

The Anatomy of Secretion in the Follicular Cells of the Thyroid Gland

I. The Fine Structure of the Gland in the Normal Rat*

By STEVEN L. WISSIG,† Ph.D.

(From the Department of Anatomy, Yale University School of Medicine, New Haven)

PLATES 227 TO 237

(Received for publication, August 21, 1959)

ABSTRACT

The paper contains a description of the fine structure of the thyroid gland of the normal rat.

The follicular colloid, a homogeneous substance of faintly granular texture, is bounded by cuboidal or low columnar epithelial cells. Numerous pleomorphic microvilli, often permeated by small vesicles extend from the apices of the epithelial cells into the colloid. Many small, membrane-limited vesicles lie in the superficial cytoplasmic layer just below the apical plasmalemma. The ergastoplasmic sacs of the follicular cells are dilated and contain a substance resembling colloid. They are of irregular outline, and the larger sacs tend to be located in the base of the cells. The Golgi apparatus lies in the vicinity of the nucleus and consists primarily of numerous small, membrane-bound droplets with a homogeneous content. Droplets, similar to the Golgi vesicles but larger, lie in the same vicinity and are tentatively identified as colloid droplets. The colloid droplets contain an extremely fine, dense particulate material. Other droplets with a denser, more heterogeneous content are also present.

Both the follicular cells and the perifollicular capillaries are bounded by a continuous basement membrane. The capillary endothelium is in certain regions extremely attenuated and is pierced by numerous patent pores, 450 Å in diameter.

The marked similarity between the presumptive colloid droplets and vesicles of the Golgi apparatus suggests that the droplets arise from this organelle. On morphological grounds alone no relation can be established between any of the organelles of the follicular cell and the process of colloid resorption.

INTRODUCTION

Evidence from diverse lines of experimental approach indicates that the follicular colloid plays an essential role in the function of the thyroid gland. In earlier morphologic studies the scarcity or abundance of colloid was considered as a measure

of the physiologic activity of the gland (Bargmann, 1939). More recent biochemical and autoradiographic studies have demonstrated that the colloid is specifically concerned in important stages in the synthesis and storage of thyroid hormone. For example, if the binding of iodine to protein in the gland is blocked by suitable agents, inorganic iodide can, nevertheless, be stored in the colloid at many times its concentration in plasma (Pitt-Rivers and Trotter, 1953; Doniach and Logothetopoulos, 1955). In untreated animals, the inorganic iodide that has been removed from the circulation is rapidly incorporated into thyroid hormone or its precursors within the framework of protein in the colloid (Wollman and Wodinsky, 1955). The final stages in the release of hormone into the general

* This study was supported by research grants (B849 and H4512 (CB)) from the National Institutes of Health. Its contents are included in a thesis submitted to the Graduate School of Yale University in partial fulfillment of the requirements for a Ph.D. degree. A portion of this study was completed while the author was a Predoctoral Research Fellow of the United States Public Health Service.

† Present address: Department of Anatomy, University of California School of Medicine, San Francisco.

circulation involve hydrolysis of its linkage to the protein and its passage across the layer of follicular epithelial cells into the perifollicular capillaries.

It is apparent that the endocrine function of the thyroid gland is superimposed upon a unique exocrine secretory apparatus. In order to effect the measured release of thyroid hormone into the blood stream, the follicular cells are required initially to synthesize and subsequently to resorb the constituents of colloid. The present study is the first in a series undertaken to examine the morphologic aspects of each of these processes.

Methods

Specimens of thyroid gland from 13 normal adult male and 2 normal adult female rats of the Sprague-Dawley strain were examined for this study. The rats were maintained in steam-heated quarters which were subject to seasonal variations in temperature. They were fed Purina lab chow in pellet form and given tap water *ad libitum*.

Under nembutal¹ anesthesia, both lobes of the thyroid gland were exposed through a midline incision in the neck. Approximately 1 ml. fixative was infused with a hypodermic syringe into each lobe. Immediately thereafter, the rat was killed by thoracotomy. One lobe of the gland, usually the right, was excised and cut into small blocks. The blocks were placed in fixative solution at 4°C. (Rhodin, 1954) for approximately 5 hours. The fixative solution was 2 per cent osmic acid buffered at pH 7.5 with acetate-veronal (Palade, 1952). After fixation, the blocks were rinsed in distilled water, dehydrated through a graded series of methanol solutions, and embedded in methacrylate (Newman, Borysko, and Swerdlow, 1949).

Thin sections, cut with a Servall Porter-Blum microtome equipped with a glass knife, were examined with an RCA EMU-2B or 2E electron microscope. Sections 2 to 4 μ thick from the same blocks were mounted on glass slides and examined with the phase contrast microscope.

OBSERVATIONS

Phase Contrast Microscopy:

The follicles of thyroid glands from normal rats are generally oval or circular in outline and are surrounded by a richly vascularized capsule of connective tissue. In the unstimulated gland, follicular branchings or evaginations are uncommon, and each follicle has a simple outline. The follicular cells vary in height from cuboidal to short col-

umnar, and frequently their bases are indented by the perifollicular capillaries (Figs. 1 and 2). The apical margin of each cell bulges in the form of a shallow dome into the colloid filling the lumen of the follicle. The nucleus appears as a large, translucent vesicle lying most often in the basal half of the cell. The cytoplasm appears diffusely fibrillar or granular and may contain a variety of inclusions.

By this method of examination, the colloid appears as a homogeneous mass with relatively little optical density. The colloid bordering the follicular cells appears lighter than the remainder, but this is apparently a halo effect produced by the apical cell margin. In all of the specimens that were examined, no vacuoles were observed at the periphery of the colloid.

From the latter part of the 19th century until two decades ago, morphologic research in the thyroid gland was hampered by erroneous conceptions concerning "colloid vacuoles," *i.e.*, vesicular, non-staining structures that appear at the periphery of the colloid in specimens of thyroid tissue fixed by immersion in the common aqueous fixatives. Early workers, who observed their absence from specimens fixed with osmium tetroxide (Baber, 1881; Langendorff, 1889), considered them artifacts. In fact, Baber gave an extremely plausible explanation for their mode of origin. He suggested that during fixation the colloid shrinks, and, in so doing, draws water from the follicular cell to form the colloid vacuoles. Later, as the function of the thyroid gland became more clearly understood, an increase in number of such vacuoles during periods of increased colloid resorption was observed, and they were referred to by some authors as "resorption vacuoles" (Aron, 1930). Their artifactitious origin was generally conceded only after the demonstration of their absence from living thyroid follicles (Williams, 1937) and from specimens of thyroid tissue fixed by freeze-drying (Gersh and Caspersson, 1940; De Robertis, 1941 *a*).

When examined under high magnification, the apical surface of the follicular cells appears covered by numerous filamentous projections or microvilli. Because their width approaches the limit of resolution of the light microscope, they can be only barely discerned (Fig. 1). In the normal rat, they appear short, but, as they are so poorly resolved, estimates of their length are of limited reliability. The existence of the microvilli was first noted during examination of thyroid tissue with the electron microscope (Monroe, 1953; Braunsteiner *et al.*, 1953). However, Monroe (1953) calls attention to a short, little known paper by Traina (1910) in which he reported having seen with the aid of a

¹ Nembutal is the registered trade mark of sodium pentobarbital produced by Abbott Laboratories, North Chicago, Illinois.

special staining technique a brush-like border on the apices of follicular cells in well preserved thyroid specimens from turtles, rats, and many other species.

Dark granules, which may be either mitochondria or some other type of inclusion, occur occasionally within the poorly resolved, fibrillar cytoplasmic ground substance (Fig. 1). Rarely, small numbers of pale spherical droplets of varied size are clustered in the apical cytoplasm of a single cell. Certainly some of these granular structures should correspond to the colloid droplets of stained histological preparations. On the basis of their appearance in the phase contrast microscope, however, the colloid droplets cannot be positively identified, much less subdivided into the chromophil and chromophobe categories observed in stained preparations.

The occurrence of stainable droplets of colloid within the follicular cells was first noted by Biondi (1888, 1892) who postulated that they were the intracellular precursors of the follicular colloid on the basis of tinctorial similarities between the two substances. Andersson (1894) confirmed Biondi's observation of the presence of chromophil secretory products in the follicular cells, and in addition described a second, chromophobe type of secretion consisting of spherical, non-staining vacuoles which appeared in the apices of actively secreting follicular cells. These he interpreted as the intracellular precursors of the colloid vacuoles despite the earlier assertions of Baber (1881) and Langendorff (1889) that the latter were artifacts. The most recent cytological investigations of the thyroid gland still do not furnish an explanation for the presence of vacuolations within the follicular cells. The vacuolations, even though they may not actually contain a secretory product, occur with a frequency dependent upon the physiological status of the gland; they are much more numerous during periods of hyperactivity (Ponse, 1951).

Occasionally, fibrillar strands subdivide the cytoplasmic ground substance in the base of the follicular cell into clear areas of diverse size and shape (Fig. 2). It is interesting to note that in light microscope studies of the thyroid gland, Bensley (1916) observed in this region secretory droplets which stained much less intensely than those found in the apex. He thought that this basal pale-staining colloid represented material destined to be secreted directly into the perifollicular space rather than into the follicular lumen. It will become obvious later that the basal vesicles seen with phase contrast microscopy correspond to voluminous internal elements of the ergastoplasm.

Although the follicular basement membrane is not readily distinguished with the phase contrast microscope, it appears to circumscribe each follicle and separate it from the surrounding, richly vascularized connective tissue.

Electron Microscopy:

Colloid.—Under electron microscopic examination, the colloid appears as a mass with medium electron-scattering properties and faintly granular texture. It exhibits practically no variation in over-all density from one area to another (Figs. 4, 7, and 8). Its periphery extends up to the plasma membrane surmounting the apical surfaces of the epithelial cells where it is indented by their microvilli. Vacuoles are completely absent from the colloid. The pleomorphism of the microvilli as seen in the electron microscope (Figs. 3, 7, and 8) strongly suggests that they are motile in the living animal. Therefore, although the colloid in life may be a gel of substantial viscosity, it appears to be neither rigid nor impenetrable as far as the follicular cells are concerned. This is best illustrated in the case of the large processes which are infrequently observed extending from the apical surface of a follicular cell (Fig. 5). Cellular or other inclusions dispersed in the colloid are not uncommonly observed in light microscopic studies of the thyroid gland, but they have not been encountered in this study.

Apical Border of the Follicular Cells.—The dome-shaped apical surfaces of the follicular cells are covered by an uninterrupted plasma membrane and are provided with numerous microvilli (Fig. 3). In longitudinal section they are usually finger-like in outline, approximately $0.35\ \mu$ tall and $0.07\ \mu$ broad. However, quite often they may be far more irregular in shape (Figs. 4 and 8). Their number varies from cell to cell and from one region of the surface of a particular cell to another. They seem to congregate preferentially in the depressions where the apical surfaces of adjacent follicular cells meet. That they are not merely solitary finger-like projections but are frequently linked to one another by web-like extensions of cytoplasm is clearly demonstrated when they are viewed in sections cut tangential to the surface of the epithelium (Fig. 7). The tendency of the microvilli of the thyroid epithelium to be linked together distinguishes them from the clearly individual and much more regularly oriented microvilli on the surface of the intestinal lining cells (Zetterqvist, 1956) and the proxi-

mal convoluted tubule cells of the kidney (Rhodin, 1954). The fact that in tangential section they appear isolated from the follicular cells is not considered evidence that they are extruded into the colloid for they are never found any significant distance away from the epithelium. It has already been reported in several articles that the plasma membrane covering the microvilli is composed of three layers: two dark layers separated by a single pale layer (Zetterqvist, 1956; Ekholm and Sjöstrand, 1957*b*). The layers have been observed in this study in sections examined under high magnification in which the plasma membrane is sectioned normal to its surface.

Often vesicular structures approximately 60 $m\mu$ in diameter appear within the substance of the microvilli (Figs. 4, 7, and 8). They are bounded by a single membrane of the same thickness as the plasma membrane. The density of their content is not appreciably different from that of either the colloid or the cytoplasmic matrix. Occasionally, indentations of the apical plasma membrane extend a short distance into the cytoplasm (Figs. 4 and 7). The width of the indentations is of the same order of magnitude as the diameter of the vesicles, and their content is directly continuous with the follicular colloid. The morphological appearances of the indentations suggest that they are vesicles either forming at the surface of the follicular cell or discharging their contents into the lumen of the follicle. Currently, we have no means to determine what is their origin and fate. If it should prove to be the case that the vesicles form at the apical surface membrane, then the phenomenon can be considered an example of pinocytosis occurring in thyroid follicular cells.

Near the apices of the follicular cells the apposed plasma membranes of adjacent cells are more ad-electronic than elsewhere on the cell surface (Figs. 7 and 8). The cytoplasmic matrix bordering this region of the membrane also appears denser than elsewhere in the cell. Near such areas of the lateral cell border one or several localized areas may occur in which the plasma membrane and the adjacent matrix appear extremely dense. The membrane itself is somewhat thickened and may be divided into two dense layers along its length. These differentiations of the plasma membrane resemble the adhesion plates or desmosomes described by other authors (Fawcett, 1958) which are presumed to anchor adjacent cells together. Direct proof that they have this function is, however, lacking.

