**Relationships of the Ovular Surface with Follicle Cells and Origin of the Zona Pellucida in Rabbit Oocytes.** BY O. TRUJILLO-CENÓZ AND J. ROBERTO SOTELO. (*From Instituto de Investigación de Ciencias Biológicas Departamento de Ultraestructura celular, Montevideo, Uruguay.*)*

In mammals, immature oocytes are surrounded by a layer of flattened cells. At this early stage of development, these "follicle cells" and the plasma membrane of the oocyte are in close proximity. As the oocyte matures, the follicle cells multiply and become cuboidal or radially elongated, and a jelly-like substance containing polysaccharides (1) appears between the oocyte cortex and the follicle cells. When fully developed, this jelly will constitute the zona pellucida (ZP).

Although separated by the zona pellucida, the bodies of the follicle cells and the mature oocyte maintain a close relationship throughout the life of the latter. Two cellular extensions effect this relationship: the multibranched prolongations of the follicle cells, and slender microvilli arising from the oocyte surface. Clearly, the zona pellucida contains both cellular extensions and amorphous substance.

The present observations on rabbit follicles are intended to elucidate two problems that arise in considering the nature of these two components—the relationship between follicle and oocyte surface, and the origin of the amorphous substance.

The material examined in this work was obtained from recently matured female rabbits and fixed in $OsO_4$ dissolved in a solution of polyvinylpyrrolidone ("periston" Bayer), according to a method outlined in a previous communication (10).

*Cellular Extensions and the Relationship between Oocyte and Follicle Cells.*—Many authors have interpreted the follicle cell prolongations as anastomotic bridges connecting the cytoplasms of follicle cell and oocyte (6, 8); others consider them to be extensions of the follicle cells that end at the oocyte surface, thereby forming a contact relationship only (2, 5, 7). Histologists seem to favor the second opinion, but Kemp's recent observations in the electron microscope (4) have caused new interest in the first concept. In a recent EM study of rat oocytes at various stages of growth, Sotelo and Porter state that they could find no evidence of continuity between oocyte and follicle cell cytoplasms in their electron micrographs (9).

In the present study as well, oocytes at different stages of growth were examined; the accompanying figures show only two of these stages. In the youngest oocytes examined, the oocyte membrane and the follicle cell membranes face each other over the entire oocyte surface but are always separated by an intervening space. Deep interdigitations between membranes occur at many points, but in every case the individual membranes of each cell appear to be separated by the same intervening space. At this stage, there is no evidence of membrane fusion or of continuity between the respective cytoplasms.

In more advanced stages, the follicle cells are further away from the oocyte surface, but not all of them at the same distance. The separation is at first incomplete, due to the persistence of the broad ameboidal prolongations from the follicle cells to the oocyte surface (Fig. 2). The amorphous intercellular substance usually becomes discernible at this same stage (Text-fig. 1).

The free intercellular spaces formed in this manner are partially occupied by long, slender, ovular microvilli, which constitute the second type of extension. Many of these extensions end freely in the intercellular space, but others are found in close contact at their distal ends with the surface of the follicle cells (Fig. 2, Text-fig. 1).

Full-grown oocytes (near maturation) are separated from the surrounding follicle cells by a thick layer of amorphous substance which by this time forms the zona pellucida. Multibranched prolongations emanate from the follicle cells in direct contact with the zona pellucida and, traversing the latter in oblique or tangential paths, extend toward the oocyte. (Under the light microscope these appear to run perpendicularly toward the oocyte.) As they approach the oocyte membrane, these prolongations interdigitate with the ovular microvilli. Fig. 1 shows part of the length of one such branch and the terminal portion of another. Similar observations have been made in rat oocytes by Sotelo and Porter (9).

Careful interpretation of electron micrographs of the oocyte cortex is necessary if the observer is to avoid mistaking as fused membrane that which,

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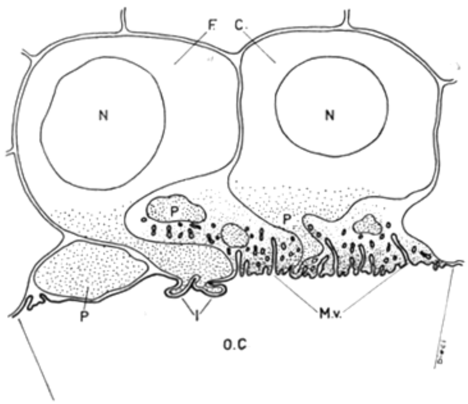


