METABOLIC AND ULTRASTRUCTURAL CHANGES IN THE FROG OVARIAN FOLLICLE IN RESPONSE TO PITUITARY STIMULATION

JOHN WALBERG ANDERSON and MILTON B. YATVIN

From the Department of Anatomy and Radiobiology Research Laboratories, University of Wisconsin Medical School, Madison, Wisconsin 53706

ABSTRACT

Frog ovarian fragments were prevented from ovulating in vitro by the addition of actinomycin D up to 3 hr following pituitary stimulation; but addition of Actinomycin D 6 hr after stimulation was far less effective. Puromycin, on the other hand, effectively inhibited ovulation when added as late as 6 hr after pituitary stimulation. Although actinomycin D reduced uptake of uridine-3H, and puromycin reduced uptake of leucine-4H and lysine-14C by pituitary-stimulated ovarian tissue minus oocytes (OTMO) in vitro, it was found that pituitary stimulation did not significantly increase uptake of these compounds by OTMO. Radioautographs of ovarian follicles fixed 6 hr after the addition of pituitary extract and uridine-3H in vitro revealed increased RNA synthesis in the peritoneal surface epithelium, compared with unstimulated controls, while the ovarian sac epithelium showed no increase. Gross ultrastructural changes occurred in the peritoneal area of ovarian follicles following pituitary stimulation in vivo, including loss of collagen fibrils, and general disorganization of the connective tissue theca. Changes in the rough endoplasmic reticulum of the peritoneal epithelial cells, while frequently encountered, were less pronounced. None of these changes was observed in the ovarian sac area, or in the interfollicular region. The above data are consistent with the hypothesis that pituitary stimulation of the frog ovary results in increased synthesis of RNA and protein by the peritoneal epithelial cells, and that the protein may be collagenase.

INTRODUCTION

It has been shown that induced ovulation can be prevented in the frog (1, 2) and the rabbit (3) by such antibiotics as actinomycin D and puromycin. The implication of these studies is that the ovulation process requires synthesis of ribonucleic acid (RNA) and protein. Because of known (4–6) or unknown side effects of the antibiotics used in the above studies, other inhibitors of RNA synthesis, whose side effects were presumed to differ from those of actinomycin D, were shown to inhibit ovulation (2). It is still necessary to establish whether the inhibitory pattern is time dependent, in a way that is consistent with current understanding of the sequence of events involved in RNA transcription and translation in protein synthesis. It is further important to understand which cells respond to pituitary stimulation by increased synthesis of RNA, and to elucidate the ultrastructural changes which occur in the follicular wall during the interval between pituitary stimulation and ovulation.

The present study yielded data which relate to all of the above points, showing that the antibiotics do indeed inhibit RNA and protein synthesis,
that their effect on ovulation is time dependent, that the cells of the peritoneal lining of the follicle respond to pituitary stimulation by increased RNA synthesis, and that profound ultrastructural changes occur in the peritoneal wall of the follicle prior to ovulation.

Some of these data have been reported in abstract form (7-9).

MATERIALS AND METHODS

Time Course of Antibiotic Effects

Ovaries were obtained from anesthetized adult, female frogs (Rana pipiens) that had been maintained at low temperature (3-5°C). The ovaries were excised, placed in frog Ringer's solution, and cut into clusters of 20-25 follicles. These clusters were then placed into glass vials containing 8 ml of frog Ringer's solution. In each experiment, egg clusters from a single ovary were randomly distributed throughout the various treatment groups and controls. Ovulation was induced by the addition of either frog pituitaries (10) or progesterone (11) to the vials. The pituitaries used for in vitro stimulation of ovulation had been previously collected and kept frozen at -20°C. As needed, these were homogenized in a glass homogenizer at 4°C with sufficient frog Ringer's solution so that 1 ml of homogenate contained 10 pituitaries. The equivalent of two to five female frog pituitaries per vial were required to induce ovulation. In the early winter, more pituitaries were required, and a more variable response was elicited, than in the experiments performed in late winter and early spring. When progesterone was used, 16 µg were dissolved in 25 µl of 50% ethanol and added to each vial; and 25 µl of 50% ethanol were added to each control vial.

