CHROMOSOME ABERRATIONS IN MOUSE SPERMATOCYTES AND OOCYTES EXPOSED TO 300R γ-RADIATION: A COMPARISON

CHROMOSOME ABERRATIONS IN MOUSE SPERMATOCYTES AND OOCYTES EXPOSED TO 300R γ-RADIATION: A COMPARISON

By

WILLIAM SHIGERU TSUCHIDA, B.Sc.

A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Master of Science

McMaster University

September, 1973

MASTER OF SCIENCE
Medical Sciences

MCMASTER UNIVERSITY
Hamilton, Ontario

TITLE: Chromosome Aberrations in Mouse Spermatocytes and Oocytes Exposed to 300R γ-Radiation: A Comparison

AUTHOR: William Shigeru Tsuchida, B.Sc.

SUPERVISOR: Dr. Irene A. Uchida, Professor of Pediatrics

NUMBER OF PAGES: vii - 35

SCOPE AND CONTENTS: It has been repeatedly demonstrated that radiation induces chromosome aberrations in mouse oocytes and spermatocytes. However, the results of previous studies, in which the frequency of aberrations recovered from irradiated oocytes and spermatocytes were compared, are conflicting (reviewed by L. B. Russell, 1962; L. B. Russell, 1968). The development of new techniques for making chromosome preparations from oocytes (Edwards, 1965; Tarkowski, 1966) and spermatocytes (Evans et al., 1964) has made it possible to reinvestigate the radiosensitivities of spermatocytes and oocytes.

In the present study, the frequency of chromosome aberrations recovered from irradiated dictyotene oocytes was compared to the frequencies of aberrations recovered from irradiated pachytene and diplotene spermatocytes.

Oocytes and spermatocytes were collected from mature mice one day and five days after exposure to a single acute dose of 300R γ-radiation. At the time of irradiation, all of

the oocytes were in dictyotene while the spermatocytes collected one day and five days post-irradiation were in diplotene and pachytene, respectively.

The average number of oocytes collected from irradiated mice was no different from the average number collected from controls. Although the ability of oocytes collected one day post-irradiation to mature in vitro (58.0%) was not affected, significantly fewer (53.6%) of the oocytes cultured five days post-irradiation matured in vitro. Since the frequency of abnormal cells was the same in oocytes cultured one day and five days post-irradiation, it is unlikely that oocytes with chromosome aberrations were selectively inhibited from maturing in vitro.

The frequencies of chromosome aberrations in dictyate oocytes cultured one day and five days after irradiation were not significantly different from the frequency of aberrations in diplotene spermatocytes (20.7%). However, significantly more chromosome aberrations (32.0%) were recovered from irradiated pachytene spermatocytes than from either dictyate oocytes or diplotene spermatocytes. Some variation in the relative frequencies of aberrations was found.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

My deepest appreciation is extended to Dr. Irene A. Uchida for suggesting the area of research and for her invaluable assistance and guidance throughout the course of this investigation.

I would also like to thank Dr. C. C. Lin for his excellent technical assistance and encouragement during this study.

I am very grateful to Mrs. Jane Vesentin for typing this thesis.

Financial support for this research was supplied by the Medical Research Council.

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Beginning with the pioneer studies of Snell, Hertwig and Brenneke, an extensive body of publications has emerged concerning the cytogenetic effects of radiation on the germ cells of mice and, to a lesser extent, other mammals. This work has been reviewed by W. L. Russell (1954); W. L. Russell, Russell and Oakberg (1958); L. B. Russell (1962); Mandl (1964); Oakberg (1968) and Leonard (1972).

Most of these experiments used indirect methods of ascertaining chromosome aberrations. Mice were exposed to radiation and then mated after an appropriate amount of time had passed permitting cells, exposed at a particular stage, to develop into mature germ cells. Chromosome damage was detected as dominant lethals, partial sterility or sex chromosome anomalies (detected by genetic markers) among the conceptuses or progeny.

The radiosensitivity of chromosomes to breakage from a single, acute dose of X- or γ-radiation was found to be dependent upon the gametogenic stage at which they were irradiated. For a given dose of radiation, the frequencies of recovered chromosome aberrations were highest in spermatids and lowest in spermatogonia while spermatozoa and spermatocytes yielded about the same frequencies.

The chromosomes of oocytes in diakinesis/metaphase I are much more sensitive to radiation than those of oocytes in dictyotene - a modified diplotene stage in which oocytes remain from shortly after birth to just prior to ovulation (L. B. Russell and Spears, 1954; L. B. Russell and Russell, 1954: L. B. Russell, 1956). The radiosensitivity of chromosomes in pre-dictyate oocytes is difficult to determine but it appears, from preliminary results, that they are less sensitive than those of dictyate cells (L. B. Russell, 1968).

The results of previous studies, in which the frequencies of radiation-induced chromosome aberrations in spermatocytes and oocytes were compared, are conflicting. Dominant lethal studies have shown that dictyate oocytes are as sensitive as spermatocytes (L. B. Russell and Major, 1953; Bateman, 1958). L. B. Russell (1968), however, found that irradiated oocytes have a greater tendency to lose sex chromosomes than irradiated spermatocytes. In contrast, it is easier to induce translocations in spermatocytes than in dictyate oocytes (L. B. Russell and Wickham, 1957; Leonard and DeKnudt, 1968).

The development of new techniques for making meiotic chromosome preparations has made it possible to reinvestigate and compare the radiosensitivities of spermatocytes and oocytes.

An air-drying technique, developed by Evans et al. (1964) for obtaining meiotic chromosome preparations from mammalian testes, has been used extensively to study radiation-induced translocations in the spermatogonia of mice and other rodents (Ford et al, 1969; Leonard, 1971; Searle et al, 1969; Evans et al, 1970). The effectiveness of the treatments was estimated from the frequency of multivalents in spermatocytes derived from irradiated spermatogonia.

Wennstrom (1971) used the same technique to study the effects of X-irradiation on spermatocytes as well as on spermatogonia. Since the duration of each stage of spermatogenesis is constant (Table 1), all cells at a given stage reach metaphase I synchronously (Oakberg, 1956). Because radiation does not alter this timing (Oakberg and DiMinno, 1960) the radiosensitivity of each stage can be estimated by scoring the frequency of abnormal metaphases at various times after irradiation. Thus, by sacrificing mice at one, three and seven days post-irradiation, Wennstrom was able to estimate the frequencies of chromosome aberrations induced in diplotene and early and late pachytene.

