

A CYTOLOGICAL STUDY OF THE ALBUMIN-SECRETING CELLS OF THE HEN OVIDUCT

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PLATES 105 TO 108

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INTRODUCTION

Recent work from several laboratories has resulted in the chemical and biochemical characterization of many cell components following their separation by differential centrifugation of tissue homogenates (17). The internal structures of the cell have thus been separated into essentially three general categories: nuclei, mitochondria, and "microsomes." It has become clear that while nuclear and mitochondrial fractions may be prepared which possess consistent biochemical and morphologic properties, the so called "microsomal" fraction may vary widely in both of these respects even within a single cell type depending on conditions of homogenization and the centrifugal fields employed (10).

Investigations with the albumin-secreting region of oviducts from laying hens have yielded results which show that material having chemical (ribonucleic acid distribution) and biochemical (amino acid-incorporating ability) similarities to liver "microsome" preparations sediments in centrifugal fields of about 600 *g* (as opposed to 20,000 *g* for the corresponding material from liver (7)). In the present paper, electron and light microscopic techniques have been applied in a study of the fresh intact tissue and the preparation obtained in low centrifugal fields. The results obtained further establish the identity of a constituent in the pellet obtained at low centrifugal speeds with microsome preparations obtained from liver. At the same time they further elucidate the nature of the intracellular organization of a tissue which is extremely active in protein synthesis.

These studies emphasize the fact that any attempt to characterize a specific cellular constituent of one tissue on the basis of conditions of centrifugation standardized with another is suspect unless carefully controlled by means of other techniques.

Materials and Methods

Laying hens were killed by decapitation and their oviducts quickly removed. The albumin-secreting region was obtained by cutting off the two ends at the easily observed lines of demarcation. This tissue was minced, washed, homogenized, and fractionated in sucrose as previously described (7).

Methods for Light Microscopy.—Portions of the intact albumin-secreting tissue, homogenate, and fractions obtained by centrifugation were immersed immediately in Carnoy's solution and fixed for 18 hours at 0° C. (11). Whole tissue was embedded directly in paraffin. The homogenate and cell fractions were centrifuged after fixation and then also embedded in paraffin. All paraffin specimens were cut at 5 μ on a rotary microtome and mounted on slides.

Control sections were stained with Azure A Eosin B at pH 5.5 and with Azure A at pH 5 to demonstrate basophilia. Alternate sections were incubated in a buffered pH 6.0 solution containing 10 μ g/ml. ribonuclease (11) for 1 hour at 37° before staining. These sections when compared with the untreated controls established the specific sites of ribonucleic acid.

Smears made from the homogenate were stained with a Giemsa solution to determine the percentage of fragmented cells.

Methods for Electron Microscopy.—Small pieces of the albumin-secreting tissue of the hen oviduct were fixed by immersion in chrome-osmium (pH 7.2) (6) for 1 hour. The tissues were then washed for ½ hour in running tap water, dehydrated through a graded series of alcohols to absolute alcohol, and transferred to a mixture of 1 part methyl to 9 parts *n*-butyl methacrylate monomer. Following two changes in this mixture the tissues were transferred to partially polymerized methacrylate of the same proportions and embedded at 65° C. Aliquots of the sediment from the centrifuged homogenate were resuspended and fixed for ½ hour in 1 per cent osmic acid containing 8.5 per cent sucrose. Following fixation the sediment was centrifuged, the resulting pellet washed in tap water, and embedded in 2 per cent agar. Agar blocks containing the pellet were then dehydrated and embedded in the same manner as the solid tissues.

Sections were cut on a Porter-Blum microtome (16) at 250–500 A and analyzed with an RCA model EMU 2C electron microscope using a 30 micron objective aperture.

