AN ELECTRON MICROSCOPE STUDY OF
RADIATION DAMAGE IN THE MOUSE OOCYTE

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ABSTRACT

Cytological changes occurring in young oocytes of the mouse, following whole-body x-irradiation, have been examined by light and electron microscopy. Two minutes after a dose of 200 r, a drop occurs in the number of mitochondria per oocyte. The normal number of mitochondria is restored in the next few minutes. Five to 6 hours after a dose of 7 r, and 30 to 45 minutes following a dose of 200 r, all the oocytes are markedly contracted. In the next 2 hours (at a dose of 7 r), some cells shrink further and become pycnotic, while others expand and show signs of karyolysis. Of the expanded cells, some (about 50 per cent of total young oocytes) become morphologically normal. In both pycnotic and karyolytic nuclei, the nucleolus was contracted but without loss of its fibrillar structure. The contracted nucleolus appeared similar to those seen in some nearly mature normal oocytes (these oocytes have a high natural death rate). No other cell type in the ovary was affected by doses of x-rays up to 200 r. Observations on the nucleus of the normal oocyte are included. In the dictyate stage of meiotic prophase the chromosomes were dispersed into bundles of 100 A microfibrils. The main component of the nucleolus was found to be tightly coiled fibrils of 60 to 100 A, which appear to have a close relationship with the microfibrils of the chromosomes.

INTRODUCTION

The extraordinary sensitivity of the young oocyte of the mouse ovary to ionizing radiation has been known for many years (1-3). Recently the irradiated mouse ovary has been studied with the light microscope in more detail (4) and the quantitative effects of varying the dose and dose rate on the killing of mouse oocytes investigated (5, 6).

In the present report previous light microscope observations are extended by the use of the electron microscope. Mouse ovaries have been observed after irradiation with doses of x-rays of from 7 to 200 r. The ovaries have been fixed at intervals from less than 1 minute to 18 hours following the irradiation. Most work has been carried out with 4-day-old mice where a large proportion of the oocytes are in the radiosensitive, dictyate phase (7-9) of meiotic prophase, but the ovaries of the young adult mouse have also been studied. The effects of x-rays on oocytes in earlier or later stages of meiotic prophase have not been determined in the present work. Normal mouse oocytes were also examined and some new observations on the structure of the nucleoli and chromosomes are reported.

MATERIALS AND METHODS

The ovaries of 110 normal and 120 irradiated 4-day-old and 10- to 20-week-old RF/Up, C3H/Cum and (C3H/Anf Cun X 101)F1 mice were used for the electron microscope and light microscope work and for the quantitative studies of structural changes in oocytes following irradiation.
The ovaries were fixed in osmium tetroxide containing sucrose (10) and dehydrated in acetone; the tissue blocks were stained with potassium permanganate (11) or phosphotungstic acid (0.2 per cent in acetone for 35 minutes) and embedded in Epon 812 (12) or methacrylate (13). For work at microscope magnifications of above 10,000 times the sections were stained with lead subacetate (14, 15). A Siemens Elmiskop I was used operating at an accelerating voltage of 60 kv.

Thick (1-micron) sections of the ovary embedded for electron microscopy were stained for light microscopy. Methacrylate sections were stained with Feulgen reagent (16) and Epon sections for 20 minutes in 2 per cent Toluidine Blue (17) at 60°C.

The irradiation was carried out using a G. E. Maxitron 300 machine with a 0.3 mm filter, a voltage of 250 kv and a current of 15 to 20 mA. X-ray doses of 7, 20, 30, 50, and 200 r were given at rates varying from 40 to 800 r/minute depending upon whether the ovary was to be fixed rapidly after irradiation or after a long interval. No portion of the body of the mouse was shielded in these experiments. Control mice were handled in the same way as the irradiated mice but without switching on the x-ray machine.

**RESULTS**

The different stages of growth of oocytes in the dictyate phase of meiotic prophase will be described with the aid of Table I (modified from Pincus (18)). Oocytes of stages I and II were found to be sensitive to x-rays while stages III and higher were radioresistant. The normal oocytes at stages I and II were structurally different from more mature oocytes in several respects, as will be described in the following sections. However, for oocytes at the same stage of maturation, no differences in normal structure, or differences in their response to x-rays, were seen between oocytes from 4-day-old mice and adult mice, or between oocytes of mice of the different strains examined. Unless otherwise stated the following results refer to the oocytes of 4-day-old RF mice.

