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Oocyte specific regulation of PGK-1 isozyme activity in female germ cells of the mouse

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SUMMARY

The expression of electrophoretic variant forms of the X-linked enzyme phosphoglycerate kinase (PGK-1) was examined during the ontogeny of the female germ-line following *X*-chromosome reactivation. Non-growing oocytes from foetal and neonatal ovaries of heterozygous females show a higher PGK-1A isozyme activity, a reflection of prior non-random X-inactivation favouring activity of the *X*-chromosome carrying the *Pgk-1*^{a} allele and *Xce*^{c} locus. On oocyte growth, the total PGK-1 enzyme activity increases 40–50 fold and the pattern of PGK-1 isozyme expression changes giving an electrophoretic pattern now skewed in favour of the PGK-1B isozymic form. Activity of the PGK-1B isozyme exceeds that of PGK-1A in all growing oocytes with a total activity greater than 0·075 nmol h^{-1} oocyte^{-1}. Mice homozygous for either *Pgk-1* allele show similar PGK-1 specific activities in somatic tissues where one *X*-chromosome is active, but oocytes of *Pgk-1*^{b} homozygotes show a higher specific activity compared to those of *Pgk-1*^{a} homozygotes. Thus increased activity of the PGK-1B isozyme relative to the PGK-1A isozyme is specific to growing oocytes.

1. INTRODUCTION

Recent evidence from analyses of the expression of a number of X-linked enzymes has shown a cycle of *X*-chromosome inactivation and reactivation in the female germ cell lineage of the mouse. Initially *X*-chromosome inactivation occurs in an early embryonic cell lineage, the epiblast, prior to delineation of the germ-line and the three definitive germ layers (McMahon, Fosten & Monk, 1983). Subsequently, the inactive *X*-chromosome is reactivated early in oogenesis around the time of entry of female germ cells into meiosis (Johnston, 1981; Monk & McLaren, 1981; Kratzer & Chapman, 1981) and both *X*-chromosomes remain active throughout oocyte development to produce eggs with two doses of X linked enzyme activity (Epstein, 1969, 1972; Kozak, McLean & Eicher, 1974; Mangia, Abbo-Halbasch & Epstein, 1975; Monk & Kathuria, 1977). Thus the oocyte is a unique

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cell in which the expression of both *X*-chromosomes may be monitored over a period characterized by considerable growth and specialization.

Specific patterns of enzyme regulation restricted to oocytes have been observed for two autosome coded enzymes, lactate dehydrogenase (LDH-1; E.C. 1.1.1.7) and glucose phosphate isomerase (GPI-1, E.C. 5.3.1.9). Unfertilized mouse eggs (Auberach & Brinster, 1967) and foetal oocytes (Brinster, 1979) express only the LDH-B subunit while other embryonic and adult tissues examined contain predominantly LDH-A subunits (Markert & Ursprung, 1962). In the case of GPI-1, differential expression of two alleles coding for electrophoretic variant forms of the enzyme is observed in ovulated oocytes (Peterson & Wong, 1978). The differential expression is under the control of a regulator locus active during oocyte growth in the neonatal ovary (McLaren & Buehr, 1981; Buehr & McLaren, 1983).

This paper reports an investigation of the activity and expression of electrophoretic variant forms of the X-linked enzyme phosphoglycerate kinase-1 (PGK-1; E.C. 2.7.2.3; Nielsen & Chapman, 1977) during the ontogeny of the female germ-line, from foetal stages to the ovulated oocyte. We present evidence for an oocyte specific regulation in PGK-1 isozyme activity associated with oocyte growth.

2. MATERIALS AND METHODS

(*i*) *Mice*

Ovulated oocytes were collected from several strain combinations of heterozygous females. In all crosses the *Pgk-1*^{a} allele was contributed by mice congenic with the inbred C3H/He strain. Inbred C3H/He, C57BL/6, BALB/c and random bred 'Swiss' albino CFLP mice were used as donors of the *Pgk-1*^{b} allele. When heterozygous mice are referred to, the first named allele of the genotype is the maternally inherited *Pgk-1* allele.

(ii) *Isolation of oocytes*

Oocytes from foetuses 15·5 days post-coitum (p.c.) and from newborn females 1 to 3 days post-partum (p.p.) were isolated by slitting gonads with electrolytically sharpened tungsten needles in PB1-PVP (McMahon, Fosten & Monk, 1981). Oocytes, identified by their large size relative to somatic cells, were collected in a finely pulled-pasteur pipette and washed three times in PB1-PVP.

Oocytes from the ovaries of females 6–42 days p.p. were isolated according to the method of Epping (1976). Adhering follicle cells were removed by pipetting oocytes in and out of a finely pulled Pasteur pipette. Oocytes were then washed three times in PB1-PVP.

Ovulated oocytes were collected from 28 to 42 days p.p. females following superovulation. Mice were injected with 5 international units (i.u.) of pregnant mare serum (PMS) at 17:00 hr, followed by an injection of 5 i.u. of human chorionic gonadotrophin (HCG) 48 h later. Sixteen to 19 h after the injection of HCG, oocytes in their surrounding cumulus mass were released from the dissected oviduct by puncturing the oviduct wall with a needle in an embryological watch-glass containing PB1-PVP. Cumulus cells were removed by 5 min incubation

in 300 i.u. of hyaluronidase in albumin free M2. Oocytes were then washed three times in PB1-PVP. For spectrophotometric assay of PGK-1 activity, oocytes were collected in 5 μl of PB1-PVP in 10 μl Drummond microcaps.

