AGE-DEPENDENT RADIOSENSITIVITY OF MOUSE OOCYTES

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(B. A. Thesis)

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AGE-DEPENDENT RADIOSENSITIVITY OF MOUSE OOCYTES

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ABSTRACT

The oocytes of small follicles in immature mice exhibit an extremely high radiosensitivity which varies with age. To aid in the interpretation of experiments using this biological system, the changing oocyte sensitivity has been quantified for the first 3 weeks of life in the Swiss-Webster mouse.

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INTRODUCTION

Meiosis and oocyte development--In normal development, a mammalian oocyte passes through a complex sequence of events as it matures. The primordial germ cells divide mitotically until, at a certain point in embryological development, they enter meiotic prophase I. Beginning meiosis with four times the haploid amount of DNA, the cells will eventually divide twice, resulting in three or four division products: one oocyte and two or three abortive polar bodies. However, these divisions do not take place until the development of sexual maturity in the animal, concurrent in each case with ovulation.

Meiotic prophase I has for descriptive purposes been divided into five stages (Griffen, 1966; Rugh, 1968; Wolfe, 1972). Following the premeiotic interphase, the primary oocytes enter into leptotema, where the first signs of chromosome condensation can be seen. Initially appearing as faint threads, the chromosomes continue to condense and shorten as the cell enters the second stage, zygotema. Here the homologous chromosomes begin to pair, or synapse. The completion of synapsis signals the entrance into the third stage, pachytene. The chromosomes remain very closely paired and continue their condensation. They are descriptively referred to here as bivalents or tetrads, in reference to their paired nature as two chromosomes made up of four chromatids. The individual chromatids become more recognizable as the cell enters into the fourth stage, diplotema. The synaptonemal complex, which apparently functions in the close pairing of pachytene chromosomes, disappears and the diplotene chromosomes have a noticeably greater separation. The chiasmata resulting from recombination become visible. In most if not all mammals, it appears that the oocytes enter diplotema prenatally and remain there until shortly before ovulation. During this period there is a marked uncoiling of the chromosomes, which can give the oocyte nucleus the appearance of being in interphase. Shortly before ovulation, the oocyte enters diakinesis, the fifth and final stage of prophase I, and the chromosomes recondense in preparation for the first division. Following this, the oocyte goes through the normal meiotic phases of metaphase I, anaphase I, and telophase I,

separating the homologous chromosomes. The first polar body separates from the oocyte (now a 'secondary' oocyte) at about the time of ovulation, as the second division is immediately initiated. This division is similar to a mitotic division without the elaborate, long-lasting prophase characteristic of meiosis I. Pairing cannot occur for the obvious reason that the homologous chromosomes have already been separated. However, the second division is often arrested at metaphase II until the oocyte is fertilized, at which time the second polar body is formed. Sometimes the first polar body, having the same chromosome constitution as the secondary oocyte, also divides, forming a fourth haploid division product.

Meiosis in the mouse--In the laboratory mouse, meiosis begins at about the 13th or 14th day of gestation. The primordial germ cells enter premeiotic interphase on about the 13th day, and by day 14 most are in leptonema and zygonema. By day 15, zygonema and pachynema predominate. Though the leptotene and zygotene stages last only a matter of hours, the pachytene stage lasts three or four days. By day 18, some of the oocytes at the center of the ovary have entered diplonema, and by day 19 (day of birth), about half of the oocytes are in pachynema, half in diplonema. In most mammals, the oocytes remain in the diplotene state until shortly before ovulation. In mice and rats, the oocytes continue into another phase, called the 'dictyotene' or 'dictyate' stage (sometimes referred to as 'late diplotene'). Whereas diplonema is often characterized by a certain amount of uncoiling of the chromosomes and the appearance of lampbrush configurations, dictyotene nuclei appear nearly completely diffuse, with no distinct organization. The first dictyate oocytes appear a few hours after birth, and by four or five days after birth, virtually all of the oocytes are dictyotene (Borun, 1961).

