

Testing for Nondisjunction in the Mouse

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Tests for nondisjunction have been carried out in male and female mice. Ten-day fetal progeny of control and treated adults have been karyotyped to establish spontaneous and induced levels of aneuploidy. In males, the effects of 100 rad x-rays on type A spermatogonia and early primary spermatocytes, and the effects of Mitomycin C (2 mg/kg) on early primary spermatocytes, have been tested. The results show insensitivity of primary spermatocytes to both agents, but a 3.5-fold increase in nondisjunction following spermatogonial irradiation. In females, comparisons have been made between young controls, young x-rayed (5 rad), aged controls and aged x-rayed (5 rad) animals. The "ageing effect" on nondisjunction is observed, but too few fetuses have been analyzed to reach conclusions regarding enhancement of nondisjunction levels by low doses of x-rays.

Introduction

Data concerning the frequency of spontaneous and induced nondisjunction at meiosis in the mouse, and the resulting levels of aneuploidy found among fetal and adult offspring have been obtained by using the following methods: (1) counts of second meiotic metaphase in oocytes (1-5) or spermatocytes (6-8); (2) karyotypes of pre-implantation embryos at the one-cell stage (9-11) or in morulae and blastocysts (12-14); (3) karyotypes of post-implantation embryos (13-18); (4) karyotypes of live newborn offspring (19); (5) genetic testing in adults by methods based on the complementation of unbalanced gametes (20) or examination of the progeny of matings with sex-linked markers (21-23).

Each method has its merits and its disadvantages as far as application to screening is concerned. Methods 1 and 2 can obviously provide a better indication of the primary incidence of nondisjunction, but methods 3, 4, and 5 give a more realistic estimate of the surviving nondisjunctional load with which we should be concerned when extrapolations to man are being considered.

In our own laboratory, we have chosen method 3 as a suitable means of screening for non-disjunction in the mouse. Our procedures are essentially those described by Yamamoto et al. (14, 15) for the karyotypic analysis of 9-10 day old fetuses among the

offspring of control and treated animals. Screening at this stage of gestation will lead to the detection of most trisomic offspring, but many monosomics will go undetected: Gropp et al. (24, 25) have shown that while most trisomics in the mouse survive at least until day 10 of gestation, the majority of monosomics have degenerated by this time. This loss of information is compensated for, however, by the fact that the technique (26) for obtaining good quality metaphase spreads from 9-10 day fetuses or their membranes is extremely simple and many fetuses can be processed in a relatively short space of time. Once a numerical abnormality has been detected, precise information on the identity of the particular chromosome involved in the nondisjunctional event can be obtained by application of a suitable banding technique (27, 28). Thus, the relative contributions made by sex chromosomal and autosomal aneuploidy can be estimated, and the involvement of specific chromosomes assessed.

Three different experiments have now commenced in our laboratory.

X-Ray Induced Nondisjunction in Males

The first was designed to test the sensitivity to x-rays of two different stages of spermatogenesis. From early work concerning the killing effects of x-rays on mouse germ cells (29-32), a dose of 100 rad was chosen as one which would ensure continuous fertility of irradiated males and minimal sper-

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Table 1. Time required for each stage of processing of 9-10 day old fetuses or their membranes.

Process	Time required, min
Dissection of fetus from uterus	5
Removal of membrane	2
Incubation in medium plus colchicine	60
Hypotonic treatment	15
Fixation	30 (minimum)
Slide-making (two slides)	10
Staining	5
Karyotype analysis on five cells	10

matogonial and spermatocyte death, while giving what we hoped would be a detectable increase in nondisjunction. It is known that preleptotene is a particularly sensitive stage to the killing effects of x-rays in the mouse, only about 50% of cells in that stage surviving a dose of 200 rad (32).