Oocyte diameters were measured using an eyepiece graticule.

(iii) *Spectrophotometric assay of PGK-1 activity in ovulated oocytes*

Groups of 20–25 ovulated oocytes were used for each determination. Samples were freeze-thawed three times in liquid nitrogen and centrifuged at 1500 rev./min for 5 min in a Beckman model TJ-6 centrifuge. The PGK-1 activity in the supernatant was assayed spectrophotometrically according to the method of Harris & Hopkinson (1976) in a Cecil CE 272 spectrophotometer.

(iv) *Cellogel electrophoresis and fluorometric quantitation of PGK-1 activity and isozyme expression in oocytes from heterozygous females*

The Cellogel (Whatman) electrophoresis and fluorometric quantitation systems were developed by Professor T. Bücher and are described in detail elsewere (Bücher *et al.* 1980; McMahon, 1981). Single oocytes or groups of oocytes were collected using a finely pulled Pasteur pipette in no greater than 0·06 μl of PB1-PVP. Samples were rapidly freeze thawed three times above liquid nitrogen before applying directly to the surface of the gel. Great care must be taken to ensure that the sample is applied to as small an area of the gel as is possible, so that the PGK-1 activity is localized after electrophoresis. The proportions of PGK-1 isozymes following electrophoresis were measured as described by McMahon (1981) and McMahon, Fosten & Monk (1981, 1983). Total PGK-1 activity in oocyte samples was calculated from the calibration curve correlating activity with peaks recorded after 29–31 min of staining (see Results).

(v) *Determination of PGK-1 specific activity in somatic tissue samples from homozygous females*

Brain, kidney and liver tissues were dissected from three homozygous *Pgk-1^a/Pgk-1^a* and three homozygous *Pgk-1^b/Pgk-1^b* females. Tissues were placed into a 0·1 ml glass homogenizer on ice with an equal volume of PBS and thoroughly homogenized. Tissue extracts were centrifuged for 10 min at 4 °C and 1500 rev./min in a Beckman model TJ6 centrifuge, the supernatant removed, diluted 1/100 with PBS and PGK-1 activity assayed spectrophotometrically. Protein contents were determined according to the method of Lowry, Rosenbrough, Farr & Randall (1951).

3. RESULTS

(i) *PGK-1 isozyme expression in ovulated oocytes from females heterozygous for Pgk-1*

PGK-1 isozyme expression was measured in single, ovulated oocytes from heterozygous females. In heterozygotes from matings between parents sharing a

Table 1. *Mean PGK-1B isozyme expression in unfertilized ovulated oocytes from heterozygous females 28 to 42 days p.p.*

Genotype of female*	Strain of mice donating the *Pgk-1^b*† allele	No. of individual oocytes scored	Mean % PGK-1B expression
Pgk-1^b/Pgk-1^a	C3H/He	11	60·6 ± 1·1§
Pgk-1^a/Pgk-1^b	C3H/He	11	58·2 ± 0·9§
Pgk-1^b/Pgk-1^a	C57BL/6	4	62·7 ± 1·9
Pgk-1^b/Pgk-1^a	BALB/C	6	67·6 ± 2·1
Pgk-1^a/Pgk-1^b	CFLP	8	61·5 ± 3·4

*First named *Pgk-1* allele is maternally inherited.
† The *Pgk-1^a* allele was donated by mice congenic with C3H/He strain in all cases.
§ These values were found not to be significantly different ($t = 1·66$, $P = >0·1$).
Data is shown ± standard error.

