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Reproductive lifespan in irradiated and unirradiated chromosomally *XO* mice

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SUMMARY

Lifetime reproductive performance was studied in 50 sib-pairs of female mice, one member of each pair chromosomally *XX* and the other *XO*. Twenty-five pairs were irradiated with 25 rad X-rays at 10 days of age and 25 were unirradiated. In both the irradiated and control series the *XO* mice had a significantly shorter reproductive life than the *XX* ones, but unirradiated *XO* bred longer than irradiated *XX*. The median age of unirradiated *XO* at birth of last litter was 280 days and they had $6{\cdot}5 \pm 0{\cdot}80$ litters, whereas for *XX* the figures were 420 days and $12{\cdot}6 \pm 0{\cdot}74$. The mean litter-size of *XO* mice was only about 55 % and their lifetime productivity 34 % of that of their *XX* sibs. Similarly, the lifetime productivity of irradiated *XX* females was only 31 % of that of unirradiated *XX*. Histological studies showed that in the unirradiated *XO* mice reproduction ended through shortage of oocytes, and the resulting secondary ovarian changes were similar to those in irradiated mice. Thus, the differences between human and mouse *XO* types are to some extent reconciled. Both become sterile through death of oocytes, in humans before puberty and in mice after. This difference may be connected with different times from oogenesis to puberty in the two species.

1. INTRODUCTION

The *X*-chromosome of mammals appears to be involved in some way in determining the survival of germ cells. Chromosomally *XX*, *XXY*, *XXXY*, etc., human males typically have testes lacking spermatogonia, apparently owing to the death of these cells before puberty (Mikamo *et al.* 1968). Similarly, in the ovaries of *XO* human females, germ cells are present at foetal and neonatal stages (Conen & Glass, 1963; Singh & Carr, 1966), but have disappeared before puberty, leaving a streak gonad. These observations are consistent with the idea that germ cells with a single *X*-chromosome survive in a testis but not in an ovary, whereas the converse applies for those with more than one *X*. In other mammals the picture in male gonads is similar. In all species in which they have been found, *XX* and *XXY* males are sterile with absence of spermatogenesis and at least for *XX* males of the mouse and goat it is known that this is because the germ cells die at an early stage (Cattanach, Pollard & Hawkes, 1971; Short, 1972). Among females, however, the only mammalian species other than man for which the *XO* type is well known is the mouse,

and here the *XO* animals are fertile (Welshons & Russell, 1959; Cattanach, 1962; Morris, 1968).

Morris (1968) showed that, at least when young, *XO* mice shed as many eggs at each ovulation as their *XX* sisters. He did not study their lifetime reproductive performance in detail, but reported that *XO*'s could have up to ten litters.

For a variety of reasons, it seemed of interest to study the length of reproductive life of *XO* female mice more fully. From studies with radiation, Mole (1959) showed that lifetime reproductive productivity is the most sensitive indicator of ovarian competence. A normal mouse has a vast excess of oocytes in the ovary at puberty, and despite a continuous process of atresia the reproductive life ends with many ova still present (Jones & Krohn, 1961). Thus any factor which caused moderate depletion of oocytes might have relatively little effect on fertility. If the number of oocytes is depleted to 4 % of normal before puberty, a mouse may still have several litters (Oakberg, 1966; Peters, 1969). It thus seemed possible that in *XO* mice ovarian atresia might indeed be more rapid than in *XX* animals but not sufficiently so that the ovaries were devoid of oocytes before puberty, at 5 weeks of age. If this proved to be the case it might reconcile the fertility of *XO* mice with the concept that two *X* chromosomes are necessary to control ovarian atresia in mammals (Hamerton, 1968).

Further reasons for interest were that if the reproductive lifespan of *XO* mice was indeed shortened through lack of oocytes these animals might prove sensitive test organisms for agents, like radiation, which could cause oocyte death, and also that *XO* mice might be useful in studies of non-disjunction. In humans the incidence of trisomic offspring, arising through non-disjunction, increases with age of mother, rising sharply in the pre-menopausal years. Since the human ovary becomes completely depleted of oocytes at the end of reproductive life, a mouse in which this occurred might prove a better model in this context than the normal mouse, which ends reproductive life with many oocytes still present.

