FURTHER STUDIES ON THE THETA CELL OF THE MOUSE ANTERIOR PITUITARY AS REVEALED BY ELECTRON MICROSCOPY, WITH SPECIAL REFERENCE TO THE MODE OF SECRETION

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ABSTRACT

Theta cells reported previously as a new cell type in the anterior pituitary of the mouse were examined with the electron microscope. This type of cell is distinguished by the presence of pleomorphic secretory granules, a characteristic arrangement of the rough surfaced variety of endoplasmic reticulum, a well developed Golgi complex, and an eccentrically located nucleus. The secretory granules are seen at first as small granules of low density within the Golgi vesicles. While they are within the Golgi vesicles they become larger and denser. Simultaneously they move from the proximal to the distal part of the Golgi region and finally emerge from the Golgi area as mature granules in the cytoplasm. Thus, secretory granules are always enveloped by a limiting membrane which originates from the wall of the Golgi vesicle. At the stage of granule-extrusion, the cell membrane fuses with the limiting membrane of the granules and openings in the cell membrane appear at the place of extrusion. The granules then appear to lie within inpocketings of the cell membrane. They lose their density within these inpocketings or within the cytoplasm and occasionally show fragmentation. After complete loss of density, the granules are extruded as amorphous materials to the territory outside of the cell.

INTRODUCTION

Previously, the author reported the existence of a cell type (17) in the mouse anterior pituitary and designated it "theta cell" following the terminology of Romeis (16). In light microscope studies (17), theta cells were characterized by an eccentrically located nucleus, a cap-like limb showing marked basophilia at the cell periphery, and an extensive supranuclear region suggestive of the Golgi zone. No secretory granules were detected. In histogenetic studies (25, 27), male mice were found to have few theta cells during their lifespan, but in females the theta cells began to appear at about 40 days of age and were always recognized after 60 days of age. Moreover, it was observed that this type of cell showed cyclic variations in number with the estrous cycle, being abundant in proestrus and estrus, scarce in metestrus and diestrus (17), and that pregnant and lactating mice possessed a great number of theta cells (8, 26). Together with the results of experimental studies dealing with ovariectomy (18) and follicle- (7) and lutein-hormone administration (19, 28), these data suggest that this type of cell may be a source of luteotrophic hormone.

In the present electron microscope study, the fine structure of theta cells was observed and their mode of secretion was established.

MATERIAL AND METHODS

The anterior pituitary specimens were obtained from mature non-pregnant, pregnant, and lactating female mice, which were killed by chloroform anesthesia. Fixation was carried out in 1 per cent osmium tetroxide solution adjusted to pH 7.3–7.5 with Palade's veronal acetate buffer (13), containing sucrose (1), at 0°–4°C for 1 hour. The specimens were then washed briefly with distilled water, dehydrated in a graded series of ethanol (50 to 100 per cent), and embedded in a mixture of polyester resins, Rigolac 2004 and Rigolac 70F, recommended by Kushida (10), and in epoxy resins following the methods of Kushida (9) and Luft (12). Polymerization was achieved by heating in an oven. Sections were cut on a thermal expansion ultramicrotome (JUM-4 type manufactured by Japan Electron Optics Laboratory Co., Tokyo) equipped with glass knives. Thin sections were mounted on copper grids covered with a collodion film and stained with uranyl acetate (21), lead hydroxide (22), or potassium permanganate (11). Electron micrographs were taken in a JEM-5G type electron microscope (Japan Electron Optics Laboratory Co.) at initial magnifications of 1500 to 9000 times and enlarged photographically.

OBSERVATIONS

Non-Pregnant Mice

The nucleus (Fig. 2) of theta cells contains a prominent nucleolus and is eccentrically located in the cytoplasm. The nuclear envelope consists of two parallel membranes enclosing a narrow space in between. Nuclear pores, formed by fusion of the inner and outer nuclear membranes, constitute the "pore complex" of cylindrical formations reported by Watson (23). The outer membrane of the nuclear envelope is continuous, in some places, with the component membranes of the endoplasmic reticulum studded with ribonucleo-

Explanation of Figures

Bm Basement membrane
D Desmosome
End Capillary endothelium
Er Endoplasmic reticulum (rough surfaced variety)
G_1 to G_7 Sequence of transition from granule-formation to granule-extrusion
Gol Golgi complex
M Mitochondrion
Mv Multivesicular body
N Nucleus
Np Nuclear pore
Nuc Nucleolus
RBC Erythrocyte

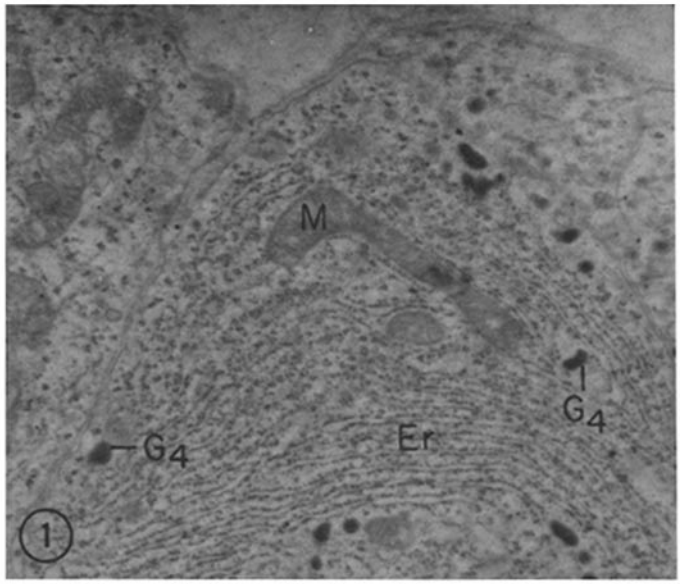
Figs. 1 and 2 are not parts of the same cell, but topographically such a combination represents the entire appearance of a theta cell.

