THE FINE STRUCTURE OF ANNULATE LAMELLAE*

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Plates 138 to 141

This paper discusses a class of lamellar structures found in the cytoplasm of several cell types. These lamellae possess a characteristic "annulate" structure resembling the nuclear membrane in the possession of numerous rings or annuli. Some, if not all, lamellae of this type are basophilic, containing a high concentration of ribose nucleic acid (9, 10). They can thus be considered as one form of the ergastoplasm, if the term is used broadly to include those lamellar or membranous components of the cytoplasm containing RNA and presumably associated with protein synthesis.

Annulate lamellae have been previously described in the literature several times. Structures probably of this type were demonstrated by McCulloch (4) in the cytoplasm of *Arbacia* oocytes. Afzelius (1) showed micrographs of annulate lamellae in *Psammechinus* oocytes, but considered them to be fragments of the nuclear membrane remaining from the maturation divisions. Structures from rat spermatids, probably also of this kind, were described by Palade (7). Lamellae from salivary gland cells of *Drosophila* (2) and from frog oocytes (3) are possibly also annulate, but magnification is insufficient for an analysis of their structure. Annulate lamellae from oocytes of the clam *Spisula* and the snail *Otala* have previously been described from this laboratory (9, 10). This paper supplements these previous observations, and demonstrates similar structures from acinar cells of amphibian pancreas, and from rat spermatids.

Materials and Methods

Tissues studied were as follows: ootestis of the helicid pulmonate snail *Otala lactea*, ovary of the surf clam *Spisula solidissima*, pancreas of 2 cm. larvae of *Ambystoma opacum*, and testis of a 4-month-old Sprague-Dawley rat. Tissues were fixed in 1 per cent osmic acid adjusted to pH 7.4 with veronal acetate (6) or citric acid phosphate buffer for 1 hour at 5°C. They were embedded in butyl methacrylate and sectioned with a Porter-Blum microtome. Observations were made with a RCA EMU-2D microscope.

OBSERVATIONS

In early oocytes of *Otala* a number of large lamellae were observed in the cytoplasm, parallel to the nuclear membrane, or irregularly arranged in the cytoplasm (Figs. 1 and 2). These lamellae in some regions appeared highly

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organized, and in others consisted largely of loose and irregular collections of cytoplasmic vesicles. The organized regions resembled the nuclear membrane. In transverse section they consisted of two membranes, 200 to 400 mμ apart, containing the perforations and vertical electron-dense projections seen in the nuclear membrane when the plane of section transects an annulus (Figs. 3 and 4). In oblique section annuli were clearly visible (Figs. 2 and 6). These did not differ significantly from annuli in the nuclear membrane, except that in some regions they appeared more regularly packed in a hexagonal arrangement. In later oocytes of both *Otala* and *Spisula* lamellae were frequently arranged in parallel arrays (Figs. 3, 4, and 6). In some cases they were so aligned that annuli in one lamella were connected to those in the next adjacent to it by tubular extensions of the annulus wall, forming a three dimensional lattice of highly complex morphology (Fig. 5). Small single lamellae also were seen, apparently in contact with the nuclear membrane (Fig. 9), which occasionally was highly convoluted and folded back upon itself (Fig. 7).

The disorganized regions showed considerable variation. In several young *Otala* oocytes they consisted largely of small vesicles with clear centers, of 100 to 200 mμ diameter, associated with amorphous masses of electron-dense material (Figs. 2 and 8). In several older oocytes larger vesicles, up to 2 μ in diameter, were also present (Figs. 3 and 4), occurring both at the ends and in the middle of lamellae. Some lamellae appeared to be composed entirely of a collection of attached vesicles (Fig. 10), and vesicles with similar wall structure also occurred singly in the cytoplasm, showing no evidence of attachment.

In acinar cells of larval *Ambystoma* pancreas annulate lamellae of somewhat similar structure were found (Figs. 11 and 12). These occurred only rarely in the tissues studied, the ergastoplasm of the usual type (12) being most evident. Small lamellar arrays were also found associated with the chromatoid body of rat spermatids (Figs. 13–15). These are almost certainly the same component described by Palade (7) also from rat spermatids. In Fig. 14 the interconnection of adjacent lamellae probably by annular tubules is evident.

