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Studies on Metatherian Sex Chromosomes VII.* Glucose-6-phosphate Dehydrogenase Expression in Tissues and Cultured Fibroblasts of Kangaroos

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Abstract

Expression of the sex-linked enzyme glucose-6-phosphate dehydrogenase (G6PD) was examined electrophoretically in tissues and cultured fibroblasts of female kangaroo heterozygotes ranging in age from 26 days *post partum* to adult. All tissues expressed only the maternally derived allele irrespective of which allele was maternal or paternal in origin.

By contrast, cultured fibroblasts derived from these same heterozygotes displayed unique G6PD phenotypes not found in the living animal. These phenotypes consist of a broad band which either completely or partially overlaps the allozymes produced by the maternally and paternally derived alleles. *In vitro* hybridization of G6PD from species showing greater differences in electrophoretic mobility indicate that G6PD is a dimer in marsupials. The cultured fibroblast G6PD phenotypes are consistent with different proportions of homodimer and heterodimer formation. The appearance of the heterodimer indicates that both alleles are active within the one cell. This conclusion is reinforced by the finding that clones from cultured fibroblasts of one of the heterozygotes had an identical phenotype to that of the mass culture from which the clones were derived.

It is concluded that some heterozygotes express both *Gpd* alleles equally in each cultured fibroblast while other heterozygotes show normal activity of the maternally derived allele and partial expression of the paternally derived allele.

Introduction

Studies on the sex-linked enzyme glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G6PD) in kangaroos have shown that the paternally derived allele is not expressed in erythrocytes (Richardson *et al.* 1971; Johnston *et al.* 1975) nor in various tissues of pouch young (Johnston and Sharman 1975). Although paternal X chromosome inactivation at the *Gpd* locus appears to be the rule for these tissues there is evidence that it may not be complete in cultured fibroblasts of female kangaroos known to be heterozygous for this locus (Cooper *et al.* 1975).

In this paper we present data on the expression of G6PD allozymes in tissues of adult kangaroo heterozygotes. In addition we describe electrophoresis of cultured fibroblasts from kangaroos heterozygous for G6PD, and confirm by means of cloning that both alleles are active in these cells.

Materials and Methods

Animals

The species and species hybrids used in this study are listed in Table 1. Captive animals were maintained at Macquarie University, North Ryde, N.S.W., and the Division of Wildlife Research,

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CSIRO, Canberra. G6PD genotypes of hybrids and backcross hybrids of *Macropus robustus* Gould were determined on the basis of pedigree data and X chromosome constitution (see Johnston and Sharman 1975). Pedigrees of some of the *Macropus rufogriseus* (Desmarest) individuals are shown in Johnston *et al.* (1975).

A sample of *M. rufogriseus* was collected under licence from the Warwick district (28°13′S., 152°02′E.) in Queensland. This area was selected because the animals were at a high density and were known to be polymorphic for G6PD with the variant Gpd^S allele reaching a frequency of 0·43 (Johnston *et al.* 1975). Heterozygous females from this population were detected by two methods: (i) the possession of pouch young with a G6PD phenotype different from their own, and (II) the expression of the unique intermediate G6PD phenotype characteristic of heterozygotes in cultured fibroblasts (Cooper *et al.* 1975).

Table 1. Marsupial species and species hybrids used in this study

Species and species hybrids	Identification symbol	Number examined ♀	Number examined ♂
(*a*) Species			
Macropus rufogriseus (red-necked wallaby)	n	21	6
M. robustus robustus (wallaroo)	w	2	2
M. r. erubescens (euro)	e	1	2
Petrogale xanthopus (yellow-footed rock wallaby)	x	—	1
Trichosurus vulpecula (brush-tailed possum)	v	—	1
(*b*) Subspecies and species hybrids[A]			
M. r. erubescens × *M. r. robustus*	ew	5	—
(*M. r. erubescens* × *M. r. robustus*) × *M. r. erubescens*	ew/e	3	—
(*M. r. erubescens* × *M. r. robustus*) × *M. r. robustus*	ew/w	1	—
[(*M. r. erubescens* × *M. r. robustus*) × *M. r. erubescens*] × *M. r. robustus*	ew/e//w	1	—
M. r. erubescens × *M. robustus*[B]	ez	1	1
(*M. r. erubescens* × *M. robustus*[B]) × *M. r. erubescens*	ez/e	2	—
(*M. r. erubescens* × *M. robustus*[B]) × *M. r. robustus*	ez/w	1	—
M. r. robustus × *M. r. erubescens*	we	7	2
(*M. r. robustus* × *M. r. erubescens*) × *M. r. robustus*	we/w	1	—
M. r. robustus × *M. rufus*	wr	1	—
M. r. robustus × *Wallabia bicolor* (swamp wallaby)	wb	—	1
M. rufogriseus × *M. giganteus* (eastern grey kangaroo)	ng	—	1
M. rufogriseus × *M. agilis* (agile wallaby)	na	1	—
M. rufogriseus × *M. eugenii* (tammar wallaby)	nt	—	1