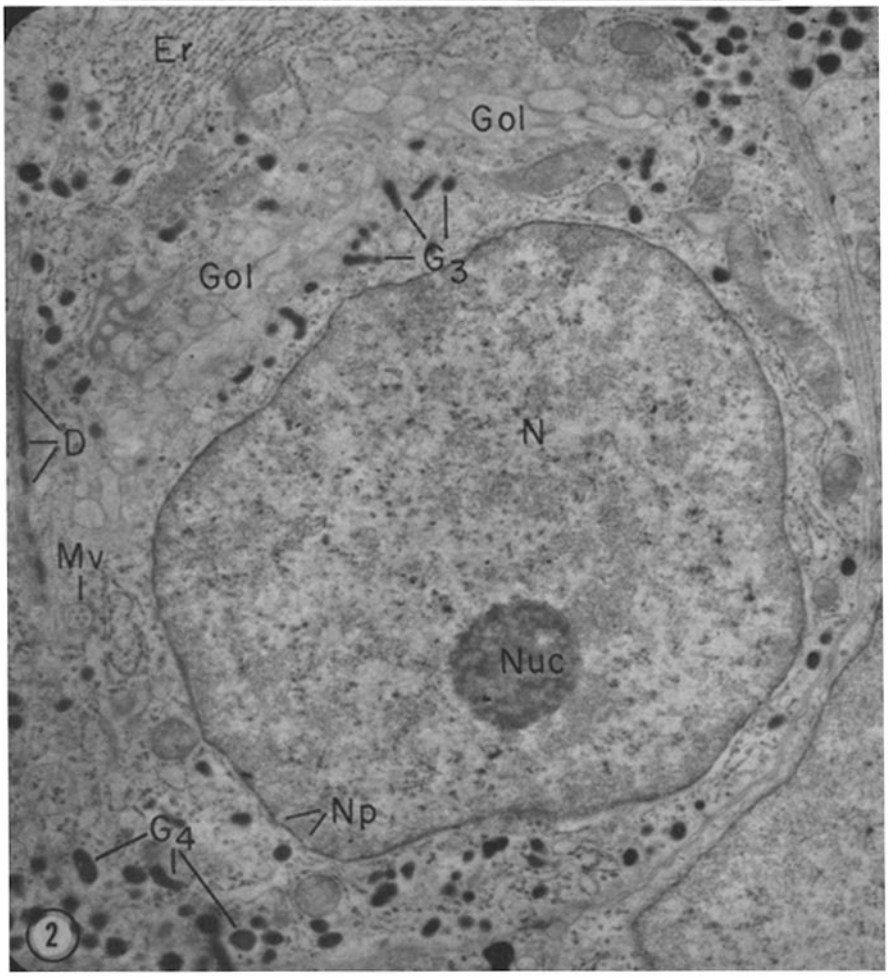
FIGURE 1

Piles of abundant membrane-bounded cavities of the endoplasmic reticulum (*Er*) are seen at the cell periphery. Paired membranes enclosing these cavities are studded with RNP particles on their outer surface. These flattened cavities are arranged parallel to one another at more or less regular intervals. Some mitochondria (*M*) and mature cytoplasmic granules (G_4) are inserted among them. From a non-pregnant mouse. Epon-embedded material. Stained with lead hydroxide. × 12,000.

FIGURE 2

A nucleus (*N*) with a prominent nucleolus (*Nuc*) is seen in its eccentric location. In the supranuclear region, the well developed Golgi complex (*Gol*) with its chain system of groups of vesicles is shown. Electron-opaque mature cytoplasmic granules (G_4) varying in form and size are found mainly at the cell periphery. Somewhat smaller and less dense granules (G_3) are gathered in the vicinity of the Golgi area. At the top left, parts of components of the endoplasmic reticulum (*Er*) are seen. Desmosomes (*D*) and a multivesicular body (*Mv*) are observed at the left side. Many nuclear pores (*Np*) are also seen. From a non-pregnant mouse. Epon-embedded material. Stained with lead hydroxide. × 12,000.


M
G4
Er
G4
1


Er
Gol
G3
Gol
D
N
Mv
Nuc
Np
G4
2

protein (RNP) particles, and the inner surface of the inner nuclear membrane closely faces a row of small dense particles in the nucleoplasm.

Theta cells show dense aggregations of the endoplasmic reticulum at the cell periphery corresponding with the basophilic region observed by light microscopy (Fig. 1). In this area, membrane-bounded cavities of the endoplasmic reticulum are very flattened and extend to distant areas. The contents of the cavities are of homogeneously low density and appear electron-transparent. A number of these flattened cavities are tightly piled upon each other, in parallel fashion, more or less at regular intervals. They are concentrically arranged with respect to the nucleus and are parallel to the cell surface. Since such parallel arrangements of membrane-bounded cavities of the endoplasmic reticulum, extending for long distances, are commonly observed in every section, it can be said that the limiting membranes of the cavities are of lamellar structure tridimensionally. Anastomosis and branching of these flattened cavities are occasionally encountered. Slight dilatations of the cavities are also observed frequently. Thus, round, oval, elongated, or cisternal profiles are seen as well as flattened vesicles. The limiting membranes enclosing the cavities of the endoplasmic reticulum are studded with RNP particles on their outer surface. Although RNP particles are generally attached to the outer surface of the walls of the endoplasmic reticulum, frequently they are found to be free within the ground-cytoplasm as individual particles or small masses of particles. Thus, the cap-like limb observed by light microscopy appears to be similar in many respects to the Nissl body reported by Palay and Palade (14) at the electron microscope level.

