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Morphodynamic aspects of the ovarian superficial epithelium as revealed by transmission, scanning and high voltage electron microscopy

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Summary. As observed by SEM, TEM and Hivo E/M the superficial epithelium of different mammals was usually composed of a single layer of polyhedral cells. The free surface of those elements was generally covered with a large number of microvilli and isolated cilia. In other cases, the cells of the superficial epithelium displayed several features such as blebs, ruffles and lamellipodia as well as smooth surfaces with small pits and cavities. Those morphodynamic changes seemed to be related not only to the cell cycle but also to the phase of the reproductive cycle and to events occurring within the ovary. On a more macroscopic level, the ovarian surface was evaginated into a series of papillae and invaginated into crypts and/or cords which, depending upon the species, might vary widely in size, shape and number. It is likely that the presence of papillae, crypts and cords was a simple proliferative expression of the superficial epithelium under normal, cyclic physiological conditions. One interpretation of these observations suggested that the surface epithelium with its cyclic morphodynamic changes and proliferative abilities might serve as a source of ovarian components during the adult life of several mammals.

Introduction.

The exposed surface of the mammalian ovary is covered by a continuous layer of epithelial-like cells commonly referred to as the (« germinal ») superficial epithelium, as it was once considered to be capable of generating new germ cells (Waldeyer, 1870).

Recently, the superficial epithelium of the mammalian ovary has been investigated using different electron microscopic techniques (Wischnitzer, 1965; Weakley, 1969 ; Motta, Cherney and Didio, 1971 ; Jeppesen, 1975 ; Anderson *et al.*, 1976), particularly in the areas of the ovary in which an ovulatory process can be expected (Motta, Cherney and Didio, 1971 ; Nilsson and Munshi, 1973 ; Cajander and Bjersing, 1975 ; Motta and Van Blerkom, 1975 ; Rawson and Espey, 1977). The purpose of this paper is to present some results obtained by scanning electron microscopy (SEM)

on different areas of the ovarian surface of mature cyclic mice and rats, and of rabbits and cats in estrus and at different post-coitum intervals. The SEM were compared with parallel transmission (TEM) and high voltage (Hivo) electron microscopy results in an attempt to make a three-dimensional reconstruction of this cellular population.

Materials and methods.

Ovaries were removed from mice and rats at different phases of the reproductive cycle.

The ovaries of rabbits and cats, which are reflex ovulators, were removed at estrus and at intervals after copulation ranging from a few hours to several days (see also Van Blerkom and Motta, 1978). All the ovaries were perfused or fixed by immersion in a solution of 2.5 p. 100 glutaraldhyde in 0.18 M cacodylate buffer, pH 7.3 (Sabatini, Bensch and Barnett, 1963). After a period of from one to several days in this solution, the tissues were washed in the same buffer for one-half hour and then cut into small blocks with a razor blade.

Dehydration was carried out rapidly through graded concentrations of acetone. After dehydration the specimens were transferred to liquid CO_2 for critical-point drying. The dried samples were mounted on aluminum studs using conductive silver paint and coated with a thin layer of carbon and gold in a high vacuum evaporator (DV-502, Denton Vacuum) under continuous rotation and with the appropriate tilt of the stud.

All specimens were examined and photographed using a Cambridge Stereoscan Model S4, a Jeol-35 and a Cambridge 150, all operated at 10 to 20 kV. For transmission