At birth the female mouse normally possesses in its ovaries some 10,000-20,000 oocytes. Of this supply, a large proportion is depleted by atresia (cell death) before the animal ever reaches sexual maturity between 4 and 7 weeks. Of those that do not die, the number is then slowly depleted by further atresia and by maturation and ovulation. Pedersen and Peters (1968) have proposed a classification scheme to describe the maturing process whereby the oocyte grows and follicle

cells attach to its surface. At birth most of the oocytes are without follicle cells; within a few days they are surrounded by a ring of flattened follicle cells--this is called a 'primary follicle.' Pedersen and Peters label these initial developmental stages types 1, 2, and 3a. Those which have not yet begun to mature remain at this resting stage. As they mature, the follicles become larger, gaining many cuboidal follicle cells, eventually developing antra and becoming mature Graafian follicles (type 8) in which the oocyte finally enters diakinesis and is ovulated. At the end of the reproductive lifespan, the ovary is either depleted of oocytes (Lindop *et al.*, 1966) or the unused primary follicles may remain without ever maturing (e.g. Oakberg, 1966), owing to strain differences.

Throughout the life of the normal mouse, the majority of the oocytes in its ovary are in the small primary follicle stage, their development arrested in the dictyotene phase. Once the follicle begins to mature (type 3b), it appears committed to development, either maturing to ovulation or becoming atretic and dying. As the oocytes begin to develop, much of the previously 'resting' chromatin becomes active, synthesizing RNA in preparation for early zygote development following fertilization. It is the relatively inactive oocyte stages (types 1, 2, and 3a) on which the primary emphasis of this study is focussed.

Radiation studies--The convenience of using primary oocytes in a study of radiation sensitivity becomes readily apparent. From a well-defined, finite population of cells, one can with little difficulty determine survival percentages uncomplicated by cell division. There have been numerous investigations in the past which have determined that the dictyate rodent oocyte is unusually radiosensitive. Only some of the more recent studies are highlighted here.

There are several possible ways of approaching this problem. One that has been often used is a reproductive capacity experiment. The animal is irradiated, usually at a prepubertal age, and allowed to breed freely until the end of its natural reproductive lifespan. The litters are carefully recorded, and one can then look at total number of offspring, average litter size, and number of litters. However, these

results become complicated as it appears that the ovary somehow compensates for a loss of primary oocytes, using those which do survive more efficiently. In one such experiment (Oakberg, 1965), 25 R of x-irradiation at 10 days of age reduced the oocyte supply to only 4% of control (measured at 56 days of age), yet the total number of offspring remained a much higher 35% of control. The apparent explanation for this paradox is that nearly all of the surviving oocytes mature to ovulation; in controls, only a small fraction of the total oocyte population is ever ovulated.

Another often-used method of looking at oocyte radiosensitivity is to look directly at the oocytes, allowing a long enough period for death and atresia to remove those immediately affected, but without allowing enough time for other conservation-type mechanisms to show significant effect. One can also look at a later date to specifically detect either conservation or increased atresia of oocytes. Peters et al. (Peters and Borum, 1961; Peters and Levy, 1964; Peters, 1961, 1966) have taken this approach. In their experiments, 20 R of x-irradiation were given on days 0, 7, 14, 21, 28, 35, 42, and 49, and the ovaries were examined at different intervals after irradiation (usually 24 hours post-irradiation and at age 40 days). Examining some groups at 1, 2, 3, and 4 days post-irradiation, a rapid destruction and removal of degenerating oocytes was observed. Similar observations were made by Dobson, Arrington, and Kwan (1976), who showed that by 24 hours all of the oocytes immediately killed by irradiation had been removed; therefore quantitative observations of surviving oocytes could accurately be made by counting at 24 hours post-irradiation. Peters observed a wide range of radiation sensitivity: on the day of birth, 20 R destroyed 50% of the small oocytes, yet at days 7, 14, and 21, 95% or more were destroyed. The cells slowly became more radiation-resistant, so that by day 49, 10% survived irradiation. These figures show a trend of changing radiosensitivity, which Peters attempts to explain in light of other qualitative observations. These observations indicate that the pachytene oocytes (which constitute about 45% of the total oocyte population at day 1) are highly radiosensitive, while the other early diplotene oocytes are radioresistant. As the oocytes pass from early diplotene to late diplotene (dictyotene), they again become highly

radiosensitive. Those which have already started to grow (type 3b and above) again show resistance to radiation