Superficial Cytoplasmic Layer.—Beneath the apical cell border of the follicular cells lies a band of cytoplasm roughly 0.5 μ in width devoid of the organelles usually found in the remainder of the cytoplasm (Fig. 4). The functional significance of this layer is not understood. Similar layers occur in the apices of various ciliated and non-ciliated lining epithelia, and in some they contain a meshwork of extremely fine fibrils (Fawcett and Porter, 1954; Palay and Karlin, 1959*a*). The layer may represent a zone in which the cytoplasmic matrix is more viscous or gelated than in other regions of the cell, and thus may correspond with the peripheral ectoplasmic layer of protozoa. A characteristic group of granules or vesicles, 50 to 150 $m\mu$ in diameter, is found just within the layer (Figs. 3 and 4). Since in sections the vesicles are always circular in outline, in three dimensions they must have the shape of small spheres. Their aspect varies in other respects from cell to cell and within individual cells. In some cells their content is pallid, and a single membrane limiting each vesicle can be readily discerned. In other cells, the vesicles contain a much denser substance which renders it impossible to distinguish a limiting membrane with certainty. In sections cut normal to the surface of the follicular cell, the vesicles appear to be few in number. When the plane of section passes more nearly parallel with the surface of the cell, the superficial cytoplasmic layer appears much broader and usually seems to be heavily populated with the vesicles. Their occurrence has been noted in a previous study (Dempsey and Peterson, 1955), but they have not yet been ascribed any functional significance.

Ergastoplasm.—Ergastoplasmic vesicles are distributed throughout the cytoplasm of the follicular cells from the lower border of the superficial cytoplasmic layer to the base of the cell (Figs. 4, 6, 9, and 14). The vesicles are limited by a single membrane approximately 70 A thick whose outer surface is studded with many fine particles 130 A in diameter, which presumably contain ribonucleoprotein (Palade and Siekevitz, 1956). The ergastoplasmic vesicles of the follicular cell are not usually arranged in an ordered pattern (Fig. 13) but display a much greater degree of pleomorphism than in other exocrine cells such as those of the pancreatic acinus (Sjöstrand and Hanzon, 1954). The smoothly curved outline of the vesicles bears no obvious orientation to neighboring structures, although their surface may occasionally be indented by mitochondria. Also, on occasion, groups of flat

tened vesicles appear in which the vesicles achieve a more or less parallel arrangement with one another and form a chain of ellipsoids reminiscent of the rouleaux formed by erythrocytes (Figs. 4 and 9). All of the vesicles are more dilated than are the ergastoplasmic vesicles of most other cells, and they contain a homogeneous material of faintly granular texture. This substance under electron microscopic examination has all the morphological properties of the follicular colloid. In fact, because of the presence of this colloid-like material within the vesicles, they have been identified previously as colloid droplets (Dempsey and Peterson, 1955). The density of the intravesicular material can vary markedly in adjacent cells. Although its morphologic resemblance to colloid is undeniable, its actual composition is unknown.

The relative size of the ergastoplasmic vesicles varies according to their location within the follicular cell. The smaller vesicles lie near the apical surface of the cell; the larger vesicles are most often located in the base. The smallest vesicles near the apical surface are only incompletely covered with ribonucleoprotein particles (Figs. 3 and 8). In the basal portion of the cell, the vesicles are coated by abundant particles and their dense concentration becomes evident when sections in which the surface of the vesicles is cut tangentially are examined (Fig. 14).

The ergastoplasmic vesicles correspond to a subdivision of the endoplasmic reticulum which has been described in other cells as a system of interconnected vesicles and cisternae (Palade and Porter, 1954). Occasional indications of these interconnections can be seen in the follicular cells in the form of richly branching vesicles. However, the degree of interconnection can be accurately evaluated only by the use of three-dimensional reconstructions based on serial sections, a method which has not yet been applied to follicular cells. In view of the fact that the isolated appearance of the majority of the vesicles may be misleading and we may actually be observing a network of branching tubules, one should bear in mind that the difference in size between the vesicles in the apex and base of the follicular cell can result as well from a greater degree of branching of the network at the apex as from a greater distention of the vesicles at the base.

Small granules appear free in the cytoplasmic matrix and are morphologically indistinguishable from the particles affixed to the ergastoplasmic vesicles (Figs. 3, 4, 6, and 8). When the surface of

an ergastoplasmic vesicle is sectioned tangentially so that the distribution of particles on its surface can be seen in full-face view, some of the particles appear to be arranged in the form of rosettes, chains, or spirals (Fig. 14). This characteristic distribution of the particles occurs in cells of other tissues (Palade, 1955*a*; Palay and Palade, 1955), even among particles not affixed to membranes. Presumably forces exerted by the particles themselves or by the surrounding cytoplasmic matrix bind the particles together in such regular arrays.

Secretion Droplets.—Droplets or granules which are presumably secretory in nature occur in the follicular cells (Figs. 9, 12, and 13). They are usually confined to the apex of the cell, and their number varies from one cell to another. In some cells they are abundant, whereas they may be absent from sections of others. They have a circular or elliptical outline, from 50 $m\mu$ to several micra in diameter. They share the common characteristic of being enclosed by a single-layered membrane approximately 50 \AA in thickness. The membrane is never coated with fine particles, and on the basis of this property they can always be distinguished from ergastoplasmic vesicles. The membrane cannot always be discerned either because of unsuccessful preservation or because of the obliquity of the plane of section.

The content of the droplets is extremely variegated. Most contain a relatively homogeneous granular material of varied electron density (Figs. 9, 11, and 12). When the material is extremely dense, the limiting membrane of the droplet is obscured. Droplets having a pallid content are generally large and lie near the apical border of the follicular cells. Some droplets have a composite content; they may contain granules of varied density as well as vacuoles (Fig. 12). Fragments of membranes may also occur in this type of droplet. Such inclusions lie within a matrix material similar to that found in the homogeneous droplets. A unique property of the majority of the droplets is that they contain numerous minute, extremely dense particles (inset, Fig. 13), 75 \AA or less in diameter. The composition of the minute particles in the droplets is not known, but their appearance is similar to that of iron-containing compounds that have been identified in the liver and spleen (Kuff and Dalton, 1957; Richter, 1957; 1959).

Golgi Apparatus.—In comparison with other secretory cells, the follicular cells of the normal rat thyroid have a relatively inconspicuous Golgi ap-

paratus located in nearly all instances just above or lateral to the nucleus (Figs. 4, 9, 10, and 12). It consists predominantly of small vesicles of varied diameter ranging from 50 to 175 m μ . Aside from their being assembled in a cluster, the vesicles bear little sign of preferred orientation with one another. The stacks of parallel flattened vesicles, so prominent in the Golgi apparatus of other cells, occur only infrequently and consist of only a relatively small number of vesicles. Occasionally, the layers appear to be arranged in arcs about an imaginary point in the supranuclear cytoplasm (Fig. 10). Each vesicle is limited by a single membrane devoid of ribonucleoprotein particles. In contrast to the ergastoplasmic vesicles, the vesicles of the Golgi apparatus are closely bunched, and free ribonucleoprotein particles do not occur in their interstices. A prominent feature of the Golgi vesicles is the variability in density of their content. The vesicles with the most dielectronic contents are larger than the others, and they appear empty (Fig. 10). Their appearance suggests that they may have been artifactitiously dilated during preparation of the specimens, but no concrete evidence to support this assumption can be offered. The content of the remainder of the vesicles ranges from pale to moderately dense. In all cases, the content of individual vesicles is homogeneous. There appears to be no correlation between the size of these vesicles and the density of their content. The lumina of the vesicles of the Golgi apparatus have not been observed in direct continuity with those of the ergastoplasm as occurs occasionally in other tissues (Palay and Palade, 1955; Palay and Karlin, 1959 a). As far as their localization within the follicular cells is concerned, they share a common pattern of distribution with only one other cytoplasmic organelle, the secretory droplets described in the previous section, to which they bear a striking resemblance except for differences in size and minor differences in content. Because of this overlap in distribution and morphologic appearance, it is not always possible to distinguish the small secretory droplets from the large vesicles of the Golgi apparatus. In fact, on the basis of size and density of content, one can select examples from both species of organelles that form a continuous series ranging from the smallest vesicles of the Golgi apparatus to the largest secretory droplets. Under these circumstances it is tempting to assume that the large secretory droplets evolve from the

small Golgi vesicles.² As mentioned previously, many of the secretory droplets contain fine, dense particles, and it appears that the small Golgi vesicles do not. However, rather than separate the two structures into separate categories on the basis of this solitary difference, one may postulate that the secretory droplets acquire the particles at some point in their development from Golgi vesicles.

Mitochondria.—Mitochondria are relatively numerous in the follicular cells and are distributed evenly throughout the cytoplasm except for their absence from the superficial cytoplasmic layer. The majority appear to be of an elongated form which is rarely straight but most often sinuous throughout its length (Fig. 14). Occasionally even such bizarre forms as doughnut-shaped or Y-shaped mitochondria are encountered. The mitochondria usually lie close to the walls of ergastoplasmic vesicles. In comparison with the mitochondria of other cells, those of the follicular cells are small. They have an average diameter of approximately 0.2 μ which changes little along their length. Their internal structure conforms to the general pattern characteristic for this organelle (Palade, 1953 a; Sjöstrand, 1953). However, the orientation of the cristae with respect to the long axis of the mitochondrion varies. At one extreme, they are oriented in the conventional cross-wise manner; at the other, they are aligned parallel to the long axis of the mitochondrion. All gradations between these extremes occur, and there are often wide differences in the orientation of cristae within a single mitochondrion. Since neighboring cristae in general run parallel with one another, the changes in orientation along the mitochondrion are gradual. In the interstices between the cristae, the mitochondrial matrix is adielelectronic. Thus the cristae do not stand out prominently against this backdrop, and each mitochondrion as a unit appears as a dark structure. The dense granules described by Rhodin (1954) in mitochondria of the epithelial cells of the

² The number of secretory droplets in the follicular cells increases markedly a few hours following a single injection of thyrotrophic hormone. At the same time, vesicular components of the Golgi apparatus of the follicular cells also increase in number. Examination of these cells provides abundant evidence to support the contention that the secretory droplets originate as vesicles of the Golgi apparatus. A detailed report of these studies will be published later.

proximal convoluted tubule occur in small numbers in thyroid mitochondria.

Nucleus.—The nucleus is enclosed within a double-walled envelope whose layers are separated by a distance of approximately 200 Å. The layers are not always visible, presumably because they can be visualized only if they are normal to the plane of section, and this occurs only when the section passes close to the center of the nucleus. Watson (1955) originally observed that small porous openings occur in the nuclear membrane of follicular cells. They have also been observed in this study, but they are relatively rare. The nucleus is roughly spherical with occasional indentations in its margin. Although the outer lamella of the nuclear membrane in acinar cells of the pancreas (Palade, 1955 *a*) and in other cells is coated with ribonucleoprotein particles, it is free of such particles in follicular cells. Under examination at low magnification, the nuclear sap appears to be of medium density with a slightly mottled texture (Fig. 9). Under higher magnification it is apparent that the nucleus contains multitudes of small granules (Fig. 6). The granules are of varied size and density, and the characteristic mottling of the nucleus can be attributed to localized aggregations of granules which seem to be larger and denser than the rest. Nucleoli are not prominent in the nuclei of follicular cells. All of the nuclei appear to be in the resting phase; no indications of nuclear changes suggestive of mitosis have been observed.

Plasma Membrane.—The plasma membrane covering the apical portion of the follicular cell has already been described. The membrane around the remainder of each follicular cell appears in sections as a single straight dense line. The three layers which comprise the plasma membrane at the apical border have not been discerned elsewhere in the membrane. The plasma membranes of adjacent cells closely parallel one another. They are approximately 70 Å in thickness and are separated by a space approximately 150 Å in width which remains constant along their length. The space contains a substance that is of the same density as the follicular basement membrane and probably represents the intercellular cement substance. The membranes themselves, apart from the alterations they display at the apex of the follicular cells, undergo no further variation in thickness or density over the remaining portion of the cells. From apex to base, the apposed membranes of adjacent cells pursue a rela-

tively straight course. Occasionally, their path becomes more tortuous where a projection from one cell forms an indentation in the wall of another. Fawcett (1955) encountered somewhat similar formations in the borders of adjacent hepatic cells, and suggested that they may serve as an anchoring device. The apposed plasma membranes display an even higher degree of contortion in regions where the edges of three adjacent follicular cells come in contact. In such corners, the profiles of paired membranes billow out in numerous small graceful arcs into the adjacent cytoplasm. It is quite conceivable that the tortuosities that are observed within sections are not merely occasional finger-like projections of one cell extending into its neighbor. More complete examination of their configuration by means of serial sections might reveal that they are ridge-like structures of considerable length. If this proves eventually to be the case, their function as an anchoring device between cells would seem even more plausible.

