ELECTRON MICROSCOPY OF BASOPHILIC STRUCTURES OF SOME INVERTEBRATE OOCYTES

II. Fine Structure of the Yolk Nuclei*

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Plates 24 to 27

(Received for publication, December 1, 1955)

INTRODUCTION

The yolk nuclei of animal oocytes usually arise in previtellogenesis (37, 42), and often undergo changes of shape, organization, and position during oocyte development. Thus, in the spider *Tegenaria*, an RNA-containing crescent arises in contact with the nuclear envelope and later gives rise to an intricate yolk nucleus (4). In the oocytes of some teleosts the yolk nucleus gives rise to a "pallial" layer and after peripheral migration breaks up to supply the cortex of the oocyte with material (8). In *Rana pipiens* flocculent basophilic yolk nuclei arise in contact with the nuclear envelope just before primary yolk formation and, after a peripheral migration, contribute material to the basophilic cortex (22); while in *Triturus helviticus*, Wittek (45) showed that basophilic masses also arise in the perinuclear region just preceding the period of secondary yolk synthesis. In other amphibians, instead of discrete basophilic yolk nuclei arising before secondary yolk formation, a perinuclear basophilic ring is formed. Many yolk nuclei disappear after the beginning of yolk synthesis (7, 9, 12, 14, 16, 18, 23, 25) although others persist into early embryonic stages (42, 44, 45). They universally arise, however, before vitellogenesis. In many species yolk granules have been reported as arising within or closely associated to the yolk nuclei (17, 42, 43). In other species where yolk nuclei break up to form basophilic regions of the cell it is usually within these regions that yolk first arises (10, 15, 22, 26, 43). It is for these reasons that yolk synthesis has been thought of as intimately associated with yolk nuclei, in those species which possess yolk nuclei.

Since yolk is in large part protein (5, 19, 35) these observations support the evidence implicating RNA in protein synthesis (3, 6, 11). It is, therefore, of great im-

* Most of the work in this paper was completed while the author held a National Science Foundation Predoctoral Fellowship. Support for part of the work, as well as funds for materials and equipment for it, came from United States Public Health Service Grant 1612 and from an Abbott Memorial Fund Grant to Dr. Hewson Swift, Department of Zoology, The University of Chicago.

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portance to study these oocyte structures with the electron microscope since the precise location of the basophilic material and the association of a formed secretory product with it offer advantages in studying the morphological changes in basophilic structures occurring during synthesis.

The structure of the basophilic regions of somatic cells has been studied by many and reviews of this work are given by Porter (36) and Bernhard (2). The earlier work indicated that these regions consisted of an interconnected set of flattened vesicles and canals in varying degrees of extension and organization. There is evidence to suggest that this system is involved in the synthesis of certain secretory products of the cell. Thus, Weiss (41) found vesicles appearing at the ends of the "ergastoplasmic sacs" and traced the origin of zymogen granules to these vesicles. Porter (36) showed the variation of these systems in the parotid gland with pilocarpine injections which empties the gland of secretion granules. Herman showed that this system arises in resting adult thyroid cells of the salamander after injection with thyrotropic hormone and is correlated with hypertrophy of the cells and the appearance of basophilic caps at either end of the nucleus (39) of the polarized cell. Evidence exists, therefore, which tends to implicate the ergastoplasm with synthesis of some cell constituents (see, however, Sjostrand (38)).

The important question of the localization of the RNA in this system has been dealt with by Palade (34) and it appears that small particles (100 to 150 A) which are often associated with the vesicles and canals of the endoplasmic reticulum are the RNA-containing bodies. Thus, this later work separates the ergastoplasm of the somatic cell into two constituents—an endoplasmic reticulum, probably, largely lipoprotein in nature, organized as described above and the small granules which can exist alone in the cytoplasm, but which are usually associated with the endoplasmic reticulum. In embryonic cells and in fast growing tumor cells, however, the main constituent seems to be the small granules (20, 34).

A description was given in a previous paper of certain of the highly basophilic yolk nuclei in the oocytes of the pulmonate snail *Otala lactea* and the surf clam *Spisula solidissima* (37). It was found, with the electron microscope, that these regions consist of many parallel lamellae, each of which can be conveniently thought of as a flattened vesicle perforated by hexagonally arranged pores the walls of which form thickened annuli. Some evidence exists that the annulus is actually composed of a set of small vesicles arranged in a circle and the possibility seems high that these small vesicles correspond to the granules of Palade (34).

In the present paper we shall describe basophilic yolk nuclei in *Spisula* which have a different form from the forms previously discussed and we shall attempt to relate all of these bodies in a model and point out evidence which suggests the synthesis of formed yolk within these yolk nuclei.

Materials and Methods

Ovaries of *Spisula solidissima*, the surf clam, and ovotestes of *Otala lactea*, a pulmonate snail were used. Methods were identical to those described in reference 37.

OBSERVATIONS

A. Light Microscopy.—

Figs. 1 and 2 are micrographs of Carnoy-fixed, azure B-stained sections of *Spisula* oocytes. The concentration of RNA in each of the dark, azurophilic bodies is over four times that of the remaining cytoplasm (39). This RNA is removable with RNA-ase used according to Kaufmann *et al.* (21).

One may roughly divide these bodies into two classes although transitional stages appear to exist. The disc-shaped bodies (represented by the rod of basophilia in Fig. 1), forming the first class have been dealt with in (37). Members of the second class of bodies are generally larger and seem to show all transitional stages from elongated, cylindrical objects to roughly spherical or ellipsoidal ones. Many of them appear to have lightened interiors, often quite eccentric, which take less stain than the surrounding cytoplasm (Fig. 1 *c*). Occasionally, one sees large, almost non-basophilic vacuoles abutting the ends of the solid cylindrical regions.