OBSERVATIONS

The structure of the intact albumin-secreting glands has been previously investigated (4, 19). The glandular epithelium is composed of polygonal cells filled with numerous secretory droplets. We have observed throughout the cytoplasm minute basophilic bodies of the order of 1 μ . These bodies appeared to be concentrated along the periphery of the secretory droplets as if held in an elastic but taut network (Fig. 1). Following ribonuclease digestion these basophilic bodies disappeared (Fig. 2) though other cellular components remained intact. It is apparent, therefore, that these minute particles were rich in ribonucleic acid.

The washed, easily sedimentable fraction (cell debris) had the highest concentration of this ribonucleic acid-rich material, which was present as particles and clumps (Figs. 3 and 4). The remaining material in the supernatant fraction showed negligible basophilia. It is, therefore, apparent that the ribonucleic acid-containing particles of the hen oviduct were sedimented in the low centrifugal field (600 *g*) used to obtain the cell debris.

The smears of homogenate indicated the extent to which the glandular epithelial cells were fragmented (Fig. 5). Cell counts of the homogenates revealed over 90 per cent fragmentation in groups of 1,000 cells, *i.e.*, cytoplasmic dispersion and/or enucleation. Validity was, therefore, established for homogenization as an effective means of fragmentation for cells in this tissue.

Electron Microscope Studies.—Satisfactorily thin sections of the glandular cells of the hen oviduct were difficult to obtain since more than the usual amount of compression was encountered. Once usable sections were obtained, however, their examination revealed the presence of a highly organized cytoplasm in these cells. At low magnification the cytoplasm appears to consist of a two-phase system, the more electron dense component constituting the continuous phase, and the less electron dense, the discontinuous phase (Fig. 6). At higher magnification the electron dense phase can be seen to consist of well developed ergastoplasm (combination of endoplasmic reticulum (16) and small granule components of the cytoplasm (13)), a situation characteristic of the majority of cells possessing a strongly basophilic cytoplasm (Fig. 8). In some cells the relatively electron-lucent areas forming interstices in the ergastoplasm are small near the base of the cell and are larger distally (Fig. 6), while in other cells some of them are larger basally, then distally (Fig. 7). Those areas contain an amorphous precipitate which is thought to represent albumin or its precursor.¹ In most instances this precipitate has approximately the same electron density in all areas of the cell and is similar to that of the secreted material in the lumen of the gland. In these cases the large vacuoles of the Golgi complex stand out sharply because of the absence of any precipitate in them (Figs. 6 and 7). In some cells, however, the concentration of this precipitate varies greatly from one area to another (Fig. 9).

For the most part the mitochondria and the components of the Golgi complex tend to be located distally in the cells (Figs. 7 and 10). There is no evidence that either of these components is directly involved in the laying down of albumin.

Frequently thin strands of cytoplasm apparently separated from the rest of the cell can be identified in the glandular lumen (Figs. 6 and 10). These are encountered often enough to suggest the possibility that secretion in these cells is partly apocrine in character.

Examination of thin sections of pellets obtained from low speed centrifugation of homogenates shows that in addition to the whole cells, parts of cells, and free nuclei ordinarily found in such a fraction, mitochondria and fragments of ergastoplasm are present in large amounts. Many of the membranes of the ergastoplasm continue to surround precipitated material considered to represent albumin¹ (Fig. 11).

¹ For ease of discussion this material will be referred to as albuminous material in this paper.

DISCUSSION

In a previous study of the hen oviduct Hendler (7) has observed that the heavier components appeared to be the most active in protein synthesis. This material incorporated radioactive amino acids linearly from zero time and exhibited the greatest specific activity throughout the incubation, whereas the other protein fractions had a lag period before reaching a linear incorporation rate and had incorporated less radioactivity per unit weight of protein.