The present paper compares the life-time reproductive performance of 50 *XO* mice and their *XX* sibs. Half the animals were untreated. The remaining half were irradiated with 25 rad X-rays at 10 days of age. At this age mouse oocytes are very easily killed by radiation, and a dose of 25 rad kills 90–95 % of small oocytes (Oakberg, 1966). The animals' fertility is then restricted by the number of oocytes available and is reduced to 35 % of normal (Oakberg, 1966; Peters, 1969). The aim of this treatment in the present experiments was thus to enhance any slight difference between *XO* and *XX* females. As previously mentioned, a moderate deficiency in oocyte number in unirradiated *XO*'s might have no detectable effect. However, in irradiated animals, with reproduction limited by the number of oocytes available, a comparable percentage deficiency might have a much more noticeable result. Thus, providing radiation killed a comparable proportion of oocytes in *XO* and *XX* females it should tend to make clearer any reproductive differences between them.

2. MATERIALS AND METHODS

In order to breed the experimental females, *XO* female mice carrying the *X*-linked tabby gene (*Ta*) were crossed with males of the HT strain, homozygous for the six autosomal recessive genes *a*, bp^H, *fz*, *ln*, *pa* and pe^H. Males of this particular type were chosen for the purposes of other observations, not to be described here.

The *XO* and *XX* daughters of this cross were recognized by their phenotypes, wild-type and striped (*Ta*/+) respectively, and saved for the experiment. In some litters all the females were given a dose of 25 rad X-rays (250 kV: HVL 1·2 mm Cu; 9 rad/min) to the whole body, when 10 days old. The remaining litters were left untreated, as controls, and all litters were weaned at 18–20 days. At ages of 7–12 weeks *XO* females and an equal number of *XX* sisters from the same litters were mated singly to the HT males up to a total of 25 irradiated and 25 control *XO*'s and their *XX* sisters.

All pairs were observed daily for birth of litters, except at weekends. The young were classified for tabby and for the six recessive genes of the HT stock, and discarded when 18–22 days old.

When a lapse of 9 weeks had occurred since a female's last recorded litter or palpable pregnancy she was judged to have stopped breeding, and was killed. The ovaries of a sample of the animals were kept for histology. They were fixed in Heidenhain's Susa and stained with haematoxylin and eosin. In addition, a sample of ovaries from younger females, which had not been bred, were kept.

Another sample of young females were mated to HT males and then dissected when pregnant, in order to find the causes of any differences in litter size. Twenty pairs of sibs, *XO* and *XX*, were used, ten of which were irradiated and ten controls. Numbers of corpora lutea and of live and dead embryos were counted so as to give estimates of preimplantation and post-implantation embryonic death.

3. RESULTS

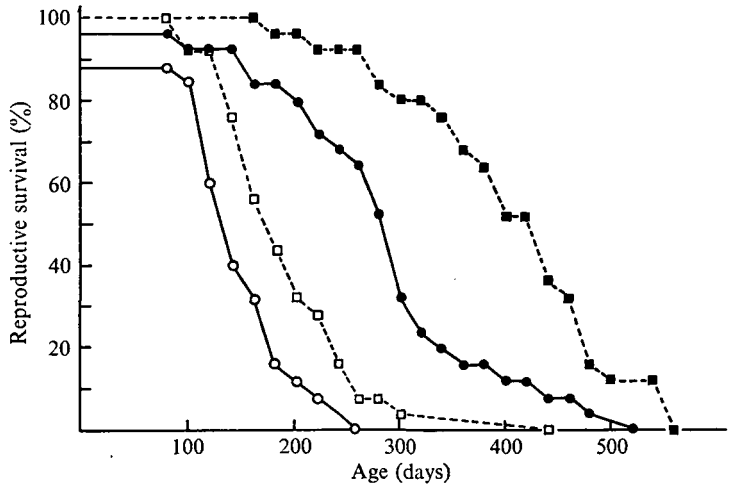
(i) *Breeding*

The end of an animal's reproductive life was taken as the date of birth of its last litter, and the reproductive 'survivors' at any age were those animals which had litters after that age.