**I. Normal Mouse Oocytes**

Oocytes at stages I and II were located peripherally in the ovary, next to the tunica albuginea, while more mature oocytes occupied the central region. Stage I oocytes were sometimes seen in the outer peritoneal-cell layer ("germinal epithelium") but no evidence of recent division or structural similarity to the peritoneal cells was obtained (19).

The tunica albuginea in ovaries from 4-day-old mice is incomplete in some areas so that oocytes lying adjacent to peritoneal cells could, perhaps, be pushed into the outer layer.

**A. The Cytoplasm**

The main features of the mammalian oocyte cytoplasm have been described by other workers (20-25). Some differences, mainly quantitative, were observed between the cytoplasm of young oocytes (stages I and II) and the cytoplasm of more mature oocytes. These differences are summarized in Table II.

**TABLE I**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Follicle cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia.</td>
<td>No follicle cells in contact with the plasma membrane of the oocyte.</td>
</tr>
<tr>
<td>Ib.</td>
<td>A few follicle cells are in contact with more than one oocyte.</td>
</tr>
<tr>
<td>IIa.</td>
<td>One or two flattened follicle cells appear in the section and independently encircle the oocyte.</td>
</tr>
<tr>
<td>IIb.</td>
<td>Portions of three or four flattened follicle cells appear in the section.</td>
</tr>
<tr>
<td>IIIa.</td>
<td>A complete ring of cuboid follicle cells surrounds the oocyte.</td>
</tr>
<tr>
<td>IIIb.</td>
<td>The follicle cells are closely packed and separation of the cytoplasm of oocyte and follicle cells with the formation of rudimentary microvilli is beginning.</td>
</tr>
<tr>
<td>IV</td>
<td>Two layers of follicle cells and a well developed zona granulosa with microvillus-like projections from oocyte and follicle cells are present.</td>
</tr>
</tbody>
</table>

**B. The Chromosome Material and the Nucleolus**

Feulgen staining of light microscope sections of ovaries from 4-day-old RF mice showed that nearly all the nuclei were in the dictyate phase of meiotic prophase. The nucleus stained lightly and evenly and no distinct chromosomes could be seen.
Occasional nuclei, near the periphery of the ovary, were seen to be in pachytene or diplotene.

After fixation with osmium tetroxide, and examination of thin sections in the electron microscope, the nuclei showed a fine granularity at low magnification, similar to that of resting somatic-cell nuclei. At higher magnification (Fig. 1), the granularity was seen to be an illusion. Apart from the nucleolus, all the intranuclear material (assumed to be derived from chromosomes) was arranged into coiled bundles ($A_1$, $A_2$ in Fig. 1), which branched and joined frequently to form a network. Bundles were seen in contact with, and usually perpendicular to, the nuclear membrane.

The main components of these coils were fine units about 100 Å in diameter that were irregularly coiled and ran together along the length of the bundles. The bundles also contained some other electron-opaque material with no resolvable structure. Occasionally, the 100 Å units, which are presumed to be microfibrils or "elementary chromosome fibrils" (26), were seen to contain two parallel filaments about 40 Å in diameter. The chromatin at stages III and IV had the same basic arrangement as has been described for stages I and II, except that the loose bundles of coiled microfibrils were more widely separated in the nuclear sap (Table II).