Parallel studies on oocytes have not been done because of technical difficulties in obtaining sufficient numbers of cells at appropriate stages for analysis. However,

TABLE 1

Duration of meiotic stages in the male mouse (Oakberg, 1957).

Meiotic stage	Duration (hours)
Preleptotene	31.0
Leptotene	31.2
Zygotene	37.5
Pachytene	175.3
Diplotene	21.4
Diakinesis + Metaphase I	10.4
Secondary spermatocytes	10.4

with the development of recent techniques for culturing oocytes in vitro (Edwards, 1965; Evans, 1969; Chandley, 1971; Donahue, 1968, 1970) and fixing and mounting them on slides (Tarkowski, 1966), it is now possible to obtain adequate numbers of meiotic cells for such studies. These techniques have been used in cytogenetic studies of normal (Donahue, 1968) and abnormal (Henderson and Edwards, 1968; Donahue, 1970) oogenesis. Although they have also been used to study the mutagenic effects of certain drugs (Jagiello, 1967; Jagiello and Lin, 1973; Jagiello and Polani, 1969), they have not previously been applied in studies of the mutagenic effects of radiation.

Investigations of the effects of radiation on the meiotic chromosomes of mature mice must be limited to oocyte stages between dictyotene and anaphase II. Of these stages, dictyotene is the most accessible to study and, because of its duration, is the most likely to be affected by environmental insults such as radiation.

Although there is no stage of meiosis in males that is strictly comparable to dictyotene, it is of some value to compare the radiosensitivity of pachytene and diplotene spermatocytes with that of primary oocytes. Pachytene is by far the longest meiotic stage in the male, being longer in duration than the rest of the meiotic stages combined (Table 1).

As a result, it is the most likely stage to be exposed to acute radiation and would accumulate the highest radiation dose from chronic exposures. However, diplotene in the male is the most comparable, chronologically, to dictyotene.

The purpose of the present cytological study is to compare the radiosensitivity of primary oocytes with that of mid-pachytene and diplotene spermatocytes.

MATERIALS AND METHODS

Mice of the ICR/Swiss strain were used. A plexiglass cage with pie-shaped divisions held the mice while they were exposed to 300R from a Cs^{137} source (29 R/min.). This dose was chosen because it produced an appreciable frequency of chromosomal aberrations without causing a high incidence of lethality. Fourteen males and 41 females were irradiated between the ages of two and three months. After irradiation, the mice were caged in groups of five or six and kept under identical conditions. Half the mice were sacrificed at one day and the rest at five days post-irradiation.

Females were sacrificed at these time intervals to evaluate any changes in the frequency of chromosomally aberrant oocytes over the period of the experiment. Although all oocytes were at dictyotene, selective ovulation, degeneration or differences in radiosensitivity due to the stage of follicular maturation could have resulted in changes in the frequency of abnormal oocytes with time.

Ten male and sixteen female control mice were handled in the same manner, except for the actual irradiation, and housed under the same conditions as the experimental mice. Half were sacrificed on the same days as the irradiated mice. Although fewer animals were used in the control group, a

sufficient number of cells were obtained to establish the frequency of spontaneous aberrations in both sexes. Because of the low number of analyzable cells recovered per female, many more females than males were required. All mice were sacrificed by cervical dislocation and meiotic preparations were made as described below.

COLLECTION AND PREPARATION OF OOCYTES

The following technique is modified after the method of Evans (1969). After the mice were killed, their ovaries were removed and placed in embryological watch glasses under 2-4 ml of a 1:1 mixture of Hanks solution and TC 199 medium. Two fine needles were used to shred the ovaries and release the oocytes from their follicles. A dissecting microscope was used to find the oocytes. The oocytes were cleaned by repeated pipetting through a finely drawn glass pipette and transferred to a dish of fresh medium. They were then washed once in fetal calf serum and transferred to a fresh dish of serum for culturing. Only oocytes containing germinal vesicles were cultured. They were incubated for 5 hours at 37°C in a 5% CO_2 environment.

At the end of the culture period, the oocytes were transferred from the culture medium to a dish of hypotonic solution (1% sodium citrate). They were examined and those that

had not matured were discarded. Oocytes that resumed meiosis or "matured" could be distinguished morphologically because they were round with a fairly uniform cytoplasm and had no germinal vesicle (Fig. 1). The absence of the germinal vesicle is due to breakdown of the nuclear membrane as the oocytes proceed from the dictyate stage to diakinesis (Edwards and Gates, 1959).

Slides were prepared after the oocytes had been treated for 20 minutes with the hypotonic solution. No more than five oocytes were placed on a clean, dry microscope slide. Excess hypotonic solution was drawn off leaving just enough to prevent the oocytes from drying out. A little fixative (3:1 absolute methanol and glacial acetic acid) was dropped on the oocytes and dried quickly by blowing on the slide under the warmth of a 25 watt light bulb. The cells were stained with carbol-fuchsin (Carr and Walker, 1961) or giemsa. The chromosomes were scored at metaphase I and photographs were taken with a Zeiss photomicroscope.

PREPARATION OF SPERMATOCYTES

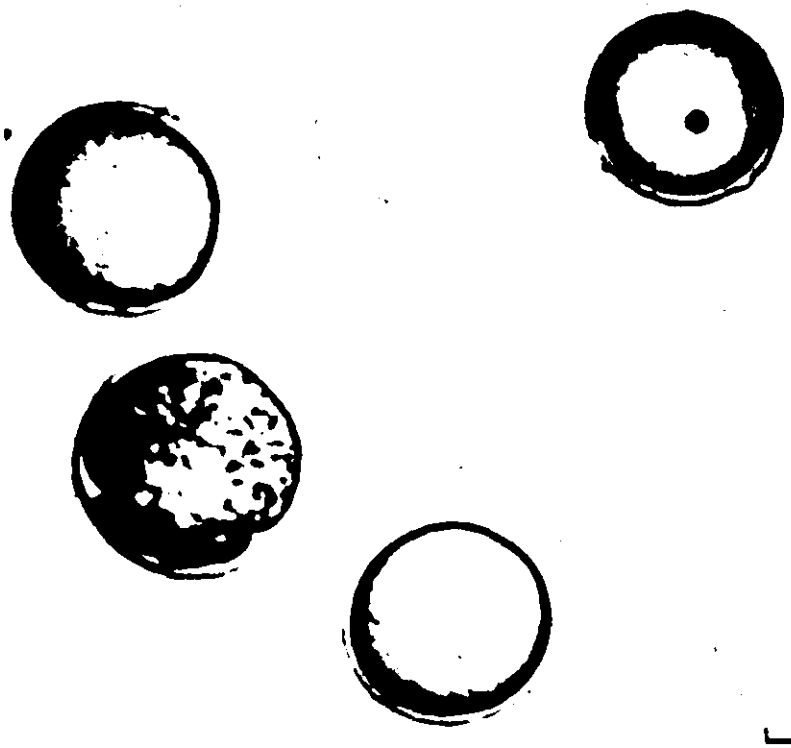
The testes were removed and placed in a dish of isotonic solution (2.2% sodium citrate). The tunica albuginia was stripped away with a pair of forceps. The seminiferous tubules were transferred to a small beaker containing just enough isotonic solution to prevent the tubules from drying