Much of the recent emphasis of this laboratory (R. L. Dobson, Lawrence Livermore Laboratory) has been the quantification of the toxicity and RBE (relative biological effectiveness) of tritium at low-level exposure (Dobson and Cooper, 1974; Dobson, Arrington, and Kwan, 1976; Dobson and Kwan, 1976a, 1976b). For many of these experiments, chronic exposures to ^{60}Co gamma rays and to $^3\text{H}\text{OH}$ in body water were used. These exposures lasted in some from conception to 14 days old (nearly 5 weeks), in others for only one or two weeks. It is readily apparent that with changing sensitivity, similar doses given at different ages will have different effects, and a given, chronic dose will have varying effects at different times during administration. Dobson, Arrington, and Kwan (1976) observed in mice irradiated from days 20 through 25 a significantly higher resistance to radiation than observed in protracted exposures through day 14. This indicated that the oocytes of this strain of mice (Swiss-Webster) could be much less sensitive than those of Peters' 'Street' mice, which showed greatest sensitivity at 21 days.

Purpose of the present investigation--It became apparent from such results that it would be necessary to investigate specifically the changing sensitivity in the Swiss-Webster strain and attempt to quantify it. Between the ages of 0 and 21 days there are not only great changes in experimental radiation sensitivity, but also a very high rate of natural atresia in the control animals. In developing a greater working knowledge of this strain, it was of interest to determine more accurately this rate of natural atresia and how it changes with time. Because of great strain variations, this information cannot be elicited from others' data. The magnitude of these differences is illustrated by a comparison of two papers. Peters and Levy (1964) noted that "the Street mouse is born with 12000 oocytes, this number decreases exponentially, and is halved in 32 days." Lindop et al. (1966), describing the SAS/4 strain, noted that "in the newborn, there are about 20,000 cells in both ovaries of the mouse (which) show a rapid exponential decrease with a half-life

of 5 days." The present experiment would also provide a basis for dose normalization, allowing more accurate intercomparisons of data from previous experiments (Dobson and Kwan, 1976b). It was also expected that this experiment would provide the basis for future investigations. A continuation of the experiment investigating longer-term effects of irradiation has already begun in this laboratory.

Recognizing that the greatest changes in sensitivity appear to take place during the first week of life, most of the data were collected from this age range. Dobson and Kwan (1976a) had shown that protracted exposure from conception to birth had very little effect compared to post-natal exposures, so the gestational period was ignored. Peters (1961) showed in her strain a marked increase in sensitivity between 0 and 7 days of age. It was decided therefore that mice would be irradiated on days 0, 2, 5, and 7 to provide data on changes in the first week of life, and animals would be irradiated also on days 14 and 21 to estimate the peak period of sensitivity. The exposed mice would then be killed 2 days post-irradiation and the ovaries examined. Control animals would be killed and examined on days 0, 2, 4, 7, 9, 16, and 23. On the basis of an earlier experiment (Dobson and Kwan, 1976b), dose was chosen to be 18 rads of gamma radiation from a ^{60}Co source. By irradiating 18-day-old mice (near the supposed peak sensitivity) at different doses, it was determined that 18 rads would result in 10% survival. This dose then would not totally deplete the oocyte supply, but would also kill enough in each age group to provide results significantly different from the controls.

MATERIALS AND METHODS

Experimental animals--Non-inbred Swiss-Webster mice were used in this experiment. They were raised in this laboratory from stock originally obtained from Simonsen Laboratories.

Females were checked for litters in the mornings. Delivery time varied from 5:00 the previous evening to 10:00 in the morning; a span of 17 hours. Realizing that there would be differences in radiosensitivity resulting from these varying birth times, and that there may be familial

differences, litters were randomized on day of birth. The mothers readily accept each other's offspring, so it was possible to give each mother a standard-sized litter of 11 or 12 females, containing only 2 or 3 of her original litter. The animals then did not require individual marking; entire litters could be used as experimental groups and control groups.

⁶⁰Co gamma-ray exposures--Experimental mice were put into individual, perforated plastic capsules for restraint, then placed on a semicircular lucite shelf. The shelf was constructed such that the capsules would be a uniform distance from a small, pneumatically-delivered ⁶⁰Co source. The mice received a dose of 18 rads, delivered over a period of 18 minutes at a dose rate of 1 rad/min, known with precision from measurements with calibrated Victoreen R chambers and LiF thermoluminescent dosimeters (TLD's).