Text-fig. 1 shows that *XO* mice had a clearly shorter reproductive life than their *XX* sibs. The difference was obvious and statistically significant in both the irradiated and the unirradiated groups, thus showing that radiation and the *XO* condition were acting cumulatively in this respect. Unirradiated *XO* mice, with a median age at last litter of 280 days, bred longer than irradiated *XX* ones (median age at last litter 170 days), indicating that the factors responsible for shortening reproductive life in *XO* females were less powerful than the effect of 25 rads at 10 days of age.

The mean number of litters per female varied in parallel with the length of reproductive life (Table 1). The unirradiated *XO*'s had an average of 6·5 litters per female, and since half of them were still breeding at 280 days of age, in routine

stock maintenance their shortened reproductive life might well pass unobserved. Our results confirm Morris's (1968) finding that *XO* mice could have up to ten litters; indeed, they had up to 14 litters and four animals were still breeding when over a year old.



Text-fig. 1. Reproductive lifespan of female mice, expressed as the proportion of animals which had litters after a given age. ——, *XO*; - - - -, *XX*; ○, □, irradiated; ●, ■, control.

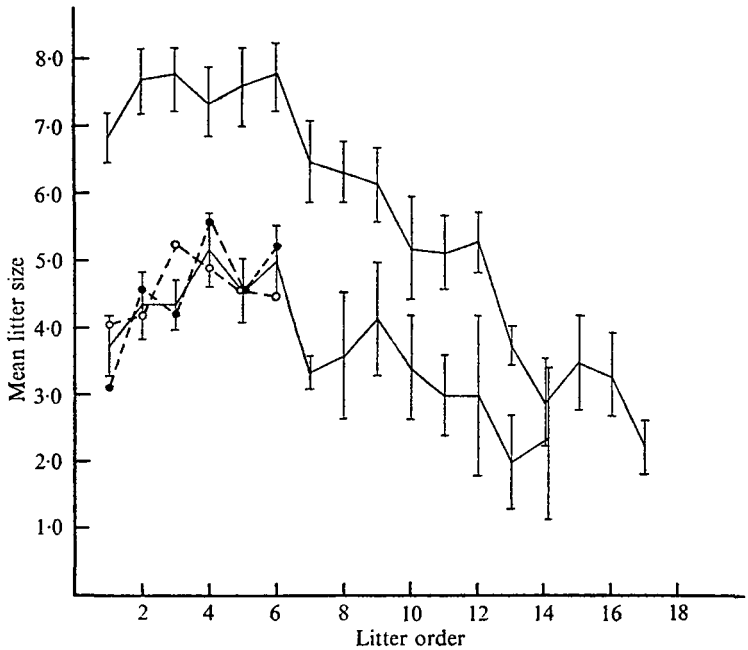
Table 1. *Reproductive performance of irradiated and unirradiated* XO *and* XX *female mice*

	Irradiated		Control		Irradiated / Control	
	XO	*XX*	*XO*	*XX*	*XO*	*XX*
Median age at last litter (days)	130*	170	280***	420	0·46	0·40
Number of litters						
Mean	2.8*	4.6	6.5***	12.6	0.43	0.37
S.E.	± 0.37	± 0.59	± 0·80	± 0·74	—	—
Range	1–6	1–9	2–14	5–18	—	—
Litter-size (mean)	3·5***	5·2	4·2***	6·3	0·83	0·83
	± 0·26	± 0·25	± 0·17	± 0·16	—	—
Lifetime productivity (mean number born per female)	9·8	24·1	26·8	78·8	0·37	0·31
	± 1·9	± 4·4	± 3·4	± 4·3	—	—

* Difference between *XO* and *XX* significant at 5 % level.
*** Difference between *XO* and *XX* significant at 0·1 % level.

