The Origin and Fate of Annulate Lamellae in Maturing Sand Dollar Eggs*

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PLATES 42 TO 46

ABSTRACT

Electron micrograph evidence is presented that the nuclear envelope of the mature ovum of *Dendraster excentricus* is implicated in a proliferation of what appear as nuclear envelope replicas in the cytoplasm. The proliferation is associated with intranuclear vesicles which apparently coalesce to form comparatively simple replicas of the nuclear envelope closely applied to the inside of the nuclear envelope. The envelope itself may become disorganized at the time when fully formed annulate lamellae appear on the cytoplasmic side and parallel with it.

The concept of interconvertibility of general cytoplasmic vesicles with most of the membrane systems of the cytoplasm is presented.

The structure of the annuli in the annulate lamellae is shown to include small spheres or vesicles of variable size embedded in a dense matrix.

Dense particles which are about 150 A in diameter are often found closely associated with annulate lamellae in the cytoplasm. Similar structures in other echinoderm eggs are basophilic. In this species, unlike other published examples, the association apparently takes place in the cytoplasm only after the lamellae have separated from the nucleus. If 150 A particles are synthesized by annulate lamellae, as their close physical relationship suggests, then in this species at least the necessary synthetic mechanisms and specificity must reside in the structure of annulate lamellae.

INTRODUCTION

In observations on echinoderm eggs with the polarizing microscope, Monné (20) noticed that birefringent "fibrillar elements" could be found in the cytoplasm. Such elements were made more obvious by increasing the tonicity of the sea water. McCulloch (17), working with centrifugally stratified *Arbacia* eggs, also found birefringent "fibrillar elements" in the upper non-granular layers. Electron micrographs of the same layers revealed the presence of "fibers" with periodic blobs of material lined up along their lengths. Electron microscope observations of these elements were also made by Lansing *et al.* (15) in the same material and these authors made serial sections which characterized the elements as sheets rather than fibers.

In 1955 Runnström (26) published further

studies on echinoderm eggs with the polarizing microscope. He noted that in hypertonic sea water immature oocytes showed an indentation of the germinal vesicle and that birefringent strands appeared within this concavity as well as at the opposite side of the nucleus in close association with the nuclear envelope. As the egg matured, these birefringent components became scattered throughout the cytoplasm. Adding sea water extract of sperm caused an increase of birefringent elements near the nucleus.

Better techniques with the electron microscope initiated a series of descriptions of lamellar structures associated with the nuclear membrane. Dalton and Felix (6) published pictures of cytoplasmic lamellae of mouse epididymal epithelium which they briefly described as morphologically identical to and closely associated with the nuclear envelope. The cytoplasm of immature *Dendraster* oocytes also has been shown by Merriam (19) to

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contain double membranes which are structurally similar to the nuclear envelope and "yolk nucleus" membranes. They are often found parallel with and closely applied to the nuclear envelope but do not have the regular annular arrangement of annulate lamellae. Gay (11) has published low magnification pictures of *Drosophila* salivary gland cells in which regular cytoplasmic lamellar systems closely resembled the same type of structure. In an electron microscope study of developing echinoderm oocytes, Afzelius (1) drew attention to cytoplasmic membrane systems which were morphologically identical to the nuclear membrane. A tentative hypothesis was presented that these cytoplasmic membranes were the remains of the germinal vesicle membrane after the maturation divisions.

The term "annulate lamellae" was introduced by Swift (27) to describe similar structures in snail ootestis, clam oocytes, and developing amphibian pancreas. In these materials he noted that the annuli of adjacent membranes were often lined up in register with a diffuse material between the annuli of adjacent membranes. He speculated that such membranes were too extensive to be remnants of the nuclear envelope and were probably formed in juxtaposition to it. Rebhun (25) described the same structures in egg cells of a clam and a snail. He called them "periodic lamellae" and found them to be basophilic. Structures with a similar morphology were also reported by Yasuzumi and Tanaka (31) in eggs of *Cipangopaludina* but interpreted differently. In a further contribution, Afzelius (2) pointed out that annulate lamellae of sea urchin eggs were often associated with masses of small granules in the cytoplasm. The granules showed an affinity for toluidine blue and were morphologically similar to the Palade granules of more familiar vertebrate cells. The annulate membranes and associated granules were termed "heavy bodies" because they were found at the centrifugal pole after centrifugation.

Materials and Methods

Eggs of the sand dollar, *Dendraster excentricus*, were collected from mid-June until mid-August by paraoral injections of isotonic KCL solution. The eggs, collected in fresh sea water, were largely immature early in the season but showed over 90 per cent fertilizability and cleavage late in the season. Aliquots of the egg suspensions were fixed in 1 per cent OsO_4 which was buffered with veronal to pH 7.4. Sucrose was added to a concentration of 0.23 M. The eggs were fixed 30 to 45

minutes at 0°C., dehydrated in graded ethanols, and embedded in a mixture of *n*-butyl and methyl methacrylate. Thin sections, with the methacrylate in place, were observed with an RCA-2A or 3 C microscope. Both had compensated pole pieces and aperatures of 50 microns.

