Origin and Release of Secretory Granules in the Anterior Pituitary of the Mouse—An Electron Microscopic Study[1]

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Only three decades have passed since progress in physics provided for the development of the first electron microscope. With its commercial availability and the application of new techniques, cytology is making a resurgence that has been unparalelled since the Cell Theory revolutionized the study of biology. We find ourselves speaking in angström (Å) units, a level of measurement which unifies biochemistry and physiology into the framework of morphology.

The mitochondrion is no longer studied by the beginning biologist as a simple tiny rod or filament, but as an important organelle with its own characteristic structure. The Golgi complex is no longer a possible artifact of fixation, but an organelle consisting of paired lamellar membranes, clusters of closely approximated vacuoles or cisterne, and small vesicles and granules. The ergastoplasm is no longer an amorphous basophilic component of the cytoplasm. Electron microscopy has shown it to be composed of a loose aggregate of double membranes often vesiculated and containing 100 Å particles. These are essentially the organelles with which our study has been concerned.

The early electron microscopic work of Farquhar and Rinehart on the anterior pituitary of the rat proposed distinct morphological cell types to be associated with the principal trophic hormones (3). These authors noted that true chromophobic cells classed as such with the light microscope are few, for when studied with the electron microscope they contain variable numbers of granules of characteristic size. In addition, these cells have a Golgi complex which is either close to one side of the nucleus or lying separated from the nucleus in the cytoplasm. This location of the Golgi complex has been used by many workers to distinguish acidophilic from basophilic cells. Farquhar and Rinehart, and Hedinger were the first to identify cell types by the difference in size of the secretory granules they contain (6). Through experimental physiological variation seven distinct types of cells have been characterized as to function; three basophils, two acidophils, one undifferentiated basophil-like cell and the true chromophobes. The basophils are thyrotrophs with 100 mμ granules, or gonadotrophs of the F.S.H. or I.C.S.H. type with 150-200 mμ granules. The acidophils are the S.T.H. cells with 350 mμ granules or the L.T.H. cells with 600 mμ granules. The undifferentiated basophil-like cells have been associated with A.C.T.H. Our observations using the pituitary of the

[1] This research was supported by the Indiana Elks' Cancer Fund number 40-821-39, and the U. S. Atomic Energy Commission.

[2] Acknowledgment is gratefully made to Dr. L. E. Roth, Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois, for his aid while a guest research associate in his laboratory.

[3] Further acknowledgment is made to Bernetta Mason and Virginia Stott for their technical assistance.