Methods

The cell stages chosen for testing were primary spermatocytes in early meiotic prophase (including S-phase) and type A spermatogonia, the cell stage most at risk as far as long term genetic effects are concerned (33). To sample those two stages, males were mated in week 5 (spermatocytes) and week 7 (type A spermatogonia) after irradiation, spermatogenic timings being based on the findings of Oakberg (29, 30), Sirlin and Edwards (34), and Bateman and Chandley (35).

In each run of the experiment, four random-bred Q strain males were irradiated with 100 rad x-rays on day 1 of week 1. Four other Q males were kept as untreated controls. On day 1 of week 5, each male was mated to two Q females and each female examined daily for the presence of a vaginal plug. On day 7 of week 5, males were removed from females and kept unmated until day 1 of week 7. Throughout week 7, the males were again mated to two fresh Q females and plugs again recorded. At the end of week 7, all males were destroyed.

Pregnant females from the week 5, week 7 and control matings were killed at 9-10 days gestation and chromosome preparations made from all viable fetuses or their membranes. Dead implants or deciduomata unlikely to give a satisfactory chromosome preparation were excluded.

Two slides were made from each fetus, one being stained for immediate analysis with Giemsa stain, the other being held in reserve for banding analysis should a chromosome abnormality be detected. A minimum of five well-spread metaphases was analysed per fetus, and a further 10 or 20 cells were scored if mosaicism was suspected.

In general, it was found that about 40 fetuses could be comfortably processed up to and including the slide-making stage in an average working day. The time in minutes taken for each stage of processing from dissection to karyotype analysis is shown in Table 1. One run of the experiment, yielding approximately 100 fetuses from the week 5 matings, 100 from the week 7 matings, and 100 control fetuses, could be completed in about 10 weeks by one individual. Costs incurred included the treatment and maintenance of the animals, small amounts of medium, hypotonic solutions, fixatives, stains and slides. The most expensive factors were the microscope for cytogenetic analysis and the salary of the individual carrying out the processing and scoring of the slides.

Results

The results are shown in Table 2. The overall frequency of abnormalities in the controls was $0.89 \pm 0.40\%$, compared with $1.75 \pm 0.40\%$ in week 5 and $2.51 \pm 0.78\%$ in week 7. The increases in weeks 5 and 7 were, however, not statistically significant.

Since mosaicism is a phenomenon produced by two cell lines developing in a single zygote, and polyploidy a phenomenon probably arising near the time of fertilization (36), they will not be considered in the following section. For aneuploidy alone (Table 3), the level was 0.36% in controls, 0.41% in week 5, and 1.26% in week 7. However, if one assumes that

Table 2. Karyotype analysis of 10 day fetuses from control and treated (100 rad x-rays and Mitomycin C) male mice.

Treatment	No. of males	No. of viable fetuses	Mean litter size	No. of fetuses analyzed	Karyotype analysis										Total abnormal	% abnormal
					Others	39	39/40	40	40/41	41	43	60	80			
Control	35	572	11.2	561			2	556		2		1		5	0.89 ± 0.40	
Week 5, 100 rad ^a	69	1007	8.9	972	2	2	3	955	2	1	1	4	2	17	1.75 ± 0.40	
Week 7, 100 rad ^b	24	410	10.3	398		1	2	388	1	4			2	10	2.51 ± 0.78	
Week 5 Mitomycin C, (2 mg/kg)	20	333	8.5	325		2		321	2					4	1.23 ± 0.61	

^aWeek 5 represents sampling of treated early primary spermatocytes; week 7 represents sampling of treated type A spermatogonia.

Table 3. Percentage aneuploid, polyploid, and mosaic fetuses from x-ray (100 rad) and Mitomycin C (2 mg/kg)-treated male mice and controls.

