Kalow and Lindsay (*11*), the enzyme activity was very slight—only 5 to 10 units. Moreover, this activity was not inhibited by $2 \times 10^{-5}M$ quinidine sulfate (*12*). Both results indicate that the hydrolysis was not due to plasma cholinesterase.

When families were studied by this method (Fig. 2), the variation in bands 2, 3, and 4 of the zymograms was shown to represent the phenotypic expression of two alleles—*AChE*^{1} and *AChE*^{2}—at a single gene locus. The homozygous phenotypes are arbitrarily designated AChE 1 and AChE 2, and the heterozygous type is called AChE 2-1. The two-allele hypothesis was consistent with data from three additional families.

Human erythrocyte AChE activity can be resolved into two components by cellulose-acetate electrophoresis and by anion-exchange chromatography; the two components differ in molecular charge, but not in molecular weight (*13*). The major zone of activity reported here (Fig. 1, band 1) appeared as two discrete bands when a sample diluted 1 : 25 was applied to the gel. This dilution was not used routinely, however, because at this dilution bands 2, 3, and 4 could not always be seen.

We believe that this is the first report of polymorphic variation in a stromal enzyme. With the use of this technique, it may be possible to examine other membrane-bound enzymes for genetic variation.

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Expression of the Mammalian X Chromosome before and after Fertilization

Abstract. *The activity of hypoxanthine-guanine phosphoribosyltransferase in unfertilized mouse ova and in mouse embryos at the two-cell stage is proportional to the number of X chromosomes present during oogenesis. This indicates that the enzyme is X-linked in the mouse and that inactivation of the X chromosome does not occur during oogenesis. However, the genetic dosage effect of the X chromosomes is not present after the increase in hypoxanthine-guanine phosphoribosyltransferase activity in the late morula and the blastocyst stages. These results indicate that the X-linked enzyme locus is expressed sometime after fertilization but before the morula stage.*

We have reported (*1*) that glucose-6-phosphate dehydrogenase (G6PD) is synthesized in a manner that is dependent on the genetic dosage of the X chromosome during oogenesis in the mouse and that inactivation of the X chromosome does not occur in the developing oocyte. These conclusions were based on experimental results showing that the activity of G6PD in ova of XX mice is twice that in ova of XO mice; no difference was observed for autosomally controlled lactate dehydrogenase. Investigations on another enzyme known to be X-linked in man, hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (E.C. 2.4.2.8.), indicate the same genetic dosage effect of the X chromosome on the enzyme's activity in mouse ova. Furthermore, measurements of the activity of HGPRT in the later stages of development of the embryo, but before its implantation in the uterus, indicate that the increase in the enzyme activity (*2*) is determined by events that occur after fertilization.

Mouse embryos at ages of 1 day (two-cell) and 3 days (morula and blastocyst) were obtained from mothers with XO (genotypes *Ta*/*O* and +/*O*) and XX (genotype *Ta*/+) chromosomes (*3*) (Jackson Laboratory). The mice were injected with pregnant mare's serum and human chorionic gonadotropin to induce ovulation and were mated with albino Swiss males (*4*). Groups of embryos were assayed simultaneously for HGPRT and guanine deaminase (E.C. 3.5.4.3) activity (*2*, *5*); the results are shown in Table 1. Guanine deaminase, not known to be X-linked in any species, was used as a control enzyme. The activity of guanine deaminase was nearly the same in both the two-cell embryos from XO mothers and those from XX mothers (XO/XX = 0.90). In contrast, the activity of HGPRT in the two-cell embryos from XO mothers was almost half of that in the embryos of XX mothers (XO/XX = 0.48). There was almost no difference, however, in activity of HGPRT in erythrocytes of XO and those of XX adult mice (XO/XX = 1.08). Thus, the activity of HGPRT at the two-cell stage of development is proportional to the number of X chromosomes present during oogenesis; synthesis of HGPRT in the oocyte appears to be controlled in the same manner as that of G6PD.

Experience indicated that the HGPRT assay was more reliable with two-cell embryos than with fertilized or unfertilized ova. The foregoing experiments were therefore carried out with fertilized two-cell embryos. However, there is a chance that the YO and some of the XO embryos, from XO mothers, destined to die before reaching the blastocyst stage (*6*), might be metabolically abnormal and, as a result, have lower HGPRT activity. Although the likelihood of this is not great [embryos of XO mothers appear normal until after the two-cell stage (*7*)], we nevertheless studied the dosage effect in unfertilized eggs. Reliable results were obtained if the ova were washed six times in medium (*8*) to remove follicular cells and other contaminants before assay. Unfertilized ova (from unmated mice primed with hormones) were treated with hyaluronidase (bovine testicular, Sigma type I, 75 units per milliliter of medium) to remove cumulus cells, were washed six times, and were assayed for activities of HGPRT and guanine deaminase. As in the two-cell embryos, the activity of HGPRT in the unfertilized eggs from XO

Table 1. Activities (mean ± S.E.) of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and guanine deaminase in unfertilized ova and in 1-day (two-cell) and 3-day [morulae (mor) and blastocysts (bl)] embryos from mothers with either XO or XX chromosome constitutions. The number of samples is shown in parentheses. Activities are expressed in picomoles per hour per embryo or per egg.

