THE ANATOMY OF SECRETION IN THE FOLLICULAR CELLS OF THE THYROID GLAND

II. The Effect of Acute Thyrotrophic Hormone Stimulation on the Secretory Apparatus

STEVEN L. WISSIG, Ph.D.

From the Departments of Anatomy, Yale University, New Haven, Connecticut, and The University of California, San Francisco, California

ABSTRACT

This paper reports a study by phase contrast and electron microscopy of changes observed in the thyroid gland of the rat at 1, 2, 12, and 24 hours following an injection of thyrotrophic hormone. Examination by phase contrast microscopy reveals that follicular cells contain numerous colloid droplets 1 and 2 hours after injection. By 12 and 24 hours, the colloid droplets are no longer present, and individual follicles appear to be subdividing into smaller units. The droplets are assumed to contain newly synthesized colloid, and their development was studied by electron microscopy. During the period of active secretion, increase in number of the Golgi vesicles leads to enlargement of this organelle. At its periphery small colloid droplets appear to form from large Golgi vesicles. As they form, their content becomes more adielectronic, and fine dense particles less than 75 A in diameter appear in their matrix. Small- and medium-sized droplets lying in the apical region of the cell contain numerous dense particles scattered in their moderately adielectronic content. Large, mature droplets in the same region have a relatively dielectronic content resembling follicular colloid and no longer contain dense particles. The follicular cells appear to utilize apical pseudopodia to release the content of mature droplets into the follicular lumen. Other droplet-like inclusions occur in follicular cells, but they do not seem to be directly concerned with secretion.

INTRODUCTION

From a physiologic standpoint the thyroid is justifiably classified as an endocrine organ. It, nonetheless, utilizes what appears from examination by light microscopy to be an authentic exocrine secretory apparatus in performing its endocrine function, *i.e.*, the follicular cells secrete colloid into the lumen of the follicle, a cavity completely lined with epithelium. The role played by the colloid in the gland's function has been outlined as a result of recent studies employing radioactive iodine (see the review by Gross, 1957). The tyrosine constituents of thyroglobulin in the follicular colloid are first iodinated to form mono- and diiodotyrosine. Pairs of these molecules, while still bound to the protein, then condense to form the iodinated thyronines which comprise the thyroid hormones. The hormones are released into the circulation when thyroglobulin undergoes proteolysis. The present study was undertaken to observe the sequence of changes that takes place in the structure of the thyroid gland during acute stimulation with thyrotrophic hormone, and, on

the basis of these changes, to attempt to ascertain the role of various cytoplasmic organelles in the elaboration of colloid.

The composition of colloid has been analyzed by a variety of techniques. Some years ago it was shown by absorption spectrophotometry that colloid exhibits the characteristic absorption spectrum of thyroglobulin in the ultraviolet range (Ginsel, 1939; Gersh and Caspersson, 1940). Colloid also gives an intensely positive periodic acid–Schiff reaction (McManus, 1946), and there has been considerable discussion concerning the nature of the carbohydrate groups it contains. Gersh (1950) postulated that, in addition to thyroglobulin, a second carbohydrate-containing protein occurred in colloid. He believed it to be a mucopolysaccharide since it was extracted with hyaluronidase. Furthermore, Levine (1950) demonstrated that extracts of thyroid gland contained hyaluronidase activity. Gersh postulated that this enzyme as well as the proteolytic enzyme detected by De Robertis (see below) act in consort to regulate the viscosity of colloid. However, the existence of the second glycoprotein has been questioned by Fisher (1953) because he was unable to extract any PAS-reactive material with hyaluronidase. It is now generally believed that the intense PAS-positive reaction of colloid can be accounted for solely by the presence of thyroglobulin with its abundant carbohydrate moieties (Ujejski and Glegg, 1955). This assumption is strengthened by the fact that hexosamine-containing compounds in thyroid extracts and thyroglobulin have identical electrophoretic mobilities at alkaline and acid pH (Hooghwinkel, Smits, and Kroon, 1954).

Some observers contend that still other proteins occur in follicular colloid, but at this point their occurrence is still open to question. After careful analysis of the basophilic properties of colloid, Dempsey and Singer (1946) reported that it contains nucleoprotein, and their observation was supported by the demonstration that colloid basophilia can be extracted with crystalline ribonuclease. However, Fisher (1953) was unable to confirm their results with this enzyme. Moreover, he noted that the basophilia of colloid can be suppressed by methylation, a procedure that preferentially blocks carboxyl groups. For this reason,

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Figs. 1 to 5 are photomicrographs of sections 1 to 2 μ thick viewed by phase contrast microscopy. The sections were mounted in glycerol without prior removal of the embedding medium. The remainder of the figures are electron micrographs.