RESULTS

Of the eggs obtained for this study, the earliest stages were free in the ovary and could be extruded after KCL injection. Their structure shows cortical granules scattered throughout the cytoplasm and a cell surface with a highly irregular outline. At this early stage the cytoplasm also contains many "yolk nuclei" which consist of concentrically arranged membranes with adhering particles about 150 A in diameter. Many scattered Golgi elements (Fig. 13) are also seen and the cytoplasm shows a high concentration of yolk platelets. A profuse scattering of vesicles of diverse size but almost entirely spheroidal can be seen in the cytoplasm of both the immature and mature cell. Occasionally, but rarely, a small set of annulate lamellae may be seen in the cytoplasm of an egg which still contains a germinal vesicle. In the mature egg the "yolk nuclei" are gone, only relatively few Golgi elements remain, and the cortical granules form a layer under the outer cell membrane.

After the maturation divisions, the regular appearance of the nuclear envelope changes. Many areas of its surface have closely applied and parallel lamellae in the cytoplasm. Sometimes vesicles appear in the nucleus close to the nuclear envelope (Fig. 1). The vesicles inside the nucleus are in clusters with diffuse, dense material or vague membranous structures between them. They are often located opposite annulate lamellae in the cytoplasm. The nuclear envelope when adjacent to the cytoplasmic lamellae or nuclear vesicles often exhibits a poorly defined appearance which no doubt, is sometimes due to folds in the membrane so that it is cut obliquely. In some instances, however, it seems to be a case of a diffuse "disorganization" of the envelope as shown in Fig. 2. Structures which appear to be small vesicles about 200 to 500 A in diameter are frequently seen in the cytoplasm in association with the diffuse areas of the nuclear envelope, as demonstrated in Figs. 1 and 2. The general appearance of matured nuclei, as shown in Fig. 1, suggests that in this stage of oogenesis the nucleus is highly "active" with respect to membrane dynamics.

It seems reasonable to suggest that such nuclear

membrane phenomena have something to do with the formation of annulate lamellae. This concept is supported by the observation that paired membranes like these found in the nuclear envelope are found in close association with the intranuclear vesicles. The paired membranes will be called "secondary membranes." Figs. 3 and 4 show secondary membranes in continuity with intranuclear vesicles and closely applied to the nuclear envelope. These secondary membranes are always found inside the intact nuclear envelope and closely applied to it. They do not yet exhibit the regular arrangement of annuli characteristic of the cytoplasmic lamellae.

In the mature egg the cytoplasm is full of scattered annulate lamellae. They occur as single membranes or as stacks of paired membranes (Fig. 5). When in stacks, the annuli of adjacent membranes are lined up on register. Often a diffuse, dense material is seen between annuli of adjacent lamellae. As can be seen in Fig. 6 where three lamellae have been cut obliquely, the annuli of a single lamella are regularly arranged in a hexagonal packing. The annuli probably have raised rims which extend slightly above and below the membrane. The rims, however, cannot be considered tubes of any great length, because in oblique section (Fig. 6) the annuli are found to be rather closely confined to the areas of electron-dense membrane between annuli. If the annuli were actually tubes of appreciable height above the membrane, one would expect to see outlines of annuli at the periphery of the oblique section in apparent isolation from the membrane. In addition, it should be noted that the centers of the annuli consistently show as much density as the membrane surrounding them.

The fine structure of the individual annuli can be made out in Figs. 6 to 8. At the arrows marked A the annuli appear to contain small structures, about 110 to 200 A in diameter, which sometimes appear to be vesicles. In a few cases a "vesicle" can be seen in the center of the ring. In most cases annuli appear to consist of an electron-dense ring which contains small electron-transparent areas or "vesicles" whose walls sometimes cannot be distinguished.

Shortly after the appearance of annulate lamellae in the cytoplasm one begins to see masses of tightly packed granules in loose association with some of them (Figs. 9 and 10). Afzelius (2) has termed these composite structures "heavy bodies"

and has shown that they are basophilic. Granules of similar size and density can be found free in the cytoplasm. These free granules are thrown toward the centrifugal end of the egg during centrifugation to form a concentration gradient which tapers off toward the light end of the stratified egg. Staining of stratified eggs fixed in osmium shows a gradient of basophilia which parallels the gradient of the granules (unpublished observations).

One cannot help noticing the morphological similarity between the masses of particles in the cytoplasmic "heavy bodies" and the masses of particles in the "minor nucleoli" of the nucleus (Fig. 11). "Minor nucleoli" are seen only in the germinal vesicle of sand dollar oocytes and usually in close association with the nuclear envelope. Heavy bodies are found only in the cytoplasm after completion of the meiotic divisions.

The fate of the heavy bodies is obscure. They are numerous in the fully matured egg and no breakdown stages have been observed. It is possible that they are structurally disintegrated later in embryogenesis. Annulate lamellae, which do not form heavy bodies, seem to disintegrate into vesicles which are morphologically indistinguishable from vesicles found abundantly in the cytoplasm during all stages examined. In Fig. 12 parallel rows of individual vesicles can be seen near rows of intact lamellae. It seems more likely that formed lamellae degenerate into vesicles than that independent vesicles line up and then fuse into a lamella independently of any preformed structure. Terminal continuity between fully organized lamellae and independent vesicles can be seen at the ends of lamellae in Figs. 5, 6, and 12.