In the supranuclear region of the theta cell, a well developed Golgi complex is encountered (Fig. 2). The Golgi complex, as stated by Dalton and Felix (2, 3), consists of "Golgi lamellae," pairs of membranes enclosing extremely flattened cavities, and "Golgi vesicles" varying in size and form. A chain system of groups of Golgi vesicles is roughly arranged in the form of a horse-shoe or a crescent with which the Golgi lamellae are closely associated. These elements of the Golgi complex are embedded in "Golgi ground substance," named by Sjöstrand and Hanzon (20), which shows a somewhat higher density than the rest of the cytoplasm. The walls of the Golgi vesicles are not studded with RNP particles, in contrast to the rough surfaced elements of the endoplasmic reticulum. Although the contents of the Golgi vesicles appear electron-transparent and homogeneous in general, some vesicles contain small, round, or oval granules of low density (Fig. 3). These granules are ill defined and their size and density are slightly different from vesicle to vesicle. As a rule, each of these Golgi vesicles encloses a single small granule, but sometimes granules with several cores are found within a single Golgi vesicle.

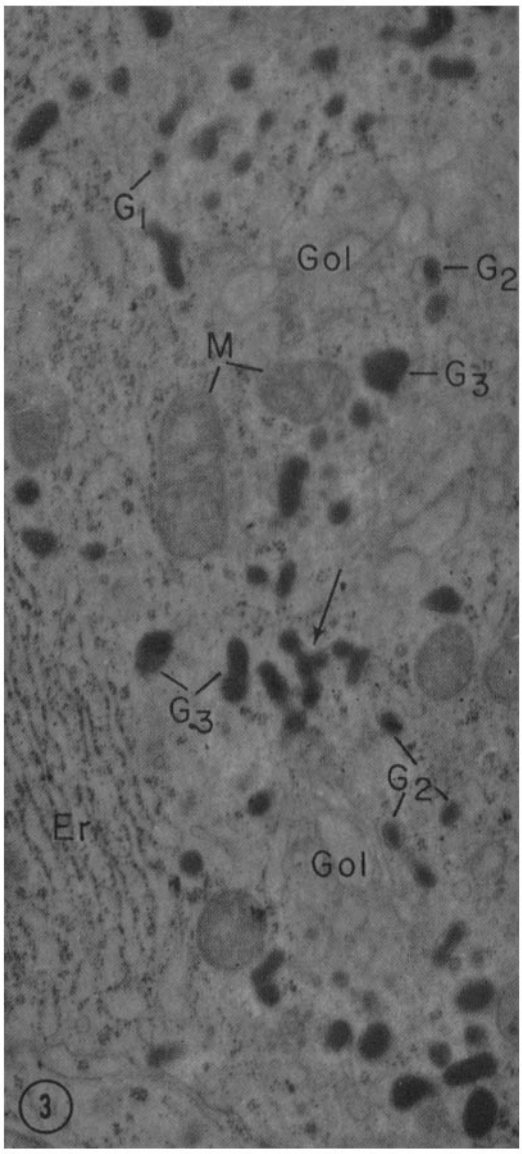
Theta cells have abundant cytoplasmic granules of this type. These granules show uniformly high density and vary considerably in size and form (Fig. 4). They are oval, rounded, triangular, rod-shaped, dumbbell-shaped, kidney-shaped, or irregularly shaped and their sizes range from 250

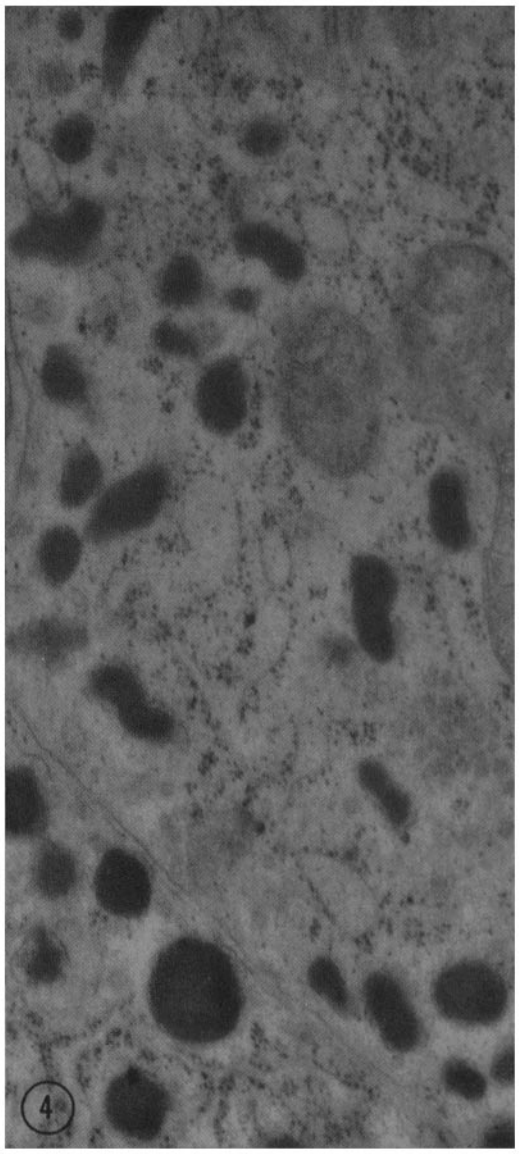
FIGURE 3

A number of Golgi vesicles of variable size are seen. Small (G_1) and medium-sized (G_2) granules contained within the Golgi vesicles are observed near the chain system of groups of the Golgi vesicles. Cytoplasmic granules (G_3) smaller in size and of lower density than mature granules are also numerous around groups of the Golgi vesicles. They are tightly enveloped by a limiting membrane. At the center, an irregularly shaped granule with several cores is seen (arrow). The granule is tightly applied to a limiting membrane. From a non-pregnant mouse. Epon-embedded material. Stained with lead hydroxide. × 22,000.

FIGURE 4

Pleomorphism of cytoplasmic granules of the theta cell is shown. All the granules are of uniform density. Each is tightly enveloped by a limiting membrane. At the bottom left is seen an acidophile cell. From a non-pregnant mouse. Epon-embedded material. Stained with lead hydroxide. × 34,000.