The antibiotics actinomycin D and puromycin were added to vials containing ovarian segments to obtain final concentrations of 1 µg/ml and 3 µg/ml, respectively. The antibiotics were added either at the time of pituitary stimulation, or 1, 2, 3, 6, or 9 hr after stimulation.

Effects of Hormonal Stimulation, and of Antibiotic Treatment, on RNA and Protein Synthesis by Ovarian Tissue

A series of experiments was performed to determine the effect of pituitary or progesterone stimulation on incorporation of RNA and protein precursors into ovarian tissue, with and without treatment with actinomycin D or puromycin. In these experiments, RNA was labeled with uridine-3H, and protein was labeled with either leucine-3H or lysine-14C. The vials were stoppered with cotton plugs and kept in the dark at room temperature (23 ± 2°C) for the duration of the experiment. Those vials containing ovarian fragments without labeled compound were allowed to stand for 24 hr, at which time they were poured out into a Petri dish and the per cent ovulations recorded. The labeling experiments were terminated at various intervals by the addition of ice-cold distilled water, and the pieces of ovary were rapidly frozen on dry ice and kept at -20°C until analyzed.

For analysis, the ovarian fragments were first thawed and then gently pressed against a stainless steel wire screen to rupture the eggs. The remaining ovarian tissue was removed from the screen, placed on filter paper, and repeatedly washed with a stream of cold distilled water from a plastic wash bottle, which removed most of the characteristically dark gray, entrapped egg material. Finally, any obvious traces of egg that remained were removed manually with fine forceps. Because of the small amount of tissue present in the frog ovary relative to oocytes, the ovarian tissue minus mature oocytes (OTMO) from two or three clusters was required, depending on the biochemical analysis to be performed. The combined OTMO was then homogenized in a motor-driven McShan-Erway (Norman Erway, Madison, Wisc.) homogenizer with 10 strokes in 3 ml of 0.5 N per-chloric acid.

Isolation, determination of concentration of RNA and DNA, and counting of radioactive RNA were accomplished by a modification of the method of Wannemacher et al. (12). Protein isolation and radioactivity determinations were also performed by the above procedures (12), and the protein concentration was measured by the method of Lowry et al. (13).

Radioautographic Studies

Ovarian segments were prepared as above, and incubated for 6 hr at room temperature in frog Ringer's solution containing 4 µCi/ml uridine-3H, and the equivalent of two pituitaries per vial. Actinomycin D (1 µg/ml) was added to some of the vials in one experiment; and control vials contained the uridine-3H but not the pituitary homogenate.

Following incubation, the tissues were fixed and processed for paraffin embedding by the method of Moore (14), with 2% MgCl2 added to the original fixative to minimize the possible distortion of the segments by smooth muscle contraction (15). 5-µ sections were decorated, hydrated, and covered with Kodak NTB-3 radioautographic emulsion. After drying, they were kept in a light-tight desiccator at -20°C for 3 wk, developed in Kodak D-19, fixed, washed, and dried. They were then stained in methylene blue at pH 3, or by a modification of the Dominion method (16), following which they were dried and mounted with Permount. All comparisons
of grain counts were made among tissues from the same ovary, which were processed together through the entire radioautographic procedure. Comparisons were made of numbers of grains overlying (a) cells of the peritoneal lining (the presumptive site of emergence of oocytes), and (b) cells lining the ovarian sac. Cells were not counted when they were either too close together or too heavily labeled to be clearly distinguished. Only those cells showing overlying grains were included in the counts.