The latter regions vary in size from 2 x 6 μ to 4 x 18 μ in the plane of the section (Fig. 1 and 2, *r*). The dimension perpendicular to this plane is difficult to measure, but it appears to be comparable to the smaller of the above dimensions. The regions appearing approximately circular to the viewer run from 4 x 4 μ to 10 x 10 μ. It will be noted in Fig. 1, that some of those with non-basophilic interiors appear to be cup-shaped with one end of the structure more or less open to the cytoplasm, although a thin line of basophilic material closes the cup. Some of these regions are similar in size and shape to the nucleolus, and many are tangent to the nucleus as in Fig. 1. If tangent to the nuclear envelope where the latter shows an indentation, the appearance is sometimes that of a body half in and half out of the nucleus but careful focusing indicates that the bodies are always outside of the often highly convoluted nuclear envelope.

Most of the eggs used in this study were ready for maturation, and fertilization of a part of the eggs from some of the ovaries was obtained by the method of Allen (1). Thus, most of the eggs had completed the growth stage. However, some earlier stages were found in parts of the ovary walls. In these early oocytes, circular, hollow bodies with thin basophilic rims were seen. No large particulate basophilic bodies were seen in oocytes with a diameter with less than 30 μ; *i.e.*, less than half of the diameter of mature eggs. These young stages were, however, intensely basophilic (37).

B. Electron Microscopy.—

Large Basophilic Regions.—Fig. 3 is a low power view of a *Spisula* oocyte. Two groups of small, approximately spherical bodies can be seen. Those about 0.5 μ across are dense and irregular in shape. The larger ones, about 1 μ across,

are paler and more regular (Fig. 8). These bodies undoubtedly are yolk platelets, and the presence of two different entities in this general size category suggests that more than one type of yolk particle is present. Other spherical bodies about 1 μ across can be seen in Fig. 8 (a higher power view of a part of Fig. 3) and the presence of cristae identifies them as mitochondria (32, 33).

The largest and most conspicuous object in the cytoplasm of the oocyte in Fig. 3 is the elliptical region composed of what appear to be "fibers" arranged in a whorl. Comparison of this region with the spherical basophilic bodies in Fig. 1 leaves little doubt that they are similar objects. Fig. 5 and 10 also represent objects falling into this class. Fig. 8 indicates that the "fibers" in Fig. 3 are actually double walled structures the composition of which will be discussed in detail later.

The regions in Figs. 5 and 10 have some yolk platelets in their interiors and many sections of bodies like this have been seen. Fig. 3 (and, of course, Fig. 8) do not show this detail. Since the double walled structures run for a considerable distance in the section it is clear that they must be plate-like rather than rod-like. We shall call them double walled lamellae.

The double walled lamellae shown in Fig. 10 can be seen to run almost completely around the whole object, at least, those in its interior. In Fig. 8, this is not as clear since many of the lamellae turn into the section and, therefore, present a surface view. When this happens it is difficult to trace them from these regions. Nevertheless, the inner lamellae appear to circle the entire region, although some of the peripheral ones possibly do not.

A region similar to that in Fig. 8 is shown in Fig. 9. Here, the double walled lamellae appear to run across the entire field although again there is difficulty in tracing them for their full length. Some of the clear, irregular areas bounded by the double walled lamellae appear to end in blind sacs at the right end of this region (marked *b*). The region in Fig. 4 is similar to that just discussed except that it does not show the curving that the lamellae of Fig. 9 do at their left end and the lamellae are somewhat more irregular. Further, the lamellae in Fig. 4 are roughly parallel to the nuclear membrane while those in Fig. 9 appear to be out further in the cytoplasm.

Fig. 11 shows a region, part of which resembles that in Fig. 4 and part of which consists of periodic lamellae (37). The double walled lamellae extend into the region of the periodic lamellae and can be seen to be lined up beneath and parallel to the latter, at the arrows in Fig. 11.

It is not clear as to what the over-all 3-dimensional shapes of the regions depicted in Figs. 4, 9, and 11 are. If, however, these correspond to the more elongate basophilic bodies, *r*, in Figs. 1 and 2, then we may think of them as banana- or cucumber-shaped. On this interpretation, the region of double walled lamellae in Fig. 11 would be an approximate cross-section and that

in Fig. 4 a longitudinal section of these regions. A more detailed interpretation will be given later.

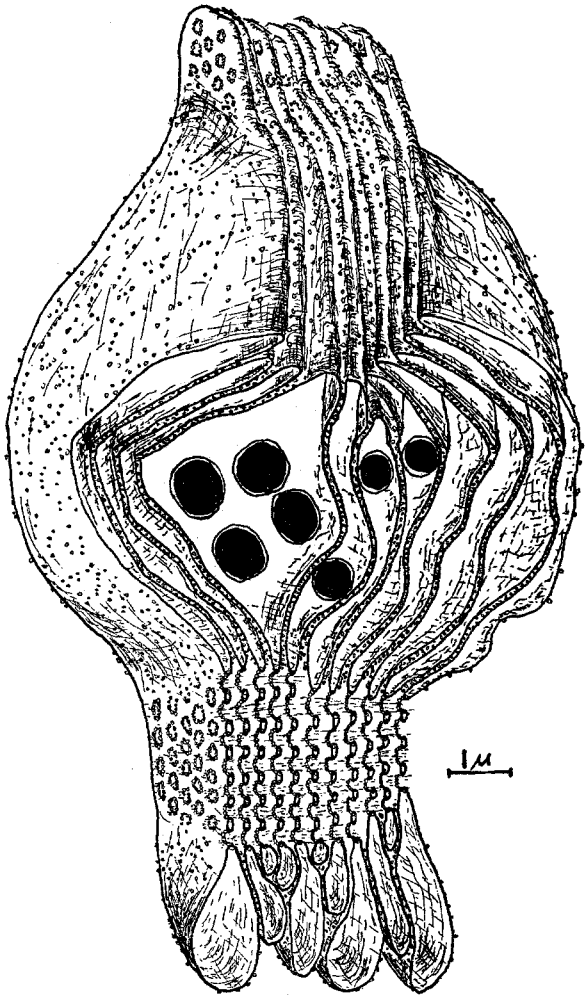
Fig. 12 is a higher magnification view of a part of a region similar to that in Fig. 10. Each of the walls of the double walled lamellae can be seen to have a "rough" and a "smooth" side (40). The total thickness of the lamellae is about 200 A across and can be seen to be reasonably constant (except, of course, for the widening where the lamellae turn into the section). The "rough" side of one of the bounding walls of the double walled lamellae faces the "rough" side of the other wall, and, though the pictures are not good enough to decide this definitely, it appears as if small granules are included between the single walls or are attached to them. The clear spaces bounded by the smooth sides of the walls of the lamellae have various shapes. In Figs. 8 and 10 they probably run around the whole region although the same uncertainty attends this statement as that pertaining to the lamellae themselves. In Fig. 9 at *b*, as has been pointed out, these spaces are bounded by a wall and thus appear as blind sacs. At *O*, in Fig. 11, a clear area appears to be bounded by a single wall over one portion of its surface. Fig. 13, a higher power view of part of Fig. 9, shows similar features to those in Fig. 12.

