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TEM Observation of Thin and Thick Tissue Sections and Comparison of Respective Images of the Golgi Structure

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ABSTRACT

The structure and functional activity of the Golgi complex in cells of the chicken pituitary gland were studied by preparing specimens in plastic sections with a thickness of 70 nm and of 1.0 um. The thin sections were observed with TEM operated at 120 kV while thick sections were observed at 200 kV.

Complimentary information regarding of the Golgi complex is obtained when images of thin and thick tissue sections were observed. Images of thin sections show that developing granules are limited to inside the Golgi cisternae while mature granules are free in the cytoplasm. Stereo images of thick sections show that in those cells which are active in synthesis, the Golgi complex extends throughout the cytoplasm, hormonal granules of different sizes are being formed and stored in different parts of Golgi complex. Few granules are free in the cytoplasm, being principally inside Golgi cisternae.

The structural comparsion between stereo images obtained from thin and thick tissue sections is valuable for better interpretation of intracellular organelle configuration.

KEYWORDS

Transmission electron microscopy, Stereo images, Pituitary gland, Golgi structure.

INTRODUCTION

Since the introduction of Transmission Electron Microscopy (TEM), it has demonstrated to be an essential optic tool for studies of intracellular structure. The resolution offered by TEM permits one to visualize those complex intracellular configurations and allows researchers to obtain a reliable data bearing information about intracellular activities. TEM and its associated sample preparation techniques such as ultramicrotomy, replicas, cryo-techniques, autoradiography, immuno-labelling etc. provide a number of alternative approaches to study cells. These studies can not only provide informations on cytological aspects but also for biochemical and physiological studies. Information relevent to a TEM operated at accelerating voltages below 100 kV is generally useful to project images from tissue sections of not more than one hundred nanometers. Because of such limitations, several sections are used in order to provide additional 3 dimensional information from structures of interests. With serial sections, a series of five tissue sections is required for information from a depth of 0.5 um. Obtaining these images in the exact same orientation can be a challenge.

An alternative solution for the mentioned limitation is to use thicker tissue sections and a TEM operated at higher accelerating voltages permitting increased

penetration through the thicker specimens and the ability to obtain greater 3 dimensional information of various structural features via stereo pair images from a single tissue section.

Recently there have been attempts to develope so called "automatic electron tomography" for 3-D reconstruction of biological specimen images. This refers to the utilization of a microprocessor-controlled TEM, large-area slow-scan CCD camaras and fast image-processing computers to collect a full data set by taking images from a specimen at a set of different tilt angles distributed over a large angular range [1]. This modern instrumentation has been able to use on-line images and produce 3-D reconstructed images of some samples such as isolated protein molecules [2, 3].

In earlier publications [4, 5, 6, 7], we have used different microscopic techniques including confocal microscopy, transmission electron microscopy and high resolution scanning electron microscopy to study the Golgi complex. We have demonstrated that the Golgi complex can be a single structure with interconnected cisternae [5]. However, it has also been demonstrated [8] that, after the treatment of cells with nocodazole, a drug that induces microtubule break down, the Golgi cisternae appear separated one from the other and become randomly distributed in the cytoplasm.

The Golgi structure is complex and is very difficult to reconstruct using 3-D automatic electron tomography. Therefore, for the present work, we decided to take a series of stereo images of thin and thick pituitary tissue sections and to look for different intracellular informations which were projected by respective stereo pairs of Golgi cisternae at different tilt angles.

MATERIALS AND METHODS

For the present work, 5 chicken (***Gallus domesticus***) at ages of 20 weeks were used. The pituitary gland were dissected immediately after decapitation and processed by using reduced osmium method [9]. Freshly excised pituitaries were fixed first with 2.5% glutaraldehyde in phosphate buffer, 0.1M, pH 7.2, for 3 hours at 4° C and postfixed with 2% OsO_4 plus 3% phosphate ferrocynate in the same buffer and same temperature for another hour. After throughly dehydrated in graded ethanol and propylene oxide, specimens were infiltrated with the mixture of propylene oxide and Epon 812 (1:1 ratio) for overnight and finally embedded and polymerized in Epon 812 (Ladd Research Ind. Inc., Burlington, VT. USA).

