

Basophilic Lamellar Systems in the Crayfish Spermatoocyte*†

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ABSTRACT

Histochemical procedures for the demonstration of RNA have shown the presence of intensely basophilic bodies in the cytoplasm of spermatoocytes of the crayfish, *Cambarus virilis*. The staining of thick sections, cut alternately with thin sections for electron microscopy, has permitted identification of the basophilic bodies with two types of lamellar systems. One of these, a set of straight annulate lamellae, is restricted to meiotic prophase. The second type of lamellar systems has been found from late prophase to early spermatid stages. It consists of an ellipsoidal lamellar set which intersects a number of straight lamellae. Within the region of intersection, the ellipsoidal lamellae break up into an array of small tubules of about 150 Å diameter. The term *tubulate lamellar system* was chosen to designate this type of lamellar complex. Small RNA-containing granules could not be detected in annulate lamellar systems. While there are a few granules in the marginal regions of the tubulate lamellar system, their distribution cannot be responsible for the basophilia which is intense within all regions of the lamellar body.

INTRODUCTION

Studies on cellular fine structure as revealed by the electron microscope have shown that the ground substance of the cytoplasm of many cell types is extensively partitioned and contains numerous membrane-bound cavities. The portion of the whole cytoplasmic volume occupied by such cavities or cisternae, their arrangement and degree of branching, is variable and frequently characteristic of the cell type. This whole spectrum of small vesiculate elements, limited by what appears to be a single membrane at the present levels of resolution, and characterized by a homogeneous content, has been subsumed under the name endoplasmic reticulum (19). Morphologically, two varieties of endoplasmic reticulum are recognized today, depending upon the presence

or absence of small granules of about 150 Å diameter on or near the outside of the vesicles. Granular endoplasmic reticulum, first described from exocrine cells of the pancreas (16, 21), is found to be extensively developed in cells engaged in protein synthesis. There is a substantial body of evidence which points to the small granular component as the possible site of cytoplasmic ribonucleic acid (RNA). Palade and Siekevitz have recently isolated these small particles from guinea pig pancreas by differential centrifugation (18). They found the postmicrosomal fractions containing the granules to have a higher RNA/protein ratio than samples containing both granules and small vesicles of the endoplasmic reticulum. Clermont (7) found the cytoplasmic RNA in the rat spermatid confined to dense granular aggregates. Both results indicate that at least some of the cytoplasmic RNA is localized within the small granules. There are, on the other hand, indications that a membrane-bound RNA fraction may exist in the cell (4). Recent work by Chaveau, Moule, and Rouiller (6) showed that the RNA-protein ratio of microsomal isolates from rat liver is independent of the abundance of small granules in the pellets.

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The French authors conclude that important quantities of RNA exist in the membranes of the cytoplasmic vesicles or their contents.

The present paper describes two classes of basophilic bodies found in the cytoplasm of crayfish spermatocytes. As the structures in question have dimensions of several micra, a correlative study of their histochemical staining reactions and of their fine structure as revealed by electron microscopy is possible. This approach was chosen to contribute toward answering the question where, in terms of ultrastructure, cytoplasmic RNA may be localized.

The first basophilic body to be described consists of a stack of cytoplasmic lamellae, oriented more or less parallel to each other and frequently spaced at regular intervals. Sections traversing lamellar sheets parallel to the surfaces show annuli similar to those described for the nuclear membrane (5, 10, 23). Essentially the same type of lamellar system has been described previously from different tissues and organisms (20, 22). Following Swift's (22) usage, this type of structure will be called an "annulate lamellar system" in the present paper.

The second body consists essentially of two intersecting lamellar systems and a set of small tubules which are oriented at right angles to the surfaces of one of the lamellar sets. The resulting aspect is that of a three-dimensional lattice of almost crystalline regularity. As the small tubules appear to be derived from one of the lamellar sets, the term *tubulate lamellar system* will be used to designate the whole structure composed of lamellae and the tubular array. The term "tubule" is intended to designate an elongated structure which has a circular cross-section and is composed of a dense outer region (wall) surrounding a transparent core or lumen.

Materials and Methods

Specimens of *Cambarus virilis*, the species used for this study, were procured from the vicinity of Minneapolis, Minnesota. For electron microscopy, the testis was removed from the decapitated animal and fixed immediately in 1 per cent osmium tetroxide buffered to a pH of 7.4 with veronal acetate (15). The tissues were embedded in *n*-butyl methacrylate (14), and the plastic containing 1 per cent 2,4 dichlorobenzoylperoxide as initiator was polymerized by ultraviolet light. Ultrathin sections were cut with a glass knife mounted on a Porter-Blum microtome. The electron microscope used was a RCA EMU-2 model

equipped with an intermediate lens, an externally centrabable 50 μ objective aperture and a self-biased electron gun.

Histochemical tests for the presence of RNA were conducted both with paraffin sections and 2.5 μ sections cut from methacrylate-embedded tissue. Both toluidine blue at pH 6 and azure B at pH 4 (9) were used as stains. Successive sections were mounted on different slides to serve as controls and experimentals in a set of tests involving crystalline ribonuclease. In the case of sections cut from methacrylate-embedded tissue, the plastic was removed by immersion in xylene prior to staining in azure B. Since the osmium used as a fixative inhibits enzymatic digestion, it was removed by immersion in 3 per cent hydrogen peroxide for 30 minutes. The enzyme, crystalline ribonuclease (Worthington Corp., prepared by the method of McDonald) was dissolved in twice-distilled water to obtain a concentration of 20 mg. of enzyme per 100 ml. solution. Slides were incubated in this solution at 50°C. for 1 hour.