G1
Gol
G2
M
G3
G3
G2
Er
Gol
3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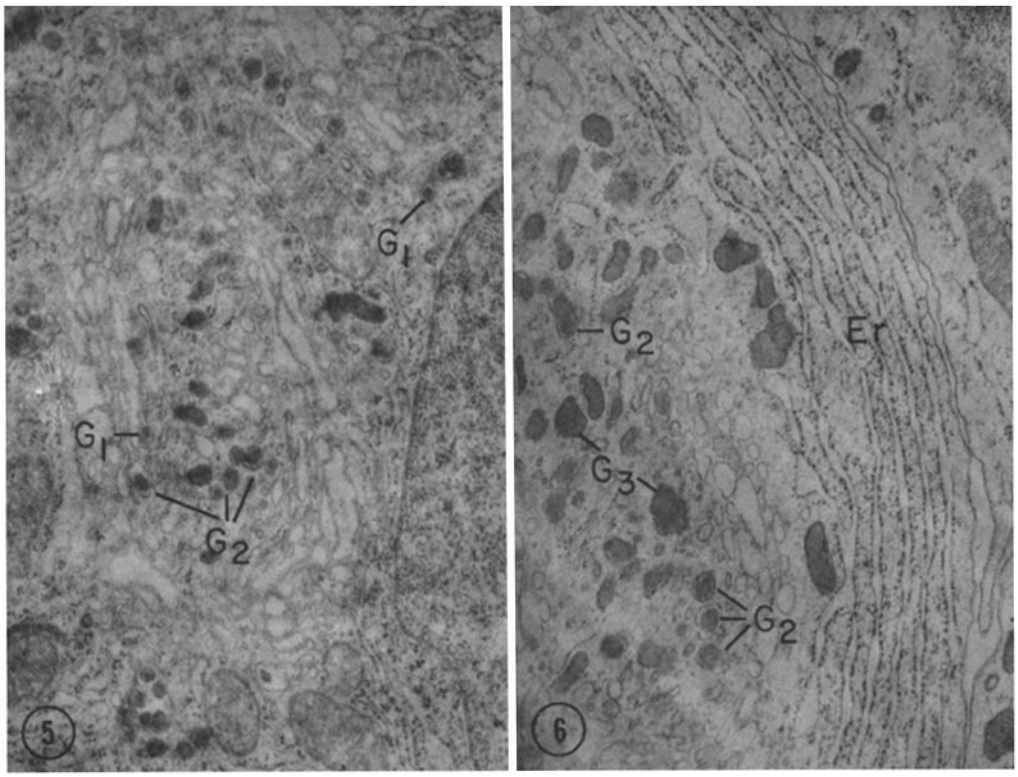


FIGURE 5

A number of Golgi vesicles are roughly arranged in the form of a ring. In the Golgi area many small (G_1) and medium-sized (G_2) granules are seen. Each is contained within a Golgi vesicle. From a mouse at 7 days of pregnancy. Epon-embedded material. Stained with potassium permanganate. × 20,000.

FIGURE 6

Medium-sized granules (G_2) contained within the Golgi vesicles are seen abundantly. At the periphery of the Golgi area cytoplasmic granules smaller in size and of lower density than mature granules are also encountered (G_3). From a mouse at 20 days of pregnancy. Polyester-embedded material. Stained with uranyl acetate. × 20,000.

to 500 mμ in maximal diameter. Occasionally, they may be 600 mμ or more in diameter. Cytoplasmic granules are sharply outlined in general, although the periphery of the granules usually shows a somewhat lower density than the central core. Each granule is closely surrounded by a limiting membrane, but in some granules this membrane is not clearly revealed. The granules are randomly dispersed, individually or in small clusters, in the cytoplasm. At the cell periphery some of them are in close apposition or in contact with the cell membrane. In the area adjoining the Golgi zone the granules are smaller in size and of lower density than mature granules (Fig. 3). Each of these granules is vaguely delineated and separated by a thin clear halo from the limiting membrane. Thus, a sequence of transition from a small granule enclosed within a small Golgi vesicle to a mature cytoplasmic granule with a tightly applied limiting membrane can be found.

Mitochondria are randomly distributed in the cytoplasm. In sections, they show circular, oval, elongated, or rod-shaped profiles. Also, Y-shaped profiles are occasionally observed. Cristae mitochondriales arranged parallel to one another are

abundant and lie perpendicular to the long axis of the mitochondria.

The cell membrane shows a roughly straight course and the formation of desmosomes can be recognized at places where thickened cell membranes of adjacent cells are in close apposition (Fig. 2).

Pregnant Mice

No marked changes in theta cells are observed in mice in the early stage of pregnancy as compared with non-pregnant mice. In middle and late stages of pregnancy, however, conspicuous changes are found in the Golgi complex (Figs. 5, 6) and cytoplasmic granules (Figs. 7 to 9). During these stages small Golgi vesicles enclosing small, rounded granules of low density are observed frequently in close proximity to the chain system of groups of the Golgi vesicles. These small granules are indistinctly outlined and applied tightly to the wall of the Golgi vesicle. In addition, the Golgi area contains abundant medium-sized Golgi vesicles enclosing somewhat larger granules. These granules are electron-opaque, rounded or oval in shape, not sharply outlined, and are separated from the vesicle wall by a rather wide, clear halo.

In the area adjoining the Golgi zone, cytoplasmic granules that are smaller in size and density than mature granules are numerous. They are closely applied to the limiting membrane. The sequence of transition from small granules contained within small Golgi vesicles to mature granules tightly enveloped by the limiting membrane is more clearly evident in pregnant than in non-pregnant mice.