Experimental material	Maternal genotype				Ratio of activities XO/XX
	XX	XO			
	Ta/+	*Ta/O*	*+/O*	Combined	
		HGPRT			
Unfertilized eggs*	0.29 ± 0.016 (7)	0.12 (6)	0.11 (8)	0.11 ± 0.017 (14)	0.40
Unfertilized eggs*	0.32 ± 0.024 (4)		0.17 (4)	0.17 ± 0.048 (4)	0.54
					Average: 0.47
		Guanine deaminase			
Unfertilized eggs*	26.9 ± 0.48 (8)	23.1 (6)	23.6 (8)	23.4 ± 0.53 (14)	0.91
		HGPRT			
Day 1 embryos*	1.07 ± 0.11 (4)	0.43 (2)	0.47 (1)	0.45 ± 0.072 (3)	0.41
Day 1 embryos*	0.35 ± 0.011 (6)	0.17 (6)	0.19 (1)	0.17 ± 0.012 (7)	0.49
Day 1 embryos*	0.65 ± 0.062 (5)	0.27 (1)	0.37 (4)	0.35 ± 0.024 (5)	0.54
					Average: 0.48
		Guanine deaminase			
Day 1 embryos*	26.6 ± 1.32 (4)	25.2 (2)	20.6 (2)	22.9 ± 1.13 (4)	0.86
Day 1 embryos*	18.2 ± 0.72 (6)	17.6 (6)	16.8 (1)	17.5 ± 0.22 (7)	0.96
Day 1 embryos*	18.5 ± 0.56 (5)	17.6 (1)	16.9 (4)	17.0 ± 0.67 (5)	0.92
					Average: 0.91
		HGPRT			
Day 3 embryos (bl)†	10.3 ± 0.30 (5)	9.6 (1)	8.9 (1)	9.2 (2)	0.89
Day 3 embryos (mor)†	6.9 (2)	6.6 (1)		6.6 (1)	0.95
Day 3 embryos (mor)†	1.79 ± 0.63 (3)		16.9 (3)	16.9 ± 1.6 (3)	0.94
					Average: 0.89
		Guanine deaminase			
Day 3 embryos (bl)†	8.7 ± 0.69 (5)	7.6 (1)	6.9 (1)	7.2 (2)	0.83
Day 3 embryos (mor)†	11.7 (2)	10.0 (1)		10.0 (1)	0.85
Day 3 embryos (mor)†	14.1 ± 1.8 (3)		12.2 (3)	12.2 ± 1.9 (3)	0.86
					Average: 0.85

* Ten per sample. † Three to five embryos per sample.

mothers was about half of that from XX mothers (XO/XX = 0.47), while the guanine deaminase activities were nearly the same in both groups (XO/XX = 0.91) (Table 1). Therefore, the HGPRT activity differential cannot be attributed to any difference in the viability or the metabolic functioning of the embryos from mothers with different chromosome constitutions.

During the third day of embryonic development there is normally an increase in the activity of HGPRT and a moderate decrease in that of guanine deaminase (*2*, *5*). If the HGPRT increase were the result of events which occurred during oogenesis, either by the synthesis of "masked" maternal messages that are later transcribed or by the synthesis of inactive enzyme precursors that are later activated, the HGPRT activity in day 3 embryos should show the same dosage effect as does the activity in the day 1 embryos. The results obtained in day 3 morulae and blastocysts (Table 1) are not consistent with this expectation. The ratio of XO/XX activities is 0.89, quite far from 0.50 and nearly the same as that for guanine deaminase (XO/XX = 0.85). Therefore, the increase in HGPRT activity probably results not from events occurring before fertilization but from gene activity (most likely, specific messenger RNA synthesis) sometime after fertilization but before the third day of development. This is the earliest developmental change in the mouse for which direct evidence exists for gene activation after fertilization. The expression in the mouse of the HGPRT gene precedes that of other genes studied in this and other systems; for example, (i) paternal mouse isocitrate dehydrogenase first appears, without any overall increase in activity, in late blastocysts (*9*); (ii) several dehydrogenases are first synthesized at the hatching stage in *Drosophila* (*10*) and at the tail bud stage in frogs (*11*); and (iii) newly synthesized 6-phosphogluconate dehydrogenase does not appear in the Japanese quail embryo until after the primitive streak stage (*12*).

In theory, it should be possible to determine from these results whether X-chromosome inactivation had occurred by the time of assay. Inactivation prior to synthesis of the messenger RNA that produced the increase in HGPRT would cause the enzyme activity to be equivalent in all embryos since only one X chromosome would be functional in XX, XY, or XO embryos; YO embryos are believed to be nonviable (*6*). Therefore, the ratio XO/XX would be 1.0. If inactivation has not occurred before synthesis of the messenger RNA and if all X chromosomes are functional during gene expression, then the ratio of HGPRT activity in the embryos from XO mothers (composed of equal numbers of XO, XX, and XY embryos) to that in embryos of XX mothers would be 0.89. If XO embryos are low in number (*6*), the ratio would be somewhat higher. These three values are too close to be reliably distinguished from each other by present methodology. Therefore, although the ratio obtained in the present study is identical to that predicted if gene expression occurred prior to X-chromosome inactivation, it is not possible to assume this is true on the basis of our data. More direct methods involving the use of X-linked biochemical markers, the determination of enzyme activities in embryos of known sex (*13*), or the transplantation of embryonic cells carrying X-linked markers (*14*) are required to determine the time of functional X-chromosome inactivation.

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