The mean litter-size of *XO* females was less than that of *XX*, as expected from the death of *OY* embryos and from previous work (Cattanach, 1962; Morris, 1968). The curves of change of litter-size with litter-order for the unirradiated *XX* and *XO* females were roughly parallel (Text-fig. 2). For both types of female the litter-size

increased between first and second litters, remained fairly constant until the sixth litter and then began to decline. In the early litters the mean litter-size of *XO* females was only about 55 % of that of *XX* females, a figure fairly close to that found by Morris. The question arises whether the apparent change with litter-order in *XO* mice was a true effect of increasing reproductive age, or was due to differing fertilities of females which had few or many litters. Accordingly, we considered



Text-fig. 2. Change of litter-size with litter-order in unirradiated *XX* and *XO* mice. Solid lines, upper *XX* and lower *XO*; - - ○ - -, *XO* females with up to six litters; - - ● - -, *XO* females with > six litters. Bars indicate standard error of mean.

separately the litter-sizes of *XO* females with various numbers of litters. Of the 25 *XO* females, 15 had less than the mean number of litters (6·5), i.e. up to six litters, and nine had more (one had no litters). The sizes of the first six litters of these two groups of females were very similar (Text-fig. 2). In particular, the litter-size of those with a short reproductive life did not fall noticeably towards the end of their fertile period, as occurred in those which had many litters. Thus, it seems that the relation of litter-size to litter-order in *XO* mice is indeed similar to that in *XX* ones and, therefore, that the factors acting to reduce litter-size in old females were not the same as those tending to shorten reproductive life in *XO* mice. There is no reason to suppose that *XO* females became sterile because their litter-size fell too low for normal pregnancy.

The litter-size was also lower in the irradiated series than in the controls. Dissections of pregnant females of each type showed that the differences in litter-size, both between *XX* and *XO* and between irradiated and control series were due to differences in number of implanted embryos per female, rather than in the

proportion of implants that were viable (Table 2). There is therefore no reason to suppose that any appreciably large number of dominant lethal mutations had been induced by the radiation. In both series the number of corpora lutea was higher in the *XO* than in the *XX* females, but further work is needed to determine the repeatability of this difference.

As a result of both their low litter-size and their short reproductive life the life-time productivity of *XO* mice was only a fraction of that of their *XX* sibs. The mean number of young born to unirradiated *XO* (26·8) was 34 % of those born to *XX* (78·8). Similarly, the life-time productivity of irradiated *XX* females (24·1) was only 31 % of that of unirradiated *XX*.

Table 2. *Results of dissections of pregnant irradiated and unirradiated* XX *and* XO *female mice, ten pairs of sisters in each series*

	Irradiated		Control	
	XO	*XX*	*XO*	*XX*
Corpora lutea	121	98	125	101
Implantations	52	57	53	89
Live embryos	47	53	48	80
Moles	5	4	5	9

The effect of irradiation on the *XO* ovary may be measured by considering the various components of reproductive productivity in irradiated animals as a proportion of that in controls, within the *XO* and *XX* groups. It is clear that, for each of the four components in Table 1, the effect of irradiation on *XO* mice measured in this way was very similar to that on *XX* ones.

(ii) *Ovarian histology*

The sample of animals killed at the end of their reproductive life comprised four irradiated *XX* females aged 4, 6 (two) and 10 months, three irradiated *XO* females aged 6, 8 and 12 months and six unirradiated *XO* mice aged 4, 6, 9, 10½ and 12 (two) months. In addition, there were five unirradiated virgin *XO* animals aged 6 weeks, 2, 3½ and 7 (two) months.

(*a*) *Irradiated animals*

All seven ovaries were histologically very abnormal. The ovary of the youngest *XX* female aged 4 months contained some growing and Graafian follicles, although fewer than in a normal ovary. It also contained structures similar to those which Peters (1969) termed 'empty rings', and which were presumably atretic follicles. As well as this there were haemorrhagic follicles, luteal tissue and hypertrophied interstitial tissue.

