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AUTHOR - AUTEUR

Full Name of Author - Nom complet de l'auteur

Barbara G. Brennan

Date of Birth - Date de naissance

May 19, 1953

Canadian Citizen - Citoyen canadien

Yes / Oui

No / Non

Country of Birth - Lieu de naissance

Canada

Permanent Address - Résidence fixe

256 Duke Street Apt. 507,  
Hamilton, Ontario.  
L8P 1Y3

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Name of Supervisor - Nom du directeur de thèse

Dr. I. Uchida

AUTHORIZATION - AUTORISATION

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POSSIBLE REPAIR OF RADIATION-INDUCED  
NONDISJUNCTION IN MOUSE OOCYTES

BY

BARBARA GAYLE BRENNAN, B.Sc., M.Sc., M.D.

A Thesis

Submitted to the School of Graduate Studies  
in Partial Fulfilment of the Requirements  
for the Degree  
Doctor of Philosophy

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POSSIBLE REPAIR OF NONDISJUNCTION

IN MOUSE OOCYTES

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AUTHOR: Barbara Gayle Brennan, B.Sc. (University of Western  
Ontario)

M.Sc. (McMaster University)

M.D. (McMaster University)

SUPERVISOR: Dr. Irene Uchida

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## ABSTRACT

There are some data from human epidemiological studies which suggest that radiation has a small but significant effect in causing aneuploid gametes. Alberman et al. (1972) suggested that much of this radiation occurred more than ten years before the conception of the abnormal child. Mice have been used to study experimentally radiation effects on chromosome segregation. Radiation induces nondisjunction in several strains, including (C<sub>3</sub>HxICR/Swiss)F<sub>1</sub> females. The present study was designed to investigate the possibility of repair of the mechanisms which results in nondisjunction following irradiation.

Female (C<sub>3</sub>HxICR/Swiss)F<sub>1</sub> mice were randomized into 4 experimental groups at 6-8 weeks of age. Some were irradiated at 3 months with 20R gamma rays from a <sup>137</sup>Cs source. Young mice were sacrificed at 3 months of age, within 24-48 hours of irradiation. Others were irradiated then housed until 9 months old. At the time of sacrifice ovaries were removed and the oocytes obtained were cultured to the metaphase II stage. Chromosomes were then analyzed.

Oocytes from 974 mice were studied. The frequency of spontaneous nondisjunction in oocytes from young mice was quite low (0.2%) and this increased significantly after irradiation to 1.6% (p=0.001). The spontaneous frequency of nondisjunction in oocytes increased with age from 0.2% to 1.2%. In contrast the nondisjunction frequency in oocytes from

irradiated-aged animals was 0.0%. In other words, following irradiation at a young age, no aneuploid oocytes were recovered. This is significantly different from the frequency in control aged animals,  $p=0.002$ .

The mechanism by which radiation causes nondisjunction is not known. Possibilities include damage to the centromere region or radiation could act by causing premature or accelerated terminalization of chiasma.

The disappearance of aneuploid oocytes in irradiated-aged animals in this study suggests that in addition to causing nondisjunction immediately there is a delayed effect somewhere between 48 hours and 6 months which results in an elimination of abnormal cells. This experimental finding in mice is quite different from epidemiological information from humans. It provides an avenue for further research into agents causing chromosome abnormalities and the factors involved in germ cell selection and survival.

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## INTRODUCTION

The most common class of chromosomal defects in humans is aneuploidy (having an irregular number of chromosomes), which results from unequal distribution of homologous chromosomes during meiosis. This type of error is usually caused by nondisjunction and can occur during the first or second division of oogenesis or spermatogenesis. Nondisjunction leads to the formation of abnormal gametes which upon fertilization can produce trisomic or monosomic progeny. The incidence of human aneuploidy is approximately 0.5% of all livebirths (Smith, 1976). Among spontaneous abortions aneuploidies are extremely common representing about 40% of those occurring before the twelfth week (Boué et al., 1975).

The development of chromosomal banding techniques (Caspersson et al., 1971) and the identification of inherited chromosome polymorphisms (Paris Conference, 1971) made it possible to determine the parental origin of certain trisomies. For example, if the mother were heterozygous for a polymorphic feature and the segregation error occurred during the first meiotic division of the oocyte, the gamete would possess two homologous chromosomes with different markers. If nondisjunction occurred during the second division the gamete would have two chromosomes with identical markers. Using this approach to study the parental origin of trisomy 21, Langenbeck et al. (1976) estimated that significantly more errors occurred during oogenesis than spermatogenesis and that

errors in meiosis I were 5-10 times more common than during meiosis II.

Specific genes may also be used as markers. The X-linked locus for the Xg blood group (Mann et al., 1962) enabled the origin of the extra chromosome in the XXY syndrome to be determined. In 50% of informative cases the meiotic error occurred in the first division of the oocyte, in 20% during the second division and 30% arose by an error during spermatogenesis (Race and Sanger, 1969; Sanger et al., 1977).

In vivo experiments to examine factors causing aneuploidy are not possible in humans for obvious reasons. Consequently, most experiments on mammalian nondisjunction have been done with the mouse as the model. In the study of aneuploidy attention has been concentrated on the female because before the advent of chromosome banding it was thought that the majority of aneuploids arose by an error in oogenesis due to the prolonged time that oocytes spend in the dictyotene stage.

Since mice are being used as an experimental model the similarities between mouse and human oogenesis must be considered. Female mice are born with a finite population of germ cells (Borum, 1966) as are human females (Manotaya and Potter, 1963). In mice, the oocytes are arrested in the dictyotene (diffuse diplotene) stage of the first meiotic prophase between the 1st and 5th day after birth (Borum, 1961). Human oocytes are arrested in dictyotene (Baker, 1963) from the 7th month of gestation. Oocytes remain at this stage until ovulation which may be up to 40 years later.

Follicle growth in the mouse begins during the first week after

birth (Borum, 1961) and continues throughout the life of the animal. Complete follicular development takes from 20-40 days (Pedersen, 1970; Oakberg, 1979) with growth starting on any day of the hormonal cycle (Peters et al., 1975).

Pedersen and Peters (1968) suggested a useful classification for mouse follicles based on the size of the oocyte and the size and morphology of the follicle:

- |         |                                 |  |
|---------|---------------------------------|--|
| Type 1  | - small oocyte (<20 $\mu$ )     | - No follicle cells                                |
| Type 2  | - small oocyte                  | - Few follicle cells                               |
| Type 3a | - small oocyte                  | - One ring (<20) of follicle cells                 |
| Type 3b | - growing oocyte (20-70 $\mu$ ) | - One ring (21-60) of follicle cells               |
| Type 4  | - growing oocyte                | - 2 layers of follicle cells                       |
| Type 5a | - growing oocyte                | - 3 layers of follicle cells                       |
| Type 5b | - large oocyte (70 $\mu$ )      | - Many layers of follicle cells                    |
| Type 6  | - large oocyte                  | - Follicle cells separated by small areas of fluid |
| Type 7  | - large oocyte                  | - Follicle has single cavity with follicular fluid |
| Type 8  | - large oocyte                  | - Same as 7 but with cumulus stalk                 |

The frequency of aneuploidy in mice can be estimated by counting chromosomes from oocytes (Edwards, 1962). Other methods include the study of pronuclei before the first cleavage division (Donahue, 1972), and karyotyping tissue from preimplantation embryos (Hansmann, 1973) and newborn animals or fetuses (Ford and Woollam, 1963; Evans et al., 1972). Of these methods, the examination of germ cell chromosomes is the most direct way to study the incidence of actual meiotic error.

### Oocyte culture techniques

One method for obtaining large numbers of oocytes is by the administration of gonadotropic hormones (pregnant mare serum and human chorionic gonadotropic hormone) to female animals and then recovering the ovulated oocytes from the oviduct 14 hours after HCG treatment (Edwards and Gates, 1959). This method is very tedious when large numbers of oocytes are needed. In addition, the oocytes are subject to the influence of the hormones. Although aneuploidy is not known to be increased with hormone treatment (Maudlin and Fraser, 1977; Golbus, 1981), there is an increase in polyspermy when the oocytes are fertilized (Maudlin and Fraser, 1977).

Another method for studying oocytes was originally described by Pincus and Enzman in 1935. Rabbit oocytes were removed from their follicles and cultured in vitro in a mixture of serum and Ringer's solution. Under these conditions the first polar body formed normally. Following the same procedure Edwards (1965) cultured oocytes from a number of mammalian species, including mice and humans. Neat fetal calf serum was found to be a suitable medium for stimulating mouse oocytes to resume meiosis and proceed to metaphase II (MII) (Henderson and Edwards, 1968).