DISCUSSION

Annulate lamellae show certain morphological similarities to the nuclear membrane, and also are frequently in close proximity to the nucleus. Both of these facts suggest that annulate lamellae may owe their structure somehow to the nuclear membrane. If so, lamellae could arise either by fragmentation of the preexisting membrane, or possibly they are synthesized upon it. The third possibility, that annulate lamellae are not directly associated with the nuclear membrane, but that similar forces shape the morphology of both, must also be considered.

Afzelius (1) considered that annulate lamellae were actually fragments of nuclear membrane, remaining after nuclear breakdown in metaphase. In the *Otala* and *Spisula* ooctyes this seems unlikely. In the larger oocytes several

months had certainly elapsed since the last oogonial division. In the oogonia the nuclei are small, and, it seems, could not possibly account for the large array of membranes seen in the later stages. Instead of fragmentation during mitosis, however, the nuclear membrane could continually bud off fragments, as indicated by Gay (2) in the salivary gland cells of *Drosophilia*. The elaborate folds in the nuclear membrane, frequently seen in young oocytes (Fig. 7), might be considered as one stage in this process, but in such folded nuclei no evidence for breaks in the nuclear membrane was seen. Although such fragmentation could account for the presence of single lamellae, it cannot account for the highly oriented three dimensional lattice seen in some cells (Figs. 5 and 14). If these structures arise solely from nuclear membrane blebs, one would also have to postulate that complex forces of aggregation caused alignment of a very precise kind. In many cells, *e.g.* in the pancreas (Fig. 12), lamellae have only been seen in arrays of the type shown.

It thus seems most likely that lamellar arrays are formed as units, and are synthesized somewhat as certain crystals, on the vertical extension of annular tubules and the horizontal extension of lamellae. Since the spacing between lamellae is in many cases quite regular (Fig. 5), and since small fragments of lamellae occur at the edge of the arrays (Fig. 3), it seems likely that lamellar aggregates increase in size by adding on material at the free margins. From observations with the light microscope, it also seems probable that the arrays are, in some cases, synthesized on the nuclear membrane (9, 10), and thus might be considered as replicas of it. In young frog oocytes Pollister *et al.* (8), Ornstein (5), and Kemp (3) have all demonstrated extensions into the cytoplasm from the nuclear membrane. From the dimensions of these structures it seems likely that they represent the annular tubules first described by Afzelius (1), but in a more elongate form. It should also be emphasized that these annular tubules can often be traced a considerable way into the nucleus, where they may become lost in a tangle of chromosomal material (10). These preliminary observations thus suggest a mechanism for the transmission of genetic specificities across the nuclear membrane regions by continuous structures associated with the annular tubules. This is, however, a highly speculative and provisional hypothesis, supported as yet by only a comparatively meager set of observations. Also, since annulate lamellae have been described from only comparatively few tissues, in many cells transfer of specificity must occur by other mechanisms.

Although changes in the morphology of cell structures associated with function are obviously difficult to analyze with the electron microscope, it seems likely that annulate lamellae arise at the nuclear membrane, move out into the cytoplasm, and break down eventually into numerous isolated cytoplasmic vesicles. Small vesicles, as shown in Fig. 8, appear to arise, at least in some cases, directly from the annulus, possibly by a pinching off of the annular tubules above and below the plane of the lamella. The annulus center thus

would become the center of the vesicle, and the annulus wall would be incorporated into the lining membrane. In the formation of larger vesicles both annular and interannular regions must be involved, the annuli apparently being disrupted by separations of the lamellar membranes (Fig. 4). Fig. 10 suggests a row of vesicles alternately formed by interannular and annular regions. The vesicles we have tentatively considered to be formed from annuli, are smaller with denser limiting membranes. Evidence that the particles lining cytoplasmic vesicles may be derived from the annulus wall has been obtained from other tissues, and will be presented elsewhere (11).

SUMMARY

Certain lamellar structures have been described from snail (*Otala*) and clam (*Spisula*) oocytes, the acinar cells of amphibian (*Ambystoma*) pancreas, and from rat spermatids. These structures are alike in possessing numerous rings or annuli, resembling those in the nuclear membrane. Thus the name "annulate lamellae" has been proposed for them. It is suggested that they may function in the transfer of specificities from nucleus to cytoplasm.