FIGURE 1

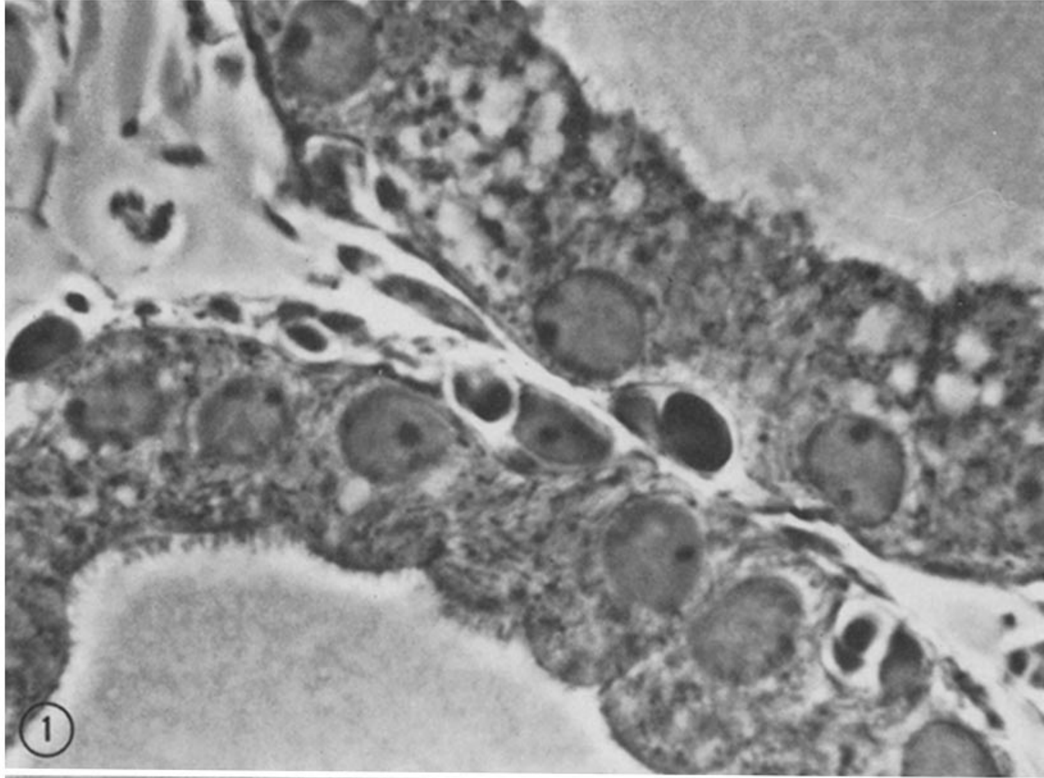
Thyroid gland of a rat that received 0.5 USP unit thyrotrophic hormone 1 hour before autopsy.

The follicles are lined by low columnar epithelial cells. Their cytoplasm is for the most part of a fibrillar texture interspersed with small granules. The apical borders of the cells in the upper follicle are markedly irregular. Some of its cells contain many clear, spherical droplets dispersed throughout their length from apex to base. These are believed to be colloid droplets. The cells of the lower follicle show less evidence of active secretion. The microvilli along their apical borders are longer than normal and are readily visualized even at this resolution. The basement membranes of both follicles can be distinguished in certain areas as a sharp line at the base of the follicular cells, and the follicles are separated from each other by a zone of perifollicular connective tissue. The peripheral margin of the follicles is frequently indented by perifollicular capillaries. $\times$ 2,300.

FIGURE 2

Thyroid gland of a rat that received 0.5 USP unit thyrotrophic hormone 2 hours before autopsy.

The follicular cells are considerably taller than normal. Microvilli cannot be discerned on their apical surfaces. Many of the cells contain an abundance of colloid droplets in their apical cytoplasm. A number of cells extend protoplasmic tabs into the follicular colloid, and at least one of these (*arrow*) appears to contain a small droplet. $\times$ 2,700.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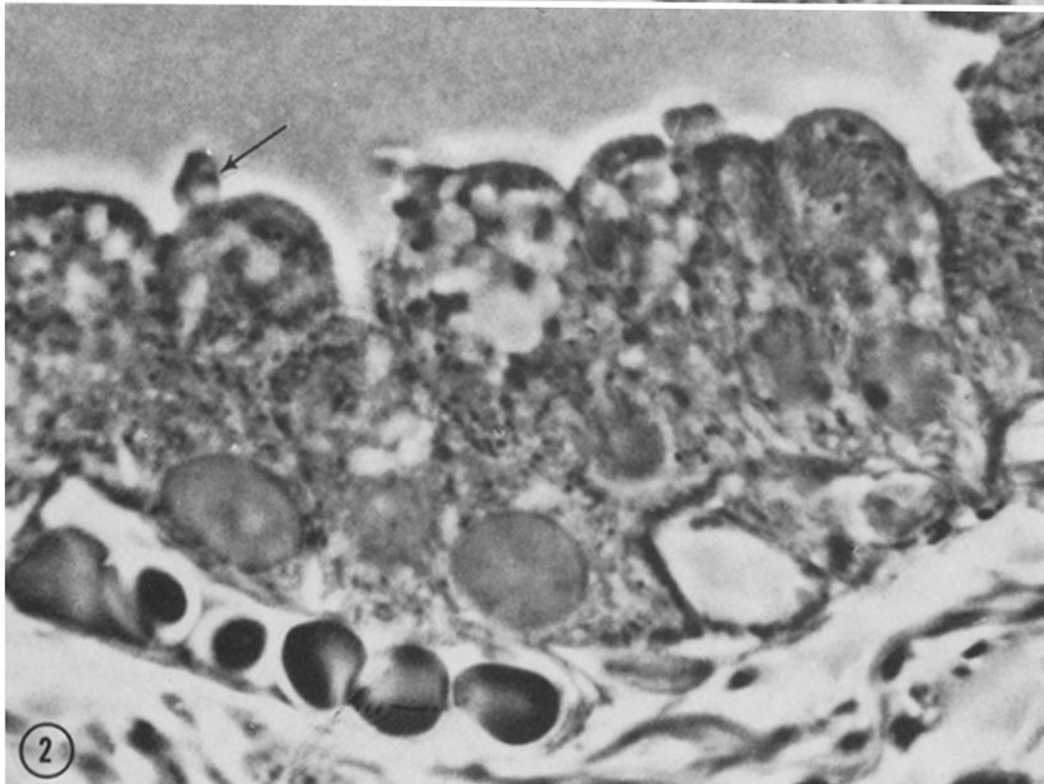
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he believed that the basophilia more likely stems from—COOH and—OSO_3H groups rather than phosphate groups of nucleic acids. De Robertis (1941 *a*) and De Robertis and Nowinski (1946) showed that droplets of colloid extracted from the lumens of follicles by micropipettes are capable of digesting gelatin films, and they considered this evidence that colloid contains a proteolytic enzyme. Thyroglobulin is thought to be the actual substrate for the enzyme which, by freeing the thyroid hormones from the parent protein, could release them from storage in the gland. De Robertis and Grasso (1946) were able to detect peroxidase activity in colloid, but only in glands activated by cold or TSH. Previously Keston (1944) had noted that a peroxidase should be capable of oxidizing iodide to iodine, and, if this reaction occurred in the thyroid gland, the iodine liberated could iodinate tyrosine, the precursor of thyroid hormone. Alexander (1959; 1961) recently verified this supposition by demonstrating the existence in thyroid homogenates of an iodide peroxidase which can perform the iodination reaction. It seems safe to conclude at this point that follicular colloid may contain a varied assortment of constituents and that a full and accurate description of its composition still remains to be achieved.