[A] Female parent placed first.

[B] Putative backcross hybrid from Taronga Zoo (see Johnston and Sharman 1975).

Blood and Tissue Collection

Blood samples were collected from the heart or throat of animals shot in the field and from the caudal vein of animals maintained in captivity and were treated following the methods of Johnston and Sharman (1975). The following tissues were collected: liver, lung, spleen, kidney, brain, nerve cord, eye, foregut, hindgut, heart, leg muscle, diaphragm, tongue, oesophagus, ear, bladder, lateral vagina, urogenital sinus, uterus, and ovary. Tissues were placed on dry ice immediately after dissection and later transferred to a freezer where they were stored at −30°C until used.

Cell Culture

Explants for tissue culture were obtained from ear biopsy of live animals and from ear, kidney, and body wall of recently killed animals. Details of collection, culture initiation, growing conditions, and cloning by the feeder layer method are described in Cooper *et al.* (1977).

Electrophoresis

Tissues were homogenized in a lysing solution containing 10 mg NADP, 0·1 ml of 2-mercapto-ethanol made up to 10 ml with distilled water, in a ratio of one part by weight of tissue to two parts by volume of lysing solution. Cultured fibroblasts were lysed by freezing and thawing in 5–10 μl of lysing solution. Lysates were applied directly on cellulose acetate (Cellogel, Chemetron, Milan) and electrophoresis was carried out in 0·1 M lithium borate–0·0024 M EDTA buffer, pH = 9·0, for 1·5 h with a voltage gradient of 14 V cm^{-1}. The stain for each cellogel sheet (17 by 10 cm) consisted of 1·0 ml stain buffer (0·1 M tris–maleate buffer + 0·01 M $MgCl_2$ + 0·01 M EDTA, pH = 8·0), 0·3 ml NADP (10 mg ml^{-1}), 0·3 ml glucose-6-phosphate (25 mg ml^{-1}), 0·3 ml MTT Tetrazolium (5 mg ml^{-1} of ethylene glycol), and 0·3 ml of phenazine methosulfate (3 mg ml^{-1}).

In vitro *G6PD Hybridization*

In vitro hybridization of partially purified G6PD from erythrocytes was carried out according to the methods of Yoshida *et al.* (1967). G6PD was converted to enzymically inactive monomer by removing NADP from the G6PD–NADP complex with acidic ammonium sulphate. Individual samples and mixtures of inactive G6PD from the various species were reactivated by incubation at 37°C with NADP at neutral pH.

Results

Two electrophoretically distinguishable forms of G6PD were found in erythrocytes of kangaroos used for examining G6PD expression in tissues and cultured fibroblasts. The slower migrating form, designated G6PD-S, occurs in *Macropus robustus erubescens* Sclater, *Macropus giganteus* Shaw, *Macropus rufus* (Desmarest), *Macropus eugenii* (Desmarest), *Macropus agilis* (Gould), and *Wallabia bicolor* (Desmarest), whereas the faster migrating form, G6PD-F, is found in *M. r. robustus*. *M. rufogriseus* phenotypes are G6PD-S or G6PD-F depending on which allele is carried by the active X chromosome. Table 2 shows the results of electrophoresis of tissues of G6PD heterozygotes ranging in age from 26 days *post partum* to adult. In each individual, erythrocyte and tissue phenotypes were the same and only the maternally derived allele was expressed.