The membrane at the base of the follicular cell forms a smooth cover for each cell (Figs. 6 and 17). Its outer surface is always coated by the dense layer identified as the basement membrane. This same layer may form an individual cover for occasional blunt cytoplasmic processes originating from the base of the cell. There are no structures homologous with microvilli at this surface of the cell. Small vesicles are not found in close proximity to the plasma membrane as they are at the apex, nor do any indentations of the membrane occur that might suggest the formation of pinocytotic vesicles. The formed constituents of the basal cytoplasm, which in this area of the cell consist principally of ergastoplasmic vesicles and mitochondria (Fig. 6), often lie immediately adjacent to the plasma membrane. This close association is most readily seen within the basal processes.

Follicular Basement Membrane.—At the peripheral limit of each follicle lies a thin, moderately dense band presumed to be the follicular basement membrane. It is homogeneous in appearance and measures 400 Å in width (Figs. 6 and 17). It is closely applied to the base of each follicular cell and proceeds uninterrupted from one cell to the next. As it passes over the basal processes of the follicular cells the membrane is not evaginated outwards. Instead, the bases of the follicular cells are indented to accommodate the processes (Fig. 6). In contrast, the perifollicular capillaries, which

are not included within the confines of the follicular basement membrane, often severely indent the membrane and the adjacent epithelial cells as well. These capillaries lie in cup- or tunnel-shaped depressions in the follicular wall which are lined by the follicular basement membrane. No fibrillar component has thus far been observed in the substance of the membrane. However, a thin layer of fibers is often applied to its external surface (Figs. 6 and 14). In cross-section the fibers have a circular outline and are 400 Å in diameter. Their fibrillar nature becomes obvious when the plane of section is parallel with their long axis. Although periodicity along their length has not been seen in this study, they are presumed to be either collagen or reticulin fibers. They are not distributed uniformly over the surface of the follicle; substantial areas of its surface are entirely devoid of them.

Perifollicular Capillaries.—As observed with the electron microscope the endothelium of the perifollicular capillaries consists of a single continuous layer of cells which varies considerably in thickness along the capillary wall. It is broadest in the region of the nucleus where it protrudes markedly into the capillary lumen. The nucleus is elliptical in section with its broad surface faced toward the lumen of the capillary. It is enclosed by a double-layered envelope, and a thin layer of cytoplasm surrounds it on all sides. The endothelial wall at the lateral margin of the nucleus tapers gradually to a very thin layer. The wedge-shaped region of cytoplasm lateral to the nucleus contains a variety of organelles, among them mitochondria, ergastoplasmic vesicles, and small vesicles of the Golgi complex. Beyond the apex of the wedge, the thin endothelial wall is intermittently interrupted by short segments of considerably greater thickness. Only infrequently can organelles such as mitochondria be observed in the thick regions. More commonly they contain only cytoplasmic matrix and vesicles (Fig. 17).

Where the plasmalemmae of adjacent endothelial cells or of the same endothelial cell meet (the latter occurs where the entire circumference of the capillary wall is formed by a single cell), the plasmalemmae in the zone of apposition display a morphologic differentiation similar to that seen in the lateral plasma membrane of the follicular cells near their apices. The space between the membranes is occupied by a homogeneous material of slight density. In the region of apposition, each plasma membrane appears denser than elsewhere as does

the cytoplasmic matrix adjoining it. Pinocytic vesicles apparently do not form at this boundary of the cell.

The thinnest portions of the endothelial wall, approximately 350 Å thick, consist only of the parallel membranes of the opposite surfaces of the endothelial cell separated by a thin layer of cytoplasmic matrix. At relatively frequent intervals the wall is interrupted by porous discontinuities, approximately 450 Å wide (Fig. 15). Where the endothelial wall is cut in cross-section, it can be clearly seen that the plasmalemmae of the opposite surfaces of the cell join at the rim of the pore. Where the endothelial wall is sectioned tangentially, the pores in the thin regions of the wall appear in full-face view. They are easily recognized as numerous circular clear areas which have a relatively uniform diameter from one pore to another (Fig. 16). Their diameter is of the same order of size as the discontinuities observed in cross-sections. The appearance of the pores in tangential section suggests that they are free openings linking one surface of the endothelial cell with the other. However, in transverse section the openings of the pores sometimes appear to be bridged by a single membrane or diaphragm continuous with the plasma membrane along the lateral margin of the pore. The occurrence of a diaphragm across the pores has been reported by previous investigators (Ekholm, 1957; Ekholm and Sjöstrand, 1957 *a*; 1957 *b*) who believe that the pores are not true openings in the endothelial wall but represent localized regions where the absence of cytoplasmic matrix separating the plasmalemmae of opposite surfaces of the endothelial cell allows the two to fuse. However, since many of the pores do not display evidence of having such a diaphragm, it is of some importance to account for its inconstant occurrence.

To begin with, the appearance of the pores in tangential section does not corroborate the existence of such diaphragms. In full-face view, the pores appear to be of pronounced transparency (Fig. 16), and they stand out in sharp contrast against the background formed by the endothelium. The sharpness of the interface at the border of the pore is not what one would expect if the plasma membrane of the endothelial cell continued across the lumen of the pore. A plausible explanation for the appearance in perpendicular sections of a diaphragm can be provided on the basis of the relationship between the diameter of the pores and the thickness of the sections. The results of a care-

ful study by Peachey (1958) show that the thickness of sections of methacrylate-embedded specimens commonly utilized for electron microscopy ranges from 50 to 150 m μ . As the diameter of the endothelial pores averages 45 m μ , one would expect on statistical grounds that, when the endothelium is sectioned perpendicularly, one cut of the microtome may pass through the lumen of a pore but the other will pass through the endothelium a short distance outside its rim. Under electron microscopic examination, the segment of the rim of the pore included in the section will appear as though it were a diaphragm extending across the lumen. Irrefutable proof of the absence of true diaphragms across the pores will only be gained by further study. It should be pointed out that the interpretation presented in this report is the only one that reconciles the appearance of the pores in tangential and perpendicular section.

The localized thickenings in the endothelial wall have a structure closely resembling that of the endothelial lining of capillaries in muscle (Palade, 1953 *b*; Moore and Ruska, 1957; Bennett, Luft, and Hampton, 1959). Small vesicles, approximately 600 Å in diameter, are scattered in the cytoplasmic matrix (Fig. 17). The appearance of invaginations of the plasma membrane at either surface of the cell which are of the same order of size as the vesicles suggests that they form or empty at both surfaces of the cell. Palade (1953 *b*) has postulated that the vesicles could function as a means of transport across the cell, and evidence in support of this hypothesis has already been reported (Wissig, 1958; Alksne, 1959).

The basement membrane of the perifollicular capillaries is a thin layer which resembles in all morphological respects its counterpart at the base of the follicular cells (Fig. 17). It is 400 Å in thickness, does not appear to contain any fibrils, and forms for each capillary an uninterrupted external layer closely adherent to the endothelium. The space intervening between the perifollicular and pericapillary basement membranes is filled presumably with gel-like interstitial ground substance. It is unlikely that the two basement membranes fuse even when apposed, for, in favorable sections, the individual membranes can always be distinguished regardless of their proximity.

Presumably the pericapillary basement membrane offers mechanical support to the thin endothelium and also enables the capillary wall to retain constituents of the plasma which the porous endo-

thelium would otherwise let escape. Plasma in the perifollicular capillaries contains a particulate and fine fibrillar material which may represent either protein or lipid constituents of blood (Figs. 6, 14 to 16). Because of its size, this material should be able to leave the circulation *via* the endothelial pores, but it has never been observed in the pericapillary space. The basement membrane is the most probable barrier to its egress.

DISCUSSION

In general, apical surfaces of absorptive epithelial cells, regardless of their location, are surrounded by microvilli, and vesicles lie close to or are connected with the apical plasma membrane. The size, orientation, and distribution of both elements are characteristic for any epithelium. For example, in intestinal lining cells (Zetterqvist, 1956; Palay and Karlin, 1959 *a*) and in cells of the proximal convoluted tubule (Rhodin, 1954; Pease, 1955; Fawcett, 1958), microvilli are numerous and extremely regular in form and distribution. In the intestine of the newborn rat, invaginations of the plasma membrane at the base of the microvilli connect with large vesicles situated in the apical cytoplasm (Clark, 1959). In the adult rat the formation of vesicles at the apical plasma membrane occurs only rarely, but its frequency of occurrence increases under appropriate experimental conditions (Palay and Karlin, 1959 *a*; 1959 *b*). In the cells of the proximal convoluted tubule of the kidney, long tubular invaginations from the apical plasma membrane at the base of the microvilli extend into the apical cytoplasm (Rhodin, 1954; Pease, 1955; Fawcett, 1958). In thyroid follicular cells the microvilli are of pleomorphic outline and are unevenly distributed over the apical surface. Small vesicles and indentations of the apical plasma membrane are interspersed frequently among them.

It is fruitful to speculate on the physiologic importance of the vesicular structures. Their appearance in sections suggests that they are related to the phenomenon of pinocytosis seen in living cells (Lewis, 1931). In the intestine, the apical vesicles have already been implicated in the absorption of intact proteins (Clark, 1959) and lipids (Palay and Karlin, 1959 *b*), but little concerning the function of the tubular invaginations of the cells in the proximal convoluted tubule has been reported thus far. It would be premature to label the vesicles associated with the apical plasma membrane of the follicular cells as pinocytic, and, hence, absorp-

tive, when their origin and fate still remain to be determined. If they empty into the follicular lumen, it is conceivable that they deposit some component of colloid, *i.e.*, thyroglobulin (Gersh and Caspersson, 1940), glycoprotein (Gersh, 1950), or nucleic acid (Dempsey and Singer, 1946) in the follicular lumen, or that they are involved in the mechanism for the active transport of iodide across the follicular cells. If they form at the cell surface, they may contain derivatives from the proteolysis of colloid which is presumed to occur in the follicular lumen. Additional experiments by the author which will be described in a subsequent report show that the number of microvilli and vesicles markedly increases during accelerated colloid resorption. Thus it is more likely that they are concerned with absorptive rather than secretory functions.

The problem of deciphering the physiologic properties of microvilli even at the level of speculation is far more difficult. In the past, it has been suggested that these organelles facilitate the entry of substances into cells by increasing the area of their absorptive surface. In the case of the intestine, variations in the rate of absorption of lipid and protein are reflected in parallel changes in the size and number of pinocytic vesicles connected with the cell surface. No obvious concurrent changes in the structure of the microvilli take place. However, Sjöstrand and Zetterqvist (1957) recently reported that following ingestion of protein, lipids, or carbohydrates, the triple-layered structure of the plasma membrane covering the microvilli of intestinal lining cells becomes obscured and the thickness of the membrane changes. The significance of the latter observations awaits explanation. In some classes of cells, microvilli appear not to be essential for the absorption of material. Macrophages lack them, and under suitable experimental conditions endothelial cells can ingest material without the aid of microvilli at their surface (Wisig, 1958; Alksne, 1959). In cells which possess pleomorphic microvilli, such as the thyroid follicular cells, it is plausible to interpret the pleomorphism in sections as evidence of motility in the living cell. By the same token, the uniform appearance of microvilli of intestinal lining cells and of the proximal convoluted tubule suggests that they are stationary in life. If these assumptions are valid, we are faced with the task of explaining why

certain epithelia utilize motile microvilli whereas others utilize stationary microvilli.

The term "colloid droplet" has in the past been assigned to certain structures presumably secretory in nature purely on the basis of their morphologic properties. Neither these droplets nor the follicular colloid has ever been isolated in a form that would permit precise determination of their chemical composition. However, because it has been ascertained that the droplets and the follicular colloid have similar staining reactions and similar spectrophotometric absorption curves (Gersh and Caspersson, 1940; De Robertis, 1941 *a*), and because increased numbers of droplets appear in the follicular cells and seem to be extruded into the follicular lumen when follicular colloid is being deposited (De Robertis, 1942), it has been assumed that the chemical precursors of thyroglobulin and whatever other proteins are present in follicular colloid are also present in the colloid droplets. As yet there is only slight justification to draw a similar analogy for the large droplets with a homogeneous content described in this report on the basis of their appearance under electron microscopic examination. As far as their distribution, general shape, and frequency of occurrence are concerned, they correspond to the colloid droplets observed with the light microscope. They are not numerous in the thyroid gland of the normal rat; they are generally spherical in outline and are found largely in the apices of the follicular cells. Studies by the author which will be reported later show that these droplets increase in number and are rapidly extruded from the follicular cells when colloid secretion is accelerated. These observations augment substantially the justification for equating them with colloid droplets.

In an earlier study of the thyroid gland by electron microscopy, Dempsey and Peterson (1955) identified the ergastoplasmic vesicles as colloid droplets. In favor of such an identification, one may cite the morphologic similarity between the contents of the vesicles and the follicular colloid. However, the number of vesicles present in normal follicular cells far exceeds the number of colloid droplets observed in stained preparations examined with the light microscope. Furthermore, the vesicles do not exhibit the characteristic configuration and outline of colloid droplets, and they are distributed more or less evenly throughout the cell. The larger vesicles are localized predominantly in the base rather than the apex of the follicular cells.

Finally, despite their large number, no appearance of the vesicles ever suggests that their contents are released into the follicular lumen.