To correlate the histochemical approach with the study of fine structure, 2.5 μ thick sections and ultrathin sections were cut alternately from the same block of methacrylate-embedded tissue. The thick sections were transferred to slides and stained with azure B, while the adjacent thin sections were mounted on grids for electron microscopy. Hence, it was possible to achieve a close correlation between the distribution of basophilia and the fine structure of the same cells. A similar correlative procedure has been applied by Moses (13), who used the Feulgen nuclear reaction to study nuclear protuberances in crayfish spermatids.

RESULTS

The Endoplasmic Reticulum in Spermatocytes.—With the exception of certain regions occupied by granules of 650 to 750 A diameter, the whole cytoplasm is crowded with numerous vesicles of the endoplasmic reticulum. The different elements of the reticulum are of varying shapes and dimensions, their interior is homogeneous, and they appear to be limited by a single membrane. The individual vesicles are, in general, found to be randomly distributed in the space between nuclear envelope and cell membrane. However, instances of strings of vesicles being preferentially oriented in concentric shells around the nucleus have been encountered. The study of thick adjacent sections with the phase contrast microscope showed that the endoplasmic reticulum assumes this disposition in the pachytene stage. During the metaphase of the first maturation division, the endoplasmic reticulum is abundant both within the spindle region and in the peripheral cytoplasm.

In this case, the vesicles of the spindle region are elongated and preferentially oriented toward the spindle poles. The vesicles of the endoplasmic reticulum are of both the smooth surfaced (agranular) and the rough surfaced (granular) type. The granules are clustered between adjacent rows of cisternae or line the outsides of the vesicular borders. Individual granules are generally rather dense and measure between 100 and 180 A in diameter. Among these, as the predominant type, there are occasional elements showing a light central core (Fig. 1). The gap between rows of vesicles may be bridged by band-like granular clusters. On close inspection of the original electron micrographs, some of these particles are seen to be elongated and to have the appearance of longitudinally sectioned tubules. Vesicles of the granular and agranular type reticulum are usually not interspersed at random, but tend to occur in groups of one or the other type. The smooth surfaced variety is usually predominant.

The Intracellular Distribution of Basophilia.—After staining with azure B at pH 4, the cytoplasm of the spermatocyte shows a general basophilia of varying intensity. In dividing cells, both the spindle and the peripheral cytoplasm are homogeneously, though lightly stained (Fig. 4 a). The two types of lamellar systems present are intensely basophilic and occur in different shapes measuring from 2 to 8 micra. The annulate lamellar system (Figs. 2 and 3) has been found only during meiotic prophase. The tubulate lamellar body (Figures 4 c and 5) was traced as far as the early spermatid stage. Its ultimate fate is unknown. Comparison of stained sections with electron micrographs from adjacent thin sections (Figs. 4 a, b, and c) shows that all parts of the tubulate lamellar system are basophilic. This includes three morphologically different regions to be discussed below: the region occupied by the tubules, the curved lamellar set (ellipsoidal body), and the vesiculate portion aligned with the ellipsoidal body.

Enzymatic digestion with ribonuclease of both paraffin sections, fixed in ethanol-acetic acid (3:1), and of osmium-fixed and methacrylate-embedded material (after hydrogen peroxide treatment), prevents all subsequent cytoplasmic staining with azure B at pH 4. Only the characteristic blue-green color of the DNA is left in the meiotic chromosomes after the enzyme has acted.

Annulate Lamellae.—It has been mentioned that during pachytene large portions of the endo-

plasmic reticulum may be arranged in regular layers surrounding the nucleus. Annulate lamellar bodies are frequently encountered during this stage. The individual lamellae are aligned parallel to each other and are frequently parallel to the nuclear membrane. Each lamellar layer can usually be traced into a corresponding layer of endoplasmic reticulum which appears in transection as a chain of vesicles. A similar succession of vesicles alternating with spaces is found within each sectioned lamella of the annulate lamellar system (Fig. 2). The latter is, however, distinguished by several features. Its vesicles are flattened and alternate regularly with spaces. These characteristics are sufficiently distinctive to permit identification of single annulate lamellae and to recognize them as different from the surrounding layers of endoplasmic reticulum. In the case of compound systems consisting of a number of layers, the spaces and vesicles of the individual lamellae are often aligned with each other. Occasionally, fine filaments like those described by Swift (22) were found to connect different lamellar strata.

The number of layers as seen in sections normal to the lamellar plane ranges from a single one to as many as sixteen. It is possible that this variability in the number of lamellae is partially due to sections passing through some projecting lamellae and missing the main portion of the lamellar stack. Single lamellae are usually found next to the nuclear membrane and parallel to it. In systems composed of many layers, the interlamellar distances are found to vary between 700 and 1600 A. For any given set of lamellae, however, these distances are fairly constant.