Figs. 6 and 7 show periodic lamellae from *Spisula* oocytes, parallel to the nuclear envelope. It can be seen that vacuoles can appear at the ends of the periodic lamellae (see descriptions of Figs. 6 and 7). Note that the vacuole walls are single and, where they turn into the section they appear to be accompanied by granules of small size (approximately 100 A). Where two adjacent vacuoles meet (*e.g.* at *w* in Fig. 7) a double walled structure of about 200 A is formed, reminiscent of those discussed above (see also reference 39). The region marked *t* in Fig. 6 should be compared with those marked by arrows in Fig. 11.

Fig. 14 shows similar vacuolization at the ends of the periodic lamellae in *Otala lactea*. The vacuole appears in cross-section as an enlarged loop. These vacuoles appear to be similar to other vacuoles in the cytoplasm. End vacuoles in *Otala* never get much larger than 1000 A and therefore never get as large as those of *Spisula*.

INTERPRETATION OF OBSERVATIONS

We have discussed several types of basophilic bodies in oocytes seen with the light microscope and certain oocyte structures seen with the electron microscope and have attempted to correlate them. The yolk nuclei seen with the light microscope were divided into two classes one of which was dealt with in the preceding paper (37) and the other in the present paper. The members of the first class were called periodic lamellae for reasons given in reference 37. The yolk nuclei of the second class appear to be composed of double walled



TEXT-FIG. 1. This is a schematic diagram of the relationships between periodic lamellae and double walled ones, discussed in the text. It should be noted that the double walled lamellae are generated by the apposition of single membranes which bound the internal space of large vesicles continuous with the internal space of the periodic lamellae. A slice has been taken from the structure to show the internal arrangement. One cutting plane is perpendicular to the plane of the periodic lamellae and the other cuts across the field without intersecting them.

lamellae whose cross-sections do not show the striking alternation of loops and spaces shown by cross-sections of the periodic lamellae (37). Instead, these cross-sections resemble more closely the profiles of the ergastoplasm of

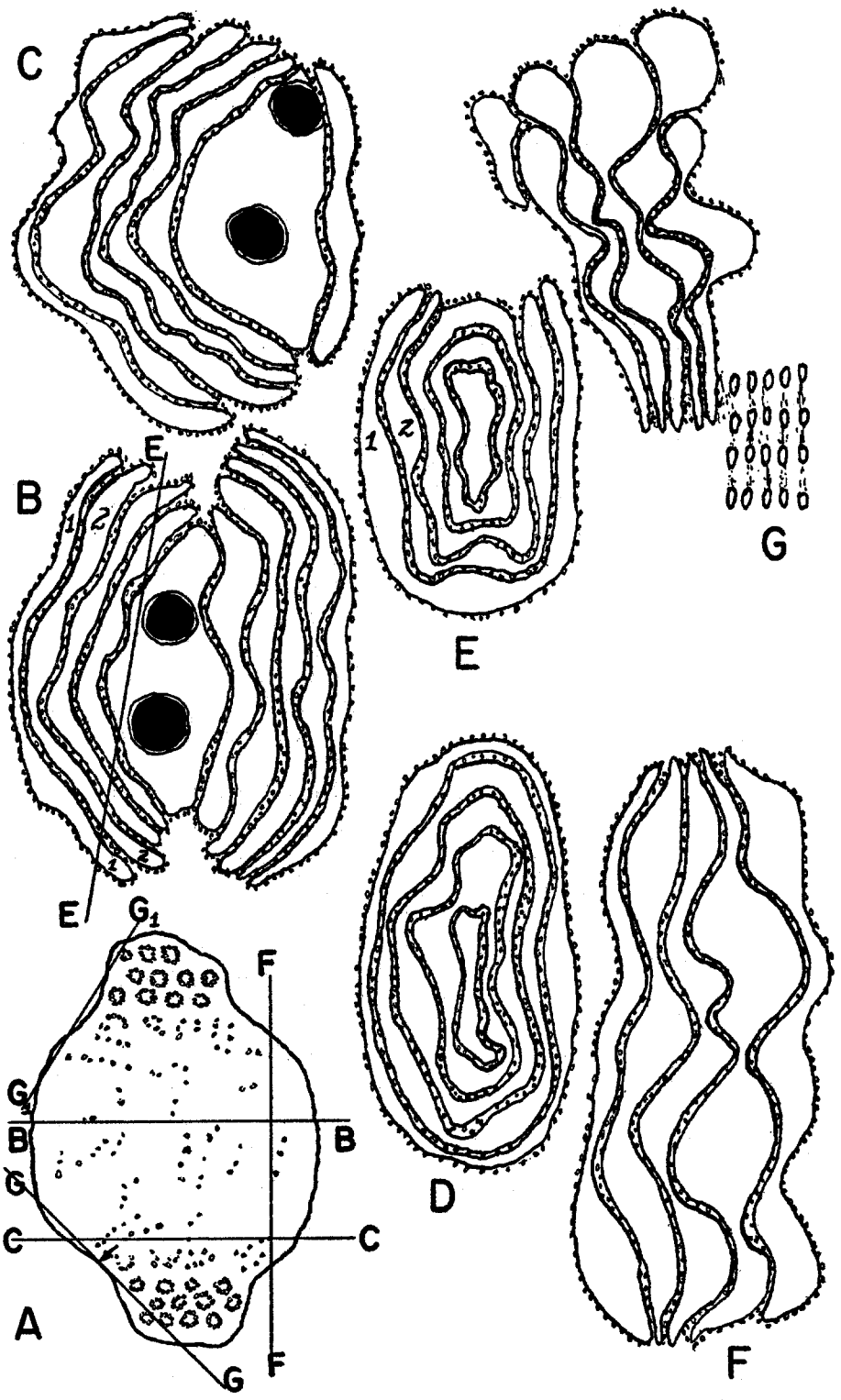
somatic cells (2, 36, 41) as will be discussed later. The double walled lamellae shown in the sections presented appear to be arranged in a variety of ways and might be thought to represent different gross structures. On the other hand, these appearances may represent sections cut at different angles from a single type of object. We believe that Text-fig. 1 represents such an object. It is drawn with a piece removed so that part of its interior is visible. It will be noted that the structure consists of two groups of periodic lamellae, separated by a central portion composed of double walled lamellae. At the juncture of the regions of periodic and double walled lamellae it can be seen that a continuity exists between the two types of lamellae which may be visualized as follows. If in Text-fig. 1 we trace along a profile of a given periodic lamella toward the central region we encounter alternating loops and spaces. These are the results of sectioning the portion of the periodic lamella not perforated by the pore and the center of the pore, respectively (37). We ultimately come to what would be a loop if the loop-space periodicity were to continue. If we think in terms of cross-sections, what occurs can be visualized as a great elongation of this hypothetical loop into a long, flat profile. If we imagine this in all the lamellae in a bundle and in the same general region, then the double walled lamellae appear to be formed by the apposition of the single walls of the adjacent elongated vesicles so formed. It should be stressed that the apposition of two such single walls does not result in fusion but in the formation of a structure quite uniform in thickness. The following points should also be noted:—

