Intercellular bridges between follicle cells and oocyte during the differentiation of follicular epithelium in Lacerata sicula RAF

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INTERCELLULAR BRIDGES BETWEEN FOLLICLE CELLS AND OOCYTE DURING THE DIFFERENTIATION OF FOLLICULAR EPITHELIUM IN *LACERTA SICULA* RAF.

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SUMMARY

Intercellular bridges first appear during lizard oogenesis when follicles are rather small (150 μm in diameter); at this stage they form connecting links between the oocyte and follicle cells, which have not yet differentiated into pyriform cells. Later on, when the follicles have become larger (1 mm) and the follicular epithelium appears constituted by 3 types of cells (small, intermediate and pyriform cells) they form connecting links between the oocyte and both intermediate and pyriform cells. The establishment of intercellular bridges between pyriform cells and the oocyte precedes the complete differentiation of the former, which excludes the possibility that the fusion between pyriform cells and oocyte occurs only after these cells are completely differentiated.

In still larger follicles (up to 2 mm in diameter), during the degeneration of the pyriform cells, the occurrence, inside the bridges, of mitochondria and other cytoplasmic material suggests that these cells at the end of their function transfer their contents into the oocyte.

INTRODUCTION

Electron-microscopic investigations of oogenesis in vertebrates have so far shown true intercellular bridges connecting the follicle cells to the oocyte only in lizards (Ghiara, Limatola & Filosa, 1968; Neaves, 1971; Hubert, 1971*b*; Taddei, 1972; Bou-Resly, 1974). The significance of these connexions has not yet been completely elucidated, although it has been suggested that they represent the path through which follicle cells transfer into the oocyte, besides other materials, ribosomes (Taddei, 1972). The follicle cells which form connexions with the oocyte are called, from their shape, pyriform cells. These cells are regularly distributed between small and intermediate cells, inside the multilayered follicular epithelium, during most of the slow pre-vitellogenic growth of the oocyte. At the end of the pre-vitellogenic phase, the pyriform cells disappear and the follicular epithelium becomes more homogeneous and single-layered (Filosa, 1973). Studies by light microscopy seem to support the hypothesis that these cells originate from the small follicle cells via the intermediate cells (Trinci, 1905; Loyez, 1906; Hubert, 1971*a*). Electron-microscopic observations have led to the view that the bridges between pyriform cells and oocyte do not

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originate by incomplete cytokinesis, as do those between prediplotenic sister germ cells, but by a secondary cell contact and subsequent fusion (Filosa & Taddei, 1976).

The data so far available have not shown at what stage of oogenesis the intercellular bridges are established, whether pyriform cell precursors (intermediate and small cells) are also involved in such connexions, or whether or not these bridges are blocked before degeneration of the pyriform cells in the later oogenetic stages, as suggested by Neaves (1971). The present investigation on lizard oogenesis was undertaken in an attempt to answer these questions, and hence gain a clearer understanding of the function of the pyriform cells.

MATERIALS AND METHODS

Adult, sexually mature females of the lizard *Lacerta s.sicula* Raf. (6–7 cm in snout-vent length) were collected in the neighbourhood of Naples. The ovaries were exposed and follicles were isolated under a dissecting microscope. The follicles were fixed in phosphate-buffered formaldehyde-glutaraldehyde, pH 7·4 (Karnovsky, 1965), postfixed with phosphate-buffered 2% osmium tetroxide, dehydrated with ethanol and embedded in Epon. Ultrathin sections, stained with uranyl acetate and lead citrate (Reynolds, 1963), were examined with a Siemens Elmiskop IA electron microscope.

RESULTS

Intercellular bridges are not detectable in early follicles (up to 100 μm in diameter). At this stage a single-layered follicular epithelium 6 to 10 μm thick surrounds the oocyte. It is composed of similarly shaped, roughly cubic cells (Fig. 1), frequently dividing. These cells have a centrally located nucleus, about 5 μm in diameter, and a thin layer of cytoplasm with sparsely distributed mitochondria (Fig. 1). The follicular cells are welded to each other and to the oocyte by numerous intercellular junctions (Fig. 2); between the cells and oocyte there is a rather broad intercellular space, into which cytoplasmic protrusions of both cell elements extend.