Within recent years it has become possible to add electron microscopy to the list of techniques by which the composition and formation of follicular colloid can be studied. When visualized with this instrument in thin sections of tissue fixed with osmium tetroxide, colloid presents a relatively undistinguished appearance: it has a uniform texture comprised of sparse fine granules or fibrils (Wissig, 1960). The task of identifying its antecedents in the cytoplasm of the follicular cell on the basis of morphologic criteria is not easily done. In the first place, secretory droplets are relatively scarce in the follicular epithelium of the normal rat, a sign perhaps that protein secretion proceeds at a slow pace. Secondly, the cytoplasmic droplets that are encountered are of diverse types and are structurally complex. None has a content identical with intrafollicular colloid. In general, they may be divided into two classes: the first contains a homogeneous matrix substance, differing in overall density from one droplet to another, in which an extremely fine, dense particulate substance is often dispersed; the second has a heterogeneous content with numerous membranes and granular constituents (Wissig, 1960). On morphologic grounds, neither can with assurance be identified as the secretory antecedent of colloid, although the first class of droplets, the smallest of which strongly resemble vesicles in the Golgi apparatus, seems the more reasonable choice. Dempsey and Peterson (1955) suggested that the dilated ergastoplasmic vesicles characteristic of thyroid follicular cells are the counterparts of colloid droplets seen by light

FIGURE 3

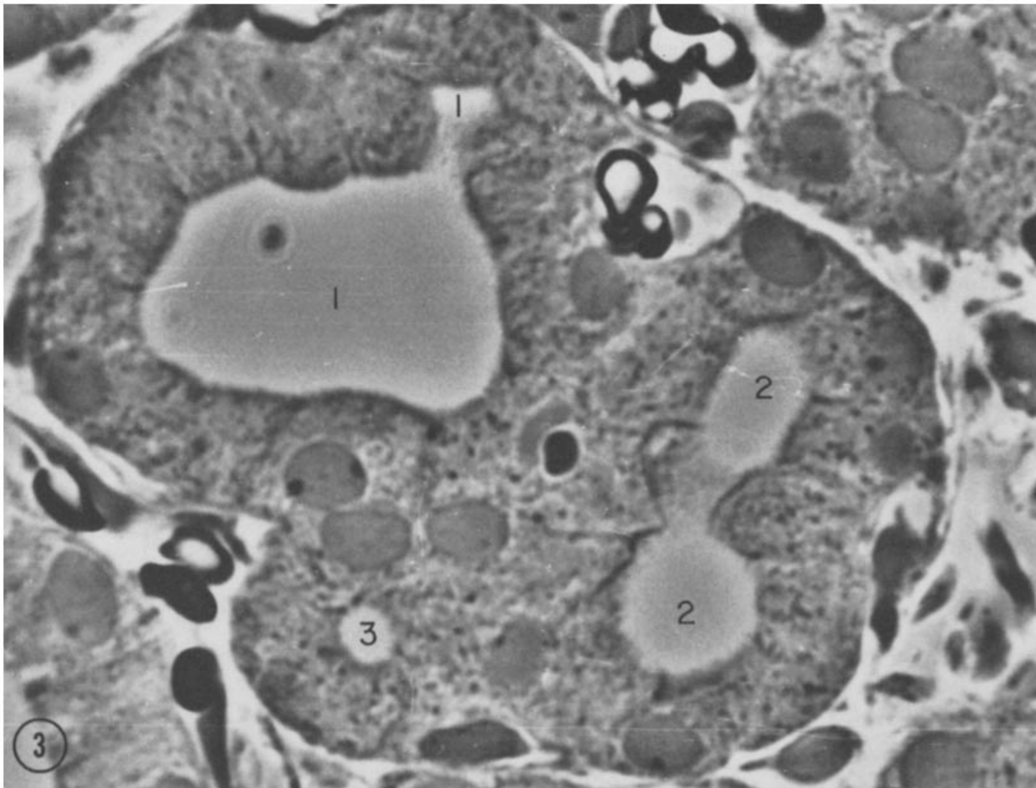
Thyroid gland of a rat that received 0.5 USP unit thyrotrophic hormone 12 hours before autopsy.

A single large follicle of irregular outline lies in the center of the field surrounded by a perifollicular connective tissue space of irregular width. Its epithelial cells do not appear to be taller than normal, nor do they appear to be actively secreting colloid for colloid droplets cannot be discerned in their cytoplasm. The follicular colloid, instead of being concentrated in a central spherical mass as in the normal gland, is subdivided into three apparently separate colloid masses (*1*, *2*, *3*). The appearance of the larger two (*1*, *2*) suggests that each was about to subdivide into smaller units at the moment it was fixed. × 1,500.

FIGURE 4

Thyroid gland of a rat that received 0.5 USP unit thyrotrophic hormone 24 hours before autopsy.

