Retarded development of XO conceptuses during early pregnancy in the mouse

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Summary. The growth and development of XO and XX mice were compared from 7½ to 18½ days of gestation. The 7½-day XO egg cylinders were retarded in development (and consequently small) when compared with XX egg cylinders, and this lag in development remained until 10½ days. By 12½ days there was a considerable degree of 'catch-up', but this was not fully maintained. A subgroup of very severely retarded XO fetuses were preferentially located near the cervix. The placentas of XO fetuses were of normal size until 18½ days when they were significantly larger than those of XX controls.

Introduction

The phenotype associated with X-chromosome monosomy is of particular interest since it indicates functions for the second X in normal females. The consequences of X monosomy have been most extensively studied in man and the mouse. XO women are typically of short stature, have streak ovaries, with consequent sexual infantilism and sterility, and are subject to a number of malformations (Simpson, 1976). In addition, there is a high fetal loss of XO females, only about 1% of those conceived surviving to term (see Polani, 1981), and those that do survive have a lower than average birth weight (Chen, Chan & Falek, 1971; Polani, 1974). By contrast, XO mice were originally described as having an essentially normal phenotype (Welshons & Russell, 1959; Cattanaach, 1962). However, more recent work has revealed deficiencies in both the number (Burgoyne & Baker, 1981a) and quality (Burgoyne & Biggers, 1976) of oocytes and there are associated reproductive shortcomings (Lyon & Hawker, 1973). XO mice also have a low birth weight and a reduced weight gain before weaning (Deckers & van der Kroon, 1981; Burgoyne, Evans & Holland, 1983).

The present study was undertaken to provide information on the aetiology of reduced birth weight.

Materials and Methods

Mice

Heterozygous In(X)/X mice were produced by mating males carrying the X inversion In(X)1H (Evans & Phillips, 1975) to outbred MF1 albino females (Olac, Oxford). These In(X)/X females were then mated to MF1 males to provide litters in which about 25% of the females are XO (Phillips, Hawker & Moseley, 1973). Litters were processed at ages ranging from 7½ to 18½ days *post coitum*. It was assumed that mating occurred in the middle of the dark period (20:00-06:00 h). The relative positions of individuals within each uterine horn were noted.

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Litters at 7½ days

The conceptuses were removed during the morning of the 8th day of gestation and the egg cylinders were dissected out in pH 7.3 phosphate-buffered saline. Individual embryos were transferred to a cavity slide, and an outline of the egg cylinder was drawn at a magnification of $\times 60$ using a Carl Zeiss microscope with camera lucida attachment. An assessment of the stage of development was recorded (i.e. early, mid or late primitive streak, or early neural plate). The diameter and length of the embryonic part of the egg cylinder were measured from the drawing and the embryonic volume was calculated by assuming approximation of the embryo to a cylinder or a cylinder with a hemisphere on one end $\left(\text{volume} = \pi a^2 b \text{ or } \frac{\pi b}{6} (3a^2 + b^2) \text{ where } a \text{ is the radius of the cylinder and } b \text{ is the length of the cylinder or cylinder and cup} \right)$.

Air-dried metaphase spreads were prepared from whole egg cylinders by treating with 1% sodium citrate for 10 min, fixing with methanol:acetic acid (3:1 v/v), and dissociating in 60% acetic acid on a slide at 60°C.

Litters at 9½, 10¼ and 10½ days

Conceptuses were removed at 14:00 h on the 10th day for 9½-day litters and at 08:00 h and 14:00 h on the 11th day for 10¼- and 10½-day litters. The fetuses were dissected out in Hepes-buffered Minimum Essential Medium (Flow Laboratories, Irvine, U.K.) and freed of fetal membranes. Somites were counted in the 9½-day group. The fetuses were transferred in medium to an aluminium weighing pan, and the fluid was withdrawn using the corner of a tissue, before weighing on a Cahn electrobalance. The placentas were mopped dry on a tissue before weighing.

Air-dried metaphase spreads were prepared from yolk sacs by the technique of Evans, Burtenshaw & Ford (1972) except that after treatment with 60% acetic acid the detached cells were washed with methanol:acetic acid (3:1 v/v) and then air-dried at room temperature. For some litters the amnions were processed for sex chromatin scoring, using the same procedure as for yolk sacs, except that the hypotonic treatment was omitted. Sex chromatin was scored following staining with toluidine blue.