In nearly every exocrine and in a number of endocrine glands which have been examined by electron microscopy, the definitive secretory droplets appear to develop from small vesicular or granular elements of the Golgi apparatus (Palay, 1958). Because of the scarcity of secretory droplets in normal follicular cells, it seems that colloid is elaborated at a relatively slow rate. However, since the few small secretory droplets present resemble vesicular elements of the Golgi apparatus, it appears likely that the origin of secretory droplets in the follicular cells conforms to the pattern already noted in other exocrine cells. A more definite conclusion cannot be drawn without additional evidence.

Among the cytoplasmic organelles, the ergastoplasm is the one most directly concerned in the synthesis of protein for secretion (see review by Haguenau, 1958). It still remains a fundamental problem of cytological research to discover the nature of the relationship between the Golgi apparatus and the ergastoplasm which permits the former to collect and store the synthetic product of the latter. No morphological evidence has been uncovered in this study to show that the ergastoplasm and Golgi apparatus are directly connected with each other. Therefore, it is possible that the transfer of secretory material from synthetic centers to sites of storage and release takes place by means of diffusion of newly synthesized materials in soluble form to the site of their accumulation in the Golgi apparatus.

The ergastoplasmic sacs of typical exocrine cells have a narrow lumen, and the composition of their content, usually a homogeneous material of slight density, is unknown. In pancreatic acinar tissue subjected to intense secretory stimulation, dense granules appear within the ergastoplasmic sacs (Palade, 1956). They have been isolated by means of differential centrifugation and shown to contain essentially the same prozymogen substances found in isolated zymogen granules. These "intracisternal granules" are postulated to represent the newly synthesized product of the ergastoplasm destined to be deposited in secretory droplets (Siekevitz and Palade, 1958 *a*; 1958 *b*). The follicular cells possess an elaborate ergastoplasmic network which extends throughout the cytoplasm of each cell but which lacks the orientation in parallel

lamellae of this organelle in other exocrine glands. Its individual sacs are distended, not flattened, and are exceedingly pleomorphic. Their silhouettes have very little relation to one another, and their content which is moderately dense forms a relatively larger portion of the whole organelle than it does in other exocrine glands. By analogy with the findings in the pancreas, it is possible that the homogeneous substance within the ergastoplasmic sacs of follicular cells represents a precursor of their secretory product.

In the lining epithelium of the rat intestine, the content of the ergastoplasmic vesicles varies with the absorptive state of the animal. In the starved rat, they are empty, whereas following a fatty meal dense lipid droplets appear in the lumina of the sacs. They are believed to represent lipid being transported from the luminal to the mucosal surface of the epithelial cells (Palay and Karlin, 1959 *a*; 1959 *b*). Again, by morphologic analogy, it is at least conceivable that the dense content of the ergastoplasm in follicular cells can reflect the involvement of this structure in mechanisms of transport.

Autoradiographic studies show that thyroid hormone is stored within each follicle as a component of the colloid. The hormone is held within the colloid by being securely bound to thyroglobulin (Nadler and Leblond, 1954). The secretion of thyroid hormone in its physiologic sense, *i.e.*, the release of thyroid hormone into the circulation, involves at a cellular level the release of thyroid hormone from thyroglobulin and its passage across the follicular cells. The process of "endocrine secretion" in the follicular cells is, therefore, most directly concerned with mechanisms of proteolysis and transport. Currently, the most widely accepted theory for the release of thyroid hormone stems from the demonstration by De Robertis (1941 *b*) of the presence of proteolytic activity in thyroid colloid. Presumably, the follicular cells secrete a proteolytic enzyme into the lumen of the follicles where it digests thyroglobulin. Thus, bound hormone is released and can diffuse across the epithelial cells into the circulation. By implication, the enzyme would be a protein, and its elaboration would represent an additional secretory chore for the follicular cells apart from the elaboration of thyroglobulin. No clear evidence of a second secretory apparatus has been uncovered in this or previous studies of the thyroid gland. However, it is possible that organelles such as the vesi-

cles of the superficial cytoplasmic layer, whose relationship to thyroid physiology has not yet been determined, may be involved in the elaboration of a proteolytic enzyme. Diffusion of soluble unbound thyroid hormone across the follicular cells should require no specific morphologic apparatus, and apart from the possible occurrence of pinocytosis at their apical surfaces, no morphologic apparatus which might facilitate diffusion of the hormone has as yet been described.

BIBLIOGRAPHY

- Alksne, J. F., *Quart. J. Exp. Physiol.*, 1959, **44**, 51.
 Andersson, O. A., *Arch. Anat. u. Physiol., Anat. Abt.*, 1894, 177.
 Aron, M., *Compt. rend. Soc. biol.*, 1930, **105**, 585.
 Bargmann, W., in *Handbuch der mikroskopischen Anatomie des Menschen*, (W. von Möllendorff, editor), Berlin, Julius Springer, 1939, **6/2**, 2-136.
 Baber, E. C., *Phil. Tr. Roy. Soc., London*, 1881, **172**, 577.
 Bennett, H. S., Luft, J. H., and Hampton, J. C., *Am. J. Physiol.*, 1959, **196**, 381.
 Bensley, R. R., *Am. J. Anat.*, 1916, **19**, 37.
 Biondi, D., *Berl. klin. Woch.*, 1888, 954.
 Biondi, D., *Arch. ital. biol.*, 1892, **17**, 475.
 Braunsteiner, H., Fellingner, K., and Pakesch, F., *Endocrinology*, 1953, **53**, 123.
 Clark, S. L., Jr., *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 41.
 Dempsey, E. W., and Peterson, R. R., *Endocrinology*, 1955, **56**, 46.
 Dempsey, E. W., and Singer, M., *Endocrinology*, 1946, **38**, 270.
 De Robertis, E., *Am. J. Anat.*, 1941 *a*, **68**, 317.
 De Robertis, E., *Anat. Rec.*, 1941 *b*, **80**, 219.
 De Robertis, E., *Anat. Rec.*, 1942, **84**, 125.
 Doniach, I., and Logothetopoulos, J. H., *J. Endocrinol.*, 1955, **13**, 65.
 Ekholm, R., *Z. Zellforsch.*, 1957, **46**, 139.
 Ekholm, R., and Sjöstrand, F. S., in *Electron Microscopy*, Proceedings of the Stockholm Conference, September 1956, (F. S. Sjöstrand and J. Rhodin, editors), Stockholm, Almqvist and Wiksell, 1957 *a*, 171.
 Ekholm, R., and Sjöstrand, F. S., *J. Ultrastruct. Research*, 1957 *b*, **1**, 178.
 Fawcett, D. W., *J. Nat. Cancer Inst.*, 1955, **15**, 1475.
 Fawcett, D. W., in *Frontiers in Cytology*, (S. L. Palay, editor), New Haven, Yale University Press, 1958, 19-41.
 Fawcett, D. W., and Porter, K. R., *J. Morphol.*, 1954, **94**, 221.
 Gersh, I., *J. Endocrinol.*, 1950, **6**, 282.
 Gersh, I., and Caspersson, T., *Anat. Rec.*, 1940, **78**, 303.
 Haguénau, F., in *International Review of Cytology*, (G. H. Bourne and J. F. Danielli, editors), New York, Academic Press Inc., 1958, **7**, 425-483.
 Kuff, E. L., and Dalton, A. J., *J. Ultrastruct. Research*, 1957, **1**, 62.
 Langendorff, O., *Arch. Anat. u. Physiol., Physiol. Abt.*, suppl., 1889, 219.
 Lewis, W. H., *Bull. Johns Hopkins Hosp.*, 1931, **49**, 17.
 Monroe, B. G., *Anat. Rec.*, 1953, **116**, 345.
 Moore, D. H., and Ruska, H., *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 457.
 Nadler, N. J., and Leblond, C. P., in *The Thyroid*, Brookhaven Symposia in Biology, 1954, **7**, 40-60.
 Newman, S. B., Borysko, E., and Swerdlow, M., *J. Research*, 1949, **43**, 183.
 Palade, G. E., *J. Exp. Med.*, 1952, **95**, 285.
 Palade, G. E., *J. Histochem. and Cytochem.*, 1953 *a*, **1**, 188.
 Palade, G. E., *J. Appl. Physics*, 1953 *b*, **24**, 1424.
 Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955 *a*, **1**, 59.
 Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955 *b*, **1**, 567.
 Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 417.
 Palade, G. E., and Porter, K. R., *J. Exp. Med.*, 1954, **100**, 641.
 Palade, G. E., and Siekevitz, P., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 671.
 Palay, S. L., in *Frontiers in Cytology*, (S. L. Palay, editor), New Haven, Yale University Press, 1958, 305-342.
 Palay, S. L., and Karlin, L. J., *J. Biophysic. and Biochem. Cytol.*, 1959 *a*, **5**, 363.
 Palay, S. L., and Karlin, L. J., *J. Biophysic. and Biochem. Cytol.*, 1959 *b*, **5**, 373.
 Palay, S. L., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 69.
 Peachey, L. D., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 233.
 Pease, D. C., *J. Histochem. and Cytochem.*, 1955, **3**, 295.
 Pitt-Rivers, R., and Trotter, W. R., *Lancet*, 1953, **265**, 918.
 Ponce, K., *Ann. Endocrinol.*, 1951, **12**, 266.
 Rhodin, J., Correlation of ultrastructural organization and function in normal and experimentally changed proximal convoluted tubule cells of the mouse kidney, Stockholm, Karolinska Institutet, Aktiebolaget Godvil, 1954.
 Richter, G. W., *J. Exp. Med.*, 1957, **106**, 203.
 Richter, G. W., *J. Exp. Med.*, 1959, **109**, 197.
 Siekevitz, P., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1958 *a*, **4**, 309.
 Siekevitz, P., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1958 *b*, **4**, 557.
 Sjöstrand, F. S., *Nature*, 1953, **171**, 30.
 Sjöstrand, F. S., and Hanzon, V., *Exp. Cell Research*, 1954, **7**, 393.

- Sjöstrand, F. S., and Zetterqvist, H., in *Electron Microscopy*, Proceedings of the Stockholm Conference, September 1956, (F. S. Sjöstrand and J. Rhodin, editors), Stockholm, Almqvist and Wiksell, 1957, 150.
- Traina, R., *Anat. Anz.*, 1910, **35**, 554.
- Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 257.
- Williams, R., *Am. J. Anat.*, 1937, **62**, 1.
- Wissig, S. L., *Anat. Rec.*, 1958, **130**, 467.
- Wollman, S. H., and Wodinsky, I., *Endocrinology*, 1955, **56**, 9.
- Zetterqvist, H., The ultrastructural organization of the columnar absorbing cells of the mouse jejunum, Stockholm, Karolinska Institutet, Aktiebolaget Godvil, 1956.

EXPLANATION OF PLATES

All of the figures illustrate specimens of thyroid glands of normal rats maintained at room temperature. The specimens were fixed in buffered osmium tetroxide and embedded in methacrylate.

PLATE 227

FIG. 1. Phase contrast micrograph of a section $2\ \mu$ thick showing portions of adjacent follicles. $\times 2,800$.

The low columnar follicular epithelium borders the homogeneous colloid (*co*). The colloid displays a noticeable density, and, at its margin, the apical surfaces of the follicular cells have a barely discernible, fringed aspect. The cytoplasm of the follicular cells contains a network of branching rod-like structures as well as occasional dense granules. The arrow indicates a solitary spherical secretory droplet of considerable density in the apical region of one of the follicular cells. The nuclei appear as voluminous spherical vesicles of moderate density lying in the basal region of the cells. Most of them contain one or two distinct nucleoli. A perifollicular capillary (*c*) lies near the right border of the figure.

FIG. 2. Phase contrast micrograph of a section $2\ \mu$ thick showing a segment of the epithelial wall of a follicle. $\times 2,800$.