FIGURE 1

(a) Oocytes after 5 hours of incubation

(b) Germinal vesicle (arrow) in a dictyotene oocyte

(c) Mature oocyte



A

50 u

C

C

20 u

out and to facilitate mincing. A fine pair of scissors were used to thoroughly mince the tubules. The mixture was diluted to 20 cc with a hypotonic solution (1% sodium citrate) and left at room temperature for 20 minutes. The suspension was filtered through several layers of gauze and centrifuged at 500 rpm for 5 minutes. The supernatant was poured off and the pellet was resuspended in the residual supernatant.

Acetic alcohol fixative (3:1) was added, one drop at a time, with thorough mixing between drops to prevent the cells from sticking together. After 4-5 drops had been added, more fixative was rapidly added to dilute the mixture to 20 ml. The suspension was left to stand for one hour. It was then centrifuged and the supernatant was discarded. The cells were resuspended in enough fresh fixative to make a milky suspension. Clean slides were prepared by rinsing them in cold tap water. Three or four drops of the cell suspension were dropped on to each wet slide from a height of about 12 inches. Excess water was blotted away and the slides were dried over an alcohol flame. The cells were stained with carbol fuchsin or giemsa.

CYTOGENETIC ANALYSIS

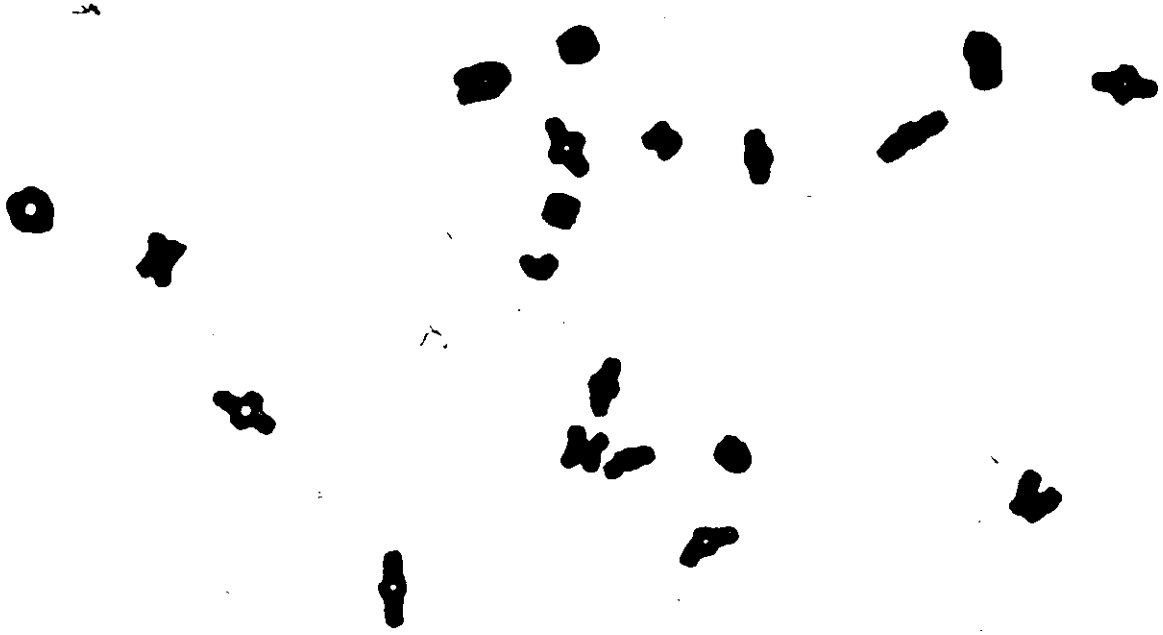
Well spread cells at diakinesis or metaphase I (Fig. 2) were scored for the presence or absence of chromatid

breaks (Fig. 3), fragments (Fig. 4) and chromatid exchanges (Fig. 5). Aneuploidy was not scored as a radiation-induced aberration because the irradiated cells had not proceeded through any divisions from the time of treatment to the time of harvest.

A maximum of 150 cells were scored per male. In the females, because of the limited number of cells that matured, all of the analyzable cells were scored; the maximum number was fifty per mouse.

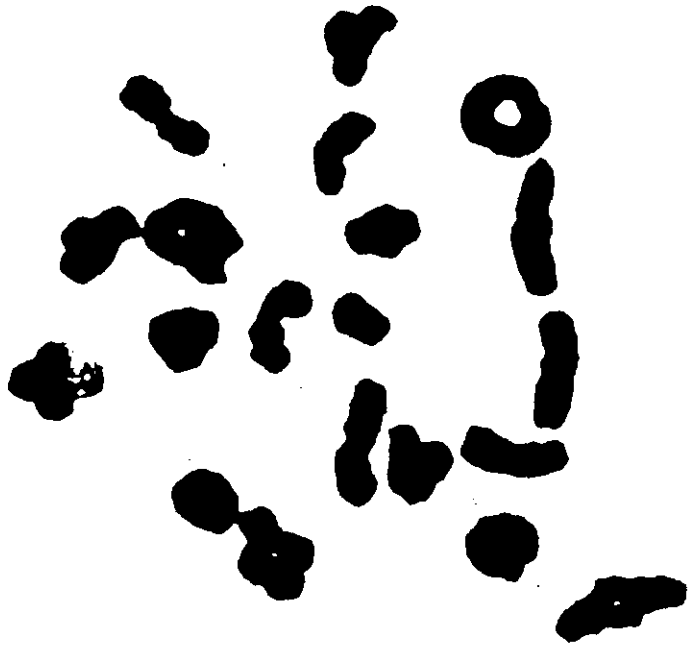
FIGURE 2

Normal diakinesis/metaphase I chromosomes in an (a) oocyte and a (b) spermatocyte