Tissue sections with thickness of 70 nm and of one micron were obtained with an ultramicrotome (Reichert-Jung, Ultracut E). They were mounted on copper grids of 200 mesh and stained with Uranyl Acetate and Lead Citrate. Two TEMs, Philips CM120 and CM200, were kindly supplied by Philips Electron Optics, Application Lab in Eindhoven, The Netherland. For thin sections observation, CM120 was used and operated at 120 kV while for thick sections, the CM200 was used and operated at 200 kV. Both microscopes were constructed with compustage, low dose illumination mode, high contrast TWIN lens system which facilitate thick biological sample observation and stereo image photography.

RESULTS

Two stereo-images of cells (Figs. 1 and 2) were taken with a TEM operated at 120 kV. These micrographs were obtained from tissue sections of two different thickness (Fig.1, 70 nm and Fig.2, one micron in thickness). The stereo-image of Fig. 1 shows that this cell is active in synthesis; the Golgi complex is extended throughout the cytoplasm, hormonal granules are developing in different Golgi cisternae. Although this stereo-pair was obtained at a tilt angle of 10°,

it can hardly produce a profound three dimensional feature of cytoplasmic structures due to the limited thickness of the tissue section which can only provide a small depth of field for 3 dimensional images. The stereo-image of Fig.2 was obtained from thicker tissue section and taken at tilt angle of 5°. This stereo pair offers not only a 3-dimensional image but also facilitate the interpretation of Golgi structure and the synthesis activity inside this organelle. The membranous structure of the Golgi complex in these micrographs initiates at the area indicated by arrow 1 where was adjacent to the nuclear membrane and extends throughout the entire cytoplasm. Hormonal granules of different sizes were being formed in the network of the Golgi cisternae (shown by arrows 2 to 7). A large granule (indicated by arrow 7) was seen located at the extreme end of a Golgi cisternae.

With a TEM operated at 200 kV, one micron thick tissue sections were examined by tilting the specimen stage and photographing at tilt angles of 10°, 20° and 30° (Figs. 3, 4 and 5). Different informations were obtained from images taken at different tilt angles. The stereo image of Fig.3 (tilted at 10°) reveals a 3-dimensional configuration of the Golgi cisternae. It can be seen that some developing granules (shown by arrows) were under formation and larger mature granules were stored in different part of the Golgi cisternae. At higher tilt angles (Figs. 4 and 5), several Golgi cisternae (a, b, c and d) bearing numerous hormonal granules were seen situated at different vertical levels, hormonal granules appear lying along the cisternae, extending toward the extreme end of the cisternae and are ready for release. If those granules were sectioned into ultrathin sections, they could be easily interpretated as granules which were encapsulated in a membrane and free in the cytoplasm.

Fig. 6 is a micrograph obtained from a pituitary section of one micron thick which was imaged at 200 kV and photographed the central area of a pituitary cell. The sample was prepared by reduced osmium method in order to outline all the membranous system. The micrograph shows a complex intracellular configuration including numerous mitochondria, abundant hormonal granules being developed inside the network of the Golgi cisternae. The rounded nucleus is shadowing underneath the rest of cytoplasmic organelles and presents a most beautiful art of the electron microscopy.

CONCLUSION

Progress in instrumentation is important to advance scientific studies. Results are limited not only by the capability of the available instrumentation but also by the individual researcher's understanding of the limits of the instrumentation.

The purpose of present paper is to utilize stereo images of thick tissue sections of the Golgi complex taken at different tilt angles to demonstrate that unique instrument characteristics in combination with specialized tissue preparation techniques can be used to optimize data acquisition.