(*a*) Text-fig. 1 indicates the small granules as being scattered *between* the walls of *each* double walled lamella.

(*b*) It is indicated that these granules may arise by the break-up of annuli in the periodic lamellae. The small granules comprising the annulus are assumed to spread over the surfaces of the elongated vesicles. The close apposition of neighboring vesicle walls to form double walled lamellae then results in the location of the granules "within" the double walled lamellae.

(*c*) Yolk platelets are depicted as being *within* the elongated vesicles although the possibility that they are *between* the vesicles has not been ruled out.

Text-fig. 2 represents a series of sections cut from objects such as those in Text-fig. 1. It should be realized that the degree of bulging of the central region may vary, as may the size and separation of the two groups of periodic lamellae. Further, the whole object may possibly be somewhat twisted or bent so that the planes of the periodic lamellae in one group may not be parallel to those in the other. On this model, the objects in Fig. 1 labelled *r* and *c* would be homologous ones in different stages of extension. This agrees with the observation that intermediate forms of yolk nuclei appear in these oocytes when examined with the light microscope.



TEXT-FIG. 2

DISCUSSION

The yolk nuclei discussed in the present paper are structurally similar to the ergastoplasm of somatic cells but have certain differences. These are:—

(*a*) The walls of adjacent vesicles are closely associated and form secondary double walled lamellae which remain remarkedly constant in width.

(*b*) No evidence has been found that the internal space of adjacent vesicles is ever connected, that is, the system is not a reticulum.

(*c*) There appears to be a continuity of walls and interiors between the vesicles of the yolk nuclei discussed here and those of the periodic lamellae (37).

The yolk nuclei are similar to the ergastoplasm in that small granules (about 100 A) seem to be associated with the outside of the vesicles; *i.e.* pressed between the two vesicle walls which form a double walled lamella. In somatic ergastoplasm small granules are also attached to the outside of the cisternae or vesicles. It is clear, then, that both the oocyte ergastoplasm and that of somatic cells are composed of vesicles (cisternae) bounded from the cytoplasm by an electron-dense layer to which small granules are attached. In oocytes, however, walls of adjacent cisternae reassociate to form secondary double walled structures, which then appear to have an identity of their own. It is probably the differences in the manner in which these two types of basophilic bodies originate that accounts for the differences in their structures.

Evidence has been mentioned in the introduction which correlated the endoplasmic reticulum and small granules (ergastoplasm) with physiological function. The correlation of the ergastoplasm with basophilic regions suggests

TEXT-FIG. 2. Text-fig. 2 A is a top view of the model of the yolk nucleus in Text-fig. 1 and the labelled lines across it represent the directions of plane sections through this model and depicted in the rest of the drawing. Text-fig. 2 B is a section perpendicular to the planes of the periodic lamellae and through the line *B-B* or any similar line. Text-fig. 2 C represents a variation of this. A structure similar to that shown in Fig. 9 results from a section through a line such as *C-C* in Text-fig. 2 A.

A section parallel to the planes of the periodic lamellae in Text-fig. 1 results in a section like Text-fig. 2 D in which the double walled lamellae completely circle the region. If this section is tipped as indicated by line *E-E* in Text-fig. 2 B, then vesicles such as those whose interiors are labelled *1* and *2*, are cut so that one end of the vesicle is below and one above the plane of the section. Then, rather than double circles, bent loops result as in Text-fig. 2 E. The inner vesicles are cut so that double circles do result. Structures such as these seem to occur in Figs. 5, 8, and 10.