Control animals were similarly restrained in individual capsules, but placed in another room, shielded from exposure. Following exposure, all mice were returned with litter-mates to their mothers.

Histological preparation--Two days post-irradiation, mice were killed and their ovaries removed. The ovaries were fixed in Zenker-formol, serially sectioned in paraffin at 5 μ m, and stained with Delafield's hematoxylin and eosin. Primary oocytes (stages 1, 2, and 3a of Pedersen and Peters (1968)) were counted in every 20th section under Leitz microscopes at 1000x magnification. One ovary from each mouse was counted.

Estimation of oocyte numbers--For estimates of the true oocyte population of the ovaries, the method discussed by Mandl and Zuckerman (1950) has been employed. Using the nucleus of the oocyte as a marker in counting, and counting every 20th section, the 'true' oocyte population (P) of an ovary is

$$P = \frac{\text{section thickness}}{\text{section thickness} + \text{nuclear diameter}} \times 20 \times \text{number of oocytes counted.}$$

Though the type 3a oocyte is in a resting stage before its enlargement to maturity, as it moves from type 1 to type 3a the nuclear diameter increases slightly. Pedersen and Peters (1968) indicate that

the oocyte (cytoplasm included) at these stages is less than 20 μ m in diameter, but give no information regarding nuclear diameter. It was felt that with such changes in growth, as well as unknown possible radiation effects at different ages, average nuclear diameters should be determined for each age group. These measurements were made on a Leitz micrometer at 1000x magnification. 18 oocytes from 3 ovaries in each group were measured and averaged.

EXPERIMENTAL RESULTS

Nuclear diameters--The measurements made on primary oocytes from each control and experimental group are shown in Table 1.

Table 1
Average Nuclear Diameter of Primary Oocytes in Unexposed
Mice and Mice Exposed to ^{60}Co Gamma Irradiation

Age (Days)	Nuclear Diameter (μ m)*	
	Unexposed (Control)	Exposed
0	5.76 $\pm$.12	----
2	6.67 $\pm$.20	6.62 $\pm$.20
4	6.85 $\pm$.16	7.17 $\pm$.23
7	7.44 $\pm$.15	7.17 $\pm$.15
9	7.83 $\pm$.15	7.28 $\pm$.11
16	7.76 $\pm$.11	8.00 $\pm$.18
23	7.77 $\pm$.14	8.02 $\pm$.15

* Mean (of 18 measurements in each case)
 $\pm$ 1 SEM (Standard error of the mean)

Slight differences are seen here between the controls and experimentals, which originally suggested that a relationship between irradiation and nuclear diameter of the surviving fraction might exist. However, statistical analysis of the data by Student's t-test showed no significant differences at the .05 level of probability, aside from

the data for 9-day-olds. On the basis of this information, the data were pooled for each age group. These results are presented in Table 2. The data for the individual groups are shown in Figure 1, however, the curve is fitted to the pooled data of Table 2. It is this pooled data from which the 'true' oocyte populations were calculated.

Table 2

Nuclear Diameter of Primary Oocytes in Mice Both
Unexposed and Exposed to ⁶⁰Co Gamma Irradiation

Age (Days)	Number of Observations	Nuclear Diameter (μm)*
0	18	5.76 ± .12
2	36	6.65 ± .14
4	36	7.01 ± .14
7	36	7.32 ± .11
9	36	7.57 ± .10
16	36	7.89 ± .11
23	36	7.90 ± .10

* Mean ± 1 SEM

Fig. 1. Average nuclear diameter of primary oocytes, shown as a function of age. Solid circles (●) are data from the unexposed group of Table 1; open circles (○) are data from the irradiated group of Table 1. The curve is fitted to the data in Table 2. Vertical bars (± 2 SEM) indicate 95% confidence limits.

Normal oocyte population--The results of the control animal oocyte counts are shown in Table 3. The data of Table 3 have been graphed in semi-logarithmic form in Figure 2 to show the apparent exponential loss of small oocytes. This graph shows a biphasic curve; analysis of the $\log_{10}$ of oocyte population gave slopes of $-.06327$ for ages 0-4 days and $-.00341$ for 4-23 days.