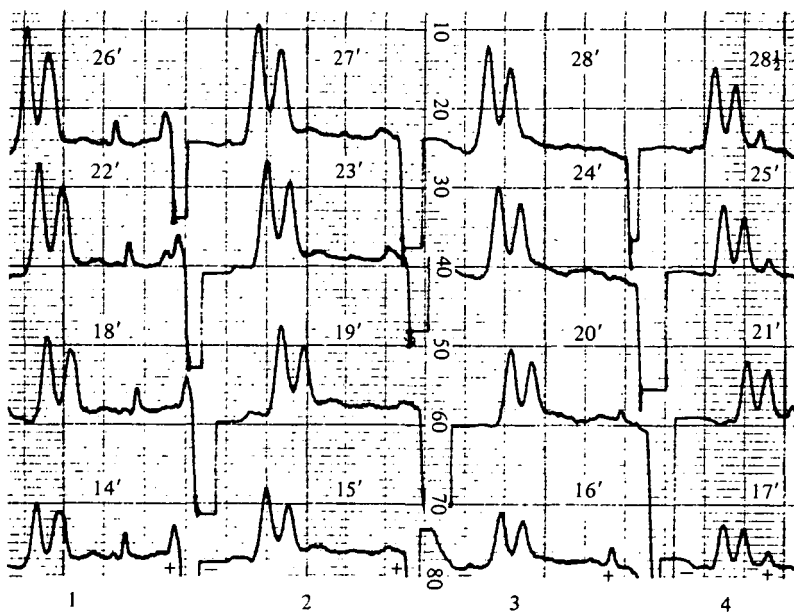

Fig. 1. Fluorometrically recorded PGK-1 isozyme activities in four, individual, ovulated oocytes from *Pgk-1^a/Pgk-1^b* female. Tracing 1 corresponds to 1 in Plate 1*a*, etc. PGK-1 isozyme activities in the oocyte samples were measured at various times after the addition of stain, as indicated. The PGK-1B isozyme contributions calculated for each oocyte are 1, 53·5 %; 2, 56·8 %; 3, 55·2 %; 4, 56·4 %. Time in minutes after the addition of stain is shown.

common C3H/He genetic background, both isozymes were expressed but the activity of the PGK-1B isozyme was greater than that of the PGK-1A isozyme. The mean PGK-1B isozyme proportions were calculated to be 60·6 % ± 1·1 and 58·2 % ± 0·9 in oocytes from *Pgk-1^b/Pgk-1^a* and *Pgk-1^a/Pgk-1^b* heterozygotes respectively (Table 1). Plate 1*a* shows a representative gel of PGK-1 isozyme expression in four individual *Pgk-1^a/Pgk-1^b* oocytes and Fig. 1 the corresponding records from which the PGK-1 isozyme proportions were estimated. Follicle cells which surround the oocytes showed the somatic pattern of PGK-1 isozyme

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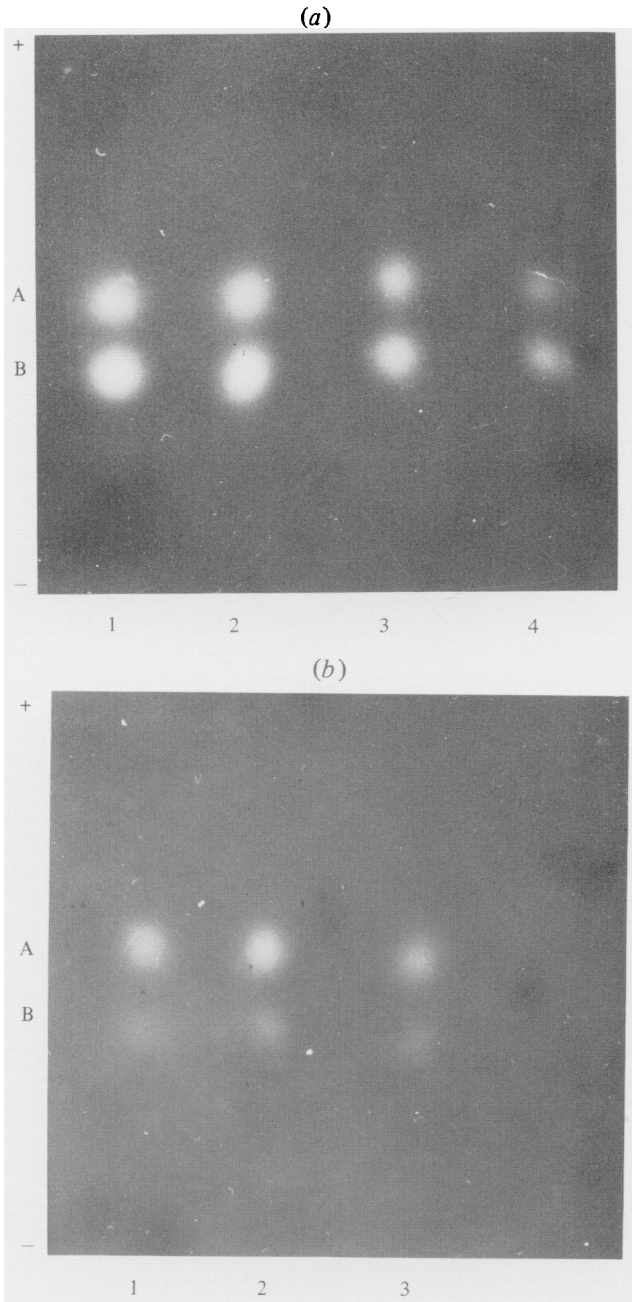

(*a*) PGK-1 isozyme expression in four individual oocytes from a *Pgk-1*^{a}/*Pgk-1*^{b} female. All oocytes show a higher PGK-1B isozyme activity. The fluorometrically recorded PGK-1 isozyme activities are shown in Fig. 1.
(*b*) PGK-1 isozyme expression in oocytes from 15·5 day p.c. *Pgk-1*^{b}/*Pgk-1*^{a} foetuses. Three samples (Tracks 1–3) each containing 33 oocytes 14–18·5 μm in diameter were applied to the gel. All samples show a higher PGK-1A activity. The fluorometrically recorded PGK-1B isozyme contribution in each sample was 1, 44·4%; 2, 42·6%; 3, 29·9%.

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expression with higher PGK-1A activity (data now shown). This is expected from non-random X-inactivation under the influence of the *Xce*^{c} allele carried on the *Pgk-1*^{a} *X*-chromosome (Johnston & Cattanach, 1981).

