MULTIVESICULAR STRUCTURES IN PROLIFERATING FIBROBLASTS OF RABBIT OVARIAN FOLLICLES DURING OVULATION

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INTRODUCTION

During ovulation and luteinization in mammals a portion of the ovarian tissue undergoes extensive remodeling. The thecal framework of a mature follicle is degraded and disrupted as the ovum is released. Immediately following ovulation the fibroblasts rapidly undergo functional reorientation (*a*) to initiate healing at the point of rupture and (*b*) to meet the changing requirements for support of the proliferating lutein cells. These features make the ovulating follicle a useful model for studying collagen resorption and synthesis.

Prior to ovulation in the rabbit the follicle wall loses tensile strength (1), and the dense collagenous tissue in the thecal layers dissociates (2). Recent data reveal that follicular fibroblasts produce multivesicular structures which protrude from the cell surface and appear to digest the ground substance surrounding them.[1]

[1] Espey, L. L. 1971. *Endocrinology*. In press.

The general features of the multivesicular structures give the impression they might have a basic function in proliferating fibroblasts. To analyze this possibility, the present report examines the distribution of these structures in postovulatory fibroblasts, which are actively proliferating during the luteinization process.

METHODS

Ovulation was induced in rabbits in estrus by mating. About 6–10 hr after ovulation the whole ovaries were fixed for 15–30 min in 6% glutaraldehyde at pH 7.4 with phosphate buffer. They were postfixed for 60–100 min in Millonig's osmium tetroxide solution. The protruding apexes of the ruptured follicles were cut from the ovary during the initial dehydration step in 50% ethanol. Following ethanol dehydration and propylene oxide substitution, the tissue was imbedded in Spurr's Epon mixture. The ultrathin sections were consistently taken from the apical region (but not at the rupture point) of the follicles by an orientation procedure which has been

described previously (2). Sections were made on a Porter-Blum MT-2 ultramicrotome and placed on 75/300 mesh copper screens. All sections were initially stained with 7% uranyl acetate (in 50% ethanol) for 45 min and then counterstained with Reynolds' lead citrate solution for 15 min. Micrographs were made with a Hitachi HU-11E electron microscope.

OBSERVATIONS

Basic Morphology

Macroscopically, the tissue of ovulated follicles is very soft and appears rather decomposed. Electron micrographs of the thecal layers show considerable dissociation of the connective tissue elements (Fig. 1). The fibroblasts display distinct multivesicular structures. Two-dimensional views of these structures usually reveal about 5–50 microvesicles in close proximity. The individual microvesicles are about 0.1 μ in diameter. It is difficult to determine whether each microvesicle has a true limiting membrane or whether its delineation results from a condensation ring. The interior of the vesicles has a diffuse electron opacity. Clusters of these microvesicles do not contain an encapsulating membrane. That portion of a multivesicular structure which protrudes from the cell is partially surrounded by the plasma membrane.

Three-dimensional examination of these structures shows that actual dissociation from the cell is rare. Serial sections of multivesicular structures which appear extruded consistently reveal that they remain attached to the cytoplasm of a fibroblast. They do occasionally appear disorganized, i.e., the microvesicles are disrupted and the plasma membrane around them is not spherical. The extracellular material surrounding disorganized structures consistently appears more digested than that around the symmetrical multivesicular structures.

Association with Cytoplasmic Processes

In fibroblasts of postovulatory tissue, the multivesicular structures appear with impressive frequency as knobs at the tips of cytoplasmic processes (Figs. 2–6). In many instances, they remain attached to the body of the cells only by a long, thin "neck" of cytoplasm.

In other observations the multivesicular structures were situated at various distances from the cell body (Figs. 7–10). Their appearance suggested that they exist at the distal end of a cytoplasmic process from the time it originates in the matrix of the cytoplasm.

Cellular Origin

The multivesicular structures could not be correlated with any specific cell organelle. In a group of 347 structures randomly examined to evaluate the cellular origin of the contents, only 1% was near a Golgi apparatus, 10% were adjacent to the nucleus (Fig. 11), 19% were in the area of rough endoplasmic reticulum (Fig. 12), and the remaining 70% could not be related to any specific cell component other than free ribosomes.