TEXT-FIG. 1. Drawing representing the cortex of an immature oocyte from the rabbit with two follicle cells (*F.C.*). The space created by the separation of follicle cells from the oocyte (*O.C.*) is occupied by ovular microvilli (*M.v.*). The amorphous substance of the zona pellucida is represented by stippling in the intercellular space. The portions of the follicle cells prolongations (*P*) in which similar material is seen in the electron micrographs are stippled somewhat more coarsely. Most of the prolongations are still wide at this stage and membrane interdigitations such as the one indicated at *I* are found over all portions of the oocyte surface. *N*, nucleus.

having been cut obliquely, simply does not show a definite outline. Only one membrane may be clearly visible in the photomicrograph—either the ovular membrane, or the follicular extension. Of course, in many of these cases even the contact relationship is difficult to demonstrate. Moreover, the possibility exists that the elements may actually be in contact at one point but fused at another point not included in the section. True cytoplasmic continuity between two different cells cannot be affirmed until a full image of the communicating bridge is clearly demonstrated. In the present study of rabbit oocytes, such an image was never encountered, although many contacts were observed. In every instance, follicle cell prolongations were found to terminate near the oocyte surface. It should be noted that young oocytes are the most favorable subjects in which to study the follicle cell–oocyte relationship.

*The Amorphous Component of the Zona Pellucida.*—As observed earlier, this substance begins to accumulate at the stage in which follicle cell bodies separate from the oocyte surface. It has not been possible to determine the site of origin of this component using the light microscope. Numerous workers have proposed variously that it is produced by the oocytes, secreted by the follicle cells, or formed by combined activity of both elements (3). The second explanation is supported by the present authors, but the stand is based solely on evidence derived from electron micrographs of immature oocytes. Additional histochemical study will probably help to interpret these findings.

Fig. 2 depicts an interesting item of evidence. Here the cytoplasm of the follicle cells facing the oocyte shows extensive zones occupied by a substance that strikingly resembles the amorphous external material. This substance was found consistently in immature oocyte specimens.

On the other hand, the limiting membrane on the zonal face and prolongations of some cells seems to have changed its physical state, appearing as alternating zones of normal densities and vaguely outlined lesser densities.

Although this work does not provide data to explain the intracellular synthetic process by which the secretion is formed, the facts gathered to date are consistent with the concept that the amorphous substance is elaborated in the cell cytoplasm and later extruded into the zonal layer. Secretion probably proceeds very actively until the zona pellucida reaches its maximal thickness. It would seem to diminish thereafter, since cytoplasmic zones loaded with amorphous material are seen less frequently in follicular cells surrounding mature oocytes. At the same time, the possibility cannot be dismissed that, at this stage, secretion is confined to the prolongations.

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EXPLANATION OF PLATE 147

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Fig. 1. Electron micrograph of the cortex of a mature oocyte from the rabbit. The picture shows part of the length of a follicle cell prolongation (*FP*) and its contact with the ovular membrane (arrow). At the left of this prolongation one can see the extremity of another follicle cell prolongation. A second arrow indicates the contact zone of this prolongation with the ovular membrane. Ovular microvilli (*MV*) are seen at several places. Profiles of vesicles (*V*) and canalicular structures (*Cs*), apparently derived from the cell membrane, are depicted in the ovular cortex. *M*, mitochondria; *ZP*, zona pellucida; *CM*, ovular membrane. × 43,500.

Fig. 2. Electron micrograph of an immature oocyte (rabbit) showing the first stage of separation of follicle cells (*FC*) from the oocyte. The follicle cell at the right shows a small prolongation (*FP*) and a wide ameboid prolongation (*FP*). The insert shows the small prolongation at a larger magnification; the zone of contact between ovular membrane and prolongation is clearly depicted (arrows). The wide prolongation contains amorphous substance similar to that constituting the zona pellucida. This material is scattered about at the proximal zone of the prolongation but is condensed in the distal region. Ovular microvilli (*MV*) are seen in the intercellular space and in contact with the follicle cells surface. *M*, mitochondria; *DM*, ovular surface. × 24,700. (Insert, × 46,800).