Intercellular bridges make their first appearance in follicles about 150 μm in diameter. Here the follicular epithelium thickens, tending to become more heterogeneous and double-layered (Fig. 3). Its cells are similar in structure to those of the previous stage and are not yet pyriform in shape. The bridges appear as rather narrow interconnexions, about 1 μm in diameter; their limiting plasma membranes do not show morphological differentiations in the form of thickenings or juxtapposed material (Figs. 3, 5). They contain numerous ribosomes and vesicles (Figs. 3, 5). The intercellular junctions are no longer visible; the intercellular space between follicle

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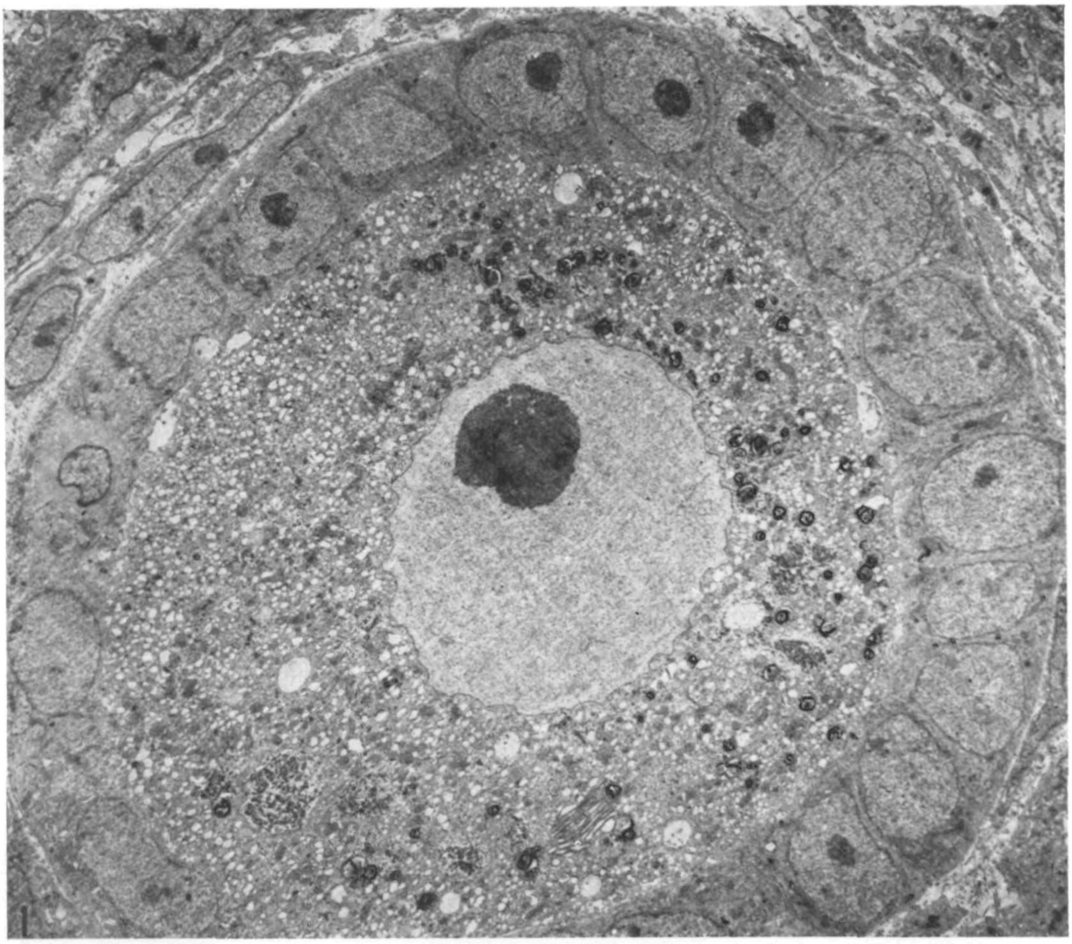
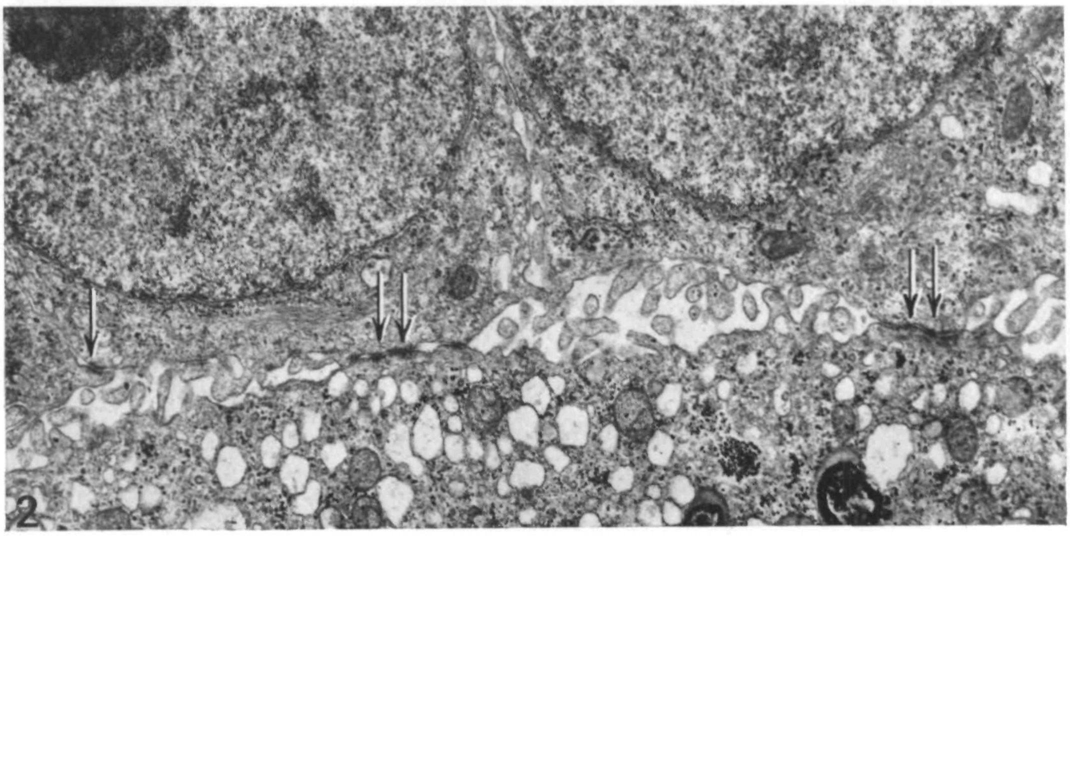
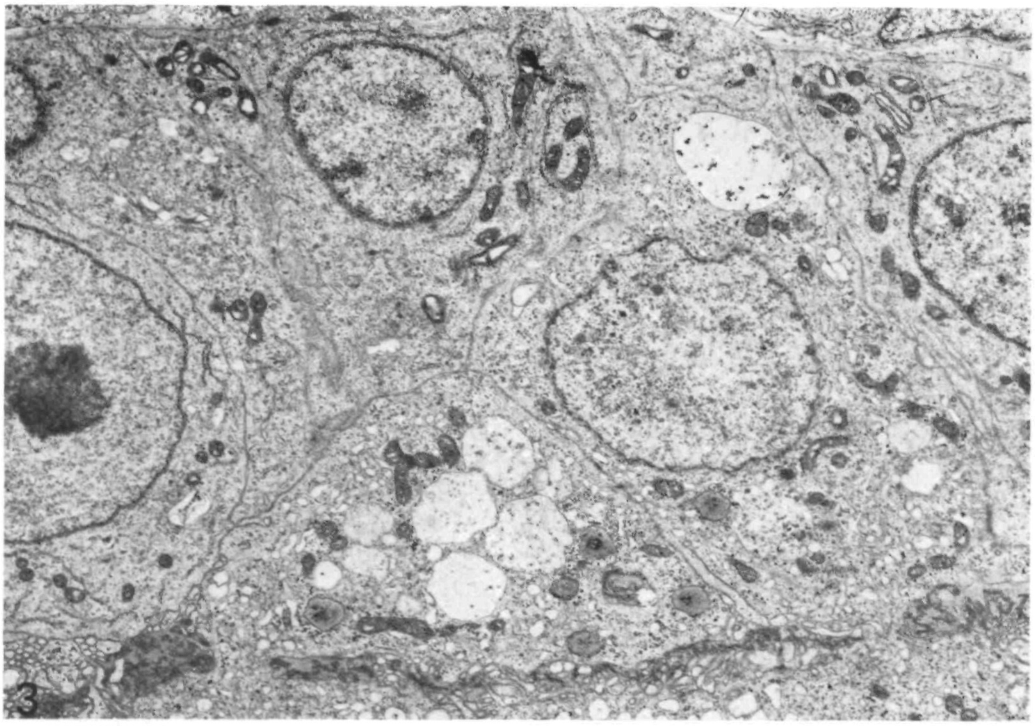
Fig. 1. Follicle 50–60 μm in diameter. Note the single-layered follicular epithelium, made of a single cell type. × 2800.