As in non-pregnant mice, sharply defined, electron-opaque cytoplasmic granules are randomly dispersed in the cytoplasm of theta cells. At the cell periphery some granules are in close proximity to the cell membrane. At these places, the limiting membranes of the granules fuse with the cell membrane and openings in the cell membrane are observed. Thus, the granules appear to lie within inpocketings of the cell membranes (Figs. 7, 8). These granules are generally less dense and smaller than mature granules. Moreover, they are irregularly shaped and so vaguely outlined that the periphery of the granules appears to fade gradually into the clear background. Such granules are located, in a strict sense, outside the cell cytoplasm. However, in a wider sense, they are always within cell territory and are not seen free in the intercellular or perisinusoidal space beyond the cell territory. The peripheral portions of the granules face the wall of inpocketings derived from the limiting membrane of the granules. The inpocketings vary in depth and in width, and the granules lie always at their base. Such figures are generally observed at places where the cell membrane faces the perisinusoidal space (the basement membrane intervening), but sometimes where two cell membranes belonging to adjacent cells appose each other without an intervening basement membrane.

Also near the cell surface, on the other hand, are found cytoplasmic granules that are loosely enveloped by a limiting membrane (Fig. 9). These granules have a homogeneously low density and are irregularly shaped. In many cases, they possess a limiting membrane, but some of them show fragmentation and several pieces of a granule are seen free or in clumps within a vesicular space surrounded by a single membrane (Fig. 8). These less dense granules found near the cell surface are sometimes observed in the middle stage of pregnancy, but very often at the end of pregnancy.

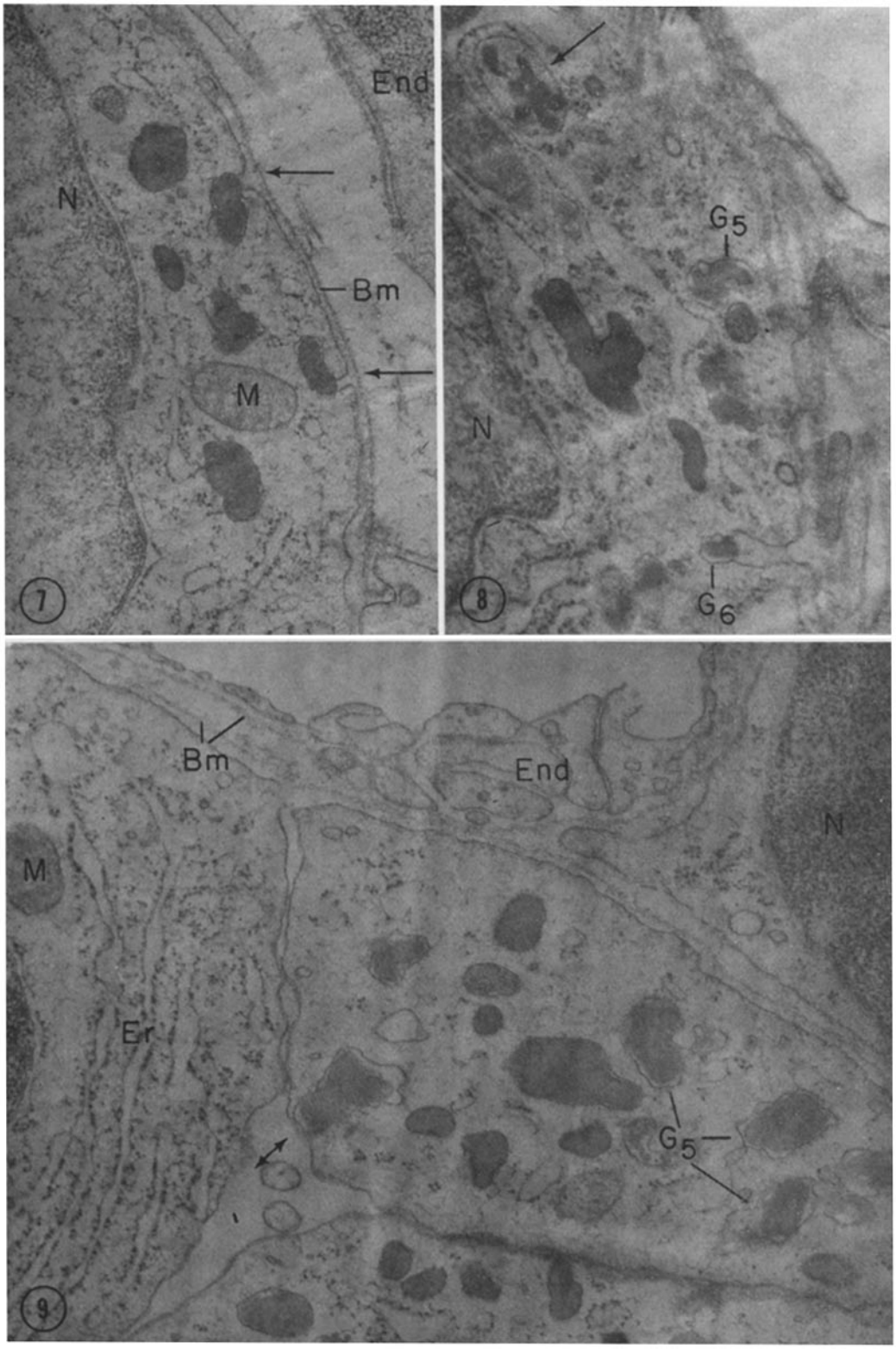
Although tightly packed, parallel arrangements of rough surfaced endoplasmic reticulum are repeatedly observed in non-pregnant mice, at the middle stage of pregnancy slight dilatations of membrane-bounded cavities of the endoplasmic reticulum are common, and oval, elongated, and cisternal profiles are frequently encountered throughout the cytoplasm. Such a tendency becomes marked as pregnancy advances.

Desmosomes observed occasionally in non-pregnant mice are scarcely encountered during pregnancy. No morphological changes are found in respect to the nucleus and mitochondria of theta cells in the pituitaries of pregnant mice.

Lactating Mice

Immediately after delivery of young, the theta cells of lactating mice present appearances similar to those seen in the late stage of pregnancy; cytoplasmic granules lying within inpocketings of the cell membrane were most frequently encountered in this group.