COMMENTS

The multivesicular structures described in this paper cannot be identified with the multivesicular bodies illustrated in various other reports. The multivesicular bodies presented by Kilarski and Jasinski (3) are clearly of nuclear origin, but none of the several thousand multivesicular structures observed in follicular fibroblasts appeared connected in any way to the nuclear surface. Nor are they comparable to the multivesicular bodies found in hepatic and neuronal cells (4, 5). Also, a variety of other cytoplasmic vesicles which are common to fibroblasts (6–8) are not the same as the multivesicular structures reported here.

The fibroblasts in a mature follicle about 10 hr from rupture are compactly distributed through the thecal layers of the follicle wall (2). The concentration of multivesicular structures at this stage is low.[1] Then, within 1 hr of ovulation, the number of multivesicular structures increases ninefold and the collagenous tissue of the follicle concomitantly begins to loosen. During this phase of the ovulatory process, most of the multivesicular structures appear relatively close to the main body of cytoplasm. The present report shows that subsequently, just a few hours after ovulation, as the tissue begins to proliferate into a massive corpus luteum, the structures are frequently visible at the apexes of extensive cytoplasmic processes. These observations imply that the multivesicular structures are involved in the formation and projection of cytoplasmic processes in motile fibroblasts. Additional support for this hypothesis comes from the frequent appearance of digested, extracellular ground substance surrounding them.[1]

Woessner (9) has thoroughly reviewed the information available on the mechanisms of col-

