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Robert R. Cardell

Funan Hu

Robert S. Knighton

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CYTOLOGY OF CELLS SYNTHESIZING SPECIFIC PROTEINS*

ROBERT R. CARDELL, JR., Ph.D.,** FUNAN HU, M.D.,*** and
ROBERT S. KNIGHTON, M.D.****

INTRODUCTION

PROTEIN SYNTHESIS has attracted the attention of many investigators in past years and continues to be of great interest. In order for cytologist to study protein synthesis it is very desirable, if not essential, that the proteins occur as specific structures, easily identifiable, within the cell. It is then possible to study the structural patterns of these cells during the various stages of protein formation.

Considerable biochemical information is available which indicates that the amino acids are linked into the protein molecule while in association with the ribonucleoprotein (RNP) particles.[1] It is believed the protein molecule is transferred into the cisternae of the rough endoplasmic reticulum (RER). Comparative cytological studies have shown that cells synthesizing proteins for secretion have an abundance of RNP particles attached to the surface of the ER; whereas cells synthesizing proteins for retention have free RNP particles throughout the cytoplasm.[2]

The fate of the proteins after segregation into the ER is less clear. In some cells protein granules are formed within the rough ER[3]; in other cells (particularly secretory cells) granule formation occurs in the Golgi zone[4,5]; some workers consider protein granules to arise as transformed or modified mitochondria[6]; the hypothesis has been presented that the granules have a nuclear origin[7]; and others believe the granules to arise in the cytoplasm from unidentified sites.[8]

We have been studying the ultrastructure of several cell types which were actively synthesizing proteins and which form protein granules. These were salamander pituitary gland cell types which were forming protein hormones; human pituitary tumor cells which were synthesizing somatotrophin or growth hormone; and mouse B-16 melanoma cells which were synthesizing melanin. It is the purpose of this paper to describe these results. Some of this work has been reported in abstract form.[9]

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**Edsel B. Ford Institute for Medical Research.
***Department of Dermatology
****Department of Neurosurgery.

Materials and Methods

The pituitary gland from the salamander (*Diemytilus viridescens viridescens*) was exposed, fixed *in situ*, and processed for electron microscopy as described below.

The human pituitary tumor was obtained immediately after surgical removal from an acromegalic patient and processed for electron microscopy.

The melanoma cells were from a cell strain (HFH-14) established in the Dermatology Research Laboratory from a B-16 mouse melanoma[10, 11]. The cells were grown as monolayers on glass in culture medium 199, supplemented with 20 per cent fetal bovine serum. The cells for electron microscopy were obtained from a fresh sub-culture after incubation of eight days and were trypsinized, washed, and processed for electron microscopy as described below.

All material was fixed in 1 per cent osmium tetroxide buffered at pH 7.4 with veronal acetate[12] or 1 per cent osmium tetroxide buffered at pH 7.4 with phosphate or 5 per cent glutaraldehyde buffered at pH 7.0 with phosphate[13]. The tissue was fixed for two hours with osmium tetroxide at 4° C. and for 12 hours with glutaraldehyde at 4° C. The glutaraldehyde-fixed material was washed for six hours at 4° C. with 0.2M sucrose solution (buffered at pH 7.0 with 0.1M phosphate). The tissue was refixed in 2 per cent osmium tetroxide for four hours. Following dehydration with a graded series of acetone the tissue was embedded in Vestopal W. as previously described[14]. Ultrathin sections were cut on an LKB ultratome and floated on the surface of distilled water. They were placed on parlodian-carbon coated grids (#100) and stained with uranyl acetate or lead hydroxide[15]. The sections were studied with an electron microscope (RCA-EMU 2B) and electronmicrographs taken of selected areas.

Observations

The cells had an abundance of rough endoplasmic reticulum in the form of paralleled arrays (Figure 1) or vesicles (Figure 18). In the cells with abundant secretory granules the rough ER appeared as tubules (Figure 3) interwoven among the granules. In the cells with few secretory granules the rough ER was in the form of stacks of vesicles (Figure 1).

Mitochondria were also present in all cells (Figures 2, 4, and 6) and they had the typical structure of this organelle. The mitochondria were most abundant in the human pituitary cells and least abundant in the melanoma cells.

The Golgi complex of the pituitary cells was made up of flattened sacs and vacuoles characteristic of this organelle (Figure 2). In normal pituitary cells the Golgi was usually located in the juxtanuclear position (Figure 9); however in hyperstimulated cells (human pituitary tumor) the Golgi extended throughout the cytoplasm and was greatly hypertrophied (Figure 11). The Golgi of the pituitary cells was more localized than that of the melanoma cells (Figures 6 and 8) and had more of the flattened sacs (compare Figure 2 to Figure 5). The Golgi of the melanoma cells was composed predominantly of vacuoles (Figures 5 and 6).

The pituitary and melanoma cells contained specific electron-dense protein granules (Figures 3, 4, 5, 6 and 7). The salamander pituitary cells contained a variety of different sized, generally spherical, secretory granules (Figures 8 and 9). Each cell contained a characteristic sized granule which is thought to contain the hormone secreted by the particular cell. In the human pituitary tumor cells secretory granules were approximately 300-400 mu in diameter (Figure 4). Another human pituitary tumor cell type was observed which had smaller secretory granules (approximately 200 mu).

All of the pituitary secretory granules were electron-dense, enclosed by a double membrane, and showed no internal structure.

The protein granules of the melanoma cells were enclosed by a double membrane and in some cells the majority of the granules were very electron-dense with no internal structure (Figure 5). Other granules were less electron-dense and were composed of rods (Figures 6, 7 and 14). These granules appeared as an oblong vacuole containing longitudinally oriented rods (Figure 13). In favorable micrographs a periodicity was noted along the rods (Figure 6). The rods varied in diameter from approximately 100A up to 4 mu (Figure 7). Granules were found which had very few rods within the vacuoles (Figures 13, 14 and 15), intermediate stages (Figures 13, 14 and 15), and very dense granules (Figures 5, 6, 7 and 14). These granules have been classified according to density from one to five (Figures 6, 13 and 15).

The Golgi vacuoles of the salamander pituitary cells contained secretory granules of various sizes up to the size characteristic of the cell under observation. Figure 8 is an electronmicrograph of cell type #4 (globular basophil) and within the Golgi zone of this cell, various-sized secretory granules were found. Similar observations have been made on the human pituitary tumor cells (Figures 10, 11 and 12). Figure 9 is an electronmicrograph of a section through the Golgi zone of an acidophil from the salamander pituitary gland and a secretory granule is shown within the Golgi vacuole of this cell type.

The Golgi zone of the mouse melanoma cells did not contain any completely formed melanin granules, but an abundance of vacuoles containing rods were observed in and near the Golgi zones (Figures 6, 13, 14 and 15). The cells which had fewer electron-dense granules had more rod-like vacuoles and these were found in closer association with the Golgi vacuoles (compare Figures 13, 14 and 14 to Figures 5, 6 and 7). The Golgi of these cells was also more prominent than that of the completely melanized cell.

Discussion

The cells under observation in this study were actively synthesizing proteins. The salamander globular basophil (cell type #4) was believed to be synthesizing thyrotrophin[16, 17]; the human pituitary tumor cells were synthesizing somatotrophin; and the mouse melanoma cells were synthesizing melanin. It is pertinent to note that three cellular organelles showed considerable activity during this process. These were: the mitochondria, the rough endoplasmic reticulum, and the Golgi complex. In all instances there was an increase in the abundance of these structures during protein synthesis.

The increase in mitochondria per cell in undoubtedly related to increased demands on the cell for energy production during protein synthesis. The abundance of the rough endoplasmic reticulum in all of these cells is well correlated with the concept that the amino acids are bound into the protein molecule at this site. It is thought that alignment of the rough ER precedes vesiculation and that the stacks of rough ER are indicative of a more actively protein-synthesizing cell. The vesiculation of the ER represents a later stage in this process. It is generally regarded that in pituitary secretory cells the protein

hormone is actually synthesized within the rough ER and transferred to the Golgi zone where it is bound into the secretory granule. The various sized granules seen within the Golgi vacuoles of these pituitary cells represents stages in the formation of the secretory granules. This process has been described for various secretory cells and appears to be a very general method of forming secretory granules.[4, 16, 18, 19]

The situation in the mouse melanoma cells is different from the pituitary cells in that the final protein (melanin) is not formed within the rough ER, but synthesis of the enzyme tyrosinase occurs there.[20] It is believed that the enzyme is transported through the rough ER to the Golgi zone and appears in the Golgi vacuoles possibly associated with the rod-like structures of stage 1 in the ontogeny of the melanin granule. At this point melanin biosynthesis begins and eventually the mature melanin granule is formed. The final formation of the melanin granule does not occur while within the Golgi zone but at different sites throughout the cytosome. This would account for the observation that various stages of melanization are not found within the Golgi zone.

Summary

The cytology of three types of cells synthesizing proteins has been studied and structural information on this process presented. Figure 16 is a diagrammatic summary of the interpretation of these results. The process appears to be similar in all of the cells studied. It is believed that the amino acids are linked into the protein molecule within the rough endoplasmic reticulum. The mitochondria supply the energy for this process. In the case of the pituitary cells the final protein hormone is synthesized and segregated into the cisternae of the rough endoplasmic reticulum and transported to the Golgi zone. Within the vacuoles of the Golgi the protein secretory granules are formed and they move out into the cytoplasm of the cell.

