

**Electron Microscopic Evidence Suggesting Secretory Granule Formation within the Golgi Apparatus.\*** BY MARILYN G. FARQUHAR AND S. ROBERT WELLINGS. (*From the Department of Pathology, University of California School of Medicine, San Francisco.*)†

Theories concerning the origin of secretory granules are diverse (1). Granules have been said to originate from nuclei, chromidia, mitochondria or from the Golgi apparatus (substance, body, etc.). The site of secretory granule formation within the cell remains controversial in spite of the large number of cytologic studies carried out by electron microscopy during the past several years. As a result of studies utilizing the electron microscope, it has been suggested that granules may be derived by: budding from the endoplasmic reticulum or ergastoplasm (2); budding or fragmentation of mitochondria (3); or conversion of Golgi material (4). Studies in this laboratory on acidophils of the adenohypophysis (5) and acinar cells of the pancreas (6) suggest that secretory granules may be formed within the Golgi apparatus in these two cell types.

The nature and significance of the Golgi apparatus has aroused considerable controversy during the last 80 years. Dalton and Felix (7, 8) and, following them, a number of workers (4, 9, 10) showed by electron microscopy that the Golgi apparatus is a characteristic cytoplasmic constituent with three principal components: (1) small granules or vesicles (*ca.* 400 Å), (2) vacuoles of variable size with little or no internal electron density, and (3) paired lamellae with electron dense walls (*ca.* 70 Å) separated by a clear area (*ca.* 70 Å). (It is generally considered that the lamellae represent

sections through flattened vesicles.) The present study describes the finding of secretory granules within components of the Golgi apparatus of pituitary acidophils and pancreatic acinar cells.

#### *Materials and Methods*

Thin sections of osmium tetroxide-fixed, methacrylate-embedded pituitary and pancreatic tissues were prepared for electron microscopy by methods described in detail elsewhere (11). Pituitaries from normal rats as well as those from rats subjected to gonadectomy (12), thyroidectomy (9), testosterone propionate treatment (5), and FSH treatment (5, 13) were studied. The pancreatic tissue was from normal mice and from mice that had been fasting for 24 hours and subsequently were given a single injection each of 0.2 mg. of a 0.3 per cent solution of pilocarpine hydrochloride. Animals were sacrificed at intervals of 15 minutes, 30 minutes, 1 hour, and 2 hours following the injection.

#### RESULTS

*Pituitary Acidophils.*—In acidophils of the normal young rat pituitary, secretory granules are readily identified as ovoid dense bodies with a maximal diameter, in the predominating type of acidophil, of approximately 350  $\mu$  (14, 12). The Golgi apparatus is located between the nucleus and the major concentration of the endoplasmic reticulum. The latter is found in abundance in the peripheral cytoplasm near the cell membrane and is present in the form of parallel rows of cisternae (15) with many associated small dense particles.

In the Golgi region of acidophils we have repeatedly observed secretory granules which are virtually surrounded by Golgi membranes (Figs. 1 to 3).

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Sometimes two membranes can be traced running parallel for a short distance before they bulge to encircle a granule. In other instances the granule is simply completely enclosed by a membrane. It seems clear that the membranes surrounding secretory granules are those of the Golgi complex, for (1) membrane-enclosed granules are seen only in the Golgi area and (2) the membranes encircling the secretory granules are free of the small granular component (16). (It has been pointed out by a number of investigators (4, 7, 8, 10, 17) that the paired membranes or lamellae of the Golgi body are always free of the small ribonucleoprotein particles that are frequently associated with the membrane surfaces of the endoplasmic reticulum (16).) Fig. 2 shows the similarity (in density and thickness) of the membranes surrounding secretory granules to those of the Golgi lamellae.

These findings suggest to us that in the rat pituitary, acidophil granules may be formed within the vesicular or lamellar components of the Golgi complex. Supporting this concept is the fact that in conditions where rapid synthesis of granules is expected, *e.g.* during the rapid growth period of the young animal (5), we have noted that the concentration of secretory granules is greatest in the Golgi area. On the other hand, in circumstances where the acidophils are considered to be primarily in a storage phase, with synthesis and release of granules at a minimum, *e.g.* following thyroidectomy (9), secretory granules are concentrated near the cell membrane. It appears to us that granules may be formed within the Golgi lamellae and when they attain maximum size move out into the peripheral cytoplasm. The mature granules may then be stored for variable periods before they are secreted by extrusion into perisinusoidal spaces (18).