When, in the case of glutamic and aspartic acids, the specific activity of the amino acids in the medium was lowered, the cell debris responded with the most rapid loss of radioactivity, indicating a greater turnover in this fraction with respect to its newly incorporated amino acids. It was further established that this fraction was richest in ribonucleic acid content. Previous work had linked the ribonucleic acid-rich granules of the cell to protein synthesis (1-3, 5, 8, 9, 12, 14, 15, 18, 20, 21), and so it appeared that in the hen oviduct similar material was behaving quite differently with respect to ease of sedimentation. The present study further establishes the similarity between the ribonucleic acid-rich material in the cell debris fraction of hen oviduct with the difficultly sedimentable granules in liver microsome preparations. It appears from the electron microscope study that microsomal material in the hen oviduct remains more organized after homogenization than does the corresponding material from liver.

Analysis of the electron micrographs of sections of intact glandular cells of the hen oviduct gives evidence from which it might be inferred that protein synthesis is occurring within the ergastoplasm.² Whether the albuminous material is released into the lumen of the gland by rupture of the cell membrane (merocrine secretion) or whether the distal cytoplasm of the cell with its contained albumin, ergastoplasm, mitochondria, and Golgi complex is pinched off into the lumen (apocrine secretion) has not been determined, although some evidence for the latter activity has been presented above. In any case, study of the normally functioning glandular cells of the hen oviduct provides evidence in support of the earlier view of Porter and Blum (16) and Weiss (20), extended recently by Palade (14), that the ergastoplasm may be directly involved in protein synthesis. There is no evidence in the present material that the Golgi complex is so involved.

The electron micrographs of sections of this material show several large

² It has been determined that thoroughly washed cell debris (obtained after incubating intact cells with a radioactive amino acid) will release into a buffer, upon subsequent incubation, proteins having a higher specific radioactivity than is normally obtained in the supernatant protein fraction from whole cells for a like period of incubation, and higher also than the original cell debris. This and other studies are taken to support the idea that the cell debris material may be directly involved in the synthesis of the soluble proteins of the hen oviduct (Hendler, unpublished material).

and many small vesicles of ergastoplasm, some containing albuminous material, some not. There are several possible reasons why so much of the ergastoplasm appears in this low speed fraction. One is that the large amount of albumin present in the original homogenate protects the membrane system from extensive fragmentation. Another is that because of the presence of large amounts of albuminous material within the membrane system of the ergastoplasm, the opportunity exists for protein to remain trapped within ergastoplasmic vesicles during and after homogenization. This situation could result in much of the ergastoplasm appearing in the sediment after relatively low speed centrifugation. Whatever the explanation, most of the ergastoplasm is present in this fraction, and such a finding is consistent with the observation that this easily sedimentable fraction is most active in the process of amino acid incorporation. Perhaps the fractionation characteristics of the hen oviduct, which at present appear to be atypical, are related to its more or less unique function of large scale protein synthesis and secretion.

SUMMARY

1. The electron and light microscope have been employed in a cytological study of the albumin-secreting cells of the hen oviduct and of fractions of this tissue obtained after homogenization and differential centrifugation.
2. These studies confirm the observation that in this tissue material corresponding to liver "microsomes" in amino acid-incorporating ability and ribonucleic acid content sediments in relatively low centrifugal fields.
3. The electron microscope studies suggest that the protein secretion of the gland is formed in intimate association with the ergastoplasm.