A

10 u

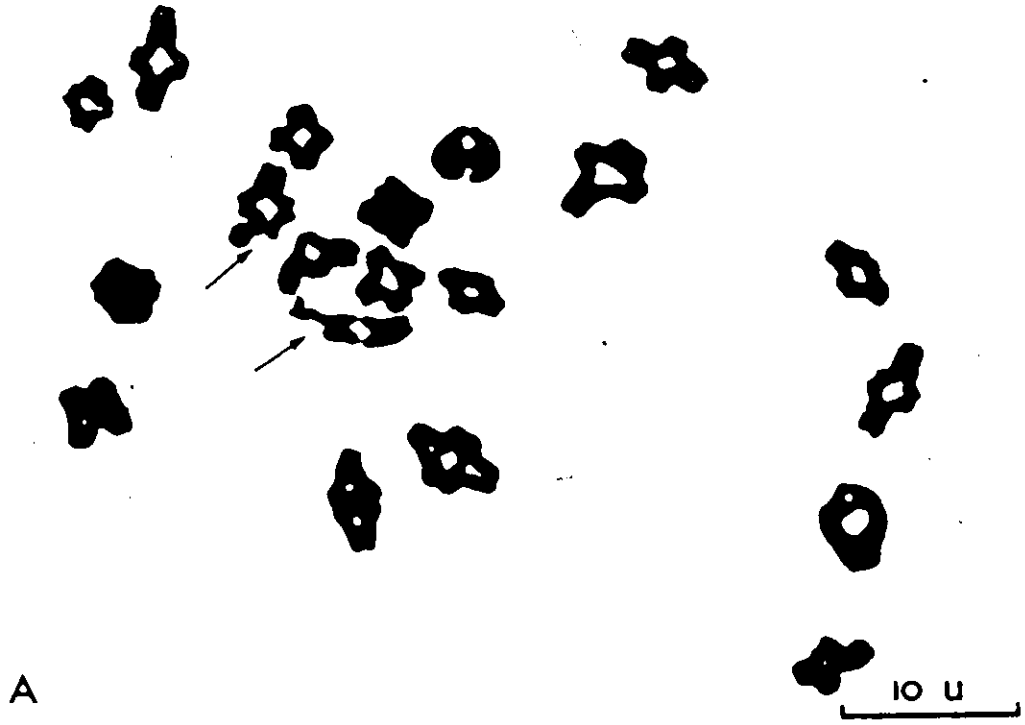


B

10 u

FIGURE 3

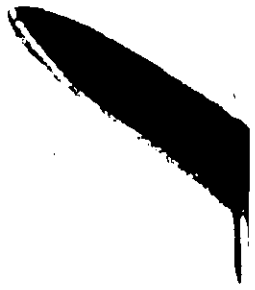
Chromatid breaks in diakinesis/metaphase I (a) oocyte and (b) spermatocyte


