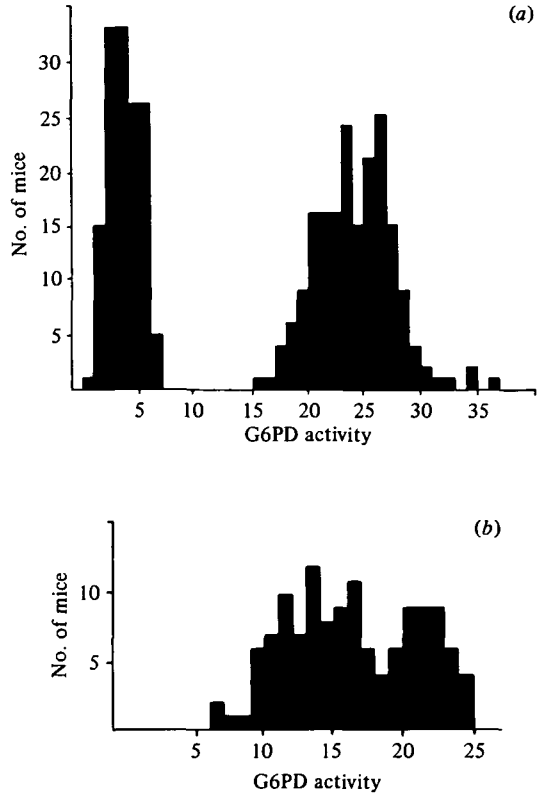

Fig. 1. Glucose-6-phosphate dehydrogenase activity in blood of mice arising from the backcross $G6pd^{a\text{-}m1Neu}/G6pd^{a} \times G6pd^{a}/Y$. Units of enzyme activity are μmol NADPH formed/min/g haemoglobin. (*a*) Males; (*b*) females.

with high G6PD activity levels (G6PD-A) than with low, because, in one experiment involving *Hq*, males carrying the marker were selected for assay rather than wild type males and in the absence of recombination *Hq*/*Y* mice were expected to have high G6PD activity (Table 2). When the data from these mice were excluded there is no excess of males which are G6PD-A. In backcross females the activity levels, as in males, tended to form a bimodal distribution, but the high end of the range for heterozygotes (9–18 units of activity) overlapped with the low end of the range for homozygotes (16–25 units of activity). Thus mice with G6PD activities between 16 and 18 units of activity could not be classified with certainty and in general were excluded from the analysis.

(ii) *Linkage of G6pd*

In Expt 1 of the two-point linkage test with *Hq*, (Table 2) both male and female offspring were tested for G6PD. Not all females could be classified with certainty, for the G6PD activities in 4 of 40 female offspring tested fell in the region of overlap of activities found in normal homozygotes and heterozygotes and the results from those four females were discarded. All males could be classified with certainty and therefore in Expt 2 only male offspring were tested (Table 2). In addition, in Expt 2, *Hq*/*Y* males were chosen preferentially for enzyme assay because an additional aim of this experiment was to find a recombinant $Hq\ G6pd^{a\text{-}m1Neu}/Y$ male that could be used in a three-point cross with *Ta*.

In Expt 1, 87 mice were scored for *Hq* and *G6pd* and four recombinants were found, giving a recombination frequency of 4·6 ± 2·3 %. In Expt 2, two recombinants were found among 79 offspring giving a recombination frequency of 2·5 ± 1·8 %. Since there is reasonable agreement between results of the two experiments, the data from Expts 1 and 2 were combined and gave a recombination frequency between *Hq* and *G6pd* of 3·6 ± 1·5 % with a 95 % confidence interval of 0·7–6·5 %.

In the two-point linkage test with *Ta* (Table 3) 181 offspring, of which 86 were females, were analysed for G6PD activity levels. Seventeen females could not be classified with certainty for G6PD because the enzyme activity levels fell in the region of overlap for activities

Table 3. *Recombination between* G6pd *and* Ta

Offspring (parental mating *G6pd$^{a\text{-}m1Neu}$* + /*G6pd^{a}Ta* × *G6pd^{a}* + /Y)			
Non-recombinant	*n*	Recombinant	*n*
G6pd$^{a\text{-}m1Neu}$ + / *Y*	54	*G6pd$^{a\text{-}m1Neu}$ Ta/ Y*	2
G6pd^{a} Ta/ Y	37	*G6pd^{a}* + / *Y*	2
G6pd$^{a\text{-}m1Neu}$ + /*G6pd^{a}* +	36	*G6pd$^{a\text{-}m1Neu}$ Ta/G6pd^{a}* +	2
G6pd^{a} Ta/G6pd^{a} +	28	*G6pd^{a}* + /*G6pd^{a}* +	4
Total	155		10

Recombination frequency ± S.E. = 10/165 = 6·1 ± 1·9 %.

found in G6PD-A and G6PD-AM1. The genotype of one of these females was established by progeny testing and so the data from this female have been included but data from the other sixteen females were excluded. Thus, only 165 offspring could be classified with confidence.