Fig. 2. Higher magnification of a portion of Fig. 1, showing the relationships between follicle cells and oocyte. Note intercellular junctions welding the adjacent follicular cells to each other (arrow) and to the oocyte (double arrows). In the rather broad intercellular space between follicle cells and oocyte microvilli and interdigitations of both cell elements protrude. Bundles of fibrillar material are present in the follicular cell cytoplasm. × 13 500.



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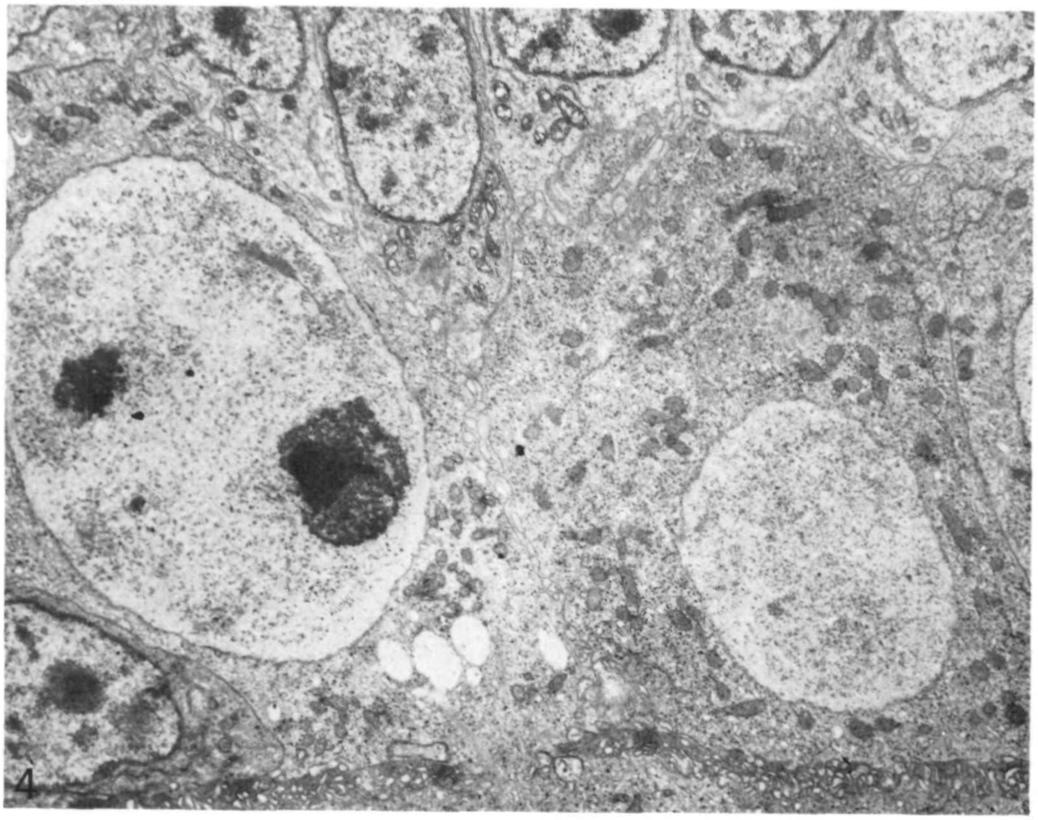
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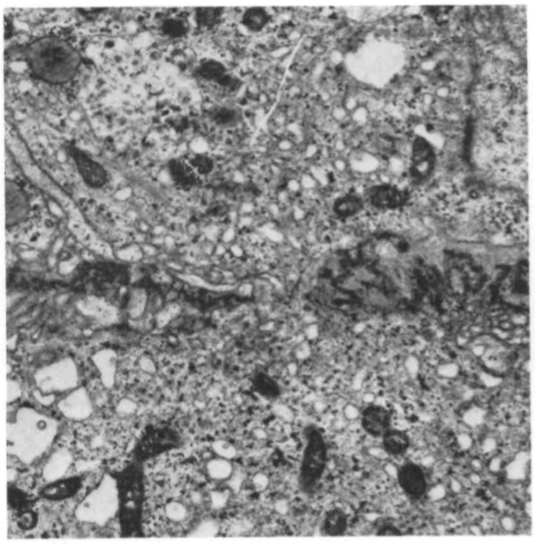
cells and oocyte, crowded with slender microvilli, is obliterated by the mutual compression of the cells and by the deposition of the zona pellucida.