Litters at 12¼–18½ days

The pregnant mice were killed at 08:00 h on the 13th day or at 14:00 h on the 13th, 15th, 17th and 19th day of pregnancy. The fetuses (freed of membranes) and their placentas were mopped dry on a tissue before weighing on an Oertling R20 pan balance.

Air-dried metaphase spreads were prepared from small pieces of liver taken from female fetuses (identified as females from gonad morphology) using the procedure described above for yolk sacs.

Scoring chromosome preparations

Four consecutive counts of 39 chromosomes were accepted as sufficient evidence for an XO. XX conceptuses could be clearly differentiated from XY conceptuses at 7½ days without recourse to banding because the Y is easily recognizable by the criteria described by Ford (1966). In 9½–10½-day litters XX females were identified by trypsin–Giemsa banding (Papaioannou, Evans, Gardner & Graham, 1979) or from their sex chromatin-positive amnion cells.

Half the XX fetuses will be heterozygous for the inversion. Trypsin–Giemsa banded preparations from a total of 104 fetuses ranging from 9½–18½ days *post coitum* were scored for the presence or absence of the inversion.

Results

Because there are major litter effects on growth during pregnancy, all comparisons have been made within litters. Preliminary analyses revealed no differences between XX females with and without the inversion, so the inversion has been ignored when making XO-XX comparisons.

In a preliminary report, Burgoyne & Baker (1981b) observed that the retardation of XO fetal mice is not uniform—occasionally individuals are encountered which are much more severely affected than the norm. Such 'XO runs' can seriously distort XO-XX comparisons, so it was considered necessary to exclude them before analysis of the XO and XX growth data. The runs were identified by calculating XO deviations in embryonic volume (7½ days) and fetal weight (12¼–18½ days) from XX litter means, and then testing the 'population' of deviations at each gestational age for outliers (Dixon, 1953). Four XO runs were identified: 2 at 10¼ days, 1 at 12¼ days and 1 at 12½ days. A check on the records of their uterine positions revealed that 3 were next to the cervix, while the 4th was only one removed from the cervix and was in a crowded horn (9 fetuses). By contrast a check for XX runs revealed 3, of which 2 were at the top end of a uterine horn and the third was halfway down.

The form of analysis used throughout for XO-XX comparisons is illustrated in Table 1, in which the mean weighted difference in volume between the embryonic part of XO and XX egg cylinders has been calculated using log-transformed data (the volumes are log-normally distributed). The mean weighted log difference (-0.2988) is highly significant ($t_{19} = 5.73$, $P = <0.005$), indicating that the embryonic volume of an XO egg cylinder was half that of the XX controls (the antilog of -0.2988 ± 0.0522 is 0.50 with 95% confidence limits of 0.41 and 0.62).

Table 1. Analysis of differences in embryonic volume between XO and XX egg cylinders at 7½ days

Litters	No. of mice		Mean log volumes		Difference (d = $\bar{X}_1 - \bar{X}_2$)	$\left(w = \frac{n_1 \times n_2}{n_1 + n_2} \right)$	Weighted difference (wd)
	XO (n_1)	XX (n_2)	XO ($\bar{X}_1$)	XX ($\bar{X}_2$)			
1	2	2	2.1343	2.2101	-0.0758	1.00	-0.0758
2	1	6	1.7993	2.0248	-0.2255	0.86	-0.1939
3	3	3	2.0345	2.3400	-0.3055	1.50	-0.4582
4	2	3	1.3802	1.9029	-0.5227	1.20	-0.6272
5	2	5	1.4831	1.7872	-0.3041	1.43	-0.4349
$\sum n_1 = 10 \quad \sum n_2 = 19$			Mean* = 1.7663	2.0530	$\frac{\sum wd}{\sum w} = -0.2988\ddagger$	$\sum w = 5.99$	$\sum wd = -1.7900$
			s.e.m. = 0.1479	0.1003	s.e.m. = 0.0522‡		
			Vol. (mm ³) = 0.0584	0.1130			

* Mean of litter means.

† 'Mean weighted difference'.

‡ Calculated using the variance within genotypes within litters.

From the observations on stage of development it was clear that the small size of XO egg cylinders was associated with retarded development. XO egg cylinders were all at the early to mid-primitive streak stages while their XX littermates were at mid- to late primitive streak stages or the early neural plate stage. At 9½ days of gestation the retardation was quantified using somite numbers. The mean weighted XO-XX difference was -2.1 ± 0.8 somites ($P = 0.025$ – 0.0125). Since somite numbers are increasing at a rate of 0.54 somites/h at this time (S. Kalmus & P. S. Burgoyne, unpublished data), the XO fetuses were 3.9 ± 1.5 h behind their XX littermates in development.

