OBSERVATIONS ON THE ANNULATE LAMELLAE OF IMMATURE AMPHIBIAN OOCYTES

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Electron microscopy has established the existence of a complex membranous-bound system of cavities, the endoplasmic reticulum, in the cytoplasm of numerous cells (1). By means of the same technique, what may be parts of another cytoplasmic organelle, the annulate lamellae (2), has also been discovered. These usually consist of a number of parallel, *double-membraned sheets*, each of which structually closely resembles the nuclear envelope. Such structural complexes, however, appear to have a more limited distribution than the endoplasmic reticulum (see review in 3), and thus far have been predominantly observed in oocytes. Among the problems associated with this new cytoplasmic entity are its ultrastructure, organization, distribution, site of origin and functional significance. The preliminary observations reported in this paper will be concerned with some of these questions.

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MATERIALS AND METHODS

Ovaries from decapitated *Triturus viridescens* were excised and placed in 0.1 M sodium chloride. Under a binocular dissecting microscope, and with the aid of McClure scissors and jeweler's forceps, small clusters of immature, yolk-free (transparent) oocytes were dissected out of the ovary. These were immediately placed in the fixative, which consisted of 1 per cent OsO_4 in 0.7 per cent sodium chloride, adjusted to pH 7.4 with McIlvaine's standard buffer solution (0.05 M). After fixation for 1¾ to 2½ hours the material was dehydrated by passage through a graded ethanol series followed by impregnation with three changes of 4 parts ethyl to 6 parts *n*-butyl methacrylate. The oocytes were then embedded in the methacrylate mixture to which 1 per cent luperco CDB (a preparation of 2,4-dichlorobenzol peroxide) has been added, and polymerization was accomplished by maintaining at 60°C. in an oven for 24 hours.

Sections were cut with a glass knife using a Porter-Blum microtome. After spreading, the sections were mounted on grids coated only by carbon films. An RCA EMU-2A microscope was used for observation and photography.

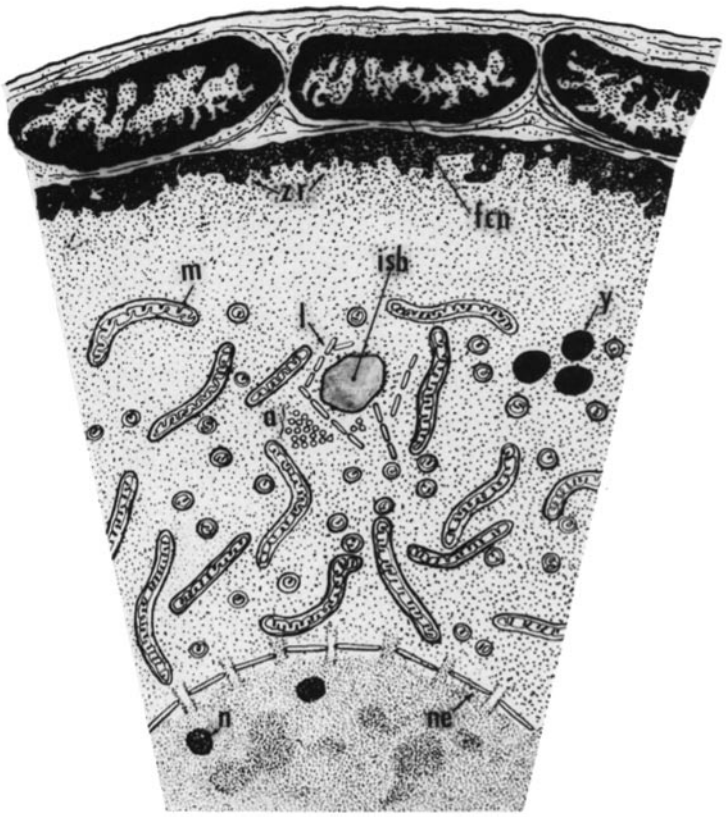


FIGURE 2

An idealized diagrammatic representation of a portion of the oocyte shown in Fig. 1 as it would appear under the electron microscope. In the periphery the follicle cell nuclei (*fcn*) are most pronounced and a complex stratum (*zr*), corresponding to the zona radiata of light microscopy, is also evident. Mitochondria (*m*), clusters of yolk platelets (*y*), and an irregularly shaped body (*isb*) are located in the cytoplasm. The unoriented annulate lamellae are seen in perpendicular section (*l*) as two discontinuous membranes, and in oblique section (*a*) as a collection of annuli. The nuclear envelope (*ne*) is resolved into its components and the nucleoli (*n*) are seen as compact granular masses.