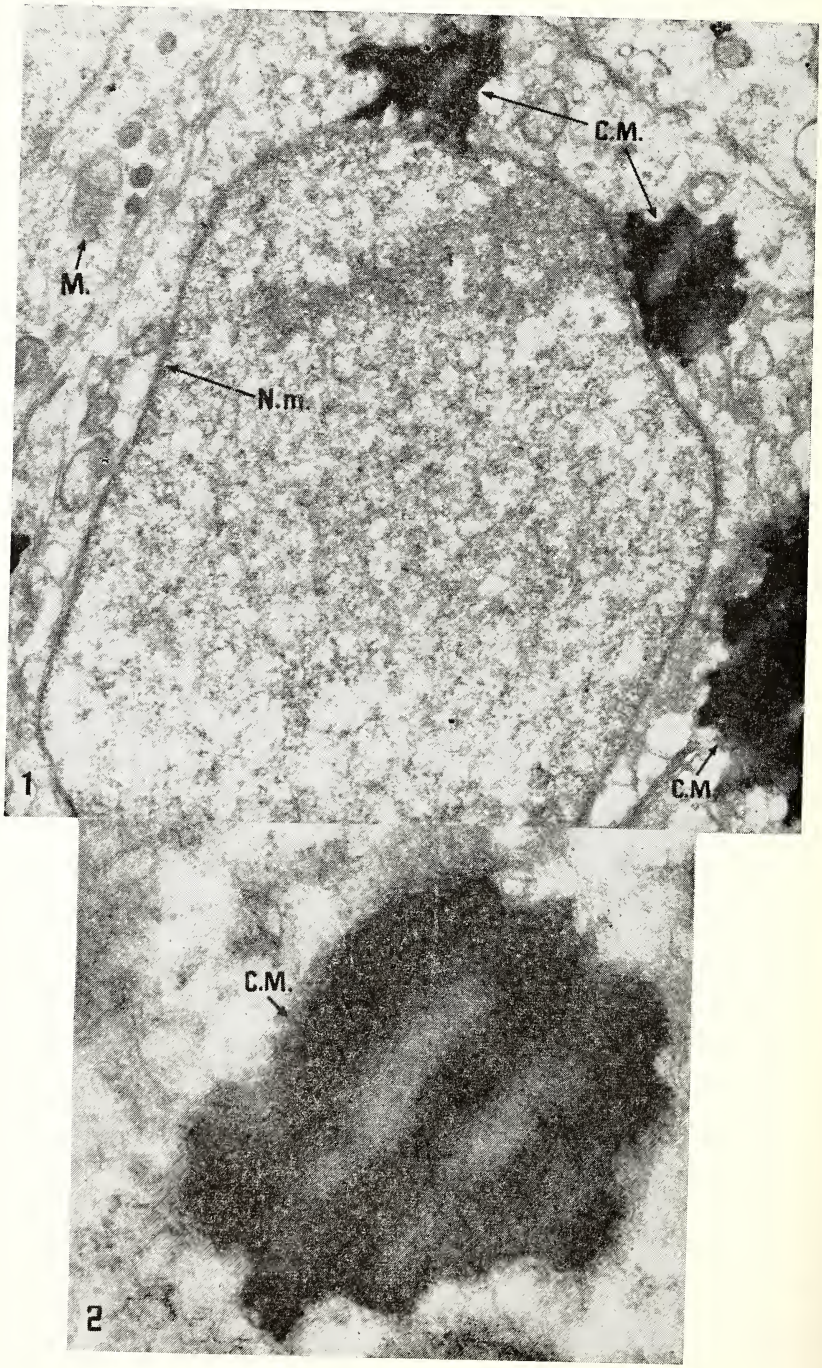
mouse and the recent study of others tend to support, at least in part, this morphological concept.

The site and mechanism by which a cell forms, segregates, concentrates, and extrudes its secretory product remains one of the basic unsolved problems of high resolution cytology (11). In an endeavor to gain insight into this secret of complex activity we have subjected the anterior pituitary of the mouse to various hormonal conditioned states. Alterations in the homeostasis of the animal by gonadectomy, thyroidectomy, inhibition of lactation and hormone administration have permitted observations on the characteristics of its cells not readily seen in an equipotential state. In mice studied at varying intervals of time after castration there is a progression of cytophysiological changes which are encountered in the anterior pituitaries (8). Until about 4-6 months there is an increased vesiculation of the endoplasmic reticulum of the gonadotrophic cells. The Golgi complex of these cells becomes more apparent than in the non-castrate control. The acidophils appear fewer, or at least less heavily granulated. With extended periods of castration numerous gonadotrophs contain a colloid of high electron density. The number of these colloidal masses increases steadily up to about 10 months castration. The size varies with the cell and often within one cell, especially in older castrates, more than one mass may be present. Since the appearance of castration basophils is obvious when these colloidal masses first appear it is easily established that the colloid is formed in gonadotrophic basophile (fig. 1).

High magnifications have suggested that these colloidal masses are formed by the coalescence of secretory granules. In the castrate mouse the lack of certain circulating steroidal hormones causes first an increased F.S.H. activity, evidenced by a high level in blood plasma. After about 4 months castration the gonadotrophs seem to be unable to release their secretory granules in a normal pattern causing what we refer to as "intracellular" secretion. In intracellular secretion the secretory granules coalesce, forming the colloid masses (8). In such an animal cycling of F.S.H.-I.C.S.H. levels ceases since the reciprocal gonadotropic-gonadal steroid relationship has been disrupted.

In the pregnant mouse there is a moderate increase in numbers of an acidophil cell type, which can be characterized by its content of very large secretory granules. At about the time of parturition, however, there is approximately a 100% increase in their proportion. In the 2-4 day lactating mouse the cell number has reached its zenith, nearly every field containing one or several of these acidophils. It has been repeatedly demonstrated that these acidophils, sometimes called pregnancy cells, are associated with the secretion of mammotrophic or lactogenic hormone. Experimentally, by preventing lactation, we have altered the degranulation of these cells (fig. 2). Our observations in these experiments have led to some interesting speculations, not only as to the release of secretory granules, but also to the formation of the secretory product of the cell. How might the ergastoplasm or endoplasmic reticulum, the mitochondria and the Golgi complex be vested in the phenomenon of cell secretion?