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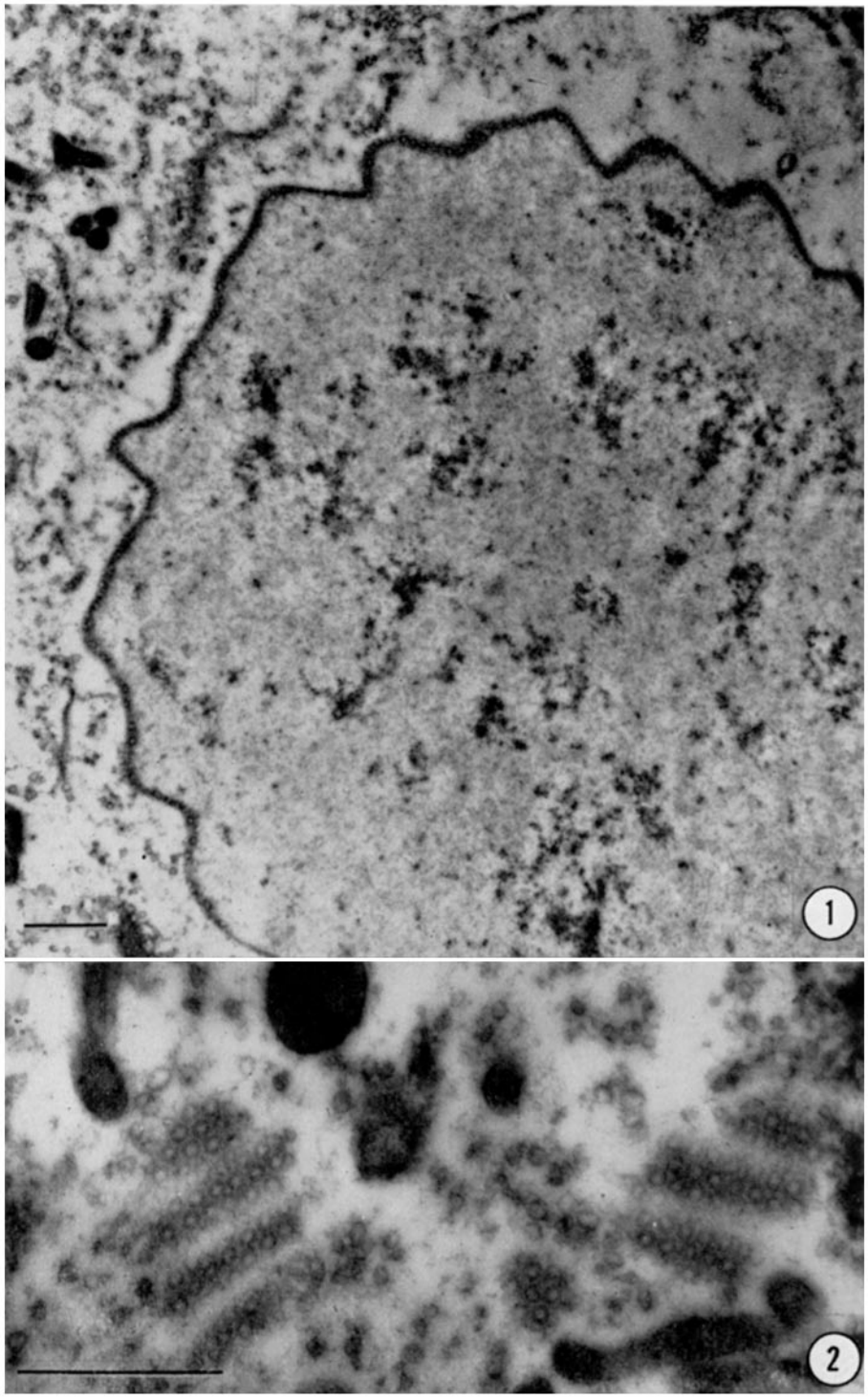
EXPLANATION OF PLATES

PLATE 138

FIG. 1. Portion of a young oocyte of *Otala*, showing arrangement of disorganized lamellae in the cytoplasm. × 12,300.

FIG. 2. Annulate lamellae from another section through the same cell. Two small lamellar arrays are visible in oblique section. × 28,500.