The *continuity in time* between membranes and general vesicles is apparently not confined to annulate lamellae. Golgi elements are numerous in the cytoplasm in late germinal vesicle stages but largely disappear as the egg approaches maturity. Fig. 13 is a demonstration of a typical Golgi element in structural continuity with independent vesicles at a time when Golgi elements are disappearing.

Yolk platelets, consisting of masses of yolk particles, are surrounded by a distinct membrane (Figs. 12 and 13) which also may undergo similar changes. As the egg approaches maturity the yolk platelet membranes begin to rupture, releasing yolk particles into the cytoplasm. The membranes do not just disappear but tend to curl up initially and then seal off into independent vesicles as seen

in Fig. 13 at the arrow marked A. Morphologically, in osmium-fixed eggs, it is difficult to see any differences in the vesicles derived from annulate lamellae, Golgi systems, or yolk platelets. They all are bounded by single membranes of about equal thickness and will be referred to here as "general vesicles."

<center>DISCUSSION</center>

Hodge et al. (12) found that formation of chloroplast lamellae in Nitella and Zea cells apparently takes place by the coalescence of numerous small vesicles into the higly organized lamellae. The suggestion was made that the primordial small vesicles were structures for the transport of lipoprotein membranes to places of more elaborate organization. During the formation of the vertebrate photoreceptor rod cell, De Robertis (8) likewise noted that fine vesicular elements disappeared during the formation of the regular system of stacked lamellae. Conversely, Hodge et al. (13) have observed that organized lamellar systems degenerate into vesicles as a result of osmotic shock. Chemical injury also was found by Anderson and van Breeman (3) to cause regular endoplasmic lamellae to change into many small vesicles in nerve cells. Porter (24) has found, in his wide experience with the endoplasmic reticulum, that the structure is labile and may exist as a reticulum or as a system of separate vesicles. In similar vein, the observation herein reported on the apparent organization of annulate lamellae from vesicles and the disorganization of annulate lamellae, Golgi systems, and yolk vesicle membranes into vesicles agrees well with the concept of membrane-vesicle interconvertibility.

Palade (21) has postulated that the membrane systems of the plasma membrane, endoplasmic reticulum and cisternae, Golgi elements, and nuclear envelope are all physically continuous. In the sand dollar egg there is scant evidence of a physical continuity between the different membrane systems of the cytoplasm. There is, however, evidence of continuity of invaginations of the immature oocyte plasma membrane with the general vesicle system of the cytoplasm (unpublished observation). In addition, it has already been indicated that annulate lamellae, Golgi elements, and yolk platelet membranes are all probably continuous with the general vesicles at the time of their disorganization in the cytoplasm. This is another way of stating that the different membrane systems, in

having a common relationship to the vesicles, may be physically continuous in time if not at a given moment. The vesicles might be homologous to the endoplasmic reticulum of other cells. Such an arrangement suggests a dynamically changing membrane system.

Recent evidence that 150 A particles of Palade are rich in RNA (16, 21, 22), and active in protein synthesis (e.g., 16, 32), has caused morphologists to view these structures with great interest. The possibility that they might contain the specificity necessary for synthesis of proteins in the cytoplasm makes their association with nuclear structures of possible significance to the question of nuclear control over cytoplasmic events. Thus, the occurrence of particulate structures inside the nucleus which are morphologically similar to particles of Palade have been occasionally described in diverse materials. For example, Porter (23) saw 150 A particles in "nucleoli" of Amblystoma larval cells and remarked about their similarity to particles in the cytoplasm. De Robertis (7) saw similar particles in "chromatin masses" in nuclei of frog nerve cells. Cohen (5) described similar particles in diffuse "nucleolar" material inside the nuclear membrane of Amoeba, and Afzelius (2) pictured granule-filled outpocketings of the nuclear membrane in echinoderm eggs. In this study, too, masses of similar particles have been seen in the germinal vesicle of the oocyte, usually in close proximity to the nuclear envelope.

There are in the literature numerous observations on the transfer of basophilic nucleolar material from nucleus to cytoplasm in developing oocytes (for critical reviews see 4 and 29), but the present study contributes little to the question. In Psammechinus eggs (2) the particles of "heavy bodies" first appear inside outpocketings of the nuclear membrane and thus are apparently of nuclear origin. In Dendraster eggs similar particles become associated with annulate lamellae only after they have moved into the cytoplasm. This means that if the 150 A particles of the "heavy body" are synthesized by annulate lamellae and if they carry specific genetic "information," that information must somehow reside in the structure of annulate lamellae.

Actually, the structure of annulate lamellae is not simple. The present study has indicated that vesicles or spheres of about 110 to 200 A are embedded in the electron-dense material of the annuli. The resolution thus demonstrated at least elimi-

nates the possibility that the annuli are simply un-resolved 150 A solid granules. (9, 10, 25, 28). Similarly, Wischnitzer (30) has recently shown that annuli in the nuclear envelope of immature oocytes of *Triturus* are composed of about 8 tubular structures arranged in a ring. It is difficult to understand how the tiny vesicles described in this study fit into the structure of the annuli but it may be pertinent to call attention to the close morphological and size similarities of these structures to the small vesicles described in nucleoli of *Allium* and *Vicia* cells (14).