Ten recombinants between *G6pd* and *Ta* were found among these 165 offspring, giving a recombination frequency of 6·1 ± 1·9 % with a 95 % confidence interval of 2·4–9·8 %. Overall there was a deficiency of offspring carrying *Ta* ($\chi^2 = 4{\cdot}42$, $p = 0{\cdot}036$) and the reason(s) for this are unclear.

By comparing the recombination frequencies of *Hq–G6pd* and *G6pd–Ta* with the findings of Isaacson *et al.* (1974) for recombination between *Hq* and *Ta*, the most likely gene order is *Hq–G6pd–Ta*.

The results of the three-point test indicated that the order of the three loci was *Hq, G6pd, Ta*. With this order all the offspring could be explained by the presence of single crossovers.

There was good agreement between the linkage test data of Table 4 and Tables 2 and 3. The combined data give a recombination between *Hq* and *G6pd* of 15/297 = 5·1 ± 1·3 % with a 95 % confidence interval of 2·9–8·2 %. The estimated map distance between *G6pd* and *Ta* is 14/296 = 4·7 ± 1·2 % with a 95 % confidence interval of 2·6–7·8 %. Thus *G6pd* is about halfway between *Hq* and *Ta*. The recombination between *Hq* and *Ta* from the current data is 13/131 = 9·9 ± 2·6 % with 95 % confidence limits of 5·4–16·4 %, somewhat less than the value of 20·5 ± 2·1 % found by Isaacson *et al.* (1974). The reason for this is unclear.

(iii) *Linkage of* mdx

Earlier experiments had located *X*-linked muscular dystrophy, *mdx*, to the *Hq–Bpa* region of the mouse *X*-chromosome (Bulfield *et al.* 1984). Further two-point and three-point crosses were carried out here in order to position *mdx* more precisely. In the linkage test with *Hq* (Table 5) there was a deficiency of hemizygotes carrying *Hq*(36 *Hq*:67 + ; $\chi^2_1 = 8{\cdot}74$, $p = 0{\cdot}0031$) and an excess of males carrying *mdx* (64 *mdx*: 39 + ; $\chi^2_1 = 5{\cdot}59$, $p = 0{\cdot}018$). The reasons for these deviations from expected 1:1 segregations are not clear. Overall, considering both male and female offspring, 33 recombinants were found among 205 mice scored giving a recombination frequency of 16·1 + 2·6 %, with a 95 % confidence interval of 11·3–21·9 %. Among female offspring the segregation at both *Hq* and *mdx* does not deviate from 1:1, and the estimate of recombination was 18/102 =

Table 5. *Recombination between* Hq *and* mdx

Offspring (parental mating *Hq* + / + *mdx* × + *mdx*/ *Y*)			
Non-recombinant	*n*	Recombinant	*n*
Hq + / *Y*	30	*Hq mdx*/ *Y*	6
+ *mdx*/ *Y*	58	+ + / *Y*	9
Hq + / + *mdx*	35	*Hq mdx*/ + *mdx*	9
+ *mdx*/ + *mdx*	49	+ + / + *mdx*	9
Total	172		33

Recombination frequency ± S.E. = 33/205 = 16·1 ± 2·6 %; 95 % confidence interval, 11·3–21·9 %.

