SOME OBSERVATIONS ON THE FINE STRUCTURE OF THE LUTEIN CELLS OF X-IRRADIATED RAT OVARY

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ABSTRACT

The ovaries of 10- to 13-week-old rats were exteriorized and irradiated with sterilizing doses of X-rays. Following treatment, the animals entered a phase of constant vaginal cornification. Animals were killed 8 to 12 wk after the onset of this phase, and their ovaries were prepared for electron microscopy. Tissue was fixed in glutaraldehyde, postfixed in osmium tetroxide (Millonig's, phosphate-buffered), and embedded in Epon. Lutein cells from these ovaries were compared with those from sham-irradiated controls. The cytoplasm of lutein cells from experimental animals was characterized by an increase in the amount of agranular endoplasmic reticulum and by an increase in the number of mitochondria. These mitochondria are more variable in external form and often possess increased numbers of villiform cristae. Other features noted were a decrease in the amount of cytoplasmic lipid granules and an increase in cell size and surface irregularity. The significance of the morphological findings is discussed in relation to ovarian hormone production in animals sterilized by X-irradiation.

INTRODUCTION

Mandl and Zuckerman (9) have shown that adult rats enter a phase of constant vaginal cornification after irradiation of their ovaries by sterilizing doses of X-rays which produce complete destruction of follicles and oocytes and that, in these circumstances, the resulting constant vaginal cornification can be terminated by total ovariectomy. It has been suggested that even normal cyclic estrus can occur in the absence of the follicular system of the ovary (12–16).

It would appear, then, that the cells remaining in the ovary, after treatment with large doses of X-rays, must be responsible for production of the factor which causes constant vaginal cornification. This view is supported by the work of Westman (18) which involved parabiosis between normal and X-ray-sterilized rats.

The present study was undertaken in order to observe what morphological changes were present, in the ovarian tissues remaining after X-irradiation, concurrent with constant vaginal cornification.

MATERIALS AND METHODS

The animals used were albino rats of a Sheffield Wistar strain, bred at the Medical School in Leeds England.

At 10 to 13 wk of age, rats were subjected to bilateral exteriorization of their ovaries, using a dorsal approach, under sodium amytal anesthesia. The technique of Mandl and Zuckerman (9) was followed,
in which the remainder of the animal's body is shielded by lead. Irradiation of the exposed ovaries was then carried out, with unfiltered soft X-rays at 200 kv and 15 ma, having a half-layer value of 0.5 mm of copper. The focus-to-source distance was 20 cm.

The animals received total doses of 4700 ± 300 R, during a continuous period of 9 min, at a dose rate of approximately 325 R per minute. Dose rate was measured with a Baldwin-Farmer substandard X-ray dosimeter (Baldwin Instrument Company, Ltd., Dartford, England), and the total dose with Con-Rad LiF, type N and Con-Rad TLD thermoluminescence dosimeters (Controls for Radiation, Inc., Cambridge, Massachusetts) (2). Control animals were sham-irradiated by subjecting them to the full surgical procedure, but the X-ray source was not activated.

Following operation, vaginal smears were taken daily in order to monitor vaginal cornification. Experimental animals were sacrificed 8 to 12 wk post-operatively, several weeks after they had entered the phase of constant vaginal cornification.

The controls were sacrificed when at full estrus, as shown by vaginal smear. At necropsy the ovaries were removed for processing for both light and electron microscopy. For electron microscopy, the tissue was cut into 1 mm³ blocks and fixed for 4 hr at 0 to 4 °C in 5% glutaraldehyde (6), buffered with 0.067 M cacodylate at pH 7.2. After being washed for a minimum period of 16 hr with cold 0.25 M sucrose in 0.1 M cacodylate buffer, the tissue was postfixed in cold, phosphate-saline and embedded in paraffin wax. Sections were stained with 1% toluidine blue in ethanol, and examined by light microscopy so that the sections examined in the electron microscope could be correlated with the histological appearance of the tissue under investigation.

