Acta Microscópica, Vol. 1, Nº 1, May 1992, pp 74-86

The 3-D architecture of the Golgi Complex of chicken pituitary cells

Susan Tai and Ralph M. Albrecht,*
Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas,
Universidad de Oriente, Cumaná, Venezuela.
* School of Veterinary Science, University of Wisconsin- Madison. U.S.A.

ABSTRACT

Correlative microscope studies, combining conventional transmission electron microscopy (CTEM), high voltage electron microscopy (HV-TEM), high-resolution scanning electron microscopy (HR-SEM) and laser scanning confocal microscopy, reveal a recognizable polarity of the Golgi complex of chicken pituitary cells and the granule formation associated with golgi structure.

In the CTEM micrographs, it has been noted that the structure of the Golgi complex varies from cell to cell. 2-D images of thin sections of the pituitary cell provide only partial information about the activity in the Golgi complex. Stereomicrographs of HV-TEM of thick pituitary sections and 3-D images of HR-SEM of cryo-fractured cells allow observation of the 3-D configuration of the Golgi complex. Confocal microscopy and computerized image reconstruction system provide a series of scanned images which demonstrate the continuous structure of Golgi complex throughout the cell. Thus the Golgi complex in the chicken pituitary cells appears to be a single structure with all cisternae interconnected.

KEY WORDS:
Golgi complex, Transmission Electon Microscopy, High Resolution Scanning Electron Microscopy, Confocal Microscopy.

INTRODUCTION

The Golgi complex is an very important element in glandular cells. The function of glandular cells is to synthesize and secrete their specific secretory products, with the Golgi apparatus being responsible for the final processing of newly synthesized protein [8,14,25,26,34,35,36]. Through a combination of morphological and biochemical methods [e.g. cell fraction analysis [1,2,3,15,16,22,23,30], biochemical and histochimical methods [9,10,31,32], LM and TEM autoradiography [13,29] and immuno-labeling techniques [6,17,18,19,20,27,33] it has become apparent that intracellular protein synthesis is a sequential multistep process. Protein molecules destined for export are synthesized exclusively on bound polyribosomes attached to the membrane of the rough endoplasmic reticulum (rER). Those secretory materials are then released from the rER, incorporated into transport vesicles, and merged into the cisternae of the cis-face of the Golgi complex [13]. Several investigators [12,13,24], using TEM, have demonstrated clearly that in most glandular cells, concentration and packaging of secretory products occurs in the trans cisternae of the Golgi complex. However, there is evidence that in a few cell types [8,25,26], these events occur in special condensing vacuoles which are separated from the Golgi cisternae.

Cytochemical and immunological technique have suggested specific orientation and sidedness of active sites for different enzymes [7,11,38,39,40,41]. From these reports it is clear that functional specialization and differentiation occurs across the Golgi cisternae. Farquhar [12,13] has described membrane traffic and many of the functions of the Golgi complex, including the demonstration of structural features of the Golgi complex in animal and plant cells, compositional differences in Golgi cisternae, and methods for studying Golgi structure and it's contents. In

addition, packaging of secretory materials, glycosylation of glycoproteins, proteolytic processing of proproteins, segregation of multiple secretory products in the Golgi, and biogenesis of membrane components related to the Golgi has been described.

Historically, the Golgi complex has been described as a single structure when viewed by the light microscope [4,5]. However, Novikoff et al [31] and Rambourg et al [40] showed that Golgi cisternae are extensively interconnected and suggested that each Golgi complex consists of a single set of stacked cisternae. Using histochemical methods, several investigators [12,13] demonstrated that secretory proteins are formed in all cisternae of the Golgi stack, although large secretory granules are only associated with the trans-most cisternae. This has been confirmed by Hashimoto et al [21] who applied anti-prolactin antiserum to ultrathin frozen sections to show that immuno-labeling was present on granules inside the condensing vacuoles or cisternae at the trans-most Golgi region (also reported by Farquhar et al, 1981).

In a previous morphological study of Golgi cisternae in chicken pituitary cells [42], HR-SEM 3-D imaging revealed that secretory granules developed from the inner membrane of the Golgi cisternae. Granules of different sizes were observed and were thought to represent different stages in granule formation. Using TEM, trafficing between the rER and the Golgi via transport vesicles and granule formation inside the Golgi region were observed. Unfortunately the polarized characteristics of the cis-trans arrangement of Golgi cisternae can not be easily discerned in most ultrathin (70 nm) sections. In order to elucidate the structural orientation of the Golgi complex in the pituitary cell and its function in sorting of hormone granules, we initiated a correlative study using Low-and High-Voltage TEM, HR-SEM and Confocal Microscopy. In this paper, we report the ultrastructural feature of Golgi cisternae in chicken pituitary cells and suggest a role of cisternae in development of secretory hormone granules.

MATERIALS AND METHODS

1. MATERIALS

18 chickens (*Gallus domesticus*) of age between 17 to 20 weeks of age were used. 4 for Conventional TEM, 4 for High-voltage TEM, 8 for HR-SEM and 2 for Confocal Microscope study. Chicken pituitaries were dissected following decapitation. They were then prepared for different microscope studies.

2. SAMPLE PREPARATION

A. For Conventional TEM and High Voltage TEM. Freshly excised pituitaries were prefixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 3h at 4 °C and postfixed in 1% OsO4 in the same buffer for 1h at 4 °C. After dehydration in graded alcohol, samples were infiltrated with propylene oxide and Epon (1:1 ratio) and subsequently embedded in Epon.
For conventional TEM, ultrathin sections (70nm) were used. For high Voltage TEM, 1 μm thick sections were used. The TEM (Hitachi H-600 installed in the Universidad de Oriente) was oprated at 100 KV accelerating voltage. The High Voltage TEM (HVEM, AEI, Mark II Installed in IMR, University of Wisconsin - Madison) was operated at 900 KV accelerating voltage.

B. For High Resolution SEM
Sample preparation for HR-SEM followed the procedure described by Tai [42]. Chicken pituitary glands were fixed in 0,5% glutaraldhyde in 0.2M Phosphate Buffer (pH=7,2) for 1/2 h, rinsed in the same buffer, post-fixed in 0.5% OsO4 for another 1/2h. After have been rinsed throughly, specimens were passed through 25% and 50% dimethyl sulfoxide (DMSO) solution for 30 minutes each. Specimens were lyed on a piece of styrofoam box and pre-chilled with liquid nitrogen, and then fractured logitudinally with a dissection knife and hammer. The fractured pieces were immediately placed in 50% DMSO solution and thawed at room temperature. Specimens were well-rinsed with buffer and post-fixed again in 1% OsO4 for 1h. They were then left in 0.1% OsO4 for 72h at 20°C, and fixed again in 1% OsO4 for 1h; treated with 2% tannic acid overnight; and once more in 1% OsO4 for 1h.