Sections nearly parallel to the lamellar surface reveal a pattern of relatively dense annular, or disk-like structures (Fig. 3). Rebhun (20), who studied the annuli of basophilic lamellar systems of some invertebrate oocytes, showed that the pore of each annulus corresponds to the space between successive vesicles as seen in transected lamellae. In the oocyte material, the annulus is clearly demarcated as a ring of dense material surrounding a light center, or pore. Swift's and Rebhun's pictures show rings of about 180 to 200 A width (20, 22). In our material, the pore is occupied by rather dense material. Consequently, the annulus is not as clearly delimited, and measurements are rendered difficult. Transected annuli show the material filling the pores as more

or less dense plugs. It is the latter which seem to give rise to the fine filaments occasionally seen to connect adjacent lamellae. An approximate value for the density of annular packing, measured as the distance between the centers of adjacent annuli, is 900 A units. Maximum diameters of the annuli themselves amounted to about 600 A. Owing to the great density of the annular plugs, the width of the annulus itself is hardly measurable. Occasionally, the dense plugs showed a more or less distinct rim which was taken to correspond to the actual annulus. The width of this rim was determined to be between 60 and 70 angstrom units. This agrees well with the average width of the lamellar wall (60 A), measured as the border of a vesicle in sections normal to the lamellar surface. The total width of the vesicle itself is usually about 300 A.

Tubulate Lamellar Bodies.—The second type of lamellar system to be discussed consists of two sets of lamellae intersecting each other at an approximately 90° angle. From micrographs of the same individual system cut at different levels, and from sections through the same type of system cut at different angles, it was possible to gain insight into some of the salient features of the tri-dimensional construction of this form of lamellar body.

The set of lamellae seen to pursue a roughly circular course in Fig. 4 *c* is then recognized as part of an ellipsoid lamellar body. Each individual lamella is double-walled. The variable density of the walls and the presence of what seems to be adsorbed material renders exact measurement of thicknesses difficult. Generally, the width of what appear to be normally sectioned lamellae varies between 150 to 180 A, with wall thicknesses of about 50 A and a central clearance of about 50 to 80 A units. These highly oriented regions of the ellipsoid body are generally surrounded by a zone of endoplasmic reticulum, the vesicles of which are oriented parallel to the surface of the lamellar ellipse. It is clear from a glance at Fig. 4 *c* that thin sections through tubulate lamellar bodies may entirely bypass the layers of the ellipsoidal body. Fig. 7 illustrates the resulting appearance. Similarly, sections showing the ellipsoid body only may be obtained (Fig. 9).

Both the ellipsoid body and the associated endoplasmic reticulum intersect a variable number of straight lamellae. Within the space of the latter, the lamellae of the ellipsoidal body are continued

as a system of highly oriented tubules of approximately 150 A total diameter. Sections passing obliquely through successive layers of the plane lamellar set cause the individual lamellae to be drawn out into broad areas of now lessened density (Figs. 5 and 6). Fig. 5 is of further interest, as it provides morphological evidence for the origin of the tubules. It is possible to trace individual lamellae of the ellipsoidal body into the tubular array. In the given instance, single lamellae of the central region alter their course through an angle of about 30° to align themselves with the tubular pattern before breaking up into tubules of their own. Some micrographs, presumably from favorably oriented sections, show lamellae of the ellipsoid body directly continuous with some of the straight lamellae.

The tubules are arranged in a hexagonal pattern on the surface of the set of straight lamellae. The largest distances between adjoining tubules amount to 450 A units. Smaller values, between 450 and 300 A, were frequently encountered in sections parallel to the lamellar plane. If the distances within a hexagon of tubules surrounding a given tubule taken as the center are measured, it is found that the shorter distances, say 300 A, are all parallel to each other. The remaining spacings in the hexagon measure 450 A. Comparison of different micrographs shows the values for the shorter distance to be variable, while the spacing of 450 A is fairly constant. It is, therefore, possible to account for the smaller spacing as due to compression during sectioning. Figs. 4 *d* and 8 are inserted to show the lumen of the tubules. Cross-sections show the lumen as a central core of low density measuring some 70 to 80 A in diameter. As the surrounding walls are of about 35 to 40 A thickness, the diameter of the whole tubule is about 150 A.

Relationships between the Lamellar Systems.—Ordinarily, annulate lamellar systems are continuous with the vesicles of the surrounding endoplasmic reticulum. A few instances have, however, been noted when annulate lamellae were seen continuous with the ellipsoid body (Fig. 10). No small tubules have been found in such instances. Instead, the annulate lamellae bear the same relationship of lamellar continuity to the ellipsoid body as the latter has to the straight lamellae of the tubulate lamellar system. Note in Fig. 10 that individual lamellae of the annulate system can be traced into single lamellae of the ellipsoid body.

DISCUSSION

Fasten (8) and McCroan (12), who gave accounts of crayfish spermatogenesis, mention the presence of two chromatoid bodies in the spermatocyte. Both are claimed to stay usually at the same spindle pole during both divisions and hence would pass into one of four spermatids. It is impossible to tell which, if any, of the two types of cytoplasmic lamellar systems are identical with Fasten's chromatoid body. As only tubulate lamellar bodies have been found in spermatids, it is possible that the latter structure represents the chromatoid body of the crayfish. Its dimensions agree with Fasten's description, and the lamellar systems described are indeed the only cytoplasmic differentiations of a size sufficient to account for chromatoid bodies. Wilson (24) states that chromatoid bodies frequently arise in growing spermatocytes near the nuclear membrane.