Treatment	Aneuploid		Polyploid		Mosaic		Total no. of fetuses analyzed
	n	%	n	%	n	%	
Control	2	0.36	1	0.18	2	0.36	561
Week 5 100 rad	4	0.41	6	0.62	6	0.62	972
Week 7 100 rad	5	1.26	2	0.50	3	0.75	398
Week 5 Mitomycin C	2	0.60	0	0.00	2	0.60	333

the eliminated monosomic embryos were originally as frequent as the trisomics, a corrected estimate, based on the frequency of observed trisomics only, can be derived. The method (13) is to double the number of trisomic embryos and express this as a percentage of the number of diploid embryos. ($2n = 40$) plus twice the number of trisomic embryos. (The mosaic embryos are allocated to the normal and trisomic classes according to their presumed zygotic constitution and the polyploids ignored). The aneuploid frequencies thereby obtained are 0.71% for controls, 0.83% for week 5, and 2.50% for week 7. This gives a 3.5-fold increase in aneuploidy over control levels for type A spermatogonia irradiated with 100 rad x-rays.

Table 4 shows the individual karyotypes of the aneuploid, polyploid, and mosaic fetuses found. Sex chromosome and autosomal aneuploidies appear to be approximately equally represented, and the five fully analyzed autosomal aneuploids show that there has been nondisjunction in every case of an acrocentric pair in the smaller size range of the genome (chromosomes 10-19). The most remarkable aneuploids found were a triple trisomic with a 43, XXY,+10,+17 karyotype conceived on day 5 of

week 5 and a surviving chromosome 19 monosomic conceived on day 7 of week 7. The latter fetus was litter mate to an XXYY tetraploid.

Mitomycin C-Induced Nondisjunction in Males

In Tables 2, 3, and 4, data are also given for a preliminary trial of the effectiveness of Mitomycin C (MC) in producing nondisjunction in early spermatocytes of the mouse (week 5 sampling). The rationale for the experiment was that MC, an antibiotic agent affecting DNA synthesis, produces chromosomal aberrations, chiefly in centromeric heterochromatin of the somatic cells of a number of species including man (37-41). The cytogenetic observation of particular relevance is that the induced aberrations show a unique spectrum of crosslike exchanges with an excessive involvement of homologous chromosomes. Such events, we supposed, might give rise to nondisjunction in an affected germ line cell treated at S phase.

Our results on 325 F₁ fetuses from males given a single intraperitoneal injection of MC (2 mg/kg) and mated during week 5 after injection showed, how-

Table 4. Karyotypes of the abnormal fetuses found in experiments 1 and 2.

Treatment	Aneuploid	Polyploid	Mosaic	Others
Control	41,XXY(2)	60,XXY	39/40(2) ^a	
Week 5, (100 rad)	39,X(2) 41,XY,+16 43,XXY,+10,+17	60,XXY(3) 3n = 60 ^a 80,XXXXX ^b 80,XXYY	39,X/40,XY 39/40(2) ^a 40,XY/41,XXY ^b 40,XY/41,XXY 40,XY/80,XXYY	40,XY,1q+
Week 7, (100 rad)	39,XX,-19 ^b 41,XXY 41,XY,+14 41,XX,+14 2n = 41 ^a	80,XXYY(2) ^b	39/40(2) ^a 40,XY/41,XY+8	
Week 5 (MC)	39,X(2)		40/41 ^a 39/40/41 ^a	

^aNot analyzed.

^bLitter mates.

Table 5. Karyotype analysis of 10 day F₁ fetal progeny of young (6-8 weeks) and aged (9 month) female mice in control and treated (5 rad x-ray) groups.

Treatment	No. females	No. viable fetuses	Mean litter size	No. fetuses analysed	Karyotype analysis								No. abnormal	% abnormal
					39	39/40	40	40/41	41	60	80			
Control young	51	572	11.2	561		2	556		2	1		5	0.89 ± 0.40	
5 rad young	10	108	10.8	106		1	105					1	0.94 ± 0.94	
Control aged	17	89	5.2	83		2	79			1	1	4	4.82 ± 2.35	
Aged + 5 rad	8	47	5.9	47			46		1			1	2.13 ± 2.10	
5 rad + aged	12	60	5.0	54	1		52		1			2	3.70 ± 2.57	

ever, no increase in nondisjunction over control levels (Tables 2 and 3). The only aneuploid fetuses recorded were two X monosomics (Table 4), and it is possible that these arose by chromosome loss rather than by true nondisjunction.