After dehydratation in graded alcohol, dried in a critical point dryer (Polaron), specimens were finally coated with platinum (aprox. 2nm) in vacuum evaporator (Polaron 6000) and observed in a FE-SEM Hitachi H-900.

C. For Confocal Microscopy

C-1. For studying the distribution of hormonal granules associated with Golgi cisternae, pituitary samples were freshly dissected out and frozen immediately in liquid N_2. Sections of 10 µm were obtained with a cryomicrotome (Reichert-Jung) and kept in a freezer at -20°C until labeling.

Frozen sections thawed by immersion in a drop of phosphate buffered saline (PBS) at 5°C for 5 min., then transfered to 1% Bovine albumin (BSA) in PBS for 5 min. followed by immersion in 2% goat serum for 5 min. Incubation with primary antibody was with ant-chicken Growth Hormone (GH) antiserum diluted 1 to 50,000 in BSA at 5 °C for a period of 20h. Specimens were then washed extensively in BSA for 30 min., at 5 °C, then incubated 30 min in FITC conjugated goat anti-rabbit antisera diluted 1 to 80 in BSA.

C-2.- For studying the Golgi structure was followed the method of Pagano et al [37], a ten µm thick frozen sections were thawed and incubated in NBD hexanoic ceramide (Molecular Probe N-1154), 1 µM in 0.68 mg BSA per ml of magnesium free Puck's saline (HCMF), for 30 min. at 4 °C. Back-exchanged in 3.4 mg BSA per ml of HCMF for 120 min at 37°C. Specimens were mounted with a mixed solution of glycerol and PBS (1:1) plus 0.1M p-phenylenediamine.

Fluorescently labeled specimens were imaged with a Bio-Rad, MRC-600 Laser sharp Confocal Microscope equipped with a Kr-Ar Laser A Nikon 60X NA 1.4 oil immersion planapo lens was used. Series of optical sections collected on the Confocal Microscope were reconstructed using Voxel View Program (Vital Images, Fairfield, CA) installed on a Sillicon Graphics Workstation.

RESULTS

1.- Conventional TEM observation

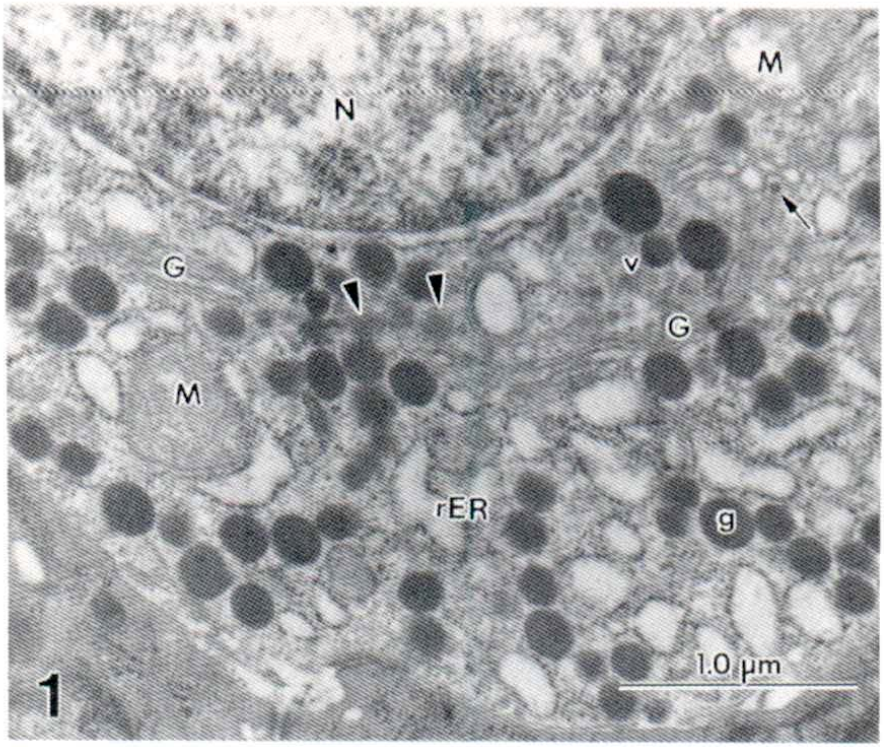
TEM observation of thin sections of pituitary tissue (Fig.1) shows that Golgi cisternae are located in the perinuclear region of the cell. They are readily distinquished from other cytoplasmic structures by their stacks of smooth surfaced cisternae. In some cells (Fig.1), the cisternae are arranged in parallel and are slightly curved at both ends. Abundant small vesicles are seen in the center of the cisternae, whereas mature granules are more prevalent at the ends of the dilated cisternae. Fig.1 also shows a small developing granule in one of the compartments of the cisternae.

In TEM micrographs, it has been noted that the structure of the Golgi complex varies from cell to cell. Fig. 2 shows two Golgi stacks which are tightly surrounded by abundant rER. One Golgi stack exhibitits regular features and some 4-5 cisternae. Granule formation is present at the ends of the cisternae, the other stack appears to be sectioned hexagonically. Transpot vesicles are seen along one side of the Golgi stack and mature granules in the middle of cisternae. Similar features are observed in other pituitary cells. For example, the cell in Fig.3 reveals at least three Golgi complexes. The one on the right side is very extensive with 4-5 cisternae in parallel arrangement. The other two Golgi are less well revealed in micrograph. One has a curved shape whereas the other is more irregular. Developing granules are seen in all three Golgi systems.

A structure which appears to be derived from an expanded cisternae is shown in Fig. 4. Many immature and mature granules are present inside this structure. This Golgi structure exhibits different orientation from those in the former micrographs because it is not parallel to the nucleus.

2.- High Voltage TEM

Stereo pairs of 1 µm thick sections taken with high voltage TEM illustrate the 3-dimensional configuration of the Golgi complex. In Figs. 5a and 5b, rER is seen communicating with the Golgi at several different positions. Transport vesicles surround Golgi region. Developing granules exhibit a large range of sizes and are located randomly in different