Cultured fibroblasts derived from either body wall, ear, kidney, heart, or lung of these heterozygotes revealed different G6PD phenotypes. These phenotypes are distinct from G6PD-S and G6PD-F and have been designated G6PD-I, G6PD-IS and G6PD-IF. The G6PD-I phenotype has been referred to previously by Cooper *et al.* (1975). It consists of a broad band of intermediate mobility which overlaps the fast and slow positions and occupies the space between them. The intensity of staining is greatest about the midline between the fast and slow positions. This distinguishes it from the G6PD-IF and G6PD-IS phenotypes where staining intensity is greatest towards the fast and slow positions respectively (Fig. 1). Individuals showing the G6PD-IF phenotype always have the Gpd^F allele active in their somatic tissues whereas G6PD-IS individuals have the Gpd^S allele active (Table 1). These phenotypes are peculiar to cultured fibroblasts of heterozygous females. They were not found in cultured fibroblasts of eight male offspring resulting from G6PD-S ♀ × G6PD-F ♂ matings or in the reciprocal crosses (Table 1). In addition to the data in Table 1 none of the six *M. rufogriseus* males examined were G6PD-I nor were any of the eight *M. rufogriseus* females known to be homozygous at the *Gpd* locus. G6PD-I was the only phenotype found in cultured fibroblasts of heterozygous female *M. rufogriseus*, whereas all three phenotypes occurred in *M. robustus* hybrids and backcross hybrids.

Table 2. G6PD phenotypes of erythrocytes, tissues and cultured fibroblasts of female kangaroos known to be heterozygous, and of males whose parents had contrasting allozymic phenotypes
E = ear, K = kidney, L = lung, B.W. = body wall, H = heart, S = skin, n.t. = not tested

Animal No.	Sex	Parents' erythrocyte phenotype ♀	Parents' erythrocyte phenotype ♂	Age at sampling (days)	Cultured fibroblasts Origin	Cultured fibroblasts Passage No. at electrophoresis	Erythrocyte phenotype	Tissue phenotype	Cultured fibroblast phenotype
ew1	♀	S	F	Adult	E, L	1–9	S	n.t.	I
ew4	♀	S	F	60	n.t.	n.t.	S	S	n.t.
ew55	♀	S	F	60	E, B.W.	1	S	S	I
ew73	♂	S	F	Adult	E	—	S	n.t.	S
ew82	♂	S	F	Adult	E	2	S	n.t.	S
ew/e1	♀	S	S	53	B.W.	3	F	F	IF
ew/e32	♀	S	S	Adult	E	3,4,6,9,13	F	n.t.	I
ew/e43	♀	S	S	Adult	E	1,2	F	n.t.	I
ew/w25	♀	S	F	165	L	2	S	S	I
ew/e//w94	♀	F	F	11	B.W.	1	S	n.t.	IS
ez22	♀	S	n.t.	Adult	E	3,4,9	S	n.t.	IS
ez23	♂	S	n.t.	Adult	E	1	S	S	S
ez/e28	♀	S	S	Adult	E	1,2,3,6,8	F	n.t.	IF
ez/e37	♀	S	S	10	S	1	F	n.t.	IF
ez/w54	♀	S	F	55	B.W.	1,6	S	S	I
we2	♂	F	S	18	H	2	F	F	F
we3	♂	F	S	63	n.t.	n.t.	F	F	n.t.
we4	♀	F	S	96	n.t.	n.t.	F	F	n.t.
we44	♀	F	S	Adult	E	1,2,4	F	n.t.	IF
we45	♀	F	S	Adult	E	1,8	F	n.t.	IF
we46	♀	F	S	60	B.W.,K	1,4,6	F	F	IF
we47	♀	F	S	60	B.W.	3,6	F	F	IF
we63	♀	F	S	26	B.W.	2,5	F	F	IF
we81	♀	F	S	69	B.W.	1	F	F	IF
we/w73	♀	F	F	60	E	n.t.	S	n.t.	IS
wr1	♀	F	S	Adult	E	1,8,16	F	n.t.	I
wb1	♂	F	S	Adult	E	1,3	F	n.t.	F
ng1	♂	F	S	Adult	E	3	F	n.t.	F
na1	♀	F	S	280	E,L,K	2,3,6,4,12	F	F	I
nt102	♂	F	S	Adult	E	1	F	n.t.	F
n2	♀	n.t.	n.t.	Adult	E,K	1	S	S	I
n3	♀	n.t.	n.t.	Adult	E	1	S	S	I
n6	♀	n.t.	n.t.	Adult	E,K	1	F	F	I
n10	♀	n.t.	n.t.	Adult	E,K	1	S	S	I
n13	♀	F	n.t.	285	E,K,L	2–3	F	F	I
n16	♀	n.t.	n.t.	Adult	E,K	1	F	F	I
n17	♀	n.t.	n.t.	Adult	E,K	1	S	S	I
n18	♀	n.t.	n.t.	Adult	E	1	F	F	I
n19	♀	n.t.	n.t.	Adult	E	1–3	F	F	I
n20	♀	n.t.	n.t.	Adult	E	1	S	S	I
n29	♀	F	S	200	E	3	F	n.t.	I
n85	♀	S	F	Adult	E	1	S	n.t.	I
n99	♀	S	F	Adult	E	1–4	S	n.t.	I

