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SHORT NOTE

XO mice

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The inactive-X hypothesis (Lyon, 1961) states that in the normal female mouse only one of the two X chromosomes is genetically active in each cell of the body other than the germ-cells, and that the choice of which X is to be inactivated occurs at an early stage of embryogenesis, and is at random in each cell. The descendants of these cells then abide by the decision so that females are mosaics for two lines of cells and may show a mosaic phenotype if they are heterozygous for a sex-linked gene. One requisite for the validity of this hypothesis is that only one X is necessary for the development of a normal female mouse, and evidence substantiating this supposition is the apparent normality of XO mice (Welshons & Russell, 1959; Cattanach, 1961*a*, *b*). On the other hand it has been suggested that XO mice are not fully viable (Russell, Russell & Gower, 1959), but no data on the comparative viability and growth of XO female mice have yet been reported. In the present communication data of this nature, collected in the process of setting up an XO stock, are presented and support the hypothesis that only one X chromosome is necessary for normal female mouse development.

The source of the XO animals was a number of exceptional females that occurred in the progeny of mice bearing an X-autosome translocation, *flecked* (Cattanach, 1961*b*). Genetical evidence had indicated that they were deficient for all or part of one X chromosome, and counts on mitotic metaphases showed that only thirty-nine chromosomes were present. The combined information was taken as evidence that the exceptional animals were deficient for a whole X chromosome.

Over twenty such XO females were used to set up an XO stock, using the sex-linked gene, *Tabby*, as marker. Unfortunately, the original animals were derived from crosses involving a random-bred *albino* stock (BC) and a strain homozygous for seven recessive genes (PCT) and were thus heterozygous for several genes, the phenotypes of which were unsuitable for the classification of *Tabby*. Crosses were therefore made to the CBA stock on which the wild-type, agouti background was most suitable for classifying *Tabby* in the heterozygous condition. The general procedure for the maintenance of the XO stock was to cross the XO females to *Tabby* and CBA males in each alternate generation, the *Tabby* males in later generations being derived from the same type of cross. Data were collected from up to six generations of XO females on an increasingly CBA background.

Theoretically XO, XX, XY and OY offspring of XO mothers should occur with equal frequency, except that the OY class can be taken to be inviable (Welshons & Russell, 1959). Table 1 shows that the number of XO females falls well short of this expectation, being only 30–37% as frequent as the XX female class. The cause of the shortage of XO females cannot be determined from the data, but a litter size of 5·3 seems rather high for a stock in which half the males and one-third of the females die in embryogenesis.

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Table 1. *Analysis of litters of XO females*

Mating	Age at which scored	Offspring XO ♀♀	XX ♀♀	XY ♂♂	(XO+XX)/XY	XO/XX	Litter size
$+/O$♀♀ × *Ta*♂♂:							
Total litters	at birth	(629)		481	1·31	—	5·39
	at 3 weeks	150	411	420	1·36	0·37	5·30
Litters containing XO daughters	at 3 weeks	150	225	254	—	—	6.29
Ta/*O*♀♀ × + ♂♂:							
Total litters	at birth	(344)		294	1·17	—	5·34
	at 3 weeks	64	212	229	1·21	0·30	4·81
Litters containing XO daughters	at 3 weeks	64	87	81	—	—	5·27

One possible explanation for the near normality of the litter size is that there is a preferential loss of the chromosome sets lacking an X to the polar bodies in the meiotic divisions of the ova, rather than death of the missing classes in embryonic development. Alternatively, an early loss of inviable embryos may be compensated by the development of all individuals of the viable classes, some of which would have been lost in larger, normal litters as a result of overcrowding in the uterus.

A most important point—illustrated by the data in Table 1—is the close agreement between the sex ratios as determined at birth and at three weeks of age. Since the frequencies of XX females and XY males are essentially the same at three weeks, it can be concluded that there is no post-natal preferential loss of the XO class up to this age. The XO class is thus as viable as the normal diplo-X class. The full viability of XO females in the first few weeks after birth makes it seem unlikely that they might be less viable before birth. This might suggest that the shortage of XO females is not due to loss in embryonic development.

Individuals of litters of XO females were weighed at three weeks of age to determine the comparative growth rates of the XO and XX classes. Allowance must be made for several factors before the weights can be compared. Firstly, there is the influence of litter size. By chance alone, XO females are less likely to occur in small litters, in which the weights of individual mice should be greater. Data illustrating the validity of this argument are shown in Tables 1 and 2. XO-bearing litters are consistently larger than the average for all litters, and the weights of the individual members are smaller. Comparison of the weights of XO and XX females can thus only be made in XO-bearing litters. Secondly, the sex-linked gene, *Tabby* (*Ta*), can influence the weight of the offspring in several ways. The effects can be determined from the data given in Tables 2 and 3 by the comparison of:

(*a*) *Ta*/*O* versus $+/O$ female offspring (Table 2) and *Ta* versus + male offspring (Tables 2 and 3)—to determine the influence of *Tabby* in the hemizygous condition;
(*b*) *Ta*/+ daughters of *Ta*/*O* mothers versus those of $+/O$ mothers—to determine the influence of *Ta* in the hemizygous mother on the weight of the offspring (Table 2), and
(*c*) *Ta*/+ versus +/+ in the female offspring—to determine the influence of *Ta* in the heterozygous condition (Table 3).