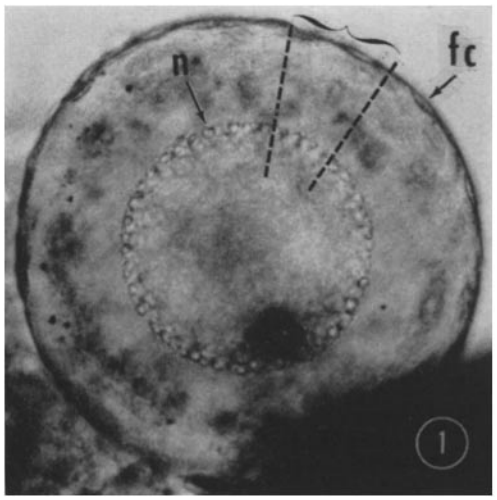


FIGURE 1

An immature oocyte from *Triturus viridescens* enclosed by its follicular epithelium containing spindle-shaped follicle cells (*fc*). The cytoplasm has variations in density, but aside from a few dark (yolk?) masses, no structural elements appear to be resolvable. The nuclear envelope and refractile nucleoli (*n*) are evident in these transparent oocytes. × 525.

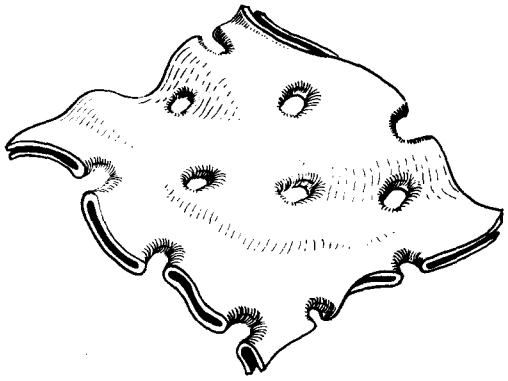


FIGURE 3

A portion of the "structural skeleton" (see text) of a double-membraned sheet from the annulate lamellae, seen in three dimensions.

OBSERVATIONS

In an immature, almost yolk-free oocyte as seen by light microscopy (Fig. 1), the nuclear envelope and numerous nucleoli can be identified. Aside from a few dark (yolk?) masses, no easily identifiable structural entities can be seen in the cytoplasm although variations in density exist. An idealized portion of such an oocyte as it would appear under the electron microscope is diagrammatically shown in Fig. 2. The nuclear envelope becomes resolved into its discontinuous double-membrane components and the nucleoli are present as compact granular masses. In the granular cytoplasm a number of different structures are evident which include mitochondria, yolk platelets, some irregularly shaped bodies (*isb*) and annulate lamellae. These lamellae exist as individual membranous sheets, the "structural skeleton" of which is shown in three dimensional form in Fig. 3, where the electron dense material associated with the discontinuities (pores) is omitted (see below).

In an electron micrograph of the peripheral portion of an immature oocyte (Fig. 4) all the aforementioned cytoplasmic organelles are evident. Numerous mitochondria can be seen in both cross- and longitudinal section as well as a cluster of small, oval, yolk platelets. Individual irregularly shaped, homogenous bodies are also present (Fig. 4, *isb*). Dark, narrow strands can be seen in close proximity to one such body. Under somewhat higher magnification (Fig. 5) the membranous character of these randomly oriented strands is somewhat more apparent.

When the area containing the annulate lamellae is magnified so that its structural components are clearly resolvable, the characteristics of the organelle become evident (Fig. 6). The individual membranous sheets that can be seen are generally found to be sectioned at an oblique angle to their surface thus revealing the annulate structure. In some of the annuli a central granule is apparent. The undulatory character of each membranous sheet in the cytoplasm accounts for the various patterns of annuli. A perpendicular section is characterized by the discontinous double-membrane nature of each sheet (Fig. 7).