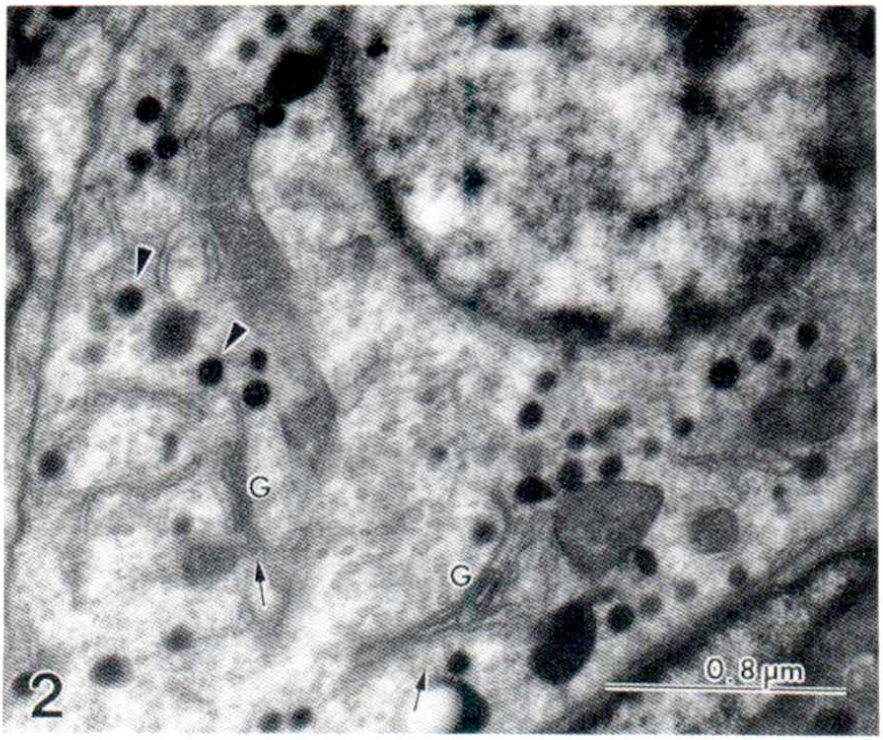
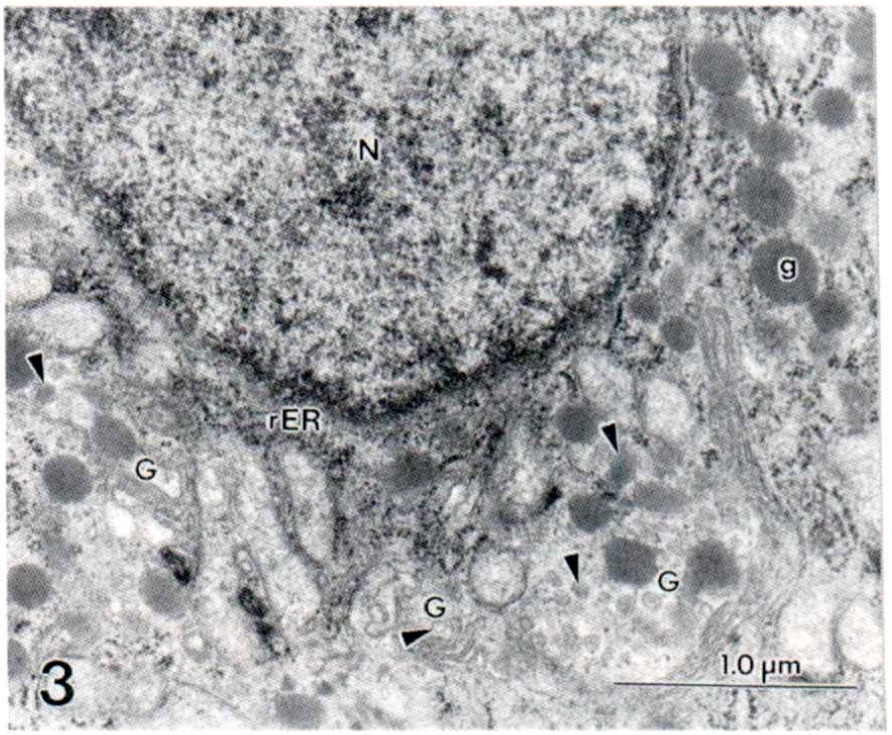


Fig. 1 Gogi complex (G) is located in the perinuclear region of the cell. Two stacks of Golgi cisternae are seen. Abundant small vesicles (v) are seen in the center of the Golgi cisternae located on the right side. Mature granules (arrowheads) at the ends of the dilated cisterane. A small developing granule (arrow) in one of the compartments of the cisternae. N : nucleus. M : mitochondria. rER : rough endoplasmic reticulum. g : hormonal granule.

Fig. 2 Two Golgi stacks are surrounded by abundant rough endoplasmic reticulum. The stack on te right side exhibits some 3-4 cisternae. Granule formation is present at the ends of the cisternae. The stack on the left side appears to be sectioned hexagonically. Transport vesicles (arrows) are seen along one side of the Golgi stack and mature granules (arrowheads) in the middle of the cisternae.



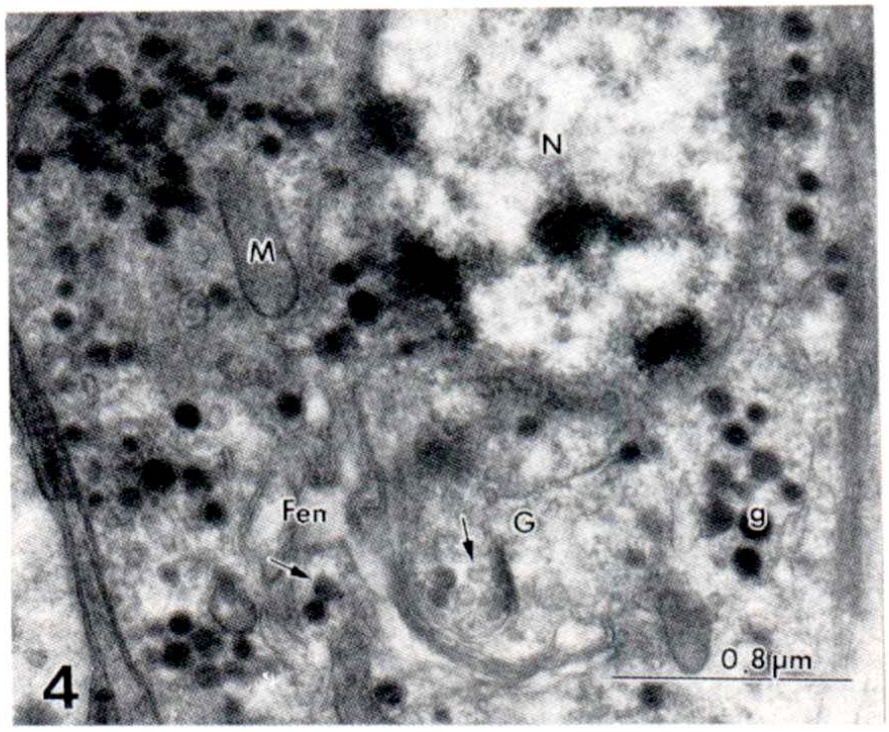


Fig. 3 A portion of the cell reveals at least 3 Golgi complex. The one on the right side is very extensive with 4-5 cisternae in parallel arrangement. The other two Golgi are less well revealed in the micrograph. One has a curved shape whereas the other is more irregular. Developing granules (arrowheads) are seen in all three Golgi systems.