*Pancreatic Acinar Cells.*—Observations on pancreatic acinar tissue from normal mice and particularly on tissue from mice injected with pilocarpine have provided evidence suggesting that zymogen may be formed within the Golgi apparatus. We have repeatedly observed material resembling zymogen in density and composition within the otherwise virtually empty appearing Golgi vacuoles of acinar cells (Fig. 4). The intravacuolar zymogen material is invariably found in the apical cytoplasm and is clearly within Golgi vacuoles. Such material is much more commonly seen in animals sacrificed 2 hours after pilocarpine injection than in normal animals. Preliminary observations (6) indicate that acinar cells from pilocarpine-injected animals show alterations of cytologic structure involving all of the common cytoplasmic constituents such as the mitochondria, endoplasmic reticulum, and Golgi apparatus. The Golgi bodies are greatly enlarged and practically every cell shows some zymogen material within Golgi vacuoles. Presumably these cells are rapidly synthesizing and releasing secretory products as a result of pilocarpine injection (1). The finding of zymogen-like material within Golgi vacuoles under these conditions indicates that the Golgi complex may be a primary site of secretory granule formation in pancreatic acinar cells.

#### DISCUSSION

It is generally agreed from studies by light microscopy that there is a close association between the Golgi apparatus and secretory processes. In many tissues a hypertrophy of the Golgi apparatus is known to accompany hypersecretion whereas atrophy is associated with hyposecretion (1). It was Cajal (19) who first suggested that secretory granules may be formed within the Golgi apparatus.

Kirkman and Severinghaus (20) proposed the idea that the Golgi apparatus may act as a "condensation membrane for the concentration in drops or granules of products elaborated in other locations that diffuse through the cytoplasm."

The status of the Golgi apparatus should now be reviewed in terms of newer concepts of cytology provided by electron microscopy. A number of workers utilizing the electron microscope have noticed the close association of Golgi components and secretory products (14, 3, 4, 12, 8-10). Sjöstrand and Hanzon (4) and Haguenau and Bernhard (10) have suggested that Golgi components may be converted into secretory products. Dalton and Felix (21) believe that the Golgi complex may be involved in the removal of water from maturing secretory substances. Our observations suggest that one function of the Golgi apparatus both in pituitary acidophils and in pancreatic acinar cells may be the formation of secretory granules. The possibility that different or modified mechanisms of granule formation may occur in other types of cells cannot be excluded. Furthermore, it even seems conceivable to us that different mechanisms of granule formation may occur in pituitary and pancreatic cells under varying physiologic and pathologic conditions. It must also be kept in mind that secretory granules may represent just one form—perhaps just the storage form—of the cell's secretory products.

We believe that the Golgi apparatus could be interpreted as functioning in the building up of secretory products from smaller particles which may be manufactured and transported by other cytoplasmic constituents such as the endoplasmic reticulum together with the ribonucleoprotein particles. In this regard, Palade (22) has suggested that the endoplasmic reticulum may function as

an intracytoplasmic transportation system. Rinehart and Farquhar (14) suggested that elements of the Golgi apparatus may communicate with the endoplasmic reticulum, and others (10, 22) have noted that the endoplasmic reticulum and Golgi components seem to merge.<sup>1</sup>

#### SUMMARY AND CONCLUSIONS

Secretory granules have been seen within components of the Golgi bodies of rat pituitary acidophils and mouse pancreatic acinar cells. The fact that secretory granules are much more frequently encountered within Golgi components under conditions of increased secretory activity suggests that granule formation may occur within the Golgi apparatus in these two types of cells.

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<sup>1</sup>In a paper which has appeared quite recently Palade (23) described the finding of granules (smaller than zymogen granules) within the cisternae of the endoplasmic reticulum of exocrine cells from guinea pig pancreas. He did not see such "intracisternal granules" in rat pancreas. Likewise we have not seen granules within the endoplasmic reticulum of exocrine cells from mouse pancreas.