In large follicles, 200 to 300 μm in diameter, those follicular cells which are connected with the oocyte through intercellular bridges, enlarge; they stand out against smaller cells located both more peripherally, close to the basal membrane, and in contact with the oocyte (Fig. 4). The enlarged cells, which are the early pyriform cells, are similar in structure to the intermediate cells of later stages (see below). They have a nucleus about 10 μm in diameter with a prominent nucleolus and dispersed



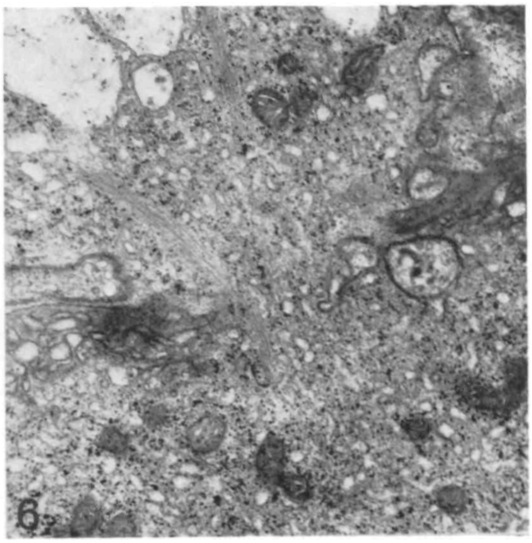


Fig. 5. Detail of Fig. 3, showing an intercellular bridge at higher magnification. × 10 000.

Fig. 6. The intercellular bridge of Fig. 4 at higher magnification; inside, ribosomes, vesicles and bundles of fibrillar material are evident. × 7200.

chromatin; their cytoplasm contains numerous vesicles and an increased number of mitochondria, preferentially located in clusters between the nucleus and the intercellular bridge (Fig. 4). Inside the intercellular bridge fibrillar material, ribosomes and vesicles are evident (Fig. 6).

In follicles 600 to 1500 μm in diameter 3 kinds of cells are observed: pyriform, intermediate, and small cells. Intercellular bridges connect the first two of these kinds of cells to the oocyte. At this stage the zona pellucida is composed of radially oriented microvilli protruding only from the oocyte surface. Dense material is dispersed