The remaining six ovaries contained no follicles and showed changes typical of those secondary to oocyte depletion, with no obvious difference between *XX* and *XO*. Two *XO* ovaries contained haemorrhagic follicles, and some apparently normal luteal tissue, together with hypertrophy of the interstitial tissue and some folding

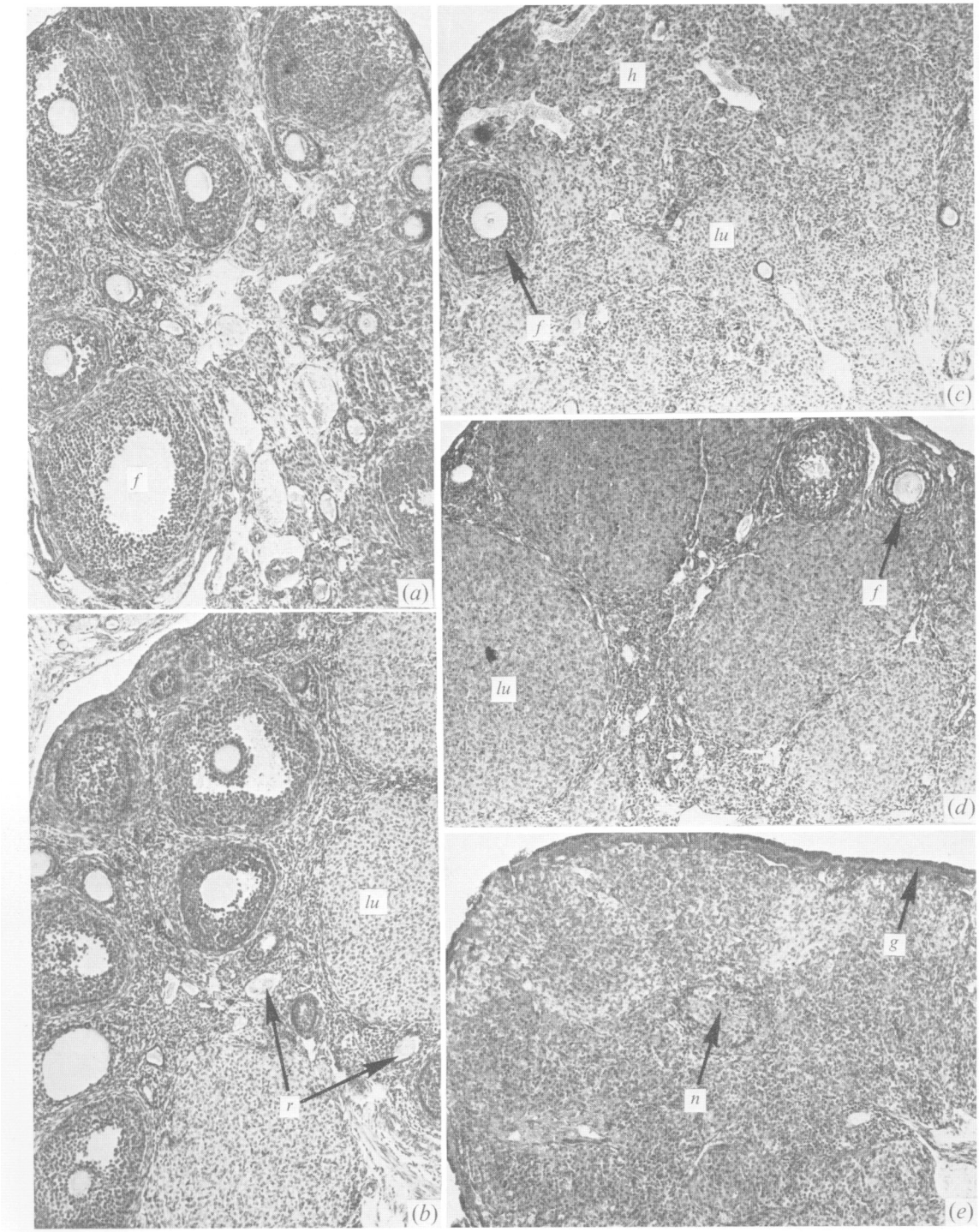


Plate 1. Ovaries of unirradiated *XO* mice. Haematoxylin and eosin; ×80. (*a*) 6-week-old animal; apparently normal numbers of oocytes at various stages. (*b*) 3½ months old. Still many oocytes in follicles (*f*) of various stages, but excess of 'empty rings' (*r*). (*c*) 7-months-old. Severe shortage of oocytes. A haemorrhagic follicle is visible (*h*), together with abnormal luteal tissue (*lu*) and 'empty rings'. (*d*) 9-months-old. Again severe shortage of oocytes but corpora lutea (*lu*) and interstitial tissue still retain normal architecture. (*e*) 12-months-old. No oocytes. Normal ovarian architecture lost. Foci of necrotic cells (*n*). Hypertrophy of germinal epithelium (*g*).

of the germinal epithelium. The remaining four ovaries showed marked folding of the germinal epithelium, combined with very abnormal ovarian architecture, foci of necrotic cells, and tumourous appearance.

(*b*) *Unirradiated* XO *mice*

The ovarian histology of the unirradiated *XO* mice was similar to that of the irradiated animals, but in general the abnormalities were somewhat less severe. The ovaries of all six of the animals which were killed after the end of their reproductive life contained some oocytes. In the two oldest mice (aged 12 months) there were very few indeed, in growing and Graafian follicles only, with no small follicles. The greater part of these ovaries consisted of hypertrophied interstitial tissue and abnormal luteal tissue. In addition, there were 'empty rings', haemorrhagic follicles, foci of necrotic cells, and hypertrophied germinal epithelium (Plate 1*e*).

The four younger animals all had a considerable number of oocytes in various stages. The 9- and 10½-month-old animals' ovaries had follicles at stages from small to Graafian (Plate 1*d*), but in addition many 'empty rings', much luteal tissue and hypertrophied stroma. The 5- and 6-month-old animals' ovaries contained much luteal tissue and many 'empty rings' but were otherwise essentially normal.