Fig. 4 An expanded cisternae, not parallet to the nucleus, exhibits different orientation from those in the former micrographs. Fen : fenestration, Arrows : developing granules.

cisternae. At the left lower of Fig. 5 and extended portion of the Golgi complex is visible with two transitional vesicles next to it's border and a mature granule inside. The length of this Golgi region is the same or longer than that of the nuclear profile.

One image corresponding to the area beneath the nuclear region obtained from serial sections of a pituitary cell is shown in Figs. 6a and 6b. This Golgi complex appears to be one single structure, of which all cisternae are interconnected. Transport vesicles are located next to the cisternae rims, and developing granules are seen in most of cisternae. Two mature granules appear at the budded end of a Golgi cisternae, possibly ready to be released to the cytoplasm.

3.- High Resolution SEM Observation

Granule formation is well visualized with HR-SEM. In Fig. 7, developing granules are seen in different parts of Golgi cisternae.One of them is enlarged more clearly reveal its formation from the inner membrane of the Golgi cisternae.Fig.8). A stereo pair of HR-SEM (Figs. 9a and 9b) demostrates similar cytoplasmic features as seen in the HV-TEM micrographs. Golgi cisternae extend from the nucleus to the plasma membrane. Developing granules are also seen in different cisternae.

4.- Confocal Microscope Analysis

Fig. 10 shows fluorescent-labelled growth hormone granules associated with the Golgi cisternae seen by confocal microscopy. A series of images through the Z-axis of the specimen were obtained by scanning from the top to the bottom of the cell. These twelve images represent different cross-sections from the area above the nucleus, through the nuclear region and then below the nucleus (Figs. 10-a to 10-1). The Golgi cisternae exibits a continuous structure from one end of the cell to the other. In addition, it shows the interconnections of the cisternae are more dense in the periphery of the nucleus than the area near the plasma membrane. Furthermore, the dilated Golgi cisternae extend to the plasma membrane. A similar continuous configuration of the Golgi complex is also seen by the preparation with NBD ceramide Fig.11.

5.- Schematic Interpretation

Using data obtained as above it was possible to develop a three-dimensional reconstruction of the Golgi apparatus in pituitary cells during their active secretory phase.

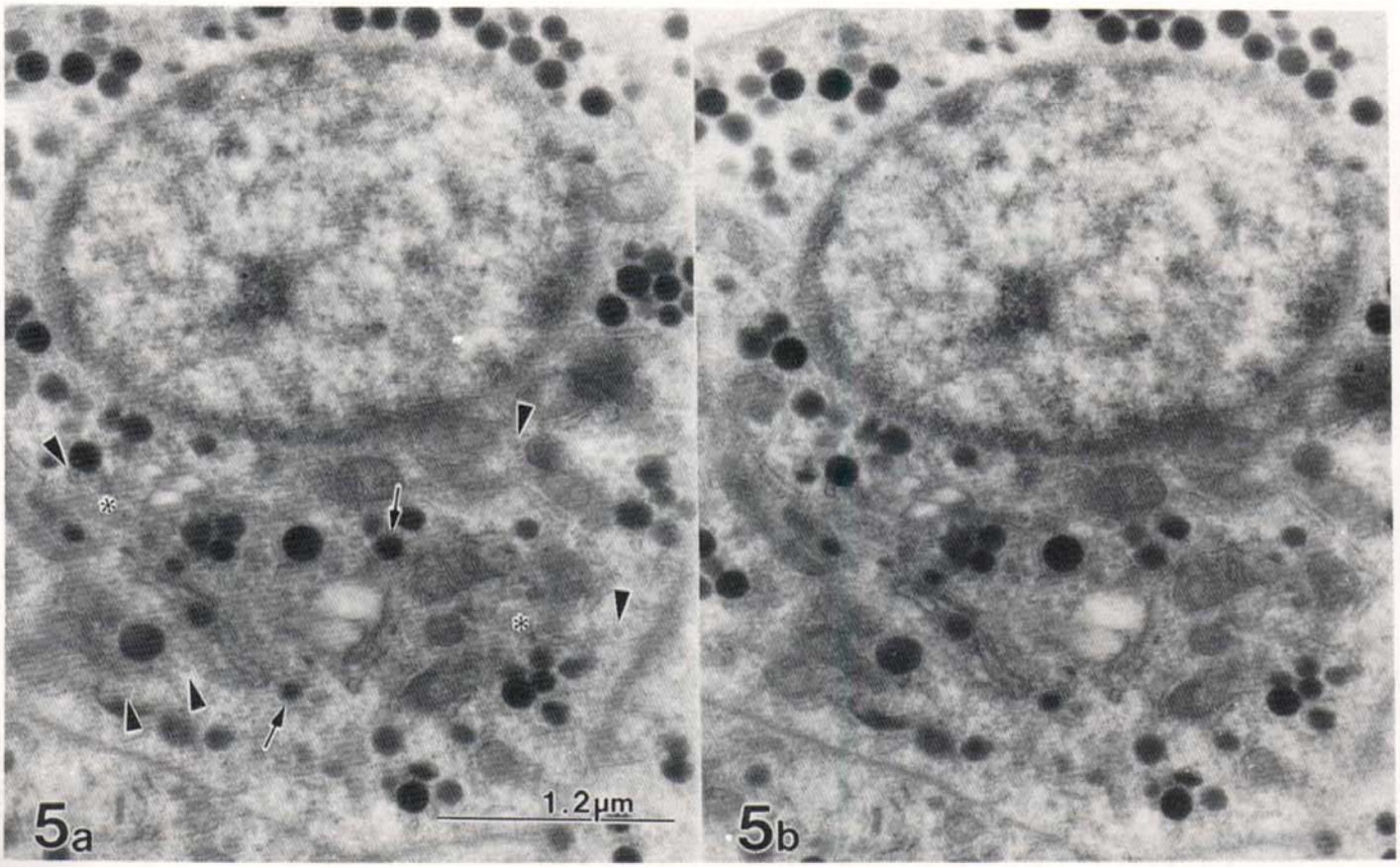
A schematic representation is shown in Fig. 12 which shows the possible relationships of structures that have been studied by the combined microscope techniques.