Finally, Text-fig. 2 G results from a section passing through the line *G-G* in Text-fig. 2 A, if the section makes an acute angle with the planes of the periodic lamellae. This condition means that the plane will intersect the periodic lamellae at a level at which it does not intersect the double walled lamellae (and *vice versa*) if the line *G-G* touches the point marked with an arrow or if it passes through line G_1-G_1. In the former case, a figure such as Text-fig. 2 G (and Fig. 11) results. In the latter, the two regions would be separate. If this plane swings out so that it misses the periodic lamellae, *e.g.* a plane passing through line *F-F* in Text-fig. 2 A, then a section resembling Text-fig. 2 F (compare Fig. 3) results, the exact form depending, of course, on the degree of bulging in the structure in Text-fig. 1. Many more situations can be predicted from this model, some of which can be followed in appropriate sections.

a relation of these regions to the process of protein synthesis. In embryonic cells and in fast growing tumor cells, the endoplasmic reticulum is reduced, in that profiles of cisternae are less in number and shorter in length as compared to normal cells, although the small granules are everywhere present (20, 34). Howatson and Ham (20) suggested, on this basis, that the association of the endoplasmic reticulum and of the small granules was necessary for the formation of *formed* secretions whereas the small granules alone might be correlated to synthesis not involving a supramolecular body; *i.e.*, a secretion granule.

The idea that yolk platelets, which can be considered as formed secretion granules, are manufactured within the yolk nuclei agrees with some classical observations, for example, Harvey's (17) on the yolk nuclei of *Antedon* or Wittek's on the basophilic regions of the period of secondary yolk formation in *Triturus helviticus* (43). In both of these organisms yolk granules appear inside the yolk nuclei and later pass out into the cytoplasm. Worley and Worley (45) and Worley (44) described yolk formation in the tectibranch *Navanax* and the lamellibranch *Mytilus* and found yolk formed *inside* bodies which were osmophilic and which stained vitally with methylene blue. They called these bodies, Golgi bodies. A comparison of their figures of *Mytilus* with ours of the related lamellibranch, *Spisula*, indicates that their Golgi bodies and the yolk nuclei described here are probably homologous. If so, then their work, based on observations on living oocytes, is strong evidence for the formation of yolk within the yolk nuclei described here. Electron micrographs of yolk nuclei, such as that in Fig. 5, often show yolk granules *within* the yolk nuclei. In Text-fig. 1 these have been put *inside* the elongated vesicles although the electron micrographs are not of sufficient quality to decide whether they occur here or between the vesicles. Nevertheless, the classical observations mentioned above, taken with the occurrence of yolk within the yolk nuclei, make us feel that the concept that formed cytoplasmic bodies of a secretory or reserve food nature tend to be formed in association with a cytoplasmic machine composed of elongated vesicles with attached granules is supported by this work.

Finally, a similarity exists between the spherical basophilic yolk nuclei and the nucleolus, when viewed with the light microscope, at least at certain stages of development. The electron microscope, however, reveals that they have very different submicroscopic structures. Many reports of extruded nucleoli exist in the literature (7, 12–16, 18, 23–25, 27–31, 40, 43) and it seems possible from the present work that these may be based on erroneous interpretations of cytoplasmic entities (yolk nuclei) which seem superficially like nucleoli.

SUMMARY

RNA containing yolk nuclei from the surf clam *Spisula solidissima* have been studied with the light microscope and with the electron microscope. A

variety of structures can be seen with both and a correlation can be made between bodies studied with the electron microscope and those studied with the light microscope. The electron microscope shows many of these structures to be composed of double walled lamellae arranged in space, in various ways. The variety of patterns seen with the electron microscope can be satisfactorily explained on the basis of a hypothetical model. The presence of yolk platelets within the yolk nuclei lends support to classical observations on the synthesis of yolk within yolk nuclei or yolk nuclei derivatives. Small granules (about 100 A) are included within the double walled lamellae and their presence probably accounts for the basophilic nature of the entire body. The presence of small granules attached to electron-dense layers relates the yolk nuclei described here to ergastoplasm discussed by others.