When mouse follicles are ruptured most oocytes obtained are 60-70 $\mu$  in diameter. These could be from type 5b-8 follicles by Pedersen's classification (1968). However, only those oocytes from mature Graafian follicles (type 8) will proceed to MII. Oocytes from types 6 and 7 follicles progress only as far as MI and type 5b remain in diplotene (Erickson and Sorensen, 1974). The average time for oocytes to progress to MI is

4-9 hours in culture and to MII 11-17 hours (Donahue, 1968; Sorensen, 1973). Maturation in culture is characterized by the dissolution of the germinal vesicle or nuclear membrane followed by extrusion of the first polar body. Oocytes and the polar bodies are easily seen with a stereodissecting microscope.

In 1966, Tarkowski described a better method for the preparation of oocyte chromosomes than the previously used squash technique (Manotaya and Potter, 1963). Instead, oocytes are placed in hypotonic solution then put on a slide and fixed. When combined with the oocyte culture techniques consistently good chromosome preparations were obtained.

When setting up an in vitro system it is important that conditions resemble as closely as possible those that occur in vivo. The number and position of chiasmata and the frequency of univalents at MI are not different between oocytes matured in vitro and in vivo (Henderson and Edwards, 1968). The time taken for an oocyte to mature in culture is similar to the in vivo time which is 5-8 hours to MI and approximately 14 hours to MII (Edwards and Gates, 1959). After reaching MII, in vitro (Edwards, 1965) and in vivo (Edwards and Gates, 1959), oocytes remain at this stage until fertilization. Electron microscopic examination reveals no difference in ultrastructure between oocytes matured in vitro and in vivo (Merchant and Chang, 1971; Calarco et al., 1972).

Factors affecting chromosome segregation and the incidence of aneuploidy can be studied using these techniques for the culturing of oocytes. Analysis of the chromosomes of MII oocytes enables the study of nondisjunctional events that occur during MI. The frequency of

metaphase II errors is estimated as the difference between the rate of nondisjunction in pronuclei and in MI.

Effect of age on the frequency of nondisjunction

In humans the association between advanced maternal age and the increased incidence of Down syndrome or trisomy 21 was reported by Penrose (1933) before the cytogenetic abnormality was identified (Lejeune et al., 1959). The risk of producing affected offspring increases from 1/2000 at age 20 to approximately 1/100 at age 40 years (Hook, 1976). This increase with age is also true for trisomies of other acrocentric chromosomes, certain non-acrocentric chromosomes for example trisomy 18 and for a sex chromosome trisomy XXY (Ferguson-Smith et al., 1964). The frequencies in spontaneous abortions (Boué et al., 1975) indicate that the increase in aneuploids with age is not entirely due to decreased selection against abnormalities but there is an actual increase in the number of abnormal conceptions.

A number of studies have been done on mice to study the effect of age on the frequency of nondisjunction. Goodlin (1965) found no aneuploid fetuses among 756 newborns from 15 month old (Balb/Cx129)F<sub>1</sub> females and Balb/C males. The chromosomes of 10.5 day fetuses from young (3-5 month) and old (11-16 month) CF<sub>1</sub> mice were examined by Yamamoto et al. (1973). When mosaics are excluded there were 4 aneuploids among 156 fetuses of old and 0/149 fetuses of young mothers (p=0.07). Fetuses of 5 inbred strains were examined between 10 and 14 days of development by Fabricant and Schneider (1978). A fetus was called a true aneuploid if more than 70% aneuploid cells were found. By this definition they found 0/239 aneuploid fetuses from young mothers 2-5 months old and about



3% from 7-10 month old mothers when the data from all strains were combined. Frequencies ranging from 7% in CBA to 0% in NZB/J for aneuploid fetuses from old mice illustrate strain differences. From these studies there is no clear relationship between age and aneuploid offspring. By examining liveborns and fetuses a number of aneuploids may have been missed by these authors because of selection against these abnormalities in the earlier stages of development. These studies also emphasize the difficulty of trying to distinguish between true aneuploids, true mosaics and culture mosaics. Those that are truly mosaics arose by mitotic non-disjunction or chromosome loss. Thus, the actual frequency of meiotic error is obscured.

Examination of MII oocyte chromosomes avoids the problems of selection and mosaicism. When MII oocyte chromosomes from CBA mice were studied, Martin et al. (1976) found a significant increase in aneuploidy only in the middle age group (0/187 in young, 0/155 in old and 6/116 in 5-8 month old females). They hypothesized that some oocytes from old female mice may contain so many univalents that they become atretic or fail to mature in culture. The increase in aneuploidy from 0 to 5% is comparable to that found by Fabricant and Schneider (1978) for CBA females. Among MII oocytes of (C<sub>3</sub>HxICR/Swiss)F<sub>1</sub> females no nondisjunction was observed in 3-6 month old females and only 0.6% in those 12 months old (Uchida and Lee, 1974; Uchida and Freeman, 1977).

Gosden (1973) studied the chromosomes of 3.5 day CBA/H-T<sub>6</sub> pre-implantation embryos. The incidence of aneuploidy increased from 1/47 when mothers were 1 month old to 3/58 when they were 8-12 months old,

a barely significant difference ( $p=0.05$ ).

Differences among these studies could be the result of examination of cells from different stages of development. In addition, the mouse strains used were not the same and the ages of the females varied. Thus, the effect of strain on the frequency of nondisjunction cannot be assessed until comparable developmental stages are examined for each strain.

### Radiation and nondisjunction

#### A. Humans

Evidence that radiation may contribute to the incidence of Down syndrome has come from three types of studies: (1) epidemiological studies both retrospective and prospective of mothers with or without exposure to radiation, (2) studies of children born in regions of elevated background radiation, and (3) children born to women exposed to radiation from the atomic bombs in Hiroshima and Nagasaki.

#### Retrospective studies

Uchida and Curtis (1961) first suggested that maternal radiation exposure may be associated with an increase in aneuploidy. They found that mothers of Down syndrome children had been exposed to significantly higher doses of diagnostic X-rays of the abdomen than control mothers ( $p=0.001$ ). In a similar study Carter et al. (1961) found no increased amount of radiation to mothers of Down syndrome children compared to mothers of children with various congenital malformations. The actual

doses involved could not be estimated and all the information was acquired by maternal recall.

No increase in the number of X-rays received by mothers of trisomies compared to mothers of normal children was found by Sigler et al. (1965) who used both hospital records and maternal recall. However, there was a significant increase in the number of fluoroscopic examinations and in the amount of therapeutic radiation to the skin of mothers of affected children. Most of these exposures occurred more than 8 years before the birth of the child. When additional subjects were included (Cohen et al., 1977) the difference was no longer apparent.

Alberman et al. (1972) examined maternal X-ray histories in groups of 0-5 years, 6-10 years and more than 10 years before the conception of the child with Down syndrome. When all years were combined there was no difference between the mothers of Down syndrome children and control mothers. However, the mean gonadal dose received by mothers more than 10 years before the conception of the affected child was significantly greater (75 mrad) than for controls (40 mrad). Most of the radiation was to mothers older than 30 years at the birth of the child.

In two other studies the authors reported no differences between mothers of Down syndrome children and control mothers in terms of radiation exposure (Lunn, 1959; Marmol et al., 1969).

There are several problems inherent in these studies which make interpretation of the data difficult.

1. All studies used interviews to obtain data. There may be a bias in maternal recall when the child is abnormal. This was partially overcome by using as controls mothers of children with other congenital malformations.

2. Most of these authors used only the total number of X-rays and not the doses involved.

3. Radiation exposures were analyzed as a preconception total. When different time periods were considered by Alberman et al. (1972) a significant difference was found. Therefore, small differences may be obscured by combining the data on radiation exposure.

#### Prospective studies

The use of a prospective study avoids dependence on maternal recall. Using hospital records to assess radiation histories, Uchida et al. (1968) found that of 972 births, 10 clinically abnormal children (7 trisomies confirmed by karyotyping) were born subsequent to maternal radiation exposure and one (not karyotyped) among the 972 births from control mothers ( $p=0.02$ ). The mean radiation dose to mothers of trisomies was 3250 mrad whereas for all mothers it was 1400 mrad.

Stevenson et al. (1970) found no increase in the number of trisomies born to irradiated mothers. However, their mean dose was only 900 mrad.

#### Background radiation

There are four sources of radiation exposure other than diagnostic X-rays: cosmic rays, external radiation from the subsoil, radon in the

air and radioactivity of food and water (Pohl et al., 1975). Nature supplies one-half of the radiation the average person is exposed to in a lifetime (Holden, 1979). Therefore, both background and diagnostic radiation should be considered when evaluating possible radiation effects.

Areas of increased radioactive environment are due mainly to thorium and radon which are gamma ray emitting elements found in the subsoil (Pohl et al., 1975).