The rough endoplasmic reticulum of the melanoma cell is involved in the synthesis of the enzyme tyrosinase. This is transported to the Golgi zone and vacuoles are seen with small rods which are interpreted as the earliest stage in the formation of the melanin granule. This is stage 1 in the diagram of the melanin-producing cell (Figure 16). Melanin biosynthesis begins and gradually the vacuole is filled with melanin (stages 2, 3, 4, and 5) which ultimately results in the formation of the mature melanin granule.

ABRREVIATIONS

ER — Endoplasmic reticulum
GA — Golgi apparatus
M — Mitochondria
MG — Melanin granule
N — Nucleus
RER — Rough endoplasmic reticulum
RNP — Ribonucleoprotein
SER — Smooth endoplasmic reticulum
SG — Secretory granule

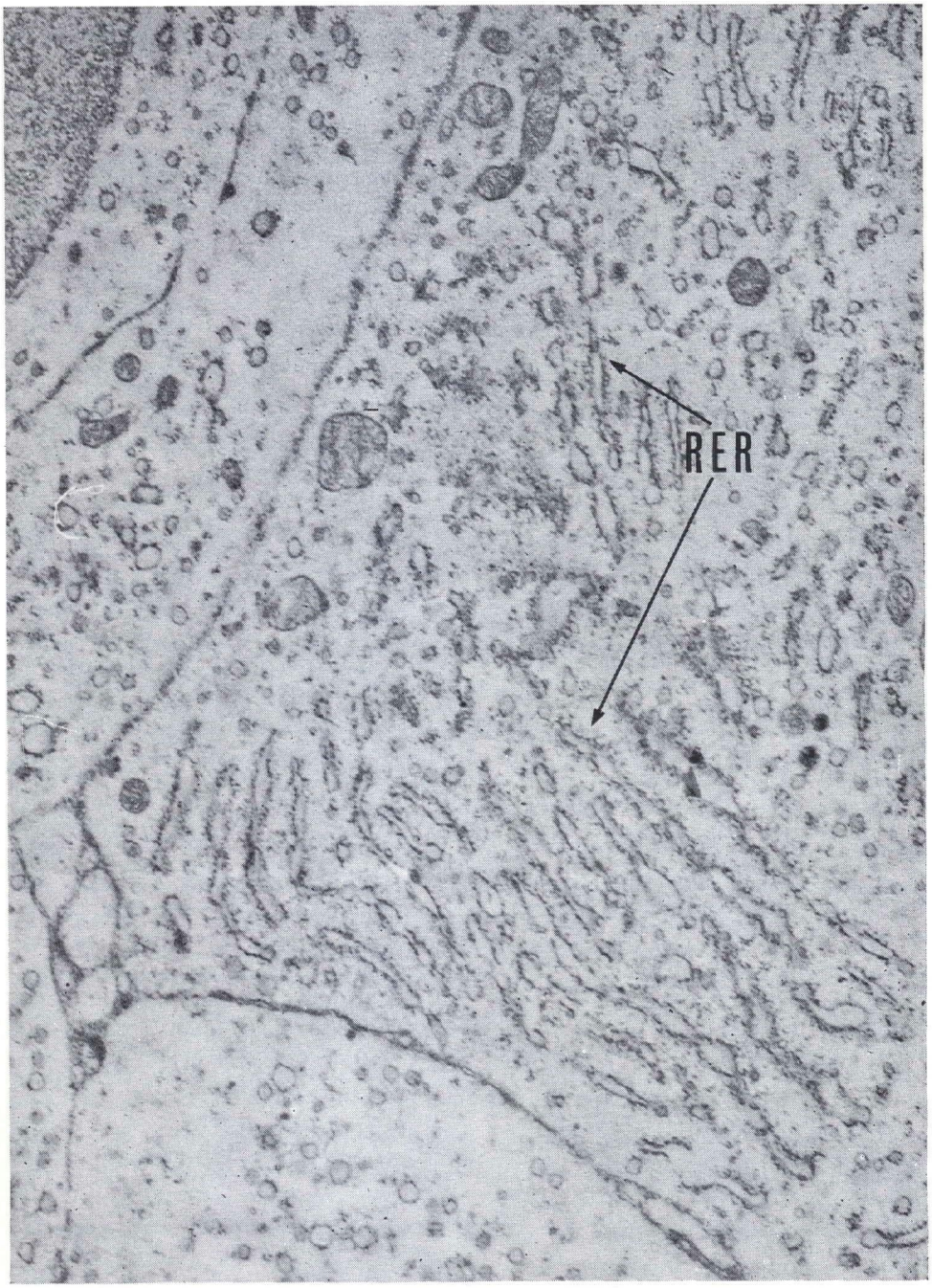

Figure 1

A portion of a human pituitary tumor cell illustrating the alignment of the rough endoplasmic reticulum (RER). Note the abundance of attached ribonucleoprotein particles to the ER. Magnification 18,000X.

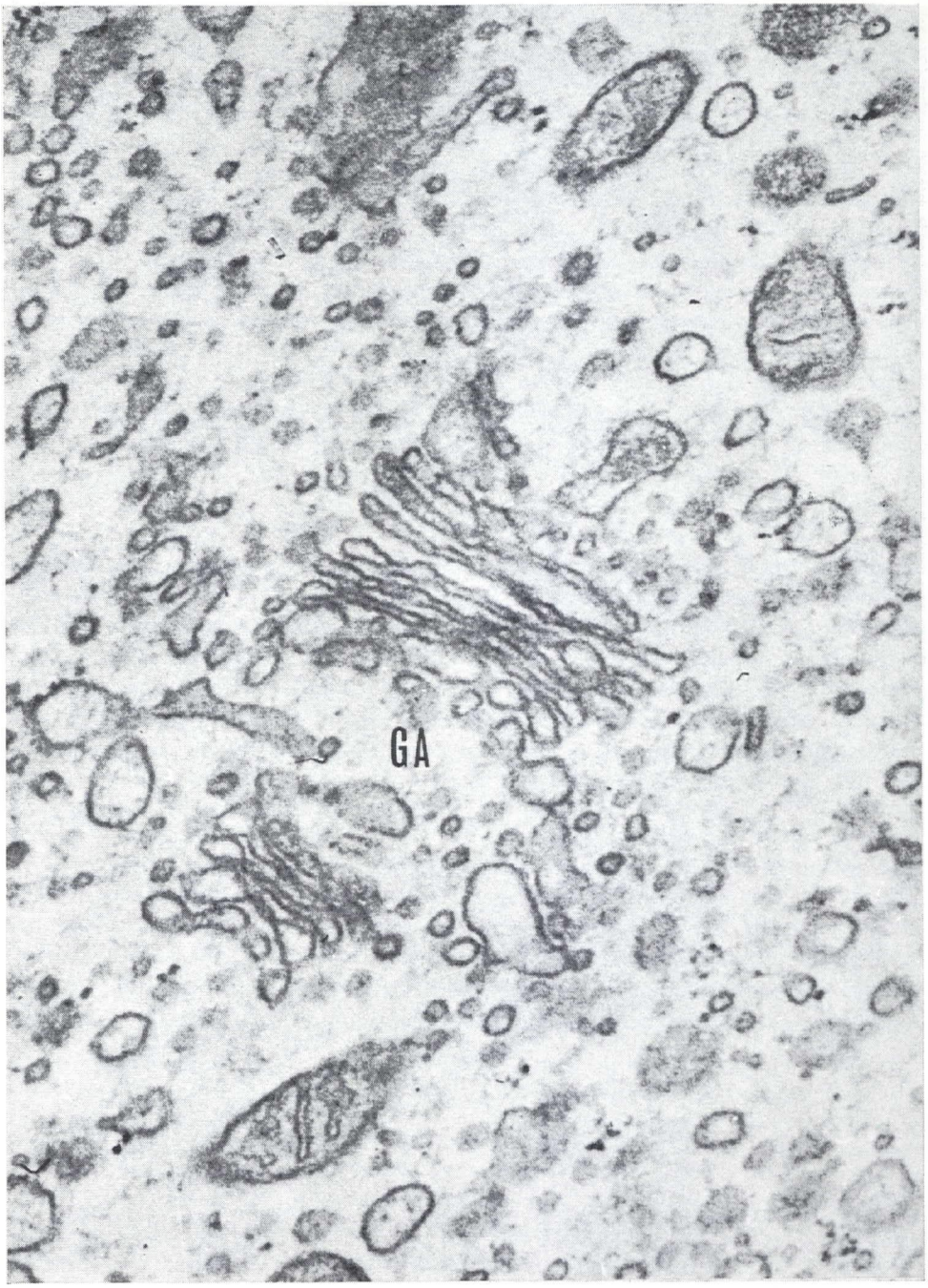

Figure 2

The Golgi apparatus in a pituitary secretory cell showing the flattened vacuoles and vesicles characteristic of this organelle. Magnification 65,000X.