The nucleolus of the normal oocyte showed considerable changes in structure as the oocyte increased in size. In oocytes of stages I to IIIa the nucleolus had the reticulated appearance shown at low magnification in Fig. 2. The largest part was the reticulated portion or nucleolonema (27), which consisted of anastomosing coils of dense material that were deceptively granular at low magnification. The density of these coils was much increased in some regions (C, Fig. 2) and especially at points of anastomosis. The second-largest portion consisted of less closely packed, granular-looking material (A, Fig. 2), which was usually arranged uniformly and did not have the appearance of coiled strands. This region was found at the periphery of the nucleolus. A third region was occasionally sectioned that lay adjacent to the main mass of the nucleolus, and often between this mass and the nuclear membrane. This region

### Table II: Structural Differences Between Young (Stages I and II) and More Mature Oocytes

<table>
<thead>
<tr>
<th>Structure</th>
<th>Stage I or II</th>
<th>Stage III or higher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Golgi zone</td>
<td>Scattered flattened sacs</td>
<td>Parallel stacks of sacculles (dictyosomes 45)</td>
</tr>
<tr>
<td>Multivesicular bodies (21)</td>
<td>Few (in Golgi zone)</td>
<td>Numerous and scattered</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>Normal distribution</td>
<td>Sparser distribution</td>
</tr>
<tr>
<td>RNA granules</td>
<td>Normal distribution</td>
<td>Sparser distribution</td>
</tr>
<tr>
<td>Plasma membrane</td>
<td>Smooth, adjacent to follicle cells</td>
<td>Microvilli and zona pellucida form</td>
</tr>
<tr>
<td>Chromosomal material</td>
<td>Network of coiled 100 Å fibrils</td>
<td>Fibrils more spread out</td>
</tr>
<tr>
<td>Nucleolus</td>
<td>Typical nucleolonema</td>
<td>Nucleolonema condenses</td>
</tr>
<tr>
<td></td>
<td>Fibrils at edge of nucleolus not distinct from chromosomal fibrils</td>
<td>Sharper demarcation from chromosomal fibrils</td>
</tr>
</tbody>
</table>
No distinct separation of the nucleolar fibrils from the chromosomal fibrils could be seen. Fibrils ran into (or out of) the nucleolus from all directions (Fig. 4). In one instance (A8, Fig. 4) a fibril was untwisted into two smaller strands; several other fibrils (A3, Fig. 4) ran between the nuclear membrane and the nucleolus.

Many of the nucleoli at stages IIb and IV had either rounded up (Fig. 5) or assumed a polygonal shape, and the coils of the nucleolonema had become very dense (Table II). The other two portions of the stage I or II nucleolus were no longer present. In nearly mature oocytes, the nucleolus was often seen in an almost completely condensed state. On a few occasions, crystal-like formations were present that appeared to be derived from the condensed nucleoli.

II. Irradiated Mouse Oocytes

In agreement with the observations of other workers (4, 5) only the younger oocytes (stages I and II) were markedly sensitive to x-radiation. In the dose range 7 to 200 r no other cell type (including peritoneal cells or "germinal epithelium," follicle cells, tunica albuginea cells, or interstitial cells) was affected. Substantial agreement was obtained for the frequency of necrosis of oocytes after different doses of x-rays, whether observed by electron microscopy or by light microscopy of stained thick, methacrylate, or Epon sections. A dose of 7 r killed approximately one-half of the stage I and II oocytes in 12 to 18 hours, while doses of 50 r or more killed 100 per cent of the young oocytes. The rate of killing increased rapidly with dose. Most of the work was carried out with the dose of 7 r since the sequence of events was spread out and more easily recognizable.

A. GENERAL COURSE OF EVENTS AFTER WHOLE-BODY IRRADIATION WITH 7 TO 200 r OF X-RAYS

Light microscopy of stained thick methacrylate or Epon sections showed no change until 6 hours.
Figure 4
The nucleolonema portion of the nucleolus of a Stage IIa oocyte. The nucleolus (NU) characteristic-
ally lies close to and probably is in contact with the nuclear membranes. Three microfibrils at A1, 100 Å in diameter, run a fairly straight course between the nuclear membrane and the nucleolus. One microfibril at A2 has untwisted into two finer strands. The nucleolonema consists mainly of coiled fibrils, of 60 to 100 Å diameter, similar to, but denser and more tightly coiled than, the micro-
fibrils. Most of the small granules appear to consist of a transverse or oblique section or projections of small coils while others are structureless. Tissue embedded in methacrylate and section stained with lead subacetate. × 77,000.