At 5 to 7 days postpartum, small granules of low density contained within small Golgi vesicles and somewhat larger granules enclosed in medium-sized Golgi vesicles are frequently found, though they are fewer in number than in pregnant mice. Cytoplasmic granules appearing to lie


End
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within inpocketings of the cell membrane are occasionally encountered. Sometimes, less dense, irregularly shaped cytoplasmic granules loosely enveloped by a limiting membrane are also observed near the cell surface.

DISCUSSION

In a previous electron microscope study on the anterior pituitary of the mouse (24), the author observed five cell types, including two types of immature cells. He reported also that the fourth type of cell was provided with pleomorphic cytoplasmic granules, a well developed Golgi region, and a characteristically arranged endoplasmic reticulum, but hesitated to identify this cell type with the theta cell because of the low resolving power of the electron microscope used in that study. When the present data are compared with the previous data, however, it becomes clear that the cells classified previously as the fourth type are identical with the theta cell.

Although theta cells showed no clear secretory granules by light microscopy, it is evident, from the morphological changes in the cells during pregnancy and lactation, that cytoplasmic granules observed in the previous and the present electron microscopic studies represent the secretory granules of this type of cell.

In the present studies, secretory granules were found to be distributed randomly in the cytoplasm (Figs. 3, 4). They showed uniformly high density and varied considerably in size and shape. The majority of them were tightly surrounded by a limiting membrane (Fig. 4). Some of them, however, revealed no clear limiting membrane. This may depend on the density of granules. When there appears to be no difference in density between the granules and their limiting membranes, provided the former are so closely applied to the latter that the perigranular halo is not recognized, the limiting membrane may not be detected. It is the author's opinion that the secretory granule, within the cytoplasm, is always enveloped by a limiting membrane.

The present data showed that a sequence of transition forms could be found between a small granule contained within a small Golgi vesicle and a mature granule tightly applied to a limiting membrane (Figs. 3, 5, 6). From the kinetic viewpoint, small granules in the area proximal to the chain system of Golgi vesicles were numerous in the early stage of pregnancy, whereas in the middle and late stages of pregnancy somewhat larger granules were abundant and were located more distally than the small granules. In the latter half of pregnancy, granules smaller in size

FIGURE 7

Continuities between the limiting membranes of cytoplasmic granules and the cell membrane, and openings of the cell membrane at these places, are seen under the basement membrane (arrows). These granules appear to lie within inpocketings of the cell membrane and are loosely enveloped by the walls of the inpocketings. From a mouse immediately after delivery. Polyester-embedded material. Stained with uranyl acetate. × 43,000.

FIGURE 8

A small, less dense, vaguely outlined granule is located at the base of a deep inpocketing of the cell membrane (G_6). Background of the inpocketing appears somewhat opaque due to the presence of the inpocketing wall within the plane of this section. In the cytoplasm, several fragments of a granule enclosed by a single membrane are seen at upper left (arrow), and at the center there is a less dense granule loosely enveloped by a limiting membrane (G_5). An irregularly shaped granule is also observed at the center. From a mouse immediately after delivery. Polyester-embedded material. Stained with uranyl acetate. × 43,000.

FIGURE 9

Several granules (G_5) are loosely enveloped by their limiting membranes. They are of low density and irregularly shaped. The limiting membrane of one of them (double-headed arrow) is about to join with the cell membrane. From a mouse immediately after delivery. Polyester-embedded material. Stained with uranyl acetate. × 45,000.