It appears that at least some of the structures subsumed under the name chromatoid body are basophilic. Those might well correspond in fine structure to one or both of the lamellar systems described in this paper. Swift (22) published some micrographs showing the chromatoid body of rat spermatids. In part, this structure consists of a small array of annulate lamellae. Connecting the transected annuli in different lamellar strata are what Swift named annular tubules. It is pointed out that the latter are not to be confused with the small tubules described in the present paper. The diameter of an annular tubule is equal to that of the lamellar annulus with which it is in contact. It is probably homologous with the tubular material noted by Afzelius (1) to be associated with annuli of the nuclear membrane. The lamellar systems described were shown to be morphologically integrated with the ground structure of the cytoplasm. From the standpoint of light microscopy they could appear "de novo" and disperse easily, due to structural rearrangement at the submicroscopic level. They may well serve specific functions during the spermatocytic growth period.

Annulate lamellae have previously been reported from oocytes of the clam *Spisula* and the snail, *Otala* (20), from the acinar cells of *Ambystoma* pancreas (22), and from rat spermatids (17, 22). Swift (personal communication) has recently obtained micrographs of annulate lamellae from grasshopper spermatocytes. Apparently similar systems have been found in-

dependently by Schulz, Weichan, and Wessel (as quoted by Afzelius (3)) in rapidly growing neoplastic cells. The features common to all these lamellar arrays are the possession of annuli similar to those found in the nuclear envelope, and the regular alignment of vesicles (lamellar cross-sections) and spaces (sectioned annuli) within the whole stack of lamellae.

The high concentrations of RNA found in those systems which were studied histochemically make it probable that the annulate lamellae are somehow connected with protein synthesis. As the latter is undoubtedly under nuclear control, the demonstration of annuli in both the nuclear envelope and in the lamellae is of special interest. Furthermore, it seems highly suggestive that the annulate lamellae are in many instances aligned with the nuclear membrane (20). It has been pointed out in the present paper, that single lamellae are almost always close to the nuclear membrane and parallel to it. It is therefore rather tempting to think of the annulate lamellae as being formed by or on the nuclear membrane, and to see in this process one manifestation of nuclear control of the formation of a cytoplasmic structure. From this point of view, it would make little difference whether the cytoplasmic lamellar system might be synthesized from material of the nuclear membrane and thence pass into the cytoplasm, or whether cytoplasmic material is organized into lamellae under the directive influence of the nuclear membrane. Tempting as this hypothesis may be, certain difficulties in connection with it should be kept in mind. For one, Rebhun's (20) measurements indicate that the nuclear annuli in *Spisula* oocytes have a larger mean diameter than those found in the lamellar system. The latter structure is also distinguished by a greater density of annular packing. Another difficulty lies in the fact that annulate lamellae have so far been reported only from a small number of cell types, while nuclear control of cytoplasmic synthesis is undoubtedly a common feature of all cells. It is quite conceivable, as Swift (22) has pointed out, that the nuclear envelope and the annulate lamellae owe their similarity of structure to like forces acting on both.

Watson (23) has advanced the hypothesis that there are basically two possible pathways of nucleocytoplasmic exchange. One would be via the pores in the nuclear envelope. The latter are regions where the inner and the outer nuclear

membrane become continuous, resulting in the formation of a ring or annulus as seen in sections passing obliquely through the nuclear envelope. The pore itself, as in the annulate lamellae of the crayfish, is frequently found to contain a plug of relatively dense material. Afzelius (1) noted tube-like extensions of the annulus extending both into the cytoplasm and the nucleus of sea urchin oocytes. Swift (22) offered the interesting suggestion that annulate lamellar systems might be formed in the cytoplasm on the vertical extension of such tubules and might conceivably transmit genetic specificity from the nucleus into the cytoplasm by virtue of this continuous structure.

The second pathway of nucleocytoplasmic exchange as suggested by Watson (23) would be provided by direct continuity of the outer nuclear membrane with the vesicles of the endoplasmic reticulum. The two pathways would connect the perinuclear space enclosed by the double-walled nuclear envelope with the interior of the endoplasmic vesicles, and the nuclear interior with the extraventricular cytoplasmic space. Connections between the nuclear membrane and the cytoplasmic vesicles are quite common in the crayfish material, and the intralamellar space of the annulate lamellar systems is definitely continuous with the intravesicular spaces of the adjacent endoplasmic reticulum.

It has been pointed out that annulate lamellar systems are apparently rather short-lived in the crayfish spermatocyte. All basophilic bodies that were found between the metaphase of the first maturation division and the telophase of the second division proved to be of tubulate construction. During meiotic prophase, both annulate and tubulate lamellar bodies occur side by side.

This timing relationship suggests the possibility that there might be, during the course of prophase, a progressive transformation of annulate lamellar systems into the tubulate type of structure. If this is so, then Fig. 10 might represent an intermediate stage in this transformation. The continuity of straight annulate lamellae with those of the ellipsoid body would precede a similar (and observed) relationship between the latter and the straight lamellae of the tubulate system. The essentially novel feature would then be the appearance of small tubules in the place of annuli. There is a further observation in favor of this point of view. In sections passing obliquely through some of the lamellar surfaces of the ellipsoidal

body, the writer has noted a distribution of dense material that is strongly suggestive of annuli. Unfortunately, even ultrathin sections are considerably thicker than the width of these lamellae (about 180 angstrom units). This leads to the inclusion of dense membrane material in sections passing through annuli, and makes their demonstration difficult due to insufficient contrast. (The 300 A distance between the walls of annulate lamellae allows one to see at least some of the annuli against the light background of the intravesicular space.) It would seem that a combination of simple geometrical transformation and lamellar growth is sufficient to accomplish the transition from an open lamellar stack to the lamellar system of the basophilic tubulate body. It is interesting to note in this connection that mitochondria are occasionally found included within the central space of the ellipsoid body (Fig. 5).