To investigate whether the uneven expression of PGK-1 isozymes was a peculiar property of the C3H/He strain, ovulated oocytes from heterozygous females from matings between other inbred and a random bred strain were examined. The results are shown in Table 1. All oocytes were found to express a higher PGK-1B activity.

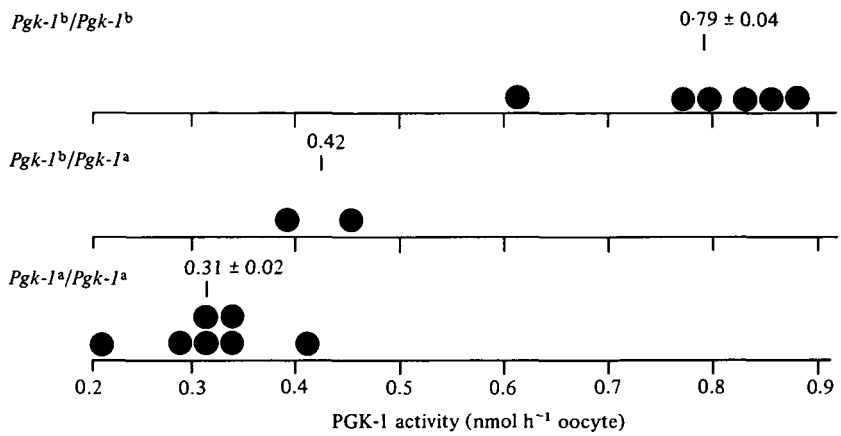

Fig. 2. Spectrophotometric assay of PGK-1 activity in ovulated oocytes. Mean PGK-1 activity for each genotype is shown ± standard error. All values as assessed by the *t* test were significantly different from their nearest neighbours (*Pgk-1*^{a}/*Pgk-1*^{a} to *Pgk-1*^{b}/*Pgk-1*^{a} $t = 2{\cdot}62$, $P = < 0{\cdot}05$; *Pgk-1*^{b}/*Pgk-1*^{a} to *Pgk-1*^{b}/*Pgk-1*^{b}/$t = 5{\cdot}10$, $P = < 0{\cdot}005$).

Table 2. *Specific activity of PGK-1 isozymes in somatic tissue samples from homozygous Pgk-1*^{a}/*Pgk-1*^{a} *and Pgk-1*^{b}/*Pgk-1*^{b} *female mice*

Tissue	Genotype of female*	Mean specific activity of PGK-1 isozyme (μmoles hr^{-1} μg protein^{-1})	
Liver	*Pgk-1*^{a}/*Pgk-1*^{a}	0·087 ± 0·017	$t = 0{\cdot}48$, $P = > 0{\cdot}5$
	Pgk-1^{b}/*Pgk-1*^{b}	0·097 ± 0·010	
Brain	*Pgk-1*^{a}/*Pgk-1*^{a}	0·115 ± 0·002	$t = 1{\cdot}46$, $P = > 0{\cdot}2$
	Pgk-1^{b}/*Pgk-1*^{b}	0·129 ± 0·009	
Kidney	*Pgk-1*^{a}/*Pgk-1*^{a}	0·144 ± 0·004	$t = 0{\cdot}59$, $P = > 0{\cdot}5$
	Pgk-1^{b}/*Pgk-1*^{b}	0·156 ± 0·019	

* Females *Pgk-1*^{a}/*Pgk-1*^{a} (congenic with C3H/He strain) and *Pgk-1*^{b}/*Pgk-1*^{b} (C3H/He strain) were used for tissue samples.

Data is shown ± standard error.

(ii) *PGK-1 specific activity in ovulated oocytes from females homozygous for Pgk-1 alleles*

PGK-1 enzyme activity was measured spectrophometrically in ovulated oocytes from homozygous *Pgk-1*^{a}/*Pgk-1*^{a} and *Pgk-1*^{b}/*Pgk-1*^{b} female mice. The mean PGK-1 activity was found to be $0{\cdot}31 \pm 0{\cdot}02$ nmol h^{-1} oocyte^{-1} and $0{\cdot}79 \pm 0{\cdot}04$ nmol h^{-1} oocyte^{-1} respectively (Fig. 2). Ovulated oocytes from heterozygous *Pgk-1*^{b}/*Pgk-1*^{a} females showed an intermediate PGK-1 activity of

0·42 nmol h^{-1} $oocyte^{-1}$, significantly different from either class of homozygous oocyte. However, the intermediate value is less than that predicted from the mean of the homozygous values (0·55 nmol h^{-1} $oocyte^{-1}$). Although only two activity values were measured for heterozygous oocytes, the mean value of 0·42 nmol h^{-1} $oocyte^{-1}$ is supported by later fluorometric assays (Tables 3 and 4) on individual oocytes and it is likely that the difference from the expected value of 0·55 nmol h^{-1} $oocyte^{-1}$ is real.