For light microscopy, tissue was fixed in formaldehyde-saline and embedded in paraffin wax. Sections were stained with haematoxylin and eosin and examined for evidence of luteinization and destruction of follicular structures.

**Observations**

**Light Microscopy**

Light microscope examination of serial sections of paraffin preparations reveals the total absence of oocytes and follicles in the X-irradiated rat ovaries. The picture presented by these ovaries is dominated by large luteinized cells usually organized in bodies resembling corpora lutea. No corpora albicantes were observed in sections.

The luteinized cells observed in X-irradiated rat ovaries resemble the granulosa lutein cells of normal corpora lutea, with respect to their large pale staining nuclei with prominent nucleoli, and abundant cytoplasm (Figs. 1 and 2).

**Electron Microscopy**

Comparison between lutein cells from irradiated and control ovaries shows a number of differences. The lutein cells of the former are considerably larger, the increase in size being almost entirely due to an increase in the amount of cytoplasm.

The cell membrane of control lutein cells (Fig. 3) shows some folds and projections. The surface irregularity is most marked on cell margins which face on perivascular spaces surrounding the endothelial cells of capillaries. The cell membranes between adjacent lutein cells are generally smooth. The extent of surface irregularity is increased in irradiated cells (Fig. 4) and often extends between adjacent cells, appearing as folds and microvillous projections.

**Lutein Cells from Control Ovaries**

The cytoplasm of lutein cells from control animals (Fig. 5) shows elements of the agranular endoplasmic reticulum in the form of both vesicles and irregularly branching tubules. In all animals examined, the amount of endoplasmic reticulum varied considerably from one cell to another. Abundant free ribosomes are present, usually aggregated into groups of four to six units. Ribosomes are also found, less frequently, attached to membranes of the endoplasmic reticulum, which then takes the form of parallel arrays of cisternal elements. The number of membrane pairs, which are arranged in this manner, is usually restricted to two or three in these cells, as seen in sections.

In control lutein cells, the Golgi zones appear to be widely distributed in the cytoplasm around the nucleus and consist of small stacks of cisternae with very few associated vesicles.

Numerous mitochondria are present in the cytoplasm of the lutein cells from the controls. Their outline is most commonly round or oval, although occasional cup-shaped or elongated forms are seen, and they possess villiform or tubular cristae,
**Figure 1** Sham-irradiated control ovary showing portion of corpus luteum. The upper part of the field shows closely packed granulosa lutein cells (GL). Photomicrograph. × 400.

**Figure 2** X-irradiated ovary showing luteinized tissue, the cells of which resemble the lutein cells of Fig. 1. Photomicrograph. × 400.
FIGURE 3  Lutein cell from ovary of sham-irradiated rat. The cell surface is seen to be relatively smooth. Large numbers of lipid granules are present. \( \times 12,000 \).

FIGURE 4  Ovary of X-irradiated rat showing portions of two lutein cells. Cell surfaces are irregular and have numerous invaginations (\( v \)). The cytoplasm has many tubules and vesicles belonging to the smooth endoplasmic reticulum. The mitochondria (\( m \)) show villiform cristae, characteristic of these cells. Their numbers appear to be increased and their shape and size more variable. \( \times 12,000 \).
Figure 5  Lutein cell from ovary of sham-irradiated rat showing smooth endoplasmic reticulum, Golgi zone (g), and numerous free ribosomes, the majority of which are associated as polysomes. The nuclear membrane is interrupted by nuclear pores (arrows). X 16,500.

Figure 6  Cytoplasm of X-irradiated lutein cell is filled with smooth endoplasmic reticulum (ser). The interiors of the mitochondria show increased numbers of villiform cristae. Numerous lysosomes (ly) are present in the peripheral cytoplasm. The cell surface (arrows) is seen to be highly irregular. X 16,500.
considered to be characteristic of the mitochondria of steroid-producing cells (1, 7). They possess a dark matrix and usually contain a number of intramitochondrial granules. Less frequently, more elongate mitochondria having lamelliform cristae are found. These form only a small proportion of the mitochondrial population of these cells.