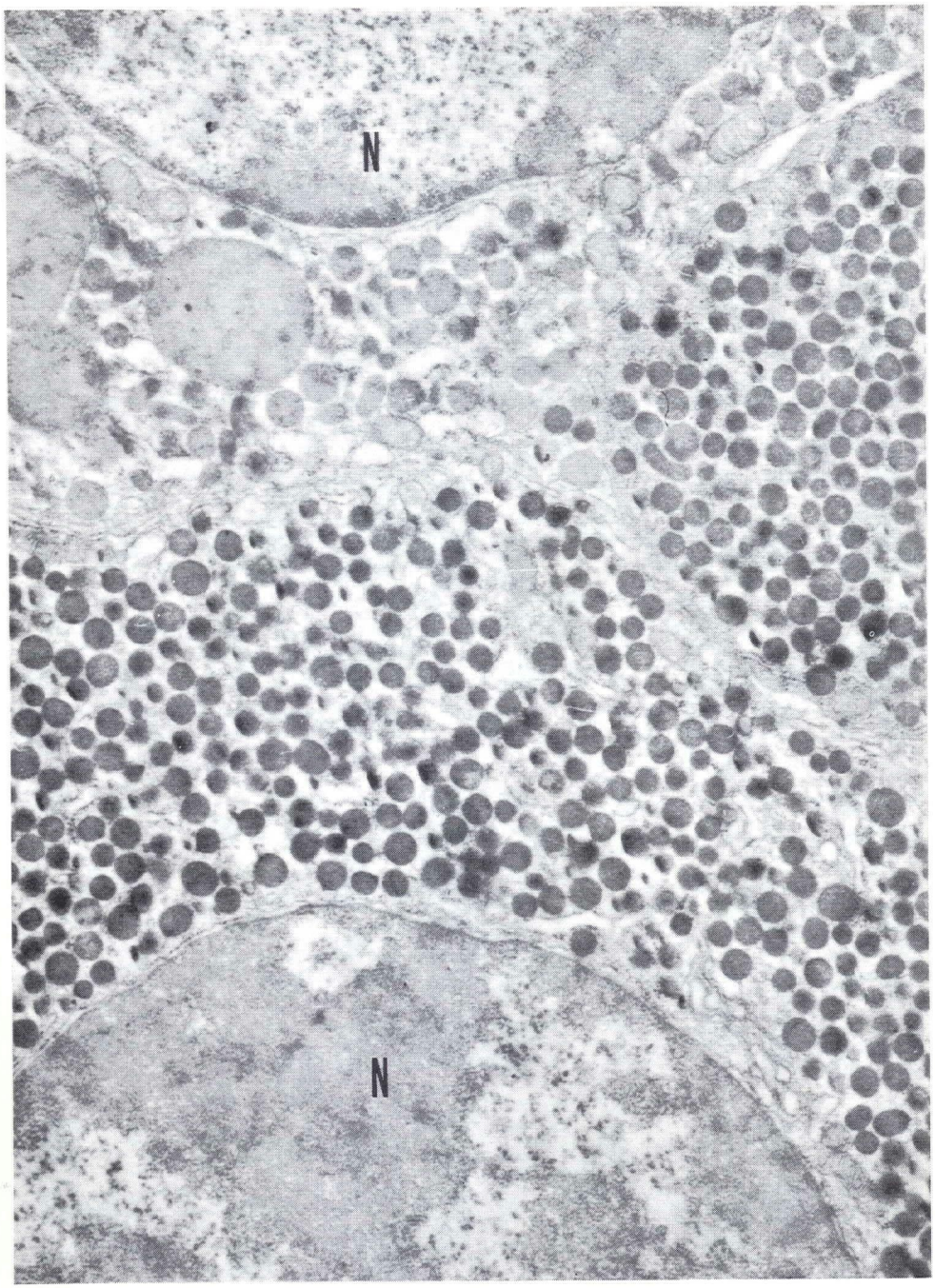

Figure 3

Three salamander pituitary secretory cells with three types of protein secretory granules. Magnification 24,000X.

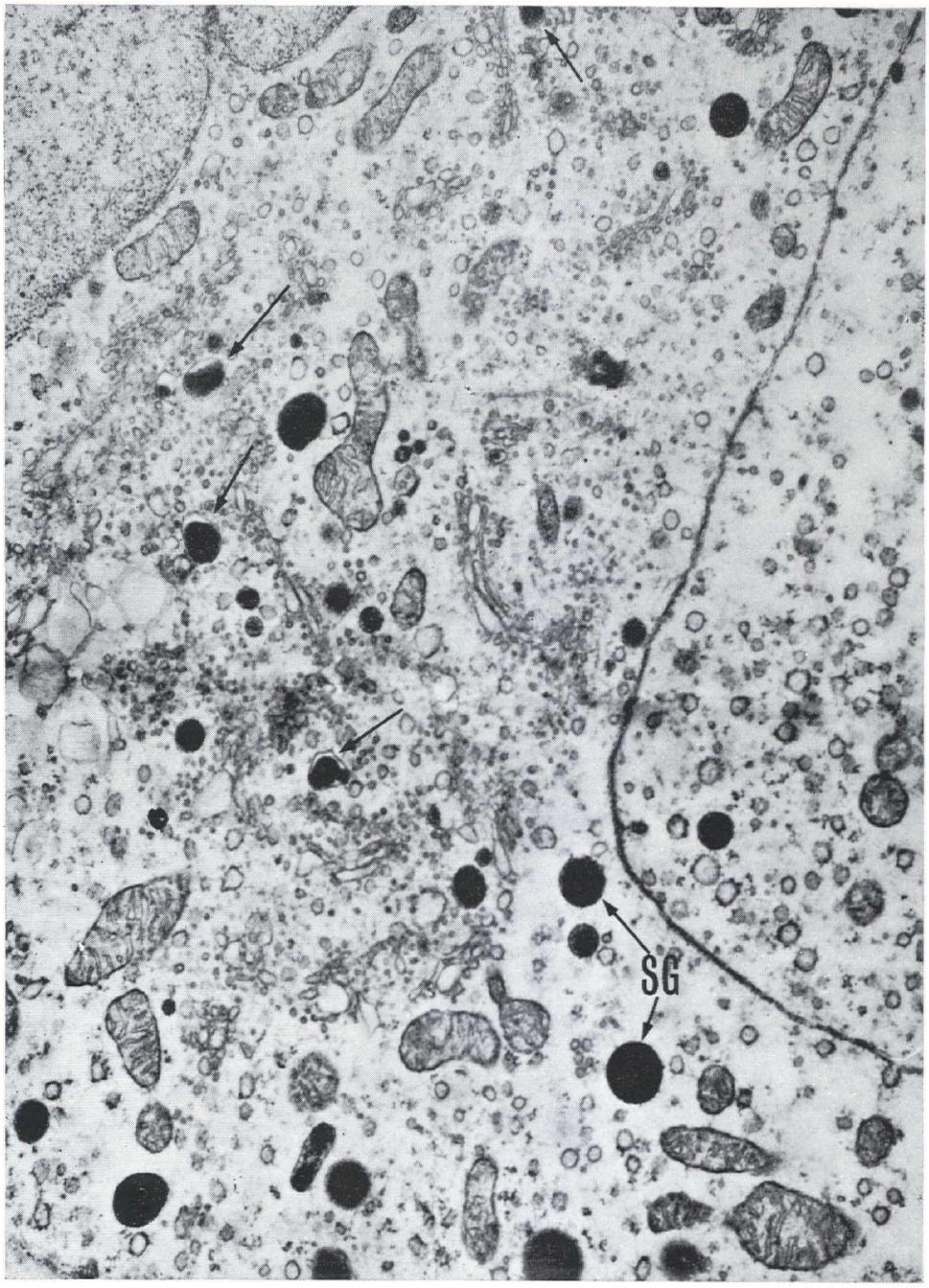

Figure 4

An electronmicrograph of human pituitary tumor cells with mature protein secretory granules (SG) and fomring secretory granules (arrows) within the Golgi vacuoles. Note the abundant mitochondria in this cell. Magnification 21,000X.

