Electron Microscopy of Oocyte-Follicle Cell Relationships in the Rat Ovary.*

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The ovary of the neonatal rat has been found to offer very favourable material for an examination of the structural relationship between an oocyte and its surrounding follicle cells, since a large proportion of the follicles are at a similar stage of development and can be examined within a single section under the electron microscope. This has made it possible to examine the changes in cell surface relationships during the development of the follicle, from a stage where the oocyte possesses an incomplete covering of flattened cells to that in which a complete layer of cuboidal cells is present.

The material used in this study consisted of the ovaries of albino rats killed from a few hours to several days after birth. The organs were fixed in osmium tetroxide and in potassium permanganate buffered to pH 7.6 with veronal-acetate, and embedded in methacrylate and araldite respectively.

Observations made on osmium-fixed ovaries from neonatal rats essentially confirm the findings of recent authors using osmium-fixed ovaries from the adult rabbit (1), mouse (2), and rat (3). The use of permanganate for fixation has, however, helped to resolve cell surface interrelationships to a finer degree.

In an early stage of follicle formation, the cell membranes of the oocyte and follicle cell are smooth and closely applied to each other. The separation between the membrane is of the order of 125 to 140 A. The follicle cells are very flattened but their extremities, which may extend for a considerable distance around the periphery of the oocyte, tend to be filiform. Owing to curvature these may appear in thin sections as a small number of isolated circular or elongated profiles. At a slightly later stage, when the follicle cells have acquired a less flattened form, small "pockets" containing larger numbers of follicle cell processes appear at intervals around the oocyte (Fig. 1). It is believed that this increase in numbers is due to the formation of fine branches from the filiform ends of the cells, although clear evidence of branching has so far been seen only in follicles at a somewhat later stage of growth. Short club-like microvilli from the surface of the oocyte also extend into these "pockets" and interdigitate with the follicle cell processes. The oocyte microvilli elongate and become cylindrical as the intercellular space widens.

A further stage in development is marked by the lateral extension of the "pockets" to form a perioocyte space (0.3 to 0.4 μ wide) which is continuous over the surface of the oocyte except at points (Fig. 2) where relatively broad areas of close contact are maintained. The cell membranes and adjacent cytoplasm in these areas show a higher degree of osmiophilia than elsewhere (Fig. 1). These dense areas probably correspond to the desmosomes to which Anderson and Beams (8) have referred.

Many more microvilli are formed on the newly separated surface of the oocyte during this stage, but there seems to be no formation of corresponding outgrowths from the adjacent follicle cell surface. The microvilli may extend across to lie in contact with the follicle cell membrane. Concurrently, an amorphous substance of low electron density fills the perioocyte space (1–4). Light microscopy of thick sections stained by the periodic acid-Schiff method indicates that a fine Schiff-positive layer, approximately 0.3 μ wide, partially or completely surrounds the oocytes in follicles at this stage of growth. With the continuing growth of the follicle this layer becomes broader. Corresponding electron micrographs show that the amorphous substance continues to fill the widening perioocyte space, and that after a certain point the oocyte microvilli no longer extend right across to the follicle cells (3). Intercellular contacts are now maintained only by the broad extensions of the follicle cells, which have become modified by elongation and branching.

It seems clear that the the Schiff-positive material corresponds to the amorphous substance seen in electron micrographs. This substance must therefore represent an initial stage in the formation of the zona pellucida which is characteristic of later stages of growth. The observation that oocyte microvilli appear before, or concurrently with, the amorphous material in the perioocyte space rather than subsequently (3) lends

* Supported by a grant made to Professor Sir Solly Zuckerman, F.R.S., by the Population Council, Inc., New York.

‡ *Received for publication, December 4, 1959.*

slight support to the view that the oocyte itself may be involved in the formation of the zona pellucida. Follicle cell processes arise initially from the tapered ends of the flattened cells and later by branching from the broad areas of contact with the oocyte. The latter are interpreted as an early stage in the existence of the cone-like processes which have been described as extending through the zona pellucida in mature oocytes (3, 5, 6). The nature of the desmosomal structures in these areas requires further investigation. It is interesting, however, to note that desmosomes normally exist at contact areas between cells of the same type, in epithelia and other tissues (9–11), and it is perhaps relevant that Dawson (12), in discussing the origin of polyovular follicles, has advanced the hypothesis that in certain instances the histochemical reactions of follicle cells suggest that they may possess some degree of the potentiality of oocytes.

In agreement with Sotelo and coworkers (1, 3) no evidence that open cytoplasmic bridges link the oocyte and the follicle cell (4, 5, 7) has been found.

Addendum.—After the acceptance of this Note for publication, communications by Chiquoine and by Odor (Abstracts of the Proceedings of the American Association of Anatomists, in *Anat. Rec.*, 1959, **133**) were noted. Both authors agree with the present findings of the sequence of events in the formation of a zona pellucida, but do not mention the occurrence of desmosomes.