Figure 5
A nucleolus of a Stage IIIb or IV oocyte is shown for comparison with that of Fig. 2. The outer loosely granular portion (Fig. 2, A) is not seen, and the coils of the nucleolonema have condensed and become uniformly dense. The nucleolus tends to assume a round or sometimes polygonal shape. At later stages, these changes are more marked and the nucleolus in some oocytes forms a uniform-looki	}

ng sphere or crystal-like body. NU = nucleolus, NM = nuclear membrane. Tissue embedded in Epon and section stained with lead subacetate. × 11,000.

after 7 hr. At this time shrinkage of the whole oocyte occurred (Fig. 6) but no necrotic cells were seen. At 7 hours after 7 hr, a few of the cells showed clumped chromatin and an irregular nuclear membrane. At 8 hours, cells of this same appearance were swollen. At this time other cells showed moderate pycnosis. At 10 to 12 hours after 7 hr, pycnosis and karyolysis were marked (Fig. 6). Intermediate types of degeneration, i.e. cells showing shrinkage but also with weakly staining nuclei, were seen. In the interval 12 to 24 hours after 7 hr, necrotic oocytes were removed and the survivors were similar to the control oocytes.

The cell volume changes were studied by measuring the diameter of the oocyte nucleus using
stained thick sections. The diameter of the whole cell was previously found to change in proportion to that of the nucleus. Frequency curves of nuclear diameter for control and irradiated (7 r) oocytes are shown in Fig. 7. At this dose 50 per cent of the young oocytes survived, and since the curves are essentially monophasic presumably all the cells are initially contracted. The volume changes for doses of 7 and 200 r are plotted against time in Fig. 8. Essentially, two populations (pycnotic and karyolytic) of cells are present. With a dose of 7 r pycnosis does not begin until 6 hours after irradiation, while it begins as early as 30 minutes after a dose of 200 r.

B. Electron Microscopy of Irradiated Oocytes

Since the time required for radiation damage (cell shrinkage, pycnosis, karyolysis, etc.) depends markedly on the dose (Fig. 8), the sequence of cytological changes will be arbitrarily divided into two phases. Phase I extends from the start of the irradiation to the onset of contraction of the oocytes. Phase II covers the interval between cell contraction and cell death.

1. CYTOLOGICAL CHANGES IN PHASE I OF RADIATION DAMAGE

Ovaries were fixed in the shortest possible time (30 seconds) from the start of the irradiation. The dose (30 or 200 r) was delivered in a short time (15 seconds). Ovaries were fixed in isotonic osmium tetroxide (10) and tissue was embedded in epoxy resin in order to eliminate artifacts, especially in mitochondria, due to swelling in a hypotonic fixative or to polymerization damage.

A. CYTOPLASMIC CHANGES: Although no damage was observed in the membranes of mitochondria, marked changes occurred in the number of mitochondria per cell and in their size following irradiation. The number of intact and abnormal mitochondria (mitochondria showing swelling, high density, or irregular membranes) was counted per unit area of the cytoplasm for oocytes at stages I and II. Efforts were made to keep the section thickness constant. The area of the cytoplasm was estimated from micrographs by cutting out its shape in tracing paper and weighing the pieces. The modal value of the diameter of the mainly spherical mitochondria (only mitochondria cut normally were counted) was also measured from frequency diagrams. The modal values were compared at different times after irradiation. The results are summarized in Fig. 9. At a dose of 200 r a significant drop in the number of mitochondria per unit area of cytoplasm occurred, at as short a time after the start of the irradiation as 2 minutes. A "rebound" followed the drop and the number of mitochondria then returned to normal values. At 30 r a drop in the number of mitochondria per unit area probably occurred but the data were insufficient to determine its statistical significance. No significant change occurred at a dose of 7 r. The modal value of the diameter of the mitochondria could be more accurately measured from some hundreds of observations for each interval after the irradiation. With a dose of 200 r, the mitochondria decreased in size just at the time when the "rebound" or restoration of the normal number of mitochondria occurred (Fig. 9).