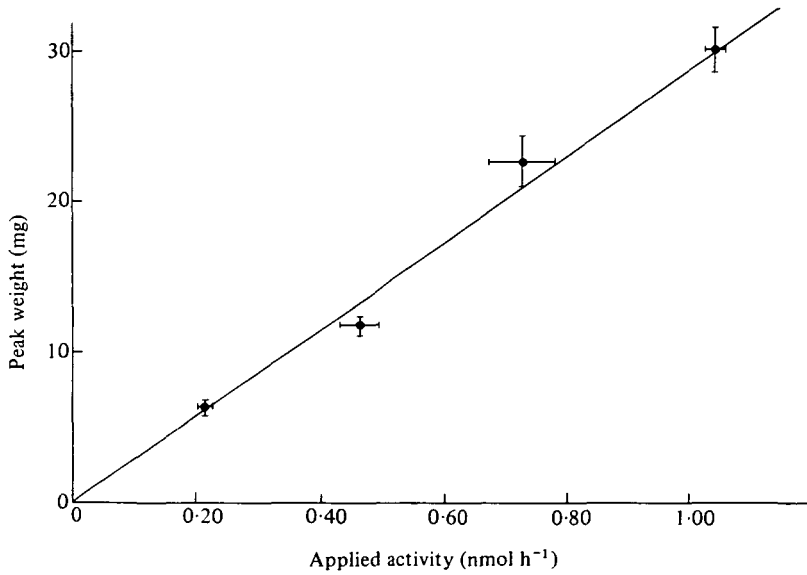

Fig. 3. Standard curve of peak weights measured 29–31 min after addition of stain against applied PGK-1 activity in serial dilutions of a brain cell extract. PGK-1 activities in these samples were assayed spectrophotometrically. A volume of 0·06 μl of each dilution was applied to the gel and recorded fluorometrically after electrophoresis. The graph shows the pooled results of three independent experiments with standard error bars. PGK-1 activity in oocytes was calculated by reference to this standard curve.

Examination of the specific activity of PGK-1 isozymes in somatic tissue extracts from brain, liver and kidney of *Pgk-1*^{a}/*Pgk-1*^{a} and *Pgk-1*^{b}/*Pgk-1*^{b} C3H/He strain female mice showed slightly higher specific activities of PGK-1 B but the differences in the specific activity of PGK-1 isozymes were not significant (Table 2) in agreement with other studies (West & Chapman, 1978; Johnston & Cattanach, 1981; Mühlbacher *et al.* 1982). Therefore the higher PGK-1B activity observed in oocytes appears to be specific to this cell type.

(ii) *Origin of differential PGK-1 isozyme expression during the ontogeny of the germ-line*

To determine when the differential activity of PGK-1 isozymes is established, oocytes at different stages of growth were collected from ovaries of *Pgk-1*^{b}/*Pgk-1*^{a} and *Pgk-1*^{a}/*Pgk-1*^{b} females of varying age. Non-growing oocytes ('resting' oocytes)

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14–18·5 μm in diameter are present throughout ovarian life. Oocyte growth commences at approximately 4 days p.p. in some oocytes and from this time the ovary contains a mosaic of different size oocytes. The largest size class of oocytes from females of different ages shown in Tables 2 and 3 represent the largest oocytes in ovaries of that particular age. Fully grown oocytes have diameters between 83 and 92 μm. Oocyte growth is also associated with growth of surrounding follicle cells (Pedersen & Peters, 1968) though most of the follicular growth occurs after the oocyte growth phase (Brambell, 1928).

Table 3. *Mean PGK-1 activity and PGK-1 isozyme expression in oocytes of varying age from Pgk-1^b/Pgk-1^a female ovaries**

Age of female†	Oocyte diameter (μm)	No. of oocytes in sample applied to gel	No. of samples scored	Mean PGK-1 activity (nmoles h^{-1} oocyte^{-1})	Mean %PGK-1B expression
15·5 d.p.c.	14·0–18·5	25–33	6	0·009 ± 0·001	40·5 ± 3·8
1 d.p.p.	14·0–18·5	25–30	2	0·012	38·7
6 d.p.p.	14·0–18·5	10–12	4	0·014 ± 0·003	44·5 ± 4·6
	23·0–28·0	5	2	0·053	40·1
	37·0–46·0	3	2	0·053	48·6
8 d.p.p.	37·0–46·0	2	4	0·114 ± 0·015	56·0 ± 0·7
10 d.p.p.	55·5–64·5	1	2	0·178	57·8
16 d.p.p.	14·0–23·0	8	1	0·043	39·7
	28·0–37·0	4	3	0·124 ± 0·014	55·8 ± 1·8
	42·0–46·0	4	3	0·145 ± 0·020	59·3 ± 3·1
	55·5–64·5	2	5	0·225 ± 0·024	57·4 ± 5·0
21–22 d.p.p.	18·5–23·0	10	1	0·049	47·6
	37·0–41·5	2–3	5	0·188 ± 0·020	53·8 ± 3·0
	55·5–60·0	2–3	4	0·288 ± 0·045	58·3 ± 2·2
	74·0–83·0	1	5	0·477 ± 0·053	57·9 ± 1·2
28–42 d.p.p.	37·0–46·0	1	4	0·369 ± 0·037	61·0 ± 2·0
	55·5–64·5	1	6	0·367 ± 0·055	57·6 ± 3·2
	83·0–92·0	1	7	0·472 ± 0·032	62·4 ± 1·7
	83·0–92·0§	1	11	0·417 ± 0·032	60·6 ± 1·1

* In this cross the *Pgk-1^b* allele is maternally inherited. *Pgk-1^b* alleles were derived from C3H/He strain mice and *Pgk-1^a* alleles from mice congenic with the C3H/He strain.