In the first study to examine the relation between congenital malformation and background radiation, regions of New York state were classified as areas of "high" or "low" radiation from geologic maps (Gentry et al., 1959). Congenital malformations were reported to be increased in the regions designated as "high", however, there was no increase in Down syndrome. Because dosage was not measured in the so-called high and low regions no firm conclusions can be drawn regarding radiation effects.

Background radiation in Vermont and New Hampshire was measured with a dosimeter and regions were divided into high (150 mrad) and low (134 mrad) radiation areas (Segall et al., 1964). Hospital records in these states were examined for cases of birth defects. The total number of trisomies or malformations was not increased in regions of high radiation. However, these differences in background radiation may have been too small to produce a detectable change.

Urban regions of three states with background radiation levels from a high of 130 mrad/yr to a low of 55 mrad/yr were selected by

(Schuman and Gullen (1970). They found a correlation between increased radiation and Down syndrome in one state. However, since the background level in this state only ranged from 70-80 mrad/yr, other factors were probably responsible for the observed increase in malformations.

In contrast to the small differences in background radiation in the above three studies an area in India has a background level as high as 1.5R-2R/yr (Gopal-Ayengar et al., 1972). The incidence of aneuploidy in this population was compared to a control region having a background of 100 mrad/yr. The populations were similar in all other variables examined. There were 12 children with Down syndrome of 12,918 total births in the region with high radiation and 0/5938 in the control region (Kochupillai et al., 1976). Because of the large numbers of births in both groups this finding was reported to be significant. This study however has been severely criticized because of variations in doses received (Sundaram; 1977).

A region in China with a background radiation of 196 mrad has been compared to a nearby control region receiving 72 mrad. Although the prevalence of Down syndrome was greater in the higher region (1.7/1000 compared to 0) the population studied was too small to show a statistically significant increase (High Background Radiation Research Group, China, 1980).

In Brazil where the average exposure from background radiation of 640 mrad/yr (Barcinski et al., 1975) is sufficient to induce a significant number of chromosome breaks in lymphocytes, the incidence of malformations has not yet been studied.

### Atomic bomb survivors

Schull and Neel (1962) calculated the mean gonadal dose received by Japanese women exposed to fallout from atomic radiation was 29R. The frequency of Down syndrome (diagnosed phenotypically) in the exposed group (0.54/1000) was actually less than in the unexposed controls (1.27/1000). Differences in exposure to relatively high radiation doses of this population compared to diagnostic X-rays may not allow comparison between this and other studies. Wald et al. (1970) suggested that high radiation doses may enhance other factors leading to ovum and fetal rejection.

### Conclusion

Some of the disagreement concerning the role of radiation in causing aneuploidy may be due to methodological differences. However, the several reports of significant findings suggest that exposure to radiation has an effect on chromosome segregation but the exact magnitude of the effect is unknown.

### Radiation and nondisjunction

#### B. Mice

X-rays and gamma rays are types of ionizing radiation with approximately the same energy level (Arena, 1971). One roentgen of X or gamma radiation from the source is equivalent to 0.93 to 0.98 rads to the gonads and can be considered for all practical purposes to be equal to the source dose (Baker and Neal, 1977). Results from studies examining the effect of X-rays or gamma rays on nondisjunction in mice are summarized in Tables 1 and 2.

Table 1

Radiation-induced nondisjunction in mouse fetuses from 9.5-12.5 days of gestation

Author	Strain	Dose	Age	Results*	
				Irradiated	Control
Yamamoto <u>et al.</u> (1973)	CF <sub>1</sub>	5R X-rays	3-5 mo.	0/111	0/149
			11-16 mo.	5/43	4/156
Lüning <u>et al.</u> (1975)	CBA	2,4,8,16,32R X-rays	5-20 wk.	230/1346	43/268
Max (1977)	CBA	2,4,8,16R X-rays	15-47 wk.	2/642	2/213
Strausmanis <u>et al.</u> (1978)	C <sub>57</sub> B1	4,8,16R X-rays	11 mo.	5/568	5/496
Hansmann and Probeck (1979)	NMRI	20R X-rays 200	8-12 wk.	0/95 2/22	0/90

\*None of these results were statistically significant at the  $p < 0.05$  level



Table 2

## Radiation-induced nondisjunction in metaphase II oocytes

Author	Strain	Dose	Age	Irradiated	Control	p Value
Uchida and Lee (1974)	(C <sub>3</sub> HxICR/ Swiss)F <sub>1</sub>	10, 20, 30R gamma rays	3-6 mo.	6/1149	0/1154	p=0.02
Reichert et al. (1975)	NMRI	22, 60, 200R X-rays	10-12 wks.	6/204	0.143	p>0.05(N.S.)
Uchida and Freeman (1977)	(C <sub>3</sub> HxICR/ Swiss)F <sub>1</sub>	10, 20, 30R gamma rays	12 mo.	15/1117	4/1306	p<0.01

Yamamoto et al. (1973) found no increase in aneuploid fetuses from young mice irradiated with 5R X-rays compared to controls. However, there was an increase in aneuploid fetuses from both old control and old irradiated mice although the difference between these groups was not significant ( $p=0.08$ ). Lüning et al. (1975) used intrauterine death as the index of abnormality and found no increase with radiation. However few mice were used so the number of fetuses was quite small. There were two aneuploid fetuses among 27 offspring of old controls and 0/46 among the progeny of old irradiated animals. Using the same strain and doses of radiation Max (1977) found no increase in aneuploid fetuses following irradiation. Strausmanis et al. (1978) used a range of low doses but found no increase in aneuploid fetuses. After a slightly higher dose (20R) no aneuploid fetuses were found from mothers 8-12 weeks old. However, 2/22 were aneuploid after 200R compared to 0/90 from unirradiated mothers (Hansmann and Probeck, 1979). Thus, when all the studies are considered in which tissue from mouse fetuses was karyotyped, there was no increase in aneuploidy following maternal irradiation.

Significant increases were found in the number of hyperhaploid oocytes in both young (3-6 months) and old (12 months) ( $C_3H \times ICR/Swiss$ ) $F_1$  females exposed to 10-30R gamma rays (Uchida and Lee, 1974; Uchida and Freeman, 1977). There was also an additional effect of age since the increase in nondisjunction in the old mice was more than twice the number induced in oocytes from young mice. Reichert et al. (1975) irradiated females 3 hours after gonadotrophic hormones were injected. The purpose was to irradiate oocytes between dictyotene and metaphase I.

The increase in nondisjunction observed after irradiation was not statistically increased over their spontaneous rate of zero nondisjunction. When NMRI females were irradiated at different stages of oogenesis (prenatally) and at dictyotene stages 1, 3 and 6 weeks after birth there were no hyperhaploid cells in either control or irradiated mice (Hansmann et al., 1975).

Tease (1982) examined the chromosomes of pronuclei after irradiating young female mice and there was a definite increase in nondisjunction over controls. This was linearly related to the dose of radiation. The author, however, found no increased sensitivity in oocytes from older females unlike Uchida and Freeman (1977).

The negative results from many of the above studies could be explained by strain differences in sensitivity to radiation damage. Because different developmental stages were examined and varying doses of radiation and ages of mice used it is difficult to make generalizations about the effect of radiation on nondisjunction in mouse oocytes.

#### Summary

Human epidemiological studies have supplied evidence that radiation is one environmental factor contributing to the incidence of aneuploidy. Some data suggest that the effective radiation is received many years before the birth of an affected child (Alberman et al., 1972). Experimental evidence, using the mouse as the model, indicates that nondisjunction increases with maternal age and radiation can enhance the frequency of nondisjunction regardless of age in certain strains.

However, there is a paucity of information concerning possible prolonged effects of radiation on chromosome segregation.

The purpose of the present study is to investigate the possibility of a repair of the mechanism which leads to nondisjunction in mouse oocytes following irradiation. In other words does irradiation at a young age cause nondisjunction that persists and accumulates with age or is the damage repaired with time? The issue of possible delayed effects of radiation is becoming increasingly important with the variety of forms of radiation to which individuals are exposed.

## MATERIALS AND METHODS

### Mice

Virgin female C<sub>3</sub>H and male ICR/Swiss mice (Mus musculus, 2N=40), 4-5 weeks old, were purchased from Roswell Park, Buffalo. They were housed in the McMaster Medical Centre animal facilities with a 10 hour light, 14 hour dark (0800-1800 hr.) schedule. They were fed laboratory chow (Purina) and allowed to drink water ad libitum. When the mice were approximately 8 weeks of age mating cages were set up containing one male and one female. The mating cages were numbered in order to distinguish litters born to different mothers. From these litters only F<sub>1</sub> females were kept and the number recorded for each litter. They were weaned at 4 weeks of age and litter-mates placed together in a new cage. Offspring from different mothers were not combined. These F<sub>1</sub> females were used in the present experiments.