At the time at which these mitochondrial changes occurred, no appreciable cell volume changes were observed, so that the decrease in concentration of mitochondria represents a decrease in the number of mitochondria per oocyte (only oocytes of the same size, stages Ib–IIa, were counted). Abnormal mitochondria were counted over the same time interval. At a dose of 200 r, an increase in abnormal forms probably was significant but the rise was very much smaller than the drop in intact mitochondria and occurred at a slightly later time. Probably, no significant change in numbers of abnormal mitochondria occurred with a dose of 30 r.

During phase I of radiation damage no changes were seen in the Golgi zone, centrioles, multivesicular bodies, or plasma membrane. Fat vacuoles were infrequently seen in the oocyte cytoplasm and were not increased after irradiation. With a dose of 200 r, a depletion in RNA granules in some areas of the cytoplasm was observed after 16 minutes. At lower doses (7 r, 30 r, 50 r) no changes in the RNA granules were seen.

B. NUCLEAR CHANGES: The chromosomal microfibrils did not appear as evenly distributed, especially around the nucleus, as in the normal oocytes. However, no large aggregates of fibrils were seen. Light microscopy of Toluidine Blue-stained thick Epon sections of ovary irradiated with 200 r showed no loss of nucleoli at any stage of cell degeneration. Toluidine Blue staining of the nucleoli was slightly less uniform in the interval 4 to 30 minutes after 200 r. At the same time, after 200 r, portions of the nucleolonema occasionally showed extra high density in the electron microscope.

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2. CYTOLOGICAL CHANGES IN PHASE II OF RADIATION DAMAGE

A. CELL SHRINKAGE: The only changes observable at this stage were an increase in density of the cytoplasm and a slight unevenness of the chromatin.

B. CELL NECROSIS; PYCNOSIS AND KARYOLYSIS: The most common type of necrosis was pycnosis. A low power view of a group of pycnotic cells 8 hours after 7 r is shown in Fig. 10. The cytoplasm and nucleus of the oocyte O2 are shrunken and dense while the adjacent follicle cells appear normal.

The nucleolus showed increased density and loss of the reticulated structure early in Phase II. At the same time, or a little later, the chromatin began to clump into dense masses, usually leaving a lighter zone around the nucleolus. In early pycnosis the cytoplasm was denser than that of the adjacent follicle cells. The mitochondria appeared normal with a dose of 7 r, but were swollen at this stage after a dose of 200 r. Fat vacuoles were sometimes present in the cytoplasm.

A late stage of pycnosis is illustrated in Fig. 11. The whole cell is very dense. The nucleolus is condensed but still shows a basic fibrillar structure at high magnification. Fibrils 200 to 500 A in diameter are recognizable in the closely packed chromatin. The cytoplasm now shows extensive changes. The mitochondria are dense and the cristae are indistinct. Vacuoles are present in the cytoplasm. RNA granules are preserved and their close packing accounts, in large measure, for the increased density of the cytoplasm. The intactness of the adjacent follicle cells (Fig. 11) is very striking.

A portion of an oocyte at an intermediate stage of karyolysis is shown in Fig. 12. The nuclear membrane is smooth and light areas are scattered throughout the nucleus. Just as with pycnotic cells, the most prominent light areas surround the nucleolus. In most areas of the nucleus the microfibrils have a normal size, shape, and distribution, while in other areas they are aggregated into small clusters (D in Fig. 12). The light areas (L in Fig. 12) contain 200 A fibrils of low density. As for pycnotic cells, condensation of the nucleolus is evident in karyolytic cells occurred early in Phase II.

The late stage of karyolysis is illustrated by Fig. 13. The nucleus is nearly empty of chromatin. In the light areas there is an even distribution of fine fibrils. Small dense aggregations (D in Fig. 13) can be found in the remaining chromatin. The nucleolus has contracted markedly and shows a very high electron opacity. However, fibrils can still be recognized throughout its structure. The nuclear membrane is irregular. At this late stage of karyolysis the cytoplasm shows more damage. Some of the mitochondria show loss of cristae and irregularity of outline. Many of the RNA granules, however, appear intact.