The cytoplasm of many control lutein cells contains large numbers of lipid droplets (Fig. 3), which appear to be accumulated in one area.

**Lutein Cells from X-Irradiated Ovaries**

The lutein cells from irradiated animals are larger than those from controls and show some striking differences in cytoplasmic appearance.

The number of mitochondria per cell appears to be greatly increased and the proportion of the cytoplasm which they occupy is also increased. The mitochondria are commonly enlarged and are more variable in form (Figs. 7 to 9) than those from control lutein cells. While many mitochondria appear to be spherical in shape, others are elongated or cup-shaped. These forms are more common than in control lutein cells. The matrix of these mitochondria is darker than the ground cytoplasm and they possess abundant villiform cristae (Fig. 8) which are often tortuous and closely packed. Those mitochondria which have this type of structure are usually of the spherical type and possess numerous intramitochondrial granules. Lamelliform cristae are also found, but are more commonly present in the elongated type of mitochondrion. Different forms of mitochondria are found in the cytoplasm of any one cell.

Lutein cells from X-irradiated ovaries contain abundant agranular endoplasmic reticulum. This frequently occupies most of the cytoplasm and is the dominating structure of these cells (Figs. 6 and 10). The amount of endoplasmic reticulum present is consistently much greater than in control cells. The reticulum takes the form of tortuous branching tubules or vesicles (Fig. 4), often closely associated with mitochondria and containing variable quantities of finely granular material. Tubular and vesicular forms of reticulum are often observed in the same cell, and on this basis no significant difference between different lutein cells from ovaries of X-irradiated animals could be found. The vesicular form is a common feature of lutein cells from all animals examined, although it is more frequently found in experimental animals than in controls. It is to be found in cells which possess well preserved lipid granules, in preparations showing well preserved erythrocytes. For this reason we do not consider the presence of vesicular agranular reticulum to be necessarily indicative of inadequate fixation.

Since there appears to be little constancy in the size of the elements of the endoplasmic reticulum, the recognition of Golgi zones is difficult. When observed, the Golgi zones do not appear to differ in structure or number from those of controls.

Free ribosomes are numerous, but their numbers and state of aggregation are similar to those of control lutein cells. Cisternal elements of endoplasmic reticulum with attached ribosomes are to be found in the cytoplasm, near the nucleus. No difference is observed between lutein cells from experimental and control ovaries, with respect to this organelle.

The number of lipid globules present in the cytoplasm appears to be reduced in lutein cells from irradiated ovaries. Those globules that remain are more variable with respect to their density and size. They are usually scattered diffusely throughout the cytoplasm, and many are found in close apposition to mitochondria (Fig. 8).

Lutein cells from both X-irradiated and control ovaries possess large nuclei which are somewhat irregular in outline. The perinuclear cisternae are perforated by numerous nuclear pores. In all cells examined, prominent skeinlike nucleoli were present.

**DISCUSSION**

The preceding observations compare the fine structure of lutein cells from ovaries of control animals with that of lutein cells from ovaries of animals that received sterilizing doses of X-rays. In both cases, ovaries were removed from animals in estrus, the criterion for this being maximal vaginal cornification.

We have used the term "lutein cells" to describe the cells observed, in this study, in both control and experimental ovaries. Clearly this term could be open to the criticism that the origin of the cells found in X-irradiated ovaries may not be from the granulosa lutein cells of normal ovaries. However, in view of the resemblance in histological preparations of the former to normal granulosa lutein cells and the similarity of their ultrastructure to that of the stimulated lutein cells described by Enders and Lyons (3), we consider our ter-
Figure 7  X-irradiation. Cytoplasmic detail from lutein cells showing interdigitating cytoplasmic processes of adjacent cells. × 15,000.