One possible objection to this theory of lamellar transformation might be based on the fact that the number of lamellae intersected by the small tubules is different from that found within the ellipsoid body. Aside from effects due to the angle of sectioning, this discrepancy can be accounted for by the observation of "stray" lamellae which diverge from the course of the main lamellar set. Such lamellae may follow an irregular course in the surrounding cytoplasm, and may ultimately end in a series of vesicles indistinguishable from the endoplasmic reticulum. Other lamellae may stay on the main course, but open up into vesicles which remain aligned with the ellipsoid body (Figure 4 c). It is generally found that the ellipsoidal membranes are surrounded by a region of associated cisternae of the endoplasmic reticulum, which probably originated by such a process of lamellar vesiculation. An alternative interpretation could be based on the view that the orientation of the vesicles might represent a first stage in the formation of additional lamellae. It is also possible that the number of straight lamellae may increase by radical growth of the material in the tubules. This might account for the short lamellae which were occasionally interspersed between full-sized membrane doublets. Whatever the actual course of events may be, it is clear that they would tend to obscure a numerical correspondence between the curved and the straight lamellae.

The small granule fraction, which is frequently thought to be the site of intracellular RNA (2, 7,

18), was not found in either the annulate or the tubulate lamellar system described in this paper. In contrast to this negative finding are previous reports of small granules associated with annuli. Thus, Gall (11) found small granules of this type in or on the annuli of the nuclear membrane. Rebhun (20) demonstrated small particulate matter in association with vesicular "end-bulbs" of annulate lamellae, and gives reasons to think that material corresponding to Palade's granules is also present in the annuli. The possibility that small granules, although present in annulate lamellae of the crayfish, might not have been demonstrated because of insufficient contrast is, therefore, considered. Destruction due to fixation is improbable, as the small granules are frequently found associated with cytoplasmic vesicles near the annulate lamellae.

The question of the absence of granules appears in a different light when the tubulate lamellar bodies are considered. Although the small granules can appear tubular, and might perhaps be homologous with the tubules of the lamellar body, basophilia is not restricted to the tubular array. Inspection of Fig. 4 *c* will show that RNA is localized in all regions of the whole lamellar system, including the ellipsoid body and the vesicular region surrounding the latter. Within the vesicular area, there is certainly no problem of differential contrast involved, as nearby cisternae of the endoplasmic reticulum may show the granules quite clearly. The "stray" lamellae of the ellipsoidal body discussed above may occasionally be found surrounded by granular clusters. The evidence discussed above leads to the conclusion that histochemically demonstrable RNA may occur independent of the small granule fraction. Possible extragranular RNA sites include, therefore, the annuli and their plugs, the small tubules, and, especially in the case of the ellipsoid body, the material between the membranes or else the membranes themselves.

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BIBLIOGRAPHY

1. Afzelius, B. A., *Exp. Cell Research*, 1955, **8**, 147.
2. Afzelius, B. A., *Z. Zellforsch. u. mikr. Anat.*, 1957, **45**, 660.
3. Afzelius, B. A., *Electron Microscopy of Sea Urchin Gametes*, thesis, Uppsala, 1957.
4. Bernhard, W., Goutier, A., and Rouiller, C., *Arch. Anat. micr. et morphol. exp.*, 1954, **43**, 236.
5. Callan, H. G., and Tomlin, S. G., *Proc. Roy. Soc. London, Series B*, 1950, **137**, 366.
6. Chaveau, J., Moule, Y., and Rouiller, C., *Exp. Cell Research*, 1957, **13**, 398.
7. Clermont, Y., *Exp. Cell Research*, 1957, **11**, 214.
8. Fasten, N., *J. Morphol.*, 1914, **25**, 287.
9. Flax, M. H., and Himes, M. H., *Physiol. Zool.*, 1952, **25**, 297.
10. Gall, J. G., *Exp. Cell Research*, 1954, **7**, 197.
11. Gall, J. G., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 393.
12. McCroan, J. E., Jr., *Cytologia*, 1940, **11**, 136.
13. Moses, M. J., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 397.
14. Newman, S. B., Borysko, E., and Swerdlow, M., *J. Research Nat. Bureau Standards*, 1949, **43**, 183.
15. Palade, G. E., *J. Exp. Med.*, 1955, **96**, 285.
16. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 59.
17. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 567.
18. Palade, G. E., and Siekevitz, P., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 671.
19. Porter, K. R., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 269.
20. Rebhun, L. I., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 93.
21. Sjöstrand, F. S., and Hanzon, V., *Exp. Cell Research*, 1954, **7**, 393.
22. Swift, H., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 415.
23. Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 257.
24. Wilson, E. B., *The Cell in Development and Heredity*, New York, Macmillan Co., 1928.