DISCUSSION

Morphologically the structure of the annulate lamellae corresponds very closely to that of the nuclear envelope. The latter was originally viewed as a double-membraned structure, having discontinuities (pores) at the points where the individual membranes come together (Fig. 3). However, observations in oocytes (4) and more recently in somatic cells (6), have shown that tube-like structures are frequently associated with each of the discontinuities. Evidence that this may also be true for annulate lamellae has been presented (2, 7) and is not contradicted by any of the observations made here. Thus, Fig. 3 can be considered to represent only the structural skeleton of an individual component of the annulate lamellae.

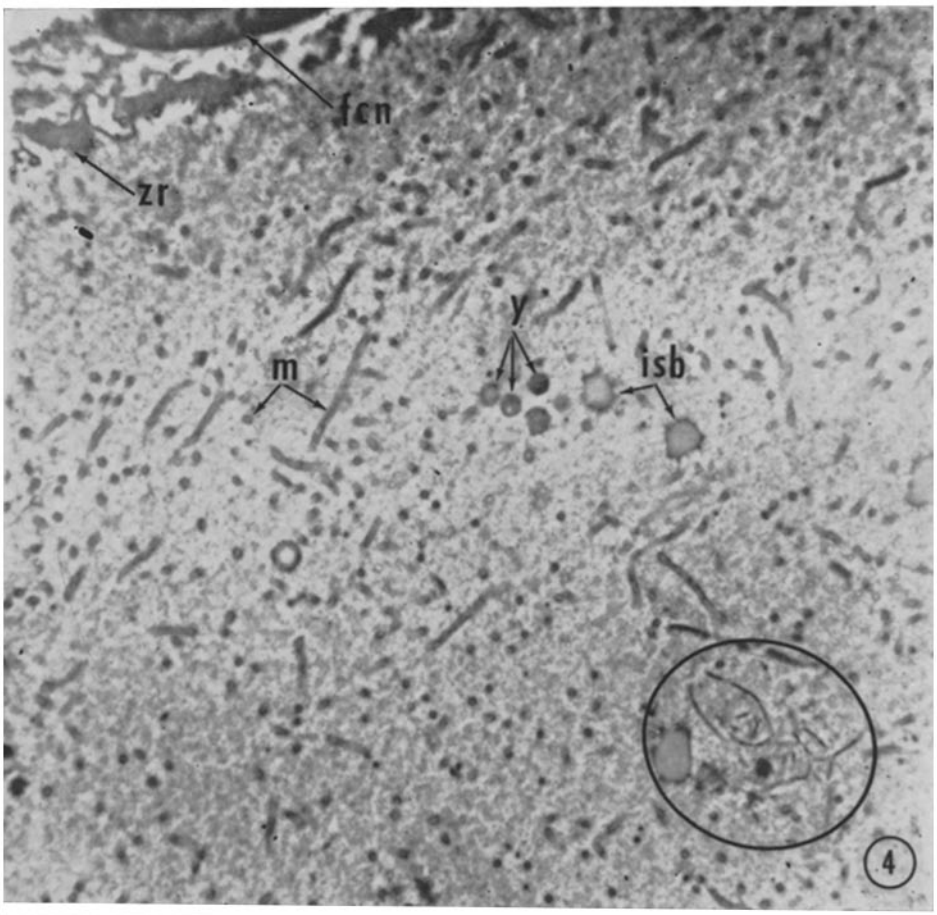
The structural similarity between the nuclear envelope and annulate lamellae is also evident from the annulae observed. A central granule is sometimes present. Such structures have been described in the nuclear envelopes of many cell types (5, 10, 11) as well as in the annulate lamellae of sand dollar oocytes (9).

FIGURE 4

An electron micrograph of the peripheral portion of an immature oocyte. A portion of a follicle cell nucleus (*fcn*) and the zona radiata (*zr*) can be seen. The granular cytoplasm contains numerous mitochondria (*m*), seen in cross- and longitudinal section, a cluster of oval yolk platelets (*y*), and some (*isb*) bodies. The region in which the annulate lamellae are located is encircled. × 5,250.