X-Ray-Induced Nondisjunction in Females

The need for experimental studies into the effect of x-rays on female germ cells has arisen because of the suggestion from a number of epidemiological and clinical studies in man, that parental (usually maternal) irradiation may increase the risk of having a baby with Down's syndrome (trisomy 21) (42-45). Alberman et al. (45) have suggested that radiation damage by small doses, added to the well known ageing effect in women, may well represent a real, albeit proportionately small, factor in the genesis of trisomy 21. These authors calculated a doubling dose of 2 rad for Down's syndrome, a risk still much smaller than the ageing effect alone, but a risk greatest in women who had received x-rays (either diagnostic or therapeutic) more than 10 years before their children were conceived (45).

The first experimental studies to test Alberman's assumptions were made by Yamamoto et al. (14, 15). They analyzed the fetal progeny, at 10.5 days gestation, from young (3-5 months) and aged (11-16 months) female mice given 5 rad x-rays or kept untreated prior to mating. They noted an increase in aneuploidy due to ageing but claimed an even greater increase in old x-rayed mice compared to old controls. They deduced that the age of the mother significantly affected the susceptibility of oocytes to radiation damage. However, the data have been severely criticized on several counts (46) and a recent attempt to repeat the experiments (18) has failed to confirm the findings.

In another attempt to test the claims of Yamamoto et al. (14, 15), we have begun experiments involving low dose x-ray exposures to female mice. Since the essential feature of the human data would appear to

be that the association between irradiation and Down's syndrome is strongest in women who conceived 10 or more years after receiving x-rays, it seemed appropriate to us to include an additional category of female and one which Yamamoto et al. (14, 15) had omitted. These were females which were x-rayed when young but mated when old. In our experiments, therefore, we have five test groups, the first four being identical to those used by Yamamoto et al. (14, 15), the fifth being our own addition: young (6-8 week) controls; young given 5 rad; aged (9 month) controls; aged, then given 5 rad; given 5 rad then aged.

Table 5 shows the results obtained so far in each group. Numbers of analyzed fetuses are still small, particularly in the aged groups, where litter sizes are halved compared with their young equivalents. Reductions in litter size of this order have been found also by Yamamoto and Watanabe (16) in matings of aged female mice. The effects of ageing on the overall frequencies of abnormalities are clearly seen from the fetal karyotyping, but there is no evidence that 5 rad x-rays enhances this frequency either in young or aged mothers. Numbers of analyzed fetuses are still too small however to permit one to reach positive conclusions regarding possible increases in levels of aneuploidy. The karyotypes of the abnormal fetuses found are given in Table 6.

Table 6. Karyotypes of abnormal F₁ fetal progeny of control and irradiated females.

	Aneuploid	Polyploid	Mosaic
Control young	41,XXY(2)		39/40(2) ^a
5 rad young			39/40 ^a
Control aged		3n = 60 ^a 4n = 80 ^a	39/40 ^a
Aged + 5 rad	41,XY,+16		39,X/40,X,(del)X
5 rad + Aged	41,XY,+3 39,X,t(6;15)		

^aNot analyzed.

Discussion

In the 1972 report of the U.N. Scientific Committee on the Effects of Atomic Radiation (47) it was stated: "future research in the field of radiation genetics should include studies on the mechanism of induction by irradiation of germ cells in experimental organisms, and on its frequency of occurrence under different conditions of irradiation of the germ cell most at risk."