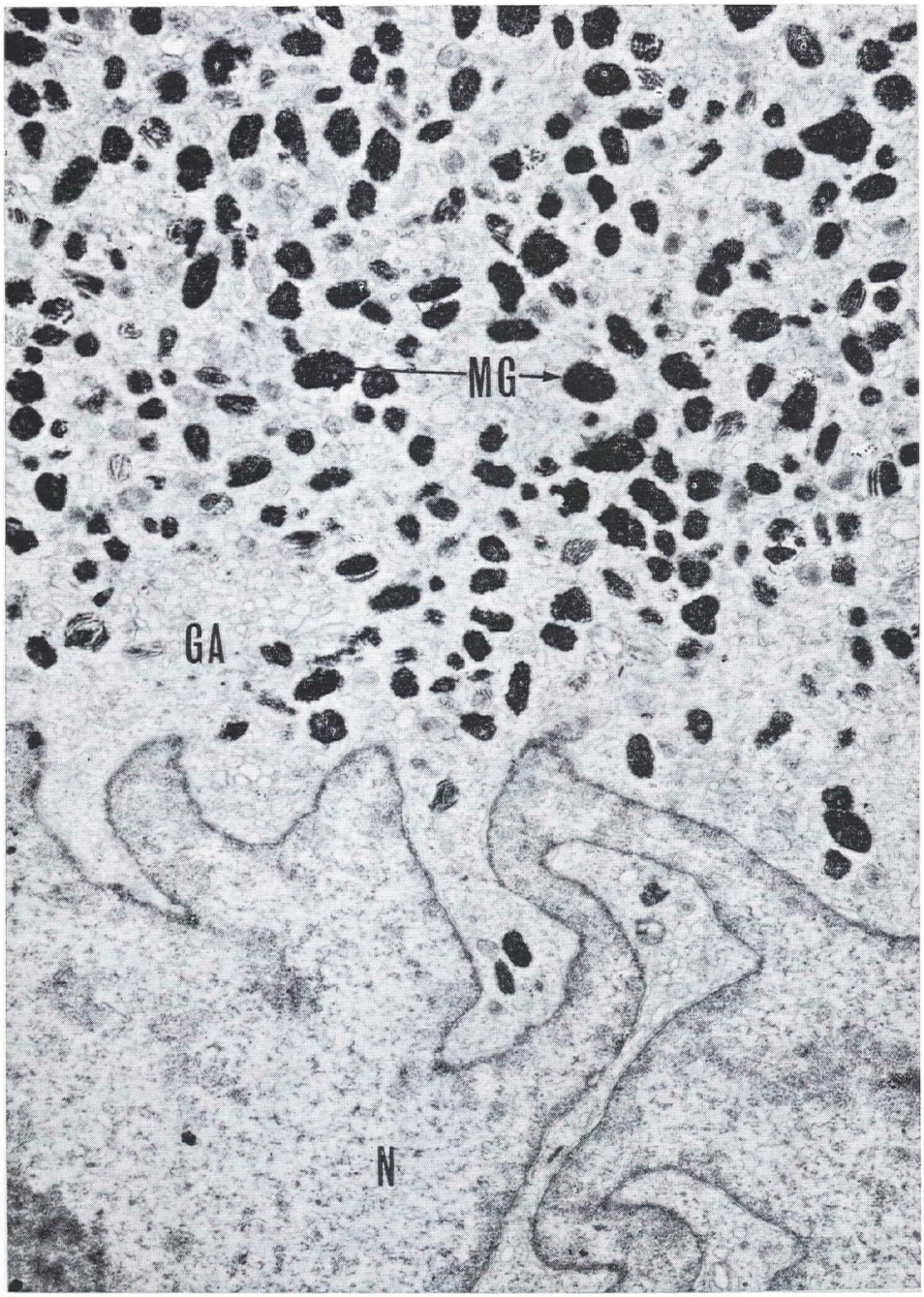

Figure 5

A portion of a B-16 mouse melanoma cell with mature melanin granules (MG) scattered throughout the cytoplasm. The rods of the forming granule are visible in some of the less-dense granules. These granules are in various stages of melaninization (see text). Note the Golgi apparatus (GA) near the nucleus (N). Magnification 13,500X.

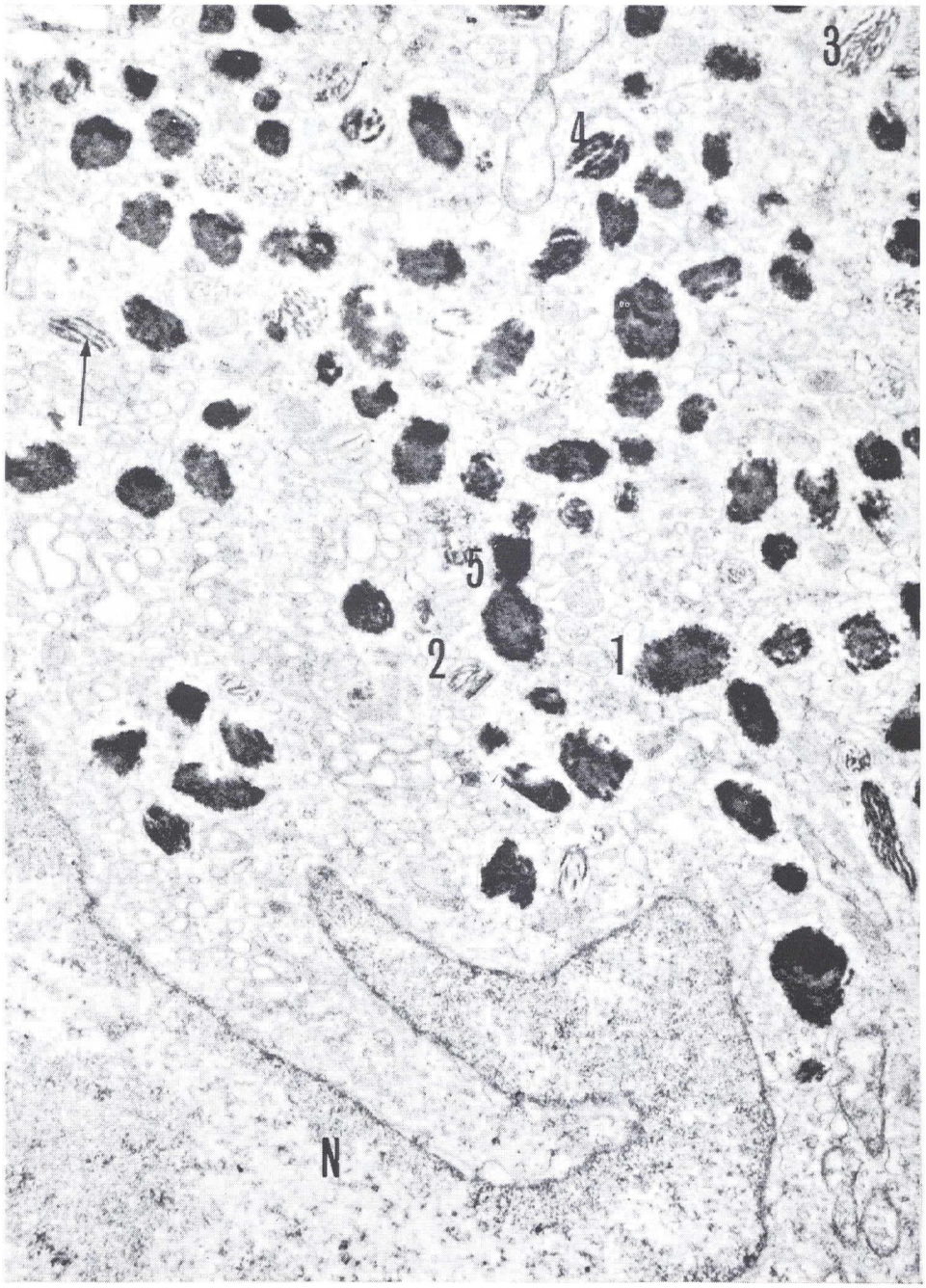

Figure 6

A mouse melanoma cell with various stages of melanin granule formation shown (1-5). Magnification 23,000X.

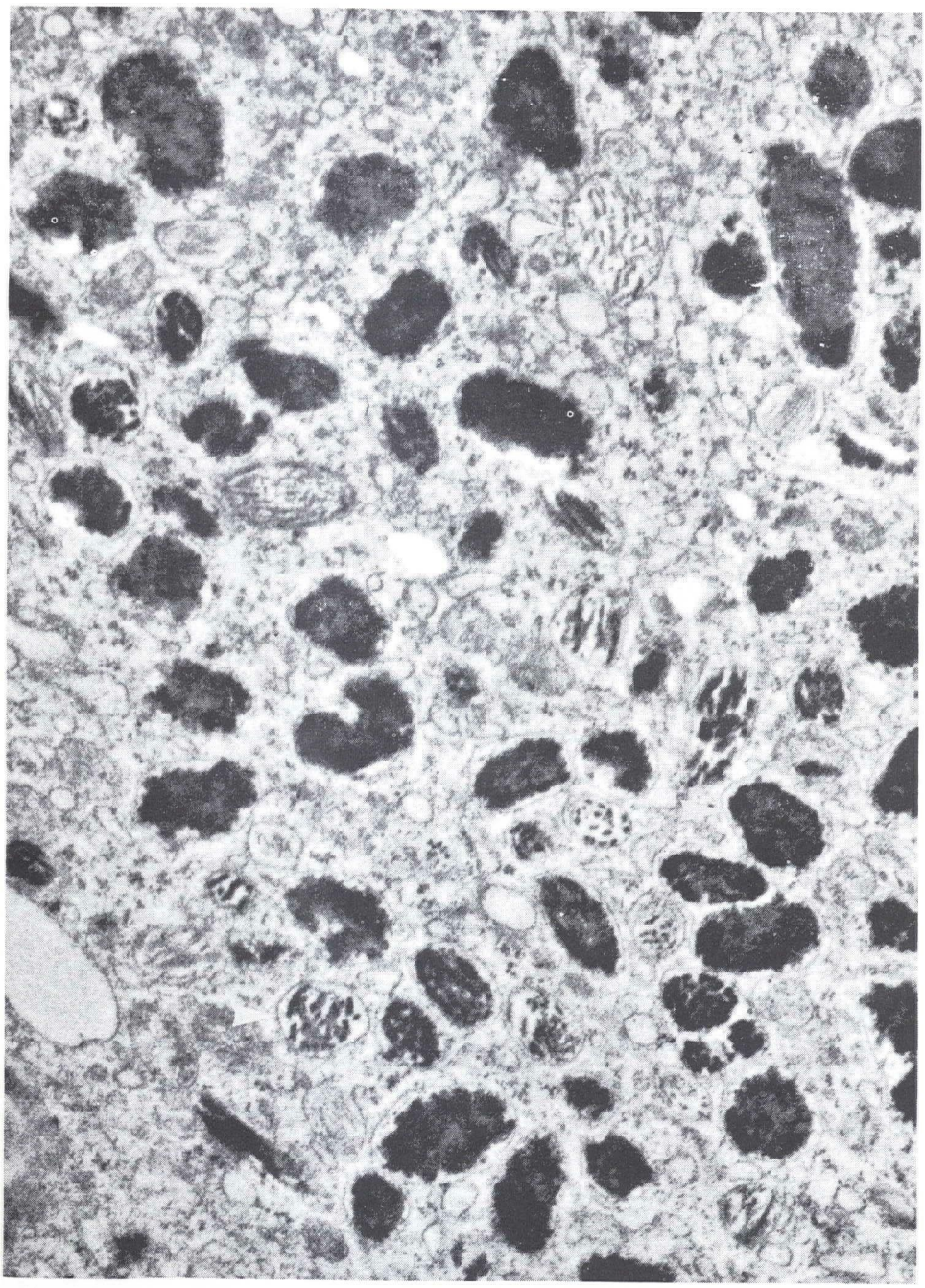

Figure 7

A higher magnification of the melanin granules showing both longitudinal and cross-sections of the granule. All of the granules are bounded by a membrane (arrows). Note the cross-sections of the rods. Magnification 35,000X.