FIGURE 5

An enlargement of the encircled area from Fig. 4. The character of some of the lamellae is evident. Those cut perpendicularly are most predominant. A few annuli (*a*) can be seen which presumably represent an obliquely sectioned sheet. × 24,250.


fcn
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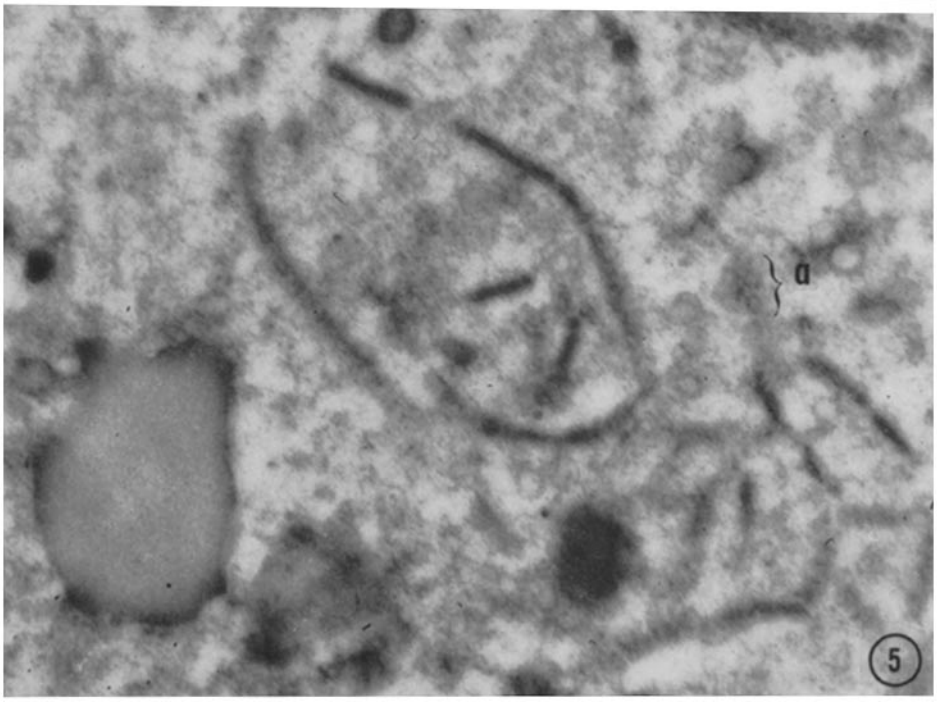
4


a
5

The annulate lamellae, as generally observed in oocytes and other material, characteristically consist of a parallel array of membranous sheets (2, 3). Such an organization is not evident in the stage of salamander oocytes which are described in the present study. Rather, they consist of individual, randomly oriented, (pairs of) membranes, grouped in a limited area of the cytoplasm. It is possible that orientation into parallel arrays takes place at a later developmental stage of the oocyte. In snail (2) and in sand dollar (9) oocytes, annulate lamellae were found to be present in the form of both organized and irregularly distributed membranous sheets.

Annulate lamellae have thus far been predominantly observed to be present in embryonic