Ultrastructural Studies

So as to obviate problems of ultrastructural changes produced by in vitro conditions, these studies were carried out on follicles stimulated in vivo. Frogs were stimulated by an intraperitoneal injection of two pituitaries. Controls were not injected, as preliminary studies indicated there was no effect produced by sham injections. At intervals from 8–16 hr following injection, the ovaries were quickly excised and placed in 2.5% glutaraldehyde in Ringer's solution. With the aid of a dissecting microscope, the large mass was cut into smaller clumps, each of which contained a ring of follicles surrounding a central one or two. Studying only the central follicles minimized any fixation distortion. Fixation was continued at 0°C for 30 min, and the clumps were rinsed in Ringer's solution and transferred to 1% OsO4 in Ringer's solution for 30 min. They were then dehydrated rapidly through an ethanol series, and embedded in Epon 812. For orientation, thick (1 μ) sections were cut on a Porter-Blum ultramicrotome, following which thin sections were cut. The latter were stained with lead citrate (17) or 10% phosphotungstic acid, and studied and photographed with a Phillips EM 200 electron microscope. In order to study large areas at sufficient magnification, it was necessary to produce montages 5–18 micrographs long.

RESULTS

Time Course of Antibiotic Effects

When administered at the proper interval following pituitary stimulation, both actinomycin D and puromycin have the ability to interfere with ovulation in vitro by segments of frog ovary (Table I). Adding actinomycin D to the vials at any time up to 3 hr after hormonal stimulation completely blocked ovulation. However, ovulation again was possible when the antibiotic was added at 6 hr or later. In contrast, puromycin was still capable of completely inhibiting ovulation at 6 hr, but not at 9 hr. It is of interest that the percentage of follicles ovulating did not return to control (pituitary-stimulated) levels, particularly in the case of actinomycin D (Table I).

Effects of Hormonal Stimulation, and of Antibiotic Treatment, on RNA and Protein Synthesis by Ovarian Tissue

Incorporation of uridine-3H into OTMO was reduced to 30% of control levels by actinomycin D (Fig. 1), and the incorporation of labeled amino acids into protein was reduced to 10% of control levels by puromycin (Fig. 2). In contrast, neither RNA nor protein synthesis by total OTMO was affected by hormonal stimulation (Figs. 1–4). Failure of hormone treatment to stimulate OTMO protein and RNA synthesis suggested that the response of the ovarian tissue might be restricted to a population of cells too few in number to produce a recognizable effect on total OTMO synthesis. Accordingly, the following study was undertaken.

Radioautographic Studies: Because of some confusion in nomenclature (18, 19), a diagram of the histological relations in the mature ovarian follicle is presented (Fig. 5). The follicular epithelium and the connective tissue theca completely surround the oocyte. These layers, in turn, are surrounded almost completely, except where the theca continues into the interfollicular area, by either ovarian sac epithelium or peritoneal epithelium. The theca underlying the peritoneal epithelium is almost avascular, except for an occasional capillary at the periphery. It is through the peritoneal surface that ovulation takes place. In interpreting the radioautographic data, the close
FIGURE 1 The effect of pituitary stimulation and actinomycin D (1 \( \mu \)g/ml) on the rate of in vitro incorporation of uridine-\(^3\)H into purified total RNA of OTMO.

FIGURE 2 The effect of pituitary stimulation and puromycin (3 \( \mu \)g/ml) on the rate of in vitro incorporation of leucine-\(^3\)H into purified total protein of OTMO.

As shown in Figs. 6 and 8, the peritoneal epithelial cells were the major locus of uridine-\(^3\)H incorporation. Much less was taken up by underlying theca, while follicular epithelial cells were essentially unlabelled. The increase in grain counts with pituitary stimulation was also confined to the peritoneal epithelial cells. One major difference between ovarian sac and peritoneal epithelia not indicated in Table II is that in the latter group almost every epithelial cell was labeled, whereas in the former group proportionally very few cells were labeled.

ULTRASTRUCTURAL STUDIES: A recent extensive study by Kessel and Panje (19) has elucidated many interesting aspects of the amphibian ovarian follicle, and of the changes subsequent to ovulation. Although they and others (20, 21) have apposition of the highly attenuated cell layers often left doubt as to whether a given mass was one or two nuclei; these were excluded from the counts.

The results of the radioautographic studies are shown in Table II, and Figs. 6-9, indicating clearly that pituitary stimulation enhanced the incorporation of uridine-\(^3\)H by the cells of the peritoneal surface, while the grain counts overlying cells of the ovarian sac were unaffected by the pituitary treatment. Actinomycin D markedly reduced the grain counts throughout the tissue.