Each of the two large follicles that appear in this field is enclosed within a layer of perifollicular connective tissue. Each is divided into several subunits consisting of a central mass of colloid surrounded by an epithelial layer. The epithelial cells are of normal height and do not show any signs of active secretion. × 1,800.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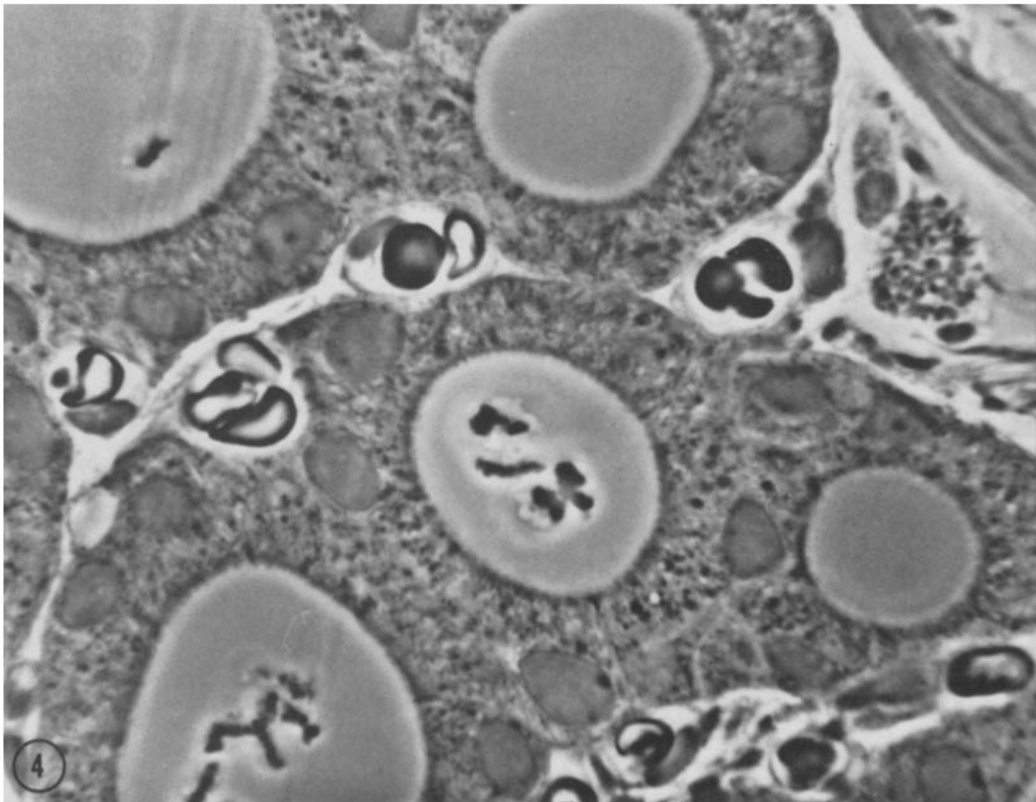
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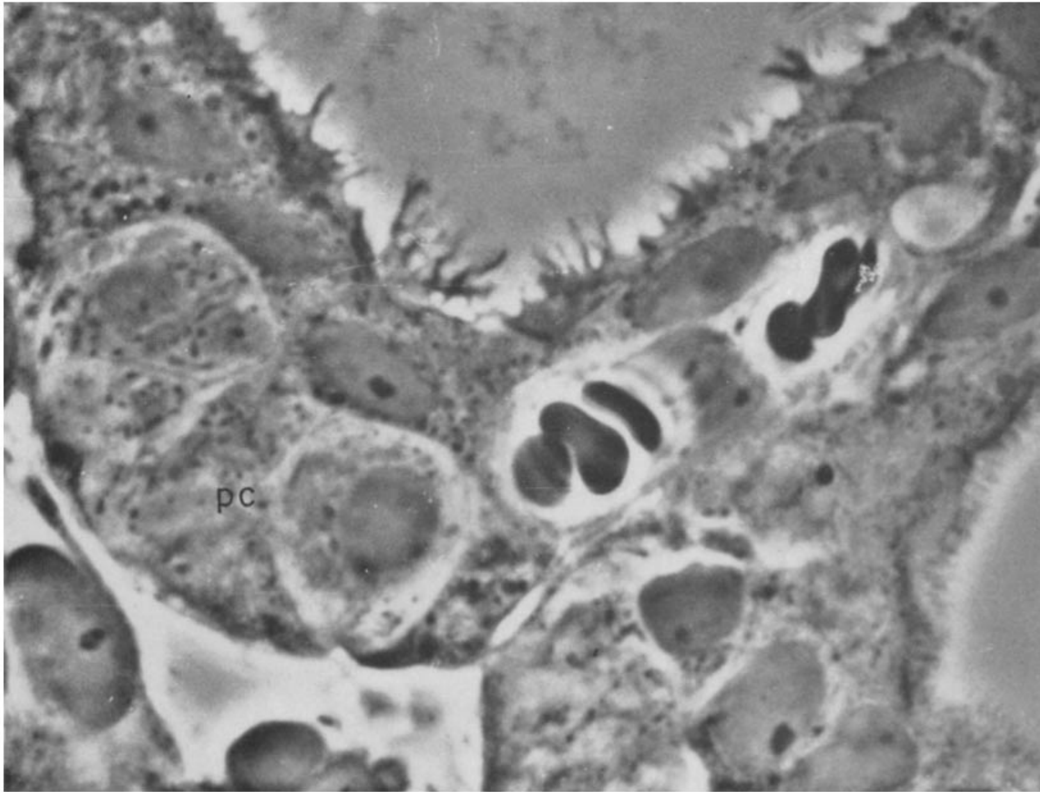


FIGURE 5

Thyroid gland of a rat that received 0.5 USP unit thyrotrophic hormone 24 hours before autopsy.