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PLATE I

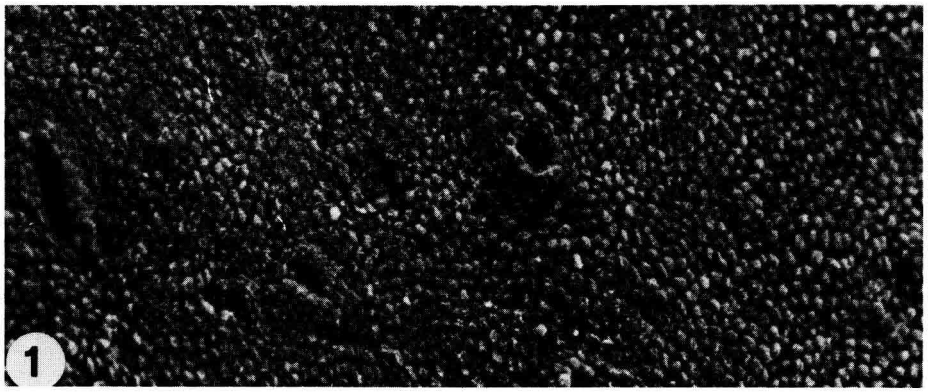
FIG. 1. — *A general view of the superficial epithelium of the ovary.* Most cells are polygonal and approximately equal in size. Even at relatively low magnification, numerous crater-like surface features of the ovary are evident. While some crypts are circular others have a more elongated shape (× 320, estrous rabbit).

FIG. 2. — *At high magnification, it is clear that the surfaces of the superficial cells contain numerous microvilli, blebs and ruffles of the plasma membrane* (× 8 200, estrous rabbit).

FIG. 3. — *High magnification scanning electron micrograph of the surface of a superficial cell.* Among the microvilli and blebs a single cilium is seen (arrow). The cilium is partially invaginated into the cortical cytoplasm (× 16 500 ; proestrous mouse).

FIG. 4. — *This scanning electron micrograph illustrates the appearance of a typical small papilla.* Papillae in the mouse and rat never attain the degree of development and relative ernormity of size observed in the rabbit. In this particular papilla, some cells display numerous microvilli and blebs of different sizes. The superficial aspects of these cells are most likely related to the phase of the cell cycle (× 4 800 ; proestrous rat).

FIG. 5. — *Papillae of various sizes are evident in this area of the ovarian surface* (× 650 ; rabbit at 6 days after copulation).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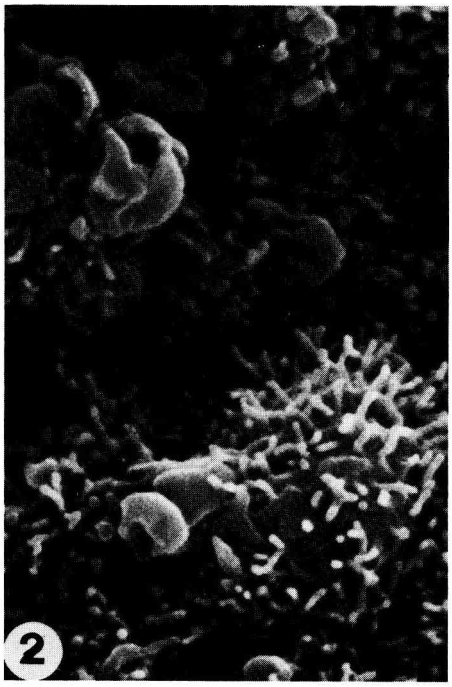
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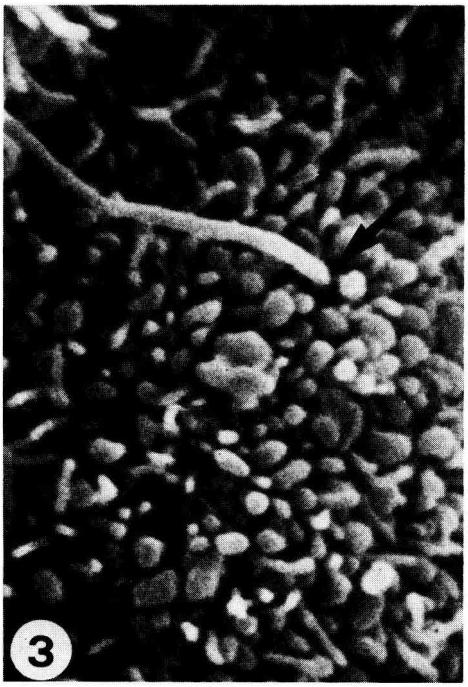
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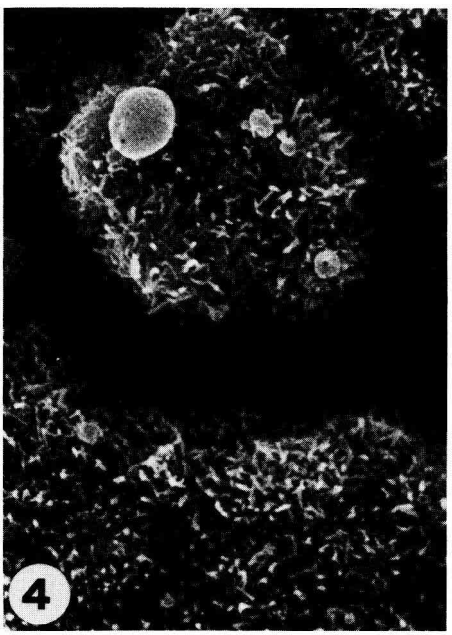
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electron microscopy (traditional and high voltage) the tissues were fixed overnight in the same fixative used for SEM. The specimens were then postfixed in 1.3 p. 100 osmium tetroxide (in 0.18 M cacodylate buffer) for two hours, washed in the same buffer and dehydrated through graded concentrations of ethanol. The blocks were embedded in Epon 812 (Luft, 1961), sectioned with glass knives in a Porter-Blum MT-1 or MT-II ultramicrotome, and stained with uranyl acetate (Watson, 1958) followed by lead citrate (Reynolds, 1963). The sections were examined with either a Zeiss EM 9 or Phillips EM 300 electron microscope.