FIGURE 3 The effect of pituitary stimulation and puromycin (3 \( \mu \)g/ml) on the rate of in vitro incorporation of lysine-\(^14\)C into purified total protein of OTMO.

FIGURE 4 The effect of progesterone stimulation (2 \( \mu \)g/ml) on the rate of in vitro incorporation of leucine-\(^3\)H into purified total protein of OTMO.

494 THE JOURNAL OF CELL BIOLOGY · VOLUME 46, 1970
mentioned the outer coverings of the follicle, primary emphasis has been placed on the relations between the follicular epithelium and the developing oocyte. Accordingly, we shall describe in some detail the ultrastructure of the normal, unstimulated follicle envelopes.

So as to permit a fuller interpretation of the data, several comments about the nature of the material are in order. Ovulation in the frog occurs as a wave passing over the ovary, so that at any time interval from 12-20 hr following pituitary stimulation there will be follicles in various stages of the process. Normally, early in the season, the first ovulations following stimulation in vivo do not occur before 12 hr after pituitary injection, while later in the season the first ovulations may occur by 10 hr, and the process is finished sooner. Further, some of the apparently ripe follicles do not rupture. Because of the above considerations, it is not possible, as it is in the rabbit (19), to know that a given follicle would have gone on to ovulate within a predictable period of time.

Great care was taken to orient the embedded follicles in such a way that sections could be taken comparing the peritoneal surface with the ovarian sac and the interfollicular area. It has not always been clear in previous reports (20, 21) whether a distinction was made between the ovarian sac and peritoneal surfaces. The preparation of montages aided greatly in establishing regional variations; but even the longest montage involved only a few epithelial cells. Thus, despite production of numerous montages, it was impossible to obtain the same comprehensive coverage of a given cell type that is possible with columnar epithelium.

In the mature, unstimulated follicles, the follicular epithelium possesses the same ultrastructural features around the entire circumference of the oocyte (Figs. 10, 12). A basal lamina intervenes between the epithelium and the surrounding theca, and the other surface of the epithelium abuts upon the zona pellucida. Occasionally, processes of the follicular epithelium penetrate into this fibrous layer (whose inhomogeneous nature is pronounced following glutaraldehyde fixation), but do not reach the plasmalemma of the oocyte. The epithelial cells show occasional desmosomes, but there are gaps between the cells, which permit ready passage of materials into and out of the oocyte. The cytoplasm of these cells exhibits significant numbers of mitochondria, rough endoplasmic reticulum (ER) profiles, and microtubules. Mitochondria are occasionally concentrated in the perinuclear region, but there is no suggestion of

Table II

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Peritoneal surface</th>
<th>Ovarian sac surface</th>
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<tbody>
<tr>
<td>Control</td>
<td>28 ± 11* (N = 59)</td>
<td>23 ± 10 (N = 68)</td>
</tr>
<tr>
<td>Stimulated</td>
<td>49 ± 15 (N = 65)</td>
<td>29 ± 11 (N = 59)</td>
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* Grain counts/labeled nucleus ± standard deviation, following 6-hr incubation with pituitary homogenate and uridine-3H.
Radioautographs of ovarian follicles fixed after 6 hr in vitro in frog Ringer's solution containing uridine-\(^{3}H\). Figs. 6 and 7 are from unstimulated control: peritoneal and ovarian sac surfaces, respectively. Figs. 8 and 9 are from pituitary stimulated follicle: peritoneal and ovarian sac surfaces, respectively. In each instance, there is a considerable number of melanin granules in the oocyte (O), increasing next to the zona pellucida. The difference between melanin granules and silver grains is pronounced with phase-contrast illumination. In Fig. 8, there is a significant increase in both the number of labeled cells, and the number of grains per cell. Although some few theca cells (T) are labeled, the great majority of labeled cells in these tissues are peritoneal epithelial cells (P). The follicular epithelium (F) was almost never labeled. Labeling of ovarian sac epithelium (S), both experimental and control, was comparable to that of control peritoneal epithelium. X 515.