The described radiation changes in the oocyte are summarized in Table III.
DISCUSSION AND CONCLUSIONS

A. Effects of Irradiation with X-Rays

It is generally assumed that cell injury or cell death following irradiation with x-rays results from damage to one, or a few, critical cell structures ("targets") or substances. In so far as structures are concerned, the application of the electron microscope to the study of radiation damage might well be expected to give more detailed and earlier evidence of affected organelles than could be obtained with the light microscope. However, where structural damage occurs, it is difficult to decide whether this represents the primary lesion or an effect secondary to it. The end effects of ionizing radiation, such as cell death, are frequently delayed hours or days after the irradiation. It is evident that the terminal effects may not be specific for radiation damage but simply represent a general reaction of the cell to injury. Furthermore, structural changes that occur rapidly after the irradiation, and that are still seen with the lowest doses which give the terminal effects, are probably more significant than late effects, or those which occur only with very high doses.

In several respects, the young oocytes of the mouse seem particularly favorable cell types for the study of radiation damage. A low dose (7 r) will kill 50 per cent of the cells so that the complex effects of high doses are avoided. Interpretation of the observed cell changes is facilitated by the homogeneity of the young oocyte cell population in the ovaries of the 4-day-old mice. Most of the oocytes are arrested in meiotic prophase and are also at the same stage of maturation. One disadvantage of radiation studies with oocytes is the susceptibility of this cell type to hormones. Because of the impracticability of shielding all the endocrine glands, the present work was carried out with unshielded whole-body irradiation.

In the present study, no cell structure has been observed to show marked damage within a short interval (a few minutes) following a low dose (7 r) of x-rays. However, within a few minutes of the start of irradiation with 200 r (and possibly with 30 r) a sharp drop in the number of mito-

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**Figure 7**

Frequency diagrams of the diameter of the nucleus of the same Feulgen-stained material as shown in Fig. 6. The ordinate gives the number of cells having diameters within 0.5 mm (magnification, X 1300) of the values given on the abscissa. The modal values (microns) of the diameter at different times after irradiation are also given. The curves are essentially monophasic at the time at which the greatest contraction of the cells occurs, indicating that all the cells are affected. Only one-half of the cells eventually became necrotic. The small peaks by the side of the large ones represent early pycnotic cells.

**Figure 8**

The dependence of the time of oocyte contraction and onset of pycnosis on the dose. At a dose of 200 r pycnotic cells appear 30 to 45 minutes after irradiation, while at a dose of 7 r they do not appear until 6 to 7 hours after irradiation. The difference in the nuclear size of the controls (0 minutes) in the two experiments is probably due to the greater shrinkage of tissues in the methacrylate-embedding medium as compared with the tissues embedded in Epon.

**Figure 9**

Changes occurring in the oocyte mitochondria within a few minutes of an x-ray dose of 30 and 200 r. The number of mitochondria per unit area (arbitrary values) of the cytoplasm drops at 2 minutes after 200 r and then rebounds to a greater than normal value. The changes at a dose of 30 r appear smaller and may not be significant. The recovery in numbers of mitochondria is accompanied by a drop in the modal value of mitochondria diameter and probably indicates the renewed fission of mitochondria. An increase in abnormal mitochondria may be significant at 200, but not at 30 r. This increase is insufficient to account for the drop in numbers of intact mitochondria.
Pycnosis begins (200r) and karyolitic cells (20Or) surviving and karyolitic cells (7r) (Epon-embedded) pycnotic cells and karyolitic cells (7r) (Methacrylate-embedded) pycnotic cells

Abnormal mitochondria (20Or) and abnormal mitochondria (3Or) Abnormal mitochondria (20Or) and abnormal mitochondria (3Or)

MITOCHONDRIA, UNIT AREA OF CYTOSOL IN SECTION

MITOCHONDRIA, UNIT AREA OF CYTOSOL IN SECTION

TIME AFTER IRRADIATION (min)

TIME AFTER IRRADIATION (min)
Figure 10

Low magnification view of outer part of ovary 8 hours after a dose of 7 r. As illustrated, all the oocytes in one "cell nest" are often found to be simultaneously affected. The oocyte at O4, which is at a slightly later stage of growth, is unaffected. The oocyte at O2 shows advanced pycnosis. The nucleolus (NU) is completely condensed, the nuclear membrane is irregular, and the cytoplasm is very dense. In spite of the complete necrosis of the oocytes at O4, O5, and O6, the follicle cells (F) appear normal. Stained with potassium permanganate and embedded in Epon. X 4,700.