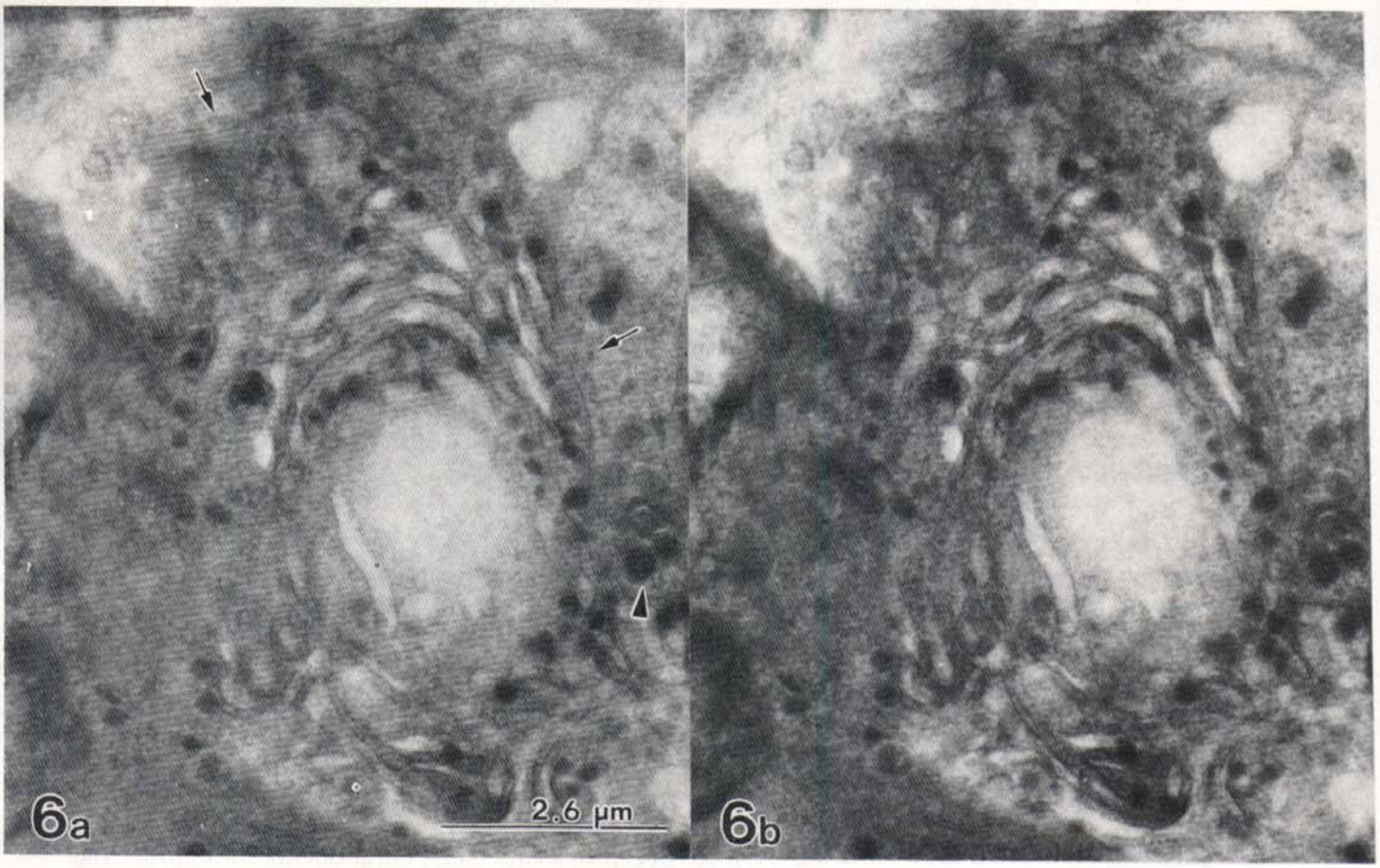
As seen in Figs. 10a-1, the interconnections of the Golgi cisternae are more numerous in the center region of the cell. This may explain why conventional TEM micrographs of thin sectioned material suggest that the Golgi complex is usually situated closer to the nucleus than to the plasma membrane. The 2-dimensional images of Figs. 1-4 and Fig. 5 correspond to the area of Fig. 12a. Fig. 6 is from the area indicated in Fig. 12b, whereas Figs. 7, 8, and 9 correspond to the area shown in Fig.12c. It is clear that had the area in Fig. 12c been processed as thin sections for TEM observation, they could be interpreted as several Golgi regions or stacks of Golgi cisternae in a single cell. Fig. 12d explains how the series of optical sections produced by confocal microscopy (Figs. 10a-1) are used to develop a three dimensional image of the cell.

DISCUSSION

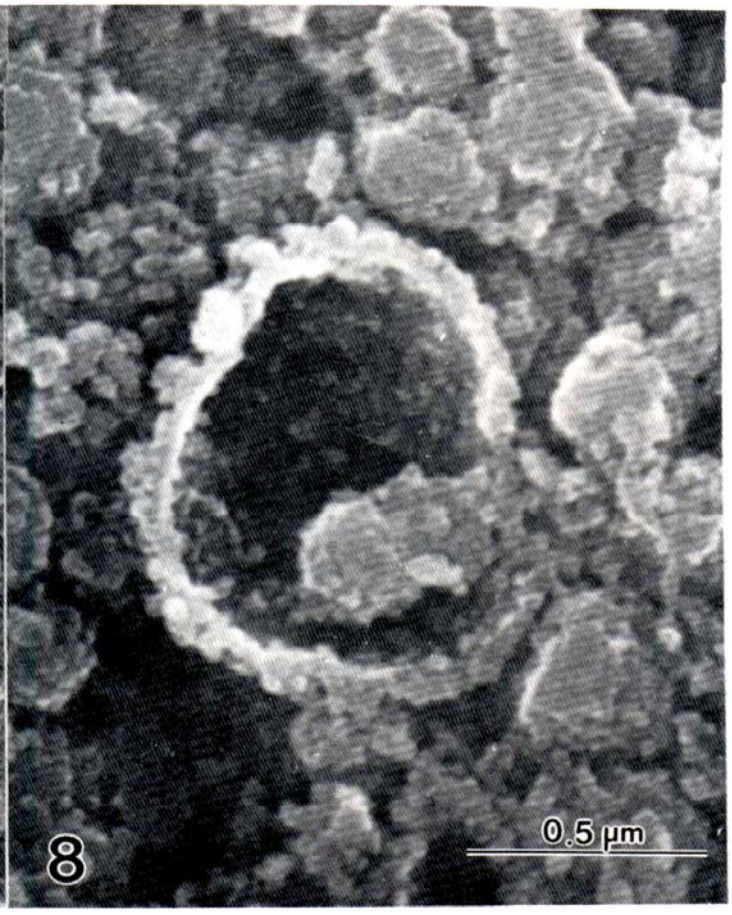
By convention, intracellular activity has been described by comparing the amount of mitochondria and rough endoplasmic reticulum, and the size and constituents of the Golgi complex. Since granule formation in the Golgi complex is observable by conventional electron microscopy, and the Golgi cisternae has been histochemically demonstrated in association with the synthesis of secreory products, the Golgi complex has become an ideal and important parameter for the indication of cell activity in most cell studies.