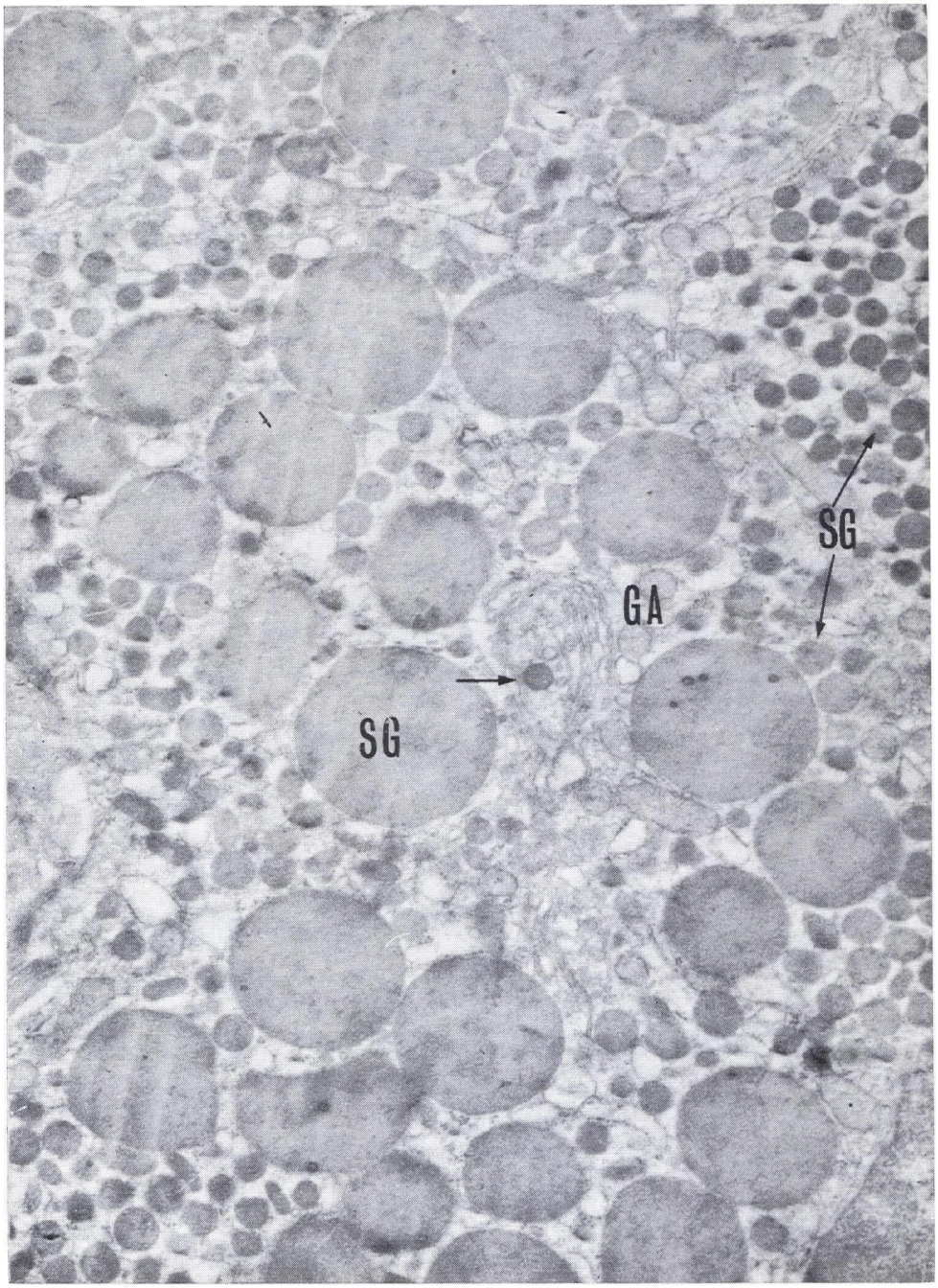

Figure 8

A section through the globular basophil of the salamander pituitary gland. Note the smaller type secretory granule (arrow) in the Golgi zone (GA). Magnification 19,000X.

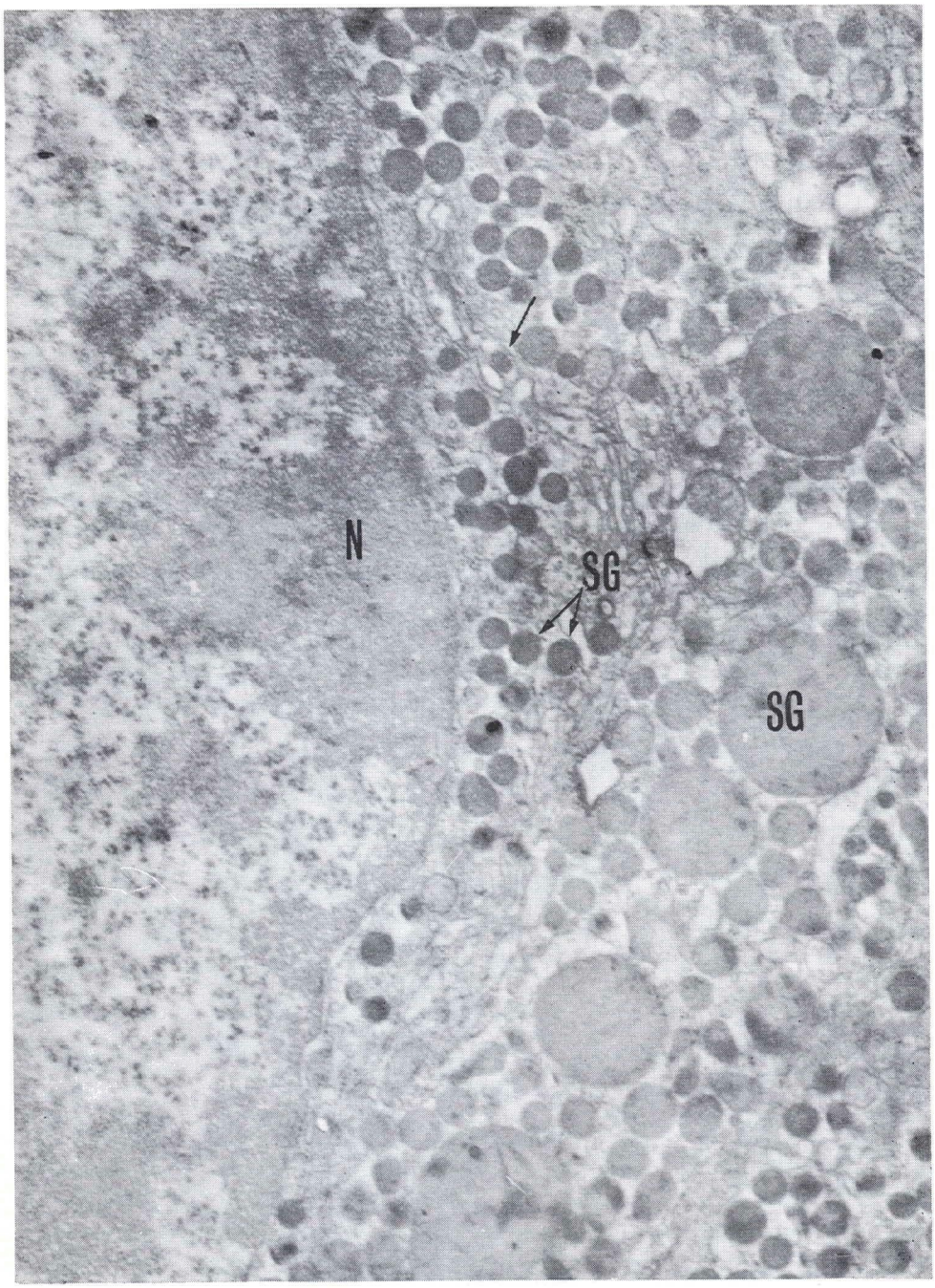

Figure 9

An acidophil and globular basophil from the salamander pituitary gland. Mature secretory granules (SG) of the acidophil are shown and a forming granule (arrow) is located within the cisternae of the Golgi vacuole. Magnification 21,000X.