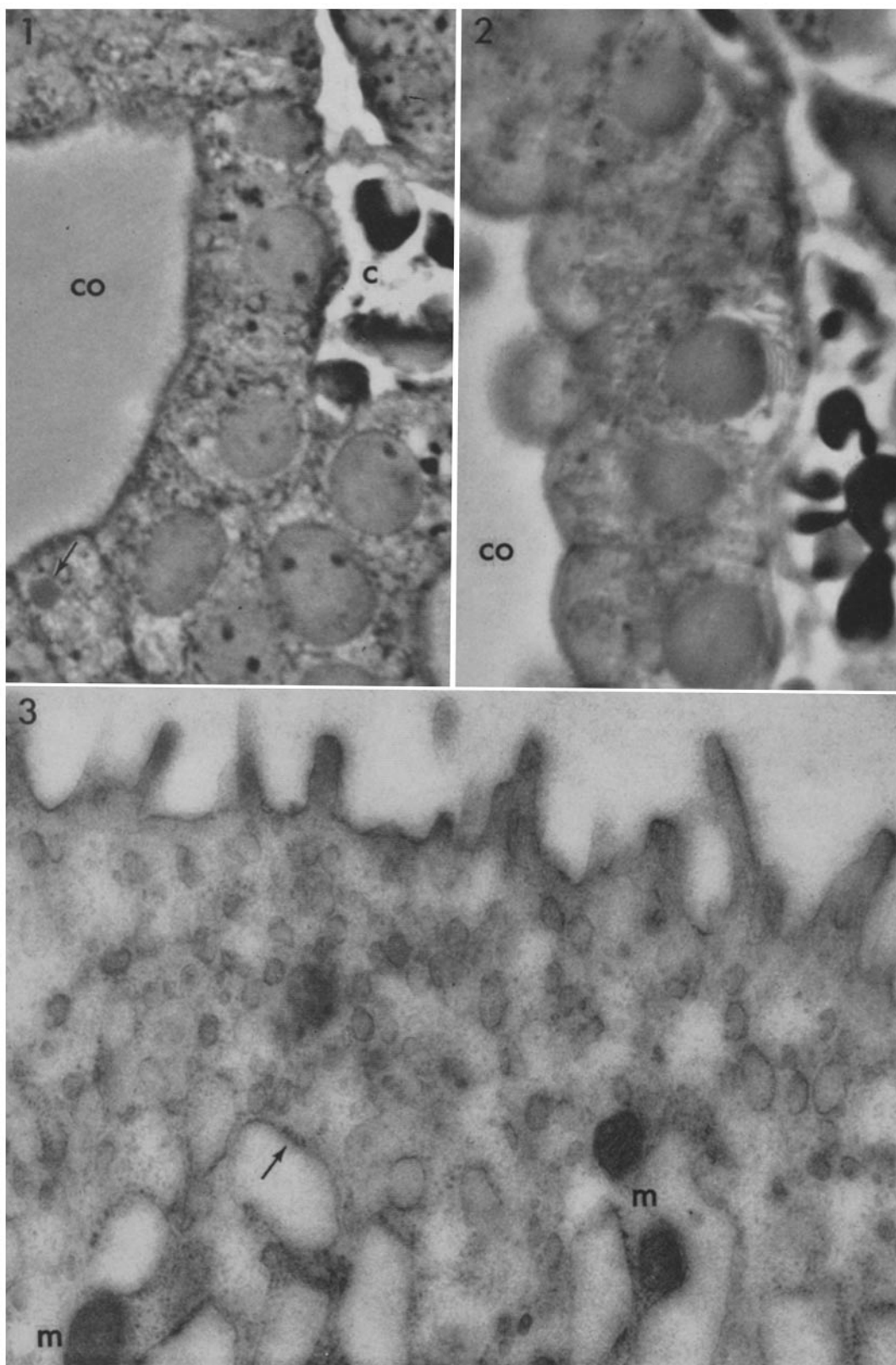
The apices of the columnar epithelial cells have a fuzzy surface that bulges into the peripheral margin of the homogeneous colloid (*co*). The cells are taller than in the previous figure, and their nuclei do not display any nucleoli. A meshwork of rods and granules lies within the moderately dense cytoplasmic ground substance. Several capillaries lie along the right border of the figure.

Between the nucleus and the basal surface of the follicular cell in the center of the figure lie a number of striations separated by clear spaces of relatively low density. A number of them are spaced equally apart and are arranged parallel to one another and to the nuclear margin. Apparently, the clear regions represent the homogeneous contents of large ergastoplasmic vesicles lying in the base of the follicular cell. The striations represent the membranous boundaries of the sacs as well as the cytoplasmic ground substance that intervenes between them.

FIG. 3. Electron micrograph of the apex of a follicular cell in perpendicular section. $\times 48,000$.

Numerous finger-like processes or microvilli of the apical surface of the follicular cell protrude into the pale, homogeneous colloid in the upper portion of the figure. The microvilli are of irregular orientation and height, and they are bounded by the apical plasma membrane. Some appear bent and fused with their neighbor. Their cores consist solely of cytoplasmic matrix. In the superficial region of the cytoplasm, below the microvilli, lie great numbers of round or oval vesicles limited by a single membrane. Their contents vary in density. Apart from these vesicles, little else can be discerned in this region of the cell.

In the zone below the vesicles of the superficial cytoplasmic layer appear a number of mitochondria (*m*). Their internal structure is obscured by their electron density. They lie among numerous ergastoplasmic vesicles of irregular outline which have a pale content similar in density to the colloid. They are limited by a single membrane which in some instances (arrow) is studded only in a restricted segment by ribonucleoprotein particles. Ribonucleoprotein particles lie free in the cytoplasmic matrix between the ergastoplasmic sacs.



(Wissig: Thyroid gland in normal rat)

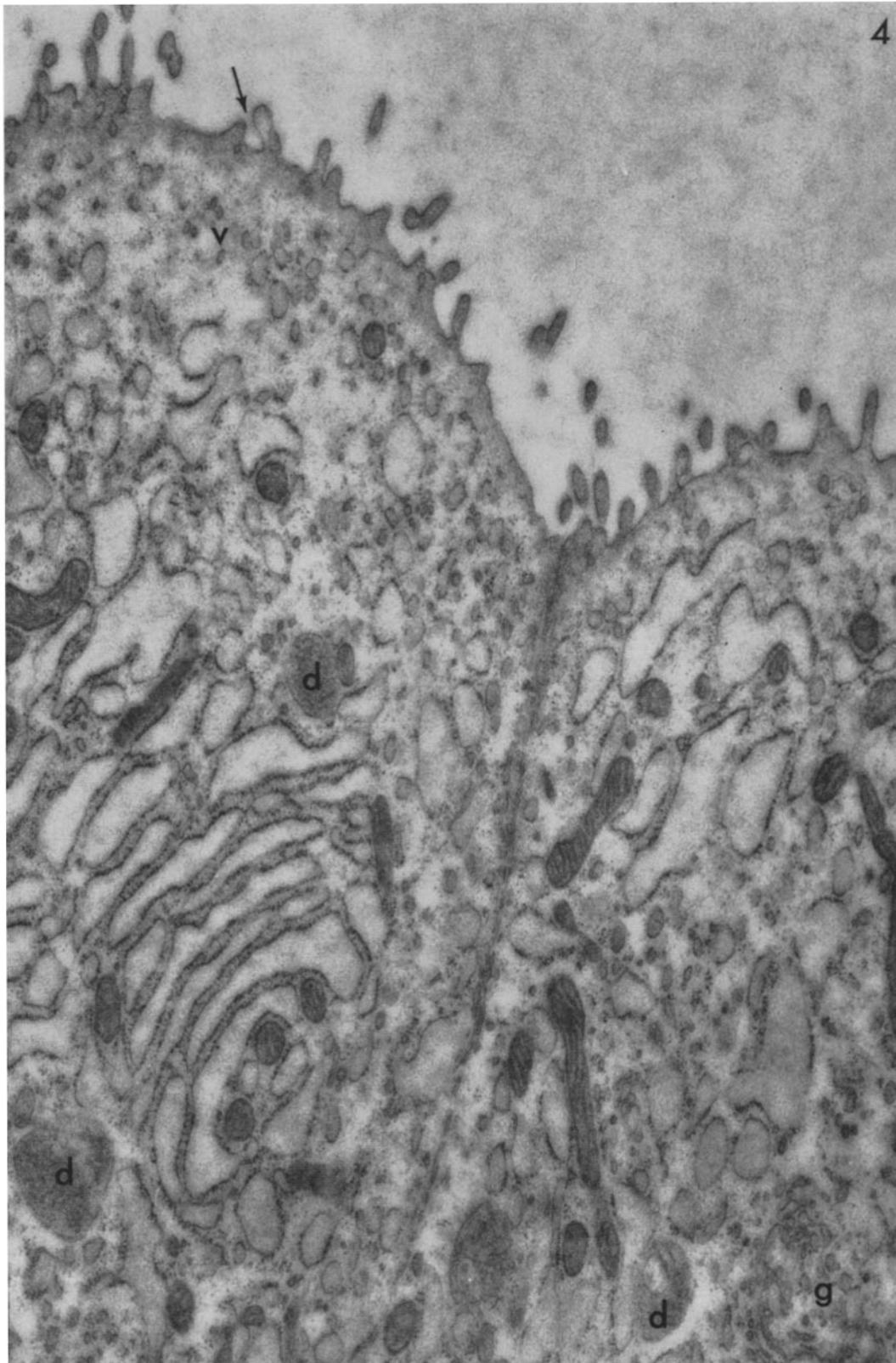
PLATE 228

FIG. 4. Electron micrograph showing portions of the supranuclear cytoplasm in two adjacent follicular cells. $\times 33,000$.

The colloid appears to have a faintly granular or fibrillar texture. Numerous microvilli of the follicular cells invade its periphery. Some appear completely detached from their apical surface presumably because their axes are oblique to the plane of section. In the upper left corner of the figure, a number of small vesicles are interspersed among the microvilli. To the right of these (arrow) the apical surface of the cell is indented by a tubular invagination of the apical plasma membrane. The cytoplasmic matrix immediately adjacent to the apical plasma membrane appears denser than elsewhere. Just below the surface of the cell to the left is a band of cytoplasm occupied by numerous small vesicles (*v*). Still deeper regions of the cytoplasm of both cells contain a mixture of ergastoplasmic sacs, mitochondria, and occasional droplets.

The mitochondria can be recognized by their internal cristae. In the cell on the left, the ergastoplasmic sacs are smaller and of irregular outline towards the apex of the cell. Farther below they are more voluminous and are flattened parallel with one another in an array resembling a rouleau. Their content resembles colloid.

Round, dense droplets (*d*) appear here and there in the cytoplasm. They are enclosed by a single membrane. In the lower right corner of the figure is a collection of small vesicles identified as Golgi apparatus (*g*). Free ribonucleoprotein particles are strewn throughout the cytoplasmic matrix.



(Wissig: Thyroid gland in normal rat)

PLATE 229

FIG. 5. Electron micrograph of portions of the apical surfaces of adjacent follicular cells. $\times 49,000$.

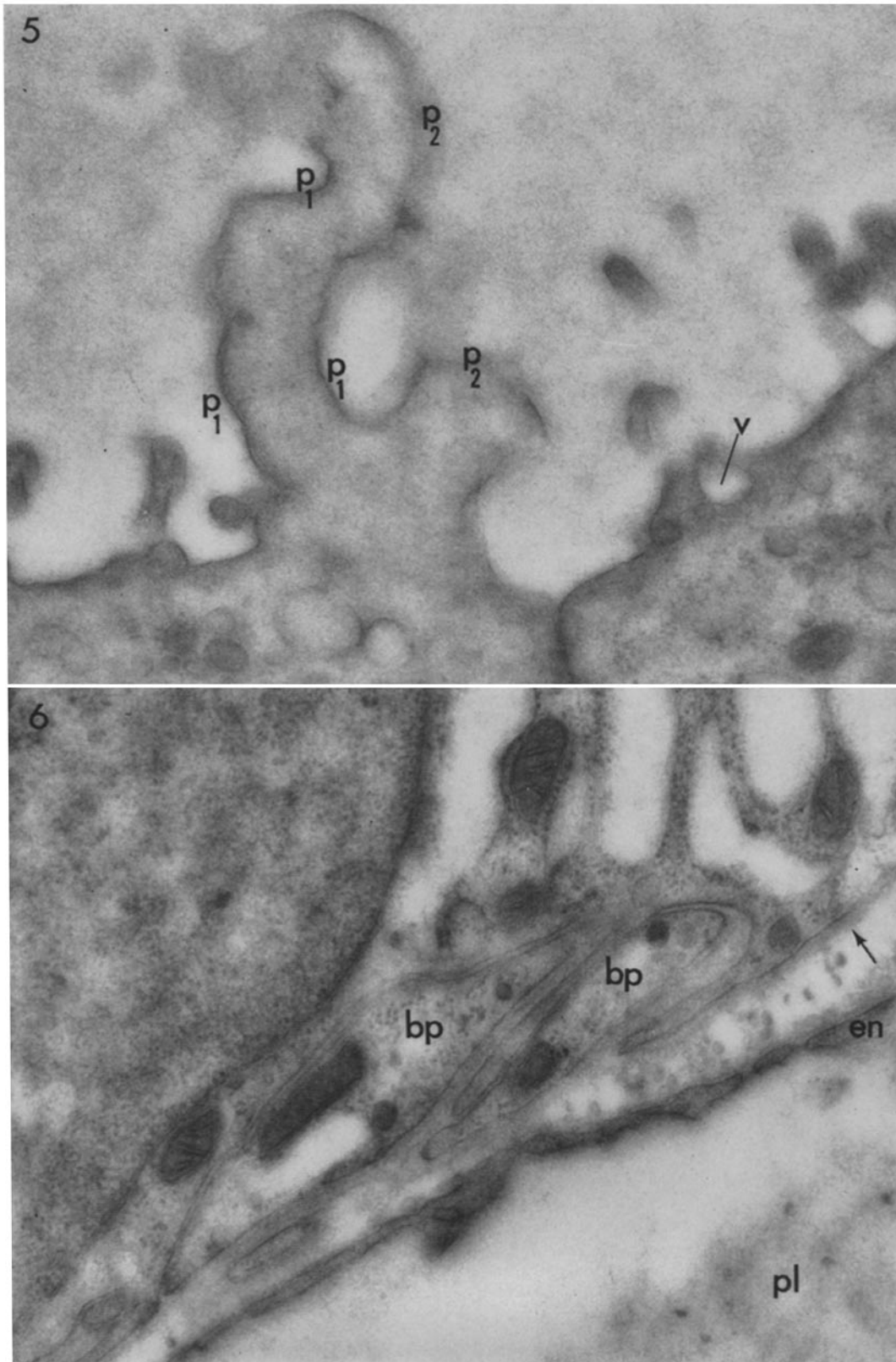
The cell on the right extends a few microvilli of diverse orientation into the colloid. A vesicular space (*v*) appears to be enclosed between two microvilli which fuse at their tips. From the cell on the left, a large serpentine pseudopod protrudes into the colloid. As evidence of its tortuous configuration, the appearance of the plasma membrane varies markedly along its length. In regions where the membrane is normal to the plane of section, it appears as a thin dark line (*p*₁). A short distance lateral to such regions, the orientation of the membrane abruptly twists to parallel that of the plane of section, and here it appears as a relatively broad band of moderate density (*p*₂).