(C<sub>3</sub>HxICR/Swiss)F<sub>1</sub> were used because the incidence of nondisjunction in animals from this cross has been determined (Uchida and Lee, 1974; Uchida and Freeman, 1977). In addition, these mice are thought to be relatively resistant to the development of leukemia following irradiation.

### Experimental groups - sample size determination

Because the low frequency of spontaneous nondisjunction necessitated large sample sizes, it was decided to use 4 experimental groups:

Group I - Young controls (non-irradiated and sacrificed at 3 months)

Group II - Young irradiated (irradiated and sacrificed at 3 months)

Group III - Old controls (non-irradiated and sacrificed at 9 months)

Group IV - Old irradiated (irradiated at 3 months - sacrificed at 9 months)

Age three months was chosen for young mice because by that time they are sexually mature and some information is available on the spontaneous rate of nondisjunction in this age group. Age nine months was chosen for old mice because a reasonable number of oocytes can still be obtained at this age.

The approximate number of metaphase II oocytes to be analyzed in each group was based on estimated averages of 30 eggs per young mouse and 15 eggs per old mouse. The frequency of nondisjunction induced by radiation is 1% in young animals. If this frequency persists into later age the expected difference between groups III and IV should be of the order of 1%. The following formula was used to calculate the required sample size per group (Fleiss, 1973).

$$n = \frac{(C_{\alpha/2} \sqrt{2\bar{P}\bar{Q}} - C_{1-\beta} \sqrt{P_1 Q_1 + P_2 Q_2})^2}{(P_2 - P_1)^2}$$

Where  $n$  = sample size per group

$P_1$  and  $P_2$  = proportions in the two populations being compared

$$Q = 1 - P$$

$c$  = value of  $z$ , test statistic

$\alpha = 0.05$ , probability of saying a result is not significant when it is significant

$\beta = 0.2$ , probability of saying there is a significant difference when, in fact, there is not

Some estimated sample sizes for varying differences between two populations are:

% Difference	Sample size
1.0	2300
1.3	1500
1.5	1200
1.7	1000

Assuming that 50% of the oocytes placed in culture would mature and the chromosomes would be countable, the number of animals needed per group was estimated as 150 for young mice and 300 for old mice. However, in calculating the number of animals needed in each group initially no account was taken of the fact that radiation kills some cells. When the magnitude of this effect was known additional mice (approximately 30% more) were randomized to group IV to compensate for this decrease.

In general, sample sizes are usually overestimates for several reasons. First, the determination is based on estimates of expected differences observed from previous experience. Second, there is no clear

mathematical way to determine by how much the sample size should be reduced when more than two experimental groups are used.

#### Randomization

To control for a possible genetic effect on nondisjunction litter-mates were randomly assigned to the 4 experimental groups when the mice were 6-7 weeks of age. A computer program for this randomization was prepared with the help of Dr. G. Anderson, Professor of Clinical Epidemiology and Biostatistics. Only mice with the same litter number from mothers of the same age were randomized together. No more than 6 mice were placed in a cage after randomization.

#### Irradiation

Mice in groups II and IV were irradiated at three months of age. They were placed in wedge-shaped compartments of a plexiglass cage and exposed to a single dose of 20R whole-body gamma radiation from a  $^{137}\text{Cs}$  source at a dose rate of 29R/min with a focal distance of 51 cm at 23°C and atmospheric  $\text{O}_2$ . The dose of 20R was chosen in order to maximize the number of aneuploid oocytes while minimizing the number of radiation-induced chromosome breaks (Uchida and Lee, 1974; Uchida and Freeman, 1977). Control mice were handled in an identical manner but were not irradiated. A small V-shaped nick was made in the left ear of irradiated mice immediately following exposure. Mice in groups I and II were sacrificed within 2 days of the experimental procedure. Animals in the other groups were housed in animal quarters until they were 9 months old.



Procedures for obtaining and culturing oocytes and chromosome preparation

Mice were sacrificed by cervical dislocation. Irradiated and control mice were alternately sacrificed. The ovaries were quickly dissected free of fat and placed in an embryological watch glass containing approximately 1 mL of 1:1 TC Hanks:TC 199 culture medium (Difco). No gonadotrophic hormones were used to induce ovulation. The oocytes, liberated by disrupting the follicles with a fine (26 gauge) hypodermic needle, were manipulated with mouth-controlled micropipettes with a bore of approximately the same size as the oocytes (60-80 $\mu$ ). A Zeiss stereo-dissecting microscope at a magnification of 15-20X was used.

Cells in the dictyate stage, identified by the presence of a germinal vesicle (Fig. 1), were transferred to a watch glass containing 1 mL of fresh medium. Any remaining cumulus cells were removed by gently sucking the oocytes in and out of the micropipette and the oocytes transferred to a sterile watch glass containing 2 mL of fetal calf serum (Grand Island Biological) and incubated at 37°C in 5% CO<sub>2</sub>. No more than 20 oocytes were placed in any one culture dish. The maximum time span between killing the animal and placing the oocytes in culture medium was 30 minutes. The number of oocytes recovered from each mouse was recorded.

After 18-24 hours the cultures were removed from the incubator. The time in culture was recorded for oocytes from each mouse. Oocytes in which the germinal vesicle had disappeared (Fig. 2A) were selected for harvesting. A polar body was usually visible (Fig. 2B). The number

of oocytes not resuming meiosis in culture, that is, those that still had a germinal vesicle, was recorded. Oocytes to be harvested were rinsed in a watch glass with 2 mL of 1.1% sodium citrate, then transferred to another watch glass containing fresh sodium citrate for 20 minutes. Slides were prepared according to the method of Tarkowski (1966) as follows: no more than 5 oocytes were placed in a small amount of sodium citrate in the centre of a grease-free slide. Then, with the mouth-controlled micropipette, excess hypotonic medium was drawn off. Approximately 20  $\mu$ L of fixative, 3 methanol:1 glacial acetic acid, were then immediately dropped onto the oocyte and blown dry under the heat of a 100 watt light bulb. All slides were coded.

The slides were stained for 3 minutes in 2% Giemsa R66 in phosphate buffer pH 6.8 (G. T. Gurr), then rinsed in distilled water. To locate the cells the slides were scanned with a Zeiss photomicroscope (planapochromat 16X objective). The verniers were recorded of all cells originally placed on each slide and the stage in meiosis was noted. Mouse MI chromosomes are 20 paired bivalents held by chiasmata (Fig. 3A) and are easily distinguished from the 20 unpaired bivalents seen in MII (Fig. 3B.). After this preliminary examination the slides were destained and the oil removed by rinsing the slide in 70% ethanol and then in warm tap water.

The chromosomes were C-banded (Figs. 3A and B) to obtain more accurate chromosome counts. A modification of the method described by Arrighi and Hsu (1971) yielded consistently good C-bands. Slide preparations were aged for 1-2 weeks then placed in 1 N HCl for 1-1.5

minutes, rinsed in distilled water, transferred to 0.04M Ba(OH)<sub>2</sub> at 60°C for 4-4.5 minutes and rinsed again in distilled water. They were then placed in 2X SSC(SSC=0.15 M NaCl, 0.015 M Nacitrate) at 60°C for 2 hours. They were rinsed again in distilled water before being stained with 5% Giemsa R-66-phosphate buffer pH 6.8 for 5 minutes.

After a second rinse in fresh buffer, they were air-dried and ready for the final analysis. Chromosomes were counted under the 100X oil 1.3 N.A. objective. All cells that were difficult to count or were presumptive aneuploids were analyzed again by at least one independent observer. All other cells on the slide were accounted for before such a cell was recorded as aneuploid.

Photographs were taken of all cells with 21 chromosomes using Kodak high contrast copy film (ASA 64). Films were developed using Kodak D-19 developer and prints made on F-2 or F-3 Kodabromide paper using Dektol developer and Kodak rapid fixer..

The code (that is, the group the animal was from) was not broken until the aneuploid cell had been confirmed and photographed.

#### Statistical tests used

The following formula was used for comparing the differences in the number of oocytes obtained and the time they spent in culture:

$$z = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$

where z=test statistic

$\bar{x}$ =mean

$\sigma$ =standard deviation

n=number in sample

When comparing the number of cells that failed to mature, remained in MI or proceeded to MII, the Chi-square test was used.

The Fisher exact test was used to compare the nondisjunction frequencies between groups. This test is to be used when the smallest expected frequency is less than 5 and yields an exact probability for the observed results rather than an estimate (Armitage, 1971; Colton, 1974; consultation with Professor R. Milner and Dr. G. Anderson, Department of Biostatistics McMaster University.)