Special regional concentration of any other organelle, nor are there any yolk platelets, lipid droplets, or membranous whorls, such as have been described in the maturing follicle cells of *Necturus* (19).

The theca of the unstimulated follicle is also the same around the entire circumference (Figs. 10-12). While the collagen fibrils are sometimes quite randomly oriented, they are often arranged in bundles in orthogonal array. There is also marked variation in the amount of interfibril matrix. Such differences are readily found in adjacent regions of the same follicle, and wider, more random spacing is more prevalent later in the season (April and May). Accordingly, the sharpest contrast between control and experimental tissues was found in material prepared in February and March. Each collagen fibril in control follicles is surrounded by a halo of decreased electron opacity. In addition, very fine, filamentous material, much smaller than the collagen fibrils, was observed, particularly in late season control specimens.

Despite the frequent statement that smooth muscle is present in the follicle wall, we have never observed such cells in any of our material. In view of the attenuated nature of all cells in this layer, it is not difficult to understand how such a concept might have arisen from light microscope studies.

The single cell type which we have observed in the theca can be classified as a fibroblast. There is a perinuclear rim of cytoplasm from which long, slender processes extend for considerable distances through the surrounding fibrous matrix. In the perinuclear region, the most prominent organelle is the rough ER; and smaller concentrations of rough ER (sometimes with expanded cisterns) are seen even in quite attenuated processes. Mitochondria are relatively few in number. In addition to the cisterns of rough ER, dilated vesicles are sometimes found, which may be invaginated lacunae of matrix.

The squamous peritoneal epithelium lies on a basal lamina; and adjoining cells usually interdigitate with each other, as well as maintaining a junctional complex (Fig. 10). Mitochondria, free ribosomes, and rough ER are all present, but not in unusual amounts. No Golgi region was observed.

The most prominent feature of the epithelium is the considerable number of vesicles which can be seen free in the cytoplasm, as well as joining with external and internal plasmalemmas. They are interpreted as evidence for pinocytosis. Fine fibrils, similar to tonofibrils, are present in the cytoplasm.

The ovarian sac epithelium (Fig. 12) is very similar to the peritoneal epithelium, except that
FIGURES 8 and 9  For legend, see opposite page.

FIGURES 10 and 11  Electron micrographs of the peritoneal surface of unstimulated control ovarian follicles fixed late in the season, showing the peritoneal epithelium (P), theca (T) with fibroblasts, and follicular epithelium (F). In Fig. 10, two adjoining peritoneal epithelial cells are seen to interdigitate, and to form a junctional complex. The significant amount of collagen is disposed in parallel bundles. The interfibril spacing of collagen is quite variable in different regions, although, in comparison with experimental follicles, the packing is quite tight. X 22,500.
FIGURE 12 Ovarian sac surface of control follicle, showing ovarian sac epithelium (S), follicular epithelium, and a process of a fibroblast in the intervening theca (T). The interfibril spacing is quite similar to that in Figs. 10 and 11. In this figure, and in Fig. 16, the inhomogeneous nature of the zona pellucida (Z) is shown. X 32,500.

Pinocytosis vesicles, while present, are far fewer in number.

The nuclei of all cell types in the follicle wall are quite similar under the present preparative conditions, although the theca fibroblasts exhibit a somewhat greater clumping and margination of chromatin.

Dramatic changes occur in the ultrastructure of the peritoneal surface following pituitary stimulation in vivo. At 8 hr after pituitary stimulation, the ultrastructural alterations have begun; and by 12 hr, the changes are pronounced (Fig. 13). The most striking change is in the nature of the intercellular materials of the theca. In contrast to the condition in the control follicles, there is gross disorganization of the fibril pattern and a considerable increase in the interfibril material, making it difficult to decide whether there is an absolute reduction in the number of collagen fibrils. Images such as Fig. 13, however, strongly suggest such a reduction. There is, among the fibrils that remain, an increase in the number of fibrils of smaller diameter; and there is also a considerable increase in the amount of fine, filamentous material (Fig. 14). The remaining collagen fibrils also retain the halo of less electron-opaque material; and, because of an apparent increase in opacity of much of the matrix, the haloes are more prominent than in the controls (Fig. 13).