For Hivo electron microscopy, 0,5-1 μm thick sections collected on grids were stained with both uranyl acetate and lead citrate and coated with a very thin layer of carbon in a high vacuum evaporator (M. Fotino, personal communication). The thick sections were studied and photographed in a Hitachi high voltage electron microscope operated at 1 000 kV.

Results and discussion.

The superficial epithelium was usually composed of a single layer of polyhedral (columnar or flattened) cells tenuously attached to the tunica albuginea. Generally, the free surfaces of these cells were covered with a large number of microvilli attaining an average density of between 300 and 350 units per μm^2 of cell surface (plates I and II). With some minor variations in length and diameter, these microvilli were rather uniform in appearance (figs. 2, 4). A single cilium, typically located in the central portion of the cell was often observed projecting above surrounding microvilli (fig. 3).

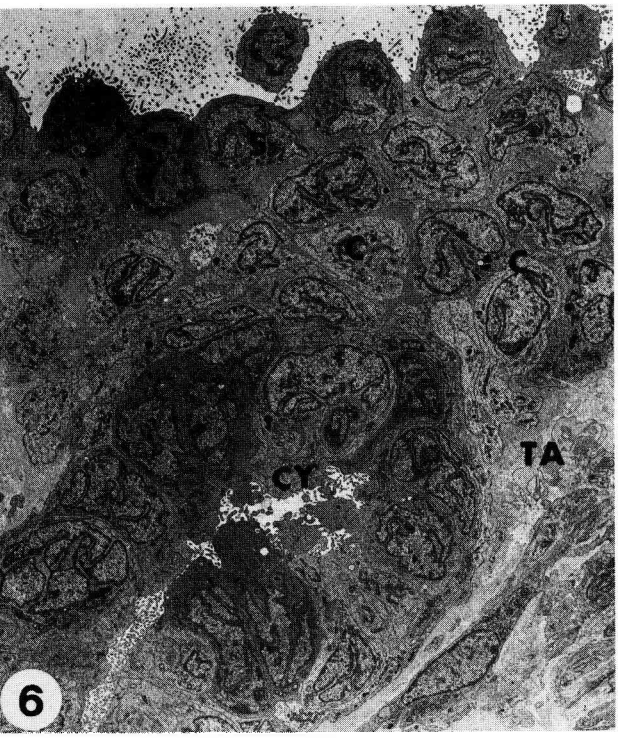
PLATE II

FIG. 6. — *Transmission electron micrograph of the ovarian cortex.* The superficial epithelium (S) penetrates into the tunica albuginea (Ta) and in the process forms cords (C) and crypts (Cy). The fine structure of the cells composing the crypts and cords is quite similar in appearance to the cells of the superficial epithelium (× 2 200 ; estrous rabbit).