† Age of female is shown in days post-coitum (d.p.c.) or days post-partum (d.p.p.).

§ Ovulated oocytes, 16–19 h post HCG injection.

Data is shown ± standard error.

PGK-1 isozyme expression during oocyte ontogeny was investigated with respect to total PGK-1 activity in oocytes and the stage of oocyte growth. The total PGK-1 activity and relative isozyme contributions were determined fluorometrically in the same oocyte sample. The enzyme activity in oocytes was obtained from the calibration curve shown in Fig. 3. This curve was prepared by comparing the PGK-1 activity in serial dilutions of somatic cell extracts, determined by spectrophotometric assay, with the fluorescence recorded 29–31 min after the addition of a stain following 'Cellogel' electrophoresis of a small sample of known volume (0·06 μl) of the same dilutions.

For the oocytes the relative isozyme contributions was determined by the relative weights of the two peaks and the total PGK-1 activity from the sum of the peak weights recorded 29–31 min after the addition of stain using the calibration curve. Tables 3 and 4 show the PGK-1 activities and isozyme proportions in oocytes of increasing diameter from *Pgk-1^b/Pgk-1^a* and *Pgk-1^a/Pgk-1^b* females of increasing age.

Table 4. *Mean PGK-1 activity and PGK-1 isozyme expression in oocytes of varying age and diameter from Pgk-1^a/Pgk-1^b female ovaries**

Age of female†	Oocyte diameter (μm)	No. of oocytes in sample applied to gel	No. of samples scored	Mean PGK-1 activity (nmoles h^{-1} oocyte^{-1})	Mean % PGK-1B expression
2 d.p.p.	14·0–18·5	20–30	2	0·010	41·1
3 d.p.p.	14·0–18·5	20–30	3	0·009 ± 0·001	37·2 ± 3·2
7 d.p.p.	14·0–18·5	20–30	3	0·016 ± 0·001	37·4 ± 1·5
	23·0–28·0	5	2	0·053	48·6
	37·0–46·0	5	2	0·090	52·2
21 d.p.p.	55·5–60·0	3	3	0·167 ± 0·009	58·3 ± 2·6
	74·0–83·0	1	3	0·358 ± 0·045	57·0 ± 3·4
28–42 d.p.p.	83·0–92·0§	1	11	0·451 ± 0·024	58·2 ± 0·9

*In this cross the *Pgk-1^a* allele is maternally inherited. *Pgk-1^b* alleles were derived from C3H/He strain mice and *Pgk-1^a* alleles from mice congenic with the C3H/He strain.

† Age of female is shown in days post-coitum (d.p.c.) or days post-partum (d.p.p.).

§ Ovulated oocytes, 16–19 h post HCG injection.

Data is shown ± standard error.

(*a*) *PGK-1 enzyme activity during oocyte growth*

Column 5 in Tables 3 and 4 shows total PGK-1 activity relative to the age of female and oocyte diameter. PGK-1 activity in foetal oocytes (15·5 days p.c.) and non-growing oocytes from neonatal ovaries (14–18·5 μm in diameter) was found to be similar, close to 0·01 nmol h^{-1} oocyte^{-1}. The smallest population of oocytes isolated at 16 and 21–22 days p.p. showed some growth (up to 23·0 μm) and higher PGK-1 activities were recorded (0·04–0·05 nmol h^{-1} oocyte^{-1}). Figure 4 demonstrates that considerable increase in PGK-1A activity occurs as oocytes grow. From the non-growing oocytes of foetal and neonatal ovaries to the fully grown oocytes of ovaries 28–42 days p.p. (83·0–92·0 μm in diameter), PGK-1 activity increased 40- to 50-fold. It may be noted that the PGK-1 activity in ovulated *Pgk-1^b/Pgk-1^a* oocytes measured by gel fluorescence (0·417 ± 0·032 nmol h^{-1} oocyte^{-1}; Table 3) is in excellent agreement with the spectrophotometrically recorded activity (0·42 nmol h^{-1} oocyte^{-1}; Figure 2).

(*b*) *PGK-1 isozyme expression in developing oocyte*

Column 6 in Tables 3 and 4 shows the PGK-1 isozyme contributions calculated in heterozygous oocytes of varying size isolated from ovaries of varying age. All non-growing oocytes (14–18·5 μm in diameter) showed a higher PGK-1A activity