The following is a statement of the Fisher exact test.

$$p(\text{probability}) = \frac{r_1!r_2!S_1!S_2!}{N!a!b!c!d!}$$

where a, b, c, d equal individual entries in the 2X2 table and r and S are row and column totals respectively. The calculated probability is the total probability of all tables which are at least as extreme as those observed.

Figure 1: Photomicrograph of mouse oocyte approximately 70 $\mu$  in diameter.  
Arrow indicates germinal vesicle. (Phase contrast x1000).

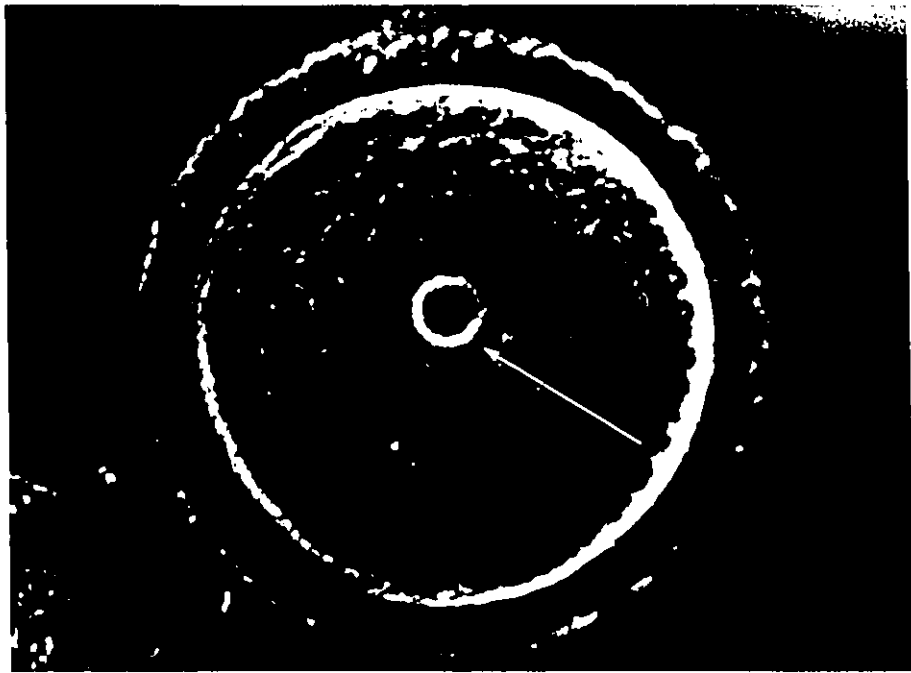


Figure 2A: Oocyte in vitro after 4-5 hours in culture. Note germinal vesicle is no longer present (x800).

- Figure 2B: Oocyte after 17-20 hours in culture. Note presence of first polar body, indicated by arrow (x1000).





Figure 3A: C-banded mouse MI chromosomes from an oocyte after 4-5 hours in culture (x2500).

Figure 3B: C-banded MII chromosomes from oocyte after extrusion of the first polar body (x3000).



## RESULTS

A total of 984 mice were randomized into the four experimental groups. Of these 10 died, 6 in group III and 4 in group IV. The remaining 974 mice were distributed among the groups as follows:

Group I	124
Group II	131
Group III	306
Group IV	413

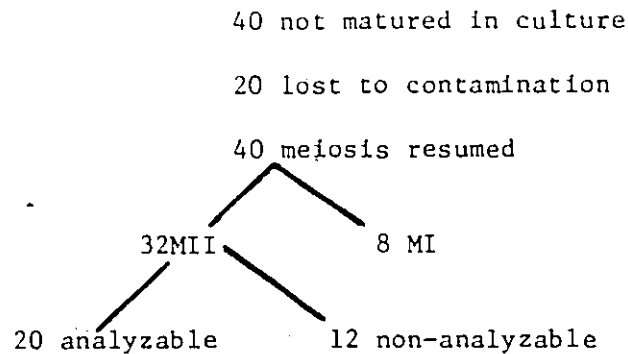
### Comparison of groups I and II (control and irradiated young mice)

A total of 4755 and 4392 oocytes were obtained from mice in groups I and II respectively (Table 3). Significantly more oocytes were obtained from control than irradiated young mice ( $p=0.003$ ).

In addition, the proportion of cells failing to mature even as far as MI was significantly greater for irradiated mice ( $p<0.0001$ ).

The number of cells that progressed to MI and MII are presented in Table 4. Of the cells which resumed meiosis, there was no difference between the two groups in the proportion of cells remaining in MI or proceeding to MII ( $p\cong 0.04$ ). Most of the cells that resumed meiosis proceeded to MII but only 64% and 62% of these could be analyzed in groups I and II respectively.

A number of oocytes were lost due to contamination. In groups I and II respectively, 1009 and 922 oocytes were lost. Given 100 oocytes the following are estimated proportions in each group:



Chromosome counts were obtained in 973 MII oocytes from group I and 762 in group II (Table 5). Only one diploid oocyte (40 chromosomes) was found among the oocytes of young irradiated animals. A cell was called diploid only if all other cells on the slide were accounted for.

One cell with a hyperhaploid number of chromosomes (21) was found in group I (Fig. 4). Six such cells were found in group II (Fig. 5). The cell shown in Fig. 5E has 22 chromosomes. Two pairs remained in the MI configuration (illustrated by arrows) which resulted in the two extra chromosomes in MII. In calculating the frequency of nondisjunction this was considered to be one event. The difference between these two groups is significant ( $p=0.03$ ), that is, there were more aneuploid cells in young irradiated animals than young controls.

During gamete formation nondisjunction results in hypo- and hyperhaploid cells. Among oocytes with less than 20 chromosomes it is not possible to distinguish between nondisjunction and chromosome loss during slide preparation. Approximately ten percent of cells had 19 chromosomes, a much higher frequency than the number of cells with 21 chromosomes. Assuming there is no preferential segregation of chromo-

Table 3: Comparison of the number of oocytes, time in culture and failure rate in culture of oocytes from animals in all groups of mice

	I	II	III	IV
Number of mice	124	131	306	413
Number of oocytes collected	4755	4392	4271	3419
Mean number of oocytes per mouse ( $\pm$ standard deviation)	37.9 $\pm$ 12.7	33.5 $\pm$ 10.7 <sup>a</sup>	14.0 $\pm$ 5.3	8.3 $\pm$ 5.6 <sup>d</sup>
Mean number of hours in culture	19.7 $\pm$ 1.1	19.9 $\pm$ 1.2 <sup>b</sup>	20.4 $\pm$ 1.5	20.4 $\pm$ 1.4 <sup>e</sup>
*Number of oocytes that failed to mature and/or degenerated	1842(49.1%)	1920(55.3%) <sup>c</sup>	1597(44.6%)	1198(42.5%) <sup>f</sup>
Failure rate per mouse	15.0 $\pm$ 10.0	14.6 $\pm$ 8.3	5.3 $\pm$ 3.7	3.1 $\pm$ 3.0

<sup>a</sup>Significant,  $p=0.003$ ,  $z=3.0$

<sup>d</sup>Significant,  $p=0.003$ ,  $z=13.9$ , 1 d.f.

<sup>b</sup>Not significant,  $p=0.09$ ,  $z=1.7$

<sup>e</sup>Not significant,  $p=1.0$ ,  $z=0$

<sup>c</sup>Significant,  $p<0.0001$ ,  $X^2=27.5$ , 1 d.f.

<sup>f</sup>Not significant,  $\chi^2=3.2$ , 1 d.f.

\*When comparing the number of cells that failed in culture the number of cells lost to contamination and during slide preparation was omitted from the total number of oocytes considered.

Table 4: Analysis of the meiotic characteristics of the oocytes from all groups of mice

	I	II	III	IV
Number of cells remaining in MI	385(20.2%)	325(21.0%)	336(17.0%)	310(19.2%)
Number of cells proceeding to MII	1519(79.8%)	1225(79.0%)	1646(83.0%)	1301(80.8%)
Number of analyzable MII cells	973(64.0%)	762(62.2%)	899(54.6%)	796(61.2%)
Mean number of analyzable cells per mouse	7.8	5.8	2.9	1.9

Table 5: Chromosome counts for analyzable MII cells in all groups

Group	<19	19	20	21	40	Total
I	166(17.1%)	109(11.2%)	697(71.6%)	1(0.1%)	0(0.0%)	973
II	77(10.1%)	68(8.9%)	610(80.0%)	6(0.8%)	1(0.1%)	762
III	98(10.9%)	92(10.2%)	699(77.7%)	5(0.6%)	5(0.6%)	899
IV	63(7.9%)	54(6.8%)	676(84.9%)	0(0.0%)	3(0.4%)	796

Figure 4: Cell with 21 chromosomes from group I (x4000).