Mitochondria in the young oocyte cytoplasm occurred, and was followed by a rapid restoration to the normal number of mitochondria. At the same time a transient decrease in the modal diameter of the mitochondria took place. The rapidity of these changes, and the absence of a sufficiently large increase in altered mitochondria, appear to rule out the possibility that mitochondria are destroyed by x-rays, and that the normal number of mitochondria are then restored by formation from mitochondrial precursors (29, 30). It seems most likely that the process of fragmentation of mitochondria, which can be observed by phase contrast microcinematography (31), is inhibited for a minute or two and then recommences. Frederic and Chêvremon (31) have shown that a spherical mitochondrion can fragment into two or more smaller mitochondria in the course of 1 minute. The present results on the transient changes in mitochondria appear identical with those reported by Noyes and Smith (32) for the reduction of the number of mitochondria in rat liver, less than 15 minutes after a dose of 1000 r of gamma rays. Mitochondria were isolated from liver homogenates. However, the conclusion of these workers, that smaller mitochondria were preferentially fragmented and the fragments absorbed by larger mitochondria, cannot be confirmed by the present electron microscope observations. The explanation offered here, of arrest in fission of mitochondria, would explain the observation by Noyes and Smith that the average unit mass of the mitochondria is transiently increased in the time that the numbers of mitochondria are decreased. The
A portion of a Stage II oocyte in an advanced stage of pycnosis (8 hours after 7 r). The nucleus is shrunken and very dense. The lighter areas (L) appear, at higher magnification, to contain mainly closely packed microfibrils. Small dense aggregates (D) are also seen. The nucleoli (NU) are just recognizable because of their outline and suggestion of the spaces of the original nucleolonema. The perinuclear space (NM) is slightly swollen. The mitochondria (M) are dense and show indistinct cristae. Some large smooth walled vacuoles (V) are present in the cytoplasm, and the Golgi vacuoles (G) are dilated. The RNA granules are unaffected. Most of the density of the cytoplasm is caused by close packing of the RNA granules. The adjacent follicle cell (F) and the neighboring portion of another oocyte (O) appear normal. Stained 30 minutes with 0.2 per cent phosphotungstic acid in acetone and embedded in Epon. X 15,000.

population sampled at this time would contain a larger proportion of undivided forms than is usual. Although it appears that doses in the range 30 to 200 r impair active movements of mitochondria, there is at present no other evidence that they are affected in a way that could lead to cell death. Structural damage to mitochondria has been reported for a number of cell types after irradiation with doses of ionizing radiation of 1000 r or more (33-36).

The earliest permanent morphological change was in the nucleolus. Possibly, an uneven distribution of chromatin developed previous to this, but this change was difficult to assess. Definite changes in distribution of chromosome microfibrils, with the formation of small spaces, particularly around the nucleolus, took place shortly after the occurrence of contraction of the nucleolus. Shrinkage of the nucleolus, with loss of the spaces between the coils of the nucleolonema and an increase in electron opacity, occurred in cells showing either karyolysis or pycnosis. These changes were similar to those seen in some unirradiated, nearly mature oocytes. Since the majority of mature oocytes die in the normal ovary and do not ovulate, it appears possible that the young oocyte nucleolus is, in
some way, in an unstable condition, and that x-rays might accelerate degenerative changes in it.

The changes in the chromosomal microfibrils are different for karyolytic and pycnotic nuclei. In the former, the formation of light areas in the nucleus presumably indicates the removal of chromatin by autolytic enzymes. Although small clumps of microfibrils also occurred, the aggregation was not sufficient to explain the formation of the light areas. This is in contrast to reported changes in the nuclei of rat spermatids after high (10^6 r) doses of 14 Mev electrons (37) where peripheral margination of chromatin occurred. The source of the proteases and nuclease in the karyolytic cell is unknown but the observation of the release of DNAse from isolated mitochondrial preparations after high doses of ionizing radiation (34) may have some relevance to the present observations.