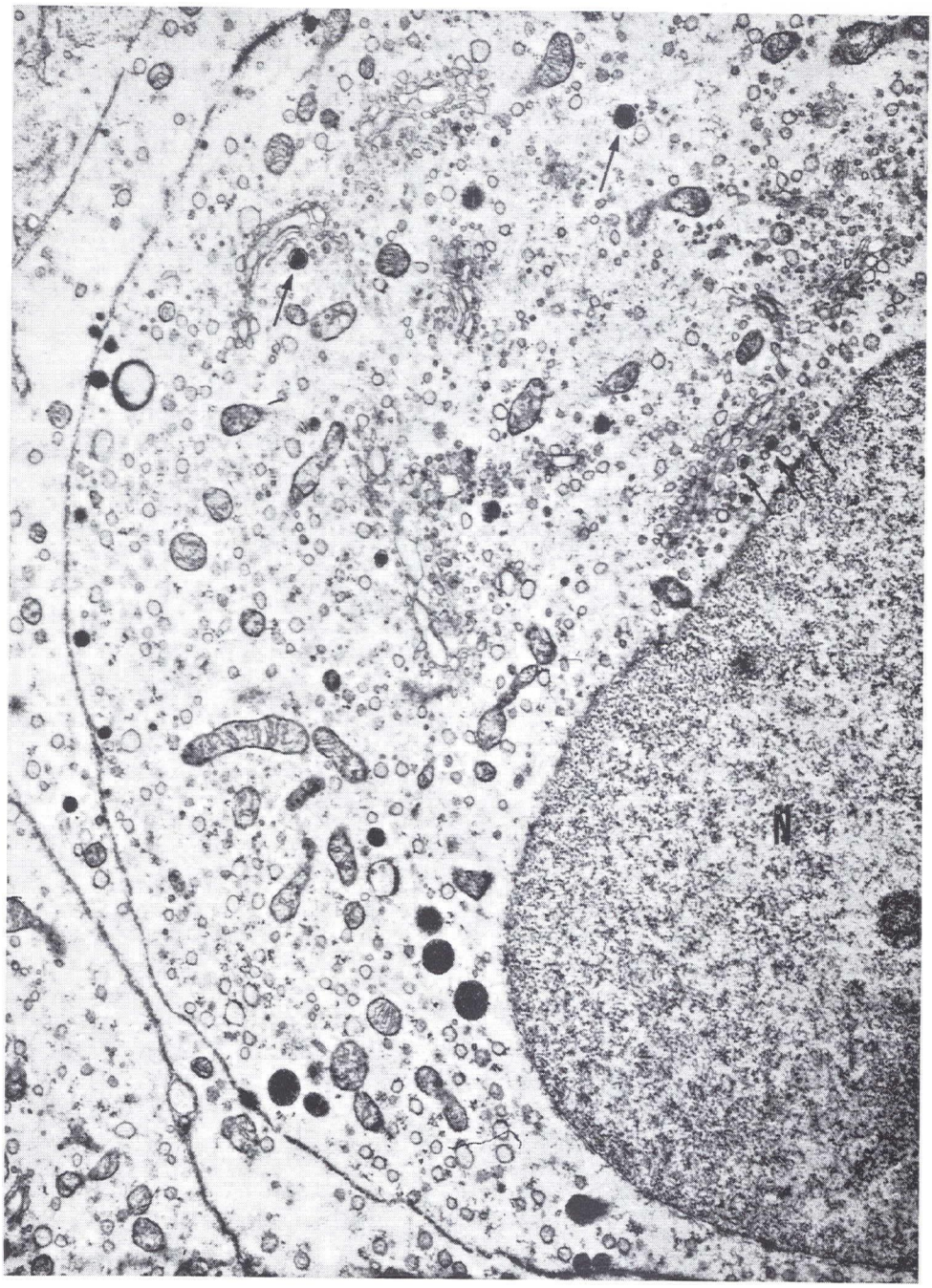

Figure 10

An electronmicrograph of a human pituitary tumor cell showing several forming secretory granules (arrows) in association with the Golgi of the cell. Magnification 15,000X.

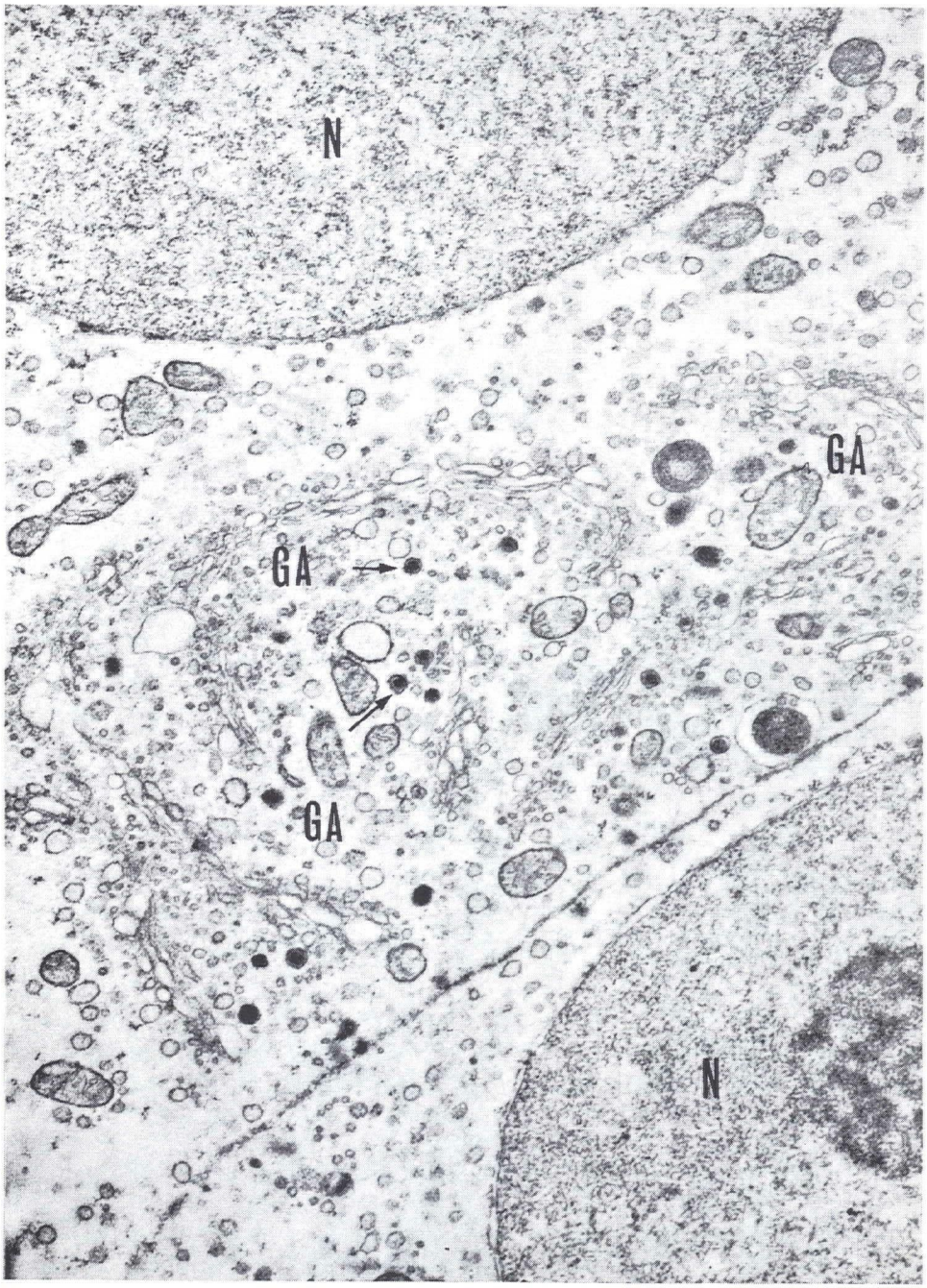

Figure 11

A section through two human pituitary tumor cells. Note the hypertrophied Golgi apparatus (GA) and the forming secretory granules (arrows). Magnification 21,000X.