The best speculation at present would picture *Dendraster* annulate lamellae as paired membranes with annuli in regular hexagonal arrangement. "Pores" in the double membrane would actually be filled with loosely organized material which sometimes is condensed into a diffuse single membrane across the "pore," and sometimes contains a particle or vesicle about 140 A in diameter. The annuli themselves would exist as low dense ridges of indefinite outline in which are embedded less dense spheres or vesicles 110 to 200 A in diameter. These are best seen only when the dense matrix is disappearing in the initial stages of structural disorganization. The matrix of the annuli has a high intrinsic density that does not disappear when the section is bleached with hydrogen peroxide (18), and hence reacts to the treatment in the same way as the 150 A particles of the heavy bodies. The possibility thus exists that the annular matrix contains nucleic acid in non-particulate form.

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EXPLANATION OF PLATES

Abbreviations

AL, annulate lamella	*N*, nucleus
Ann, annulus of "pore"	*NE*, nuclear envelope
GV, general vesicle	*NV*, nuclear vesicles
HB, heavy body	*SM*, secondary membrane
M, mitochondrion	*Y*, yolk platelet
MN, "minor nucleolus"	

PLATE 42

FIG. 1. Electron micrograph of nucleus of an egg which has completed the meiotic divisions. The nucleus is enclosed by a nuclear envelope which is closely associated with annulate lamellae in the cytoplasm, as well as with nuclear vesicles and secondary membranes within the nucleus. Yolk platelets and mitochondria can be seen scattered about in the cytoplasm. × 15,000.

FIG. 2. Electron micrograph of a portion of the nuclear envelope shown in Fig. 1. Nuclear vesicles seem to be associated with a local disorganization of the nuclear envelope. Small vesicles can be seen on the cytoplasmic side. × 29,000.
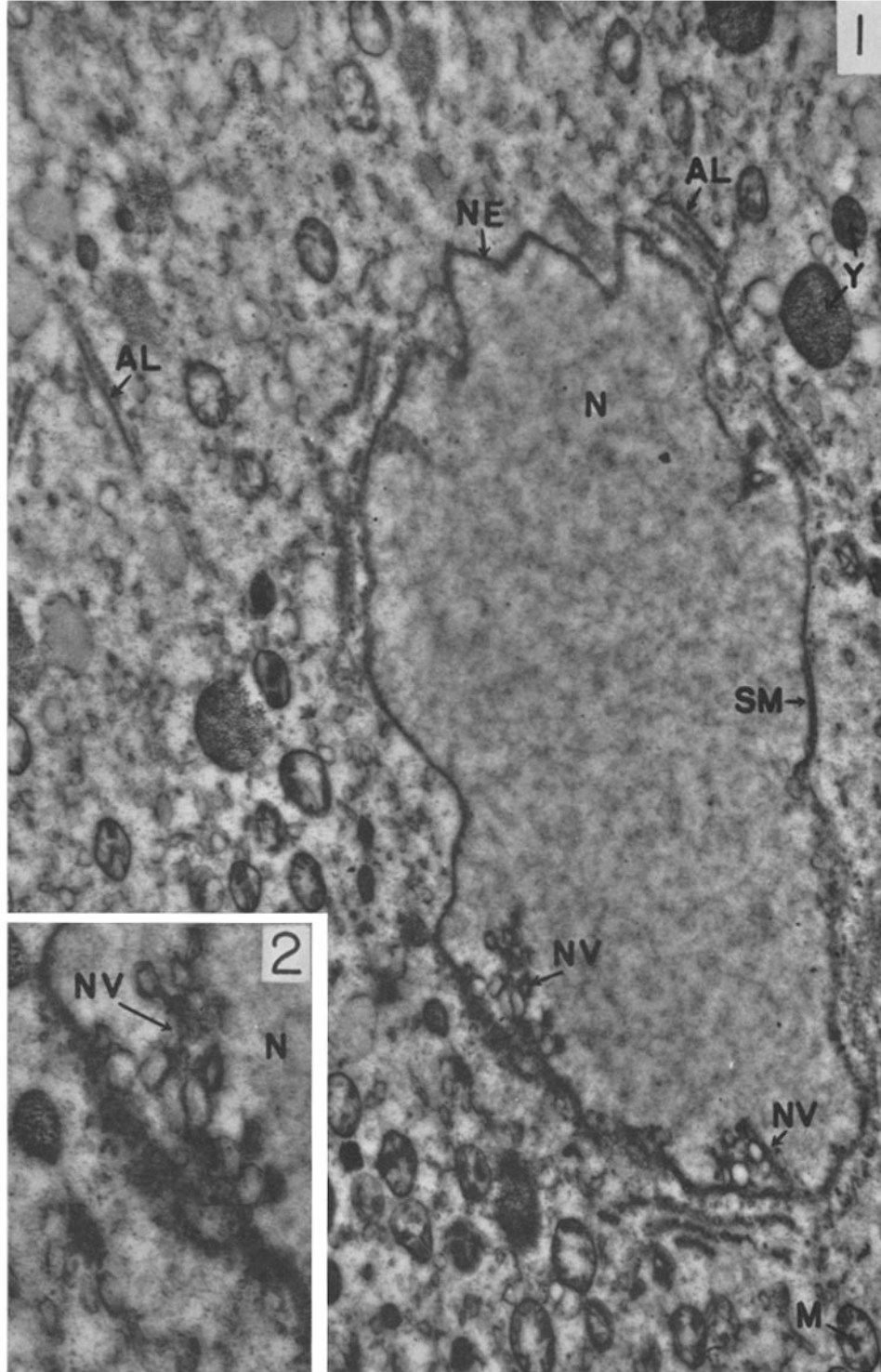
(Merriam: Annulate lamellae)

PLATE 43

FIG. 3. Electron micrograph showing terminal association of secondary membranes with nuclear vesicles. Both lie in close apposition to the nuclear envelope. × 40,500.