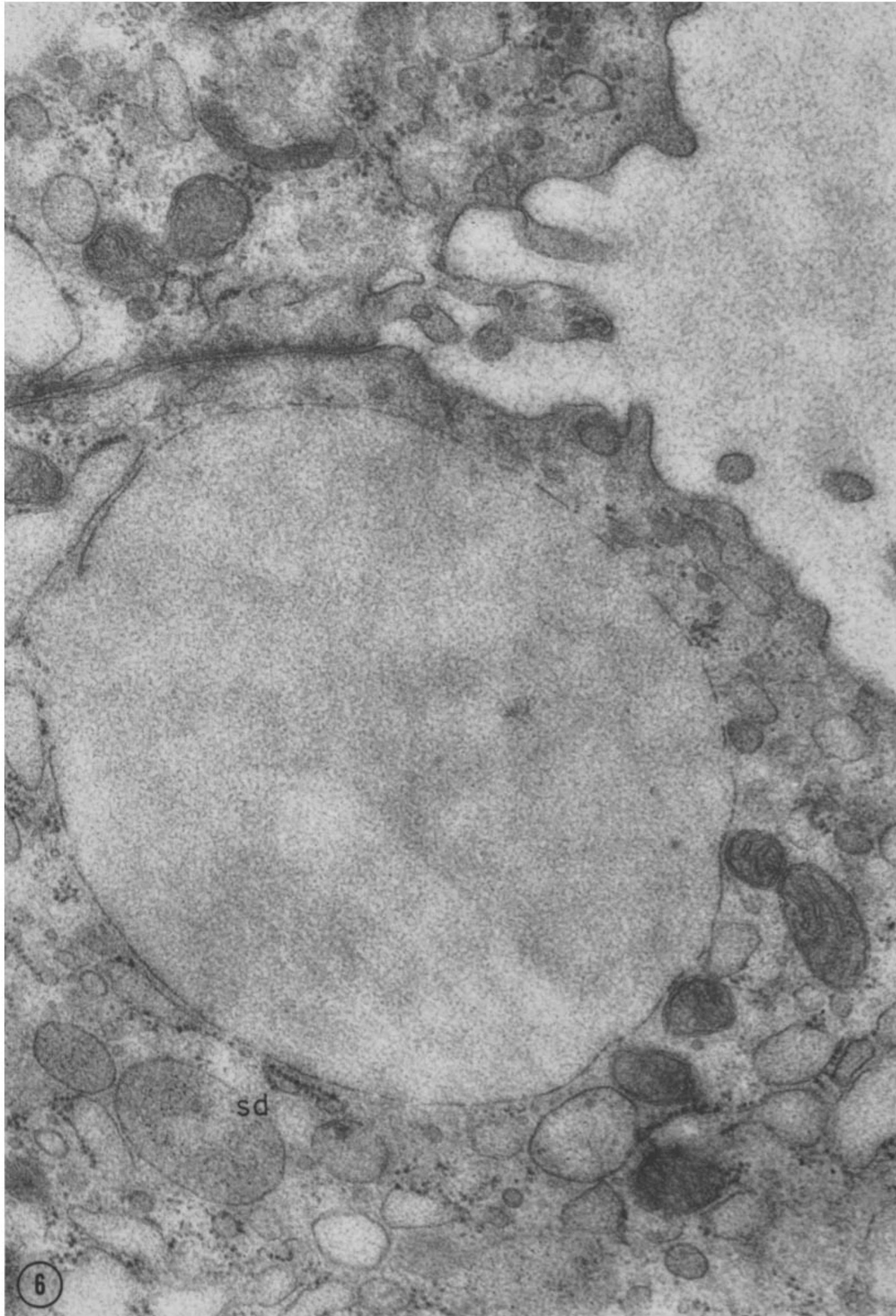
This figure illustrates the unique and extraordinary degree of elongation of microvilli that occurred in a number of the follicles of this specimen. In the region shown in this figure, some of the microvilli reach a height of 5 μ, which is more than ten times the length of microvilli in the normal gland. Furthermore, the microvilli appear as fingerlike extensions surmounting protoplasmic ruffles emitted into the colloid by the follicular cells.

The epithelial cells appear to be of normal height and do not show signs of active colloid secretion. Perifollicular capillaries lie within deep indentations in the basal regions of the follicular cells. A cluster of distinctive cells (*pc*) bounded peripherally by the follicular basement membrane and proximally by the follicular epithelium is seen to the left in the figure. Their cytoplasm is more translucent than that of the follicular cells and contains only relatively few granules presumed to be mitochondria. These cells have been tentatively identified as parafollicular cells (see Nonidez, 1932). × 2,700.

FIGURE 6

Thyroid gland of a rat that received 0.5 USP unit thyrotrophic hormone 2 hours before autopsy.

The colloid of the follicular lumen in the upper right corner of the figure is relatively dielectronic and of uniform texture. Apical regions of two adjacent follicular cells occupy the remainder of the figure. A large colloid droplet lies near the apical plasma membrane of the lower cell. Its content, although homogeneous, is slightly more adielectronic than the colloid. A cluster of small droplets (*sd*) lies adjacent to this droplet. Their content is more adielectronic than that of their larger neighbor, and, in addition, includes numerous fine, dense particles which are barely visible at this magnification. × 48,000.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microscopy. They assumed that the vesicles lose their ribosome coat before they are released by the cell. However, in the light of current knowledge of ergastoplasmic function, direct transformation of its vesicles into secretory droplets seems unlikely and requires more substantial proof before being accepted.