The relative dearth of information concerning induced nondisjunction in mammalian germ cells at the time this report was prepared stimulated our own interest in the subject. Most experimental data available at that time concerned nondisjunction in females, and the main question being asked was whether low dose x-irradiation could act over and above the ageing effect to enhance the already known high levels of nondisjunction found in older women (14, 15, 42-45). Less attention had been paid to males and the possible effects on nondisjunction which might be produced by irradiation of the testis. It was known, however, that x-rays could induce nondisjunction in spermatocytes of *Drosophila melanogaster* (48), and in man, there was suggestive evidence that aneuploid (and polyploid) abortuses occurred more frequently among the progeny of men (but not women) exposed professionally to radiation (49). Furthermore, collective data concerning the genesis of trisomy 21 in man now indicate that one fifth of cases are paternal in origin, the contribution to nondisjunction coming almost equally from the first and second meiotic division (50).

In the mouse, sporadic reports of viable primary trisomics among the sterile and semisterile progeny of irradiated and chemically treated males had been made (51-53), but doubts had been expressed concerning their possible origin (54). Lyon and Meredith (54) questioned whether the extra element in the karyotype could be a translocation product, the 41 chromosome mice in question thus being tertiary and not primary trisomics. The reports were, of course, made prior to the advent of banding methods for the identification of chromosomes, but the authors (53) were themselves convinced, from the meiotic configurations, that they were indeed dealing with primary trisomics. If they were correct in their interpretations, it is interesting in the context of this paper, to note that six out of the seven such aneuploid animals produced by x-irradiation of sires, were derived from the treatment of type A spermatogonia and only one from a treated spermatocyte. However, higher x-ray doses (350 and 700 rad) were used than in our own investigations. In another series of experiments, Russell and Montgomery (55) failed to recover XO progeny from 600 rad irradiation

of spermatogonia, and they suggested this may have been due to the inviability of XO and OY cell progeny in the testis. Indeed, Searle (20) deduced from the results available that any chromosome losses produced in spermatogonia of the mouse would not normally be transmitted to the next generation. He also suggested that complete trisomy is probably not induced in spermatogonia to any great extent since an extra chromosome has only rarely been found in mouse spermatocytes at metaphase I or II after spermatogonial irradiation (56). Our own observations, however, indicate that nondisjunction does occur following treatment of spermatogonia, a dose of only 100 rad being sufficient to produce a 3.5 fold increase over control levels for type A spermatogonia and that the progeny of such nondisjunctional events can be transmitted to the next generation. As type A spermatogonia are among the most radio-resistant of the spermatogonial generations in terms of cell killing (33) and are certainly the most important cell type from the point of view of long term genetic effects following irradiation to the male, we believe our findings are of some importance. Further investigations along the lines already commenced are therefore proposed.

We believe that 10-day fetal karyotyping provides a simple and reliable screening method for nondisjunction which could readily be applied to the testing of other mutagenic compounds. The procedures are laborious, but we believe that the great need for information on aneuploidy in mammalian germ line systems is sufficient justification for their continued use.

Finally, let us consider how our findings in the mouse relate to the situation in man. It would seem that the levels of aneuploidy recorded at conception for man are of an order of magnitude higher than for the mouse. From cytological studies on one-cell embryos of the mouse, Maudlin and Fraser (11) have estimated that less than 2% are aneuploid. In man, an estimate of aneuploidy at conception based on the known percentage of all conceptions which terminate in abortion, and the numbers of aneuploids among those abortions, would work out at around 10% (57). For postimplantation fetuses in the mouse, our uncorrected estimate based on 9-10 day fetuses is 0.36% and that of Ford and Evans (13) for 8-11 day fetuses is 0.60%. Their estimate for 12-15 day fetuses is 0.32%. Among liveborn and adult mice, the limited data available (19, 23, 55) suggest a negligible incidence of aneuploidy of perhaps less than 0.05%, while in man, the level is 0.39% among consecutive live-born babies (58). Thus there are striking differences in the levels of aneuploidy for the two species, and this should be borne in mind when extrapolations between man and the mouse are being made.

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