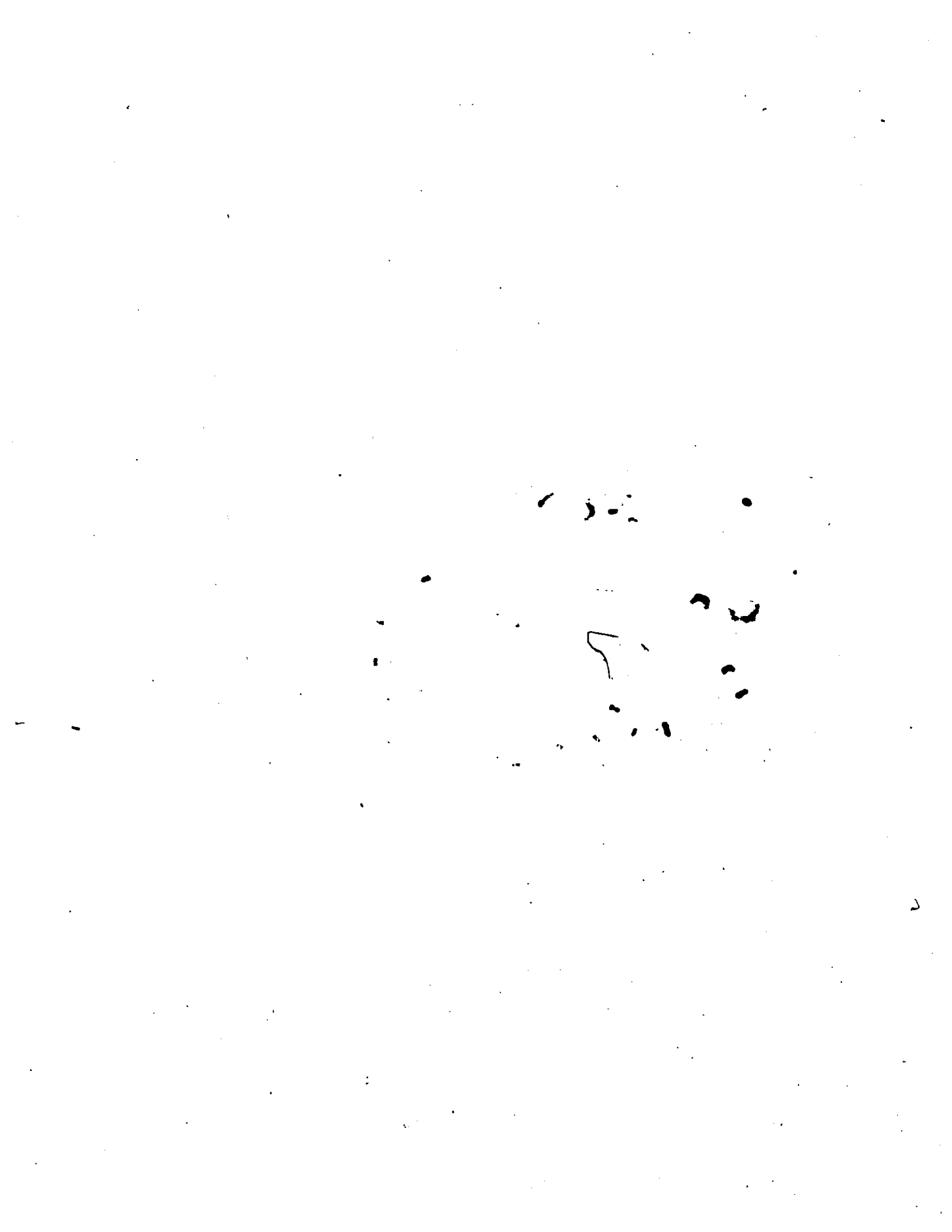


Figure 5: Six cells with 21 chromosomes from group II (x3500).  
(A - F)



A



B

C

D

W



E

F

somes into the polar body, an estimate of the total frequency of non-disjunction can be obtained by doubling the number of cells found with 21 chromosomes. This method of doubling the number of hyperploid cells to obtain the frequency of nondisjunction is an accepted practice in the field of cytogenetics (Rohrborn, 1972; Golbus, 1981). Thus, the estimated frequency of nondisjunction in group I is 2/973 (0.2%) and in group II 12/762 (1.6%), a highly significant difference,  $p=0.001$  (Fisher exact test). It is apparent that radiation increases the frequency of nondisjunction in the oocytes of young mice.

#### Comparison of groups III and IV (control and irradiated old mice)

A total of 4271 and 3419 oocytes were obtained from mice in groups III and IV respectively; an average of 14 oocytes from old control animals and 8 from irradiated-aged animals (Table 3). This difference is highly significant ( $p=0.003$ ).

The proportion of oocytes failing to proceed to MI was the same in both groups ( $p\approx 0.08$ ). There was also no difference in the proportion of cells resuming meiosis ( $p\approx 0.08$ ). The number of hours spent in culture (Table 3) was the same for these two groups. The total number of oocytes lost was 692 and 600 for groups III and IV respectively.

Table 5 presents chromosome counts for the analyzable cells in groups III and IV. There was no difference in the number of diploid oocytes found, 5 in group III and 3 in group IV.

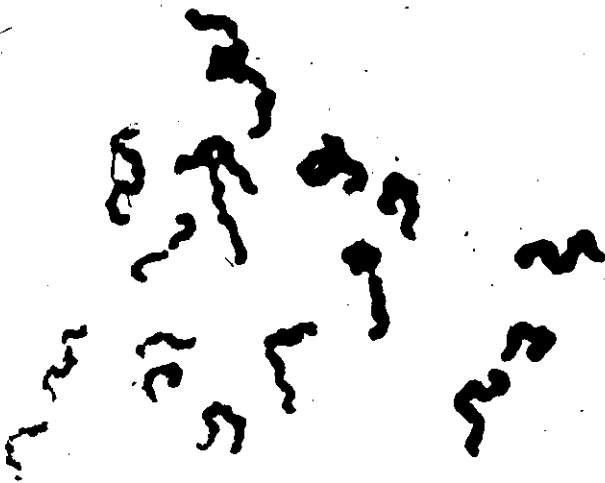
There were 5 hyperhaploid cells in group III, a frequency of 0.6%. Photographs of 4 of these cells are shown in Fig. 6. One cell with 21

Figure 6: Four cells with 21 chromosomes from group III (x2500).  
(A - D)

Li



A



B





C

D

chromosomes was destroyed by a microscope objective after the chromosome count was confirmed but before it was photographed. No cells with 21 chromosomes were found in group IV, in fact, not even the control level of 0.6%. The difference between the groups is significant at the  $p=0.04$  level, using the Fisher exact test. Thus, there were significantly more aneuploid oocytes in old control mice than irradiated-aged animals. When the numbers of hyperhaploid cells were doubled to obtain a more accurate estimate of the frequency of meiotic error this difference becomes highly significant ( $p=0.002$ ).

#### Comparison of young and old animals

Significantly fewer oocytes were obtained from old compared to young mice in both control and irradiated groups ( $p<0.0001$  for both), but the percentage decrease in the mean number of oocytes with age was greater for irradiated than control mice (77% versus 63%).

A number of cells placed in culture failed to resume meiosis or degenerated. Significantly more oocytes failed to mature from young animals than old in both control and irradiated groups ( $p<0.0001$  for both), that is, more cells from old animals resumed meiosis. Of those cells that did resume meiosis there was no significant difference in the proportion of cells in MI or MII between irradiated young and aged animals. However, significantly more cells remained in MI from young than aged controls..

The time spent in culture, although statistically greater for oocytes from older animals, was a matter of .45 to .7 hours and resulted from the random timing of cell harvesting.

The overall frequency of diploid oocytes was low in all groups (0.3%). Between control and irradiated animals of the same age there was no significant difference in the number of diploid oocytes, however more were found in old mice than young.

Using the raw data there was no significant increase in the frequency of nondisjunction with age in non-irradiated mice ( $p=0.09$ ). However when the number of cells with 21 chromosomes was doubled to account for those with 19 chromosomes, significance was attained ( $p=0.01$ ).

Comparison of the frequencies of nondisjunction between irradiated groups II (0.8%) and IV (0%) also yielded a significant result ( $p=0.01$ ), which was even more significant when the numbers were doubled ( $p=0.0002$ ). Thus the frequency of nondisjunction in young irradiated mice was significantly greater than for mice that were irradiated when they were young and then aged.