Table 3

Observed Count and 'True' Oocyte Population
of One Ovary in Unexposed Mice

Age (Days)	Observed Count*	'True' Oocyte Population*
0	883.0 $\pm$ 67.3	8206 $\pm$ 648
2	707.7 $\pm$ 53.0	6075 $\pm$ 473
4	550.3 $\pm$ 35.4	4582 $\pm$ 309
7	618.3 $\pm$ 51.6	5017 $\pm$ 426
9	628.6 $\pm$ 33.9	5001 $\pm$ 278
16	573.6 $\pm$ 26.0	4450 $\pm$ 211
23	538.7 $\pm$ 38.8	4176 $\pm$ 305

* Mean (of 10 ovaries in each case) $\pm$ 1 SEM

Fig. 2. 'True' oocyte population for single ovaries in control mice, shown as a function of age; the data are from Table 3. Vertical bars (± 2 SEM) indicate 95% confidence limits. The curve is a least squares best fit; analysis of the linear regression of the $\log_{10}$ of oocyte population gave slopes of $-.06327$ for ages 0-4 days and $-.00341$ for 4-23 days.

Irradiated ovaries--The oocyte counts for the experimental females are given in Table 4. Survival percentages have also been included; these are graphed in Figure 3. The point shown in Figure 3 for day 18 is taken from data given by Dobson and Kwan (1976b).

Table 4

Observed Count, 'True' Primary Oocyte Population, and Percent Survival of Oocytes in Mice Exposed to ^{60}Co Gamma Irradiation

Age at Irradiation (Days)	Age When Counted (Days)	Observed Count*	'True' Oocyte Population*	Percent Survival**
0	2	623.0 $\pm$ 60.1	5348 $\pm$ 528	88.0 $\pm$ 11.1
2	4	200.5 $\pm$ 23.0	1669 $\pm$ 194	36.4 $\pm$ 4.9
5	7	79.6 $\pm$ 9.1	646 $\pm$ 74	12.9 $\pm$ 1.8
7	9	77.6 $\pm$ 7.4	617 $\pm$ 59	12.3 $\pm$ 1.4
14	16	53.8 $\pm$ 6.5	417 $\pm$ 51	9.4 $\pm$ 1.2
21	23	141.6 $\pm$ 25.3	1098 $\pm$ 197	26.3 $\pm$ 5.1

* Mean (of 10 ovaries counted in each case) $\pm$ 1 SEM

** Proportion ((exposed/control) $\times$ 100) $\pm$ 1 SEP (Standard error of the proportion)

Fig. 3. Survival of primary oocytes (percentage of control), shown as a function of age at irradiation; the data are from Table 4. (The point for day 18 is from Dobson and Kwan (1976b).) Vertical bars (± 2 SEP) indicate 95% confidence limits.

DISCUSSION

The experimental results reported here demonstrate at least three distinct phases of oocyte radiosensitivity in prepubertal mice between the ages of 0 and 21 days. There is an initial period of rapidly increasing sensitivity, followed by an approximately stationary phase of high sensitivity lasting from about 5 to 18 days of age, which in turn is followed by a period of decreasing sensitivity. At 21 days, the oldest age tested in this study, the sensitivity still appears to be decreasing; it is not known from the present data how long this period lasts. In addition, two phases have been demonstrated for the decrease of the primary oocyte population in mice between 0 and 23 days of age. This decrease is due to a combination of natural atresia and maturation, and can be described by two exponentials (Fig. 2). At birth and for the first three days, loss occurs at a very high rate (half-time about 5 days), and then decreases at 3 or 4 days of age to a much lower rate (half-time about 85 days). It has also been shown that the average diameter of the primary oocyte nuclei increases from 5.8 μ m on day of birth to an apparently stationary 7.9 μ m by age 16 days.