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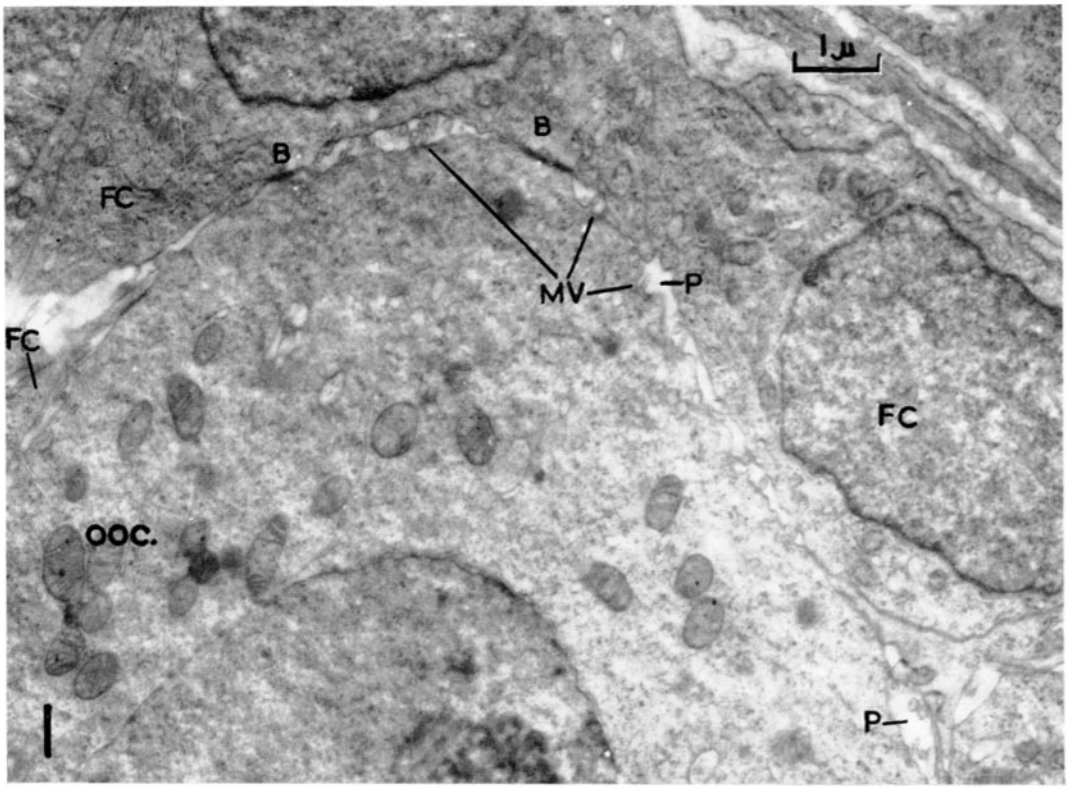
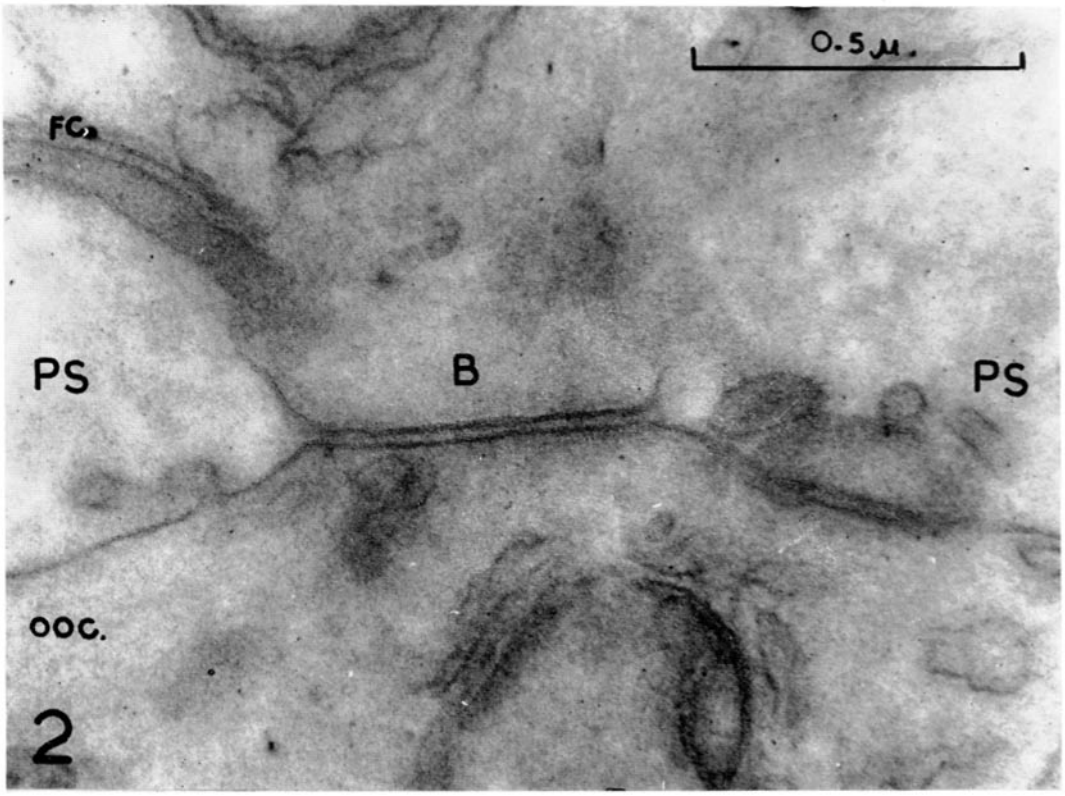
Explanation of Plate 219

Fig. 1. Electron micrograph of part of a small follicle before the stage of formation of a complete perioocyte space. Intercellular pockets (*P*), into which project microvilli (*MV*) from the oocyte (*ooc.*) and processes from the extremities of the follicle cells (*FC*), are beginning to extend around the oocyte. Broad contacts (*B*) between the oocyte and a follicle cell are marked by the greater electron density of cell membranes and underlying cytoplasm. (OsO_4 fixation). × 13,000.

Fig. 2. Electron micrograph of one of the broad areas of contact (*B*) between the oocyte (*ooc.*) and follicle cell (*FC*) at a slightly later stage of development. The perioocyte space (*PS*) has broadened and the diffuse substance of the zona pellucida can be distinguished. Small branches are arising from the follicle cell process at this point. ($KMnO_4$ fixation). × 99,000.





(Franchi: Oocyte-follicle cell relationships)