Of the five ovaries of unmated *XO* females those of the youngest animals showed no obvious abnormality (Plate 1*a*), having numerous follicles at all stages, and normal corpora lutea. The 3½-month-old ovary had an excess of empty rings (Plate 1*b*), but otherwise looked normal, whereas by contrast the ovaries of the two 7-month-old unmated *XO* mice were markedly abnormal (Plate 1*c*). The number of follicles was much below normal in both, and in one of the two there were very few indeed. Both ovaries consisted mainly of luteal tissue, and contained many 'empty rings'. There was one haemorrhagic follicle.

4. DISCUSSION

The data indicate that, at least under the conditions of this experiment, the reproductive life of *XO* mice is shorter than that of normal females. In this experiment, the median age at last litter of *XO* mice was only two-thirds that of corresponding normal animals. However, the exact degree of shortening might well vary from strain to strain (Jones & Krohn, 1961; Jones, 1970), and we have no evidence on this point.

The next question is whether the curtailment of reproduction in *XO* mice is due to shortage of oocytes, or to some other cause. The cumulative action of the *XO* condition with radiation, which is known to reduce oocyte number, suggests that *XO* mice may have fewer oocytes, but the histology provides stronger evidence. The ovaries of *XO* females were severely depleted of oocytes at an age well before the median age of last litter in *XX* mice. Therefore, it seems reasonable to suppose that *XX* ovaries could not have been so depleted at that age, and that reproduction in the *XO* animals had ended through shortage of oocytes. Although some of the *XO* mice appeared to have become sterile with some oocytes still remaining in their ovaries, it is known that this can occur after irradiation also (Slate & Bradbury,

1962), so there is no need to postulate any separate cause for this sterility. The histological changes in the ovaries of irradiated *XO* mice seemed entirely similar to those in *XX* ones, which in turn appeared to show the classic features known to be secondary to oocyte loss through various causes (Lacassagne *et al.* 1962; Orr, 1962; Murphy & Russell, 1963). The unirradiated *XO* animals appeared to show an earlier stage of the same sequence of changes (Peters, 1969), and hence there is at present no evidence of any abnormality in *XO* ovaries other than shortage of oocytes. It is not yet possible to say whether this shortage is due to reduced formation of oocytes in *XO* embryos, or to normal formation with more rapid atresia.