A number of years ago De Robertis (1941 *b*; 1942) and Dvoskin (1947; 1948) demonstrated that, from 1 to 3 hours following a single injection of thyrotrophic hormone, colloid secretion was sharply accelerated as evidenced by the increased number of colloid droplets in the follicular cells during this period. Their results indicated that the secretion of protein, like other aspects of thyroid function such as the trapping of iodide, the incorporation of iodine into protein, the release of thyroid hormone into the circulation, etc., (Taurog, Tong, and Chaikoff, 1958 *a*; 1958 *b*), is in some measure under the control of the anterior pituitary gland. In the present study an experimental protocol similar to that employed earlier by De Robertis (1941 *b*) was used to activate the elaboration of colloid. Specimens of rat thyroid glands removed at intervals following a single injection of thyrotrophic hormone were examined by electron microscopy primarily in order to identify the colloid droplets and to investigate further their mode of formation, development, and release by the follicular cells.

In the interpretation of the morphologic changes recorded in this report, colloid droplets are assumed to represent a manifestation of the secretory activity of follicular cells; *i.e.*, they contain newly synthesized protein slated for release into the follicular lumen. This interpretation assigns to them a role similar to that of secretory granules in other exocrine glands such as the salivary glands and the exocrine portion of the pancreas. However, owing to technical limitations, the deductions concerning the function of colloid droplets are based on their static appearances in fixed preparations. An alternative interpretation of the role of colloid droplets, namely that they contain resorbed colloid that is being hydrolyzed within the follicular cells, may be advanced and is not invalidated by the findings reported here. On the basis of light microscopic (Ponse, 1951), autoradiographic (Wollman and Spicer, 1961; Nadler *et al.*, 1962), and electron microscopic (Porte and Petrovic, 1961) evidence, a number of workers are inclined to accept the latter interpretation. In the author's opinion, irrefutable proof substantiating either interpretation has not yet been presented. For simplicity's sake and, since on the basis of their fine structure and their relationship to Golgi vesicles, colloid droplets are closely patterned after the secretory apparatus in a number of gland cells (Palay, 1958), colloid droplets will be assumed to be related to secretion. The justification for this assumption will be considered in more detail in the Discussion.

MATERIAL AND METHODS

Sixteen male Sprague-Dawley rats of approximately 400-gm body weight were each given 0.5 USP unit

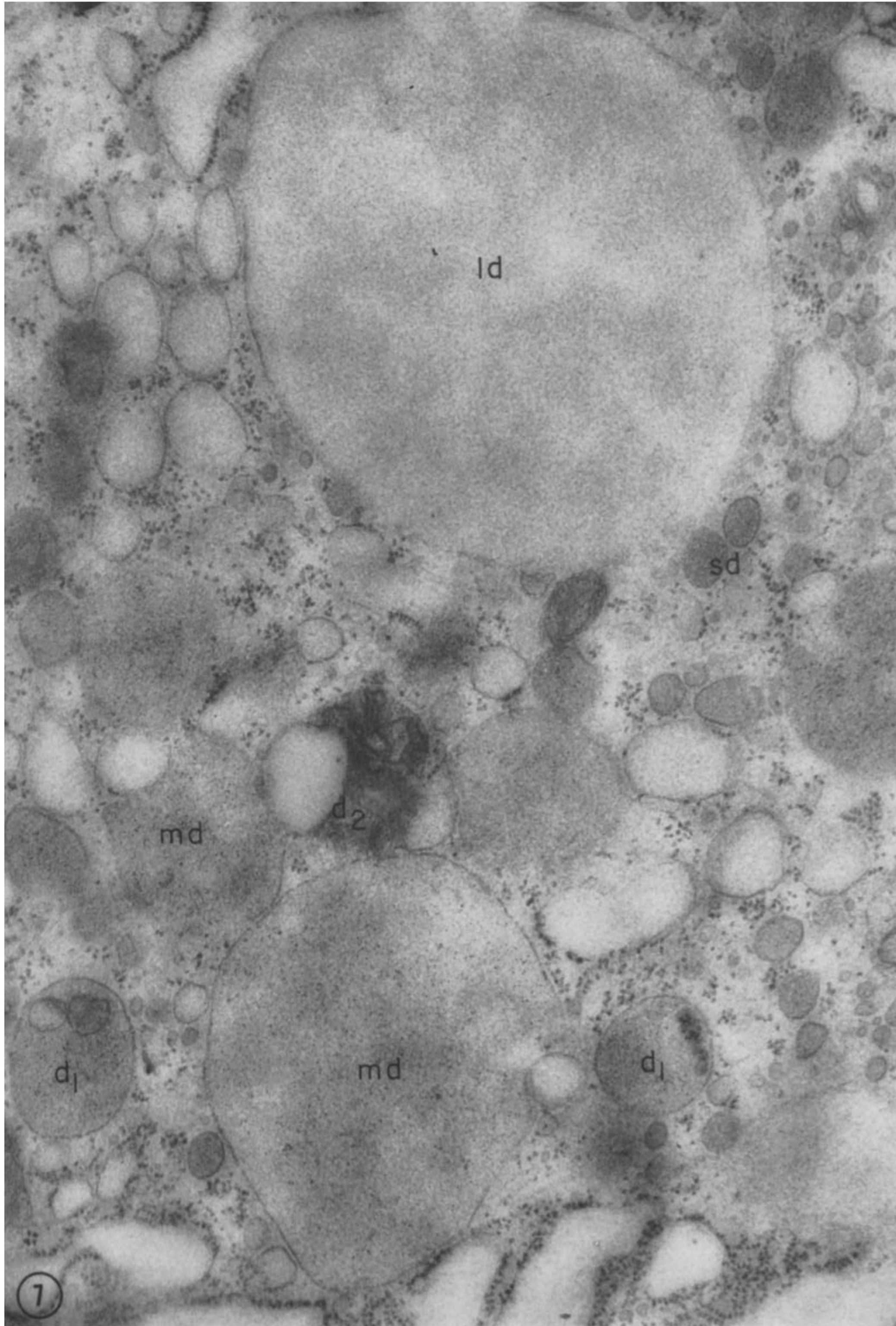
FIGURE 7

Thyroid gland of a rat that received 0.5 USP unit thyrotrophic hormone 1 hour before autopsy.