The timing of these events can be correlated with other changes in the development of the mouse. Borum (1961) examined changes of meiotic prophase in the mouse and observed the transition from a combination of pachytene and diplotene nuclei at birth to nearly all dictyotene by age 4 days. Squash preparations were not done in this experiment, therefore direct comparisons could not be made with Borum's data. However, similar timing is inferred for the Swiss-Webster strain, as the oocyte nuclei, as seen in sections, change from primarily heterogeneous meiotic figures in newborns to the characteristic dictyate appearance in 4-day-olds. The change in nuclear diameter, which is most rapidly increasing between 0 and 4 days, is probably related to this overall change from compact pachytene and diplotene nuclei to diffuse dictyotene nuclei. The increasing sensitivity over this period appears to reflect the increasing numbers of dictyate oocytes. (It should be noted that the pattern of radiosensitivity observed here does not lend support to the high radiosensitivity of pachytene oocytes described by Peters and

Borum (1961).) During this particular time period, the rapid increase in oocyte radiosensitivity is matched by both a natural rapid loss of primary follicles and a dynamic, changing cytology, in which the oocytes are progressively becoming dictyate.

From 4 days on, the small-follicle oocytes remain dictyate. During the period of high radiosensitivity (5-18 days of age), the natural loss of these oocytes occurs at a slower rate, and the average nuclear diameter reaches a temporary maximum and levels off. Thus we see that a period of relatively unchanging high radiosensitivity is marked by seemingly stable oocytes which show a low rate of atresia and a relatively unchanging appearance.

The above correlations tend to indicate that between 0 and 18 days of age there is a relationship between oocyte morphology and radiosensitivity. However, similar morphological events are not seen coinciding with the decrease in sensitivity observed between 19 and 21 days. Slizynski (1967) observed slight differences in nuclear morphology between oocytes of primary follicles in 14-day-old mice and those of mice over 6 weeks old, but it is not known when those changes occur. Beaumont (1969) raises the strong possibility that hormonal influences affect oocyte radiosensitivity at this age. After removal of the pituitary (thus stopping endogenous gonadotropin production), Beaumont observed in adult rat oocytes an increased resistance to radiation. Administration of exogenous gonadotropin reversed the effect of hypophysectomy. Rats, which show a similar though slower development than mice, also have highly radiosensitive dictyate oocytes. Examination of serum levels of gonadotropins in rats shows high levels of FSH through 20 days of age, followed by a sharp drop-off to low levels normally characteristic of the mature animal (Meijs-Roelofs et al., 1973). The implication, though it has yet to be tested in immature animals, is that the high radiosensitivity observed in mice and rats before day 20 is related to high FSH levels; when the level of FSH decreases, so does the radiosensitivity. Meijs-Roelofs et al. suggested a correlation between the drop in FSH and the sharp increase in numbers of large follicles (types 6, 7, and 8 of Pedersen and Peters (1968)) which occurs in rats at about 21 days of age. The similar increase

in numbers of large follicles occurs in mice between 14 and 21 days of age (Pedersen, 1972). This event probable causes the drop in FSH secretion (through pituitary inhibition by estrogen which is secreted by the more mature follicles), and may be closely related to the decrease in radiosensitivity reported here between 19 and 21 days of age.

Why are the oocytes of the small follicles so sensitive to radiation? What is the mechanism which results in oocyte death and removal in less than 24 hours? Why do these cells show the highest radiosensitivity of any mammalian cell thus far investigated? Varying response with age, as shown here, indicates that both cellular morphology and hormonal environment may be involved. Other experiments have shown additional variations in oocyte response to irradiation, indicating that damage does not always lead to immediate oocyte death. Oakberg (1966), irradiating at 10 days of age, measured the decrease in numbers of the small follicles over the entire reproductive lifespan in irradiated mice and compared this rate of decrease with controls. In addition to the high initial loss, he observed over time a consistently higher rate of oocyte population decline in the irradiated animals, which appears to be the result of an increased rate of atresia. Peters and Borum (1961) observed a similar effect. Mice irradiated at birth showed not only immediate oocyte degeneration, but also showed traces of damage and degeneration throughout the prepubertal period. Slizynski (1967) has hypothesized that this is the result of damage to inactive genes, which, when activated at a later stage of development, express themselves as lethal mutations. Damage does not, therefore, always result in immediate oocyte death. Dobson and Kwan (1976b) have shown that the oocyte has some, though limited, capability of recovery from radiation damage. For the same effective dose, higher dose rates result in more destruction than lower dose rates, which indicates that chronic exposures allow cellular repair of injury before a critical lethal amount of damage is inflicted.