If these factors are assumed to influence the weights proportionately and to combine multiplicatively, the expectation for the influences of these factors is then as shown in

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Table 2. *Weights (at 3 weeks) of offspring of XO females*

Mating	Offspring: XO♀♀ No.	XO♀♀ Weight	XX♀♀ No.	XX♀♀ Weight	XY♂♂ No.	XY♂♂ Weight	Litter size
$+/O$♀♀ × *Ta*♂♂:							
Total litters	57	7·63 ± 0·24	130	9·97 ± 0·14	139	10·86 ± 0·15	5·72
Litters containing XO daughters	57	7·63 ± 0·24	77	9·71 ± 0·15	86	10·61 ± 0·15	6·67
Expected value		*a d x*		*b x*		*y*	
Ta/*O*♀♀ × +♂♂:							
Total litters	26	6·85 ± 0·27	93	7·44 ± 0·13	99	5·99 ± 0·12	5·32
Litters containing XO daughters	26	6·85 ± 0·27	33	6·88 ± 0·15	37	5·65 ± 0·19	6·00
Expected value		*b d x*		*b x*		*a b y*	

x = weight of normal females
y = weight of normal males

Factor	Effect of	On weight of	Ratio of weights	Proportionate reduction in weight
a	*Ta*/*O* versus +/*O*	XO daughters	0·79	21%
a	*Ta* versus +	sons	0·75	25%
b	*Ta*/*O* mothers versus +/*O* mothers	offspring	0·71	29%
d	XO versus XX	daughters	0·99	1%

Tables 2 and 3, where *a* is the proportionate effect of factor (a); *b* that of (b), and *c* that of (c). It was calculated that (a) *Tabby* reduces the weight of hemizygotes by 21% in XO daughters and by 25% in sons of XO mothers (Table 2): this compares well with the estimate of 22% in sons of *Ta*/+ mothers (Table 3); (b) *Tabby* in the hemizygous mothers reduces the weight of individuals of their litters by 29% (Table 2), and (c) *Tabby* in the heterozygous condition only has a very slight effect on weight—2% (Table 3). The actual

Table 3. *Weights (at 3 weeks) of offspring of* Ta/+ *females*

Ta/+♀♀ No.	*Ta*/+♀♀ Weight	+/+♀♀ No.	+/+♀♀ Weight	*Ta*♂♂ No.	*Ta*♂♂ Weight	+♂♂ No.	+♂♂ Weight
30	9·17 ± 0·32	23	9·35 ± 0·37	28	7·64 ± 0·38	23	9·81 ± 0·37
	c x		*x*		*a y*		*y*

x = weight of normal females
y = weight of normal males

Factor	Effect of	On weight of	Ratio of weights	Proportionate reduction in weight
a	*Ta* versus +	sons	0·78	22%
c	*Ta*/+ versus +/+	daughters	0·98	2%

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difference in weight between *Ta*/+ and +/+ offspring was far from significant ($t_{51} = 0{\cdot}787$). This information is critical for the determination of the influence of XO versus XX, where *Ta*/+ females represent the normal diplo-X class.

Once the above allowances were made the influence of the possession of only one X chromosome on the weight of females (factor *d* in Table 2) could be calculated: it was then found to be very slight (1%). This estimate can be checked by comparing the XO and XX daughters of *Ta*/*O* mothers (Table 2). Here, there is no difference between the weights of the two classes and this situation is maintained when the data are weighted for the numbers of each class per litter. Growth of XO females up to the age of three weeks is therefore no less than that of their normal sisters.

The conclusion from the combined data is that there is a marked shortage of XO daughters in litters of XO females and that this is not due to post-natal inviability since sex-ratios at birth and at three weeks are the same. Post-natal growth is also as rapid for XO as for XX females, at least during the first three weeks. These facts suggest that the shortage of the XO class is unlikely to be due to embryonic death of two-thirds of the XO females, but probably results from a preferential loss of chromosome sets lacking an X to the polar bodies, so that most eggs carry an X chromosome.

The same facts indicate that only one X chromosome is necessary for normal development of the female mouse, at least up to the age of three weeks, and this favours the validity of the inactive-X hypothesis.

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