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## EXPLANATION OF PLATES

## PLATE 101

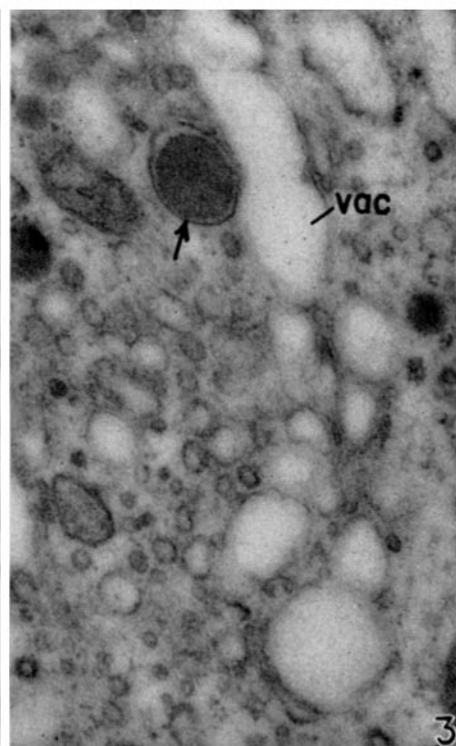
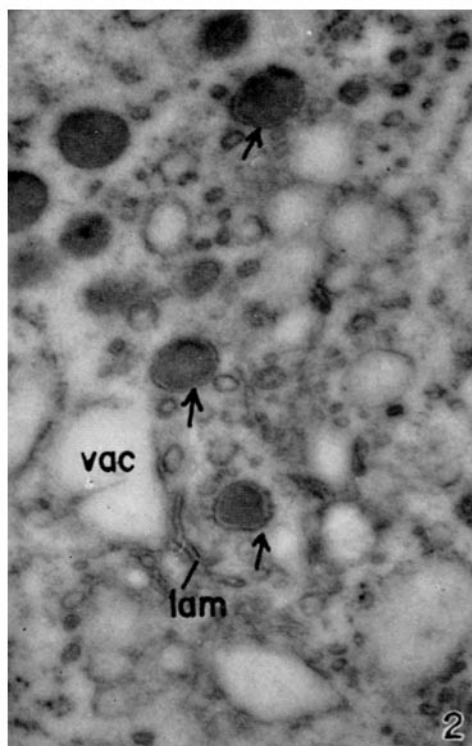
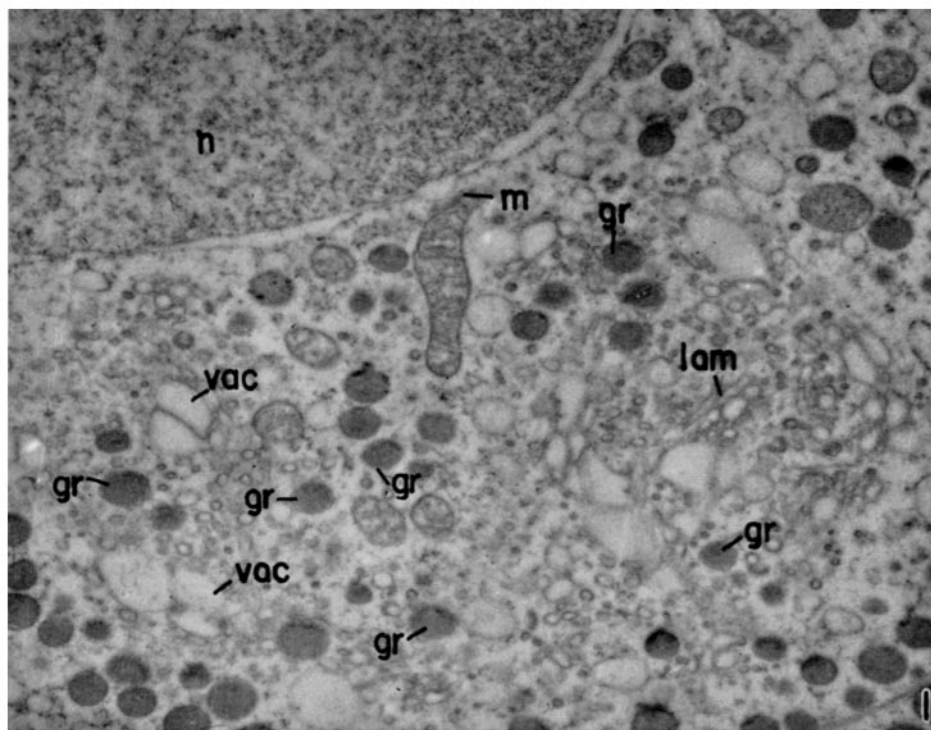
Fields from acidophils of the normal young rat adenohypophysis.

FIG. 1. Area of an acidophil showing a segment of the nucleus (*n*) above. The cytoplasm below contains mitochondria (*m*) and the Golgi apparatus. Several groups of large, empty appearing Golgi vacuoles (*vac*) and the closely associated, paired membranes or Golgi lamellae (*lam*) are present.

In the Golgi region a number of secretory granules (*gr*) are enclosed by membranes believed to be those of Golgi lamellae or vesicles. The secretory granules located more peripherally in the cell (*i.e.* lower left) are not enclosed by membranes.  $\times 20,000$ .

FIG. 2. Golgi area showing secretory granules enclosed by Golgi membranes (arrows). The rest of the field is occupied by the lamellar (*lam*), vacuolar (*vac*), and vesicular elements of the Golgi complex (*G*). It can be seen that the membranes enclosing secretory granules are similar in density and thickness to the membranes of the Golgi lamellae.  $\times 36,000$ .

FIG. 3. Secretory granule (arrow) surrounded by Golgi membrane adjacent to Golgi vacuoles (*vac*).  $\times 40,000$ .