In pycnotic nuclei the microfibrils appear to be preserved. This is in agreement with the conservation of DNA in the nucleus at the earlier stages of pycnosis, as measured by chemical analysis (38) or microspectrophotometrically (39).

As far as late changes in other cell organelles are concerned, the preservation of RNA granules in severely degenerate cells, after irradiation with doses of x-rays less than 200 r, was surprising. Basophilia of the cytoplasm is usually decreased for cells in unfavorable conditions. Liver cells showed decreased endoplasmic reticulum, including RNA granules, in rats after fasting or poisoning with carbon tetrachloride (40). In the present study a dose of 200 r caused slight depletion of oocyte RNA granules midway through Phase I of radiation damage. A marked depletion of the RNA granules of intestinal epithelial cells 3 days after a dose of 3000 r has been reported (41).

The significance of the marked contraction in cell volume following low doses (7 r) of x-rays is not clear. Pycnosis may represent simply a continuation of the loss of water content in some of the irradiated oocytes. Since very little is known about the control of water absorption and secretion by the cell, there is no indication, at present, as to where the x-rays are acting to produce this effect.

A comparison of the morphology of oocytes in the radiosensitive and radioresistant phases (Table II) indicates that the nucleoli and Golgi zone show the most striking differences. However, the number of surrounding follicle cells also increases and the zona pellucida develops. The present work gives no indication as to which, if any, of these changes is responsible for the loss of radioresistivity of the young oocyte.

B. Structure of the Normal Oocyte Nucleus

The chromosomal material of the oocyte nucleus in the dictyate phase has been shown to be present...
### TABLE III

**Summary of Radiation Damage in the Mouse Oocyte**

<table>
<thead>
<tr>
<th>Time from Start of Irradiation</th>
<th>Whole-Body Dose of X-Rays</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 r</td>
</tr>
<tr>
<td>20 seconds to 8 minutes</td>
<td>No visible change in chromatin or nucleolus. No visible change in mitochondria.</td>
</tr>
<tr>
<td>16 minutes</td>
<td>No visible change in RNA granules at any stage.</td>
</tr>
<tr>
<td>30 minutes</td>
<td>Cell contraction begins.</td>
</tr>
<tr>
<td>45 minutes</td>
<td>Nucleolus contracts and becomes dense. Chromatin uneven with a space around the nucleolus. Some cells swell and the nuclei show karyolysis with loss of microfibrils. Nuclear membrane of these cells is irregular. Other cells contract further and become pycnotic with no loss of microfibrils.</td>
</tr>
<tr>
<td>5 hours</td>
<td>Cell contraction begins.</td>
</tr>
<tr>
<td>7 hours</td>
<td>Nucleolus contracts and becomes dense. Chromatin uneven with a space around the nucleolus. Some cells swell and the nuclei show karyolysis with loss of microfibrils. Nuclear membrane of these cells is irregular. Other cells contract further and become pycnotic with no loss of microfibrils.</td>
</tr>
<tr>
<td>12 hours</td>
<td>Necrosis nearly complete.</td>
</tr>
<tr>
<td>24 hours</td>
<td>Most necrotic debris removed. Surviving cells appear identical with normal oocytes but are slightly swollen.</td>
</tr>
</tbody>
</table>

As loose bundles (of about 0.1 micron diameter) containing microfibrils of 100 A diameter similar to those reported for a variety of cells (26). The 100 A fibril appears also to contain two 40 A units.

Present observations indicate that tightly coiled fibrils of 60 to 100 A diameter are the main component of the oocyte nucleolus. No distinct separation of the nucleolar material from the main mass of 100 A microfibrils (presumably chromosomal material) could be seen. Fibrils could be traced running between the two types of structure. These observations would be consistent with the hypothesis that the nucleolus is made up by the aggregation and intercoiling of a special portion of the chromosome microfibril which is associated with RNA. While there is no convincing analytical or cytochemical evidence that the nucleolus contains DNA, autoradiographic results (42) indicate that DNA synthesis commences at the nucleolus and then spreads to the rest of the nucleus. Recent autoradiographic (43) and interferometric (44) evidence that the nucleolar material is not degraded in mitosis but is simply dispersed would also be compatible with the hypothesis.

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