In the present study of chicken pituitary cells, we have shown from conventional TEM observations that granules develop in the dilated Golgi cisternae (Figs. 2 and 3) as well as fenestrations (Fig. 1). HR-SEM images have shown granule development from the inner surface of the membrane of the Golgi complex. In addition, the formation of granules was also seen in many Golgi components throughout the


1.2μm
5a
5b

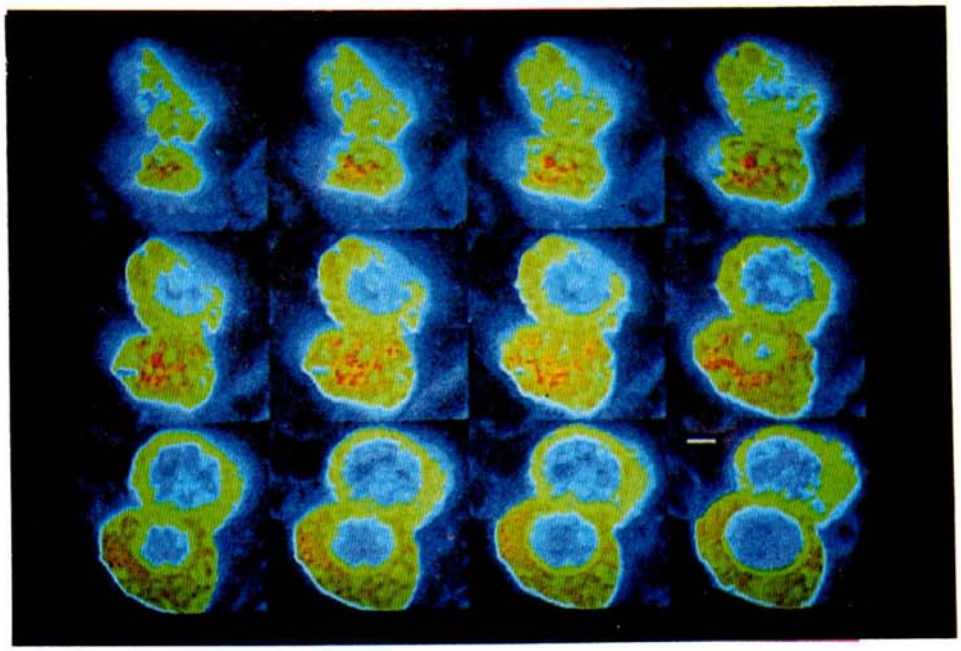

2.6 μm
6a
6b


M
N
1.6 µm
7


0.5 µm
8


G
N
9a
101120 10KV X11.0K 2.70um


9b
101121 10KV X11.0K 2.70um



Figs. 10a-1

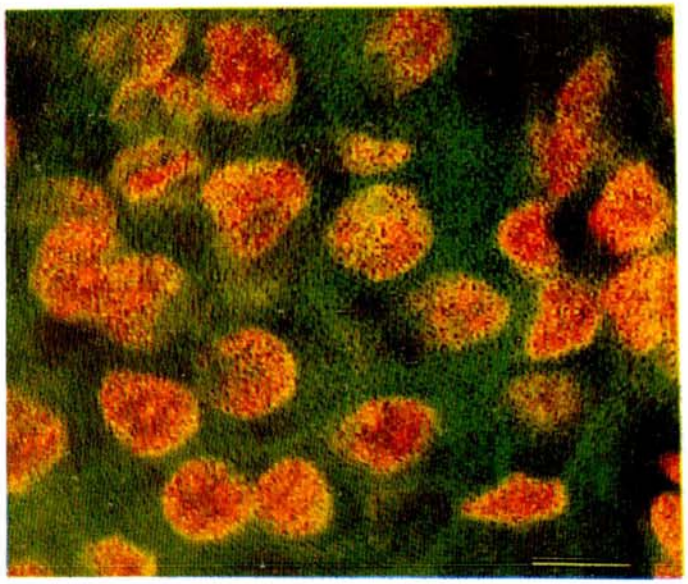


Fig. 11

Figs. 5 and 5b Stereo pairs of HV- TEM illustrate the 3-D configuration of the Golgi complex. Rough endoplasmic reticulum communicating with the Golgi at several positions (*). Transport vesicles (arrowheads) surround Golgi region. Developing granules (arrows) in different cisternae.

Figs. 6a and 6b The Golgi complex appears to be one single structure. All cisternae are interconnected. Two mature granules (arrows) appear at the budded end of a Golgi cisternae. Arrowheads : transport vesicles.

Fig. 7 HR-SEM micrograph illustrates that granule formation (arrows) are seen in different parts of Golgi cisternae.

Fig. 8 HR-SEM micrograph shows a granule is developing from the inner membrane of the Golgi cisternae.

Figs.9a and 9b A stereo pair of HR-SEM demonstrates Golgi cisternae (G) extend from the nucleus to the plasma membrane. Developing granules (arrows) are seen indifferent cisternae.

Figs. 10a-1 Confocal microscope images show fluorescent-labelled growth hormone granules associated with the Golgi cisternae (in red colour). The series of images through Z- axis of a cell were obtained by scanning from the top to the bottom of the cell. These twelve images(the order a to l is shown from top to bottom, left to right) represent different cross-sections from the area above the nucleus, through the nuclear region and then below the nucleus. (Bar=2μm).

Fig. 11 A confocal microscope image shows that the Golgi complex (in green colour) is a continuous structure from one end of the cell to the other. (Bar=7.2 μm)