Due to the sensitivity of biological specimens to beam irridiation, tissue sections are mounted on grids with supporting membranes in order to avoid possible damage and loss of the specimen. However, the beam irridiation damage is less of a problem since newly designed low dose modes can minimize the amount of electrons that strike on the sample. Using this kind of illumination mode, even a series of stereo images of cryo sections can be taken without suffering any morphological alteration [3]. Another aspect which must be considered is that the alignment for each tilt angle should be coordinated. With older instruments ajustment acts for each tilt angle were necessary. Today modern microscopes are equipped with microprocessor or computer

operated eucentric goniometer stages which permit nearly automatic stereo image photographing.

Stereo images of thin tissue sections offer 3-D features at a tilt angle of not more than + and - 10 degrees. Beyond this tilting angle, images not only do not offer more information but also give a blurred effect. TEM operated at 200 kV can project images from tissue sections over one micron. The Golgi cisternae shown in those stereo pairs at gradually decreased tilt angles under a stereoscope provided information from a greater depth in the structure. We can observe with those stereo images the relations between neighbouring cisternae or the continuity of a single cisternae in the complex. These studies also suggest that the maximun tilt angle for such thickness is 30 degree, beyond this tilt angle indistinct effect will appear in the stereoscope observation.

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RESUMEN

Se estudió la estructura y actividad funcional del complejo de Golgi, en células de pituitaria de pollo, por medio de la preparación de especímenes en cortes de resina plástica con espesores de 70 nm y de una 1.0 µ. Los cortes finos se observaron en TEM operado a 120 kV, mientras que los cortes gruesos fueron observados a 200 kV.

Cuando se observaron los cortes finos y gruesos, se obtuvo información complementaria concerniente al complejo de Golgi. Las imágenes de los cortes finos demuestran que los gránulos en desarrollo están limitados al interior de las cisternas del Golgi mientras que los gránulos maduros se encuentran libres en el citoplasma. Por el contrario, las imágenes estereoscópicas de cortes gruesos, muestran a las células que se encuentran en síntesis activa con un complejo de Golgi extendido a través del citoplasma, visualizándose los gránulos hormonales formándose y almacenándose en diferentes partes del complejo. Pocos gránulos se encuentran libres en el citoplasma, observándose principalmente en el interior de las cisternas del Golgi.

La comparación estructural entre imágenes estereoscópicas obtenidas de cortes finos y gruesos permite una mejor interpretación de la configuración intracelular de la organela.

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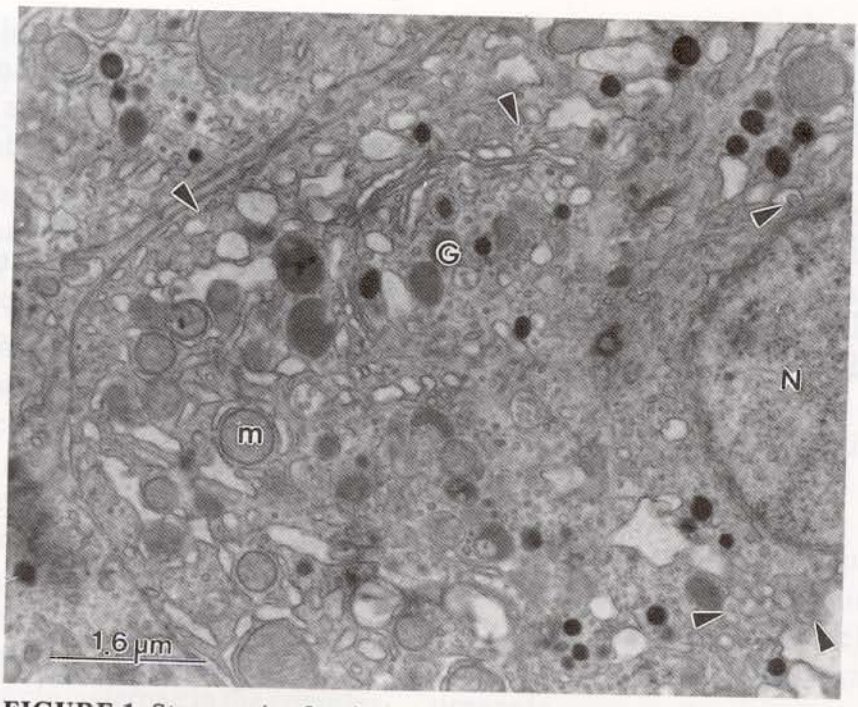