FIG. 7. — *A superficial cell is evident in this Hivo electron micrograph.* As a rule these cells are irregular in shape, possess a few microvilli and contain a highly infolded nucleus (N). The mitochondria (M) as seen under the Hivo are dense and elongated. Lipid droplets (L) and lysosomes (LY) are also frequent in the cytoplasm of these cells. At the base of the cell is seen a large intercellular space (S) in contact with the basal lamina (BL) (× 11 750 ; estrous rabbit).

FIG. 8. — *Transmission electron micrograph of a « nest » of cord cells.* These elements possess an irregular, infolded nucleus (N), lipid droplets (L), numerous free ribosomes, polysomes, and a few membranes of the endoplasmic reticulum. Also evident within the cytoplasm is a Golgi complex and occasional lysosomes. Cord cells are very similar in appearance to follicle cells in adjacent, developing follicles and also to cells of the superficial epithelium. Compare the cells of this figure with those in figures 6 and 7 (× 6 500 ; estrous rabbit).

FIG. 9. — *This scanning electron micrograph illustrates the rather characteristic appearance of the cells located on the surface of the ovary and lining the lumen of a crypt.* Typically, these cells contain few microvilli but have an irregular appearance due to the presence of numerous blebs (*) (× 2 200 ; estrous rabbit).



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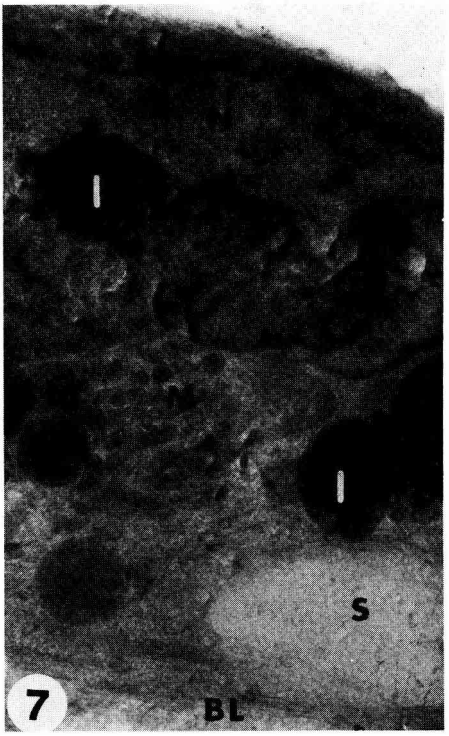

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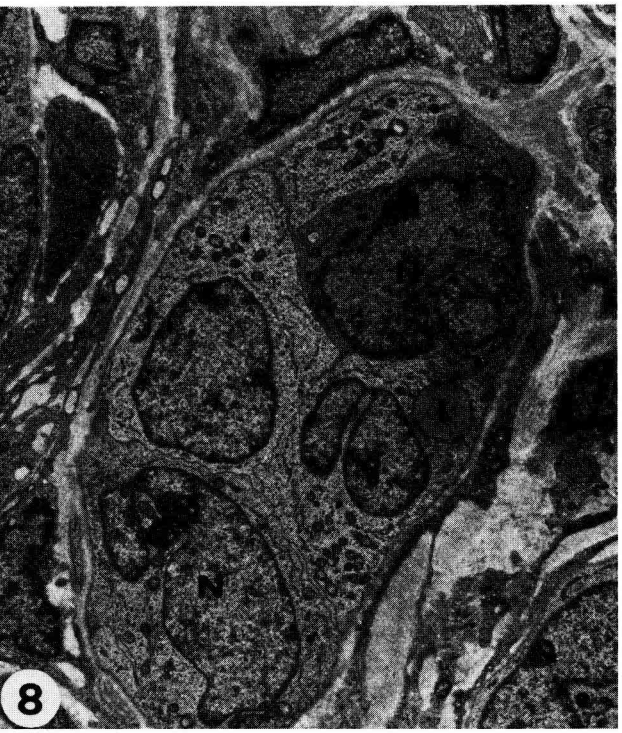

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At high magnification, the exposed surfaces of the microvilli had a rather rough appearance owing to the presence of an amorphous material similar in texture to the glicocalyx coat present on the microvilli in other tissues (the intestinal epithelium, for example). The areas of superficial cells devoid of microvilli displayed a number of small pits and cavities opening into cortical caveloae.