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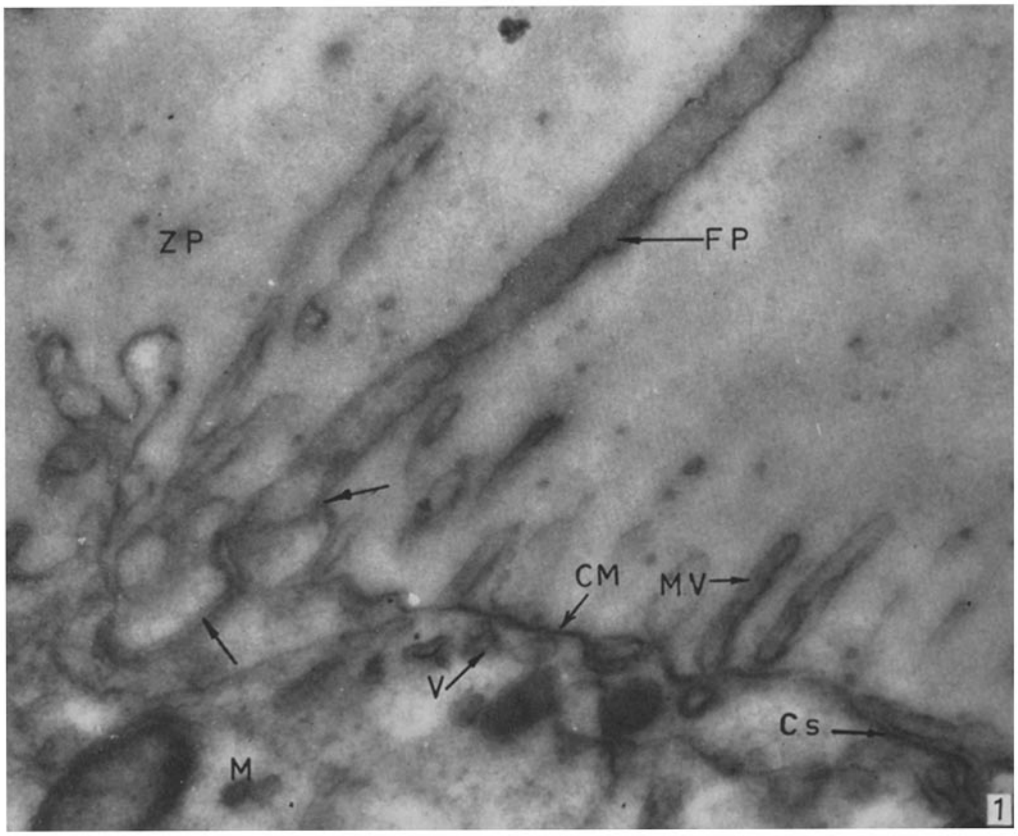


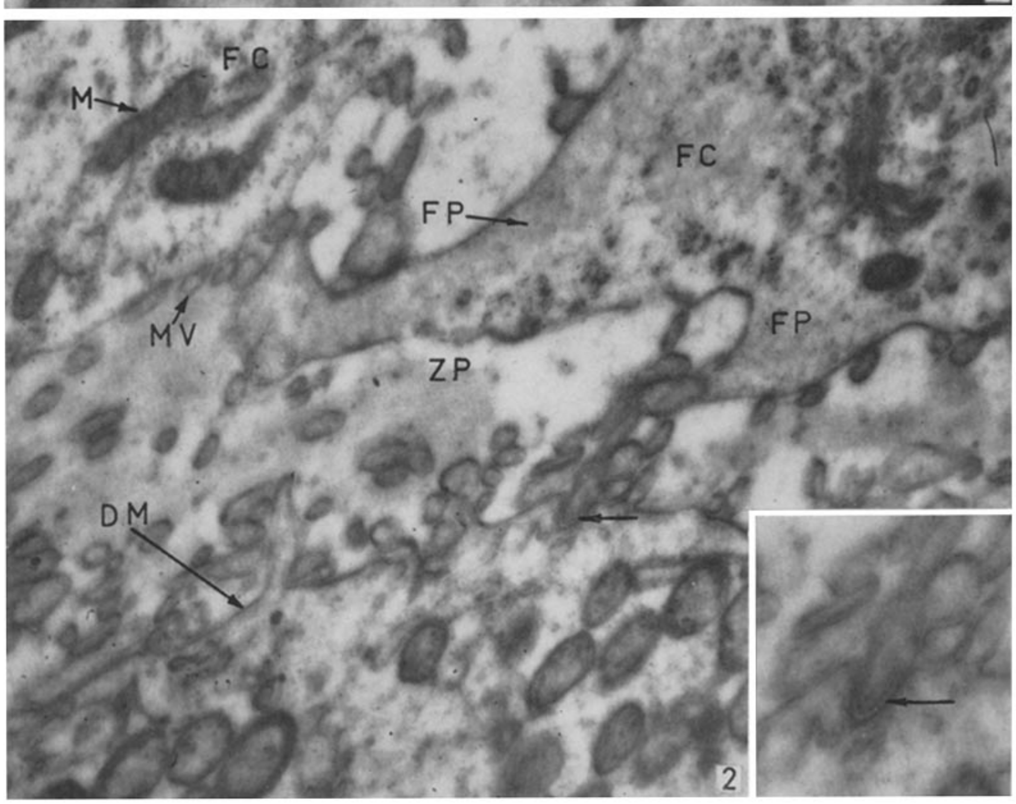


(Trujillo-Cenóz and Sotelo: Relationships of ovular surface and follicle cells)