This figure illustrates the spectrum of structural variations observed in colloid droplets. The variations seem to be correlated with the size of the droplet.

All of the droplets are bounded by a single membrane, and their matrix substance is of homogeneous texture. The content of the large droplet (*ld*) is noticeably more dielectronic than that of the small droplets (*sd*). The content of medium-sized droplets (*md*) is of intermediate opacity to the electron beam. Fine dense particles which are 75 A or less in diameter occur within both small- and medium-sized droplets, but they are relatively more numerous in smaller droplets. The large droplet does not contain these particles.

Other droplets of more complex form are also visible in the field. Some (d_1) closely resemble medium-sized colloid droplets, but they contain vesicular or granular structures not ordinarily observed in colloid droplets. Another droplet (d_2) consisting of an agglomeration of granular and membranous residues may be a lysosome. It is of interest to note that these structurally more complex droplets also have a rich content of fine, dense particles. × 41,000.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thyrotrophic hormone (Armour, lot No. 317-51, dissolved in M/15 Sörensen buffer, pH 8.1) by subcutaneous injection. Groups of four animals each were sacrificed at 1, 2, 12, and 24 hours following injection, and specimens of thyroid were fixed in a manner previously described in detail (Wissig, 1960). A solution of 1 or 2 per cent osmium tetroxide buffered at pH 7.4 with acetate-veronal (Palade, 1952) was used as the fixative. Fixation was carried out for 3 to 6 hours at 2°C. The specimens were embedded either in a mixture of 15 parts methyl: 85 parts *n*-butyl methacrylate or in a prepolymerized solution of *n*-butyl methacrylate. Sections 1 to 2 μ thick were cut with a Porter-Blum microtome and examined by phase contrast microscopy. Thin sections were also cut and examined with a Siemens Elmiskop I. The thin sections were mounted on grids coated with a carbon film and then stained with lead salts either by the method of Dalton and Zeigel (1960) or of Watson (1958). After staining, they were sandwiched beneath a film of formvar according to Watson (1957).

With the preparative techniques used in this study the limiting membranes of the colloid droplets, particularly of the larger ones, were unsatisfactorily preserved, and, in many instances, they appear ruptured. The embedding procedure is probably the cause of the artefact since the number of ruptures was greatly reduced in specimens embedded at a later date in epoxy resin.

OBSERVATIONS

Phase Contrast Microscopy

Examination by phase contrast microscopy of specimens from this study corroborated the observations of earlier workers made with the light microscope. As noted by De Robertis (1941 *b*) and Dvoskin (1947; 1948), the follicular cells of the rat thyroid gland elaborate colloid at an increased rate in the first few hours after injection of thyrotrophic hormone (Figs. 1 and 2), and the accelerated secretory response subsides by 12 to 24 hours thereafter (Figs. 3 and 4). During the early phase, De Robertis (1942) observed that the follicular cells extend pseudopodia containing colloid droplets from their apices into the colloid. He postulated that progressive thinning of the cytoplasm surrounding the droplets eventually allows their contents to merge with the colloid. Droplets can also be discerned in the pseudopodia by phase contrast microscopy, but the appearance of the pseudopodia visualized by electron microscopy suggests even more strongly that they participate in the release of secretion (see later).