Table 4. *Recombination between* Hq, G6pd *and Ta*

Male offspring (parental mating + *G6pd$^{a\text{-}m1Neu}$ Ta/Hq G6pd^{a}* + × + *G6pd^{a}* + / *Y*)		
		n
Non-recombinant	+ *G6pd$^{a\text{-}m1Neu}$ Ta/ Y*	59
	Hq G6pd^{a} + / *Y*	59
Single recombinant 1, *Hq–G6pd*	+ *G6pd^{a}* + / *Y*	6
	Hq G6pd$^{a\text{-}m1Neu}$ Ta/ Y	3
Single recombinant 2, *G6pd–Ta*	+ *G6pd$^{a\text{-}m1Neu}$* + / *Y*	2
	Hq G6pd^{a} Ta/ Y	2
Total		131
	Recombination R.F. ± S.E. (%)	Confidence interval (95 %)
Hq–G6pd	9/131 6·9 ± 2·2	2·9 – 8·2
G6pd–Ta	4/131 3·1 ± 1·5	2·6 – 7·8
Hq–Ta	13/131 9·9 ± 2·6	5·4 – 16·4

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Table 6. *Recombination between* mdx *and* Mo^{blo}

Offspring (parental mating $mdx+/+Mo^{blo} \times mdx+/Y$)			
Non-recombinant	*n*	Recombinant	*n*
$mdx+/Y$	65	$mdx\ Mo^{blo}/Y$	3
$+Mo^{blo}/Y$	43	$+\ +/Y$	5
$mdx+/mdx+$	59	$mdx\ Mo^{blo}/mdx+$	3
$+Mo^{blo}/mdx+$	43	$++/mdx+$	11
Total	210		22

Recombination frequency ± S.E. = 22/232 = 9·5 ± 1·9 %; 95 % confidence interval, 6·0–14·0 %.

17·6 ± 3·8 %, with a 95 % confidence interval of 10·8–26·4 %.

In the two-point cross with Mo^{blo} (Table 6), there was a deficiency of both male and female offspring carrying Mo^{blo} (92 Mo^{blo}:140+; $\chi^2_1 = 9{\cdot}52$, $p = 0{\cdot}0020$). Overall 22 recombinants were found among 232 offspring giving a recombination frequency of 9·5 + 1·9 %, with a 95 % confidence interval of 6·0–14·0 %. If the recombination frequency is estimated using offspring which do not carry Mo^{blo}, the recombination frequency is slightly higher (16/140 = 11·4 ± 2·7 %). Taking all the results together, the probable gene order is *Hq–mdx–*Mo^{blo}.

For the three-point test the *mdx* phenotype was identified by elevated levels of muscle creatine kinase (CK) activity in circulating blood and the distribution of blood plasma CK activities in backcross progeny is shown in Fig. 2. The levels of CK activity form a bimodal distribution with $+/mdx$ and $+/Y$ progeny less than 9·0 units of activity, and *mdx/mdx* or *mdx/Y* progeny greater than 9·0 units.

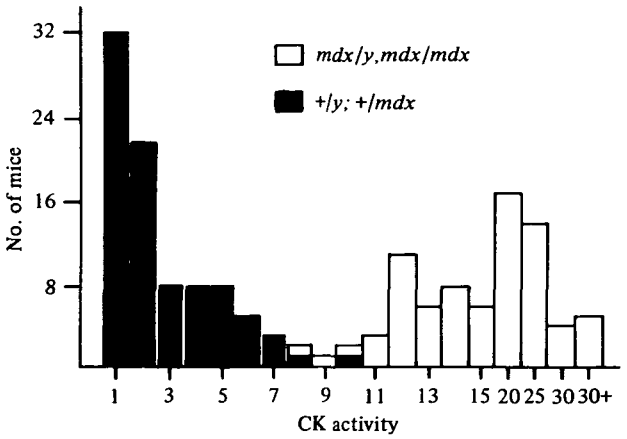

Fig. 2. Creatine kinase activity in plasma of mice arising from the backcross $+/mdx \times mdx/Y$. Units of enzyme activity are μmol NADH formed/min/μl of plasma at 30 °C. Genotype of individuals in region of overlap between the two distributions was established by progeny analysis.

The results of the three-point test with *Hprt* and *Pgk-1* (Table 7) indicated a gene order of *Hprt*, *mdx*, *Pgk-1*. With this order all offspring except one could be explained by the presence of single crossovers. The remaining one would require a double crossover, and with any other order of loci the number of double crossovers would be greater. The recombination frequency between *Hprt* and *mdx* is 14/162 = 8·6 ± 2·2 % with a 95 % confidence interval of 5·3–13·1 %. The recombination frequency between *mdx* and *Pgk-1* is 22/162 = 13·6 ± 2·7 % with a 95 % confidence interval of 9·4–18·8 %. The recombination between *Hprt* and *Pgk-1* is 34/162 = 21·0 ± 3·2 % with a 95 % confidence interval of 15·8–26·9 % which does not differ from that previously reported for these two loci (Chapman *et al.* 1985).

