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Mammalian Oocytes: X Chromosome Activity

Abstract. *The glucose-6-phosphate dehydrogenase and lactate dehydrogenase contents of oocytes from XO and XX female mice have been measured. The activity of the former in the oocytes of XO mice is half of that in the oocytes of XX mice, whereas the lactate dehydrogenase activities in the two groups of ova are the same. These results indicate that glucose-6-phosphate dehydrogenase from a mouse oocyte is an X-linked enzyme, that its synthesis occurs in the oocyte and is dosage dependent, and that inactivation of the X chromosome does not occur in the mouse oocyte.*

Cytological and biochemical evidence indicates that only one X chromosome functions in any somatic cell of female mammals, so that males with only one X chromosome and females with two are functionally equivalent with regard to the gene products of the X chromosome (*1*). However, the two X chromosomes of mammalian oocytes do not behave in the same manner as those in the somatic cells. Rather than one of the X chromosomes appearing heteropycnotic (inactive) and the other isopycnotic (active), both are isopycnotic (*2*). If the cytological appearance of the chromosomes can be translated into physiological terms, both X chromosomes in the mammalian oocyte should be metabolically active. To test this hypothesis it is necessary to have a dosage-dependent X-linked marker and oocytes differing in the number of X chromosomes which they possess. These requirements are met by oocytes from the mouse *Mus musculus,* and our results indicate that both X chromosomes do function in mammalian oocytes.

Of the enzymes known to be present in the mouse ovum, the two present in the largest amount are lactate dehydrogenase (LDH) (E.C.1.1.1.27) and glucose-6-phosphate dehydrogenase (G6PD) (E.C.1.1.1.49) (*3*). The B subunits of LDH in man, deer mouse, and pigeon are under autosomal control, and it may be inferred that the B subunit present in the mouse oocyte is also under autosomal control (*4*); LDH thus is a useful index of autosomal activity.

Ohno has recently argued that all mammalian G6PD's are under control of the X chromosome since erythrocyte G6PD is X-linked in several quite remotely related mammalian orders, including man (*5*). Furthermore, G6PD from both deer mouse and human erythrocytes is functionally homologous, and that from rat and human erythrocytes is hybridizable in vitro, indicating structural homology (*6*). The X-linked G6PD must be distinguished from the autosomally controlled and less specific microsomal hexose phosphate dehydrogenase or glucose dehydrogenase (*7*). The G6PD in mouse oocytes has the same substrate specificity as erythrocyte G6PD, and both differ quite markedly from the hepatic microsomal hexose phosphate dehydrogenase (*8*).

Gene dosage holds for many autosomal loci of man and other mammals, and investigations of polyploid cells in mammalian liver have indicated that dosage probably holds for whole sets of chromosomes as well as for individual loci (*9*). If gene dosage applies to the synthesis of oocyte G6PD, and if oocyte X chromosomes are not inactivated, the content of G6PD should be proportional to the number of X chromosomes in the oocyte. This is what has been observed.

To obtain mouse oocytes differing in the number of X chromosomes they contain, XX and XO female mice were used (*10*). The XO and XX females are produced by crossing *Ta*/O females with B6CBAF_1(+) males. Tabby (*Ta*) is a marker used to follow segregation of the X chromosome. The female offspring of this cross are either *Ta*/+(XX) with a variegated phenotype or +/O (XO) with a wild-type phenotype. The XO females are also obtained by crossing *Ta*/Y males with +/O females to produce *Ta*/O females with the full tabby phenotype. Although the ovulated ova from XO females are either X or O in genotype after extrusion of the first polar body, oocyte LDH and G6PD are synthesized before this time, and all oocytes from XO females may be considered to have been XO in geno-

Table 1. Enzyme activities (mean ± S.E.) of mouse oocytes. The numbers of individual assays are noted in parentheses. Activities are expressed as nanomoles of substrate per hour per oocyte; *P* was calculated by Student's *t*-test.

Experiment	Genotype				Ratio of activities XO/XX	*P* for XO vs. XX
	XX	XO				
	Ta/+	*Ta*/O	+/O	Combined		
			Glucose-6-phosphate dehydrogenase			
1	1.64 ± .07 (17)	0.83 ± .05 (4)	0.83 (1)	0.83 ± .04 (5)	0.50	<.001
2	1.67 ± .10 (9)	.92 ± .03 (4)	.97 (1)	.93 ± .02 (5)	.56	<.001
3	1.54 ± .05 (10)	.80 (1)	.82 ± .06 (4)	.81 ± .04 (5)	.53	<.001
All experiments	1.62 ± .04 (36)	.86 ± .03 (9)	.84 ± .04 (6)	.86 ± .02 (15)	.53	<.001
			Lactate dehydrogenase			
2	34.5 ± 3.7 (7)	38.9 ± 1.4 (4)		38.9 ± 1.4 (4)	1.13	>.40
3	45.4 ± 2.1 (9)	45.5 ± 5.7 (2)	46.8 ± 2.7 (4)	46.4 ± 2.7 (4)	1.02	>.70
All experiments	40.7 ± 2.4 (16)	41.1 ± 2.2 (6)	46.8 ± 2.7 (4)	43.4 ± 1.9 (10)	1.06	>.40

type during the period of enzyme synthesis (*3*).