There are therefore at least three responses by the primary follicle to irradiation: 1) immediate oocyte death (within 24 hours), 2) delayed oocyte death (which takes place over the entire lifespan of the animal), and 3) recovery from damage. Is the damage which

results in death the same in the first two cases? Several theories of radiation-induced cell death have been put forward, with most if not all indicating that broken nuclear DNA is the lethal damage. However, in the case of a resting, non-dividing primary oocyte, one must question the applicability of these hypotheses.

The widely-held theory of radiation-induced death in dividing cell populations (see, e.g., Chadwick and Leenhouts (1973)) states that the critical radiation damage to a cell is the production of a lethal number of DNA double-strand breaks. These breaks manifest themselves at mitosis or subsequently when chromosome imbalance leads to 'reproductive' death. However, in the non-dividing primary oocyte a simple break can hardly result in such a chromosome imbalance. In either immediate or delayed death, the oocyte dies a nonreproductive or 'interphase' death long before it would normally divide (just before ovulation). The hypothesis suggested by Slizynski (1967) of damage to presently inactive genes provides a reasonable, possible explanation for the delayed oocyte death, but leaves the immediate death unresolved. Baker (1971) postulates that the extreme radiosensitivity of rodent dictyate oocytes is a direct result of their nuclear morphology. Lampbrush chromosomes, which are observable in other species at comparable stages of development, are much less radiosensitive. Baker's hypothesis maintains that a thick sheath of ribonucleoprotein holds the lampbrush DNA rigidly in place, and therefore facilitates DNA repair by holding broken ends together. Dictyate oocytes of the mouse, not having this thick sheath, would be less capable of repair. However, it is not clear how such damage in a 'resting' cell would lead to immediate death. The dormant oocyte of a primary follicle has been shown to have a low rate of metabolic activity, synthesizing very little RNA (Moore *et al.*, 1974). Such damage therefore would probably not be manifested to a significant degree as defective protein; nor would it result in a chromosome imbalance. How, then, does such damage cause cell death? It hardly seems possible that the oocyte could recognize the damage and self-destruct. Is it not reasonable to also consider other possible targets, which might be cytoplasmic or membranous? Damage affecting the lysosomes

or plasma membrane could result in significant changes which might destroy the oocyte. It is of interest to note here that Parsons (1962), using electron microscopy, reported changes in mitochondria of mouse oocytes following x-irradiation, and described shrinkage, followed by swelling, of the whole oocyte within 24 hours of irradiation. This has yet to be confirmed by other investigators, but suggests that elements of the cytoplasm, as well as the nucleus, may be affected by radiation. This is currently under study in this laboratory.

An explanation of immediate radiation-induced oocyte death must account for several phenomena. Age-dependent changes in radiosensitivity may reflect both structural changes within the oocyte and endocrinological changes within the animal. While the delayed death may be plausibly explained by DNA damage, the mechanism of immediate death remains unresolved. Further investigation is necessary before definite conclusions can be made about either the target or the mechanism. The author is currently involved in investigating the longer-term effects of radiation on mouse ovaries, and the above-mentioned electron microscope examination is taking place in this laboratory. Further investigations which are being planned include a determination of the gross target volume using neutron irradiation, and further studies into the hormonal factors affecting radiosensitivity.

SUMMARY

It has been shown that there are three distinct phases of radiosensitivity in oocytes of prepubertal mice: 1) a period of rapidly increasing sensitivity between 0 and 4 days of age, 2) a period of consistent, high sensitivity between 5 and 18 days of age, and 3) a period of decreasing sensitivity from 19 to at least 21 days of age. Two distinct phases have been demonstrated for the rate of population decline of the oocytes of primary follicles: 1) an initial period of rapid loss from 0 to 4 days of age, and 2) a period of much slower loss from 5 through 23 days of age. Correlations have been drawn between the first two phases of radiosensitivity and morphological changes in the oocyte, and between the third phase of radiosensitivity and

endocrinological changes in the maturing animal.

The reaction of oocytes to radiation has been separated into two categories: 1) immediate death (within 24 hours), and 2) delayed death (over the entire lifespan of the animal). These reactions have been discussed in relation to previously-published theories of radiation-induced cell death. Though a plausible explanation has been discussed providing an explanation for delayed death, the mechanism resulting in immediate death is probably different and as yet unexplained. It has been concluded that further investigations are necessary before this extreme sensitivity can be understood, and future lines of investigation have been presented.

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