One of the most semantic controversies of cytophysiology is the involvement of the ergastoplasm in the biochemical processes of the cell. With the electron microscope the ergastoplasm has been described in


C.M.
M.
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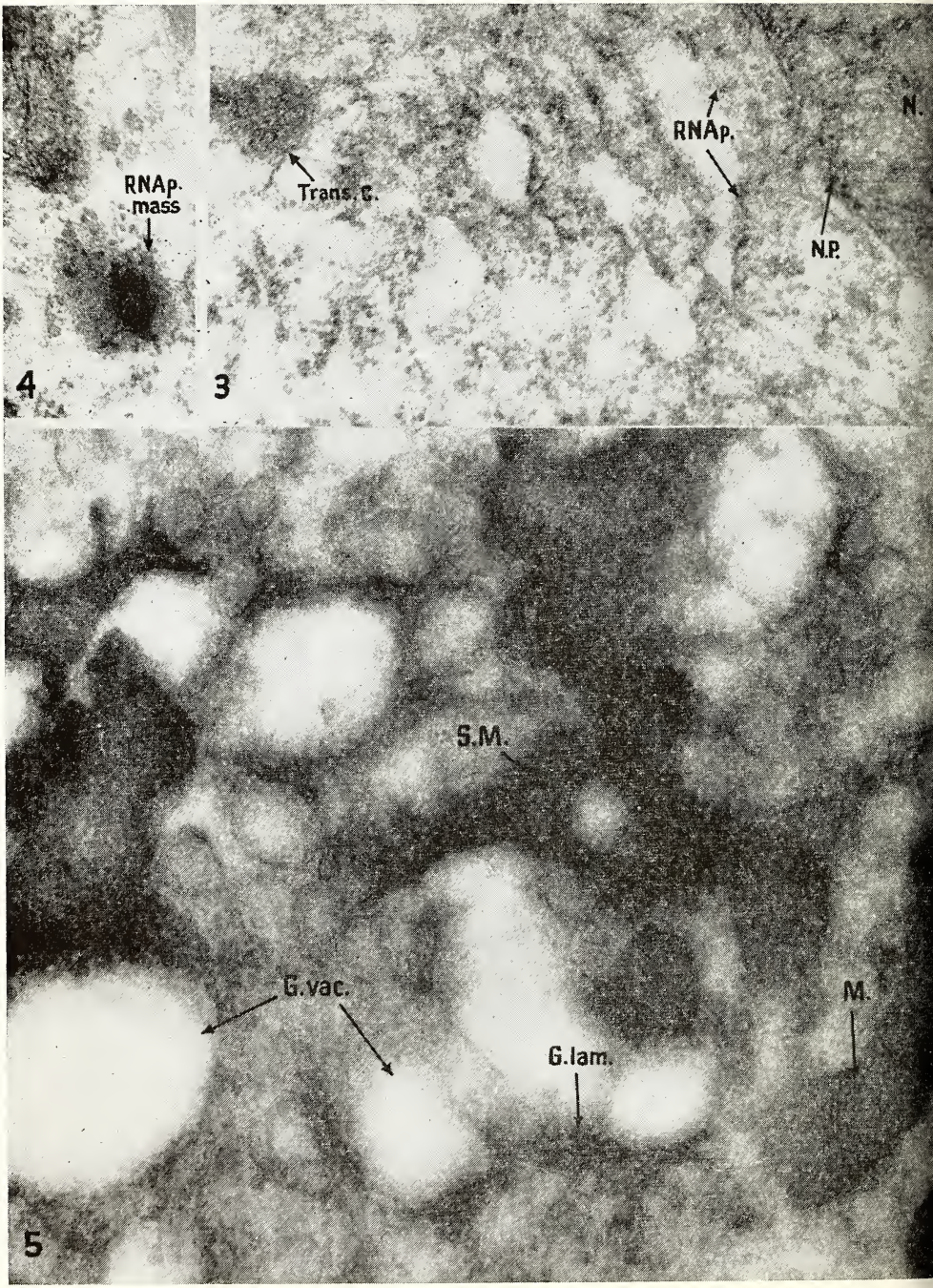
1
C.M.
2