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EXPLANATION OF PLATES

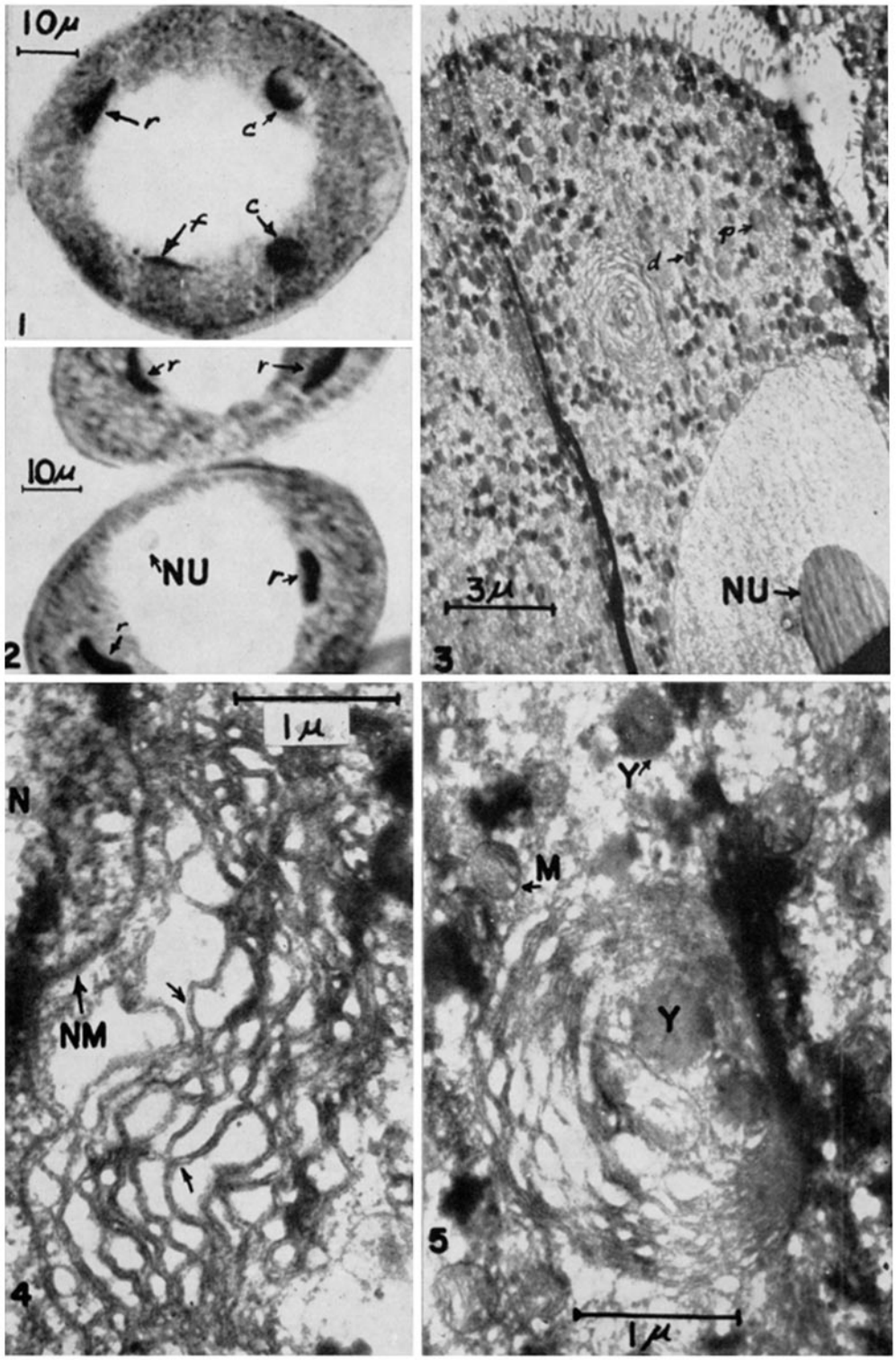
PLATE 24

FIGS. 1 and 2. Carnoy-fixed sections of *Spisula* oocytes stained with azure B to show RNA. The three types of basophilic bodies are represented at points labelled *c*, *f*, and *r*. The forms such as *f*, have been discussed in reference 39. The thicker, elongate forms, *r*, show a concavity facing the nucleus. Focusing through the section indicates that they are about equal in height and width. They are, therefore, shaped roughly like a cucumber. The forms appearing round in cross-section, *c*, are actually spherical or ellipsoidal. The upper one has an eccentric, non-staining center whereas the lower one appears relatively solid although there is some variation in density within it. The nucleolus, *NU*, at this stage stains more lightly than in smaller oocytes (39).

FIG. 3. Low power electron micrograph of a *Spisula* oocyte. A fold runs through the section which was compressed during cutting in a direction perpendicular to the fold. The whorl-like region in the cytoplasm is of the right size and shape to represent the spherical basophilic bodies, *c*, of Fig. 1. The granules in the cytoplasm appear to be of two sizes: a smaller denser, spherical one, *d*, and a larger paler one, *p*. They probably both represent types of yolk granules or platelets.

FIG. 4. A roughly rectangular lamellate region of a *Spisula* oocyte is shown. The double walled (see arrows) structures (lamellae) running across the field, though wavy, are roughly parallel to the nuclear envelope, *NM*. Irregular spaces, devoid of dense material, separate the dense lamellae. *N* is the nucleus.

FIG. 5. This circular region resembles that in Fig. 3 and again, can be compared to the bodies labelled *c* in Fig. 1, although it appears to be smaller than them. Its component lamellae resemble those in the structure in Fig. 4, but are here arranged in an irregular circle. A yolk platelet, *Y*, appears in the center of the object, while a fold distorts one side of it. The mitochondria are labelled *M*.



(Rebhun: Basophilic structures of invertebrate oocytes. II)

PLATE 25

FIGS. 6 and 7. These regions from *Spisula* oocytes show periodic lamellae (37) parallel to the nuclear envelope. The regular alternation of small loops, *l*, separated by spaces, *s*, along the lamellae is clear, especially in Fig. 7. It can be seen that vacuoles appear at the ends of most of the lamellae. Where the (single) vacuole wall turns tangent to the section, it can be seen to contain some small granules, *g*. Where two single vacuole walls meet, a double walled structure about 200 A thick is formed, *w*. Thus, vacuole walls appear to maintain a definite minimum distance apart where the vacuoles meet.

FIG. 8. A view at higher magnification of the circular region in Fig. 3. It can be seen that double walled lamellae, about 200 A thick run approximately, concentrically about the region. Although, it is difficult to trace these structures throughout their length, they appear to encircle the whole region. The smaller, darker granules in Fig. 3, *d*, here appear somewhat irregular in shape and are possibly disrupted lipide granules. The larger granules show, in some views, a thin, light layer, about 200 A in thickness around the rim. They are probably spherical in form. Mitochondria are labelled *M*.