Because of the small mobility difference between the G6PD-S and G6PD-F positions it was necessary to select species showing greater electrophoretic separation in order to determine the subunit structure of G6PD in marsupials. Of the species surveyed *Trichosurus vulpecula* Kerr possessed the fastest migrating form, with a

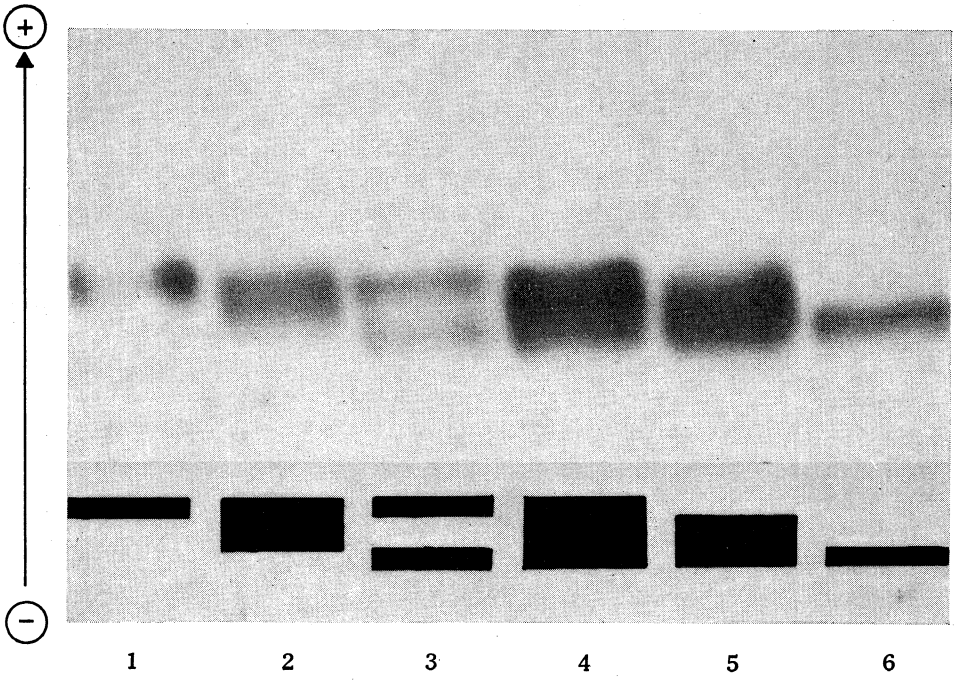

Fig. 1. Photograph and diagram of G6PD phenotypes of kangaroo cultured fibroblasts. *1*, G6PD-F; *2*, G6PD-IF; *3*, G6PD-S + G6PD-F mixture; *4*, G6PD-I; *5*, G6PD-IS; *6*, G6PD-S.