FIG. 6. Electron micrograph of the base of a follicular cell. $\times 99,000$.

The substance of the nucleus appearing at the left border of the figure consists of multitudes of small particles embedded in a homogeneous, pale matrix. The nucleus appears mottled because of localized aggregations of particles which are larger and denser than the remainder. In the cytoplasm at the base of the cell lie numerous ergastoplasmic sacs with mitochondria mingled among them. A large number of ribonucleoprotein particles are distributed in the matrix between the sacs.

A thin, homogeneous basement membrane coats the basal surface of the follicular cell (arrow). A number of processes (*bp*) indent its base and are also coated by the follicular basement membrane. The processes apparently arise from follicular cells as they have essentially the same components as the main body of the cell. The substance of the basement membrane extends into the spaces surrounding the processes.

The capillary in the lower right corner of the field is bordered by a thin endothelial cell (*en*), and within its lumen lie particulate and filamentous remnants of plasma (*pl*).



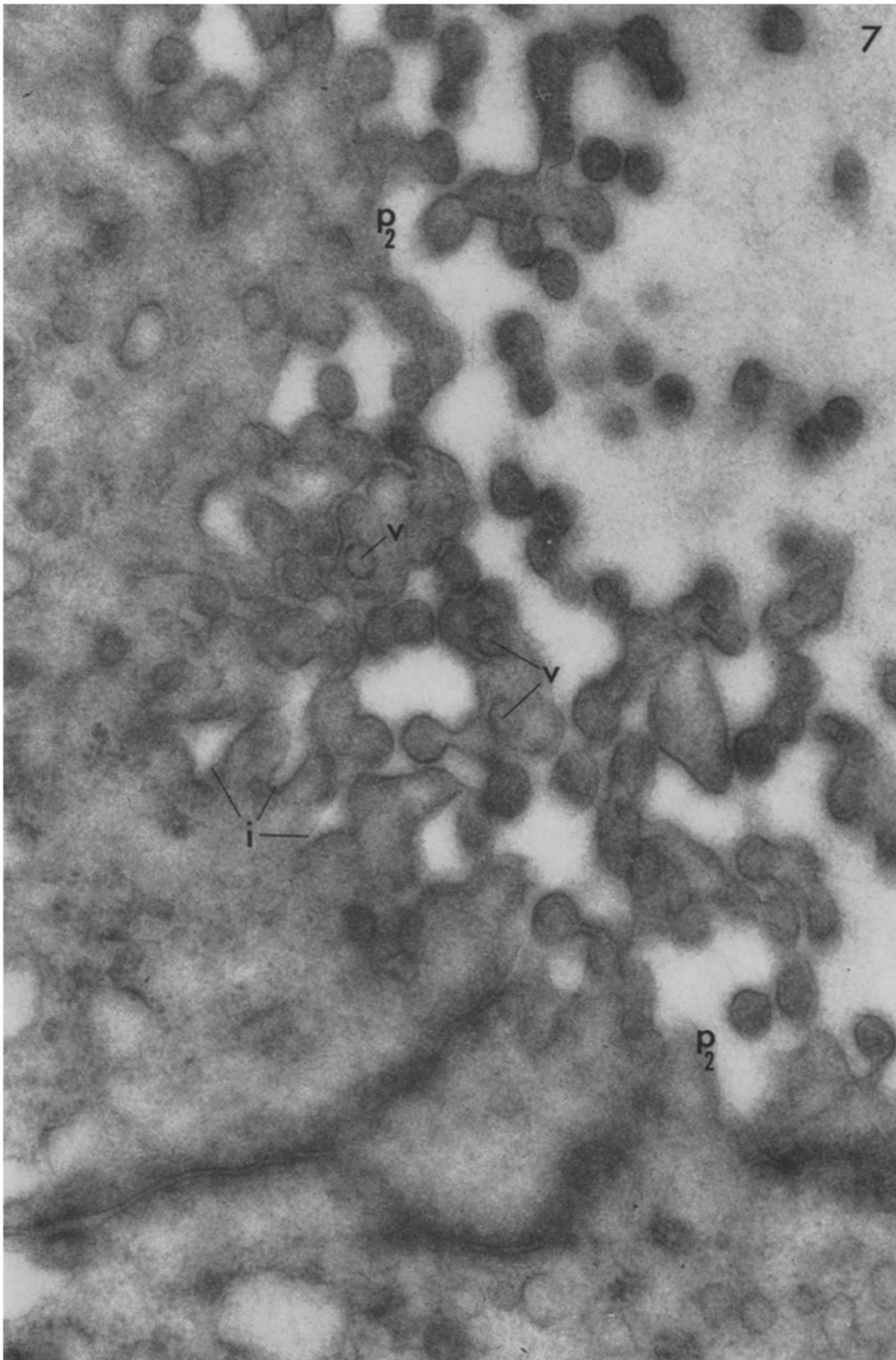
(Wissig: Thyroid gland in normal rat)

PLATE 230

FIG. 7. Electron micrograph of an oblique section through the apices of adjacent follicular cells. $\times 72,500$.

In the upper right corner of the figure lie a number of circular profiles of finger-like microvilli cut in cross-section. Between them the colloid has a mottled, granular texture. Closer to the surface of the follicular cells the circular profiles of the microvilli are joined to one another by thin, web-like isthmuses of cytoplasm. In three dimensions, microvilli joined in this manner should appear as ruffles. Because of the obliquity of the plane of section relative to the apical surfaces of the cells, the apical plasma membrane appears as an indistinct boundary (p_s). Scattered through the substance of the microvilli are numerous vesicular profiles (v). Three invaginations of the apical plasma membrane at the base of microvilli are seen at i .

In the lower portion of the figure the apposed plasma membranes of the adjacent cells follow a parallel course. The cytoplasmic matrix adjacent to the membranes is markedly denser than elsewhere. As the membranes approach the left margin of the figure, they form a desmosome in which the membranes spread slightly apart and are doubled.



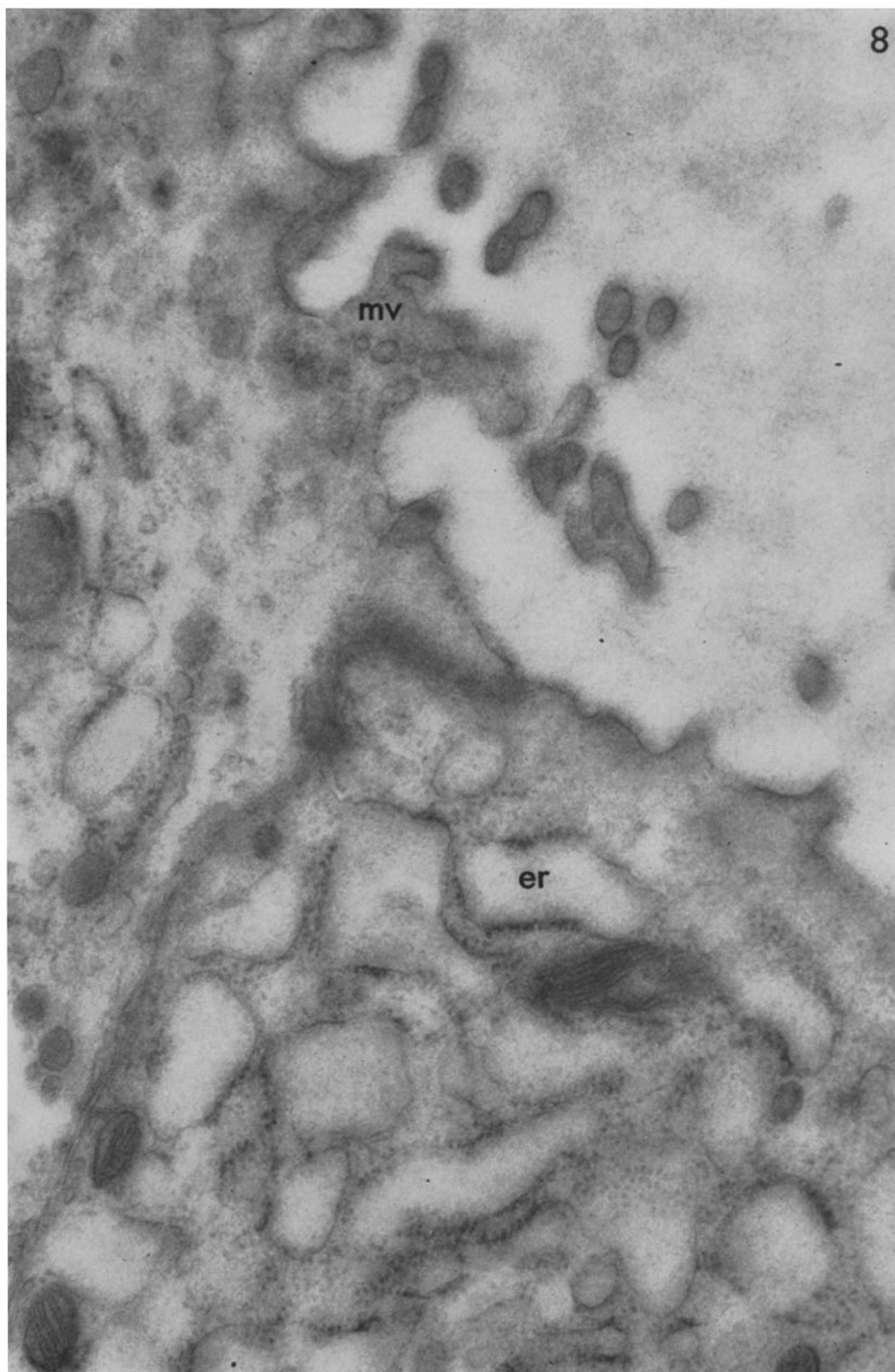
(Wissig: Thyroid gland in normal rat)

PLATE 231

FIG. 8. Electron micrograph of the apical region of adjacent follicular cells. $\times 60,000$.

The colloid in the lumen of the follicle has a granular, faintly mottled texture indistinguishable from that of the material in the ergastoplasmic sacs in the lower portion of the figure. Since the plane of section was relatively oblique to the apical surface of the cells, isolated profiles of microvilli appear detached in the colloid. A relatively broad microvillus of irregular outline is seen at *mv*. Within its substance are numerous circular profiles of small vesicles. The parallel apposed plasma membranes of the follicular cells enter the figure near its lower left corner. As they near the surface of the cells, they are no longer distinguishable, as their orientation becomes oblique to the plane of section. In this region, the increased density of the cytoplasmic matrix adjacent to the membrane is the counterpart of the terminal bar seen with the light microscope.

Numerous ergastoplasmic sacs of relatively small size occur throughout the apical cytoplasm. They are of irregular outline and appear dilated. They are limited by a single membrane which in most instances, as at *er*, has only portions of its surface coated with a heavy concentration of ribonucleoprotein particles. The remainder of its surface is particle-free. Numerous particles lie free in the cytoplasmic matrix between the sacs.