A
10 u


B
10 u

FIGURE 4

Chromosome fragments in diakinesis/metaphase I (a) oocyte and (b) spermatocyte



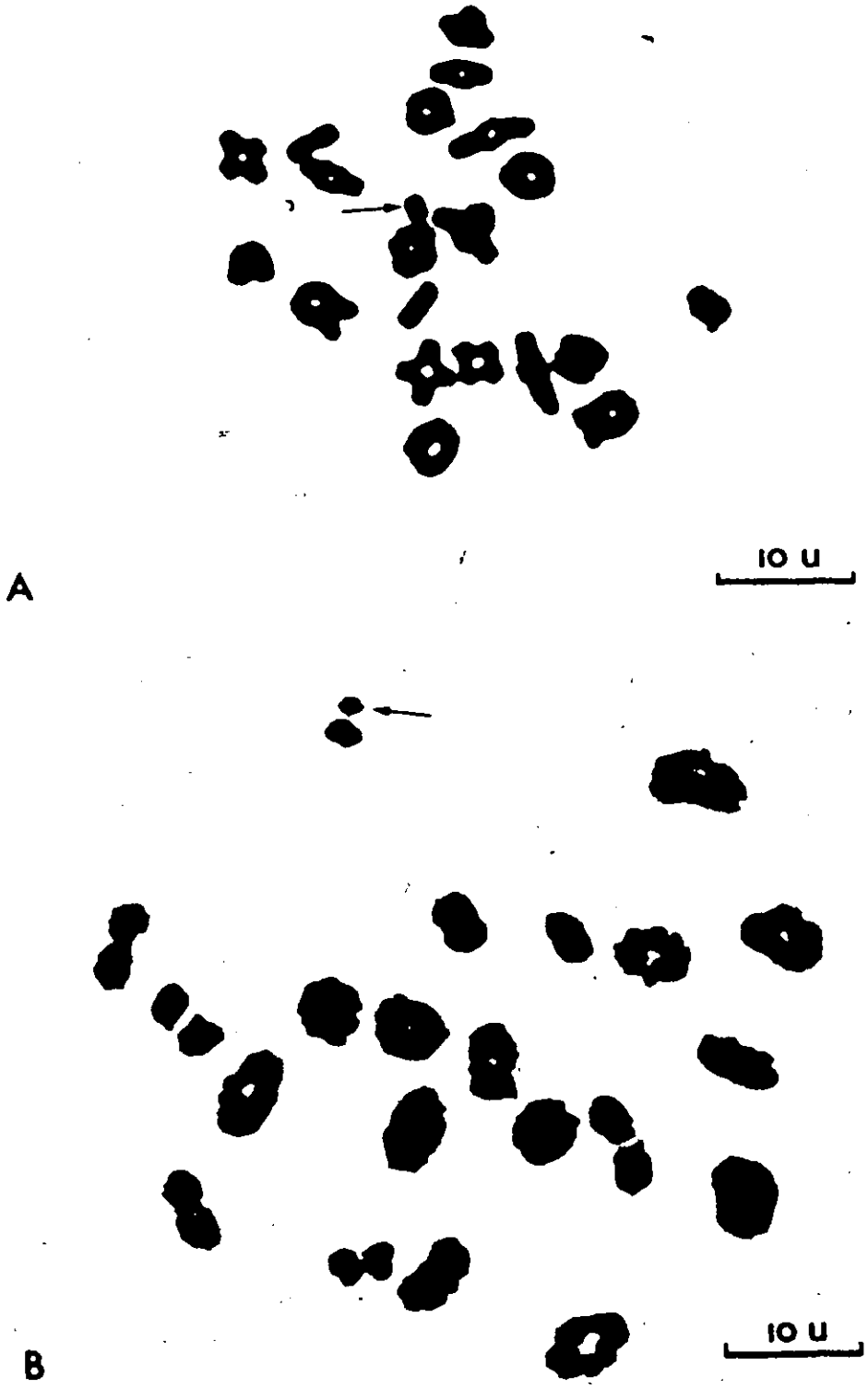
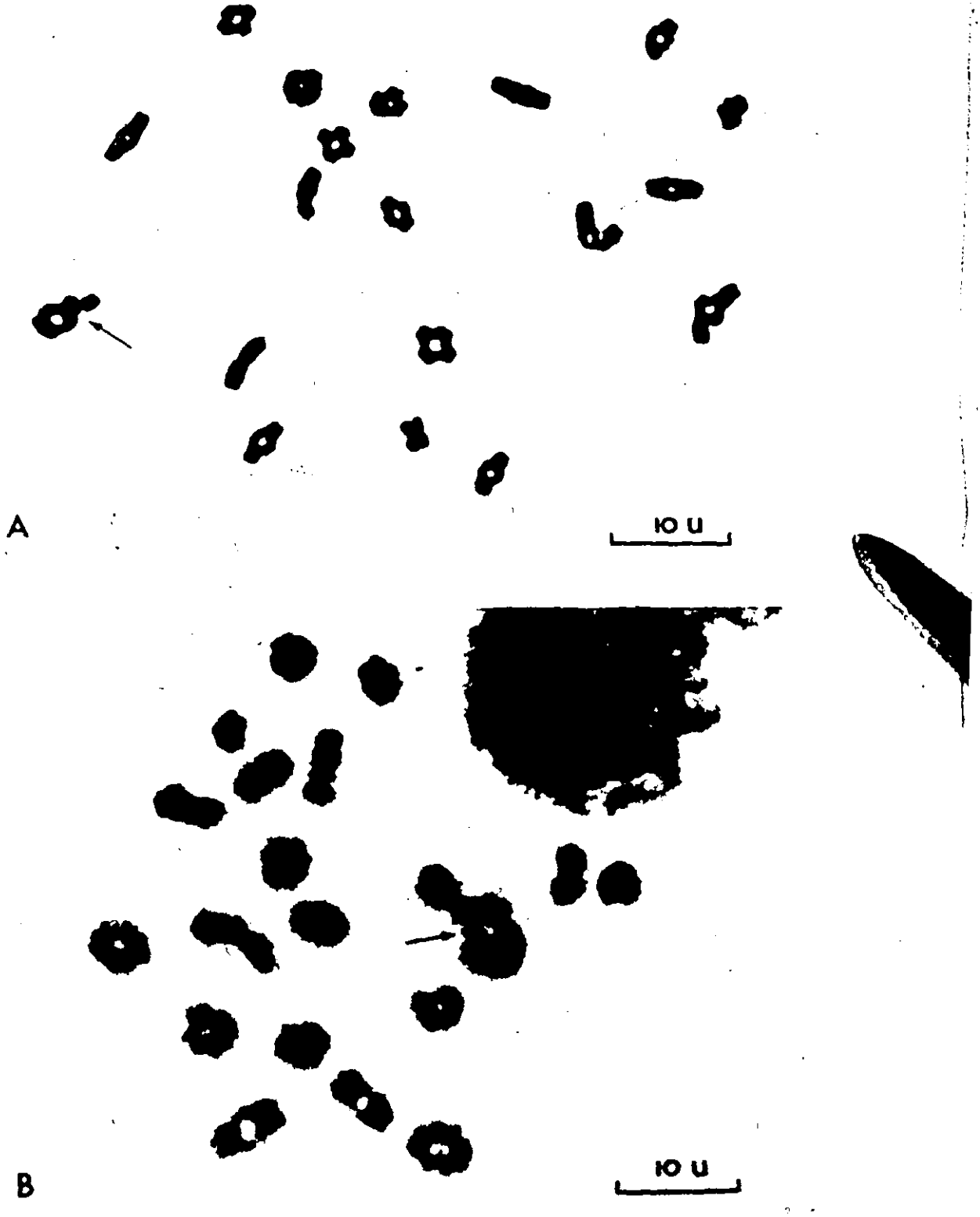

A
10 u
B
10 u

FIGURE 5

Chromatid exchanges in diakinesis/metaphase I (a) oocyte and (b) spermatocyte


A
10 U
B
10 U

RESULTS

OOCYTE COLLECTION AND IN VITRO MATURATION

The total number of oocytes collected from sexually mature mice one day and five days after exposure to 300R and from non-irradiated controls is shown in Table 2. An average number of 61.5 oocytes/mouse was obtained from controls while 57.2 and 64.7 oocytes/mouse were recovered from adults one day and five days, respectively, after irradiation. These averages are not significantly different. Exposure to 300R γ-radiation, thus, did not affect the number of oocytes recovered.

Fifty-eight per cent of the 1144 oocytes, collected one day after irradiation, resumed meiosis *in vitro* (Fig. 1). This proportion was not significantly different from the proportion of control oocytes (60.5%) that matured. However, significantly fewer (53.6%) of the 1359 oocytes cultured 5 days after irradiation resumed meiosis than either the control oocytes ($P<0.001$) or oocytes cultured 1 day post-irradiation ($P\sim0.03$).

SPERMATOCYTE PREPARATIONS

The air drying method generally yielded good metaphase preparations. None of the mice were sterile. A total of 804 cells were scored from the non-irradiated mice

TABLE 2

In vitro maturation of non-irradiated oocytes and oocytes exposed to 300R one day or five days prior to culturing

Number of mice	Post-treatment day of oocyte collection	Total oocytes collected	Average number oocytes/mouse ± S.D.	Matured	
				No.	Per cent
20	1	1144	57.2 ± 20.1	664	58.0
21	5	1359	64.7 ± 26.3	729	53.6
16	Not irradiated	984	61.5 ± 19.7	595	60.5

X^2 Tests

	df	X^2	p
1 day vs control	1	1.3	0.256
5 days vs control	1	10.8	<0.001
1 day vs 5 days	1	4.9	0.027

while 531 and 440 cells were scored from the treated mice one day and five days, respectively, after exposure to 300R.

CYTOGENETIC ANALYSES

1. CONTROLS

Of 598 unirradiated oocytes which matured in vitro, a total of 298 (50.1%) had well spread and analyzable chromosomes. Of these, 3 cells (1.0%) were abnormal. Nine (1.1%) of 804 unirradiated spermatocytes were abnormal. The frequencies of chromosome aberrations in control and irradiated cells are given in Table 3.