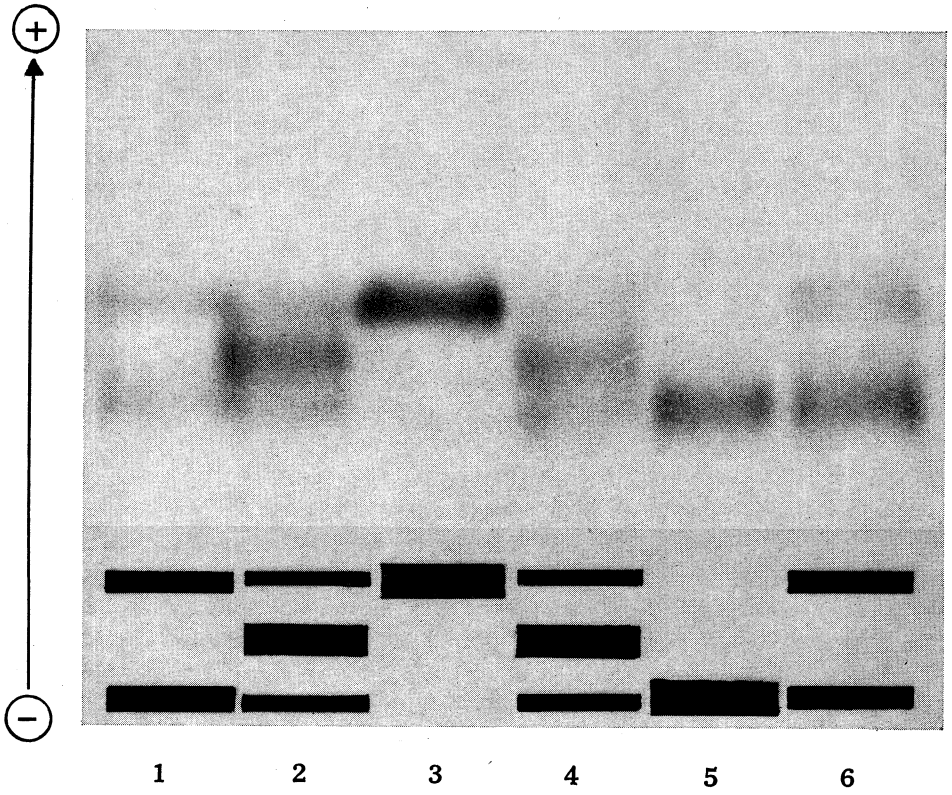

Fig. 2. Photograph and diagram of G6PD following *in vitro* hybridization. *1* and *6*, *T. vulpecula* and *P. xanthopus* G6PD mixture after separate reassociation; *2* and *4*, *T. vulpecula* and *P. xanthopus* G6PD mixture after reassociation together; *3*, *T. vulpecula* G6PD; *5*, *P. xanthopus* G6PD.

relative mobility of 1·12 compared with the standard G6PD-S position, and *Petrogale xanthopus* Gray the slowest mobility (relative mobility 0·94). Fig. 2 shows the results of electrophoresis of reassociated samples and mixtures of partially purified G6PD from *T. vulpecula* and *P. xanthopus*. When mixtures were allowed to reassociate together an intermediate band was observed in addition to the *P. xanthopus* and *T. vulpecula* G6PD bands. The intermediate band was absent when mixtures were made after the G6PD from the two species had reassociated separately. *In vitro* hybridization of G6PD from *M. r. robustus* and *M. r. erubescens* resulted in a broad band which could not be resolved into three separate bands and was indistinguishable from G6PD-I.

Mixed cultures were established by growing similar numbers of G6PD-S and G6PD-F *M. rufogriseus* cultured fibroblasts together for four passages. After each passage electrophoresis revealed distinct G6PD-F and G6PD-S bands, with no indication of a band between them.

Cultured fibroblasts from the *M. rufogriseus* × *M. agilis* female hybrid (nal) were cloned by the feeder layer method. All 67 clones expressed the G6PD-I phenotype, identical to that of the mass culture.

Discussion

Johnston and Sharman (1975) found no evidence of expression of the paternally derived *Gpd* allele in tissues of four *M. robustus* heterozygous pouch young, aged between 60 and 165 days.

We have now examined G6PD expression in tissues of an additional six *M. robustus* pouch young and 10 adult *M. rufogriseus* heterozygotes and have found no evidence of activity of the paternally derived allele irrespective of which allele is maternal or paternal in origin. This finding rules out the possibility that the paternally derived allele may be switched on later in development and it now appears that only the maternally derived G6PD allele is functional in somatic tissues of kangaroos.

These results contrast with those obtained from electrophoresis of G6PD from various somatic tissues of human heterozygotes (Linder and Gartler 1965) and mule heterozygotes (Hook and Brustman 1971). These workers observed variable expression of G6PD phenotypes in different tissues which presumably was related to the proportion of cells in the respective tissues with either the maternally or paternally derived X chromosome active. However, unlike the situation in kangaroos, it was always possible to detect activity of both the maternally and paternally derived allozymes.