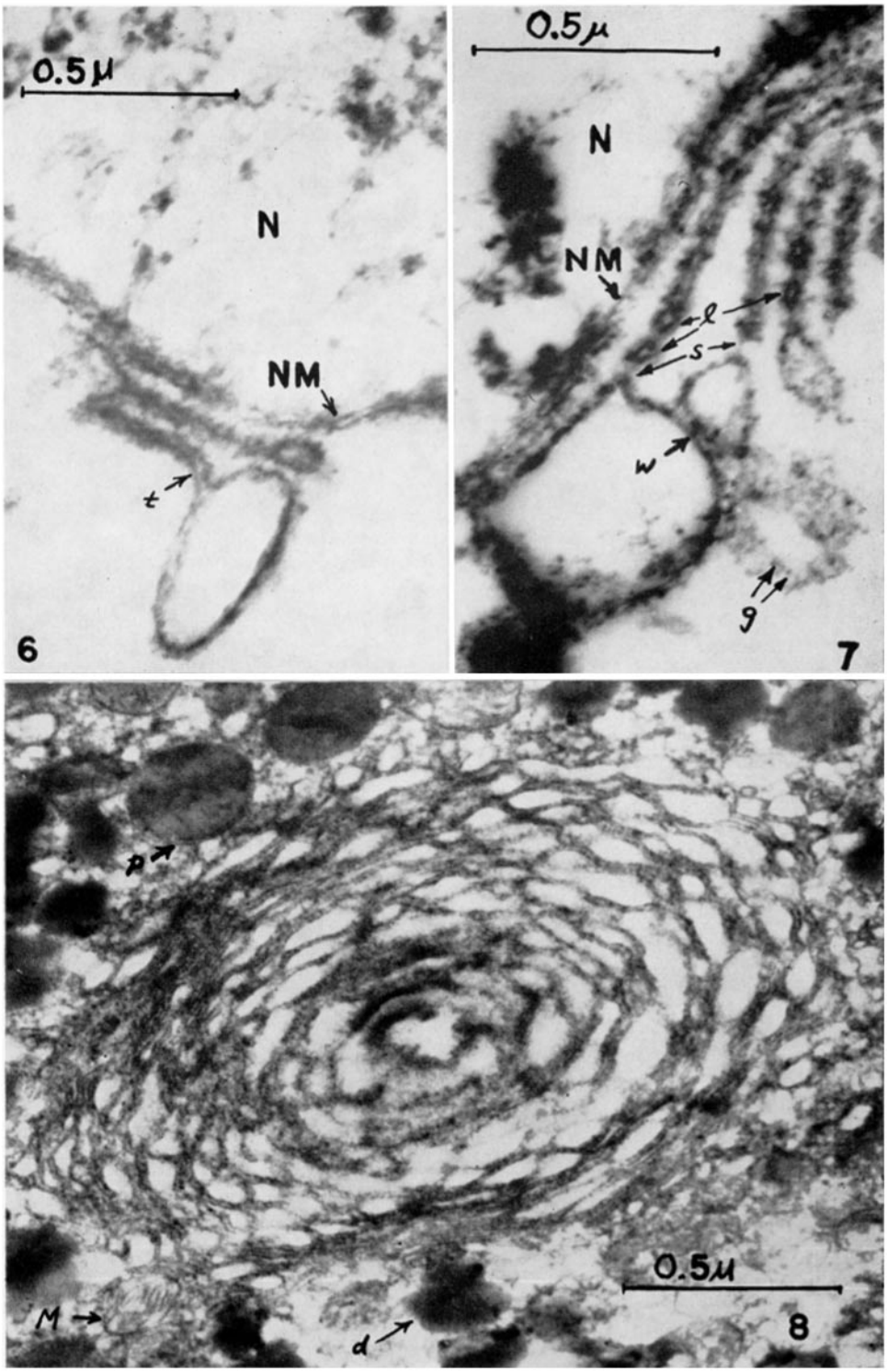


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PLATE 26

FIG. 9. This region resembles that in Fig. 8 except that the lamellae do not run completely around the region but appear to run from side to side and to terminate in blind sacs, *b*. The left hand side of the region is somewhat compressed and it is therefore difficult to make out exactly what happens to the lamellae. At the right the confluence of several single walled vacuoles appears to generate double walled structures similar to, though shorter than those running across the field. It is not possible to trace the structures throughout their length in these micrographs. Part of this field appears at higher magnification in Fig. 13.

FIG. 10. In this region from a *Spisula* oocyte the double walled lamellae can be traced around the whole field and they probably do encircle it although the details are obscured by compression of the section.

FIG. 11. Irregular double walled lamellae, similar to those in the previous figures can be seen in part of the field, whereas periodic lamellae such as seen in Figs. 6 and 7 and discussed in reference 37 can be seen in an adjacent region. Irregular continuations of the double walled lamellae back into the region of the periodic lamellae appear to line up parallel to the latter. At *O*, single walls appear to bound the clear interiors of the vesicles. For an interpretation of Figs. 3 to 11 see Text-figs. 1 and 2.