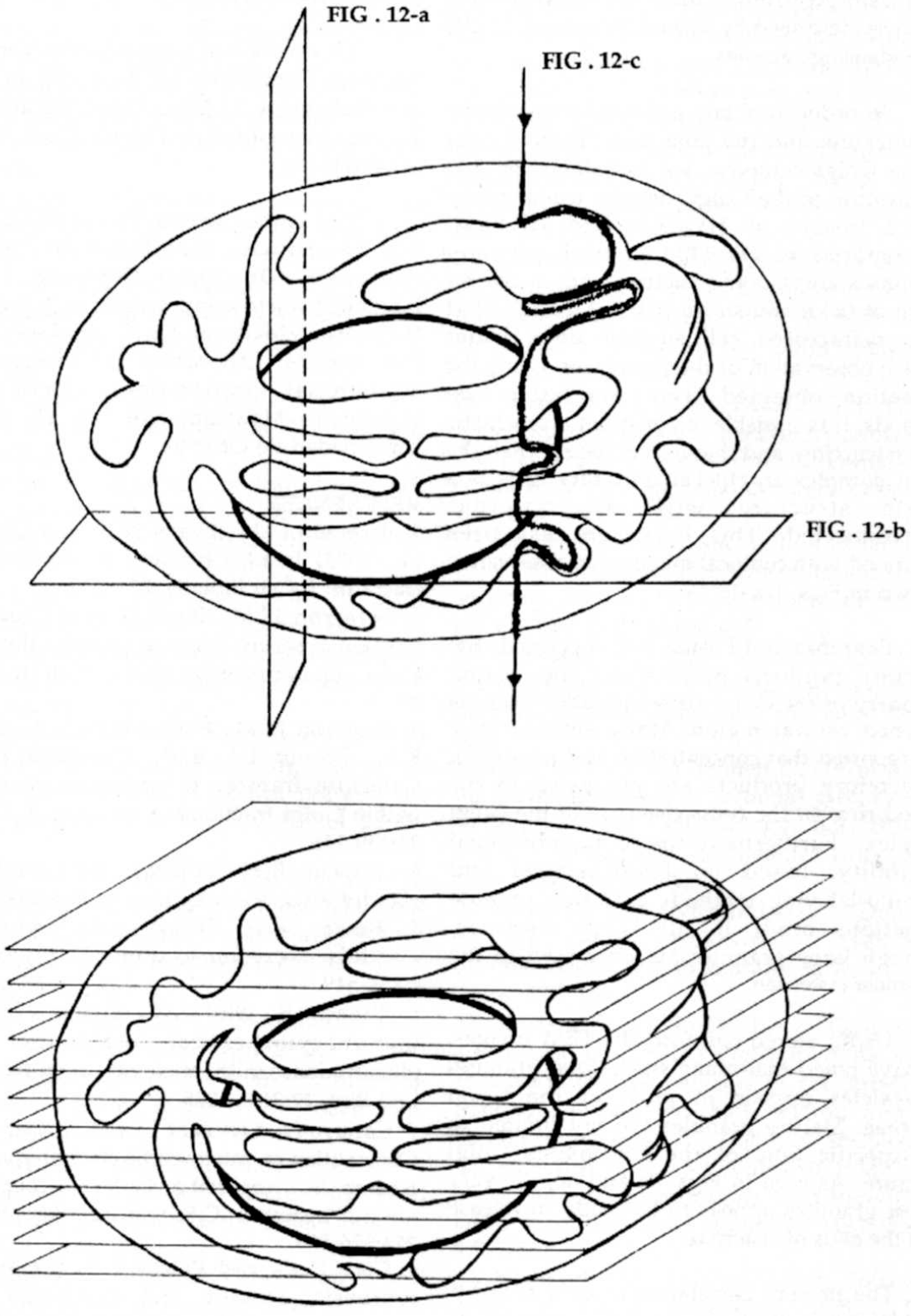


Fig. 12 A schematic representation shows the possible relationships of strictures that have been studied by those combined microscope techniques. The 2-D TEM images of Figs. 1-4 and Fig.5 correspond to the area of Fig. 12a. Fig. 6 is from the area indicated in Fig. 12b. Figs.7, 8 and 9 correspond to area shown in Fig.12c.

cytoplasm. Apparently these Golgi components are those described by several authors [8, 25, 26] as condensing vacuoles.

In order to obtain a clearer image of the organization and the polarized characteristics of the Golgi complex, we examined the 3-D architecture of the Golgi complex using stereo-paired images of HV-TEM and HR-SEM. Stereographs of HV-TEM of thick pituitary sections show Golgi structure with a greater depth of field, and stereo pairs of the HR-SEM of cryo-fractured cell surface allow wider surface observation of the cytoplasm. Using the formation obtained from the latter two methods, it is posisble to construct a schematic reconstruction and have concluded that the Golgi complex in chicken pituitary cells is a single structure with all cisternae interconnected. This hypothese has been confirmed with confocal microscope observation (shown in Figs. 12a-1).

Farquhar and Palade [13] suggested that secretory products move along the dilated periphery of the Golgi cisternae rather than its flattened central region. Many authors have also reported that concentration and packaging of secretory products are processed in the dilated rims of the trans cisternae of the Golgi complex. Furthermore there is additional possibility, based on histochemical and immuno-labeling methods [21] that granule formations occur in all Golgi cisternae, although large granules are only seen in the transmost cisternae.

Using stereo-pairs of HV-TEM images, we have noted that many small-sized granules or vesicles appear in most of the Golgi cisternae. Mature granules are not limited to any specific area of the continuous Golgi structure. As seen in Figs. 6a and 6b, the very largest granules appear to be ready to release from the ends of cisternae.

The present correlative studies reveal a recognizable polarity of the Golgi complex of chicken pituitary cells and the granule formation associated with Golgi structure. However, the mechanism of transport of mature granules from one cisternae to the other, and their release from the Golgi, remain subjects for further study.

ACKNOWLEDGEMENT

This work was supported by Venezuelan National Committee for Research in Science and Technology (CONICIT) under grant No. PI-28 and Universidad de Oriente grant No. CI-5-018-00442/90.

The author would like to thank several friends who have contributed for completing this work; Dr. Vicky Centonze Frohlich, Integrated Microscopy Resources, University of Wisconsin - Madison; Dr. C. Erickson and Mr. Paul Simms, Laboratory for Biological and Biomaterial preparation, University of Wisconsin- Madison; and Mr. D. Arrieche, Universidad de Oriente.

REFERENCES

1. Bergeron J.J.M., Evans W.H. and Geschwind I.I. (1973) Insulin binding to rat liver Golgi fractions. J. Cell Biol. **59:** 771-776.

2. Bergeron J.J.M., Borts D. and Cruz J. 1978 Passage of serum-destined proteins through the Golgi apparatus of rat liver. J. Cell Biol. **76:** 87-97.

3. Bergeron J.J.M., Rachubinski R.A., Sikstrom R.A., Posner B.I. and Paiement J. (1982) Galactose transfer to endogenous acceptors within Golgi fractions of rat liver. J. Cell Biol. **92:**139-146.

4. Bowen R.H. (1929a) The cytology of glandular secretion. Q. Rev. Biol. **4:**299-324.

5. Bowen R.H. (1929b) The cytology of glandular secretion (continued). Q. Rev. Biol. 4:484-519.

6. Brands R., Slot J.W., Geuze H.J. (1982) Immunocytochemical localization of β-glucuronidase in the male rat preputial gland. J. Cell Biol. **27:**213. - 225.

7. Cararro N., Ovtracht L. and Favard F. (1971) Observation en microscopie electronique a haute tension de l' appareil de Golgi sur coupes de 0.5 a 5 u d' epasseur. C.R.Acad Sci. Paris Series, D, **273:**876-879.

8. Caro L. G. and Palade G.E. (1964) Proteir synthesis, storage and discharge in the pancreatic exocrine cell. J. Cell Biol.**20:**473-495.

9. Cheng H. and Farquhar M.G. (1976) Presence of adenylate cyclase activity in Golgi and other fractions from rat liver. ll. Cytochemical localization within Golgi and ER membranes. J. Cell Biol. **70:**671-684.