The only eutherian tissue in which both *Gpd* alleles have been shown to be active in the same cell is human foetal ovary containing a high proportion of pre-dictyate oocytes (Gartler *et al.* 1973). Ovaries of some of the pouch young used in this study were at a similar stage of development but electrophoresis revealed no indication of paternal allele activity. This has been reported elsewhere in more detail (Johnston *et al.* 1976).

The behaviour of G6PD may be different from that of another sex-linked enzyme phosphoglycerate kinase, PGK-*A*, in kangaroos. VandeBerg *et al.* (1973, 1977) examined PGK-*A* expression in putative heterozygotes of the whiptail wallaby, *M. parryi*, and known heterozygotes of the eastern grey kangaroo, *M. giganteus*. They found that while there was some minor intra- and interspecific variation, it was possible to categorize tissues into three groups:

(1) tissues in which there is no detectable activity of the paternally derived allele, e.g. liver, lung and spleen;
(2) tissues which express predominantly the maternally derived allele and to a lesser extent the paternally derived allele, e.g. skeletal muscle, cardiac muscle and tongue; and
(3) tissues which express both alleles equally (restricted to leg muscle and combined uteri and ovaries of one pouch young and leg muscle of one juvenile *M. giganteus*).

Of these three categories only category (1) was observed for G6PD. It is not known whether the failure to observe category (2) for G6PD represents an actual difference in behaviour between the two loci or whether it reflects a difference in detectability of the two enzymes. The second alternative cannot be ruled out, firstly because some of the tissues in which PGK-*A* activity was high had low G6PD activity, e.g. cardiac and skeletal muscle (see Scrutton and Utter 1968), and secondly because in other tissues where G6PD activity was high, e.g. tongue, oesophagus and uterus, it is likely that different cell types were contributing to the levels of enzyme activity observed, e.g. glandular cells were largely responsible for G6PD activity and muscle cells for PGK-*A* activity. Results obtained for the two loci are therefore not strictly comparable for these tissues. We are confident, however, that category (3) does not occur for G6PD especially as G6PD activity is higher in pouch young tissues than in adult tissues.

An alternative explanation for the difference in behaviour between the two loci could be due to a species effect. However, it has not been possible to test this because we have not yet found an animal heterozygous for both *Gpd* and *Pgk-A*.

The G6PD results concur with limited data on DNA replication patterns of X chromosomes in tissues of kangaroos. J. D. Murray (personal communication) has carried out *in vivo* labelling studies on *M. robustus* hybrids and has found that spleen cells have a late-replicating paternally derived X chromosome.

It is not clear why some of the *M. robustus* hybrids and backcross hybrids with a maternally derived Gpd^F allele active in their somatic tissues exhibited G6PD-I or G6PD-IF cultured fibroblast phenotypes, while others with an active maternally derived Gpd^S allele showed G6PD-I or G6PD-IS phenotypes. The phenotype expressed does not appear to be related to the age at which the individual was sampled, the tissue from which cultured fibroblasts were derived, or the passage number when the cells were subjected to electrophoresis.

The intermediate band observed after G6PD from *T. vulpecula* and *P. xanthopus* was hybridized *in vitro* clearly demonstrates that G6PD is a dimer in marsupials as has been found in humans (Yoshida *et al.* 1967; Gartler *et al.* 1973). The pattern observed after *in vitro* hybridization of *M. robustus* G6PD was not distinguishable from the G6PD-I phenotype. This indicates that heterodimers are being produced in both cases but because of the small mobility difference between the fast and slow allozymes resolution of the hybrid band is not possible. The G6PD-IF and G6PD-IS phenotypes could result from different proportions of homodimers and heterodimers.

The finding that no heterodimers were formed when artificial mixtures of G6PD-F and G6PD-S cell lines were grown together for four passages rules out the possibility of exchange of G6PD polypeptides or mRNA between cells.

We therefore conclude that some heterozygotes express both *Gpd* alleles equally in each cultured fibroblast while other heterozygotes show normal activity of the

maternally derived allele and partial expression of the paternally derived allele. This conclusion is supported by two findings—firstly, the appearance of identical G6PD-I phenotypes in clones and mass cultures from which the clones were derived, and secondly the findings of Raphael and Cooper (1978) who estimated quantitative levels of G6PD in cultured fibroblasts of some of the female and male *M. rufogriseus* used in this study. They found that the ratio of G6PD activity in females to that in males was close to 2:1. In the other species they examined, including some *M. robustus* hybrids, the ratio was closer to 1·5:1 which is in accordance with the appearance of the G6PD-IF and G6PD-IS phenotypes and suggests that the paternally derived allele was not fully active. However, the 1·5:1 ratio had a large standard error, so that further data are necessary to test this suggestion.