numerous cells, appearing as a branching reticulum of the cell endoplasm. The term endoplasmic reticulum has been used by many investigators to denote such a system. In the cells of the anterior pituitary the endoplasmic reticulum may appear as a well formed organelle or it may show little organization into a reticular system. It would seem that the cells of the anterior pituitary are extremely sensitive to variations in tonicity. We have observed that chromophobic cells of basophilic or acidophilic classification have a relatively scanty reticulum which is smooth surfaced in profile. As the cell becomes larger and more differentiated the reticulum appears more organized and surface profiles are rough. Higher magnifications of the endoplasmic reticulum demonstrate that the roughness is due to the presence of small particles on the outer surface of the reticulum. As cell activities continue the endoplasmic reticulum becomes vesiculated with the particles somewhat larger and quite prominent. These particles are associated not only with the reticulum but with the nuclear membrane as well. A continuity of endoplasmic reticulum to nuclear membrane has been claimed by some authors, and our studies would lend support to this concept (7). In the cells of the anterior pituitary it would appear that these particles move along or with the reticulum to the nuclear membrane where they can be observed on both the cytoplasmic and nuclear sides. It would appear that these particles may diffuse through the nuclear membrane into the nucleus at any point in which they may come in contact.

Our attention to the changing physiologic states of the nucleoli in pregnancy cells would agree with the suggestion of Caspersson that the nucleolus is "a center" in protein synthesis. At the stage of particle diffusion the nucleoli of these cells are not markedly distinguishable. Shortly thereafter they become more electron dense, their striking change in density and their somewhat increase in size suggest a crux in the biochemical liability of the structure. Continued investigations into what we may consider the sequence of cell secretion suggest that the particles making up the nucleoli in some way, not yet morphologically understood, find their way to the pores of the nuclear membrane (9). These particles which on measurement seem to be somewhat larger than those particles which diffuse through the nuclear membrane pass through the pores into the ergastoplasm (fig. 3). In the ergastoplasm they may or may not show in profile a continuity with the reticulum. Numerous micrographs suggest that these particles coalesce in the ergastoplasm, forming homogeneous masses. Transverse cuts indicate that such masses can be directly associated with the reticulum or apparently free in the ergastoplasm. Preliminary histochemical observations on acidophils, cells which secrete a simple protein hormone, has suggested that here might be where ribosenucleoprotein becomes a templet in protein hormone synthesis (10), (fig. 4).

Fig. 1. Nucleus and surrounding cytoplasm of a gonadotrophic cell from a mouse castrated 4 months. Colloidal masses (CM) which are thought to be the coalescence of secretary granules. Mitochondria (M). Nuclear membrane (NM). X 36,000.

Fig. 2. Higher magnification of a colloidal mass. This mass was located in an acidophil from a mouse which was not permitted to lactate. Note the apparent similarity with those in fig. 1, though formed in different cell types under different physiological conditions. X 81,000.


RNAp.
mass
4
Trans. C.
RNAp.
N.
N.P.
3
S.M.
G.vac.
G.lam.
M.
5

A more dense homogeneous material has been observed in the Golgi zone of anterior pituitary cells. At this point it becomes important to remember that protein synthesis, the actual production of secretory material, and secretory granule formation are far from one and the same. The author believes they are parts of a biochemical continuum, the morphological associations which we hope to elucidate further. The accumulation of the apparently unbound homogeneous electron dense material within the Golgi complex supports the concept that the area is directly involved in the secretory processes of the cell. It has been suggested by numerous investigators of various tissues, that the Golgi complex appears to be involved in secretory granule formation (4). The existence of secretory granules surrounded by Golgi membranes in the cells of the anterior pituitary has been viewed in our laboratory, and reported earlier by Farquhar. Our findings have suggested that a major step in protein synthesis takes place in the ergastoplasm, and that it is directly associated with the coalescence of "microsomal" particles. The presence of particles different in size and texture found only in the Golgi complex are believed to be involved with the actual production of secretory material (5). The vacuoles or cisterne, as suggested by Dalton (1), are probably involved in the condensation of the secretory material. The orientation of mitochondria with densely packed cristae toward the Golgi complex suggest that they are involved in the maturation of secretory material (2); a process that would undoubtedly require high energy bonds. The finding of homogeneous electron dense material within the Golgi complex suggests a material ready to be packaged and extruded from the cell (fig. 5). The observations of Golgi membranes virtually cutting into and surrounding portions of this material suggests the formation of secretory granules.