A characteristic feature of the ovarian epithelium was the high degree of variability in the density of microvilli on superficial cells : 1) located in different regions of the same ovary, 2) of ovaries of different mammals, 3) of ovaries of different ages and 4) of ovaries in different phases of the estrous cycle (plates I and II). By SEM, TEM and Hivo EM, cells of the superficial epithelium showed several features likely related to the phase of the cell cycle (Porter, Prescott and Frye, 1973). These features included branching evaginations, blebs, ruffles or lamellipodia, as well as cells with relatively smooth surfaces (figs. 2, 3, 4). Cells in which either blebs and/or lamellipodia were evident had a reduced population of microvilli, thus suggesting that microvilli might not be a permanent aspect of these cells but rather might be continually replaced by other surface features. Changes in morphology and architecture related to the cell cycle were undoubtedly surface seemingly manifestations of both the phase of the reproductive cycle and of events occurring within the ovary (Van Blerkom and Motta, 1979). The various morphological and organizational aspects of the ovarian epithelium, probably related to the cyclic and dynamic nature of that organ, made this mesothelium somewhat different from other mesothelial linings, including the peritoneal and visceral mesothelia (Motta, Andrews and Porter, 1977).

The occurrence of a single cilium on some cells of the superficial epithelium is a feature common to mesothelial surfaces (Andrews and Porter, 1973). Cilia which are observed with difficulty by TEM, are encountered not only on the epithelium of the ovary (Wischnitzer, 1965) but also on cells within the ovary itself (Motta, Takeva and Palermo, 1971). It seems most probable that ovarian cilia are nonfunctional rudimentary organelles. However, it is nevertheless an intriguing possibility that they may have some as yet undefined role in the coordination of physiological processes during follicular growth (Motta, 1965).

On a more macroscopic level, the ovary is frequently evaginated into a series of villous-like projections or papillae which, depending upon the species, may vary widely in number, size and distribution on the ovarian surface (Harrison and Matthews, 1951). As an example, rabbit and cat ovarian papillae are generally quite numerous, occasionally visible to the unaided eye, and frequently assume intriguing and unusual arrangements (fig. 5) (Cherney, Motta and Didlo, 1973 ; Motta and Van Blerkom, 1975). In other mammals as rats, mice, dogs and humans (Sternberg, 1963), the papillae are comparatively smaller and reduced in number, at least under normal physiological conditions (fig. 4) (Jensen and Norris, 1972).

In several species, areas of the superficial epithelium are often invaginated into subjacent, cortical layers. These invaginations form small well-defined, simple or ramified crypts and cords (Harrison and Matthews, 1951 ; Mossman and Duke, 1973). As observed by both TEM ans SEM (figs. 1, 6-9), the fine morphology of the cords and crypts in the mature ovary, mainly of rabbits and cats but also in some mice and rats, clearly demonstrated that these structures were true infoldings of the superficial epithelium. The crypts were hollow, tubular invaginations in which the lumen opened

directly to the surface of the ovary (figs. 1, 9). The cells on the surface of the ovary which surround the openings of the crypts were similar in appearance to other cells of the superficial epithelium : some of these possessed numerous microvilli, whereas others were relatively flattened or elongated, displayed few microvilli, and might have either a smooth surface or a surface containing blebs (figs. 6, 7, 9). Wischnitzer, 1965 ; Weakley, 1969 ; Motta, Cherney and Didio, 1971 ; Papadaki and Belby, 1971). Crypt cells were polyhedral elements (columnar or cuboidal) with a lobulated nucleus and a cytoplasm characterized by numerous free ribosomes, polysomes, a Golgi complex, relatively small mitochondria and a few, narrow cisternae of the rough-surfaced endoplasmic reticulum (RER) (fig. 6). The exposed surfaces of crypt cells which lined the lumen showed microvilli, an occasional cilium, blebs and ruffles (fig. 9). The crypt cells were interconnected by both interdigitations of the plasma membrane and by junctional complexes (desmosomes, tight and gap junctions).