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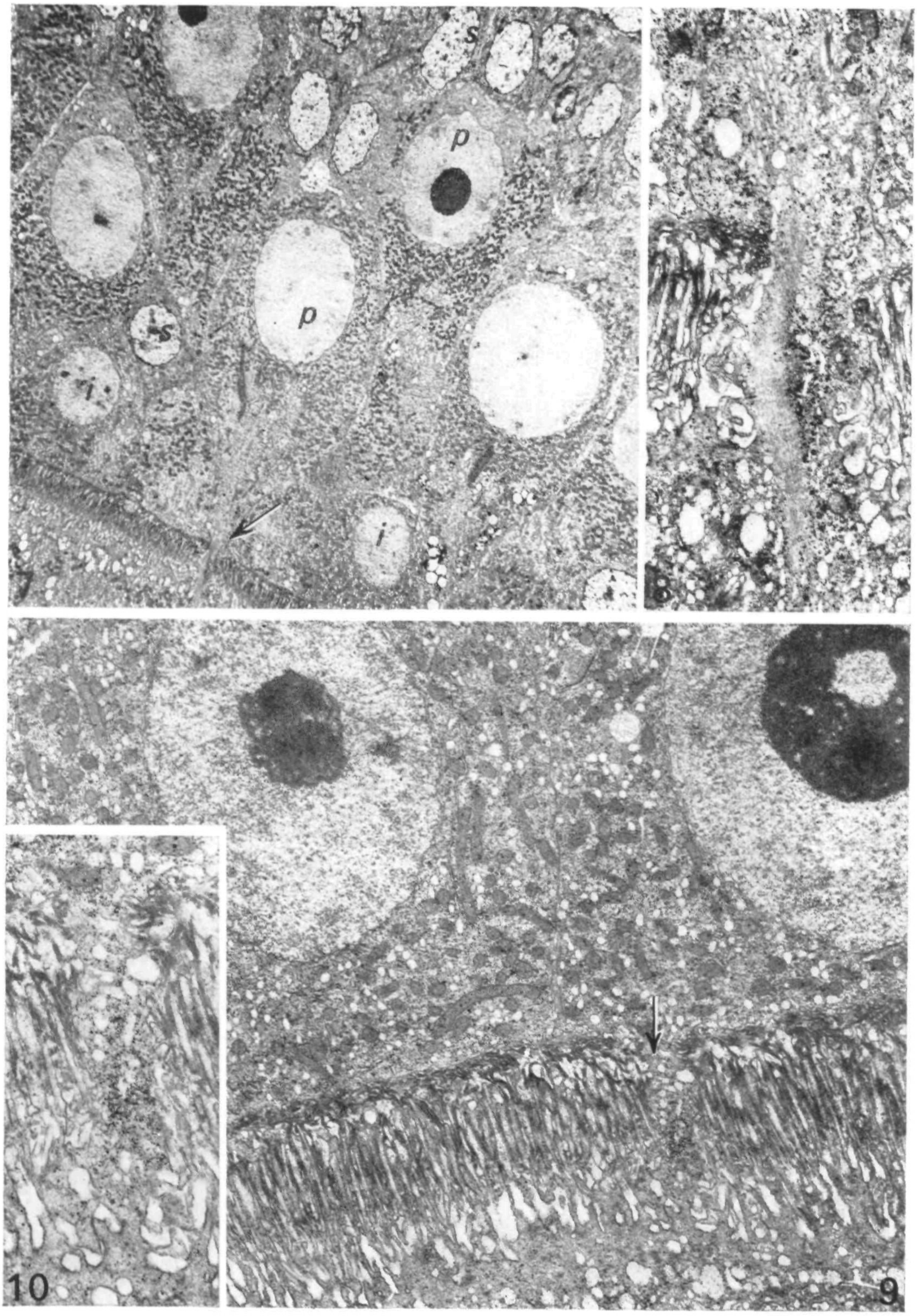
Fig. 3. Follicle 150 μm in diameter. The follicular epithelium has become double-layered. Intercellular bridges between follicle cells and oocyte are visible. × 10 000.

Fig. 4. Follicle 200–300 μm in diameter. The polymorphic follicular epithelium is double-layered and shows large and small cells. A large cell is connected to the oocyte through an intercellular bridge. The small cells are mainly located at the periphery of the follicle. × 4600.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between the oocyte and the follicle cells. The pyriform cells are fully differentiated, with the nucleus located at the distal pole and the narrower part of the cell pointed towards the oocyte. The overall length of the cell may reach 50 μm; the large, vesicular nucleus may reach 20 μm in diameter; the cytoplasm, especially in the stem region, is filled with mitochondria (Fig. 7). The bridge may contain bundles of fibrillar material, extending its entire length (Figs. 7, 8). The cells showing intermediate morphology, located among the stalks of the pyriform cells, are bell-shaped, with their base facing the oocyte. They show a vesicular nucleus, about 10 μm in diameter, containing a prominent nucleolus and scattered chromatin (Fig. 9). Their interconnecting bridges are usually observed in excentric positions: they contain vesicles and abundant ribosomal material (Figs. 9, 10). The small cells are located among the other cellular components, more frequently at the periphery of the follicle, just below the basal lamina. Their nuclei are rather small, about 5 μm in diameter, with more abundant heterochromatin patches; their cytoplasm appears fairly dense (Fig. 7).