All hyperhaploid oocytes in all groups were from different progeny and mothers. In general, the ages of the mothers and the litter number varied. They were not confined to one time of treatment or season but were spread over a period of  $3\frac{1}{2}$  years. Because individual chromosomes cannot be identified in meiotic preparations it is not possible to tell if there are particular chromosomes that are prone to undergo nondisjunction.

There was no difference in the number of cells found with 19 chromosomes when comparing groups of control ( $\chi^2=.59$ , 1 d.f.) or irradiated mice ( $\chi^2=2.62$ ). Oocytes from young animals exhibited no significant

difference in the number of cells with 19 chromosomes ( $\chi^2=2.5$ ). However, there were significantly fewer cells with 19 chromosomes from irradiated-aged than control aged mice ( $\chi^2=6.56$ ,  $.01 < p < .02$ ).

The Chi-square test was also used to compare the number of cells with <19 chromosomes between groups (Table 5). This group of cells is usually considered to be due to chromosome scatter during slide preparation. There is a significant increase in the frequency of such cells from young control compared to aged control ( $\chi^2=14.9$ ,  $p < 0.001$ ). The same is not true when young irradiated and irradiated-aged animals are compared ( $\chi^2=2.3$ ,  $.1 < p < .2$ ). When both age groups are examined, radiation was associated with significantly fewer cells with less than 19 chromosomes (young-  $\chi^2=19.2$ ,  $p < .001$ ; aged-  $\chi^2=5.13$ ,  $.02 < p < .05$ ).

## DISCUSSION

Because aneuploidy is a major cause of reproductive loss in humans and an economic burden to society, much attention has been focused on etiology. That radiation can cause nondisjunction was first observed by Mavor (1922) in Drosophila and has since been extensively studied in mice. Results from epidemiological studies suggest that radiation also has a small but significant effect in humans. The present study was designed to test experimentally the possibility of repair of the mechanism which causes nondisjunction after cells are irradiated.

### Lethal effect of radiation

In designing the experiment it was estimated that an average of 30 oocytes would be obtained from young mice and 15 from the old. These estimates were very close to the numbers actually obtained from both young and old control and young irradiated mice. However, significantly fewer oocytes were recovered from irradiated-aged mice. Jones and Krohn (1961) suggested that the decrease in the number of oocytes in both groups of older animals is probably the result of necrosis and ovulation.

In sexually mature animals the response to radiation depends on the stage of follicular growth since all oocytes are in the dictyotene stage. Small follicles are very sensitive to radiation (Peters and

Levy, 1964). Higher doses of radiation are required to kill growing follicles (Mandl and Zuckerman, 1956). It has been shown that there is no arrest of follicular development by radiation (Oakberg, 1979). It follows therefore that in aged mice the oocytes recovered from mature Graafian follicles must have been those that survived radiation when they were in small non-growing follicles.

Relatively more mouse oocytes are killed by radiation than are human oocytes. Baker (1971) calculated that a dose of 50R to adult mice would kill all oocytes. In cultured human fetal ovaries a dose of 2000-5000R was required to kill all oocytes (Baker, 1969; Baker and Neal, 1969), thus making human oocytes among the most radio-resistant of those species so far examined.

The exact mechanism of radiation-induced cell death is not known although several factors are probably involved. Cell death from radiation is thought to depend in part on metabolic activity of oocytes since those in mature follicles are the least active in nuclear RNA synthesis (Oakberg, 1968; Moore et al., 1974) and the most resistant to cell death (Mandl and Zuckerman, 1956).

Chromatin conformation may also determine radiosensitivity. Dictyotene chromosomes in arrested human oocytes have a lampbrush-type configuration (Baker, 1963; Baker and Franchi, 1967) and are extremely radio-resistant (Baker, 1969). In mouse oocytes dictyate chromatin is highly diffuse until the development of the antrum in follicles. Chromatin then becomes more compact and takes on the lampbrush appearance (Chouinard, 1975).

### Cytogenetic analysis of oocytes

There are two types of numerical chromosome abnormalities, aneuploidy and polyploidy. Polyploidy results from diploid gametes.

The overall incidence of diploid oocytes in the present study was 0.3% and most diploid cells (8/9) were from old females. This contrasts with the frequency of 1.7% diploid oocytes found in CBA mice which was not related to maternal age (Martin et al., 1976). There are no comparable data on the incidence of diploid oocytes in (C<sub>3</sub>HxICR/Swiss)F<sub>1</sub> females.

Diploid oocytes in MII probably arise by failure of extrusion of the first polar body. Fertilization of a diploid oocyte would result in a triploid zygote. The incidence of triploidy ranges from 1%-4% in mouse fetuses depending on the strain and does not increase with age (Gosden, 1973; Yamamoto et al., 1973; Max, 1977). The low incidence of diploid oocytes in MII indicates that failure of formation of the first polar body is a relatively infrequent event.

Aneuploidy, usually the result of nondisjunction, is the production of gametes with more or less than the haploid number of chromosomes. The excess of hypohaploid compared to hyperhaploid cells has been frequently observed (Rohrborn, 1972; Hansmann, 1974; Uchida and Lee, 1974; Uchida and Freeman, 1977). Some cells with 19 chromosomes probably result from chromosome loss during slide preparation. To obtain a more accurate frequency of aneuploidy the number of cells with 21 chromosomes is doubled. This method is based on the assumption that there is no preferential segregation of cells with 19 or 21 chromo-

somes into the polar body. Recently Maudlin and Fraser (1977) provided experimental evidence to support this assumption. They used the technique developed by Donahue (1972) to study the chromosomes of fertilized ova at the pronuclei stage before the first cleavage division. Since the male pronucleus is larger and the chromosomes less condensed than those in the female pronucleus the chromosomal contribution from each parent can be identified. In TO mice there was a close correlation between the total number of monosomies and trisomies of maternal origin with an overall incidence of aneuploidy of 1-2% (Maudlin and Fraser, 1977). Eggs from 4 other mouse strains fertilized with TO sperm (Maudlin and Fraser, 1978; Fraser and Maudlin, 1979) showed a slight excess of monosomies but the difference was not marked.

The above research provides some evidence to support the doubling of cells with 21 chromosomes to obtain the nondisjunction frequency. In the present study there was little variation between groups in the number of cells with 19 chromosomes except for the significant decrease in irradiated-aged than control-aged animals. In addition there appeared to be an association between radiation and decreased numbers of cells with fewer than 19 chromosomes, suggesting that cells which have been exposed to radiation are less apt to become disrupted during slide preparation. This finding has not been previously reported in studies of radiation effects on nondisjunction. The mechanism involved in this phenomenon is pure speculation. One possible explanation would be that through molecular changes, for example protein cross linking, the radiation somehow stabilizes cell membranes making them less susceptible to traumatic



damage. Alternatively, since radiation causes a significant amount of oocyte death perhaps more fragile cells are destroyed more easily.

The interaction of such unknown factors in the production of hypohaploid cells lends added justification to the use of only those cells which are hyperhaploid in the calculation of the frequency of nondisjunction.

#### Effect of age on the frequency of nondisjunction

Our frequency of spontaneous nondisjunction in the oocytes of young females (0.2%) is comparable to that observed previously in the same strain (Uchida and Lee, 1974). Similar low frequencies have been reported in the oocytes of other strains including NMRI (Becker and Schoneich, 1974; Hansmann and Probeck, 1979; Hansmann *et al.*, 1982) and CBA (Martin *et al.*, 1976). A higher frequency (4.8%) was found in C<sub>3</sub>H females (Rohrborn, 1972; Hansmann, 1974). The frequency of nondisjunction in young 101xC<sub>3</sub>H females was originally reported to be 12.4% (Rohrborn and Hansmann, 1971; Rohrborn, 1972) but this decreased to less than 1% when more oocytes were studied (Hansmann and El-Nahass, 1979).

Less than 0.05% of liveborn mice are aneuploid since most trisomies are lethal very early in fetal development (Goodlin, 1965; Lyon, 1966; Gropp *et al.*, 1975). From studies of zygotes it has been estimated that approximately 2% are aneuploid (Maudlin and Fraser, 1978). Thus, the study of chromosomes of newborn animals does not reveal the true incidence of meiotic error.

The frequency of nondisjunction increased significantly from 0.2% to 1.2% as mice aged from 3 to 9 months. Uchida and Freeman (1977)

reported an insignificant increase from 0% at 3-6 months to 0.6% at 12 months. This slightly greater frequency of nondisjunction at 9 months compared to 12 months is not statistically significant. Martin et al. (1976) reported a higher frequency of nondisjunction in middle-aged CBA females compared to old, however their middle-aged animals were 5-8 months old and comparable to the old animals in the present study.