Another variant we have met, aside from the location of the Golgi complex, is that the form the complex assumes depends on the particular physiological state of the cell. We therefore believe that the Golgi complex is an important metabolic center in the secretory activity of anterior pituitary cells. Histochemical work has demonstrated that it is more than a packaging and wrapping station, but a center of vaguely understood complex activity. What determines secretory granule size may depend on the morphological location and structure of the Golgi complex. As a general rule the further away the complex from the nucleus the smaller are the secretory granules of the individual cell. It would seem that there is little association of secretory granule size to the molecular weight of anterior pituitary hormones. If an association does exist it is probably a biophysical one, in that those hormones essential to life (i.e. A.C.T.H. and T.S.H.) have smaller secretory granules permitting their

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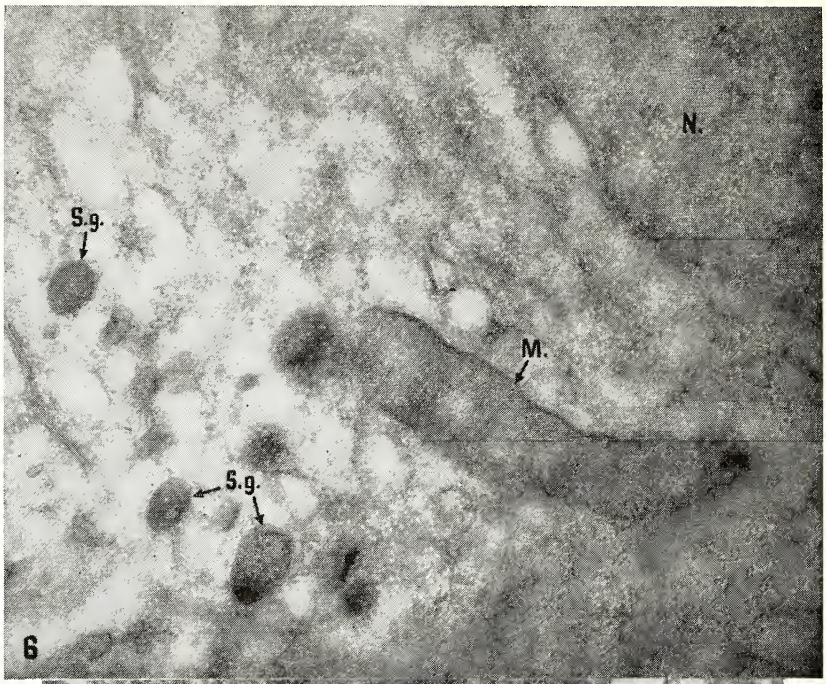
Fig. 3. Note the association of endoplasmic reticulum to nucleus by the nuclear pore (NP). RNA particles appear to be passing through the pore. Transverse cut of granules along the reticulum (Trans. C). X 144,000.

Fig. 4. A mass of RNA particles which appear to have coalesced in the ergastoplasm. X 144,000.

Fig. 5. Golgi complex of an anterior pituitary cell. The presence of homogeneous electron dense material within the Golgi complex suggests material ready to be packaged and extruded (SM). Golgi lamellae (G. Lam.). Golgi vacuoles (G. vac.). X 72,000.