At later stages, when the follicle diameter reaches 1500–2000 μm, intercellular bridges still connect the pyriform cells with the oocyte. Nevertheless, important changes modify the overall follicular texture: the follicular epithelium undergoes a gradual reduction in thickness; the pyriform cells start to degenerate and become distinctly separate from a multiple, outer layer of small cells (Fig. 11); intermediate cells are no longer seen. Furthermore, the zona pellucida is greatly thickened. The pyriform cells lose their typical shape and become smaller; their nucleus, which retains its typical texture, becomes irregularly outlined; their cytoplasm undergoes extensive vacuolation, showing vacuoles of different size and content, liposomes, and a reduced mitochondrial population; the intercellular bridges appear as long channels radially crossing the thickness of the zona pellucida (Fig. 12), and they contain mitochondria and rough endoplasmic reticulum fragments (Fig. 13).

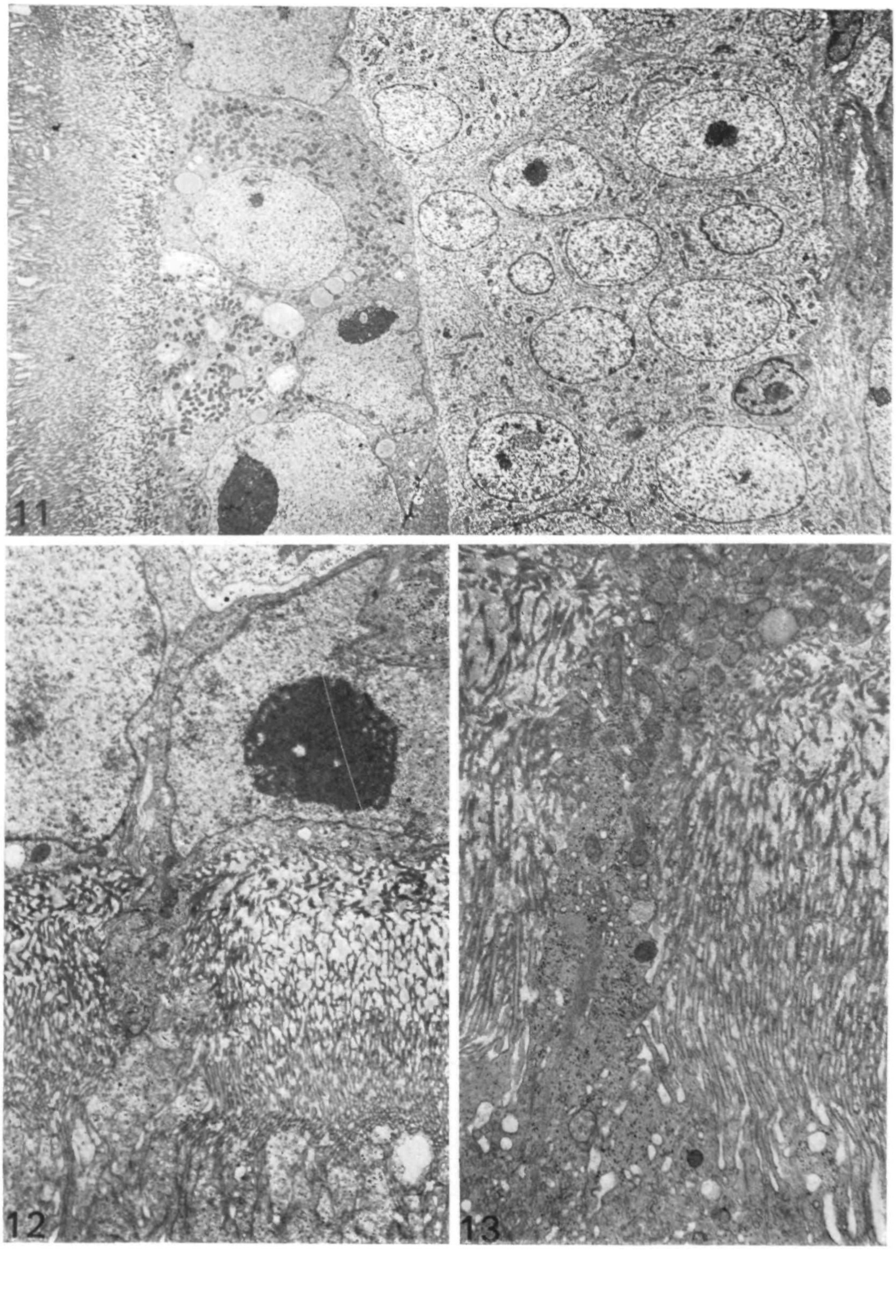
After the disappearance of the pyriform cells, in still larger follicles, the follicular epithelium again appears single-layered, composed only of small cells without intercellular bridges.