Table 7. *Recombination between* Hprt, mdx *and* Pgk-1

Offspring (parental mating $Hprt^a + Pgk\text{-}1^a/Hprt^b\ mdx\ Pgk\text{-}1^b \times Hprt^b\ mdx\ Pgk\text{-}1^b/Y$)		
		n
Non-recombinant	$Hprt^a + Pgk\text{-}1^a/Y$	32
	$Hprt^b\ mdx\ Pgk\text{-}1^b/Y$	33
	$Hprt^a + Pgk\text{-}1^a/Hprt^b\ mdx\ Pgk\text{-}1^b$	27
	$Hprt^b\ mdx\ Pgk\text{-}1^b/Hprt^b\ mdx\ Pgk\text{-}1^b$	35
Single recombinant 1, *Hprt–mdx*	$Hprt^a\ mdx\ Pgk\text{-}1^b/Y$	3
	$Hprt^b + Pgk\text{-}1^a/Y$	6
	$Hprt^a\ mdx\ Pgk\text{-}1^b/Hprt^b\ mdx\ Pgk\text{-}1^b$	1
	$Hprt^b + Pgk\text{-}1^a/Hprt^b\ mdx\ Pgk\text{-}1^b$	3
Single recombinant 2, *mdx–Pgk-1*	$Hprt^a + Pgk\text{-}1^b/Y$	8
	$Hprt^b\ mdx\ Pgk\text{-}1^a/Y$	3
	$Hprt^a + Pgk\text{-}1^b/Hprt^b\ mdx\ Pgk\text{-}1^b$	6
	$Hprt^b\ mdx\ Pgk\text{-}1^a/Hprt^b\ mdx\ Pgk\text{-}1^b$	4
Double recombinant *Hprt–mdx–Pgk-1*	$Hprt^b + Pgk\text{-}1^b/Hprt^b\ mdx\ Pgk\text{-}1^b$	1
Total		162
	Recombination R.F. ± S.E. (%)	Confidence interval (95 %)
Hprt–mdx	14/162 = 8·6 ± 2·2	5·3–13·1
mdx–Pgk-1	22/162 = 13·6 ± 2·7	9·4–18·8
Hprt–Pgk-1	34/162 = 21·0 ± 3·2	15·8–26·9

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4. Discussion

The two three-point crosses in this report involve six separate loci on the *X* chromosome. These two crosses provide direct evidence which supports the relative order of *X*-chromosome genes *Hq–G6pd–Ta* and *Hprt–mdx–Pgk-1*. Together with data from two other two-point crosses some indication about the possible ordering of these loci on the X chromosome can be ascertained. A summary of the linkage information involving seven *X*-chromosome loci is shown in Fig. 3. The relative positions of *G6pd* and *mdx* have not been directly tested and they are assigned provisionally as indicated in the figure. The relative order of *Hq–G6pd–mdx* is suggested by the recombination frequencies between *Hq* and *G6pd* of 5·1% and between *Hq* and *mdx* of 16·1%. However, this interpretation should be treated with some caution, because the recombination frequency between *Hq* and *Ta* of 9·9% in the cross involving *G6pd* is substantially less than the value of 20·5% previously reported by Isaacson *et al.* (1974). Isaacson et al. (1974) also found a recombination frequency of 24·9 ± 2·2% between *Hq* and Mo^{br}; an estimate supported by recent data of Cattanach (1988) who found the *Hq–*Mo^{vbr} recombination frequency to be 22·7 ± 1·4%. Possibly the *X* chromosome with $G6pd^{a\text{-}mlNeu}$ suppresses recombination in this region, although no evidence of a structural rearrangement could be found in the *X* chromosome of the stock carrying $G6pd^{a\text{-}mlNeu}$ (E. P. Evans, personal communication). Our data suggest that *G6pd* is located about halfway between *Hq* and *Ta* and this is in broad agreement with the finding of Martin-DeLeon *et al.* (1985) which assigned *G6pd* to the A region by *in situ* hybridization and with the linkage data of Avner *et al.* (1987); Brockdorff *et al.* (1987*a*,*b*) using a genomic probe as a marker for *G6pd*.

Muscular dystrophy, *mdx* has been located by two- and three-point crosses, using markers from the proximal half of the chromosome. Whereas preliminary work by Bulfield *et al.* (1984) had positioned *mdx* to the *Hq–Bpa* segment, the results of both the two-point and three-point tests in the present study place *mdx* slightly more distally, to the *Str striated/Phk phosphorylase kinase* region. Interestingly, *Phk* is another locus affecting skeletal muscle.