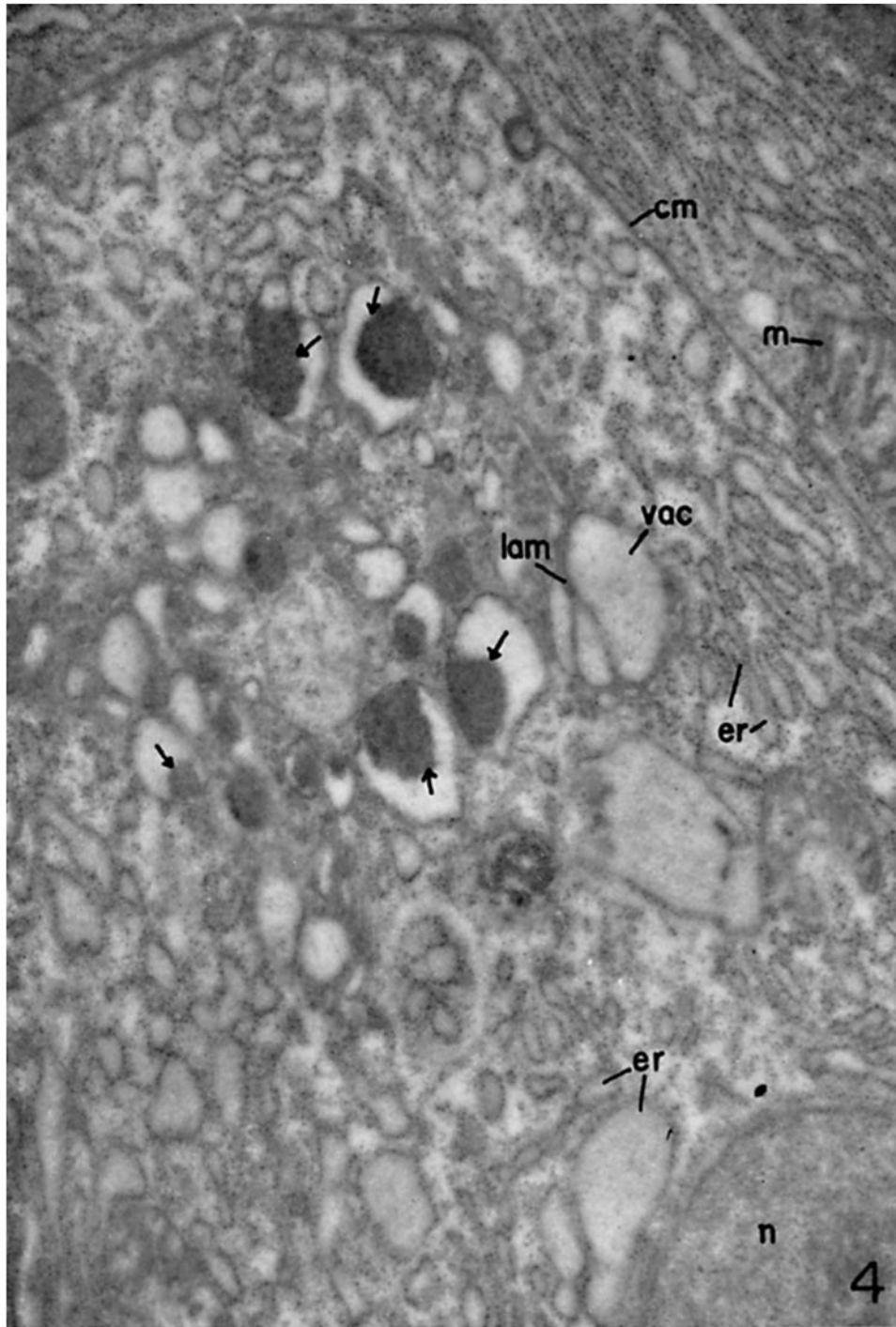


(Farquhar and Wellings: Secretory granule formation in Golgi apparatus)

PLATE 102

FIG. 4. Portion of an acinar cell from the pancreas of a mouse sacrificed 2 hours after pilocarpine injection. Part of the nucleus (*n*) is present below and a segment of the cell membrane (*cm*) is visible above. The cytoplasm between the nucleus and cell membrane contains the Golgi apparatus with lamellae (*lam*), vacuoles (*vac*), and granules (vesicles). Material resembling zymogen in density and composition can be seen within many of the Golgi vacuoles (arrows).

Surrounding the Golgi components are circular and longitudinal profiles of the endoplasmic reticulum (*er*). Numerous small ribonucleoprotein particles (16) are aggregated on the membrane surfaces of the endoplasmic reticulum. Golgi vacuoles may be distinguished from the endoplasmic reticulum by their lower internal density and relative lack of aggregated ribonucleoprotein particles on the limiting membrane.  $\times 20,000$ .



(Farquhar and Wellings: Secretory granule formation in Golgi apparatus)