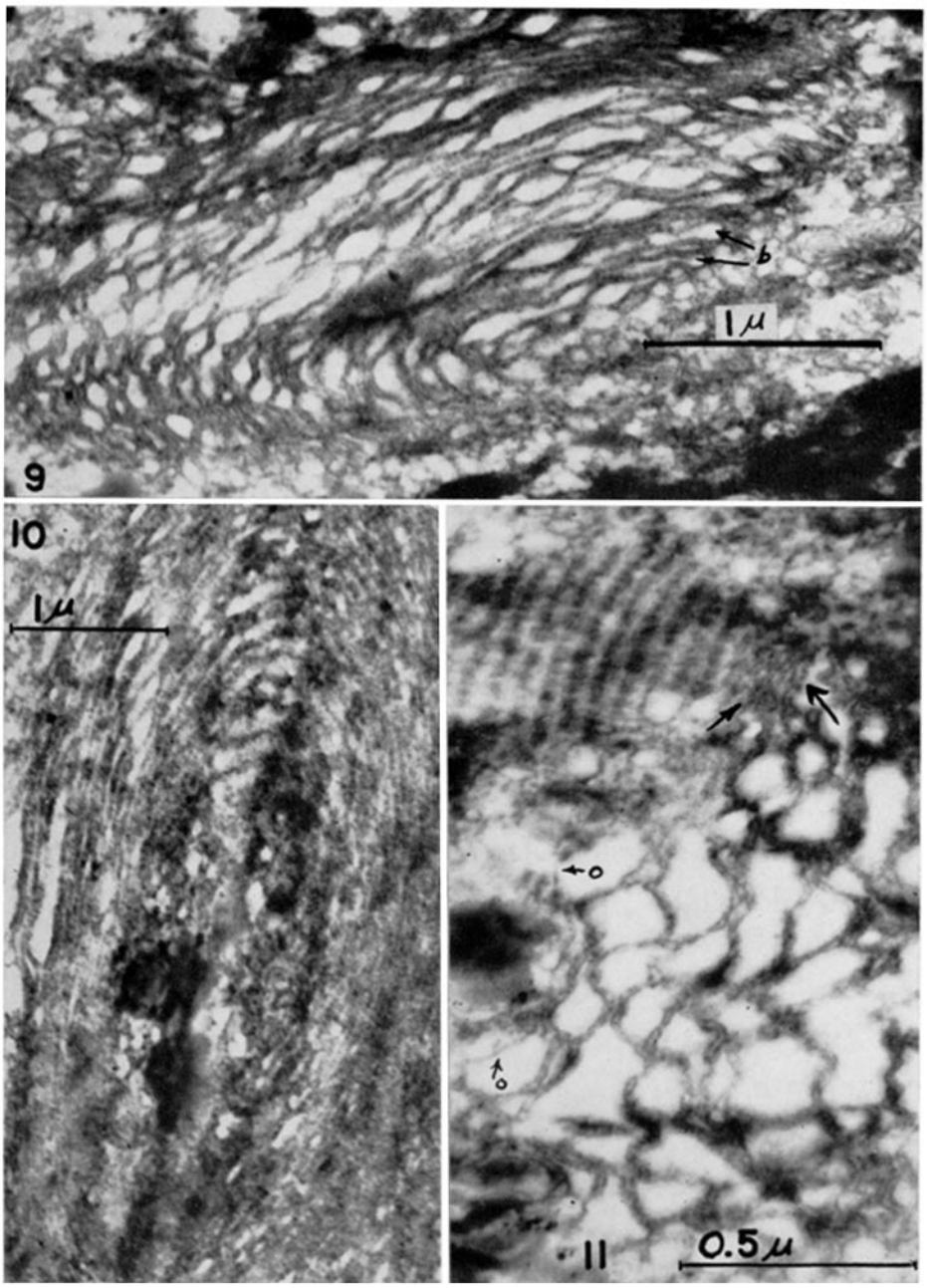

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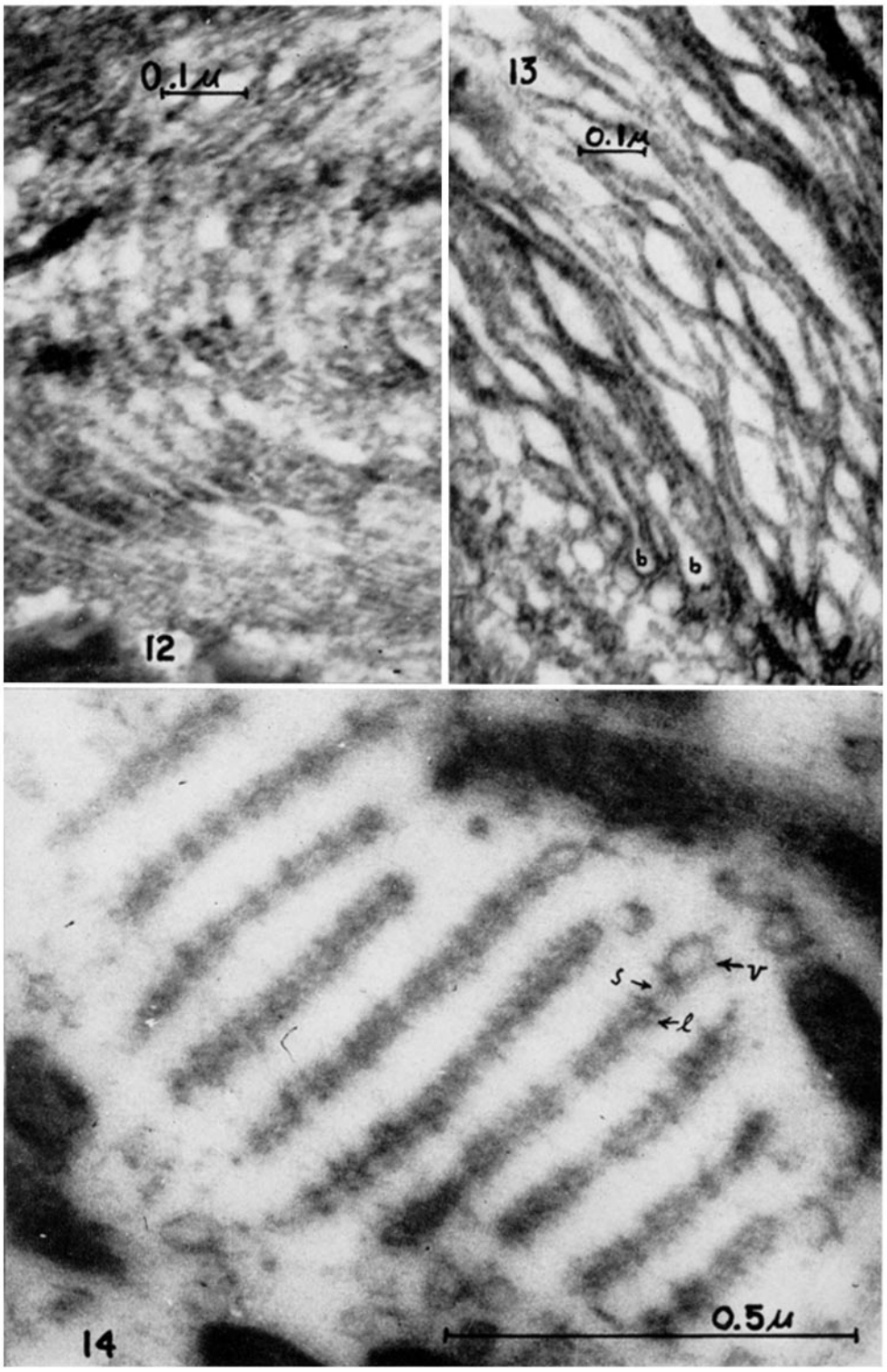
PLATE 27

FIG. 12. View at higher magnification of a region similar to that in Fig. 10. The double walled lamellae can be seen to run continuously across the field. Each such structure is composed of two parallel adjacent lines. Between these lines can be seen some evidence of granularity although the resolution is not high enough for clear viewing. This appearance probably is due to small granules attached to the single membranes or squeezed between them. This is interpreted as supporting the idea that the small granules of Palade (34) are associated with these structures.

FIG. 13. View at higher magnification of a part of Fig. 9 showing similar points to those described in Fig. 12. The smooth sides of the single membranes in both cases, face areas mostly devoid of electron-dense material. This same organization of double walled lamellae can be seen in Figs. 4, 5, 8 to 13. At *b*, can be seen a blind termination to the elongated clear space.

FIG. 14. Region from an oocyte of *Otala lactea*, showing a set of parallel, periodic lamellae. Vacuoles, *v*, can be seen adjacent to the ends of the lamellae as in Figs. 6 and 7.



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