Marked variation, both spatial and temporal, characterizes the thecal changes. Often a follicle 12 hr after pituitary stimulation will show greater ultrastructural changes than one 16 hr after stimulation (See Figs. 13 and 15). Fig. 16, on the other hand, depicts a 12-hr follicle in which the collagen bundles show no sign of change. There are, however, indications of macropinocytosis in the overlying peritoneal epithelium. Further, it was not uncommon for a montage of one part of the peritoneal lining area to differ sharply from a second montage taken a short distance away. There was no discernible pattern in these regional differences; nor was it possible to relate the changes in either ground substance or collagen fibrils to the close proximity of any one cell type.

Until very late stages, when there is frank necrosis of the entire area, the follicular epithelial cells and fibroblasts of the theca in the peritoneal area...
do not undergo significant ultrastructural changes. The peritoneal epithelial cells exhibit an increase in the number and size of cytoplasmic vesicles; but the degenerative changes in the tissue as ovulation approaches obscure the exact nature of many of the vesicles. In the perinuclear region, there is a dilation of the cisterns of the rough ER, with contents slightly more electron opaque (Fig. 17). These changes also show regional variations; and, although observed in a number of instances, the changes in the rough ER are limited.

The interfollicular area and the ovarian sac area show none of the above ultrastructural changes. This is most sharply illustrated in a follicle which was fixed during the ovulation process (Fig. 18). Here, of course, the changes in the peritoneal area of the follicle had progressed to the point at which the tissue of that area had completely broken down. Both the ovarian sac and the interfollicular area were indistinguishable from the control condition.

**DISCUSSION**

The results presented in this study can be summarized briefly as follows: actinomycin D is effective in blocking pituitary-induced ovulation in vitro if it is presented within 3 hr after stimulation commences; but it is less effective when presented at 6 hr or later. Puromycin, on the other hand, is effective when presented at 6 hr, but not at 9 hr. The two antibiotics are effective in reducing the uptake of RNA and protein precursors by OTMO. The effect of pituitary stimulation on uptake of these precursors, however, is masked by the fact that increased synthetic response appears to be confined to a small population of cells: the peritoneal epithelium (and, possibly, some few adjacent theca cells). The theca underlying the peritoneal epithelium undergoes marked changes in both the fibrous element and the interfibrillar substance, leading to apparent loss of mechanical strength of the area, thus permitting the egg to leave the follicle.
The results with the actinomycin D and puromycin confirm and extend those of Dettlaff (1). The time sequence for effectiveness of the two antibiotics is consistent with the hypothesis (1, 2) that at least one necessary result of pituitary stimulation is increased transcription of DNA to RNA, and translation of RNA to protein. Although it is tempting to consider the possibility that this is the result of synthesis of one specific messenger RNA (coding for collagenase?), further work is necessary to test this hypothesis. Had this time sequence not been found, however, it would have been more likely that some of the known (4-6) or unknown side effects of the antibiotics were preventing ovulation. The OTMO results indicate that inhibition of RNA and protein synthesis occurred as expected.

Following her demonstration that frog ovulation is inhibited by antibiotics, Dettlaff (1), without further supporting data, proposed that "M-RNA's and proteins concerned in the process of ovulation are synthesized in the follicle cells." The present radioautographic studies have provided the first direct evidence regarding cells of the frog ovarian follicle that respond specifically to hormonal stimuli, resulting in ovulation. These data dispel the theory of Dettlaff, since it is the peritoneal epithelial cells which respond to pituitary stimulation by increased RNA synthesis, while in neither the control nor the hormonally stimulated ovaries did the follicle cells show any significant incorporation of uridine-3H.