When all the results are considered it is evident that *G6pd* and *mdx* must be closely linked. It will be necessary to examine *G6pd* and *mdx* in the same cross to verify the most probable order of these loci and the relative distance between these genes.

There appears to be a conserved block of genes in mouse and man containing *Hprt* and *G6pd* and whereas in man both *HPRT* and *G6PD* are located on the long arm in Xq26–27·3 and Xq28 respectively (Goodfellow *et al.* 1985), in the mouse the homologous genes are proximal in band A (Lyon *et al.* 1987*b*; Martin-DeLeon *et al.* 1985). The homology between *Hprt* and *HPRT*, and *G6pd* and *G6PD* is unequivocal but the human homologue of *mdx* is less certain. Bulfield *et al.* (1984) found that *mdx* showed histological lesions of muscular dystrophy and this rasied the possibility that the gene could be homologous with an *X*-linked muscular dystrophy in man. Two such loci are known in man, *EMD*, Emery–Dreifuss muscular dystrophy and *DMD*, Duchenne muscular dystrophy. *EMD* has been mapped to Xq27 to Xqter, and therefore must be close to *G6PD* and HPRT (Hodgson *et al.* 1986; Yates *et al.* 1986; Thomas *et al.* 1986), and *DMD* has been mapped to Xp21 (Goodfellow *et al.* (1985). Avner *et al.* (1987) investigated the localization of five probes on the human and mouse *X* chromosomes and concluded that the human Xq26 to Xqter region, which includes *HPRT* and *G6PD*, is conserved as a continuous region of the mouse *X* lying proximal to *tabby*, *Ta*. Thus the murine homologue of *EMD* is predicted to map within this conserved segment. The mouse equivalent of *DMD* has been located proximal to *Ta* but distal to *G6pd* (Brockdorff *et al.* 1987*b*: Heilig *et al.* 1987; Chamberlain *at al.* (1987). If *mdx* is proximal to *G6pd*,

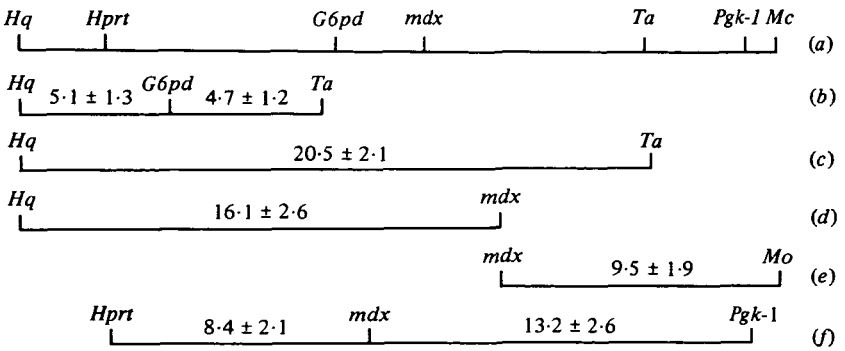

Fig. 3. (*a*) Diagram showing positions of seven loci on the *X* chromosome based on (*b*) two- and three-point crosses involving *Hq*, *G6pd* and *Ta*, (*c*) two-point cross between *Hq* and *Ta* (Isaacson *et al.* 1974), (*d*) two-point cross between *Hq* and *mdx*, (*e*) two-point cross between *mdx* and *Mo*, (*f*) three-point cross between *Hprt*, *mdx* and *Pgk-1*. For (*b*)–(*f*) recombination percentages ± S.E. are shown. For (*a*) the distance between *Hq* and *Hprt*; *Pgk-1* and *Mo*; and also *Hq* and *Ta* shown on published linkage maps (Lyon, 1987*a*) has been used to position the loci, whereas the distance found between *Hq* and *Ta* was substantially less (see text).

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then it may correspond to Emery–Dreifuss musculur dystrophy, but if it is distal to *G6pd* then it may be homologous with *DMD*. A direct test of linkage involving *G6pd, mdx* and other *X*-linked markers is required to establish the position of *mdx*. Support for the homology of *DMD* and *mdx* has come recently from the finding that the protein dystrophin is absent in DMD-affected individuals and in *mdx* mice (Hoffman *et al.* 1987*a,b*).

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