2. ONE DAY POST-IRRADIATION

One day after irradiation, 69 (18.9%) of the 366 oocytes examined had structural chromosome aberrations. This frequency is significantly higher than in controls ($P<0.001$). Chromatid breaks comprised 47.6% of the aberrations observed in the irradiated oocytes. Thirty-three per cent of the aberrations were fragments and the remainder (19.5%) were chromatid exchanges.

Abnormal metaphases were observed in a significantly higher proportion of the irradiated spermatocytes than in controls ($P<0.001$). Of 531 irradiated cells scored, 110 (20.7%)

were abnormal. Fragments were the most frequent (45.9%) and chromatid exchanges the least frequent (26.2%) of the aberrations.

Tests of significance comparing the frequencies of abnormal cells and the relative frequencies of chromosome aberrations in irradiated spermatocytes and oocytes are shown in Table 4. The frequency of abnormal spermatocytes was not significantly different from the frequency of abnormal oocytes. However, chromatid breaks were significantly more common in the irradiated oocytes than in the irradiated spermatocytes (47.6% vs 27.9%; P=0.02)

3. FIVE DAYS POST-IRRADIATION

Significantly more abnormal oocytes ($P<0.001$) and spermatocytes ($P<0.001$) were present among the cells from mice five days after they had been exposed to radiation than among control cells. Seventy-six (21.6%) of 352 irradiated oocytes and 141 (32.0%) of 440 irradiated spermatocytes had structural chromosome aberrations. The frequency of abnormal spermatocytes was significantly higher than that for abnormal oocytes ($P=0.001$).

Fragments accounted for 37.6% of the aberrations in the irradiated oocytes and 42.0% of the aberrations in

TABLE 3

Frequency of structural chromosome aberrations in the oocytes and spermatocytes of mice one day and five days after exposure to 300R and in unirradiated controls

No. days post-irradiation	Sex	Meiotic stage irradiated	Total cells analyzed	Abnormal cells		Chromatid breaks	Fragments	Chromatid exchanges
				No.	Per cent			
Non-irradiated	♀	-	298	3	1.0	1	2	-
Non-irradiated	♂	-	804	9	1.1	2	8	-
One	♀	Dictyotene	366	69	18.9	39 (47.6%)	27 (33.0%)	16 (19.5%)
One	♂	Diplotene	531	110	20.7	34 (27.9%)	56 (45.9%)	32 (26.2%)
Five	♀	Dictyotene	352	76	21.6	31 (36.0%)	32 (37.6%)	22 (25.0%)
Five	♂	Pachytene	440	141	32.0	67 (40.0%)	71 (42.0%)	31 (18.0%)

TABLE 4

Tests of significance for Table 3

Sex	Days post-irradiation		Sex	Days post-irradiation	No. abnormal cells (1 degree of freedom) X^2	No. abnormal cells (1 degree of freedom) P	Type of chromosome aberrations (2 degrees of freedom) X^2	Type of chromosome aberrations (2 degrees of freedom) P
♀	1	vs	♂	1	0.5	0.49	8.3	0.02
♀	5	vs	♂	5	10.7	0.001	1.9	0.38
♀	1	vs	♀	5	0.8	0.36	2.2	0.33
♂	1	vs	♂	5	16.1	<0.001	5.1	0.08
♀	1	vs	♂	5	18.0	<0.001	2.0	0.36
♂	1	vs	♀	5	0.1	0.75	2.0	0.37

the irradiated spermatocytes. Chromatid breaks were slightly less common, comprising 36% of the aberrations in the irradiated oocytes and 40% in the irradiated spermatocytes. Twenty-five per cent of the aberrations in the oocytes and 18% of the aberrations in the spermatocytes were chromatid exchanges. The relative frequencies of the aberrations between sexes was found not to be significantly different.

4. COMPARISONS BETWEEN ONE DAY AND FIVE DAYS POST-IRRADIATION

No differences in the frequencies of abnormal metaphases were found between spermatocytes collected one day post-irradiation and oocytes collected on either post-irradiation day (Table 4). However, significantly more abnormal spermatocytes were recovered five days compared to one day after irradiation ($P<0.001$). Chromatid breaks were less frequent among spermatocytes recovered one day post-irradiation than among oocytes collected one day ($P<0.02$) and spermatocytes collected five days ($P<0.04$) post-irradiation while no significant differences were found for fragments and chromatid exchanges. The frequency of abnormal spermatocytes recovered five days post-irradiation was also higher than the frequency of abnormal oocytes ($P<0.001$). Thus spermatocytes collected five days post-irradiation, i.e., exposure during pachytene, had the most aberrations of all the meiotic cells examined.

SUMMARY OF RESULTS

Exposure to 300R did not affect the number of oocytes obtained from mice one day or five days post-irradiation. However, fewer of the oocytes collected on the fifth post-irradiation day matured *in vitro*.

The frequencies of oocytes and spermatocytes with aberrations one day and five days after the mice were exposed to 300R were significantly increased over non-irradiated controls.

No significant difference was found between oocytes collected one day and five days after irradiation in either the total frequency of abnormal oocytes or the distribution of the three types of aberrations.

Diplotene chromosomes of the male were as sensitive to radiation as the dictyotene chromosomes of oocytes. However, the relative frequencies of the aberrations induced in the spermatocytes were significantly different from those of the oocytes.

The frequency of abnormal spermatocytes seen five days after irradiation was significantly higher than the frequency one day after irradiation. This increase was due to a significantly higher frequency of chromatid breaks in the former. Thus, the chromosomes of pachytene spermatocytes were more sensitive to radiation than those of both dictyotene oocytes and diplotene spermatocytes.

DISCUSSION

The results of previous studies, in which the frequencies of radiation-induced chromosome aberrations in spermatocytes and oocytes were compared, are conflicting. This may be due, in part, to limitations of the techniques employed in these experiments. Chromosome aberrations induced in spermatocytes or oocytes were detected as dominant lethals, partial sterility or sex chromosome anomalies among the conceptuses or progeny conceived from sperm or ova derived from the irradiated cells (L. B. Russell, 1962; L. B. Russell, 1968). The frequency of induced chromosome aberrations in spermatocytes as determined by these techniques may be inaccurate because of the possible admixture of sperm from different gametogenic stages in the seminiferous tubules.