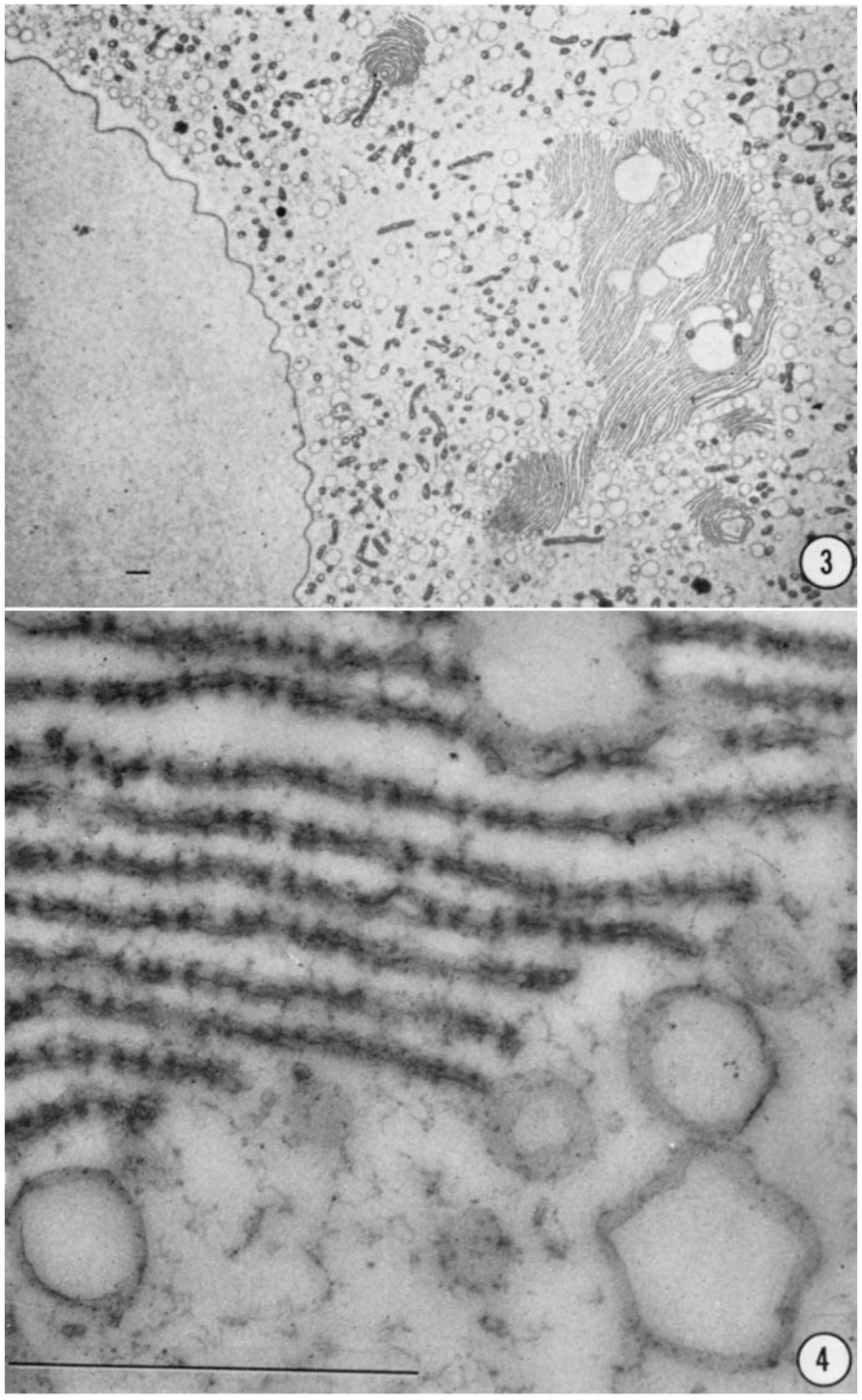


(Swift: Fine structure of annulate lamellae)

PLATE 139

FIG. 3. Part of an older oocyte of *Otala*, showing a large array of annulate lamellae in the cytoplasm. × 3,600.

FIG. 4. Detail from portion of Fig. 3 showing annuli, and vesicles both in center and at ends of lamellae. × 57,000.