One explanation for the increase in aneuploidy with maternal age relates to chiasma frequencies. Oocytes from aged female mice of several strains have been shown to have decreased numbers and more terminal location of chiasmata than young females (Henderson and Edwards, 1968; Luthardt et al., 1973; Polani and Jagiello, 1976; Speed, 1977; Jagiello and Fang, 1979). Reduction in number of chiasmata may lead to the formation of univalents followed by an increase in aneuploidy. DeBoer and Van Der Hoeven (1980) did not find a decrease in chiasmata with age but they did find a parallel between univalent frequency and frequency of nondisjunction in metaphase II oocytes.

There are two possible explanations for the decrease of chiasmata: either chiasmata prematurely terminalize during the prolonged dictyotene stage or the reduced number of chiasmata may result from decreased recombination in certain cells. There is some evidence in favour of the latter suggestion. Offspring of older female mice were found to have reduced recombination between two pairs of loci (Bodmer, 1961; Reid and Parsons, 1963). However, recombination between more pairs of loci needs to be studied.

In an attempt to explain this phenomenon of decreased chiasmata

formation with age Henderson and Edwards (1968) proposed a "production line" hypothesis: there is a gradient in chiasma formation with those cells that enter meiosis first having the most chiasmata. The oocytes with a reduced number of chiasmata would be ovulated later in life. Jagiello and Fang (1979) found that chiasmata in oocytes of 16 day mouse fetuses were significantly more frequent than in oocytes on fetal day 18 or one or nine months postnatally which also supports the idea of a gradient in chiasma formation. Greater contraction at the time of crossing over could interfere with chiasma formation. Bivalents in oocytes of old mice have been shown to be more contracted than those from young females (Speed, 1977; DeBoer, and Van Der Hoeven, 1980).

In humans the effect of aging on chromosome segregation may relate to the persistence of the nucleolus. Evans (1967) suggested that with increasing time spent in dictyotene there is a greater chance that nucleoli will not be broken down in diakinesis. As a result the acrocentric chromosomes which contain nucleolus organizing regions would be expected to have an increased risk of abnormal segregation. In fact, trisomies of the acrocentric chromosomes account for 40% of lethal human trisomies (Boué and Boué, 1977). In an attempt to evaluate experimentally whether or not irradiation could have an effect on the chromosomes involved in nucleolus organization, Stenstrand (1978) X-irradiated human lymphocytes and studied satellite associations. With 20R-50R she found an increase in the number of associations but because control frequencies are not given the data are difficult to interpret. Mirre et al. (1980) studied nucleoli in both human and mouse oocytes using electron microscopy. In human oocytes several bivalents can be joined by a bridge of material around the same

nucleolus. The persistence of this material which normally is degraded enzymatically prior to metaphase could lead to nondisjunction. In mouse oocytes the nucleolar organizers are not associated so this explanation may be valid in humans but not in mice.

#### Effect of radiation on chromosome segregation

Radiation caused a significant increase in nondisjunction in the oocytes of young mice within one day of exposure (1.6% compared with 0.2% in controls). This increase is similar to that reported by Uchida and Lee (1974). Oocytes of mice irradiated one day before they were sacrificed were from mature follicles. Thus, these oocytes were irradiated when the chromatin was in the lampbrush stage (Chouinard, 1975) comparable to that found in humans (Baker and Franchi, 1967).

Radiation-induced nondisjunction is not dose-dependent in (C<sub>3</sub>HxICR/Swiss)F<sub>1</sub> females (Uchida and Lee, 1974; Uchida and Freeman, 1977) or in NMRI females (Reichert *et al.*, 1975; Hansmann *et al.*, 1982). However, Tease (1982) described a linear relationship between radiation dose and the frequency of nondisjunction in pronuclei in strain 3H1 females. The mice in this experiment were irradiated when the oocytes were in diakinesis, a very radiation-sensitive stage (Edwards and Searle, 1963).

It is known that radiation excites molecules by imparting energy to them leading to ionization or free radical formation (Andrews, 1974). This can result in damage to the DNA, either single-stranded breaks or base changes that result in mutation or damage to other cellular molecules (Setlow and Setlow, 1972).

Nondisjunction caused by radiation is probably not due to a point mutation since the spontaneous mutation rate is many times lower than the spontaneous rate of nondisjunction (Russell, 1972). There could be a cluster of genes for chromosome segregation and mutation of any one of them results in nondisjunction. However, the meiotic genes which cause nondisjunction in Drosophila exert their effect through decreased recombination (Baker et al., 1976; Boyd et al., 1976). Recombination in mammals occurs early in meiotic prophase before dictyotene and long before exposure to radiation in the present experiment.

Agents that damage the meiotic spindle such as colchicine are capable of inducing nondisjunction in Chinese hamster oocytes when used in low doses (Sugawara and Mikamo, 1980). In an in vitro study a dose of 50,000R gamma rays was found to have an effect on tubulin polymerization but this may have little to do with the in vivo situation (Zaremba and Irwin, 1977). Spindle microtubules do not form until the time of germinal vesicle breakdown (Calarco et al., 1972). In the in vitro situation the spindle does not occur until the oocytes are placed in culture. A precursor of spindle formation would have to be damaged by an in vivo exposure to radiation. Alternatively radiation may have an effect on the centromere region of the chromosomes (Grell et al., 1966) which may cause improper attachment to the spindle microtubules. Thus, the mechanism by which radiation causes nondisjunction may involve the centromere, spindle proteins or other cellular molecules necessary for normal chromosome disjunction.

Effect of time on nondisjunction following radiation

It is now well-documented at least in  $(C_3H \times ICR/Swiss)F_x$  female mice that radiation causes an increased frequency of aneuploid gametes. This effect is greater when older females are irradiated (Uchida and Freeman, 1977). The question posed in the present study concerns the possibility of a repair of the mechanism which leads to nondisjunction. As expected nondisjunction was demonstrated in the non-irradiated aged females but the unexpected absence of any aneuploid oocytes from females that were aged after irradiation requires explanation.

Other experiments have dealt specifically with the question of prolonged effects of radiation on aneuploid frequency. When Max (1977) irradiated CBA mice at 6 weeks of age with doses from 2-16R and then mated them at 32-35 weeks only two aneuploids were found among 289 fetuses compared to 0/78 from control mothers. In two other studies Q strain females were irradiated with 5R X-rays at 8 weeks of age and then mated at 9 months (Chandley and Speed, 1979; Speed and Chandley, 1981). There was no difference in the number of aneuploid fetuses from aged control or irradiated then aged females. Possible repair mechanisms were not studied.

In the present study older animals were irradiated 6 months before they were sacrificed. Thus, oocytes recovered from these animals were irradiated in the small follicle stages. The absence of nondisjunctional events in these oocytes is in direct contrast to oocytes recovered from irradiated young animals whose oocytes were in large follicles when irradiated. Oocytes in small follicles may be

resistant to the induction of nondisjunction by radiation. However, if this is the complete explanation all older mice, whether irradiated or not, should have the same frequency of nondisjunction as the result of the aging process.

Since chromosomes synapse before reaching dictyotene, exposure to radiation would not cause asynapsis. Rather the effect would be upon processes following dictyotene. Radiation could act by causing premature or accelerated terminalization of chiasmata. The immediate effect would result in nondisjunction of smaller chromosomes. However with time enough bivalents may prematurely separate to cause cell death. this would explain the absence of aneuploid oocytes from irradiated-aged animals. In support of this mechanism Wennstrom (1971) found that autosomal bivalents in male NMRI mice seemed to separate and undergo desynapsis as a result of irradiation.

Another explanation for the present results is that radiation damage to the centromere region could lead to abnormal chromosome segregation (Grell *et al.*, 1966). This damage might then be repaired over time. There are two factors against this explanation. If such a mechanism were involved it would have to be in effect after 48 hours since before this time irradiated oocytes had significantly higher frequencies of nondisjunction than controls. Mouse oocytes have been shown to undergo some form of DNA repair after irradiation (Masui and Pedersen, 1975; Pedersen and Mangia, 1978). However in both of these experiments DNA repair was studied two hours after irradiation. The other argument against this explanation is that irradiated-aged animals would be expected to have control levels of nondisjunction.

The results in this study were unexpected and it is difficult to conceive of any simple explanation for the observations. These results are in contrast to those in humans of Alberman et al., (1972). They reported an increase in aneuploidy among children born ten years or more after mothers had been exposed to radiation. There is evidence that aneuploidy can be induced in mice and humans by radiation however the disappearance of abnormal oocytes with time indicates that the response to radiation in mice may be different from humans. Care must therefore be exercised in extrapolating results from genetic experiments from mice to humans. There is great concern about the effects of human exposure to radiation from both medical and nuclear sources and mice are still a useful experimental model for investigating basic mechanisms in radiation biology.



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