FIG. 4. Electron micrograph of nuclear vesicles and secondary membranes. Note the close parallel apposition of the secondary membrane to the nuclear envelope. × 49,000.

FIG. 5. Electron micrograph of annulate lamellae in the cytoplasm which are cut in cross-section. Annulate "pores" are seen as local interruptions of the double membranes. × 53,500.
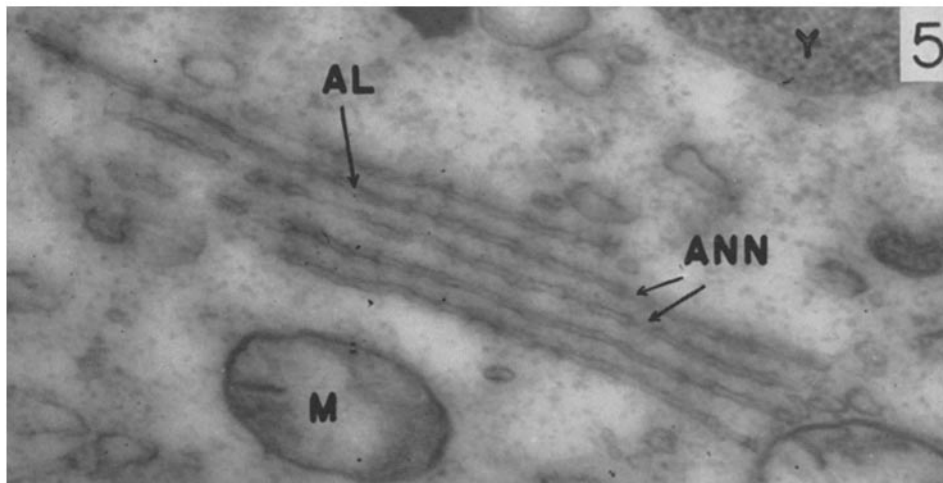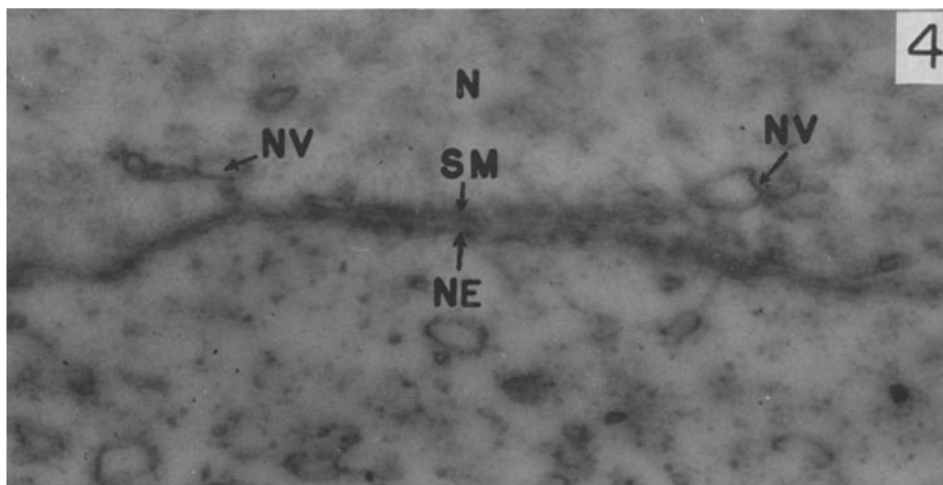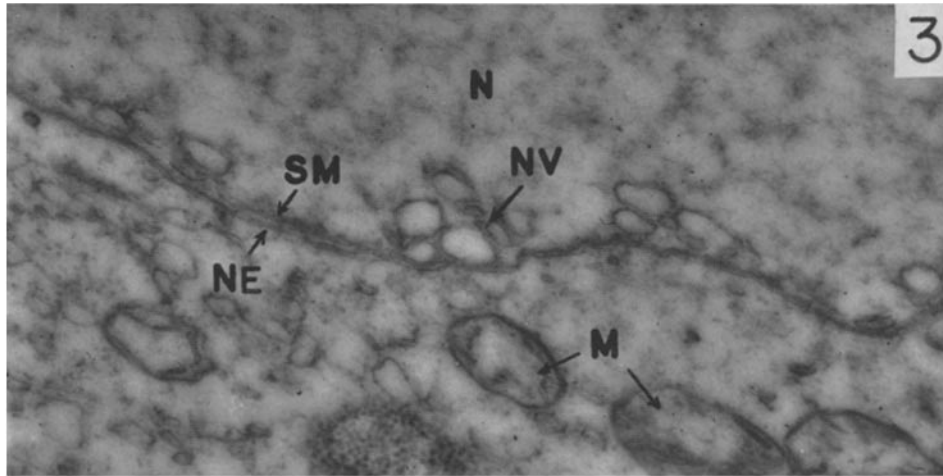
(Merriam: Annulate lamellae)

FIG. 6. Electron micrograph of annulate lamellae in the cytoplasm which have been cut obliquely or normally. Annulate "pores" are seen as complete rings whose centers are generally as dense as the membranous material between annuli. Careful scrutiny reveals density irregularities in many annuli. $\times$ 76,000.