The development of new techniques for making chromosome preparations have made it desirable to reinvestigate the radiosensitivities of spermatocytes and oocytes. Meiotic chromosome preparations can now be obtained directly from spermatocytes (Oakberg, 1957; Evans et al, 1964) and from oocytes after maturing them *in vitro* (Edwards, 1965). These techniques were employed in the present study to determine the frequencies of chromosome aberrations recovered after dictyate oocytes and pachytene and diplotene spermatocytes were exposed to 300R γ-radiation.

Although exposure to 300R did not result in a decrease in the total number of oocytes obtained from mice up to five days after treatment, it did result in a decrease in the proportion of oocytes which matured in vitro on the fifth post-irradiation day. However, since the frequencies of chromosome aberrations recovered from oocytes on the first and fifth post-irradiation days were not significantly different, it is unlikely that this decrease represents the selective inhibition of maturation in oocytes with chromosome aberrations. If such a mechanism were operating, we would expect a decrease in the average number of oocytes ovulated by irradiated mice. Previous investigations have shown that this is not the case. Russell and Russell (1956) found that up to two weeks after exposure to 400R, the mean number of oocytes ovulated per female was higher than in controls.

All oocytes collected one day and five days post-irradiation were at the same meiotic stage - dictyotene - at the time of exposure but some of the cells collected on day five may have been at earlier stages of follicular growth when irradiated than those collected on day one (Pederson, 1970). No follicular stage differences in radiosensitivity were indicated by our data. These results are in agreement with those of L. B. Russell (1955) who found that the dominant lethal incidence for females irradiated 16 hours prior to

fertilization was no higher than for females treated up to four and a half days prior to fertilization.

Although some fluctuation occurred in the relative frequencies of chromatid breaks, fragments and chromatid exchanges between the two days tested, they were not significantly different. Chromatid exchanges were the least frequent of the aberrations on both days. This latter result is expected since chromatid exchanges arise from the breakage and fusion of chromatids in two bivalents. Thus, the lack of any significant difference in the severity of damage suggests that oocytes with induced aberrations are not eliminated between one and five days after irradiation.

The radiosensitivity of diplotene and pachytene spermatocytes was estimated by scoring the frequencies of abnormal metaphases one day and five days, respectively, after irradiation. The duration of each stage and the time required for each stage to reach diakinesis/metaphase I has been determined by Oakberg and DiMinno (1962) who also showed that this timing is not affected by irradiation.

Spermatocytes remain in diakinesis/metaphase I for approximately 10 hours and in diplotene for 21 hours (Table 1). Since mice were sacrificed between 21 and 28 hours after exposure in order to catch cells irradiated at

diplotene, very little contamination from cells at other meiotic stages was expected.

Pachytene in mouse spermatocytes is a relatively long stage, lasting for about 175 hours. Accordingly, by sampling diakinesis/metaphase I cells at 5 days post-irradiation, we expected to capture a clean sample of cells irradiated during mid-pachytene.

Some spermatocytes are killed as a result of exposure to radiation. Oakberg (1968) has calculated the LD_{50} of mid-pachytene and diplotene spermatocytes to be 382R and 564R, respectively. Thus, more pachytene than diplotene cells may have been killed by irradiation with 300R. Since no correlation was found between cell death and chromosome breakage (Oakberg and DiMinno, 1962), it is not possible to determine the extent to which spermatocytes with chromosome aberrations were eliminated in each of these stages.

Our results indicate that pachytene is more radiosensitive than diplotene to the induction of chromosome aberrations. Oakberg and DiMinno (1960) came to the same conclusion. Furthermore, L. B. Russell (1968) reported that induced X-chromosome loss occurred more frequently after spermatocytes were irradiated in pachytene than in post-pachytene stages of meiosis. Wennstrom (1971), on the other hand,

found a decrease in the frequency of abnormal first metaphase cells from a maximum at one day after irradiation to a minimum seven days after irradiation which he attributed to restitution. No evidence for restitution was found in our results.

If radiation-induced cell death results in the selective elimination of cells with chromosome aberrations, we would expect more to be eliminated from spermatocytes irradiated in pachytene than in diplotene because of the difference between these two stages in radiosensitivity. Thus, pachytene may be even more radiosensitive to chromosome damage, relative to diplotene, than is indicated by these results.

Data concerning the types of aberrations induced in spermatocytes and their relative frequencies have been published only by Wennstrom (1971). Our results concerning the relative frequencies of chromatid breaks, fragments and chromatid exchanges in irradiated diplotene spermatocytes are entirely in accord with his: fragments were most common while chromatid breaks and exchanges occurred with roughly comparable frequencies. The frequencies for irradiated pachytene spermatocytes are not comparable to Wennstrom's since he used different dose levels (200R) and harvested cells three and seven days after exposure.

The relative frequencies of aberrations in pachytene spermatocytes were significantly different from those in diplotene spermatocytes. This difference was due to a significant increase in the frequency of chromatid breaks in the cells of the former group. The reason for this difference might be related to the differences between the two stages in the organization of the chromatin, however, these results are contrary to expectation. In pachytene, homologous chromosomes are tightly paired and separation of the chromatids has not yet occurred. At diplotene, the homologues have begun to separate and are held together only at the chiasmata; the chromatids are visible (Griffen, 1966). We would have expected, therefore, to recover more chromatid breaks from irradiated diplotene than irradiated pachytene spermatocytes. The possibility remains that this difference may be due to the relatively small sample size.

The frequencies of chromosome aberrations recovered from irradiated dictyate oocytes and diplotene spermatocytes were the same. However, significantly more were found among irradiated pachytene spermatocytes. Since the frequencies of spontaneous aberrations were the same in male and female controls, and since the radiosensitivity of dictyate oocytes was the same on both post-irradiation days tested, this difference must be attributed to meiotic stage variations in radiosensitivity of

the spermatocytes.

Comparisons between spermatocytes and oocytes are complex because more spermatocytes are killed by radiation than mature oocytes. About 38% of the spermatocytes irradiated in pachytene and 13% of those irradiated in diplotene are eliminated after exposure to 300R (Oakberg, 1968). The frequencies of chromosome aberrations in pachytene and diplotene spermatocytes may, therefore, have been underestimated as a result of the elimination of some affected cells. In contrast, oocytes in mature follicles are able to withstand doses of at least 400R and still be capable of ovulation and fertilization.