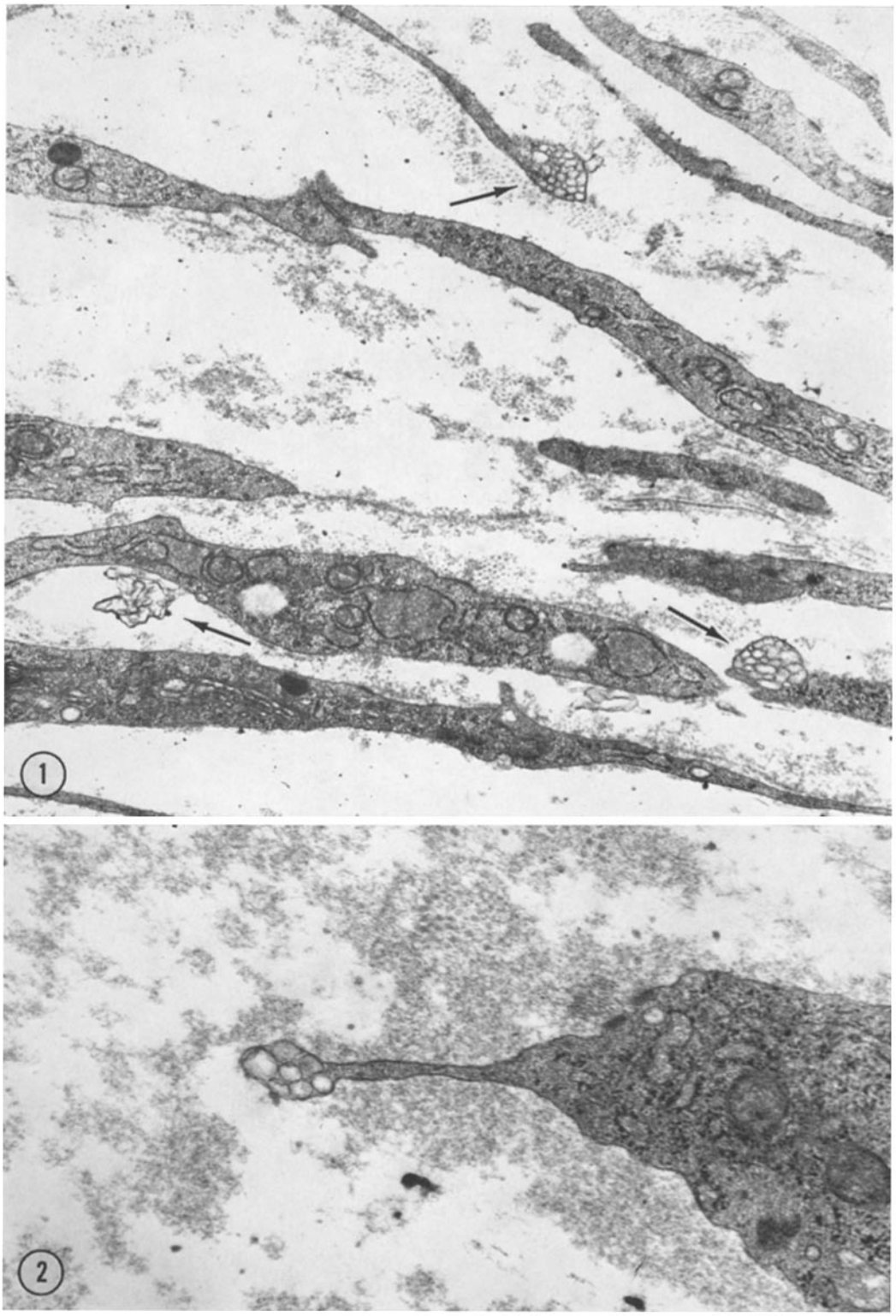

FIGURE 1 Section through the tunica albuginea near the apex of a follicle 8 hr after rupture. The fibroblasts and extracellular elements are very dissociated in comparison to those of preovulatory tissue. Three multivesicular structures (arrows) are present in this micrograph. The absence of ground substance around the disorganized multivesicular structure is typical. × 10,000.

FIGURE 2 A fibroblast containing a multivesicular structure at the tip of a long, narrow, cytoplasmic process. × 34,000.