We cannot decide whether both alleles are active in the cells of the ear or kidney tissue from which the cultured fibroblasts were derived or whether both alleles become active after the cells are grown *in vitro*. The precise origin of the cells which grow in culture is not known, but are thought by Franks and Wilson (1970) possibly to be derived from the precursors of small blood vessels. From observations of cell outgrowths from tissue explants it would appear that these cells comprise only a very small percentage of tissue. It is therefore not surprising that, even if both alleles were active in these cells of the live animal, no paternal allele activity was noted after electrophoresis of ear or kidney homogenates.

The G6PD cultured fibroblast results contrast with those obtained in eutherian mammals where cloning of known heterozygotes has shown that only one allele is active in each cell (Davidson *et al.* 1963; Hamerton *et al.* 1971). The only case where both *Gpd* alleles have been shown to be active in eutherian somatic tissue was in cells cultured from an aborted human XXY triploid (Weaver *et al.* 1975).

Some similarities exist between the expression of G6PD and PGK-*A* in cultured fibroblasts of kangaroos. Cooper *et al.* (1977) cloned cultured fibroblasts from *M. giganteus* heterozygotes and found that each cultured cell expresses the maternally derived allele completely and the paternally derived allele partially. This is consistent with some of the *M. robustus* G6PD-IF and G6PD-IS results but does differ from the other *M. robustus* and *M. rufogriseus* results where both alleles appear to be equally active.

In eutherians late replication of the X chromosome and the inactivation of many if not all of its genes have so far been found to be highly correlated—so much so that late replication is generally taken to indicate inactivity. The results presented here and in Cooper *et al.* (1977) for the *Pgk* locus show that in kangaroos late replication does not always indicate inactivity. In lymphocytes there is a late-replicating X chromosome and no activity for the paternally derived *Gpd* allele (Sharman and Johnston 1977). Likewise *M. giganteus* lymphocytes have a late-replicating X chromosome (Graves 1967) and an inactive paternally derived *Pgk* allele (VandeBerg *et al.* 1977). By contrast cultured fibroblasts may have a fully active paternally derived *Gpd* allele and a late-replicating paternally derived X chromosome (J. M. Graves, personal communication). Cooper *et al.* (1977) also found that the fibroblasts with a partly active paternally derived allele had a late-replicating X chromosome.

Whatever the relationship between gene inactivity and late replication it is clear that the *Gpd* locus in kangaroos behaves quite differently from the same locus in eutherians, where inactivation is random and complete in somatic cells.

We wish to emphasize the parental source effect and the expression of both alleles within individual cultured fibroblasts which characterizes the kangaroo *Gpd* locus. In a recent review Gartler and Andina (1976) have mistakenly attempted to use some of our earlier data to imply that the kangaroo X inactivation system is a random system modified by varying degrees of cell selection. Both the parental source effect in the cells of the living animal and the lack of inactivation for the *Gpd* locus in cultured fibroblasts are quite incompatible with their hypothesis.

Acknowledgments

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References

Cooper, D. W., Edwards, C., James, E., Sharman, G. B., VandeBerg, J. L., and Graves J. A. M. (1977). Studies on metatherian sex chromosomes. VI. A third state of an X-linked gene: partial activity for the paternally derived *Pgk-A* allele in cultured fibroblasts of *Macropus giganteus* and *M. parryi*. *Aust. J. Biol. Sci.* **30**, 431–43.

Cooper, D. W., Johnston, P. G., Murtagh, C. E., Sharman, G. B., VandeBerg, J. L., and Poole, W. E. (1975). Sex-linked isozymes and sex chromosome evolution and inactivation in kangaroos. In 'Isozymes. III. Developmental Biology'. (Ed. C. L. Markert.) pp. 559–73. (Academic Press: New York.)

Davidson, R. G., Nitowsky, H. M., and Childs, B. (1963). Demonstration of two populations of cells in the human female heterozygous for glucose-6-phosphate dehydrogenase variants. *Proc. Natl Acad. Sci. U.S.A.* **50**, 481–5.