With the exception of one study, (L. B. Russell, 1968), no data have been published which allow comparisons of radiosensitivities to be made between dictyate oocytes and specific spermatocyte stages. Russell's data indicate that dictyate oocytes are more easily damaged by radiation than pachytene spermatocytes. We conclude from our experiments that pachytene spermatocytes are more sensitive to radiation than dictyate oocytes. These observations are not necessarily in conflict since Russell measured sex chromosome loss among the progeny which may have resulted from nondisjunction or anaphase lag while we measured chromosome breakage phenomena directly in meiotic cells.

BIBLIOGRAPHY

Bateman, A. S. (1958). Mutagenic sensitivity of maturing germ cells in the male mouse. Heredity, 12: 213-232.

Carr, D. H. and Walker, J. E. (1961). Carbol fuchsin as a stain for human chromosomes. Stain Techn., 36: 233-236.

Chandley, A. C. (1971). Culture of mammalian oocytes. J. Reprod. Fert. Suppl., 14: 1-6.

Donahue, R. P. (1968). Maturation of the mouse oocyte in vitro. I. Sequence and timing of nuclear progression. J. Exp. Zool., 169: 237-249.

Donahue, R. P. (1970). Maturation of the mouse oocyte in vitro. II. Anomalies of first polar body formation. Cytogenetics, 9: 106-115.

Edwards, R. G. (1965). Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes. Nature (Lond.), 208: 349-351.

Edwards, R. G., and Gates, A. H. (1959). Timing of the stages of the maturation divisions, ovulation, fertilization and the first cleavage of eggs of adult mice. J. Endocr., 18: 292-304.

Evans, E. P. (1969). Personal communication to Dr. I. A. Uchida

Evans, E. P., Breckon, G., and Ford, C. E. (1964). An air-drying method for meiotic preparations from mammalian testes. Cytogenetics, 3: 289-294.

Evans, E. P., Ford, C. E., Searle, A. G., and West, B. J. (1970). Studies on the induction of translocations in mouse spermatogonia. III. Effects of X-irradiation. Mutat. Res., 9: 501-506.

Ford, C. E., Searle, A. G., Evans, E. P., and West, B. J. (1969). Differential transmission of translocations induced in spermatogonia of mice by irradiation. Cytogenetics, 8: 447-470.

Griffen, A. B. (1966). Nuclear cytology. In Green, E. L. (ed.), Biology of the laboratory mouse. New York, McGraw-Hill, pp. 51-85. 2nd ed.

Henderson, S. A., and Edwards, R. G. (1968). Chiasma frequency and maternal age in mammals. Nature (Lond.), 218: 22-28.

Jagiello, G. (1967). Streptonigrin. Effects on the first meiotic metaphase of the mouse egg. Science, 157: 453-454.

Jagiello, G., and Lin, J. S. (1973). An assessment of the effects of mercury on the meiosis of mouse ova. Mutat. Res., 17: 93-99.

Jagiello, G., and Polani, P. E. (1969). Mouse germ cells and LSD-25. Cytogenetics, 8: 136-147.

Leonard, A. (1971). Radiation-induced translocations in spermatogonia of mice. Mutat. Res., 11: 71-88.

Leonard, A., and DeKnudt, G. H. (1968). The sensitivity of various germ cell stages of the male mouse to radiation-induced translocations. Can. J. Genet. Cytol., 10: 495-507.

Mandl, A. M. (1964). The radiosensitivity of germ cells. Biol. Rev., 39: 288-371.

Oakberg, E. F. (1957). Duration of spermatogenesis in the mouse. Nature (Lond.), 180: 1137-1139.

Oakberg, E. F. (1968). Mammalian gametogenesis and species comparisons in radiation response of the gonads. In Effects of radiation of meiotic systems. International Atomic Energy Agency, Vienna, pp. 3-15.

Oakberg, E. F., and DiMinno, R. L. (1960). X-ray sensitivity of primary spermatocytes of the mouse. Int. J. Rad. Biol., 2: 196-209.

Pederson, T. (1970). Follicle kinetics in the ovary of the cyclic mouse. Acta Endocrinol., 64: 304-323.

Russell, L. B. (1956). Dominant lethals induced at a highly sensitive stage in mouse oogenesis. Anat. Rec., 125: 647-648.

Russell, L. B. (1962). Chromosome aberrations in experimental mammals. In Steinberg, A. G. and Bearn, A. G. (ed.), Progress in medical genetics, New York, Grune & Stratton, 2: 230-294.

Russell, L. B. (1968). The use of sex-chromosome anomalies for measuring radiation effects in different germ-cell stages of the mouse. In Effects of radiation of meiotic systems, International Atomic Energy Agency, Vienna, pp. 27-41.

Russell, L. B., and Major, M. H. (1953). Dominant lethals in mouse oocytes induced by X-rays in air and in 5% oxygen. Genetics, 38: 687-688.

Russell, L. B., and Russell, W. L. (1954). Pathways of radiation effects in the mother and embryo. Cold Springs Harbor Symp. Quart. Biol., 19: 50-59.

Russell, L. B., and Russell, W. L. (1956). The sensitivity of different stages in oogenesis to the radiation induction of dominant lethals and other changes in the mouse. In Mitchell, J. S., Holmes, B. E., and Smith, C. L. (eds.), Progress in radiobiology, Edinburgh, Oliver and Boyd, Ltd., pp. 187-192.

Russell, L. B., and Spears,R. J. (1954). X-ray induced dominant lethals in mouse oocytes and their relation to irradiation-to-ovulation interval. Genetics, 39: 991.

Russell, L. B., and Wickham, L. (1957). The incidence of disturbed fertility among male mice conceived at various intervals after irradiation of the mother. Genetics, 42: 392.

Russell, W. L. (1954). Genetic effects of radiation in mammals. In Hollander, A. (ed.), Radiation Biology, New York, McGraw-Hill, pp. 825-859, Vol. I.

Russell, W. L., Russell, L. B., and Oakberg, E. F. (1958). Radiation genetics of mammals. In Claus, W. D. (ed.), Radiation Biology and Medicine. Reading, Mass., Addison-Wesley Publishing Co., Inc., pp. 189-205.

Searle, A. G., Ford, C. E., and West, B. J. (1969). Studies on the induction of translocations in mouse spermatogonia. II. Effects of fast neutron irradiation. Mutat. Res., 7: 235-240.

Tarkowski, A. K. (1966). An air-drying method for chromosome preparations from mouse eggs. Cytogenetics, 5: 394-400.

Wennstrom, J. (1971). Effects of radiation on the chromosomes in meiotic and mitotic cells. Comment. Biol., 45: 1-60.