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EXPLANATION OF PLATES

PLATE 105

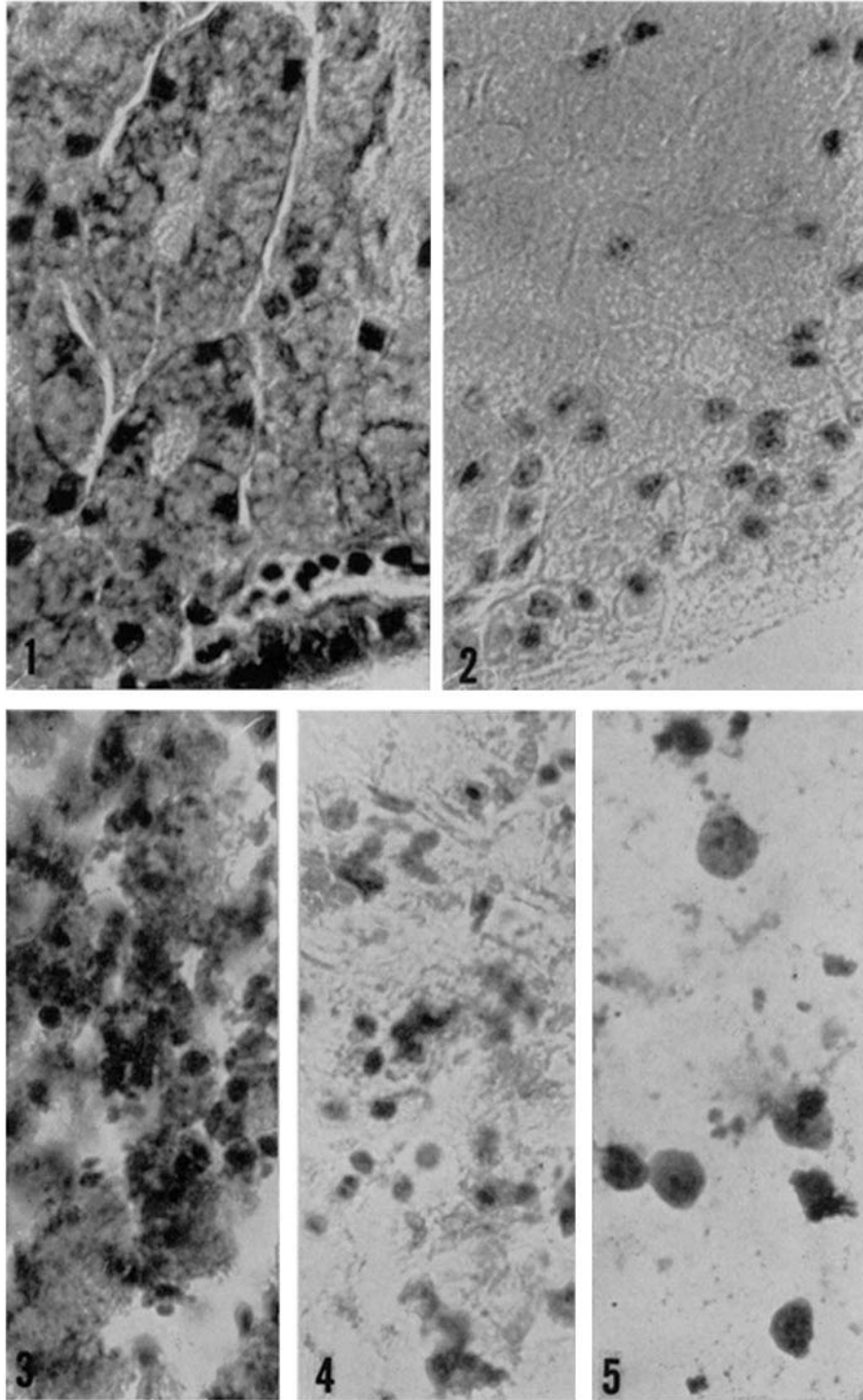
FIG. 1. Section of glands of albumin-secreting portion of hen oviduct. Note reticular pattern of basophilic granules. Azure A pH 5.0. \times 1,930.

FIG. 2. Consecutive section to Fig. 1 following ribonuclease digestion. Azure A pH 5.0. \times 1,930.

FIG. 3. Cell debris fraction containing nuclear elements and numerous minute basophilic particles. Azure A pH 5.0. \times 2,100.

FIG. 4. Consecutive section to Fig. 3 following ribonuclease digestion. Azure A pH 5.0. \times 2,100.

FIG. 5. Smear of homogenate showing extent of cell fragmentation. Giemsa stain. \times 1,650.