(Swift: Fine structure of annulate lamellae)

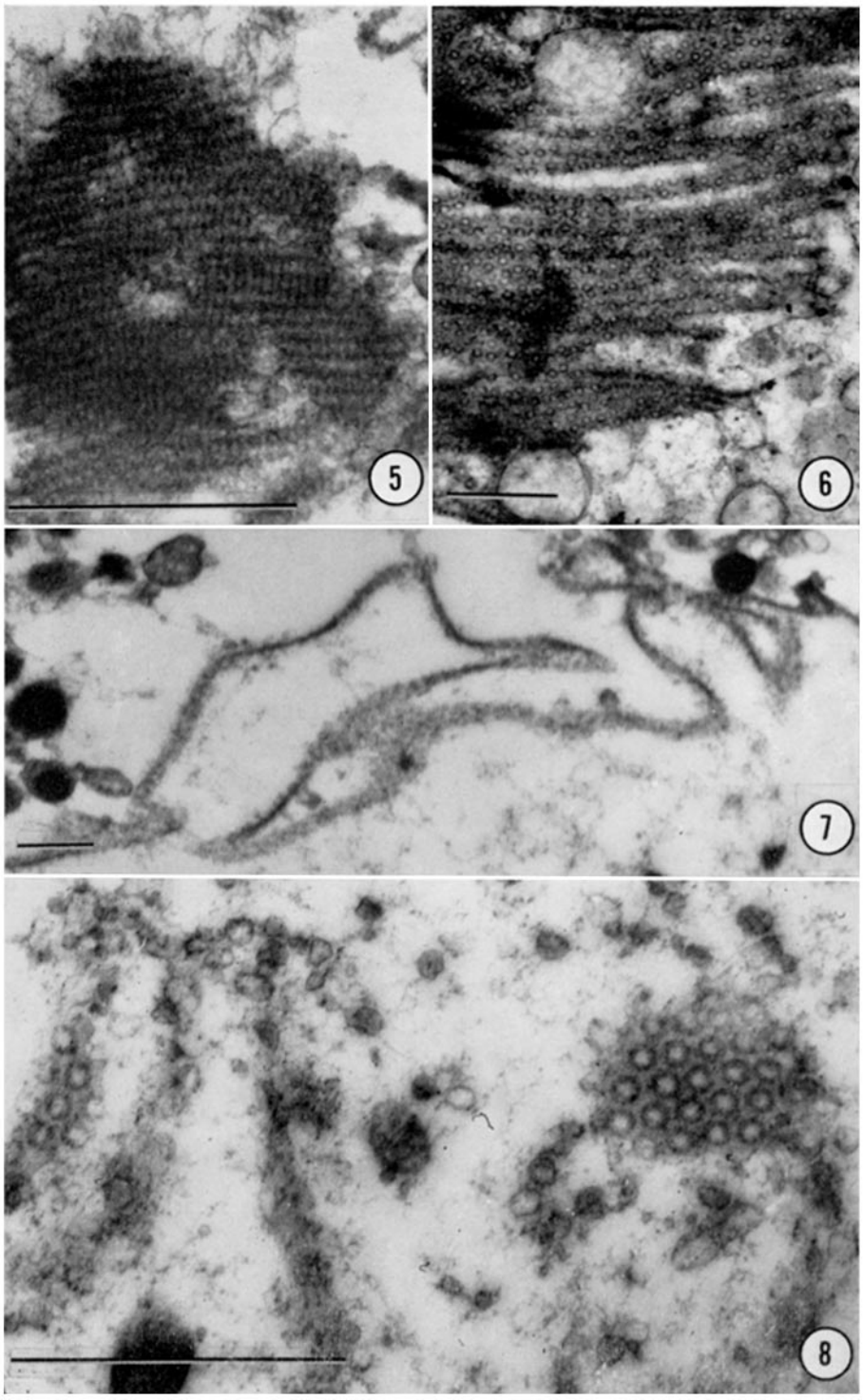
PLATE 140

FIG. 5. Arrays from a large oocyte of *Spisula*, showing interconnecting annular tubules between lamellae. × 39,400.

FIG. 6. Thick section of annulate lamellae from large *Otala* oocyte. × 15,000.

FIG. 7. Highly convoluted nuclear membrane in an oocyte of *Spisula*. × 10,300.

FIG. 8. Organized and disorganized lamellae from the same young *Otala* oocyte shown in Figs. 1 and 2. × 46,000.



(Swift: Fine structure of annulate lamellae)

PLATE 141

FIG. 9. Small annulate lamella associated with the nuclear membrane of a large *Otala* oocyte. × 38,000.

FIG. 10. A group of attached cytoplasmic vesicles from a large *Otala* oocyte. The alternate arrangement of large and small vesicles has been seen in other cells, and suggests that the vesicles have arisen by swelling of interannulate and annulate portions of a lamella. × 29,400.