10. Dalton A.J. and Felix M.D. (1954)

Cytological and cytochemical characteristics of the Golgi substance of epithelial cells of the epididymis in situ, in homogenates and after isolation. Am. J. Anat. **94**:171-207.
11. Ehrenreich B.A. and Cohn Z.A. (1967) The uptake and digestion of iodinated human serum albumin by macrophages in vitro. J. Exp. Med. **126**:941-958.
12. Farquhar M.G. (1985) Progress in unraveling pathways of Golgi traffic. Ann. Rev. Cell Biol. **1**:447-488.
13. Farquhar M.G. and Palade G.E. (1981) The Golgi Apparatus (Complex) - (1954-1981) - from artifact to center stage. J. Cell Biol. suppl. **91**:77s-103s.
14. Fawcett D.W. (1981) The Cell. W.B.Saunders Co., Philadelphia, London and Tronto. p.369.
15. Fleischer B., Fleischer S. and Ozawa H. (1969) Isolation and characterization of Golgi membranes from bovine liver. J. Cell Biol. **43**:59-79.
16. Fleischer B. and Smigel M. (1978) Solubilization and properties of galactosyltransferase and sulfotransferase activities of Golgi membranes in Triton X-100. J. Biol. Chem. **253**:1632-1638.
17. Geuze H.J. and Slot J.W. (1980) Disproportionate immunostaining pattern of two secretory proteins in guinea pig and rat exocrine pancreas cells. Eur. J. Cell Biol. **21**:93-100.
18. Geuze H.J. and Slot J.W., van der Ley P.A. and Scheffer R.C.T. (1981) Use of colloidal gold particles in doubles-labeling immunoelectron microscopy of ultrathin frozen tissue section. J. Cell Biol. **89**:653-665.
19. Geuze H.J. and Slot J.W. Strous G.J.A.M., Hasilik A. and von Figura K. (1984) Ultrastructural localization of the mannose 6-phosphate receptor in rat liver. J. Cell Biol. **98**:2047-2054.
20. Green J., Griffiths G., Louvard D., Quinn P. and Warren G. (1981) Passage of viral membrane proteins through the Golgi Complex. J. Mol. Biol. **152**:663-698.
21. Hashimoto S., Fumagalli G., Zannini A. and Meldolesi J. (1987) Sorting of three secretory proteins to distinct secretory granules in acidophilic cells of cow anterior pituitary. J. Cell Biol. **105**:1579-1586.
22. Howell K.E., Ito A. and Palade G.E. (1978) Endoplasmic reticulum marker enzymes in Golgi fractions. What does this mean? J. Cell Biol. **79**:581-589.
23. Howell K.E., and Palade G.E. (1982) Hepatic Golgi fractions resolved into membrane and content subfractions. J. Cell Biol. **92**:822-832.
24. Ichikawa M., Ichikawa A. and Watabe T. (1982) High resolution analysis of three-dimensional structure of the Golgi apparatus in rapid-frozen, substitution fixed gerbil sublingual gland acinar cells. J. Electron Microsc., **31(4)**:397-401.
25. Jamieson J.D. and Palade G.E. (1967a) Intracellular transport of secretory proteins in the pancreatic exocrine cell. I. Role of the peripheral elements of the Golgi complex. J. Cell Biol. **34**:577-596.
26. Jamieson J.D. and Palade G.E. (1967b) Intracellular transport of secretory proteins in the pancreatic exocrine cell. II. Transport to condensing vacuoles and zymogen granules. J. Cell Biol. **34**:597-616.
27. Kraehenbuhl J.P., Racine L. and Jaimieson J.D. (1977) Immunocytochemical localization of secretory proteins in bovin pancreatic exocrine cells. J. Cell Biol. **72**:406-423.
28. Kornfeld R. AND Kornfeld S. (1985) Aseembly of asparagine-linked oligosaccharides. Ann. Rev. Biochem. **54**:631-664.
29. Leblond C.P. and Bennett G. (1976) Role of the Golgi apparatus in terminal glycosylation. In International Cell Biology. B.R. Brinkley and K.R. Porter eds., Rockefeller Univ. Press, New York. pp.326-336.
30. Morre D.J. and Ovtracht L. (1977) Dynamics of the Golgi apparatus : Membrane differentiation and membrane flow. Int. Rev. Cytol. **5**:61-188.
31. Novikoff P.M., Novikoff A.B., Quintana N. and Hauw J.J. (1971) Golgi apparatus, GERL, and lysosomes of neurons in rat dorsal root ganglia, studied by thick section and thin section cytochemistry. J. Cell Biol. **50**:859-886.
32. Novikoff A.B. and Novikoff P.M. (1977) Cytochemical contributions to differentiating GERL from the Golgi apparatus. Histochem. J. **9**:525-551.
33. Novikoff P.M., Tulsiani D.R.P., Touster O., Yam A. and Novikoff A.B. (1983) Immunocytochemical localization of a-D-mannosidase II in the Golgi apparatus of rat liver. Porc. Natl. Acad. Sci. USA **80**:4364-4368.
34. Palade G.E. (1959) Functional changes in the structure of cell components. In Subcellular Particles. T. Hayashi ed. Ronald. New York.

pp.64-80.
35.Palade G.E. (1966) Structure and function at the cellular level. J. Amer. Med Assoc. 198:143-153.
36. Palade G.E. (1975) Intracellular aspects of the process of protein secretion. Science **189:**347-358.
37. Pagano, R.E. (1989) A fluorescent derivative of ceramide: physical properties and use in studying the Golgi apparatus of animal cells. In Methods in Cell Biology : Fluorescence Microscopy of Living Cells in Culture. D. Lansing Taylor and Y.L. Wang, eds. Academic Press Inc., Orlando, FL. **29:**75-85.
38. Rambourg A. (1969) L' appareil de Golgi: examen en microscopie electronique de coupes epaisses (0.5-1 μ) colorees par le melange chlorhydrique-phosphotungstiques. C.R. Acad. Sci. Paris, serie, D, **269:**2125-2127.
39. Rambourg A. and Chretien M. (1970) L' appareil de Golgi: examen en microscopie electronique de coupes epaisses (0.5-1 μ) apres impregnation des tissus par le tetroxyde d'osmium. C.R. Acad. Sci. paris, serie, D, **270:**981-983.
40. Rambourg A., Clermont Y. and Marraud A. (1974) Three-dimensional structure of the osmium-impregnated Golgi apparatus as seen in the high voltage electron microscope. Am. J. Anat. **140:**27-46.
41. Rambourg A., Clermont Y .and Hermo L. (1979) Three-dimensional architecture of the Golgi apparatus in sertoli cells of the rat. Am. J. Anat. **154:**455-476.
42. Tai S. (1992) Correlative TEM and HR-SEM studies on the fine structural organization of the chicken pituitary gland. S.E Intern. **6** (1)