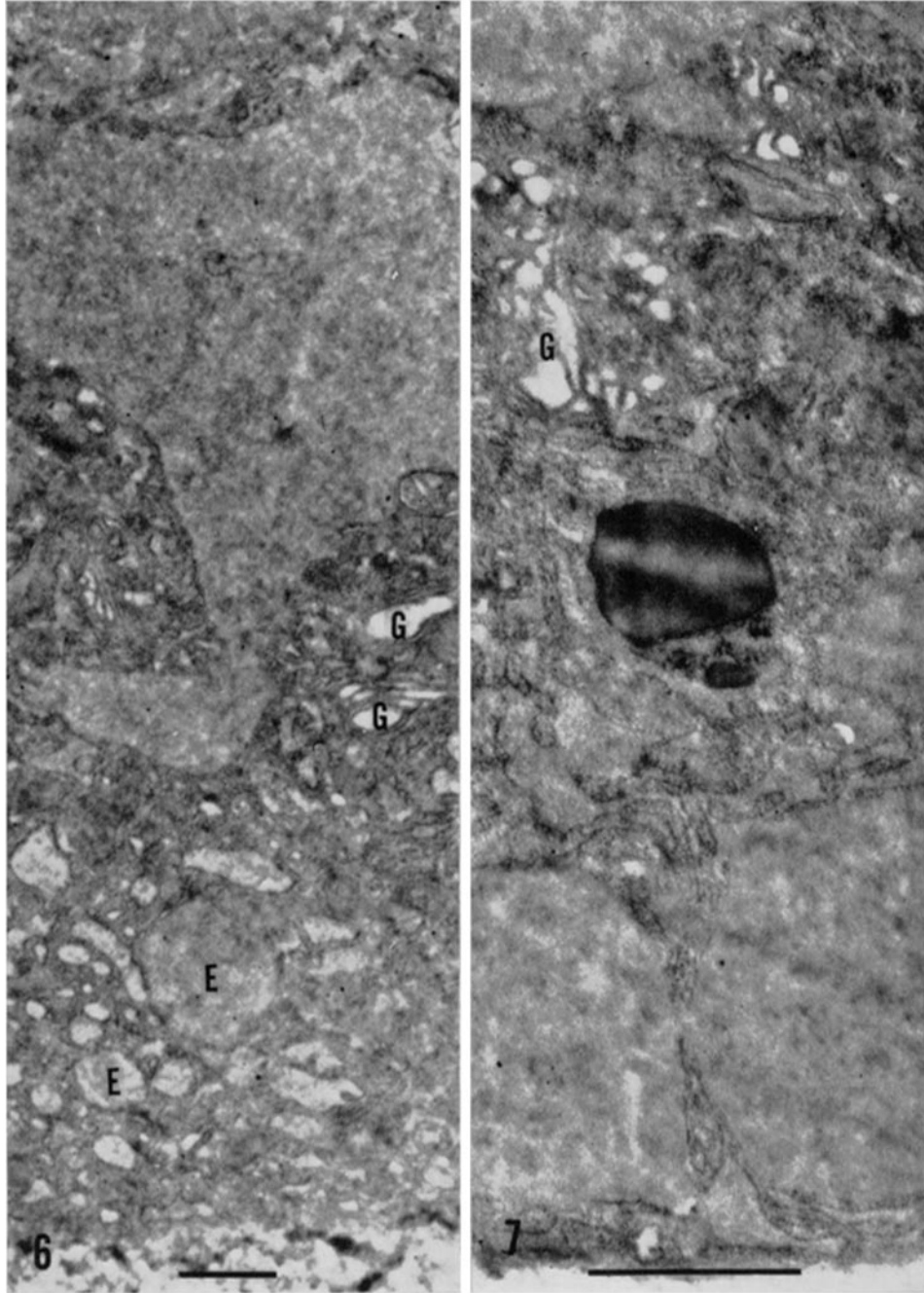


(Hendler, Dalton, and Glenner: Albumin-secreting cells of hen oviduct)

PLATE 106

FIG. 6. Low power electron micrograph of a vertical section through the cytoplasm of a glandular cell of the hen oviduct. At this magnification, details of fine structure are not evident but vesicles (*E*) containing albuminous material can be seen to be small basally and larger distally. At the right of center are a group of Golgi vacuoles (*G*) identifiable by their electron lucency. There is no distinct boundary at the distal border of the cell. (The dark line in this and the following figures represents 1 micron.) \times approximately 13,000.