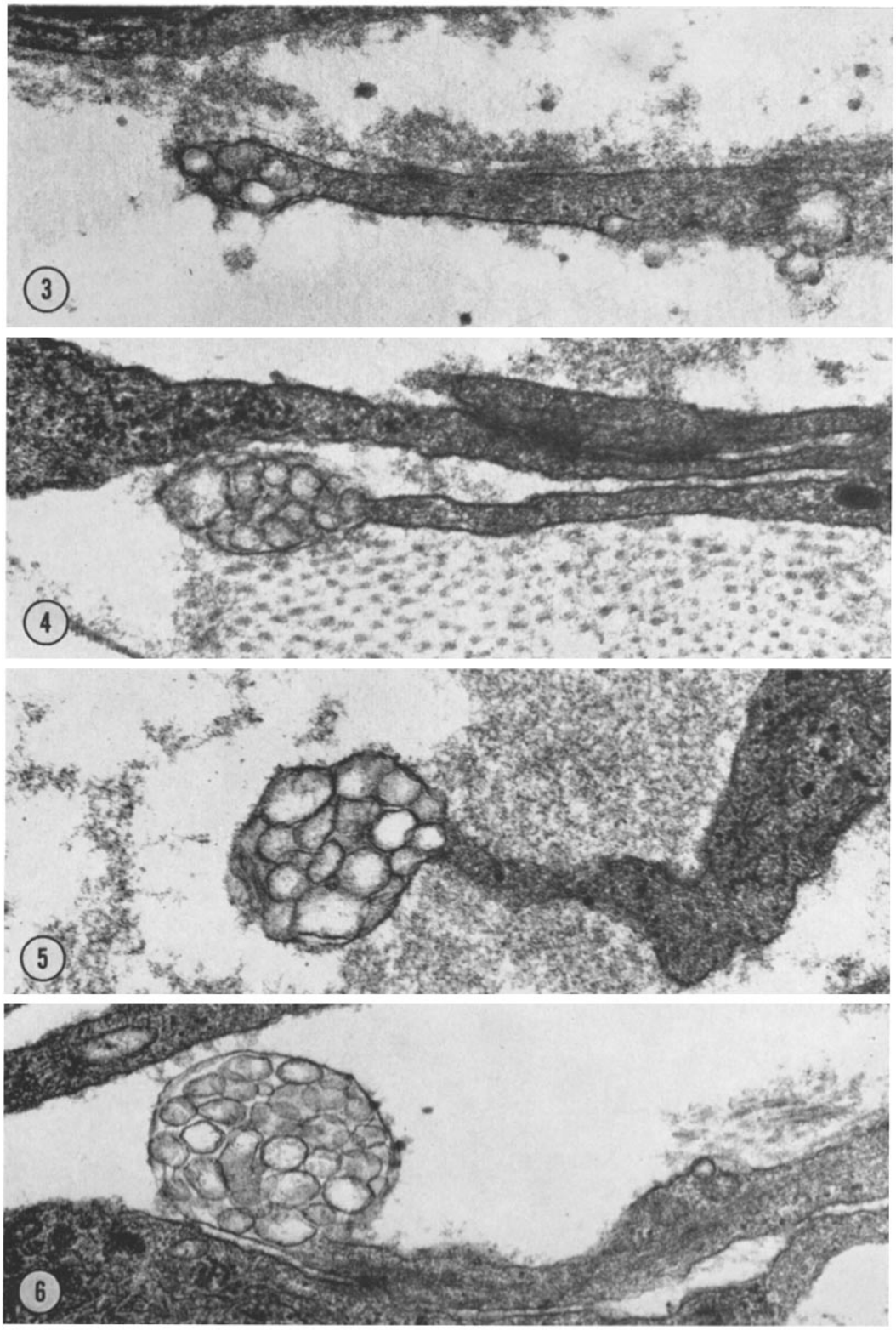

FIGURE 3 In postovulatory tissue the multivesicular structures were commonly observed at the apex of cytoplasmic processes extending from fibroblasts. × 50,000.

FIGURE 4 A multivesicular structure bounded on one side by a cytoplasmic process of another fibroblast and on the other side by a bundle of collagen fibrils seen in cross-section. × 50,000.

FIGURE 5 A multivesicular structure showing a halo of digested ground substances at its apex. × 50,000.

FIGURE 6 A multivesicular structure with numerous microvesicles. The thickness of sections allow detection of overlap between microvesicles. × 50,000.