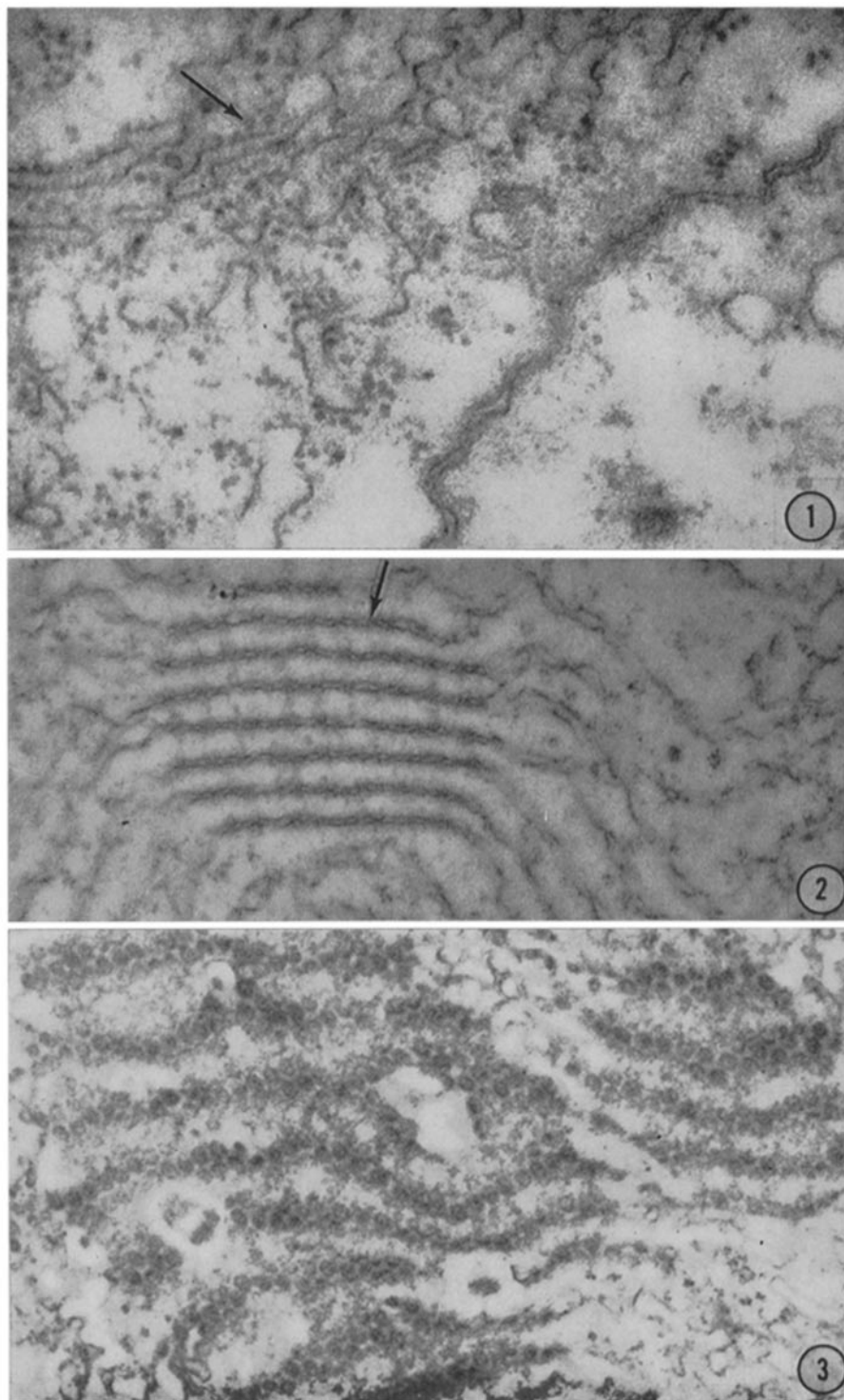
EXPLANATION OF PLATES

PLATE 144

FIG. 1. Region of the endoplasmic reticulum showing vesicles and granules. Some of the larger granules (180 to 200 A) show a central lumen (arrow). The two lines traversing the figure from the lower left to the upper right are cell membranes. $\times 74,000$.

FIG. 2. Transected annulate lamellar system. Note the alternation of vesicles and spaces, as well as the continuity with the vesicles of the endoplasmic reticulum surrounding the lamellar body. The arrow points to one of the spaces. Adjoining vesicles right and left of the tip of the arrow. $\times 28,000$.

FIG. 3. Annulate lamellar system sectioned nearly parallel to the lamellar surface and demonstrating annuli. $\times 27,000$.



(Ruthmann: Basophilic lamellar systems)