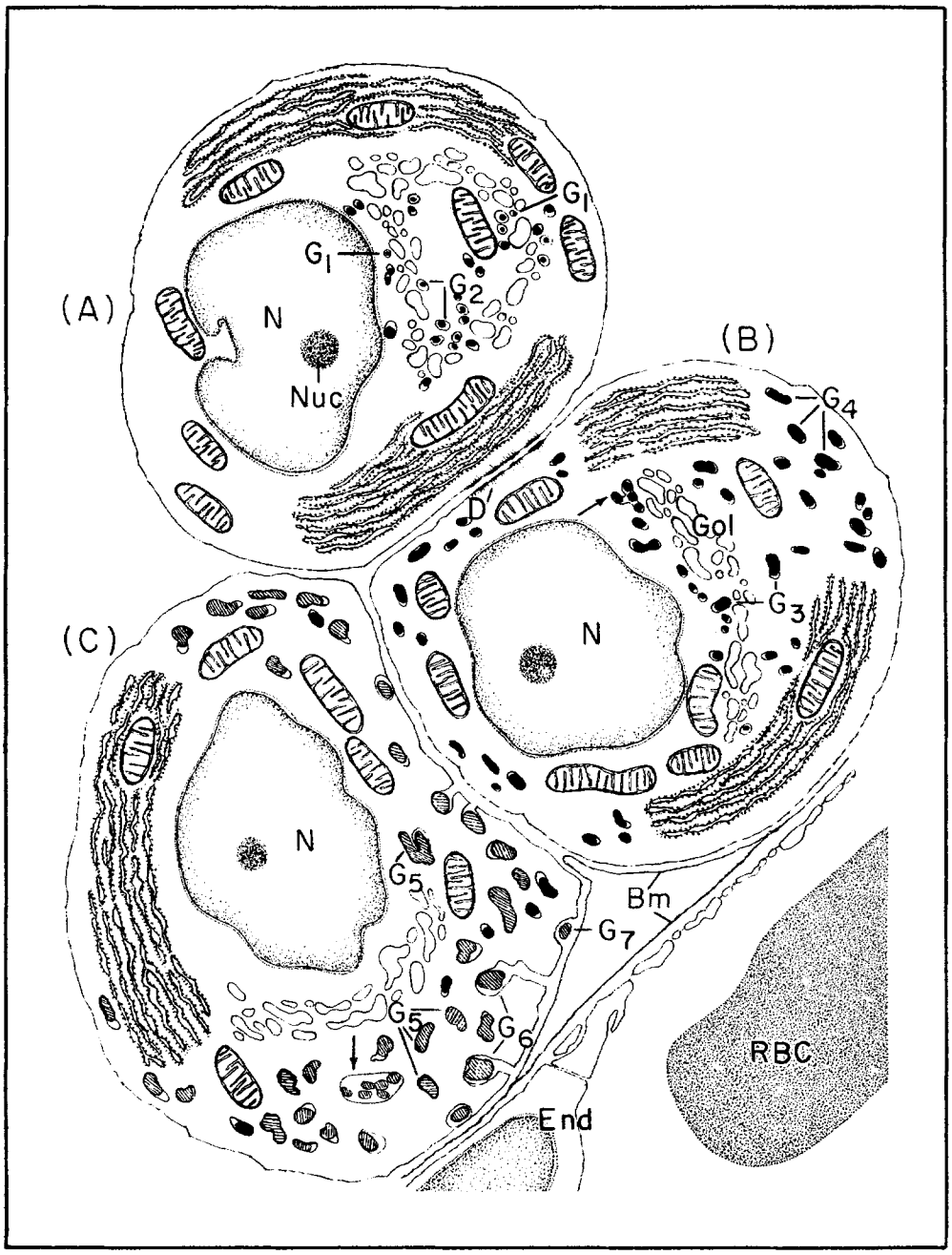

FIGURE 10

Semischematic drawing of the mode of secretion of the theta cell. (In cell (*C*), the size of the secretory granules is exaggerated).

(*A*) and (*B*) represent the stage of granule-formation. Small granules of low density (G_1) contained within small Golgi vesicles appear at first near the chain system of groups of the Golgi vesicles. These granules become larger and denser (G_2) and simultaneously move from

and of lower density than mature granules were also encountered in abundance in the areas adjoining the Golgi zone. From these observations, it can be concluded that secretory granules are at first revealed in a granular form within small Golgi vesicles. While still within the Golgi vesicles they become larger and denser. Simultaneously they move from the proximal to the distal part of the Golgi region and finally emerge from the Golgi area as mature granules in the cytoplasm. The fact that granules with several cores are sometimes encountered at the periphery of the Golgi region suggests that some of the granules are formed by the conglomeration of several cores within a single Golgi vesicle (Fig. 3). Irregularly shaped granules which are characteristic of the theta cell may be formed in such a manner.

Since the discovery of the Golgi apparatus, numerous investigators have reported on its existence and function (see Palay, 15). In a variety of glandular cells, at the electron microscope level, the Golgi apparatus, together with the endoplasmic reticulum and mitochondria, appears to be the site of elaboration of secretory products. Concerning the anterior pituitary, Farquhar and Wellings observed cytoplasmic granules contained within smooth surfaced vesicles in acidophiles of the rat and they assumed that the secretory granules are formed within the Golgi vesicles (5). Similar observations were also reported in acidophiles and basophiles of the rat (4, 6). From the present data, it is clear that the Golgi vesicle is the site of granule-formation in the theta cell.

During late pregnancy and immediately after delivery, continuity between the limiting membrane of secretory granules and the cell membrane and opening of the cell membrane at the place of extrusion were repeatedly observed (Figs. 7, 8). At these places, the granules appeared to lie within inpocketings of the cell membrane. These granules were smaller in size and of lower density than mature granules, and they were so vaguely outlined that the periphery of the granules gradually faded away, through a grey amorphous material, into the clear background (Fig. 8). It was also observed that no secretory granules were encountered outside the cell territory. From these findings, it can be deduced that prior to extrusion from the cell territory the granules lose their density and become amorphous within the inpocketings or within the cytoplasm. The presence near the cell surface of less dense, irregularly shaped granules which are loosely enveloped by a limiting membrane (Fig. 9) is favorable evidence for such an interpretation, because it is highly

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the proximal to the distal part of the Golgi region. In the vicinity of the Golgi area, granules (G_3) smaller in size and of lower density than mature granules are seen. Mature granules (G_4) are randomly distributed in the cytoplasm. The granules are always enveloped by a limiting membrane which originated from the wall of the Golgi vesicle. An irregularly shaped granule with several cores is enveloped by a single limiting membrane (arrow in (*B*)). This suggests that some granules are elaborated by conglomeration of several cores within a single Golgi vesicle.

(*B*) also represents the stage of granule-accumulation. This shows the most frequently encountered appearance of the theta cell in non-pregnant mice. Mature granules (G_4) are distributed in a relatively peripheral region of the cytoplasm, and immature granules (G_3) are seen in the vicinity of the Golgi area. In addition, the well developed Golgi complex (*Gol*), the characteristic arrangement of rough surfaced endoplasmic reticulum, and the eccentric location of the nucleus (*N*) are shown.

(*C*) represents the stage of granule-extrusion. Besides the dense, mature granules there are also many less dense, irregularly shaped granules (G_5) (marked by oblique lines). Each is loosely enveloped by a limiting membrane. One of these granules shows fragmentation within a vesicular space surrounded by a single limiting membrane (arrow). At the cell periphery, the limiting membranes of the granules become continuous with the cell membrane and the granules (G_6) appear to lie within inpocketings of the cell membrane. The granules are always located at the base of inpocketings. The granules lose their density within the inpocketings (G_7) or within the cytoplasm and are extruded to the region outside of the cell as the inpocketings are reduced in size. No granules are seen in the narrow space between the cell membrane and the basement membrane or in the intercellular and perisinusoidal spaces.

probable that these granules are in a stage immediately before extrusion and that some of them may already lie within inpocketings of the cell membrane in other sections. The granules contained within inpocketings of the cell membrane were always located at the base of the inpocketings (Figs. 7, 8). This may imply that the granules are passively extruded by reduction in size of the inpocketings.