The results of the analysis of the log-transformed fetal and placental weight data after exclusion of the runs are given in Table 2. Each line in the table is equivalent to the bottom section of Table 1. In Text-fig. 1 these results are summarized by plotting XO size throughout as a proportion of XX size. Also plotted is the predicted size for XO fetuses if they remained the 3.9 h behind in

development which was estimated from the 9½-day somite data. The fetal weight data show that XO fetuses caught up with their XX littermates between 10½ and 12½ days. Additional data were collected at 12½ days which substantiated this. By 14½ days the XO fetuses had lagged behind again, but more data are required to confirm this. In marked contrast, the placentas of XO fetuses were initially the same weight as controls, and became significantly heavier than those of controls by 18½ days.

Table 2. Mean fetal and placental log weights for XO and XX mice and the estimated differences between them for the period 9½–18½ days *post coitum*

Days of gestation	No. of mice		Mean $\pm$ s.e.m. log wt* (wt in mg)		Mean $\pm$ s.e.m. weighted XO-XX difference	Significance of XO-XX difference (<i>P</i>)
	XO	XX	XO	XX		
<i>Fetal</i>						
9½	10	18	0.356 $\pm$ 0.06(2.27)	0.484 $\pm$ 0.02(3.05)	-0.124 $\pm$ 0.053	0.025-0.0125
10½	13	40	1.276 $\pm$ 0.02(18.86)	1.345 $\pm$ 0.02(22.13)	-0.071 $\pm$ 0.020	0.0025-0.0005
12½	7	22	1.924 $\pm$ 0.01(84.0)	1.922 $\pm$ 0.02(83.5)	-0.002 $\pm$ 0.021	NS
12½	15	41	2.035 $\pm$ 0.02(108.4)	2.035 $\pm$ 0.01(108.4)	-0.005 $\pm$ 0.011	NS
14½	18	30	2.461 $\pm$ 0.03(288.8)	2.494 $\pm$ 0.01(311.7)	-0.039 $\pm$ 0.014	0.005-0.0025
16½	13	30	2.847 $\pm$ 0.03(703.1)	2.869 $\pm$ 0.02(739.8)	-0.025 $\pm$ 0.011	0.0125-0.001
18½	13	18	3.142 $\pm$ 0.02(1386.2)	3.155 $\pm$ 0.02(1429.6)	-0.010 $\pm$ 0.018	NS
<i>Placental</i>						
9½	10	21	1.473 $\pm$ 0.01(29.7)	1.473 $\pm$ 0.01(29.7)	-0.003 $\pm$ 0.016	NS
10½	13	40	1.645 $\pm$ 0.02(44.2)	1.654 $\pm$ 0.01(45.1)	-0.009 $\pm$ 0.010	NS
12½	15	40	1.933 $\pm$ 0.02(85.8)	1.908 $\pm$ 0.01(81.0)	+0.016 $\pm$ 0.018	NS
14½	18	30	2.114 $\pm$ 0.03(129.9)	2.077 $\pm$ 0.01(119.3)	+0.036 $\pm$ 0.020	NS
16½	13	30	2.141 $\pm$ 0.03(138.2)	2.103 $\pm$ 0.02(126.9)	+0.032 $\pm$ 0.022	NS
18½	13	18	2.132 $\pm$ 0.03(135.5)	2.037 $\pm$ 0.03(108.9)	+0.098 $\pm$ 0.025	<0.001

* Means of litter means.

Text-fig. 1. XO fetal and placental weights plotted as proportions of XX weights for the period 9½–18½ days of gestation. Bars indicate standard errors and the number of XOs contributing data is indicated for each point. The broken line, calculated from the XX growth data, indicates the expected size of XO fetuses if they remained the 3.9 h behind in development which had been estimated from the 9½-day somite data.

Discussion

Our results reveal that XO mice were already lagging behind their XX sibs in development by the 8th day of pregnancy. This lag continued until 10½ days of gestation, but there was evidence of catch-up growth between 10½ and 12½ days. However, this 'catch-up' was not maintained, which is in keeping with the finding that XO mice are underweight at birth (Burgoyne *et al.*, 1983).