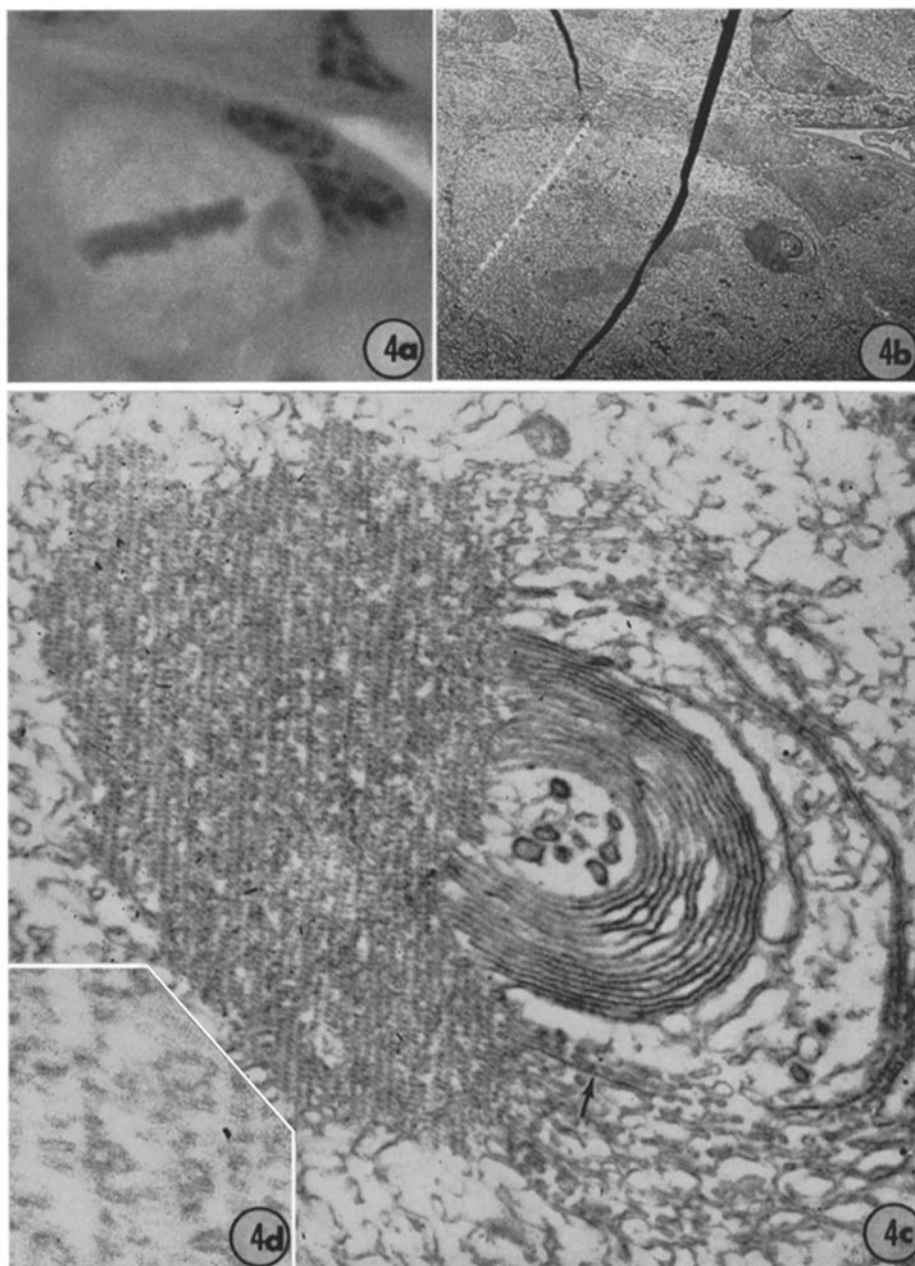
PLATE 145

FIG. 4 *a*. Micrograph from osmium-fixed and methacrylate-embedded tissue, cut at $2.5\ \mu$ thickness, and stained with 0.25 per cent azure B at pH 4 to demonstrate basophilia. The chromosomes and the follicle cell nuclei surrounding the metaphase cell are intensely blue (DNA plus RNA). Note the diffuse cytoplasmic basophilia (RNA) and the intense RNA-staining of the lamellar body next to the chromosomes. $\times 2,000$ (approximately).

FIG. 4 *b*. Low power electron micrograph ($\times 2,000$) of same area as Fig. 4 *a*, from adjacent thin section.

FIG. 4 *c*. Electron micrograph of lamellar body in Fig. 4 *b*, photographed at higher magnification. Note that the basophilic body of Fig. 4 *a* comprises three structurally different regions: the area of the fine tubules (left half of 4 *c*), the set of oriented lamellae (right half, center), and the oriented vesicles (lower and upper right of picture). The arrow points to a lamella widening into a vesicle. $\times 26,000$.

FIG. 4 *d*. Some of the obliquely sectioned tubules of Fig. 4 *c* magnified to show the light core and the dense wall of the individual elements. $\times 101,000$.



(Ruthmann: Basophilic lamellar systems)