Fig. 7. Follicle 900 μm in diameter. The epithelium is polymorphic and many-layered showing small (*s*), intermediate (*i*), and pyriform cells (*p*). Note the cytoplasm of pyriform cells, rich in mitochondria, and in continuity with the oocyte via an intercellular bridge (arrow). × 1600.

Fig. 8. Higher magnification of the intercellular bridge of Fig. 7. Bundles of fibrillar structures, and elements of rough endoplasmic reticulum are visible. × 9500.

Fig. 9. Follicle 900 μm in diameter. The bell-shaped intermediate cells are arranged along the oocyte surface. Note their cytoplasm, rich in mitochondria and ribosomes, in continuity with the oocyte via an intercellular bridge (arrow), located at the margin of the cell. × 3800.

Fig. 10. Higher magnification of the intercellular bridge shown in Fig. 9. Note the abundance of ribosomes and vesicles. The intercellular bridge crosses the zona pellucida, which at this stage is filled with microvilli arising from the oocyte surface. × 10 000.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DISCUSSION

The present observations demonstrate that in lizard oogenesis intercellular bridges between follicle cells and oocyte make their first appearance in early follicles, just as the differentiation of the follicular epithelium from a single to a double layer is beginning.

Intercellular bridges are lacking in early follicles, which are composed of a single-layered follicular epithelium welded to the oocyte by means of intercellular junctions, as in other vertebrates (Wartenberg, 1962; Bellairs, 1965; Zamboni, 1974). Their first appearance at the subsequent stage strongly supports the view that they originate by a secondary fusion of the plasma membranes of the 2 kinds of cells. The mechanism by which the fusion of the plasma membranes occurs is still obscure: intermediate steps in this process have not yet been observed, because of the very irregular outline of the oocyte plasma membrane.

The finding of pyriform cell precursors, connected to the oocyte through intercellular bridges, demonstrates that the establishment of the intercellular bridge precedes rather than follows the differentiation of the pyriform cell. An attractive hypothesis is that the oocyte cytoplasm, by transferring some factors through the intercellular bridges, exerts a direct influence on pyriform cell differentiation. Furthermore, in contrast to the data reported in *Anolis carolinensis* (Neaves, 1971), our data show that the intercellular bridges are maintained late in the previtellogenic stage of oocyte growth, up to the time when these cells disappear.

The functional significance of the syncytial organization in lizard oogenesis is still obscure. The present data demonstrate that this organization is established very early during oocyte growth and remains till the pyriform cells disappear. In a previous paper, it was suggested that the intercellular bridges represent the path through which the pyriform cell ribosomes, and other materials, are transferred into the growing oocyte (Taddei, 1972). The data reported in this paper corroborate this hypothesis, since ribosomes are very abundant inside the bridges during the oocyte growth. Furthermore, the presence of numerous mitochondria and elements of rough endoplasmic reticulum inside the intercellular bridges, during the later pre-vitellogenic stage in which the pyriform cells disappear, and the concurrent reduction of mitochondrial density in the pyriform cell cytoplasm, suggest that the intercellular bridges represent channels through which the pyriform cell organelles, and possibly all the cytoplasm, flow into the oocyte. The fate of the pyriform cell nuclear material remains

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Fig. 11. Follicle 1700 μm in diameter. Note the segregation of the outer multiple layer of small cells with light cytoplasm, from the pyriform cells. The latter are reduced in size, and have irregularly shaped nuclei and scanty mitochondria. × 2250.

Fig. 12. Follicle 1700 μm in diameter. Intercellular bridges are still present at this stage. An irregularly shaped nucleus is located near the bridge. × 6000.

Fig. 13. Follicle 1700 μm in diameter. Many mitochondria, elements of rough endoplasmic reticulum, vesicles, ribosomes and bundles of fibrillar material are present in the bridge. × 8800.

unknown: we have never found such material inside the bridges or in the cortical region of the oocyte. The observation of pyriform cell nuclei, reduced in size and modified in shape, close to the intercellular bridges, may suggest that the nuclear material is disassembled inside the cell and then, possibly, transferred into the oocyte.

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