FIG. 7. Electron micrograph of a vertical section through the cytoplasm of another glandular cell of the hen oviduct. In this case large vesicles containing albuminous material are visible near the base of the cell. Groups of Golgi vacuoles are present near the distal border (*G*). What is considered to represent a lipide droplet is present in the center of the figure. \times approximately 29,000.

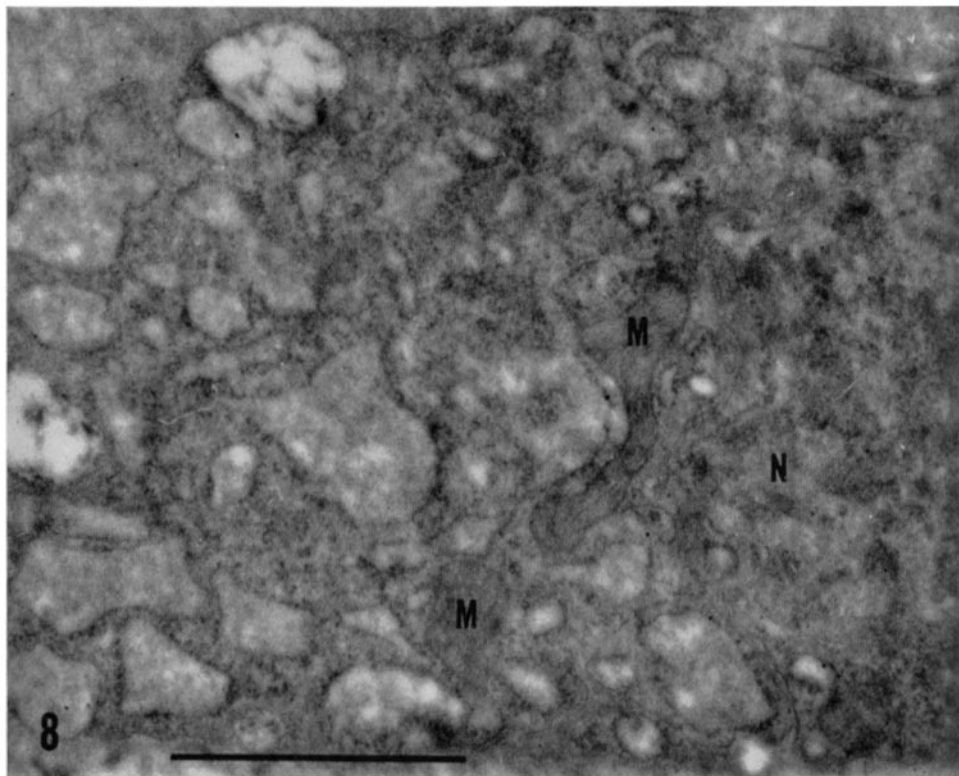
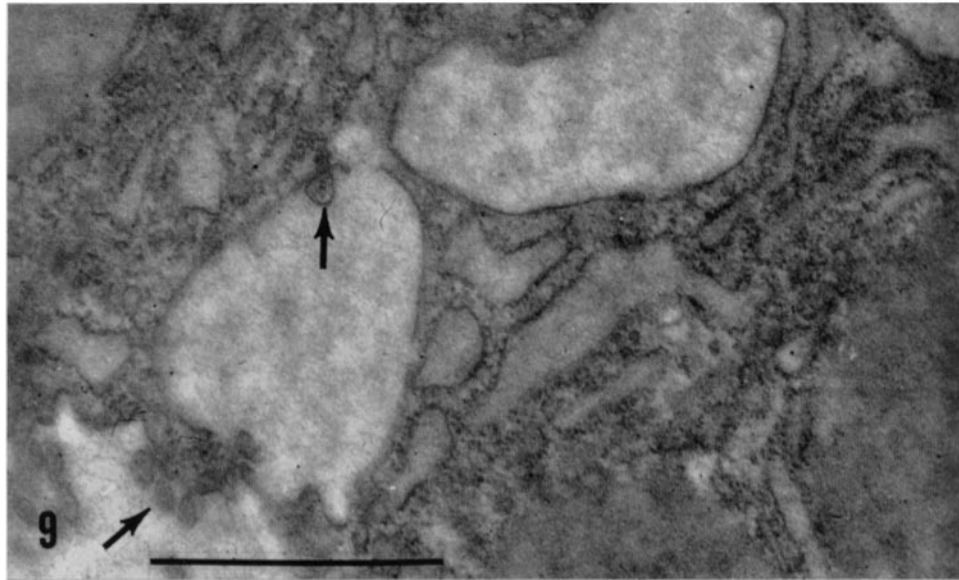