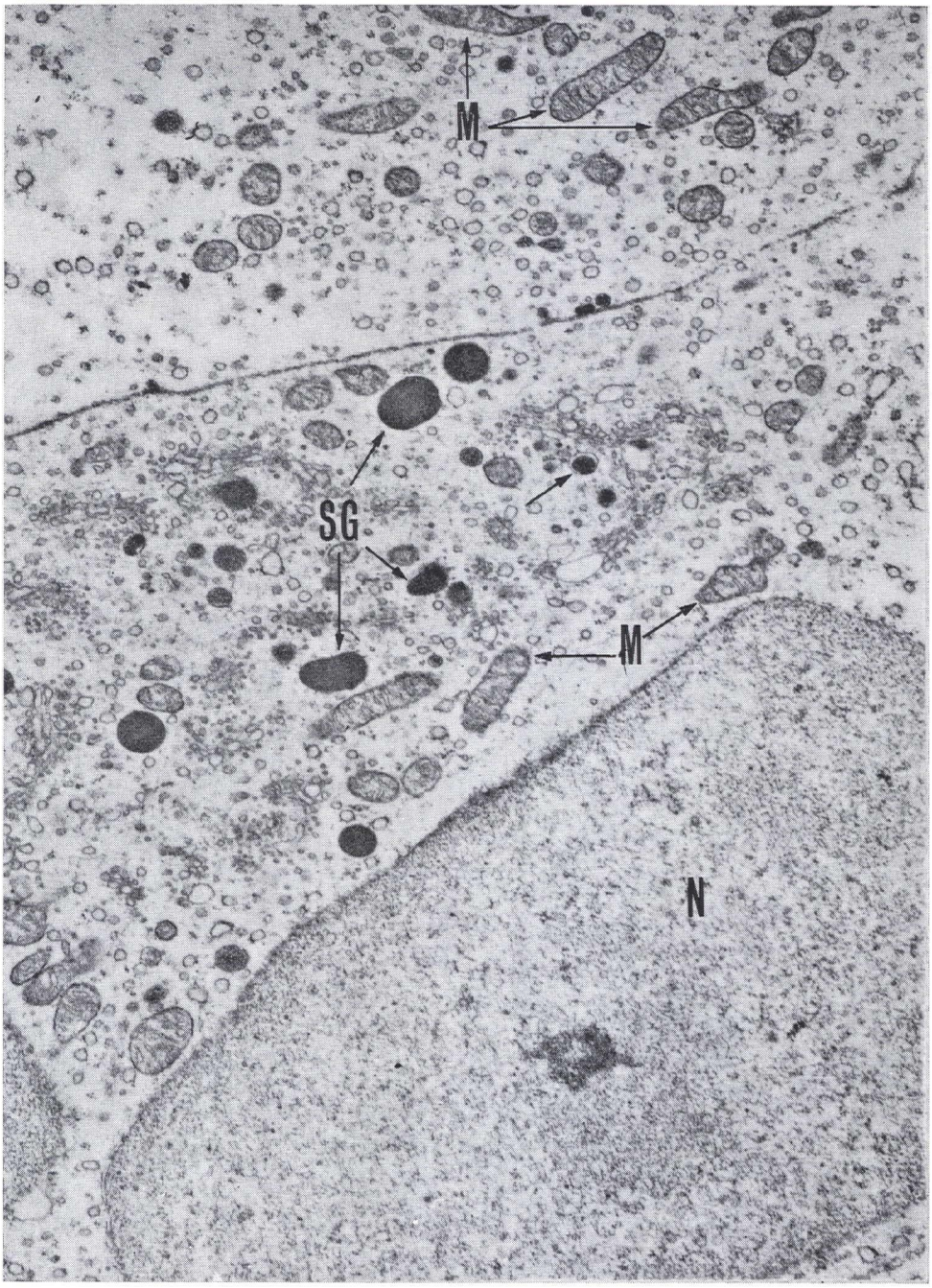

Figure 12

Several stages (arrows) in the formation of the mature secretory granules (SG) of a human pituitary tumor cell. Note the abundant mitochondria (M)). Magnification 15,000X.

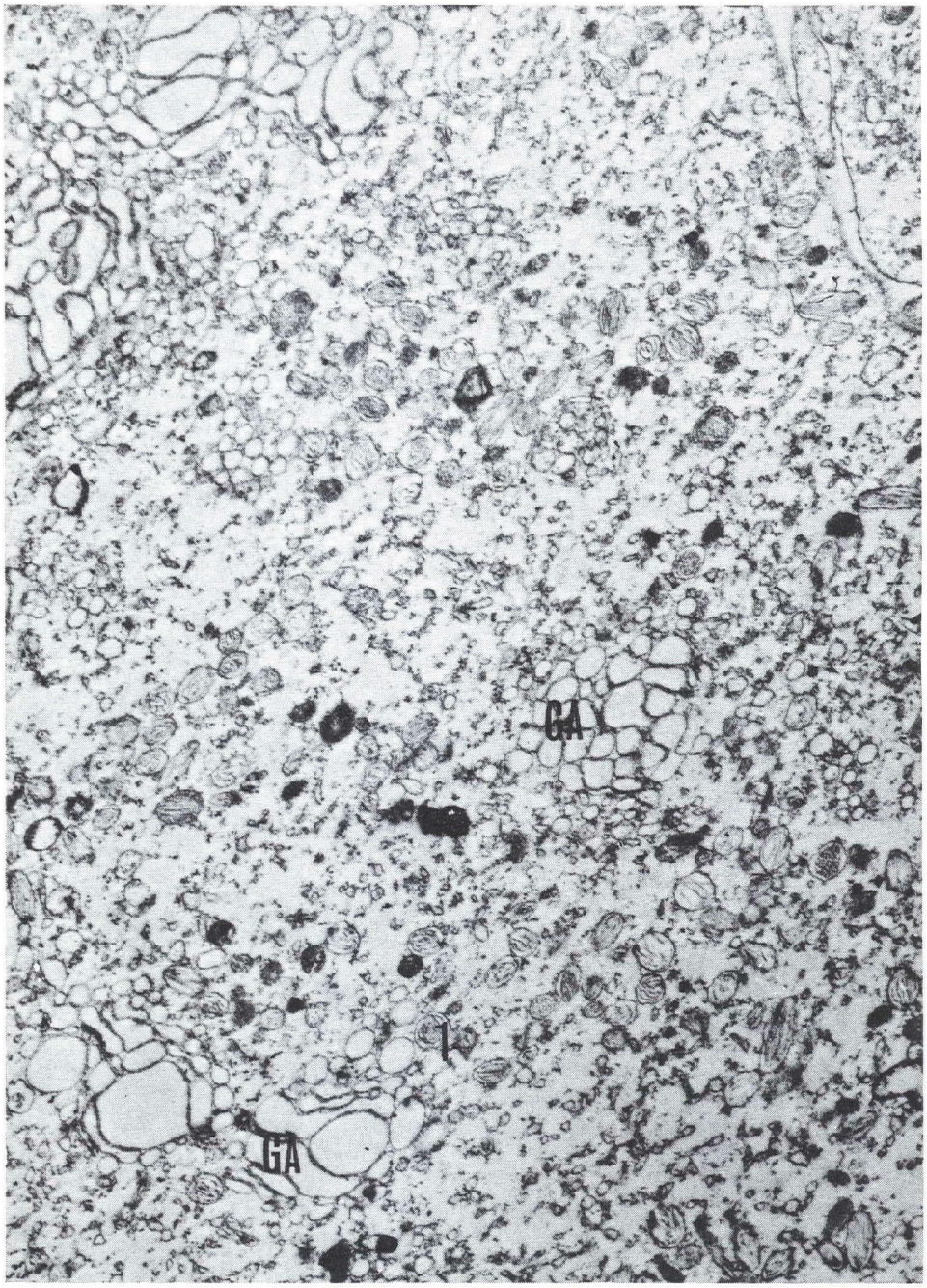

Figure 13

A mouse melanoma cell showing the early stages (1 and 2) of melanin granule formation. Note the hypertrophied Golgi (GA) of this cell. Magnification 20,000X.

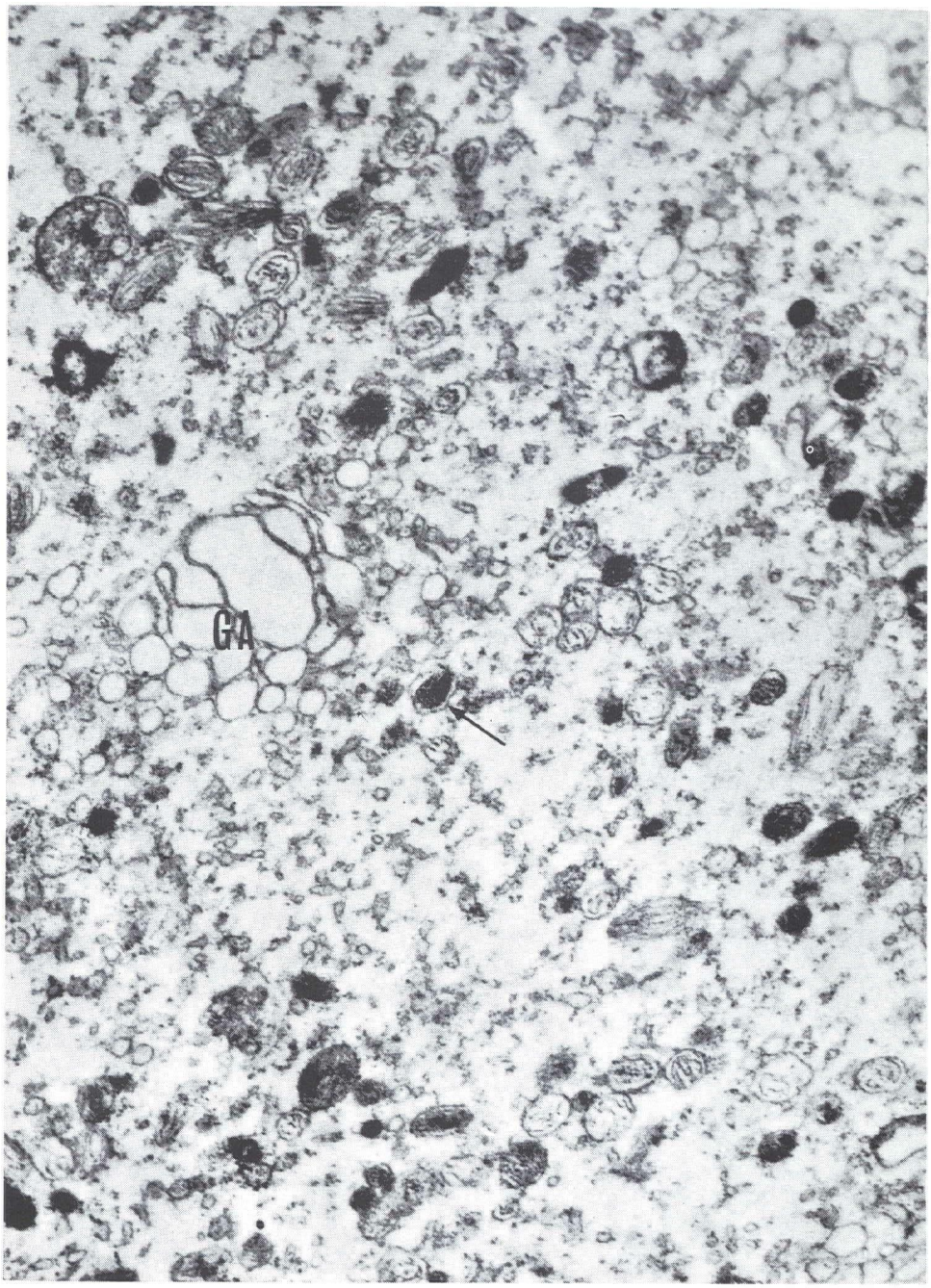

Figure 14

A cell showing later stages of melanin formation and association of melanin granule (arrow) with Golgi vacuoles (GA). Magnification 27,500X.