A similar mode of release of secretory products was reported by Ichikawa (6) in acidophiles and basophiles of the anterior pituitary of the rat. He postulated that secretory granules are extruded, without any change in form, through openings in the cell membrane which occur at places where the cell membrane fuses with the limiting membrane of the granules, and that thereafter they lose their density in the narrow space between the cell membrane and the basement membrane or in intercellular spaces. Farquhar (4) also reported similar results in acidophiles of the anterior pituitary of the rat. She stated that the dense granule cores maintain their spherical shape and are clearly visible immediately after fusion of the membranes. Further, she assumed that the dense content rapidly dissolves later. According to the present data, however, secretory granules of theta cells showed morphological changes before extrusion. They lost their density within the inpocketings of the cell membrane or within the cytoplasm and occasionally showed fragmentation within the cytoplasm. Since the Golgi vesicle wall should have participated in granule-elaboration, it may be reasonable to assume that secretory granules lose their density while they are surrounded by their limiting membranes or walls of inpocketings which originated from the walls of the Golgi vesicles. Further, it was observed that secretory granules were not found either within the narrow space between the cell surface membrane and the basement membrane or free in perisinusoidal and intercellular spaces. Thus, the author assumes that before granule-extrusion occurs there is liquefaction and/or fragmentation of the granules in the cytoplasm or within the inpocketing and that the granules are extruded as amorphous materials to the region outside of the cell after they have completely lost their density.

According to the present data, granule-formation in theta cells became marked from the middle stage to the end of pregnancy but decreased gradually during lactation. On the other hand, granule-extrusion was most conspicuous at late pregnancy and immediately after delivery. These data are compatible with the author's assumption that theta cells may be a source of luteotrophic hormone.

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BIBLIOGRAPHY

1. CAULFIELD, J. B., Effects of varying the vehicle for OsO_4 in tissue fixation, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 827.
2. DALTON, A. J., and FELIX, M. D., Cytologic and cytochemical characteristics of the Golgi substance of epithelial cells of the epididymis—*in situ*, in homogenates and after isolation, *Am. J. Anat.*, 1954, **94**, 171.
3. DALTON, A. J., and FELIX, M. D., A comparative study of the Golgi complex, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 79.
4. FARQUHAR, M. G., Origin and fate of secretory granules in cells of the anterior pituitary gland, *Tr. New York Acad. Sc.*, 1961, **23**, 346.
5. FARQUHAR, M. G., and WELLINGS, S. R., Electron microscopic evidence suggesting secretory granule formation within the Golgi apparatus, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 319.
6. ICHIKAWA, A., Electron microscope study on secretion of the rat adenohypophysis, *Acta Anat. Nippon.*, 1959, **34**, 460 (in Japanese with English summary).
7. IEDA, M., Effects of follicle hormone administration on the anterior pituitary of the male mouse, *Okajimas Folia anat. jap.*, 1959, **33**, 285.
8. KATO, T., Histological studies on the mouse pituitary during pregnancy and after parturition, *J. Nagoya Med. Assoc.*, 1956, **72**, 489 (in Japanese).
9. KUSHIDA, H., On an epoxy resin embedding method for ultra-thin sectioning, *Electronmicroscopy*, 1959, **8**, 72 (in Japanese with English summary).
10. KUSHIDA, H., A new polyester embedding method for ultrathin sectioning, *J. Electronmicroscopy*, 1960, **9**, 113.
11. LAWN, A. M., The use of potassium permanganate as an electron-dense stain for sections of tissue embedded in epoxy resin, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 197.
12. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.

13. PALADE, G. E., A study of fixation for electron microscopy, *J. Exp. Med.*, 1952, **95**, 285.

14. PALAY, S. L., and PALADE, G. E., The fine structure of neurons, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 69.

15. PALAY, S. L., The morphology of secretion, *in* Frontiers in Cytology (S. L. Palay, editor), New Haven, Yale University Press, 1958, 305.

16. ROMEIS, B., Hypophyse, *in* Handbuch der mikroskopischen Anatomie des Menschen (W. von Möllendorff, editor), Berlin, Springer, 1940, **6**, Part 2/III.

17. SANO, M., A new cell type of the mouse anterior pituitary. Its relation to the cyclic variations during the oestrous cycle, *Okajimas Folia anat. jap.*, 1958, **31**, 17.

18. SANO, M., TAWADA, T., and YOSHIDA, K., Effects of ovariectomy on the mouse anterior pituitary, with particular reference to the theta-cell, *Okajimas Folia anat. jap.*, 1959, **32**, 225.

19. SANO, M., YAMAHATA, H., and IEDA, M., Effects of progesterone administration on the theta cell of the mouse anterior pituitary, *Okajimas Folia anat. jap.*, 1960, **34**, 207.

20. SJÖSTRAND, F. S., and HANZON, V., Ultrastructure of Golgi apparatus of exocrine cells of mouse pancreas, *Exp. Cell Research*, 1954, **7**, 415.

21. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 475.

22. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals. II. Application of solutions containing lead and barium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 727.

23. WATSON, M. L., Further observations on the nuclear envelope of the animal cell, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 147.

24. YAMADA, K., and SANO, M., Electron microscopic observations of the anterior pituitary of the mouse, *Okajimas Folia anat. jap.*, 1960, **34**, 449.

25. YAMADA, K., SANO, M., and ITO, T., A postnatal histogenetic study of the anterior pituitary of the mouse, *Okajimas Folia anat. jap.*, 1957, **30**, 177.

26. YAMADA, K., SANO, M., KATO, T., and MIZUTANI, S., Histological changes in the anterior pituitary of the mouse during lactation, *Okajimas Folia anat. jap.*, 1956, **29**, 287.

27. YAMADA, K., SANO, M., OKUMURA, K., and SAKAKURA, K., Cellular changes in the mouse anterior pituitary from maturity to senility, *Okajimas Folia anat. jap.*, 1960, **35**, 107.

28. YAMAHATA, H., Cellular changes in the mouse anterior pituitary following progesterone administration, *Okajimas Folia anat. jap.*, 1960, **35**, 119.