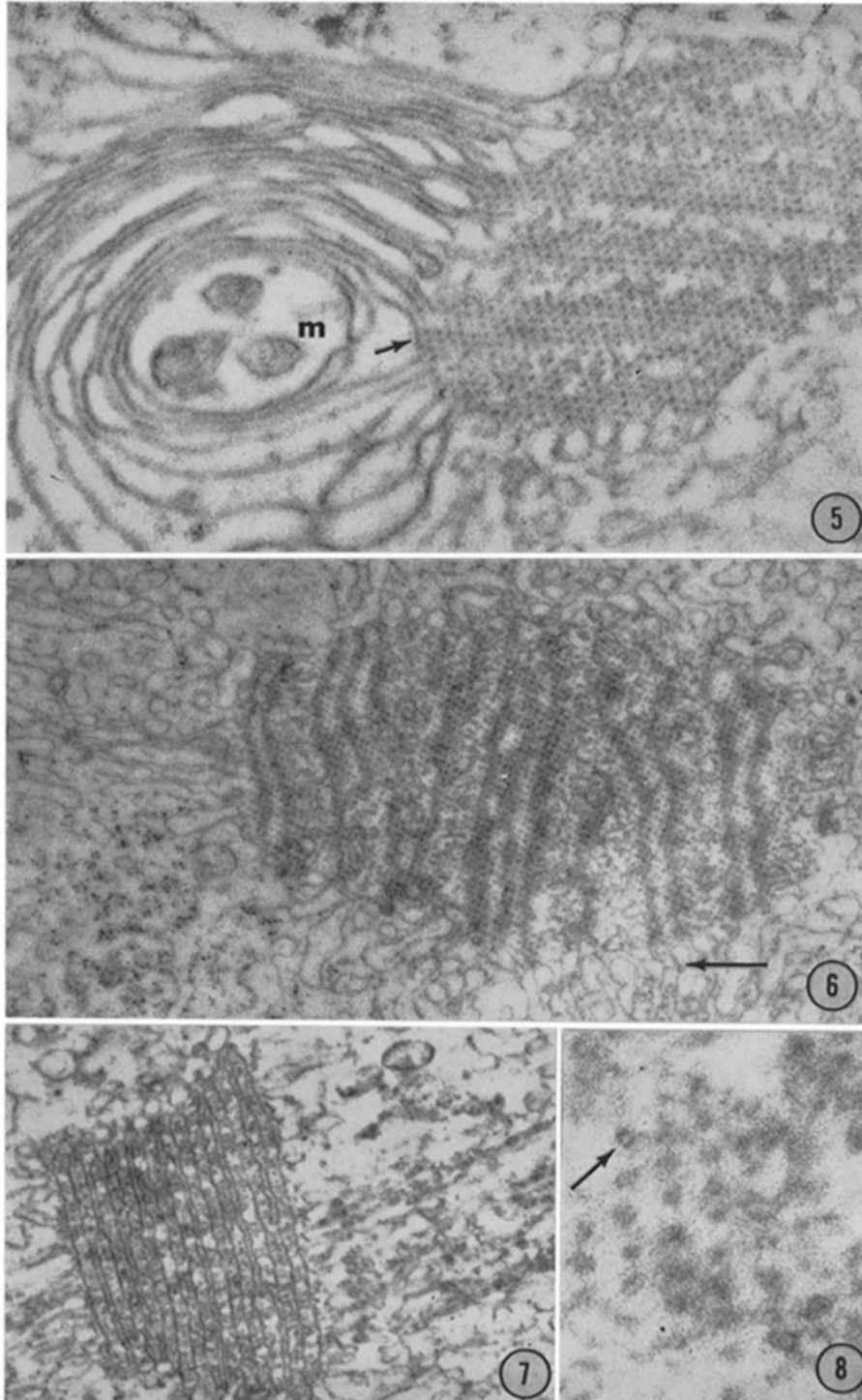
PLATE 146

FIG. 5. Section cut obliquely through successive layers of the plane lamellae. Note the cross-sectioned tubules and the alignment of the lamellar layers of the ellipsoidal body with the tubular pattern (arrow). At least one of the structures inside the ellipsoidal body is a trapped mitochondrion (*m*). $\times 35,000$.

FIG. 6. Obliquely transected system, showing the straight lamellae as broadened electron dense areas and arranged in groups of two or three. Note the highly regular spacing of the cross-sectioned tubules, and the cytoplasmic vesicles aligned with the lamellar system (arrow). The area at left shows the vesicles of the endoplasmic reticulum studded with the small granules described in the text. $\times 27,000$.

FIG. 7. Sectioned lamellar system seemingly lacking an associated ellipsoidal body. Note the reticular vesicles oriented at right angles to the straight lamellae. A micrograph like this would result from sections bypassing the ellipsoidal body. $\times 21,000$.

FIG. 8. Section of inter lamellar tubular array. Note cross-sectioned tubule at arrow. $\times 150,000$ (approximately)

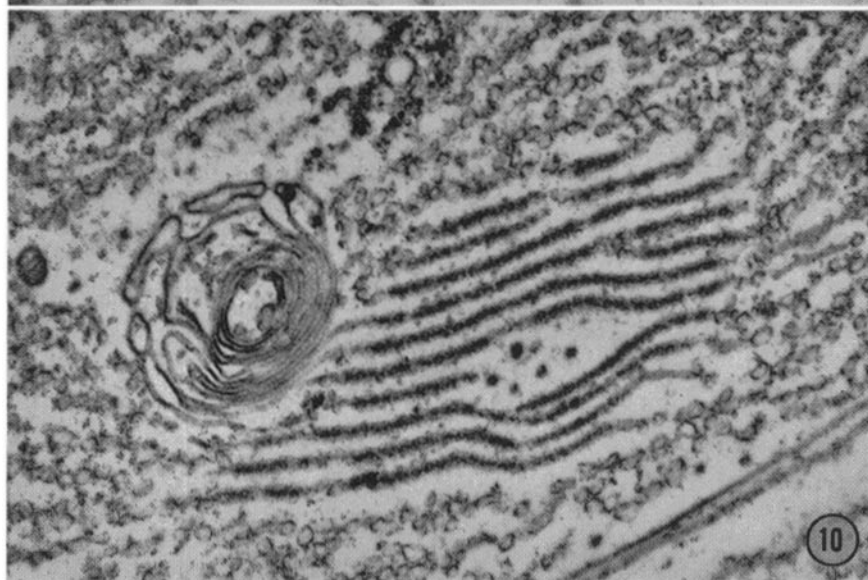


(Ruthmann: Basophilic lamellar systems)

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FIG. 9. Section through an ellipsoidal body, showing a region of folding. Note small granules on "stray" lamellae (arrow). This lamellar system was found near the metaphase plate of a primary spermatocyte. The granular material at the upper left is one of the chromosomes. $\times 36,000$.

FIG. 10. Ellipsoidal body associated with annulate lamellar system. Individual lamellae of the latter can be traced into the ellipsoidal body. $\times 15,000$.



(Ruthmann: Basophilic lamellar systems)