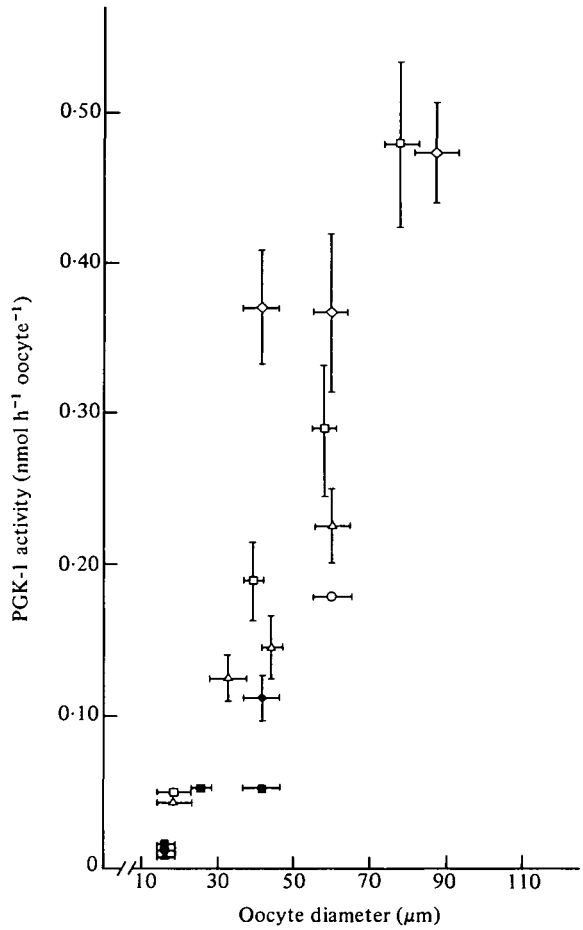

Fig. 4. PGK-1 activity in oocytes of varying diameter isolated from *Pgk-1^b/Pgk-1^a* females of various ages. Data plotted from Table 3. Oocytes from ovaries 15·5 days p.p. ▲; 1st day p.p. ●; 6th day p.p. ■; 8th day p.p. ◆; 10th day p.p. ○; 16th day p.p. △; 21st to 22nd day p.p. □; 28th to 42nd day p.p. ◇. Horizontal bars indicate range in oocyte size and vertical bars standard error for the measurement of enzyme activity.

(Plate 1*b*), while all oocytes greater than 55·0 μm in diameter showed a higher PGK-1B activity, similar to ovulated oocytes. Oocytes at intermediate stages of growth showed either a higher PGK-1A or PGK-1B activity depending on the age of the ovary from which they were isolated. The first oocytes with higher PGK-1B isozyme activity were found in females 7–8 days p.p.

Comparison of PGK-1 isozyme contribution with PGK-1 activity showed a clearer relationship (Fig. 5). All oocytes with a PGK-1 activity less than 0·075 nmol h^{-1} oocyte^{-1} showed a higher PGK-1A activity while those with a PGK-1 activity greater than 0·075 nmol h^{-1} oocyte^{-1} showed a higher PGK-1B activity. Therefore, as oocytes increase their PGK-1 activity, as described above, the pattern of PGK-1 isozyme expression changes to that seen in the ovulated oocyte.

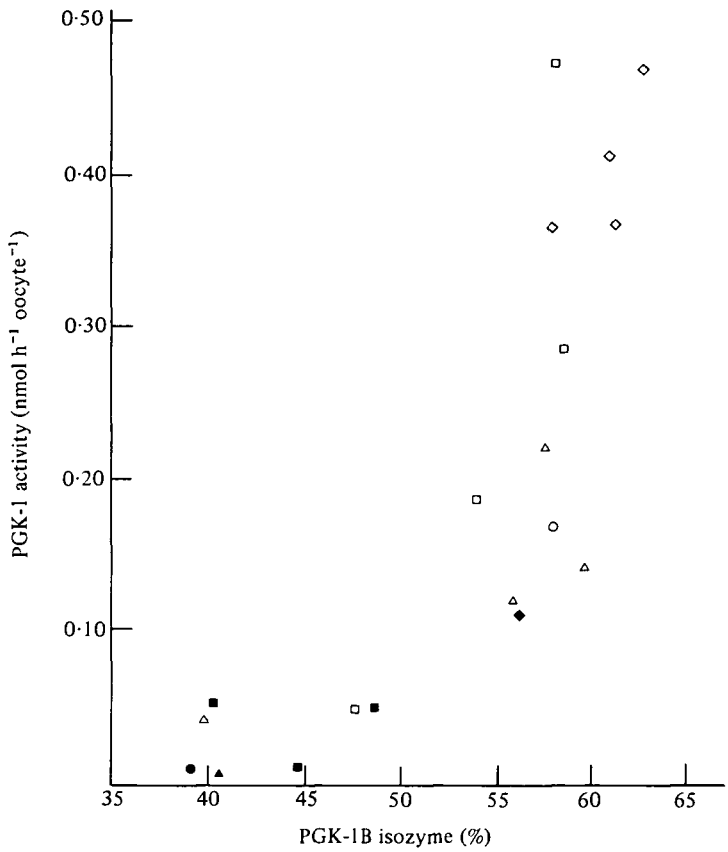

Fig. 5. Comparison of PGK-1 isozyme expression with total PGK-1 activity in oocytes form *Pgk-1*^{b}/*Pgk-1*^{a} heterozygous females. Data plotted from Table 3. Symbols used are described in legend to Fig. 4.

4. DISCUSSION

PGK-1 activity and relative PGK-1 isozyme expression were examined in oocytes from early meiotic stages to ovulation in the mouse. Both PGK-1 isozymes were expressed in single oocytes from heterozygous females confirming earlier PGK-1 enzyme gene dosage experiments (Kozak *et al.* 1974) which indicated both *X* chromosomes were active in oocytes at this time.