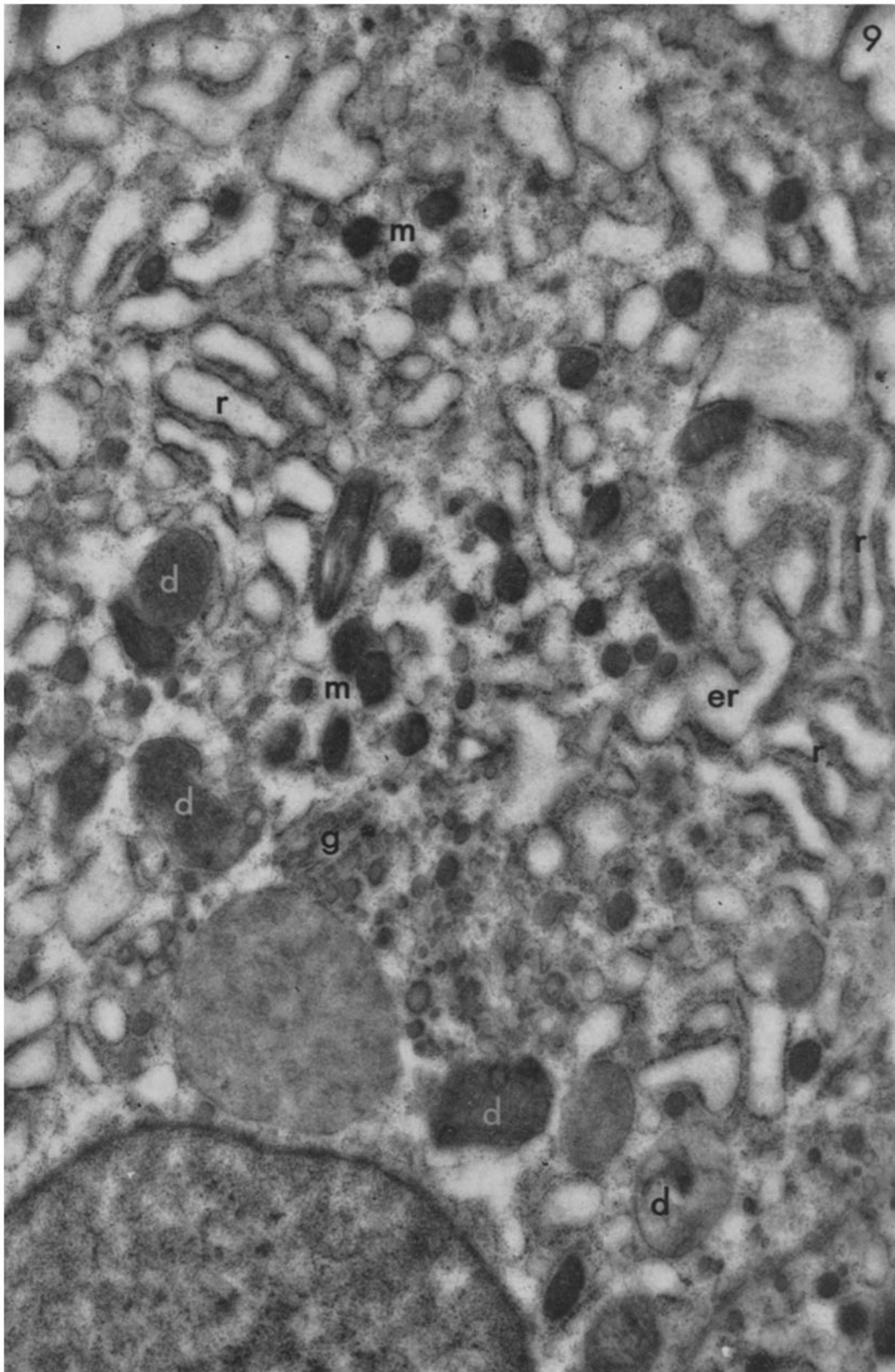


(Wissig: Thyroid gland in normal rat)

PLATE 232

FIG. 9. Electron micrograph of the supranuclear portion of a follicular cell. $\times 26,400$.

The nucleus at the lower border of the figure has a mottled appearance because of the inhomogeneous distribution throughout its substance of minute particles of various sizes and densities. Above it and slightly indenting its upper pole is a large spherical droplet which is bounded by a single membrane and contains a relatively homogeneous substance of medium density. Clustered around the droplet are numerous smaller droplets and vesicles, some of which have the characteristic appearance of vesicles of the Golgi apparatus (*g*). Other large droplets (*d*) of a distinctive character are scattered in the adjacent cytoplasm. Frequently, they are of an elongated or dumbbell shape. Their limiting membrane encloses a dense material that often contains scattered vacuoles and fragments of membranes. Throughout the remainder of the cytoplasm are scattered mitochondria (*m*) which are so dense that they are not easily distinguished from the dense droplets. Voluminous ergastoplasmic sacs are numerous. Some have a sinuous outline (*er*), and only infrequently are they gathered into clumps of flattened vesicles showing some degree of parallel orientation with one another (*r*).



(Wissig: Thyroid gland in normal rat)

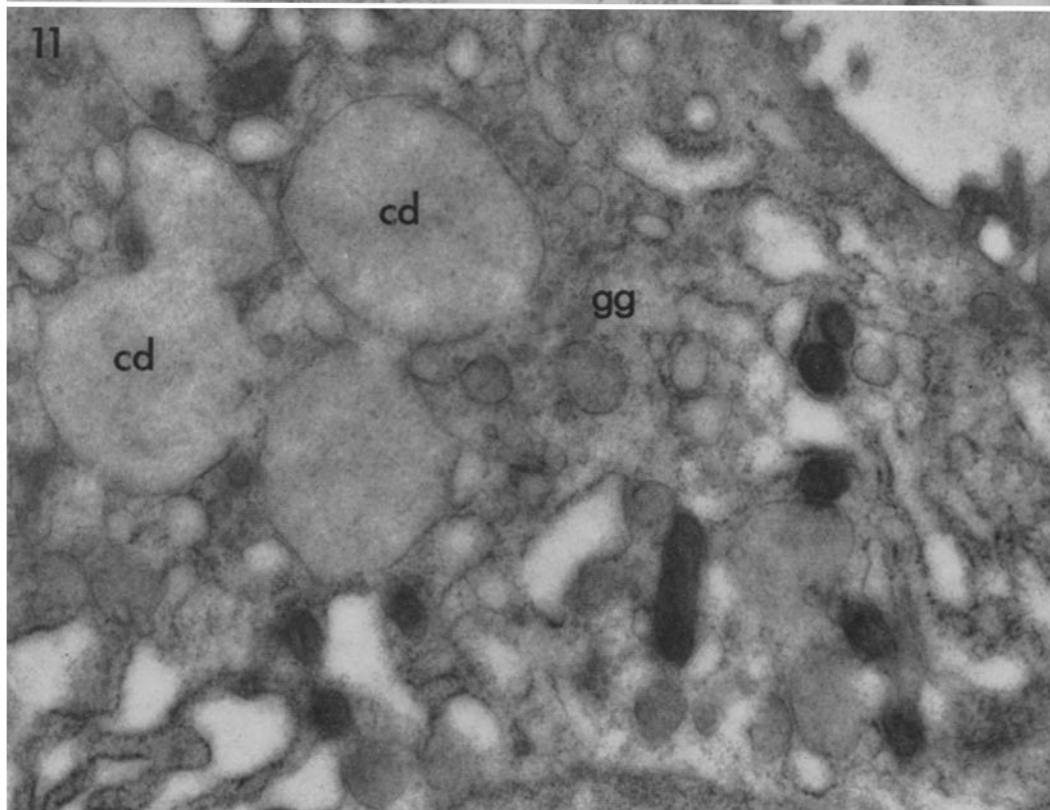
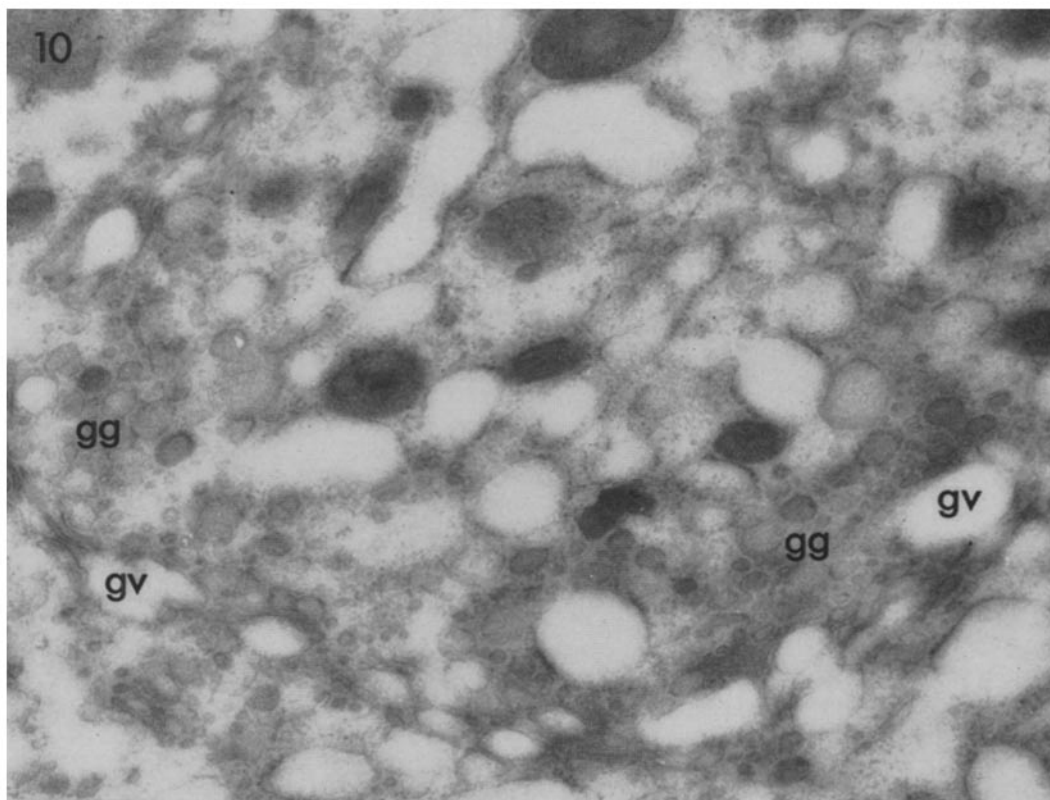
PLATE 233

FIG. 10. Electron micrograph showing the characteristic appearance of a large Golgi complex in a follicular cell. $\times 42,000$.

The center of the figure contains a number of mitochondria and small ergastoplasmic sacs. Arranged in a whorl around them are the elements of the Golgi apparatus. They consist primarily of numerous small, membrane-bound vesicles (*gg*) containing material of a homogeneous, but varied density. Scattered throughout the whorl are small groups of flattened vesicles in a relatively parallel orientation. Some of these vesicles (*gv*) appear relatively large and empty as though for some reason they had become dilated.

FIG. 11. Electron micrograph of the supranuclear region of a follicular cell. $\times 27,000$.

The upper pole of the nucleus lies just at the lower border of the figure. To the left is a cluster of large secretory droplets (*cd*) with a single membrane enclosing a homogeneous material of medium density. Where they abut on one another the intervening membranes have disappeared and their contents merge. In the cytoplasmic matrix to their right are numerous smaller droplets (*gg*) which have a morphologically similar content. The small droplets cannot be distinguished from dense, membrane-bound vesicles in the Golgi apparatus shown in Fig. 10.

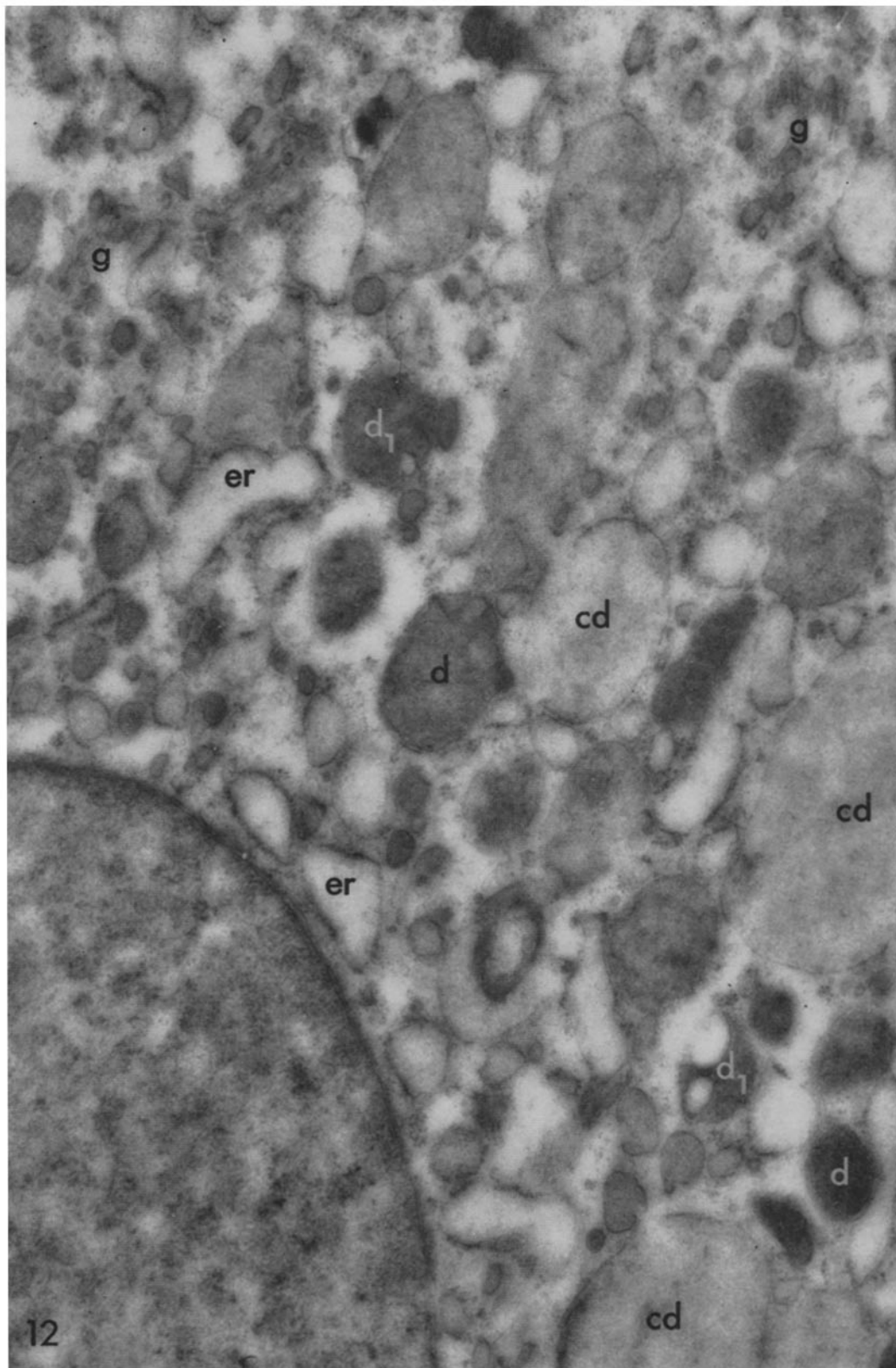


(Wissig: Thyroid gland in normal rat)

PLATE 234

FIG. 12. Electron micrograph of a cytoplasmic region bordering the nucleus of a follicular cell. $\times 38,000$.