(Hendler, Dalton, and Glenner: Albumin-secreting cells of hen oviduct)

PLATE 107

FIG. 8. An electron micrograph showing detail of the cytoplasm. At this magnification it is clear that albuminous material is present within the system of ergastoplasmic membranes. The small ribonucleic acid-rich granules tend to outline the membrane system. Mitochondria (*M*) and a portion of the cell nucleus (*N*) are visible. approximately $\times 39,000$.

FIG. 9. An electron micrograph showing detail in the cytoplasm of another cell in which the electron density of the spaces within the membrane system of the ergastoplasm appears to vary greatly. It is possible that the membranes forming the boundary of the more electron lucent spaces are continuous with and part of the plasma membrane of the cell. They differ from the usual ergastoplasmic membranes in that the small ribonucleic acid-rich granules do not exhibit a tendency to line up along their inner boundaries. Further, there is evidence of small bud-like projections of the membrane into these spaces (arrows). \times approximately 39,000.

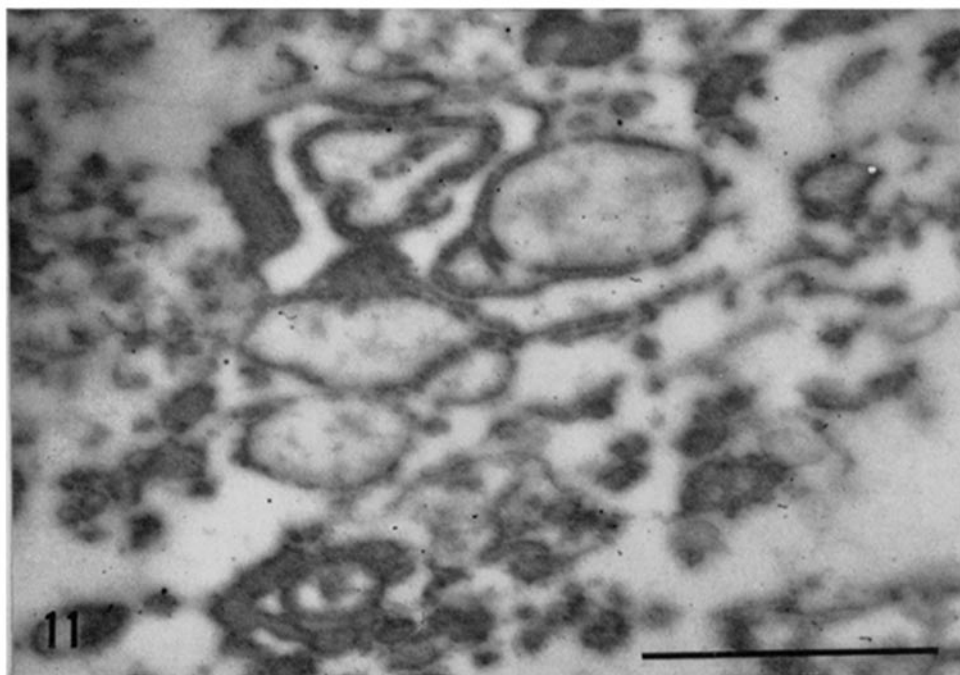
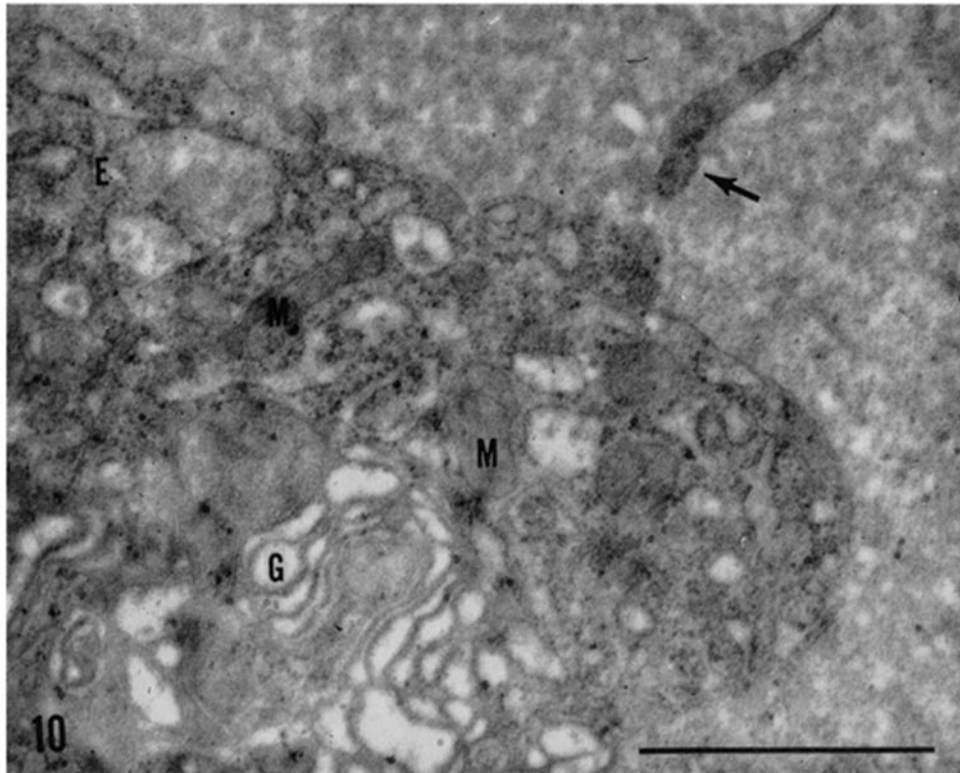


(Hendler, Dalton, and Glenner: Albumin-secreting cells of hen oviduct)

PLATE 108

FIG. 10. An electron micrograph of a part of the distal portion of a glandular cell showing evidence of the apocrine type of secretion. The cell boundary extends diagonally from the upper left to the lower right of the figure. The glandular lumen containing albumin appears at the top and right. A small strand of cytoplasm is apparently free in the lumen (arrow). The fine structure of the Golgi complex with its electron-lucent vacuoles (*G*) is apparent. Details of the ergastoplasm (*E*) and mitochondria (*M*) are also evident. \times approximately 39,000.

FIG. 11. An electron micrograph of a section of sediment obtained from centrifuging hen oviduct homogenate at 600 *g*. Ergastoplasmic membranes which appear to be intact surround spaces varying considerably in size. The electron density of the material in these spaces varies considerably also, with less density in the larger spaces. The small granules bordering the membranes are not as sharply outlined as those in intact cells. In any case, it is clear from this micrograph, which is typical of those obtained, that the components of the so called "microsome" fraction have been sedimented in this low centrifugal field. \times approximately 39,000.



(Hendler, Dalton, and Glenner: Albumin-secreting cells of hen oviduct)