The ultrastructural data reported here have striking parallels in two studies on related systems. The first of these is the work of Espey (22), who has shown that prior to coitus-induced ovulation in the rabbit, there are gross ultrastructural changes in the collagen bundles and ground substance of the follicular wall. In the mammalian ovary, the liquor folliculi and the follicular tissue show strong metachromasia in the mature follicle, turning to orthochromasia as ovulation approaches (23). Furthermore, there is present in the liquor folliculi prior to ovulation an enzyme that is capable of depolymerizing hyaluronic acid (23). Espey and Rondell (24) have studied collagenolytic activity in the Graafian follicle at ovulation, and have shown a decrease in activity of this enzyme in the follicular tissue as ovulation approaches (23). The metabolism of connective tissue during ovulation is complex, and it is evident that ultrastructural studies cannot elucidate the entire process.

A second system which parallels the present work is that of the tissue changes in the tadpole tail during thyroxin-induced re-orption. The process involves both hyaluronidase and collagenase activity (25), the collagenase being a product of the epidermis and susceptible to puromycin blockage, and the hyaluronidase being a product of connective tissue and not subject to antibiotic blockage. These data were interpreted as suggesting that the hyaluronidase exists prior to thyroxine treatment and is merely released from the connective tissue cells, whereas the epidermal cells must produce the bulk of the collagenase following hormone treatment. The data from the present study would not be inconsistent with the concept that a similar process is operating during ovulation.
Figure 15  Peritoneal surface of ovarian follicle 16 hr after pituitary stimulation. Although this follicle had been under the influence of pituitary stimulation for 4 hr longer than that in Fig. 13, the loss of integrity of collagen bundles is not so advanced. In some places, however, one can discern fine, filamentous material in close association with the fibrils. × 22,500.

Figure 16  Peritoneal surface of an ovarian follicle 12 hr after pituitary stimulation. Although there has been some development of large vesicles in the peritoneal epithelium, other changes in the tissue have been minimal. The closely spaced, regular array of collagen fibrils, even more marked than in Figs. 10 and 11, can probably be explained by the fact that this material was fixed earlier in the season. × 22,500.
Ultrastructurally, the two processes show many similarities (27): a general loss of integrity of the fibril arrangement; an increase in the interfibril distance; decrease in diameter of the individual fibrils; and increase in the amount of fine, filamentous material. Two major differences should also be noted: it takes only 10-15 hr for ovulation to occur in the frog following hormonal stimulation, while tail resorption does not show striking ultrastructural changes for several days; and the resorption process involves a great amount of phagocytosis of collagen fibers by macrophages, while no suggestion of this phenomenon has been observed during ovulation. Perhaps both differences may be related to the fact that the volume of tissue to be changed is very much less in the case of the ovarian follicle: the entire peritoneal lining of the follicle is less than 1 mm in diameter and less than 20 \( \mu \) thick. Also, tail resorption is a more complex process, involving extensive remodeling of a considerable amount of tissue.

The fact that, during the late winter and early spring, the peritoneal area of unstimulated follicles undergoes progressive ultrastructural changes implies that there is a gradual build-up of an enzyme complement, followed by a sharp rise in activity just prior to ovulation.

It should be stressed that, while our data point to some of the events which lead to breakdown of the follicle wall, they do not deal with the problem
of the motive force which causes extrusion of the oocyte. Rondell and Wright (28) have shown that in vitro ovulation in the frog can be prevented by inhibiting oxygen uptake by ovarian fragments, but they concluded that the oxygen was utilized in synthesis, since the level of KCN that was used to inhibit both oxygen consumption and ovulation was not sufficient to depress contractions of the fragments. The lack of smooth muscle fibers in the follicle wall leaves open the source of contractions that have been observed by many workers. Other reports on the ultrastructure of the frog ovarian follicle (19-21) have also failed to show any smooth muscle fibers, although it is not apparent that they were actively sought. Perhaps, there are such fibers in interfollicular septa.

Lastly, it should be pointed out that the theory of enzymatic destruction as a primary cause of release of the oocyte from the frog ovarian follicle was advanced many years ago by Rugh (29), who stated that “localized degenerative changes, possibly encouraged by enzyme action, are offered as the ultimate cause of rupture.”

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