FIGURE 1. Stereo pair of a pituitary cell was obtained from a ultrathin section of 70 nm and taken at tilt angle of 10 and accelerating voltage at 120 kV. It reveals that the cell is active in synthesis; very extended Golgi Complex with numerous developing granules in different Golgi cisternae. Transfer vesicles (arrows). Rough endoplasmic reticulum (*). m:mitochondria. g:mature granules. G:Golgi Complex. arrowheads:developing granules.

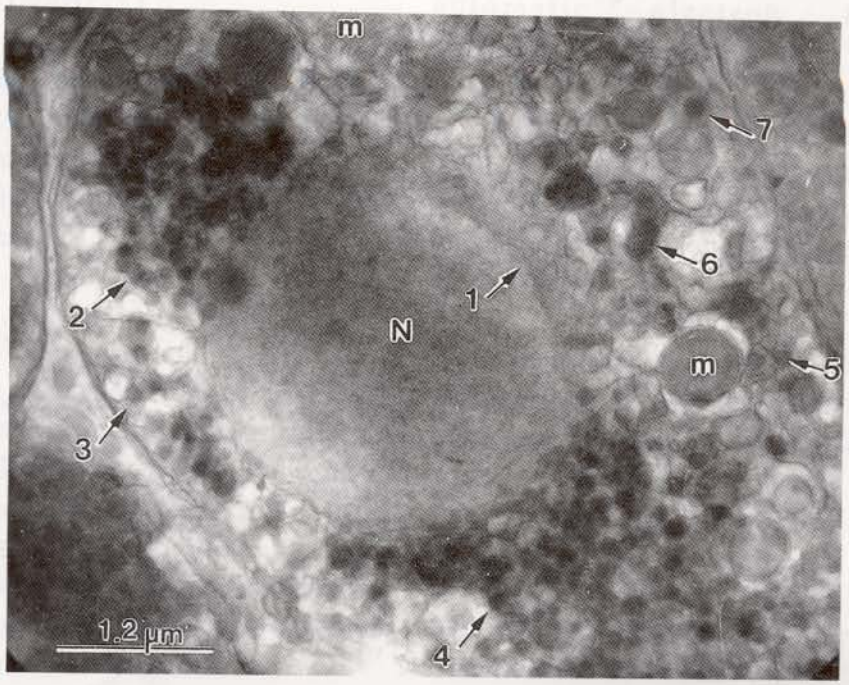
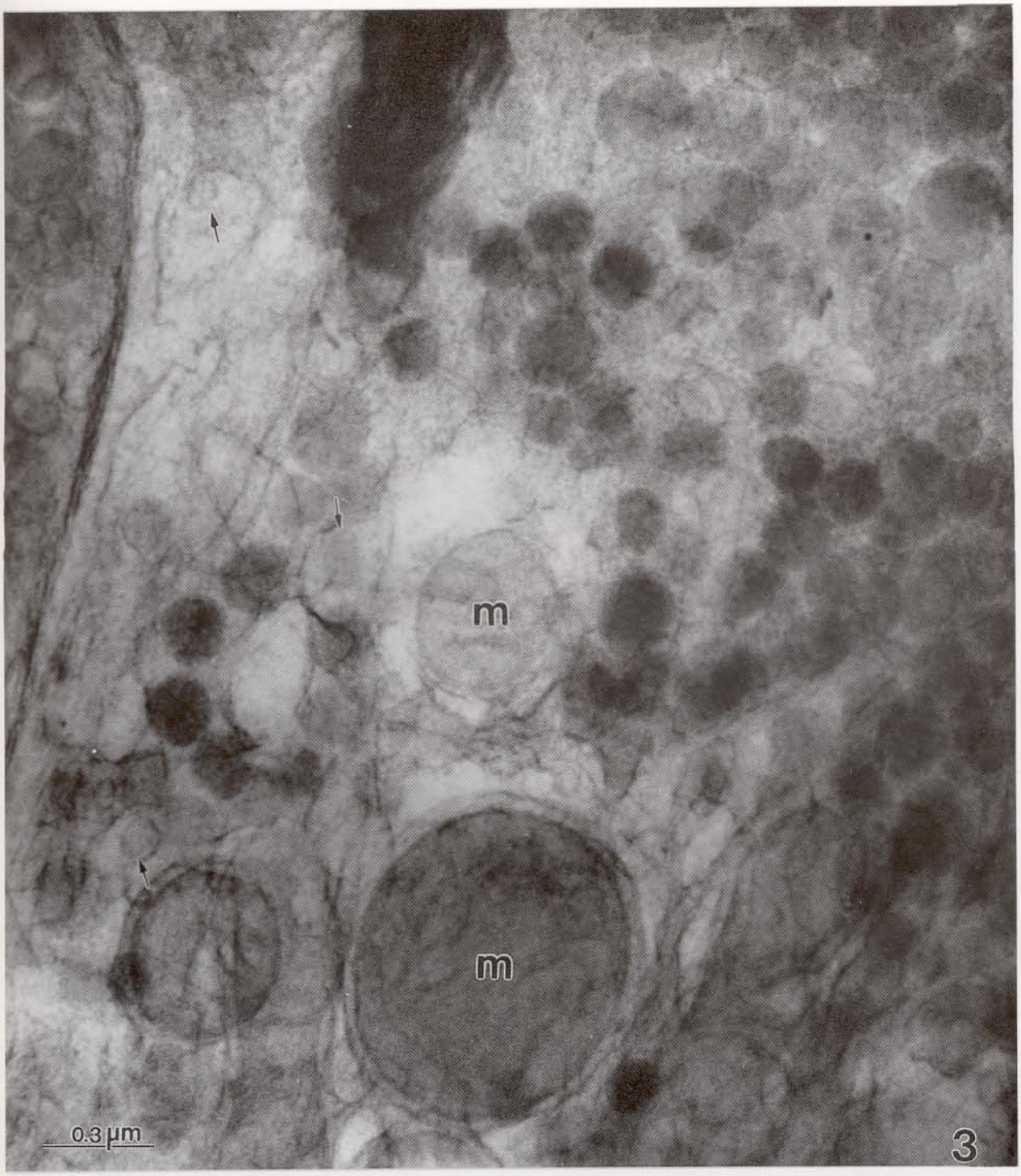
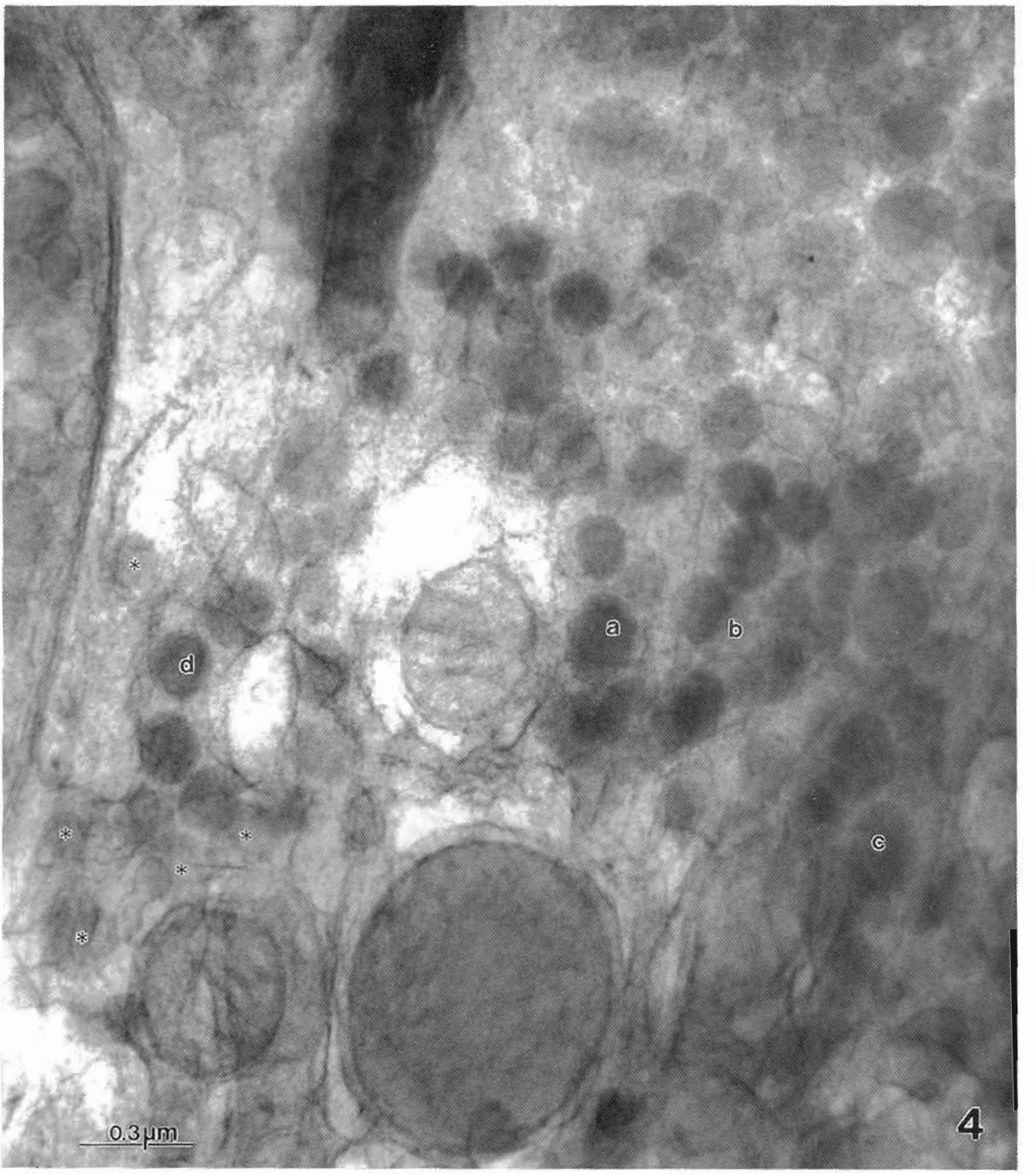