N.
S.g.
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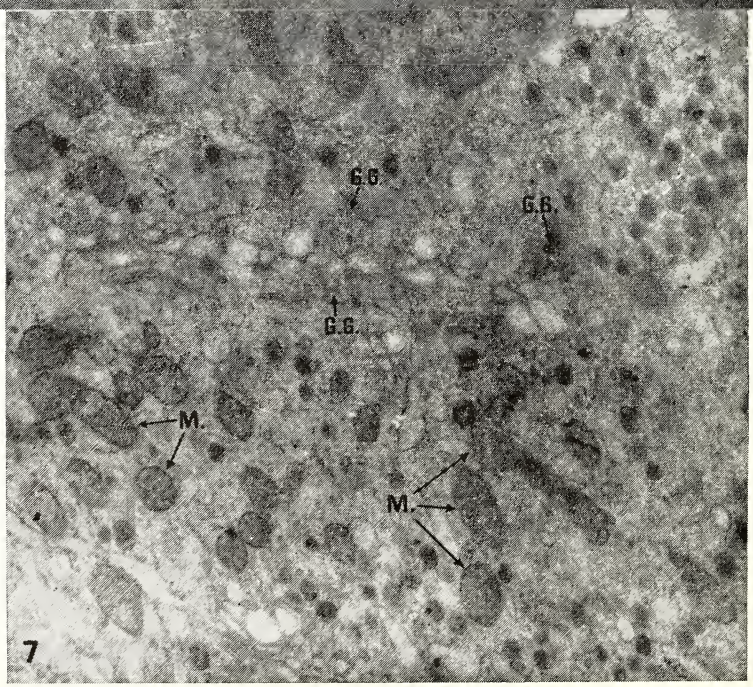
6


G.G.
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more rapid extrusion from the cell into the perisinusoidal spaces. It may be that individual secretory granule sizes are multiplies of hormone molecular weights (Fig. 6, Fig. 7).

It should be remembered that these declarations, regarding the formation and secretion within the gland, are hardly more than edified conjectures based on present experimental information. If these data prove our conjectures true, only much work and time will answer. As for conjecture, it is never worthless if it stimulates thinking. For as Dr. Jean Brachet, in his book Biochemical Cytology, states "Nowadays some scientists forget that thinking may sometimes be more useful than performing an experiment" (11).

Summary

The secretory processes of the anterior pituitary have been studied. Our observations have suggested that hormone production, as an example of the secretory process, involves the entire cell in a continuum of intergraded morphological and biochemical change.

Literature Cited

1. DALTON, A. J. and MARIE D. FELIX. 1956. A Comparative Study of the Golgi Complex. J. of Biophys. and Biochem. Cytol. **2**: 79-84.
2. DEMPSEY, E. W. 1956. Variations in the structure of mitochondria. J. of Biophys. and Biochem. Cytol. **2**: 305-312.
3. FARQUHAR, M. G., and J. F. RINEHART. 1954. Electron microscopic studies of the anterior pituitary gland of castrate rats. Endocrin. **54**: 516-541.
4. FARQUHAR, M. G., and S. ROBERT WELLINGS. 1957. Electron microscopic evidence suggesting secretory granule formation within the Golgi apparatus. J. of Biophy. and Biochem. Cytol. **3**: 319-322.
5. HAGUENAU, F., and W. BERNHARD. 1955. L'appareil de Golgi dans les cellules normales et cancereuses de vertebres. **44**: 27-55.
6. HEDINGER, VON C. E., and M. G. FARQUHAR. 1957. Elektronenmikroskopische Untersuchungen von zwei Typen acidophiler Hypophysenvorderlappenzellen bei der Ratte. Schweizerische Zeitschrift für Allgemeine Pathologie und Bakteriologie. **20**: 766-768.
7. PALADE, G. E. 1955. A small particulate component of the cytoplasm. J. of Biophys. and Biochem. Cytology **1**: 59-72.
8. SMITH, R. E. 1959. B15. Light and Electron Microscopic studies of the anterior pituitary of short and long term castrate mice. Seventeenth Annual Meeting Electron Microscope Society of America.
9. WATSON, M. L. 1959. Further observations on the nuclear envelope of the animal cell. J. Biophys. and Biochem. Cytol. **6**: 147-156.
10. WOODS, P. S., and J. H. TAYLOR. 1959. Studies of Ribonucleic Acid metabolism with tritum-labeled cytidine. Lab. Invest. **8**: 309-318.
11. Biochemical Cytology. Jean Brachet. Academic Press, New York. 1957.
12. Frontiers in Cytology. Edited by Sanford L. Palay. Yale University Press, New Haven. 1958.

Fig. 6. Area of an acidophil showing mitochondria (M), secretory granules (Sg.), located in the cytoplasm close to the nucleus (N). X 72,000.

Fig. 7. The Golgi complex of pituitary acidophil at the stage when the nucleolus of the cell is quite dense. Note Golgi granules (GG), note also orientation of numerous mitochondria to the Golgi complex. X 38,000.