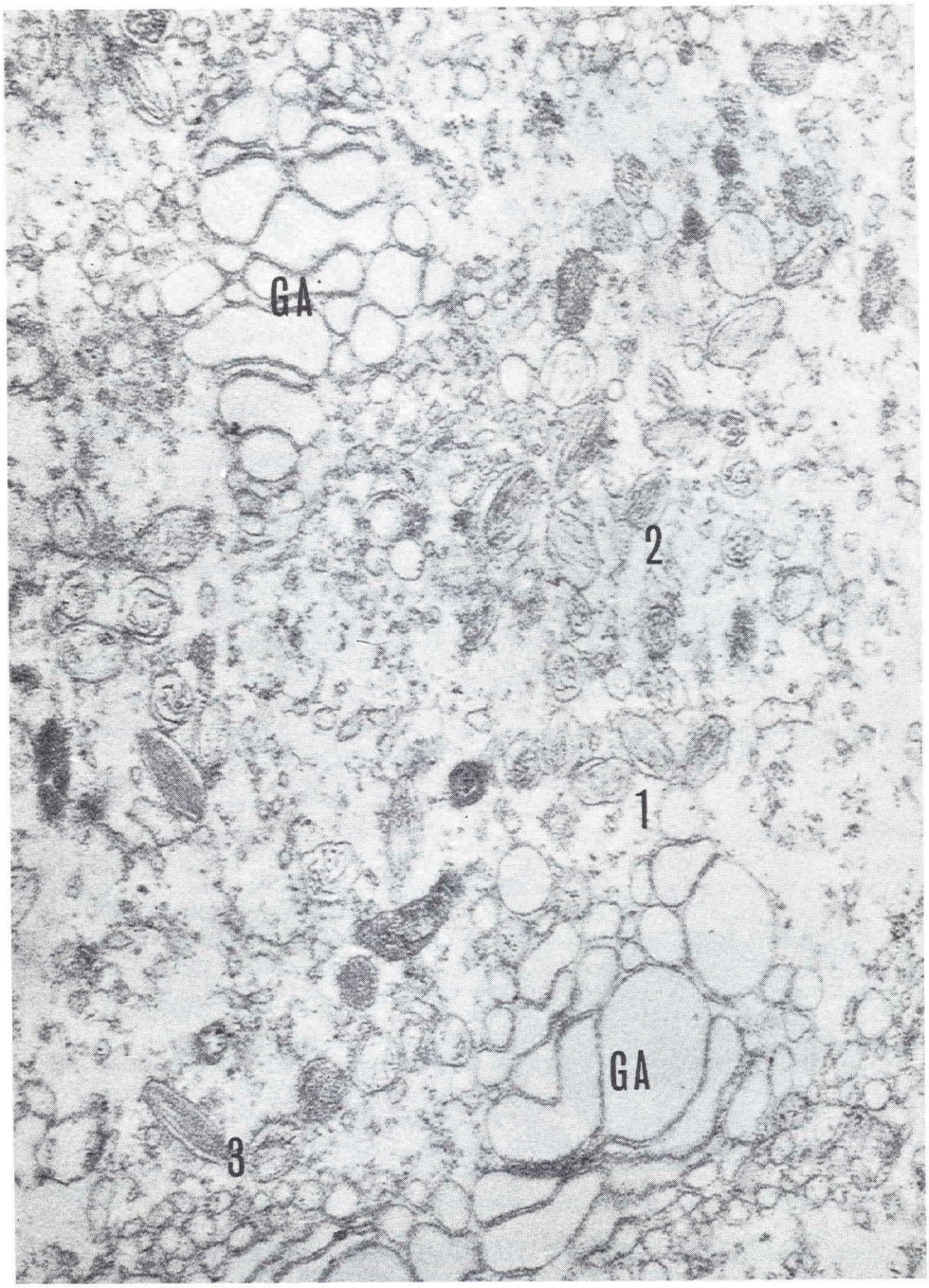

Figure 15

Higher magnification of the Golgi apparatus (GA) of a mouse melanoma cell and several stages of granule formation are shown near the Golgi vacuoles. Magnification 31,000X.

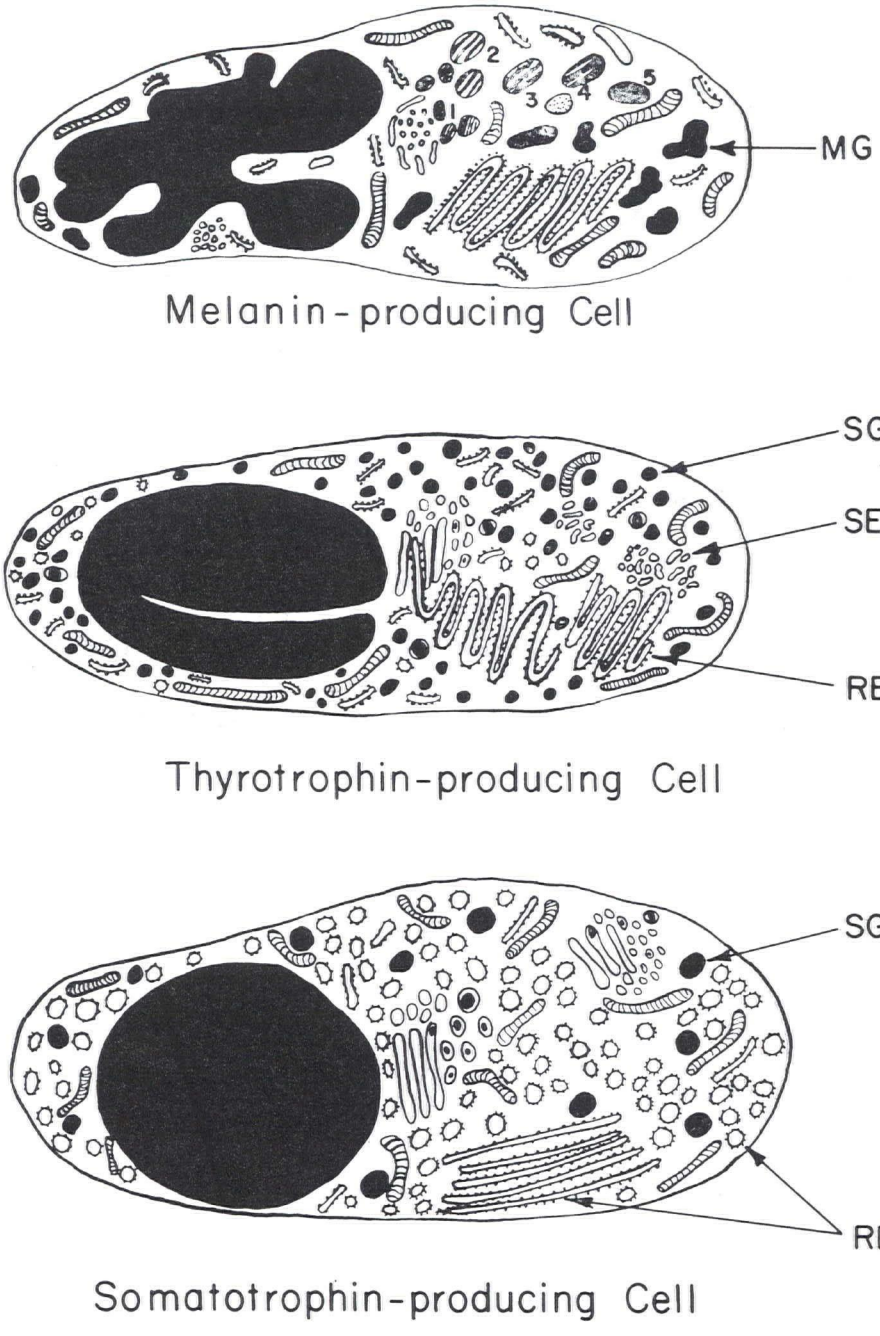

Figure 16

A diagrammatic summary of the cytology of these three types of cells during protein synthesis. See text for a discussion of this process.

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