As the specific activity of PGK-1 isozymes is equal in somatic tissue (Table 2; see also West & Chapman, 1978; Johnston & Cattanach, 1981; Mühlbacher *et al.* 1983), one might expect approximately equal expression of each isozyme in the ovulated oocyte. However, a higher PGK-1B isozyme activity (~60%) was consistently recorded. This result was true of all strains tested and was independent of the parental origin of the *Pgk-1* allele. When PGK-1 isozyme expression was investigated during oocyte growth, only growing oocytes showed a higher PGK-1B isozyme activity. Non-growing oocytes in contrast, displayed a higher PGK-1A activity.

Recent evidence has convincingly demonstrated that female germ cells undergo

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X inactivation and subsequent reactivation around the time of entry into meiosis (Gartler, Rivest & Cole, 1980; Johnston, 1981; Monk & McLaren, 1981; Kratzer & Chapman, 1981). It is suggested that the higher PGK-1A activity in non-growing oocytes reflects the past X inactivated state of the mitotic germ cell (McMahon *et al.* 1981). The result is consistent with studies on *Pgk-1* isozyme expression in oocytes of foetal mice heterozygous for *Pgk-1* and Searle's X autosome translocation (Lyon *et al.* 1964). The translocated *X*-chromosome is active in most, if not all cells after X-inactivation due to cell selection (McMahon & Monk, 1983). After the previously inactive *Pgk-1*^{a} carrying *X* chromosome is reactivated in non-growing foetal oocytes PGK-1A isozyme activity remains considerably less than the PGK-1B isozyme activity (Johnston, 1981; McMahon, 1981), reflecting the previous imbalance of X-inactivation. In the females considered in this paper the *Pgk-1*^{a}-carrying *X* chromosome is preferentially expressed following X-inactivation due to its high expression *Xce*^{c} locus (Johnston & Cattanach, 1981). The persistence of the higher PGK-1A isozyme activity in non-growing oocytes from neonatal ovaries of *Pgk-1* heterozygotes is probably a result of the little, if any, new RNA synthesis in these oocytes (Moore *et al.* 1974; Moore & Lintern-Moore, 1978; Lintern-Moore & Moore, 1979).

With the onset of oocyte growth, PGK-1 activity increases. PGK-1 activity in the largest ovarian oocytes is 40- to 50-fold that in non-growing oocytes from foetal and neonatal ovaries. The total increase in PGK-1 activity is in line with the reported 38-fold elevation in protein synthesis over this period (Schultz, Letourneau & Wassarman, 1979). In contrast, the increase in activity of two other glycolitic enzymes LDH (Mangia & Epstein, 1975; Mangia, Erickson & Epstein, 1976) and glucose-6-phosphate dehydrogenase (E.C. 1 . 1 . 1 . 49; Mangia & Epstein, 1975) are reported to be proportional to or greater than the increase in oocyte volume.

The increase in PGK-1 activity, presumably associated with the transcription and translation of new message, is correlated with a change in the pattern of PGK-1 isozyme contributions. All oocytes with a total PGK-1 activity of greater than 0·075 nmol h^{-1} $oocyte^{-1}$ show a higher PGK-1 B isozyme activity. It is interesting to note that the first oocytes found to show the oocyte specific pattern of PGK-1 isozyme expression are the largest oocytes of the 7- to 8-day p.p. ovary. McLaren & Buehr (1981) first observed the oocyte specific regulation of GPI-1 expression in similar oocytes from 6 to 7 day p.p. ovaries. Differential expression of PGK-1A and PGK-1B isozymes may also occur in embryonal carcinoma cells heterozygous for *Pgk-1* and with two active *X* chromosomes. Although McBurney & Strutt (1980) claim roughly equal isozyme expression, a closer examination of their data suggests a higher PGK-1B activity.

The higher PGK-1B isozyme activity observed in growing oocytes may result from (*a*) oocyte specific differential degradation of PGK-1A message or isozyme, (*b*) an oocyte specific regulatory gene controlling PGK-1 isozyme production. In support of the former explanation PGK-1A isozyme is known to be less heat stable than the PGK-1B isozyme in somatic tissue extracts at 49 °C (McMahon, 1981; Mühlbacher *et al.* 1983), though no difference in stability is detected at 45 °C (McMahon, 1981). In the oocyte, it is apparent that any differential stability of isozymes would be restricted to the growth phase when PGK-1 activity is

increasing, as prior to this time PGK-1A isozyme activity is higher. If differential stability plays a role it is specific to oocytes. Blood cells which one may expect to reflect differences in stability show the normal somatic cell expression, that is a higher PGK-1A activity (McMahon, unpublished observations).

Linkage analysis may be attempted to look for tissue specific regulation of alleles but in general, regulatory and structural loci are closely linked (Breen, Lusis & Paigen, 1977; Petersen & Wong, 1978; McLaren & Buehr, 1981) making this type of genetic analysis problematic. In conclusion, we have presented further evidence for regarding the oocyte as a uniquely regulated biochemical environment, although the significance, if any, of the oocyte specific variation in PGK-1 isozyme activities is unknown.

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