An unexplained point is the very reduced litter-size of *XO* mice. The death of *OY* embryos of course accounts for part of the reduction, but like Morris (1968) we found that the percentage reduction was too great to be due only to this. Whatever the reason, this low litter-size must at least in part account for the life-time productivity of *XO* mice being only 34 % of normal, although the length of reproductive life was as much as two-thirds normal.

As a result of this work the difference in reproductive ability between mouse and human *XO*'s is to some extent explained. In both, oocytes are present in early life, but the supply becomes prematurely exhausted. In the human *XO* all oocytes disappear before maturity, whereas in the mouse many persist until well after puberty. The histological differences between the ovaries of *XO* mice and humans as well as the effects on fertility may be attributed to this variation in time of oocyte loss.

It remains to explain how this difference in timing of oocyte depletion arises. There are at least two other genetic diseases, Niemann–Pick disease and nephronophthisis, which in man have their onset in childhood (at 1–10 years of age) but in the murine counterparts are not detectable until early adult life (2–4 months) (Lyon, Hulse & Rowe, 1965; Lyon & Hulse, 1971). One possibility is that in all these cases absolute time is important. In man oocytes must survive for about 14 years from oogenesis to allow normal puberty and about 20 years to permit fertility, whereas a mouse may be fertile if oocytes persist for only 6–8 weeks. Although early *XO* human foetuses have many oocytes, the number has become severely depleted by the time of birth or a few months later, and some neonatal *XO* ovaries may be completely devoid of oocytes (Carr, Haggar & Hart, 1968). Thus, it may be that the times of survival of *XO* oocytes in human and mouse are not very different. If it were true that *XO* oocytes of any species can survive only a few months one would expect the fertility or otherwise of the *XO* type in various species to be connected with generation time, but there is not enough evidence at present to say clearly whether this is so. The *XO* type has been described in the pig, where the animals were sterile with absence of oestrous cycles (Nes, 1968). Among species with shorter generation times, the female Oregon vole is normally *XO* and fertile, but its germ cells are chromosomally *XX* (Ohno, 1967), and the *XO* type has been described in the black rat *Rattus rattus* (Yong, 1971) and the field mouse *Akodon azarae* (Bianchi & Contreras, 1967), but with no detailed description of the ovaries. Thus, the evidence is too slender to support or negate the idea that a short generation time is necessary for fertility of the *XO* type in a given mammalian species.

This work does, however, clearly support the hypothesis that two *X* chromosomes are necessary to control ovarian atresia in mammals (Hamerton, 1968), and indirectly that all *X* chromosomes present in germ cells are active (except perhaps in later stages of spermatogenesis) (Lyon, 1970, 1972).

Now that the *XO* mouse has been shown to have its reproduction curtailed, but to be otherwise a reproductively fairly normal animal, it may prove to be a useful test organism. The *XO* mice had proportionately the same sensitivity to radiation as their *XX* sibs, but the sterility due to the killing of oocytes by radiation became obvious at a shorter time after treatment, and the same might well be true for other agents with similar effects on oocytes. Moreover, studies of chromosomal non-disjunction in *XO* mice would be very interesting. Since their fertility ends through depletion of oocytes they may pass through a period towards the end of their reproductive life when their oestrous cycles are irregular, and there may be hormonal imbalance. In this respect they could prove useful models of the situation in human premenopausal females, in whose oocytes chromosomal non-disjunction occurs with a high frequency, leading to the birth of abnormal children.

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