This figure illustrates the variety of granules and droplets that occur in the supranuclear zone of the follicular cell. Near the upper left and upper right margins of the figure lie accumulations of Golgi structures (*g*). Elsewhere in the figure are large, pale droplets with a homogeneous content enclosed within a single membrane (*cd*). Additional droplets (*d*), with a content of marked density, are also scattered in the cytoplasm. Often these dense droplets contain membrane-bound vacuoles or other inclusions within their substance (*d_i*). In the regions of the droplets ergastoplasmic sacs (*er*) are sparse.



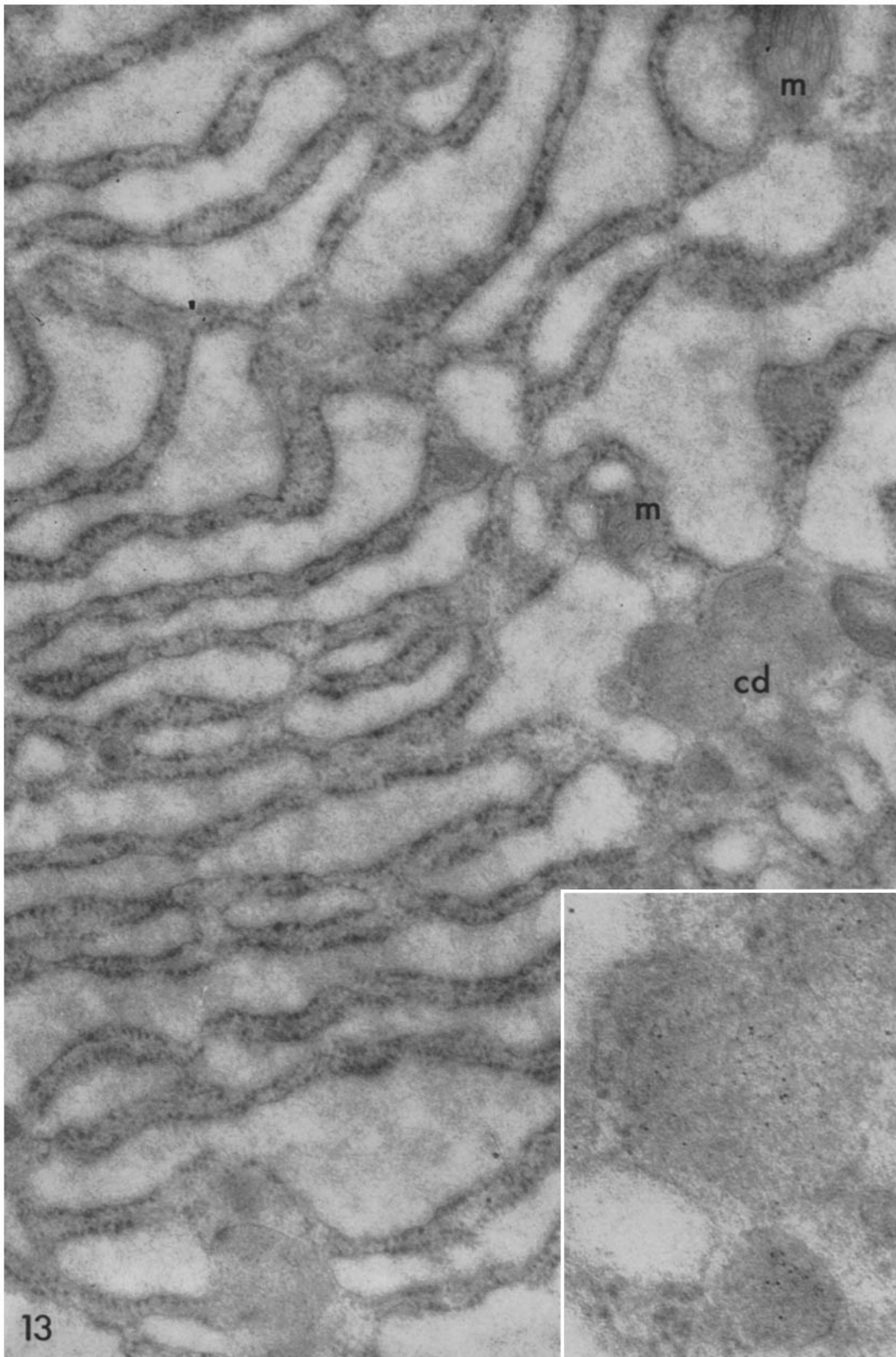
(Wissig: Thyroid gland in normal rat)

PLATE 235

FIG. 13. Electron micrograph of an area of ergastoplasm in a follicular cell. $\times 48,000$. Inset magnification, $\times 112,000$.

The figure shows a rare example in a follicular cell of a cluster of oriented ergastoplasmic sacs resembling those found in other exocrine cells. The sacs are flattened and contain a homogeneous substance of moderate density and granular texture resembling colloid. The vesicles tend to lie parallel with one another. The outer surface of their limiting membrane is covered by numerous ribonucleoprotein particles approximately 130 Å in diameter.

In the cytoplasmic matrix between ergastoplasmic sacs lie mitochondria (*m*) and groups of small droplets (*cd*). The droplets consist of a homogeneous substance enclosed within a membrane. They also contain numerous, extremely fine, dense particles. The group of droplets at the right are shown greatly enlarged in the inset. The particles are numerous and are unevenly distributed throughout the droplet. They tend to be round or angular and are of varied size. The largest is about 75 Å in diameter, and the smallest can barely be resolved.



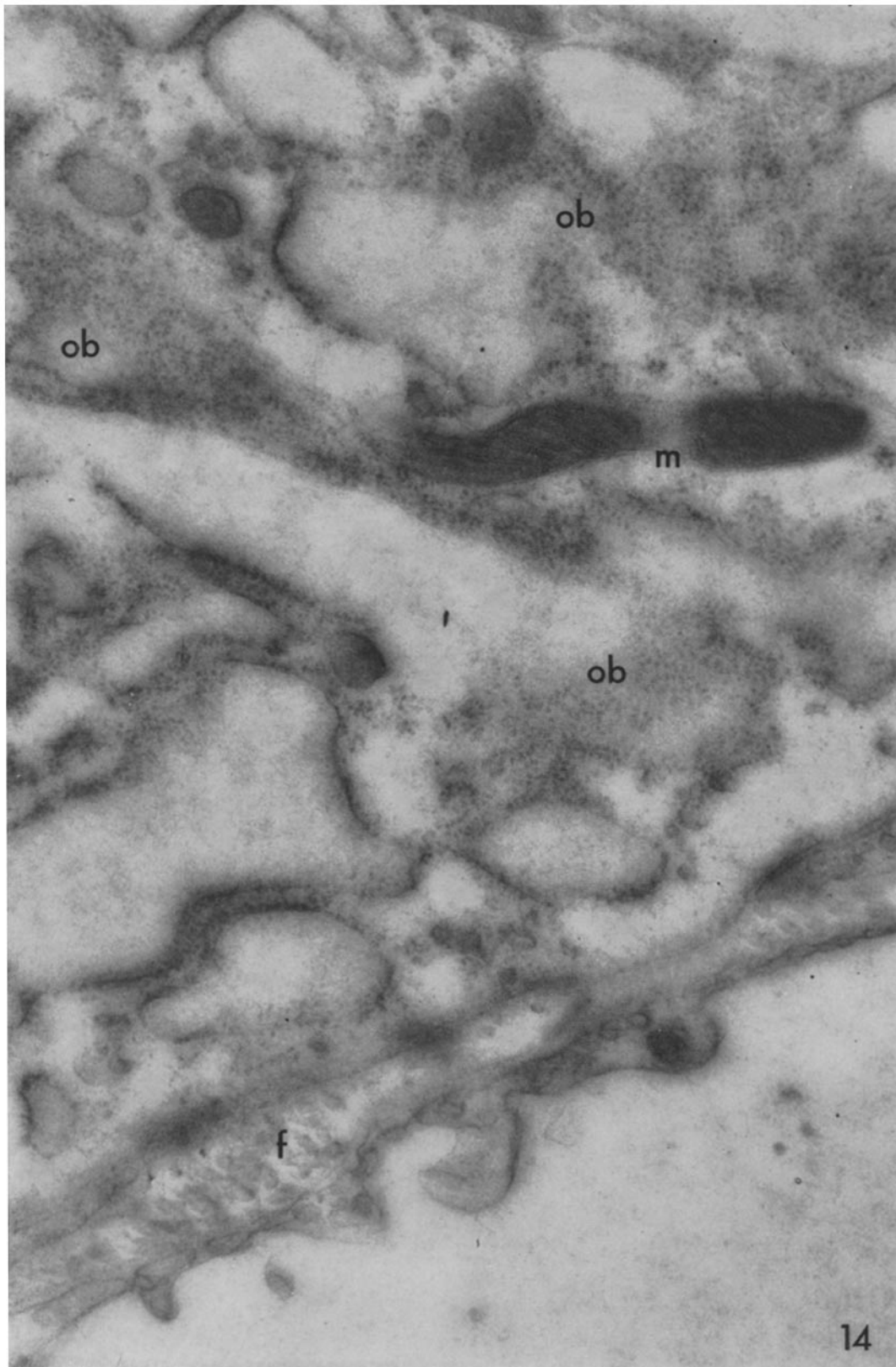
(Wissig: Thyroid gland in normal rat)

PLATE 236

FIG. 14. Electron micrograph of the base of a follicular cell bordering a perifollicular capillary. $\times 52,000$.

The base of the follicular cell contains numerous, large, pleomorphic ergastoplasmic vesicles which are richly covered with numerous ribonucleoprotein particles. Because of their frequent contortions, at many points of their surface their limiting membrane lies oblique to the plane of section and does not appear as a distinct line. Such regions (*ob*) provide a full-face view of the arrangement of the particles on the surface of the membrane. They are frequently aligned in chains, and a number of these seem to run in pairs. Rosettes and other geometric arrangements are also seen. Numerous particles are free in the cytoplasmic matrix between the vesicles. A sinuous mitochondrion passes in and out of the plane of section at *m*.

In the space between the base of the follicular cell and perifollicular capillary lie numerous connective tissue fibers (*f*) cut transversely. The endothelial wall of the capillary is alternately of substantial thickness and extremely thin. The plasma in its lumen has a faintly granular or finely fibrillar texture and contains occasional small particles of considerable density.



(Wissig: Thyroid gland in normal rat)

PLATE 237

FIGS. 15 to 17. Electron micrographs of perifollicular capillaries.

FIG. 15. The base of a follicular cell extends across the upper portion of the figure. Below it lies a portion of the wall of a perifollicular capillary cut in transverse section. The basement membranes of the follicular cell and of the capillary appear only as pale, hazy lines. In its thinnest regions, the endothelial wall is only 350 Å thick, and it is perforated by frequent pores (arrows) with an average diameter of 450 Å. $\times 55,000$.

FIG. 16. The bases of several adjacent follicular cells (*ep*) extend across the upper portion of the figure. Below these, the endothelial wall (*en*) of the capillary appears in oblique section. In the central thin portion of the endothelial wall, a number of oval and circular clear areas can be discerned (arrow). These have the same average diameter as the pores and are presumed to represent their aspect in full-face view. $\times 65,000$.

FIG. 17. The base of a follicular cell lying across the upper margin of the figure is subtended by several flat processes (*bp*). The follicular basement membrane (*bm₁*) appears as a distinct line coating the peripheral limits of the cell and the processes.

The endothelial wall of the capillary consists of alternating thick and thin areas. Occasional pores are seen in the thin areas. In the thick regions, numerous small vesicles are distributed throughout the cytoplasmic matrix.

The capillary basement membrane (*bm₂*) appears as a distinct line coating the external surface of the endothelial cell. The space between it and the follicular basement membrane is relatively free of connective tissue fibers. Note that the density of this space is appreciably less than that of the plasma in the lumen of the capillary. $\times 50,000$.