FIGURE 2. A stereo pair of a cell was obtained from a one micron thick section. These images were taken at tilt angle of 5 degrees and at 200 kV, revealing a 3-dimensional intracellular structure which shows the entire Golgi complex initiated at the area indicated by arrow 1, adjacent to the nuclear membrane, extended throughout the cytoplasm. Hormonal granules were developing in different part of the network of Golgi cisternae (shown by arrows 2 to 7). m:mitochondria. N:nucleus.



FIGURES 3 to 5. Stereo images of a one micron thick section were taken at 200 kV and with tilting angles at 10 degrees (Fig.3), 20 degrees (Fig.4) and 30 degrees (Fig.5). It is seen in Fig.3 granules(arrows) are developing in different Golgi cisternae. In Figs.4 and 5, several Golgi cisternae (a, b, c and d) were seen situated at different vertical levels. Hormonal granules (*) appear lying along the cisternae, extending toward the extreme end of of the cisternae and are ready for release (arrows).


a
b
c
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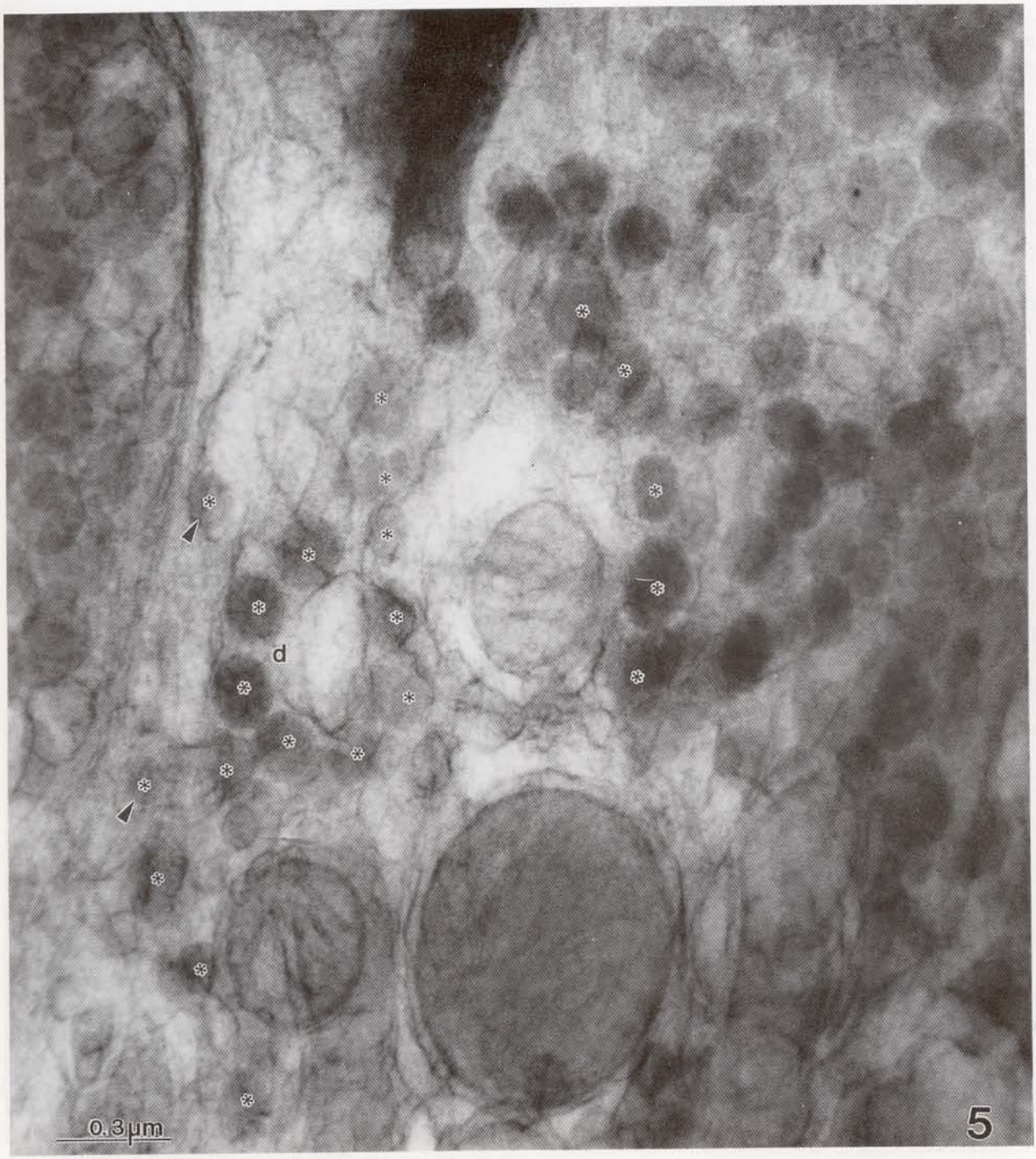
0.3 µm
4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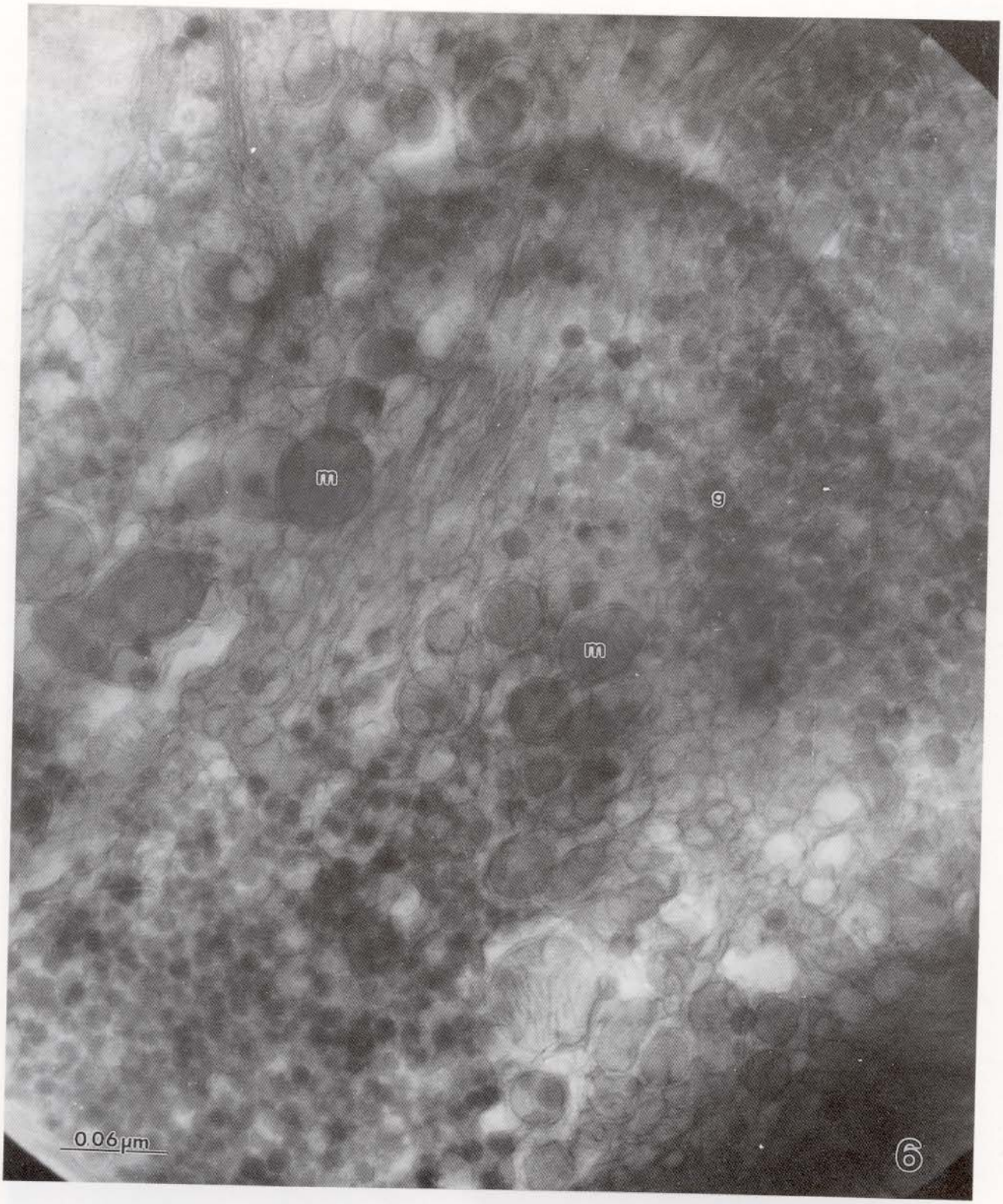

FIGURE 6. A TEM micrograph was obtained from a pituitary section of one micron thick taken at 200 kV, showing a complex intracellular configuration including numerous mitochondria(m), abundant hormonal granules (g) developing inside the network of the Golgi complex. The rounded nucleus (N) is shadowing underneath the rest of cytoplasmic organelles and presents a most beautiful art of the electron microscopy.