XO monosomy may have a continuous effect on development, or the growth profile from 7¼ to 18½ days may simply be a result of the retardation present at 7¼ days. Tarkowski (1959) has described the development of single blastomeres from 2-cell mouse embryos. These 'half embryos' continue to develop according to their original programme and consequently form half-sized blastocysts; but development subsequently lags behind that of controls with the result that by the primitive streak stage development and size have become co-ordinated. The lag remains for 2 or 3 days, and then an apparent acceleration of growth and development brings them back in line with controls by about 12½ days. Tarkowski's (1959) experiments suffered from inadequate controls, but taken at face value the development of these chromosomally normal 'half embryos' from the primitive streak stage onwards mimics XO development. Thus we tentatively suggest that XO mouse conceptuses are not being constrained by their genotype during the period studied.

The origin of the developmental lag already present at 7¼ days is clearly of interest. Why should XO embryos be affected when one X is sufficient for XY embryos? It may be that the X and Y chromosomes share some genes in common (see Burgoyne, 1982) and that these are required in double dose in the early embryo. However, the XO embryos in the present study also differ from XY embryos in having a paternal rather than a maternally derived X chromosome. A study of the development of XO embryos with a maternally derived X is needed to help resolve this question.

At first, the occurrence of XO 'runts' preferentially located near the cervix is puzzling, since XX runts in this study and the runts described by McLaren & Michie (1960) were preferentially located at the top of a uterine horn. However, it is clear from information on uterine position in a separate study (P. S. Burgoyne & S. Kalmus, unpublished data) that at 9½–10½ days a cervical location is associated with lowered XX and XY fetal and placental weights. This effect is not seen late in pregnancy, in keeping with observations of McLaren & Michie (1959) on 18½-day litters. It therefore seems that there is a genotype-environment interaction for XO runts, with the less favourable cervical position exacerbating the developmental deficiencies associated with XO monosomy.

Interpretation of the data on placental size is complicated by the fact that the placenta has fetal and maternal components. Presumably the increase in size of XO placentas relative to XX placentas in late gestation involves the fetal contribution because the fetal contribution dominates at this stage. A similar size advantage is seen in XY placentas at this time (P. S. Burgoyne & E. P. Evans, unpublished). It will require histological study to determine whether the fetal contribution to the placenta is normal at the earlier stages.

Do these observations on the early development of the XO mouse have any relevance to the development of human XO conceptuses? Boué, Philippe, Giroud & Boué (1976) have recorded the stage of development attained by human XO abortuses. The mean developmental age judged from the embryonic remnants and/or placenta was 6 weeks after fertilization. In about two-thirds of the specimens there was little embryonic tissue, but in most of the remainder there was an essentially normal embryo of 40–45 days of development. There was a discrepancy of several weeks between the developmental and gestational ages, which Boué *et al.* (1976) have interpreted as being due to retention *in utero* after developmental arrest. In the light of our observations on the XO mouse it seems likely that much of the discrepancy may in fact be due to a much earlier developmental retardation, after which development continues.

The most striking feature of human XO development is the 99% loss by spontaneous abortion during pregnancy (see Polani, 1981), which contrasts with a negligible pregnancy loss in the mouse (P. S. Burgoyne & E. P. Evans, unpublished). This difference may be related to the different strategies for the maintenance of pregnancy in the two species. In the mouse ovarian steroids are

involved throughout gestation, whereas in man placental steroids become essential towards the end of the first trimester. Boué, Boué & Spira (1974) argue that the peak of spontaneous abortion around 12 weeks after the last menstrual period is due to the cessation of ovarian support at this time, those conceptuses with placental hormone insufficiency being aborted. Our belief is that human XO embryos which reach 6 weeks of development are inherently viable, but that the placenta, possibly because of immaturity, is in most cases unable to maintain the pregnancy.

It is clear from descriptions of XO fetuses surviving beyond the first trimester (Singh & Carr, 1966; Singh, 1970; Boué *et al.*, 1976) and from information on the 1% that survive to term (Simpson, 1976) that there are a number of anomalies, in addition to small stature and sterility, which are associated with XO monosomy in man. Among the more frequently encountered are skeletal abnormalities, webbing of the neck, and lymphoedema of the hands and feet at birth. Such anomalies have not been described in the XO mouse. It is possible that the initial developmental retardation is much greater in the human XO fetus, and that the anomalies arise during a catch-up period. In the mouse severe retardation is followed by some developmental mismatch during the catch-up period (Snow, Tam & McLaren, 1981; Tam, 1981), with consequent malformations. Alternatively, the anomalies could be a consequence of damage incurred during a near-failure of the pregnancy, resulting from the same placental insufficiency which more usually results in abortion.

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