FIG. 7. Electron micrograph of tangential section through annulate lamellae. At arrows marked A note the vesicle-like structures in the dense annulus. $\times$ 95,000.

FIG. 8. Electron micrograph of tangential section through annulate lamella. Note the vesicle-like structures in the annulus at the arrow marked A. $\times$ 95,000.
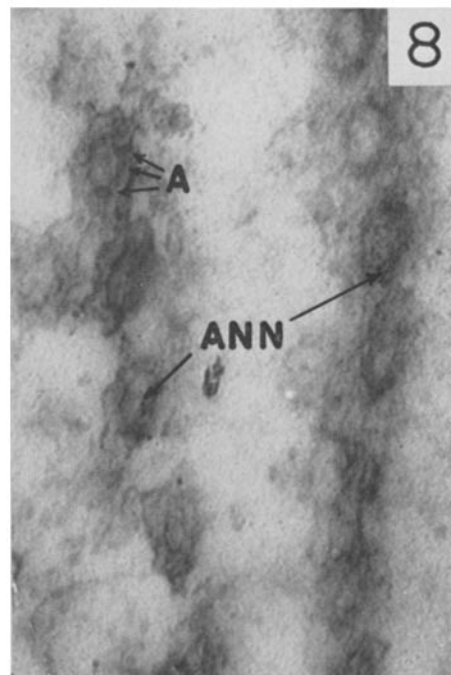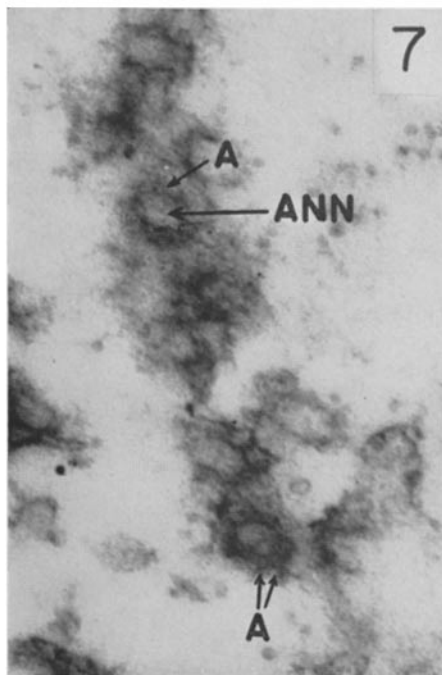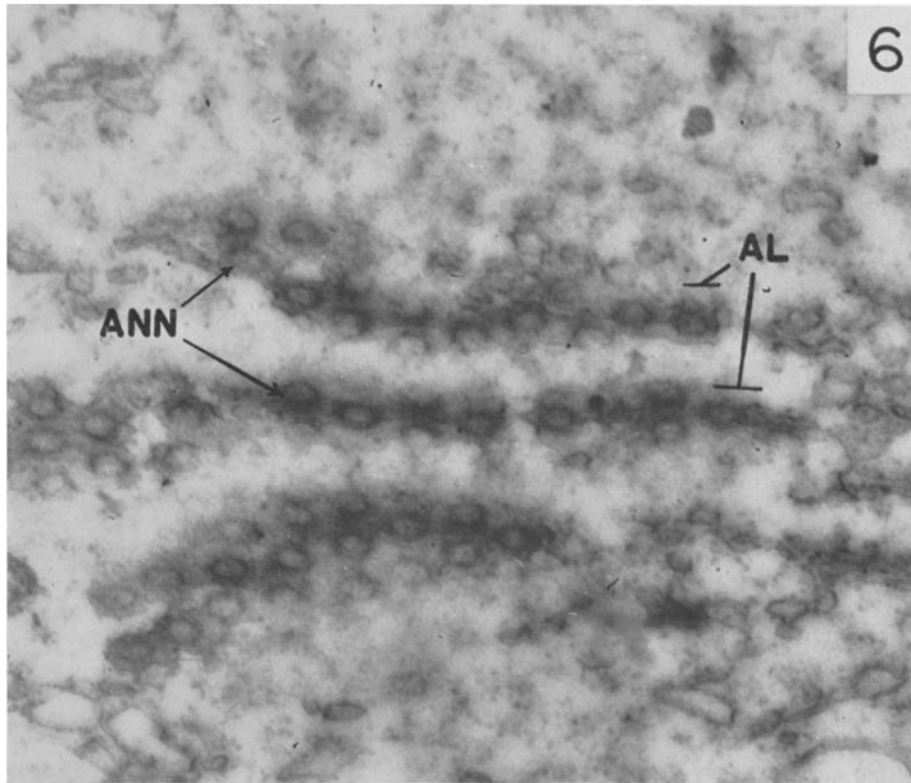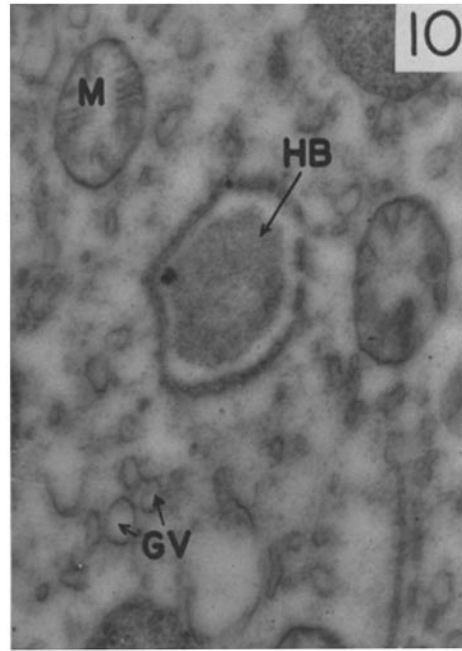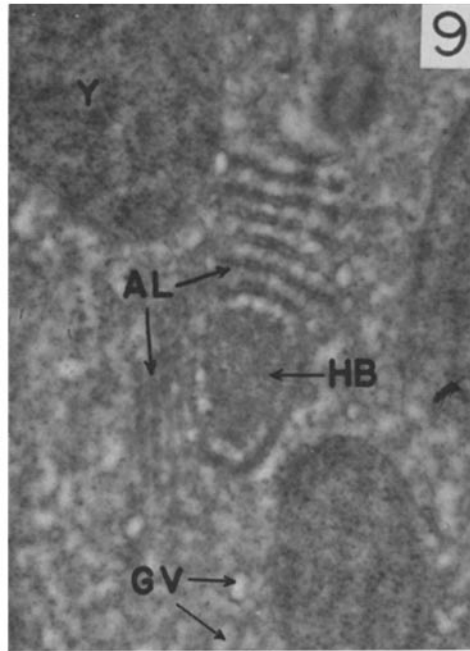
(Merriam: Annulate lamellae)