Some invaginations of the superficial epithelium terminated as small, irregularly shaped and apparenty fragmented cords which, at higher magnification, appeared as « nests » of epithelial-like cells (fig. 8). These nests lay in close proximity to developing follicles and/or to isolated clusters of interstitial gland cells. Generally, due to a more solid configuration, the cord-like structures did not have a lumen, and the cells composing the cords did not possess either large cellular projections or a sizeable population of microvilli. At the fine structural level, the cord-cells contained lipid droplets, numerous free ribosomes, lysosomes, a Golgi complex and a reduced number of elements of the smooth-surfaced endoplasmic reticulum (SER). Intercellular contact was maintained through small desmosomes and tight and/or gap junctions.

Ultrastructurally, the cord cells appeared quite similar to the granulosa cells associated with developing follicles in regions adjacent to the cords (Unsicker, 1971) and also to some groups of differentiating interstitial cells (Mori and Matsumoto, 1970 ; Motta, 1974*a*). Furthermore, cord and crypt cells were morphologically similar to the cells of the superficial epithelium. On strictly fine structural criteria, one interpretation of these observations suggests that the superficial epithelium, with its cyclic proliferative abilities, may serve as an important and continuous source of crypts and cords, and perhaps also of other new ovarian components present during adult life.

In this regard, the superficial epithelium of the ovary may represent a true « germinative » epithelium (Motta, 1974*a*). Clearly, criteria other than morphological ones are required to support this hypothesis.

The presence of both invaginations and evaginations on the ovarian surface demonstrates the dynamic nature of this superficial epithelium. It has been shown that the incidence, number size and degree of development of the superficial structures (crypts, cords, villi and papillae) may be influenced by the levels of circulating pituitary gonadotropins and/or ovarian steroid hormones (Harrison, 1962 ; Jensen and Norris, 1972). Therefore, it is possible that these structures are related to the reproductive cycle of each species in which they occur. Consequently, the formation of villi, papillae, cords and crypts may be simply a proliferative expression of the superficial epithelium under normal, cyclic physiological conditions. The demonstration of submicroscopic changes on the surfaces of the cells of the ovarian epithelium (microvilli, blebs, ruffles) strongly suggests that the superficial epithelium undergoes

a cyclic process probably similar to that which occurs in other parts of the ovary and in other regions of the genital tract during the reproductive cycle (Motta, 1974*b*).

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Résumé. L'épithélium superficiel de différents mammifères observé par microscopie électronique à balayage, microscopie électronique à transmission, microscopie électronique à haut voltage, apparaît habituellement composé d'une simple couche de cellules polyédriques. En règle générale, la surface libre de ces éléments est recouverte par un grand nombre de microvillosités et de cils isolés. Dans d'autres cas, les cellules de l'épithélium superficiel montrent plusieurs aspects tels que des gonflements, des festons et lamellipodia ainsi que des surfaces lisses avec de petits creux et cavités. Ces modifications morphodynamiques semblent être en relation non seulement avec le cycle cellulaire mais aussi avec les phases du cycle de reproduction et les événements survenant dans l'ovaire.

A un niveau plus macroscopique, la surface ovarienne est dévaginée en une série de papilles et invaginée en cryptes et/ou cordons qui, selon les espèces, peuvent varier grandement en taille, forme et nombre. Il est probable que la présence de papilles, cryptes et cordons est une expression simple de prolifération de l'épithélium superficiel sous des conditions physiologiques normales, cycliques.

De ce point de vue, une inteprétation de ces observations suggère que la surface de l'épithélium, avec ses modifications morphodynamiques cycliques et ses possibilités de prolifération, peut servir comme origine de nouveaux composants ovariens pendant la vie adulte de plusieurs mammifères.

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