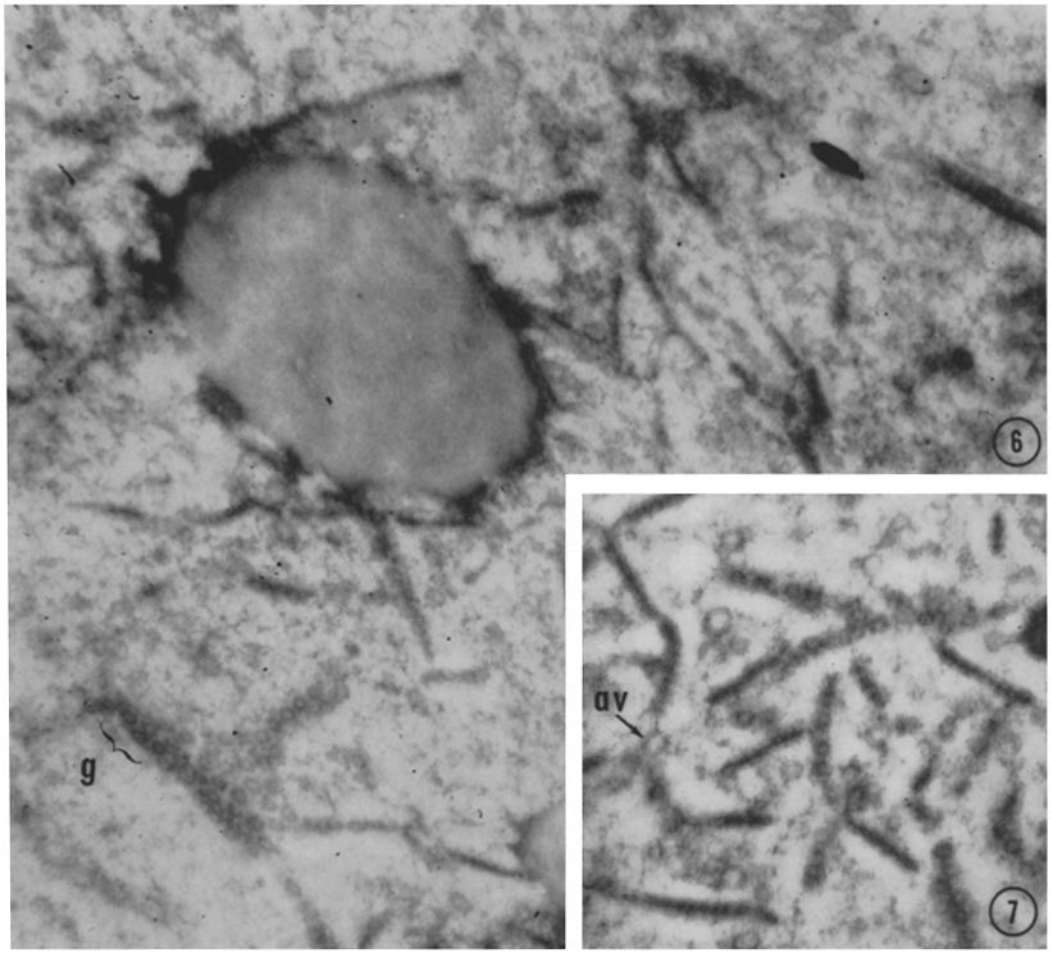


FIGURE 6

An electron micrograph showing the annulate lamellae irregularly distributed but seemingly closely associated with one of the "isb" bodies found in the cytoplasm. The annuli have different patterns of arrangement due to the waviness of the lamella. In some of the annuli a central granule (*g*) can be seen. × 24,250.

FIGURE 7

Annulate lamellae seen in perpendicular and tangential sections. A number of vesicles are present, most of them apparently are free, but one (*av*) is distinctly attached to a lamella. × 24,250.

cells, especially invertebrate oocytes (2, 8, 9, 11). It was thus not surprising to find these organelles in the cytoplasm of an oocyte taken from a vertebrate. Annulate lamellae usually are found in the vicinity of the nuclear envelope and evidence has been presented which suggests that they arise therefrom (2). This organelle has also been observed in the more peripheral regions of the cytoplasm (3) as is the case in the amphibian oocytes studied.

Functionally these membranous sheets, by virtue of their basophilia (2, 8, 12) and the fact that they are, on occasion, found to be continuous with the endoplasmic reticulum, have been considered to be a specialized form of ergastoplasm (2).

The observations reported above show that in a number of cases the annulate lamellae are in close proximity to irregularly shaped homogenous bodies. It is conceivable that the lamellae are functionally related to such neighboring bodies. Similar bodies have been called lipochondria (13), but histochemical tests are needed to confirm such an identification in this case. In this connection it can be noted that Afzelius (11) and later Merriam (9), studying invertebrate oocytes, found isolated membranous elements surrounding granular masses. The latter are basophilic and nucleolus-like in appearance. While the function of this newly discovered organoid remains as yet undetermined, it appears probable that it is in some way involved in the mechanism of protein synthesis.

SUMMARY

Annulate lamellae, in the form of randomly oriented, membranous sheets, were seen in the amphibian oocyte. In both oblique and perpendicular sections they appear to be structurally similar, if not identical, to the nuclear envelope. In some cases this organelle was found to be morphologically associated with an irregularly shaped homogenous body which is as yet unidentified. The observations were discussed from the standpoint of structure, organization, distribution, and function.

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