Franks, L. M., and Wilson, P. D. (1970). 'Spontaneous' neoplastic transformation *in vitro*: the ultra structure of the tissue culture cell. *Eur. J. Cancer* **6**, 517–23.

Gartler, S. M., and Andina, R. J. (1976). Mammalian X-chromosome inactivation. In 'Advances in Human Genetics. 7'. (Eds H. Harris and K. Hirschhorn.) pp. 99–140. (Plenum Press: New York.)

Gartler, S. M., Liskay, R. M., and Grant, N. (1973). Two functional X chromosomes in human fetal oocytes. *Exp. Cell Res.* **82**, 464–6.

Graves, J. A. M. (1967). DNA synthesis in chromosomes of cultured leucocytes from two marsupial species. *Exp. Cell Res.* **46**, 37–57.

Hamerton, J. L., Richardson, B. J., Gee, P. A., Aden, W. R., and Short, R. V. (1971). Non-random X chromosome expression in female mules and hinnies. *Nature (London)* **232**, 312–15.

Hook, E. B., and Brustman, L. D. (1971). Evidence for selective differences between cells with an active horse X chromosome and cells with an active donkey X chromosome in the female mule. *Nature (London)* **232**, 349–50.

Johnston, P. G., Robinson, E. S., and Sharman, G. B. (1976). X chromosome activity in oocytes of kangaroo pouch young. *Nature (London)* **264**, 359–60.

Johnston, P. G., and Sharman, G. B. (1975). Studies on metatherian sex chromsomes. I. Inheritance and inactivation of sex-linked allelic genes determining glucose-6-phosphate dehydrogenase variation in kangaroos. *Aust. J. Biol. Sci.* **28**, 567–74.

Johnston, P. G., VandeBerg, J. L., and Sharman, G. B. (1975). Inheritance of erythrocyte glucose-6-phosphate dehydrogenase in the rednecked wallaby, *Macropus rufogriseus* (Desmarest), consistent with paternal X inactivation. *Biochem. Genet.* **13**, 235–42.

Linder, D., and Gartler, S. M. (1965). Distribution of glucose-6-phosphate dehydrogenase electrophoretic variants in different tissues of heterozygotes. *Am. J. Hum. Genet.* **17**, 212–20.

Raphael, K. A., and Cooper, D. W. (1978). Studies on metatherian sex chromosomes. VIII. Evidence for an absence of dosage compensation at the glucose-6-phosphate dehydrogenase locus in cultured cells of *Macropus rufogriseus*. *Aust. J. Biol. Sci.* **31**, 425–31.

Richardson, B. J., Czuppon, A. B., and Sharman, G. B. (1971). Inheritance of glucose-6-phosphate dehydrogenase variation in kangaroos. *Nature* (*London*) *New Biol.* **230**, 154–5.

Scrutton, M. C., and Utter, M. F. (1968). The regulation of glycolysis and glyconeogenesis in animal tissues. *Annu. Rev. Biochem.* **37**, 249–302.

Sharman, G. B., and Johnston, P. G. (1977). X chromosome inactivation in kangaroos (Marsupialia). In 'Reproduction and Evolution: Fourth International Symposium on Comparative Biology of Reproduction'. (Eds J. H. Calaby and C. H. Tyndale-Biscoe.) pp. 67–8. (Australian Academy of Science: Canberra.)

VandeBerg, J. L., Cooper, D. W., and Sharman, G. B. (1973). Phosphoglycerate kinase *A* polymorphism in the wallaby *Macropus parryi*: activity of both X chromosomes in muscle. *Nature* (*London*) *New Biol.* **243**, 47–8.

VandeBerg, J. L., Cooper, D. W., and Sharman, G. B. (1977). Studies on metatherian sex chromosomes. V. Activity of the paternally derived allele at the X-linked *Pgk-A* locus in some tissues of the kangaroos *Macropus giganteus* and *M. parryi*. *Aust. J. Biol. Sci.* **30**, 421–30.

Weaver, D. D., Gartler, S. M., Boue, A., and Boue. J. G. (1975). Evidence for two active X chromosomes in a human XXY triploid. *Humangenetik* **28**, 39–42.

Yoshida, A., Steinmann, L., and Harbert, P. (1967). *In vitro* hybridization of normal and variant human glucose-6-phosphate dehydrogenase. *Nature* (*London*) **216**, 275–6.

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