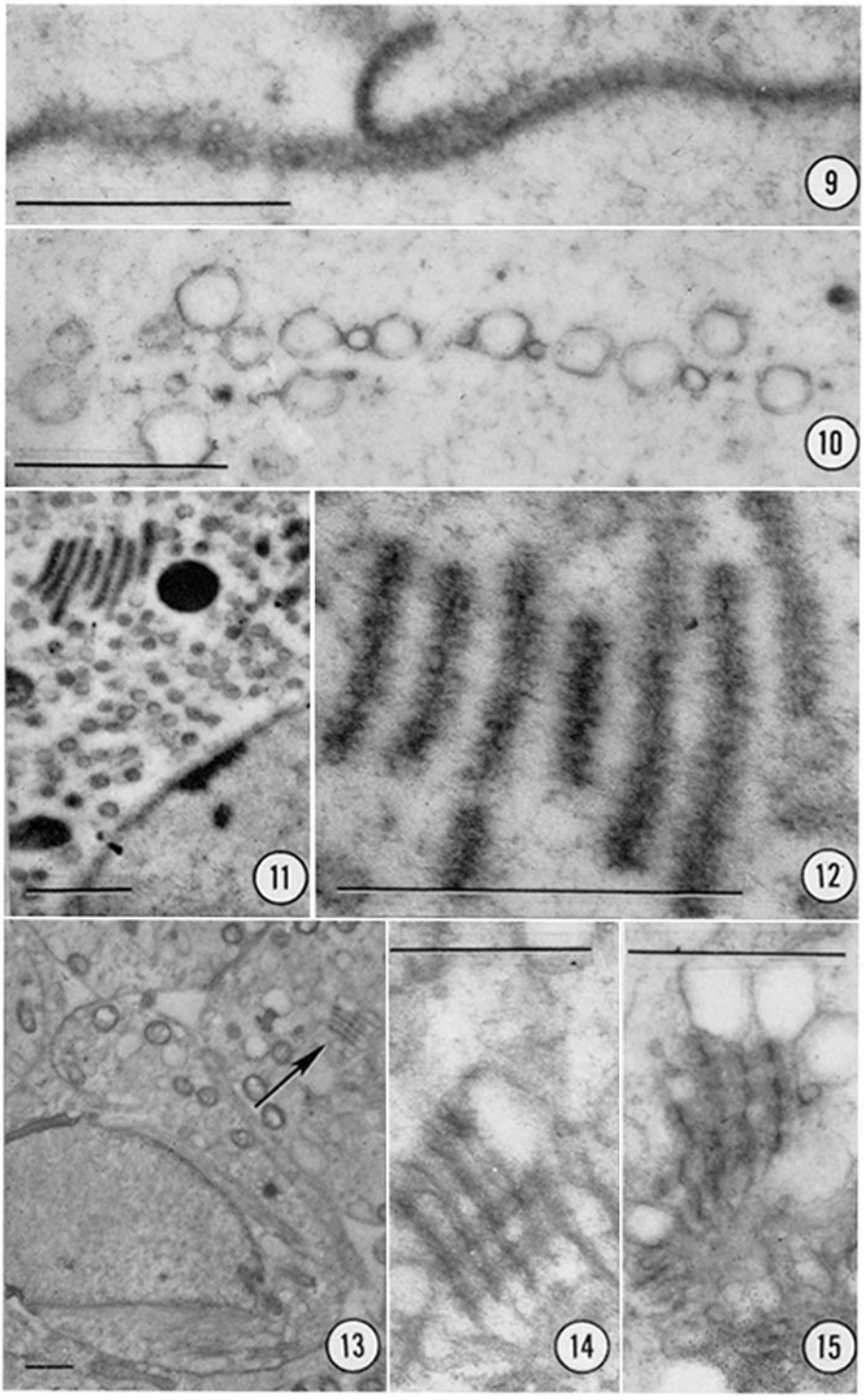
FIG. 11. Annulate lamellae from larval *Ambystoma* pancreas. × 14,200.

FIG. 12. The same structure from an adjacent section. Note attached vesicles at lower right. × 57,000.

FIG. 13. Portions of two rat spermatids, showing nucleus of one, and part of the chromatoid body of another (arrow). × 6,600.

FIG. 14. Detail of Fig. 13, showing annulate lamellae in chromatoid body. Note the continuity of annular tubules between lamellae. × 27,500.

FIG. 15. Chromatoid body from another spermatid, showing similar structure cut at an oblique angle. × 27,500.



(Swift: Fine structure of annulate lamellae)