Figure 8  Mitochondria from lutein cell of X-irradiated rat ovary are closely associated with lipid globules. The mitochondria possess abundant villiform cristae in a finely granular matrix. × 20,000.
Figure 9 X-irradiated ovary. Detail of cytoplasm of lutein cell showing mitochondria and agranular endoplasmic reticulum. \( \times 48,000 \).

Figure 10 X-irradiated ovary, showing a portion of cytoplasm filled with agranular endoplasmic reticulum. \( \times 48,000 \).
The appearance of the control lutein cells is similar to that described by previous workers (4, 7), the characteristic mitochondria with villiform cristae and dense matrix and the abundant agranular endoplasmic reticulum being features which make these cells relatively easy to recognize in electron micrographs. Enders and Lyons (5) suggested that the abundance of agranular endoplasmic reticulum may have functional significance in the lutein cells, and the relevance of this organelle to steroid production has been considered by Christensen and Fawcett (3). Enders and Lyons (5) reported an increase in the amount of agranular endoplasmic reticulum in lutein cells of rats treated with luteotrophic (or mammotrophic) hormone (LTH), comparable to the increase found in X-irradiated rat ovaries in the present study. This increase in amount of endoplasmic reticulum is, in our opinion, of particular relevance to steroid secretion in these ovaries.

Other similarities between lutein cells of X-irradiated and LTH-stimulated rat ovaries are also evident. The most notable are an increase in cell size, the more frequent occurrence of cup-shaped and otherwise “abnormal” mitochondria, and the decrease in the amount of lipid granules present. Enders and Lyons (5) considered these features to be those of active lutein cells.

It is possible that X-ray sterilization of rat ovaries, by destroying the normal structure of the ovary, may interfere with a feedback mechanism operating on the anterior pituitary and thus permit uncontrolled secretion of pituitary gonadotrophins (18). The constant vaginal cornification, which results from X-ray sterilization, could be explained if it could be shown, by biochemical studies, that the stimulated lutein cells are secreting estrus-producing hormones. Tentative support for this suggestion is supplied by the clinical observations of Smith and Emerson (17), who found an increase in urinary estrogen in postmenopausal women with mammary carcinoma whose ovaries had been X-irradiated.

Irradiated rat ovary lutein cells, in the present study, exhibit a feature which is not described by Enders and Lyons (5) in LTH-stimulated cells. This feature is an apparent increase in the number of mitochondria per unit of cell area and per cell, particularly of the enlarged and spherical types of mitochondria, and in the number of their villiform cristae. This feature suggests that these cells may be highly active, especially since lipid globules are commonly associated closely with these mitochondria. Although the agranular endoplasmic reticulum appears to be the organelle which is directly responsible for steroid synthesis in the cells under consideration, the energy and reduced pyridine nucleotides required for this process must be produced by mitochondrial activity. We therefore regard the increase in the number of mitochondria, which parallels the increase in the amount of agranular endoplasmic reticulum, as having some significance as an index of cellular activity, though not necessarily specific for steroid production. The decrease in the number of lipid globules also suggests that lipid is being utilized by the active lutein cells.

None of the changes described can be regarded as being caused specifically by X-irradiation. The general appearance of the cytoplasm of lutein cells from experimental animals resembles, in most respects, that of LTH-stimulated cells and is compatible with that of active steroid-secreting cells. It is realized that the normal lutein cells described above as controls may have been taken from inactive corpora lutea.

As the lutein cells described in this study are apparently the only cells present in X-ray sterilized ovaries which appear, morphologically, to have the capacity for steroid production, we consider it probable that these cells are responsible for the constant vaginal cornification observed. At present, the sole criterion of estrogenic activity in the rat is vaginal cornification. In order to overcome this limitation, work is now in progress that is designed to estimate the output of estrogens by normal and X-irradiated rat ovaries, using in vitro biochemical techniques.

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