Ovulation was induced in mature XO and XX females by the intraperitoneal administration of 5 international units (I.U.) of pregnant mare serum gonadotropin (Equinex, Ayerst); 48 hours later 5 I.U. human chorionic gonadotropin (A.P.L., Ayerst) was administered intraperitoneally. The mice were killed 24 hours after the second injection, and the oviducts were removed. The ova were collected from the oviducts, and the cumulus cells were stripped off by brief exposure to hyaluronidase (Wydase, Wyeth). The ova were washed three times in Krebs-Ringer bicarbonate with 0.1 percent bovine serum albumin and then assayed for G6PD or LDH activity (*3*). Both assays were carried out at 37°C in total volumes of 0.12 ml with a Gilford 2000 recording spectrophotometer. Two to four ova were used for each G6PD assay and one for each LDH assay. Oocytes were obtained from a total of 6 XX (*Ta*/+) and 7 XO (4 *Ta*/O, 3 +/O) females. In nearly all instances, fewer usable ova were obtained from XO (mean 5.6) than from XX females (mean 13.8).

In each separate experiment, as well as in the combined results, the mean G6PD activity in XO oocytes was half of that in XX oocytes (Table 1). This is what had been predicted, since XO oocytes have half as many X chromosomes as XX oocytes. The G6PD activities in the two different genotypes of XO, *Ta*/O and +/O, did not differ, an indication that the results were not influenced by the presence of the tabby mutation. By contrast to G6PD, the amounts of autosomally controlled LDH in the XO and XX oocytes did not differ significantly. Therefore, the G6PD results cannot be attributed to some general metabolic abnormality of XO ova. Furthermore, follicular and other ovarian stromal cells all manifest X-chromosomal inactivation and have only one functioning X chromosome in both XO and XX animals (*2*). Therefore the enzyme must be synthesized within the egg, and the difference in G6PD activities must reflect an intrinsic difference in the oocyte—the difference being the number of active X chromosomes.

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Leukotactic Factor Produced by Sensitized Lymphocytes

Abstract. *Lymph node lymphocytes obtained from guinea pigs exhibiting delayed hypersensitivity are stimulated in vitro by specific antigen to produce a soluble factor that is chemotactic in vitro for mononuclear macrophages. The material is nondialyzable, relatively heat stable, and elutes from Sephadex G-100 in the fraction containing molecules smaller than immunoglobulins.*

In recent years, several aspects of the mechanism of delayed hypersensitivity have been delineated with the use of an in vitro assay for cellular hypersensitivity which features the inhibition of random cell migration from capillary tubes (*1*). Lymphocytes from guinea pigs exhibiting delayed hypersensitivity produce, during incubation with specific antigen, a soluble material that inhibits the random migration of normal macrophages in vitro (*2–4*). This factor elutes on gel filtration with materials the size of albumin and has been termed migration inhibitory factor (MIF) (*5–7*). In the same system, only a few sensitized lymphocytes need be present to affect the behavior of a large number of nonsensitized macrophages (*2*, *8*). Further, studies made with the use of passive transfer of delayed hypersensitivity have demonstrated that the majority of cells present in a delayed skin lesion are nonsensitized cells of host origin, only a few being specifically sensitive to antigen (*9*). Therefore, it was of interest to determine whether sensitized lymphocytes stimulated by antigen would produce a soluble chemotactic factor (or factors) that would attract large numbers of mononuclear cells; the presence of such a factor (or factors) might well explain the predominance of nonsensitive mononuclear cells in the delayed skin reaction.

Lymph node lymphocytes obtained from guinea pigs previously injected with *o*-chlorobenzoyl chloride conjugated to bovine gamma globulin (OCBC-BGG) (*10*) in complete adjuvant (*11*) were placed in culture medium (*12*) at a cell concentration of 2.4×10^7 per milliliter, as described previously (*3*, *5*). The specific antigen, OCBC-BGG, was added to one group of cultures to a final concentration of 100 μg/ml; the control group received an equal volume of physiologic saline. The cultures were gassed with 5 percent CO_2 and air to *p*H of 7.5 and incubated for 24 hours at 37°C; the medium was then centrifuged at 17,300 *g* for 20 minutes, and the supernatant was collected. Samples of the supernatants obtained from the control and antigen-stimulated lymphocytes were supplemented with normal guinea pig serum (15 percent final volume) and were assayed for MIF activity on normal peritoneal exudate cells as previously described (*3*). Chemotactic testing was carried out within 48 hours with material kept at 5°C or frozen in dry ice (freezing and thawing one time had little effect on the chemotactic activity of a given preparation).

Chemotactic studies were performed with rabbit or guinea pig mononuclear cells from an exudate induced 4 days earlier by the intraperitoneal injection of sterile mineral oil (*13*). Only results obtained with rabbit mononuclear cells are reported here, although guinea pig cells responded in essentially the