Plate 45

Fig. 9. Electron micrograph of a "heavy body" which consists of a mass of particles about 150 A in diameter surrounded by annulate lamellae. × 24,500.

Fig. 10. Electron micrograph of a cytoplasmic "heavy body" with particles and a single remnant of an annulate lamella around them. × 31,500.

Fig. 11. Electron micrograph of a "minor nucleolus" in the germinal vesicle of an immature oocyte. It is composed of particles of the same dimensions as the particles of "heavy bodies" which are found in the cytoplasm at a later stage of development. × 38,000.
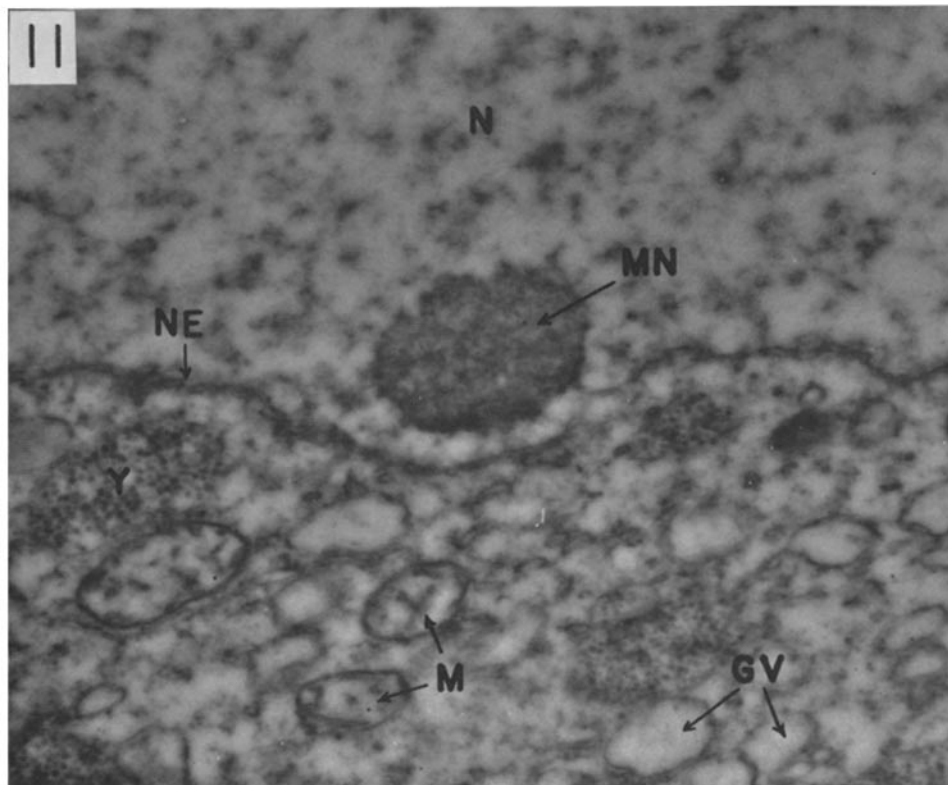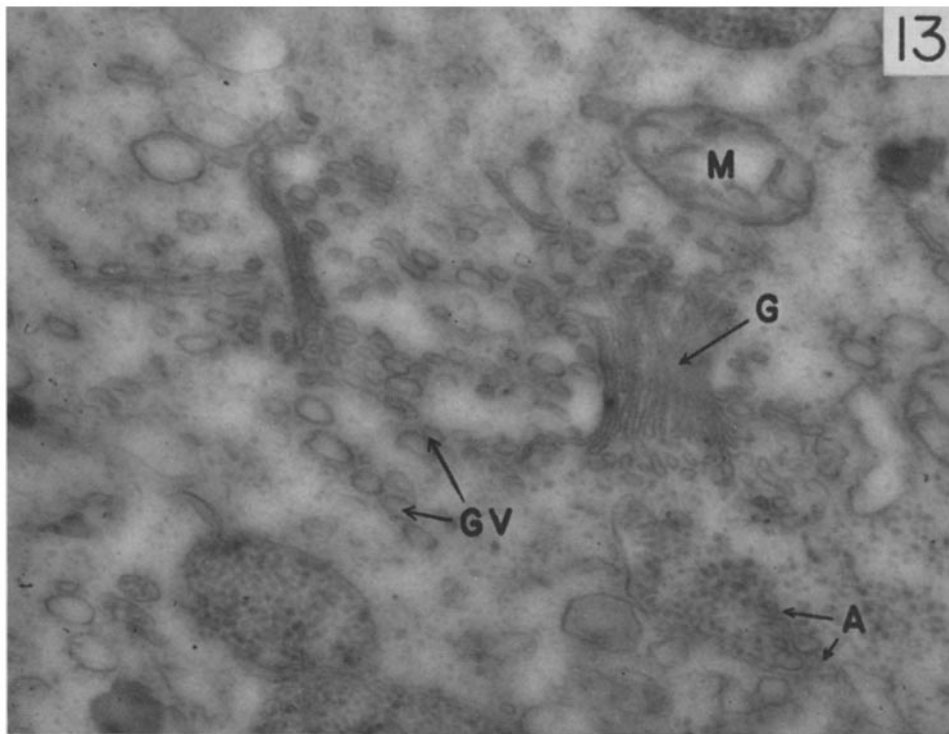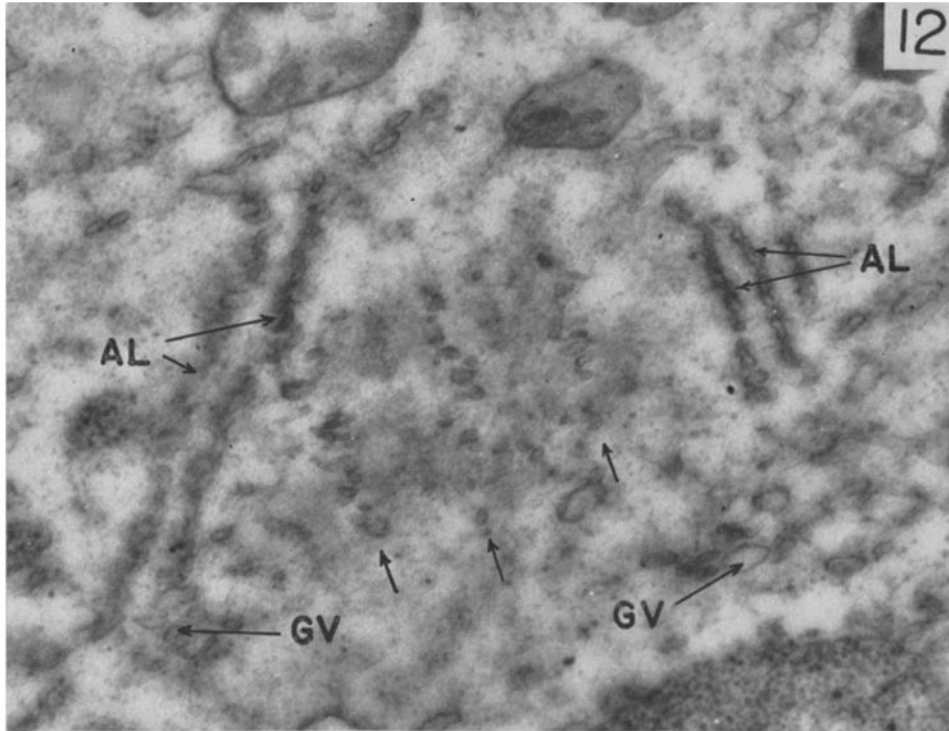
(Merriam: Annulate lamellae)

PLATE 46

FIG. 12. Electron micrograph of annulate lamellae and rows of vesicles (at the arrows). × 41,500.

FIG. 13. Electron micrograph of Golgi membranes (G) which seem to be in structural continuity with general vesicles of the cytoplasm. Rupture of the limiting membrane of a yolk platelet can be seen. At the arrow marked A note the tendency of the broken limiting membrane to curl and form vesicles. × 48,500.

(Merriam: Annulate lamellae)