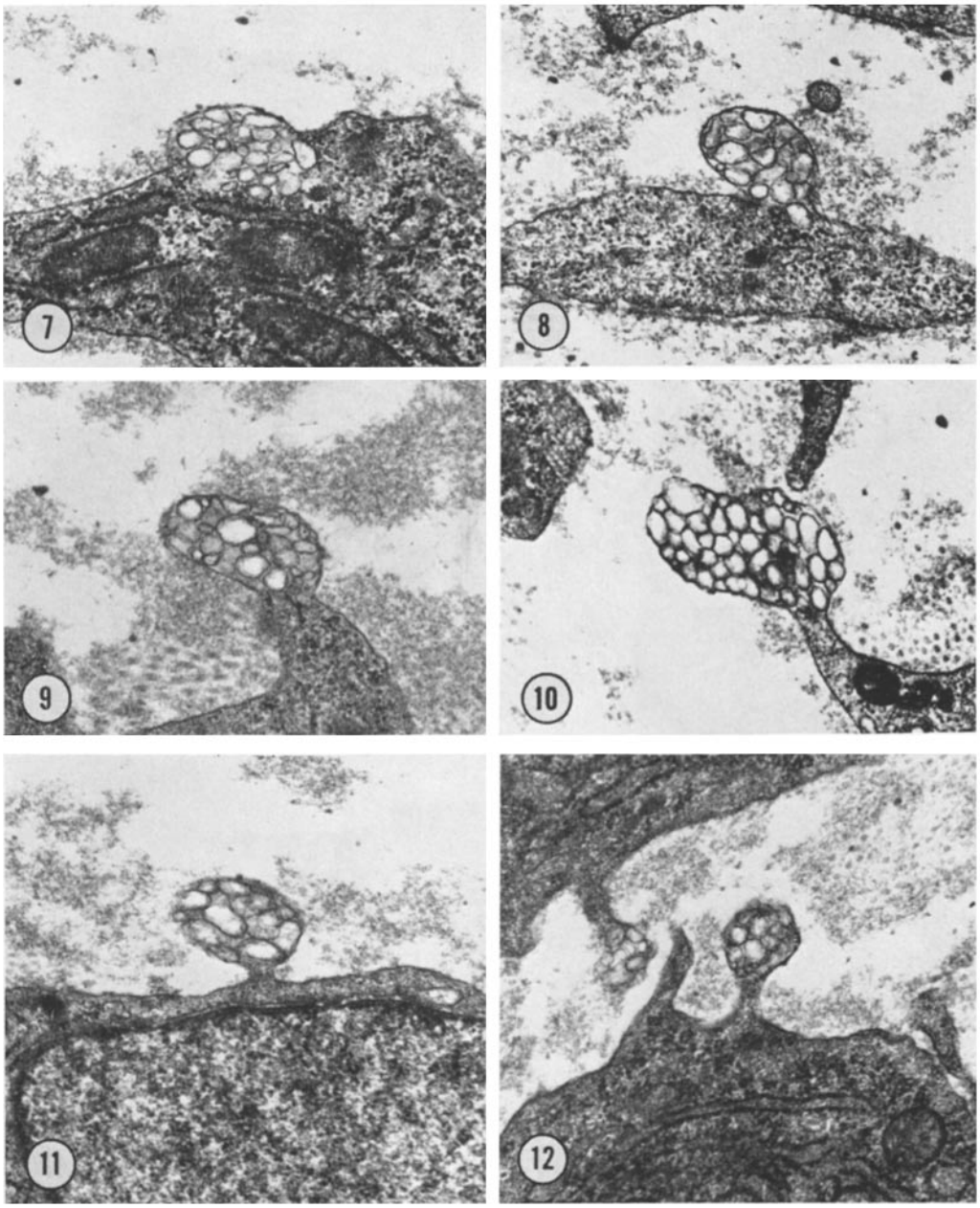

FIGURE 7 A multivesicular structure protruding half-way out of a fibroblast. These structures were rarely observed any further inside the cytoplasmic matrix. This structure was presumably in an early stage of formation. (Figs. 7–10 have been arranged to give the author's interpretation of the protrusion process.) × 30,000.

FIGURE 8 A multivesicular structure which extends above the cell mass. Note the absence of any cytoplasmic components other than free ribosomes in the area. × 30,000.

FIGURE 9 This multivesicular structure appears at the apex of a short cytoplasmic process. × 30,000.

FIGURE 10 A large multivesicular structure at the end of a more extensive cytoplasmic process. × 30,000.

FIGURE 11 The multivesicular structures occasionally developed near the nucleus, but never in conjunction with the nuclear membrane. × 30,000.

FIGURE 12 Multivesicular structures arising from regions of rough endoplasmic reticulum in two opposing cells. Note the dissociation of collagen in the area. × 30,000.

lagen decomposition. He indicates that essentially every tissue that is undergoing physiological remodeling is also undergoing collagen synthesis and breakdown in concert to bring about remodeling and repair effectively. In some instances, the rate of breakdown exceeds that of synthesis. It seems probable that, in the early stages of luteinization, an ovarian follicle enters a phase where collagen breakdown significantly exceeds synthesis. The multivesicular structures in the cytoplasmic processes of follicular fibroblasts could contain a substance that is important in the decomposition of the collagenous connective tissue. Further analysis of the ultrastructure of the numerous collagen resorption processes which Woessner has classified should shed more light on this possibility. Initial observations of the relaxed symphysis pubica in the guinea pig reveal similar multivesicular structures which clearly digest the dense collagen in this connective tissue (unpublished observations).

SUMMARY

During ovulation the connective tissue in the follicle wall undergoes extensive reorganization. This report examines the ultrastructure of the connective tissue at the apexes of follicles 6–10 hr after rupture. Special attention is given to multivesicular structures associated with the cytoplasmic extensions of fibroblasts, which proliferate during the remodeling process. The multivesicular structures contain numerous microvesicles about 0.1 μ in diameter. They appear to develop in the vicinity of free ribosomes near the plasma membrane. Evidence is presented to demonstrate that the multivesicular structures are frequently located in the leading edge of cytoplasmic processes which extend from the fibroblasts of follicular tissue. The nature of these structures indicates that they could have a major role in the mechanism of ovulation.

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