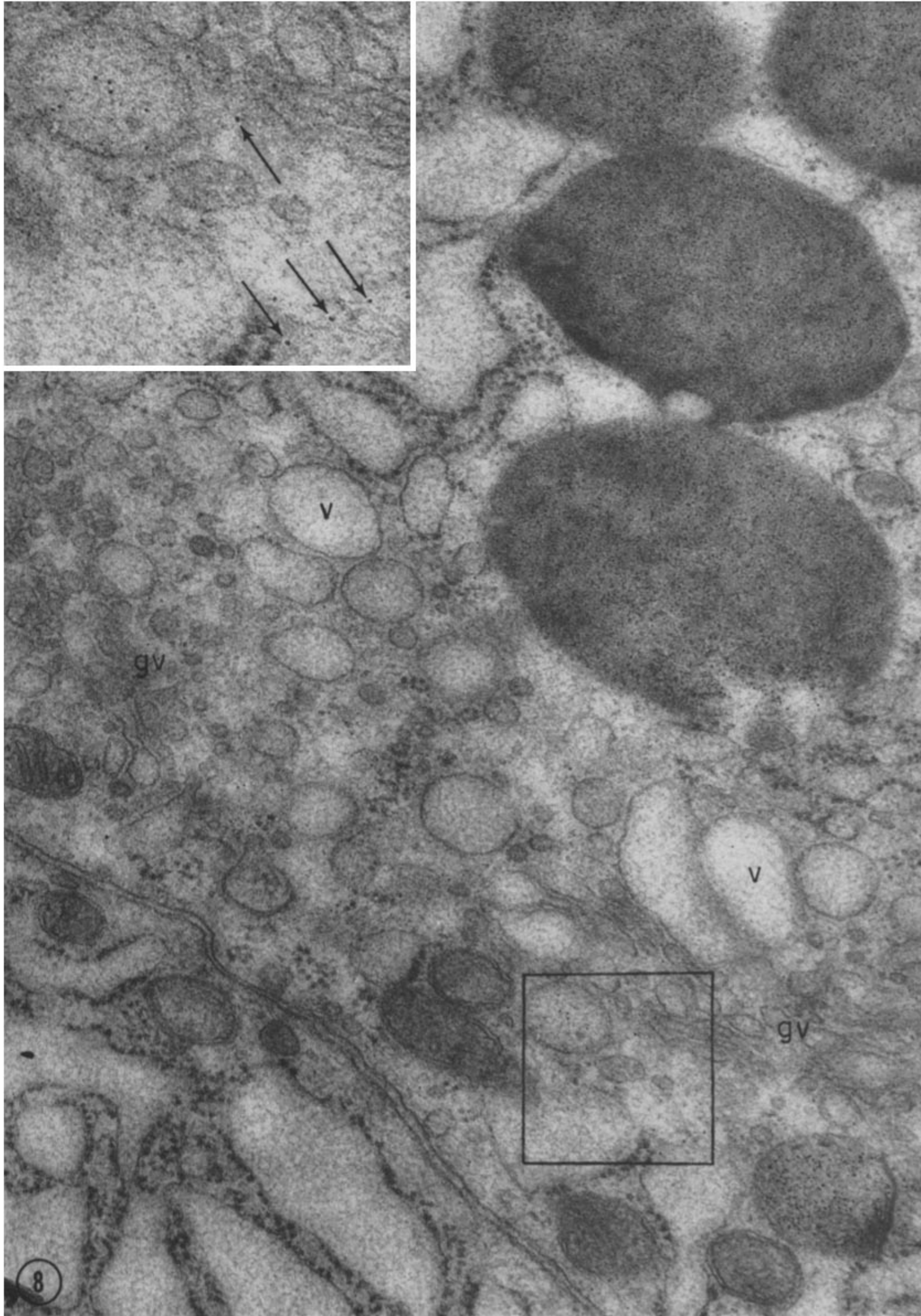
As previously reported (Wissig, 1960), the phase contrast microscope is barely able to resolve the microvilli on the surface of unstimulated follicular cells. Following stimulation by thyrotrophic hormone, they may increase in size and thus become more clearly visible. The occurrence of the enlargement is somewhat capricious for it appeared in one specimen 1 hour after injection of TSH (Fig. 1), was absent for another 2 hours after injection (Fig. 2), and was even more marked in a third specimen fixed 24 hours after injection (Fig. 5). Little is known of the function of microvilli, and it is difficult to interpret the physiological significance of their response in this particular situation.

Because colloid droplets sometimes occur in the base of follicular cells, some authors have stated that the cells are able to secrete from their base as well as their apex (Bensley, 1916; De Robertis, 1941 *b*). I have not detected any morphologic evidence by phase contrast or even electron microscopy that secretory material in the form of droplets is released from this pole of the follicular cell. It is, however, not uncommon for secretory droplets in cells containing an abundance of secre-

FIGURE 8

Thyroid gland of a rat that received 0.5 USP unit thyrotrophic hormone 2 hours before autopsy.

Clusters of Golgi vesicles (*gv*) appear in the field. They consist predominantly of small vesicles and flattened cisternae. Larger vesicles (*v*) lying near the definitive Golgi elements are also believed to be components of the apparatus. A single large vesicle enclosed by a square contains a few fine dense particles and is consequently classified as a small colloid droplet. The square is shown at higher magnification in the inset. A few fine dense particles similar to those enclosed within the small colloid droplet appear in the cytoplasmic matrix near it (*arrows*). In the upper right corner of the figure are four large droplets which contain a very dense matrix substance richly interspersed with fine dense particles. × 59,000. Inset magnification, × 112,000.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tion to extend beyond the apex into the basal region (Fig. 1). In addition, it is worthwhile to bear in mind as was mentioned earlier (Wissig, 1960) that even in the unstimulated gland voluminous ergastoplasmic cisternae frequently occur in the base of the follicular cell. Their content may conceivably have staining properties similar to colloid and may give the false impression in the light microscope of secretory material stored in the base of the cell prior to being released.

By phase contrast microscopy of these specimens, it is not possible to observe the Golgi apparatus and draw any conclusions about its relationship to the secretory droplets. The same can also be said of the ergastoplasm.

From 12 to 24 hours after the injection of thyrotrophic hormone, when the accelerated secretory response seems to have subsided, the architecture of individual follicles changes. The volume of intrafollicular colloid appears reduced, and instead of being localized in a single central mass as in the normal, it is often subdivided into several masses which appear discrete (Fig. 4). Each mass is surrounded by a layer of follicular cells, and forms an apparently separate subdivision of the original follicle. However, the individual masses are not mistaken for independent follicles for they lack separate investments of perifollicular connective tissue; *i.e.*, their epithelial mantle abuts directly against the epithelium of an adjacent subdivision of the same follicle. The appearance of some follicles suggests that they were fixed just at the time when portions of colloid were being detached from the main colloid mass to form separate follicular subdivisions (Fig. 3). Whether the follicular subdivisions actually sever their connections with one another was not determined in this study as serial sections were not examined. It would seem a logical speculation that the increased surface area of colloid exposed to the follicular cells by the subdivision of the follicular mass might serve as a mechanism to foster colloid resorption.

Electron Microscopy

The principal aim of this section of the paper is to furnish a description of the fine structure of colloid droplets during their formation, maturation, and release in follicular cells. These stages are most readily visualized during the period of accelerated colloid secretion; *i.e.*, at 1 and 2 hours following administration of TSH. The morphology of the follicular cells is essentially the same in both groups of specimens, and a single description covering both will be given. Under electron microscopic examination, the fine structure of follicular cells in specimens collected at 12 and 24 hours after administration of TSH closely resembles that of normal cells described in detail in an earlier report (Wissig, 1960) and requires no further description.

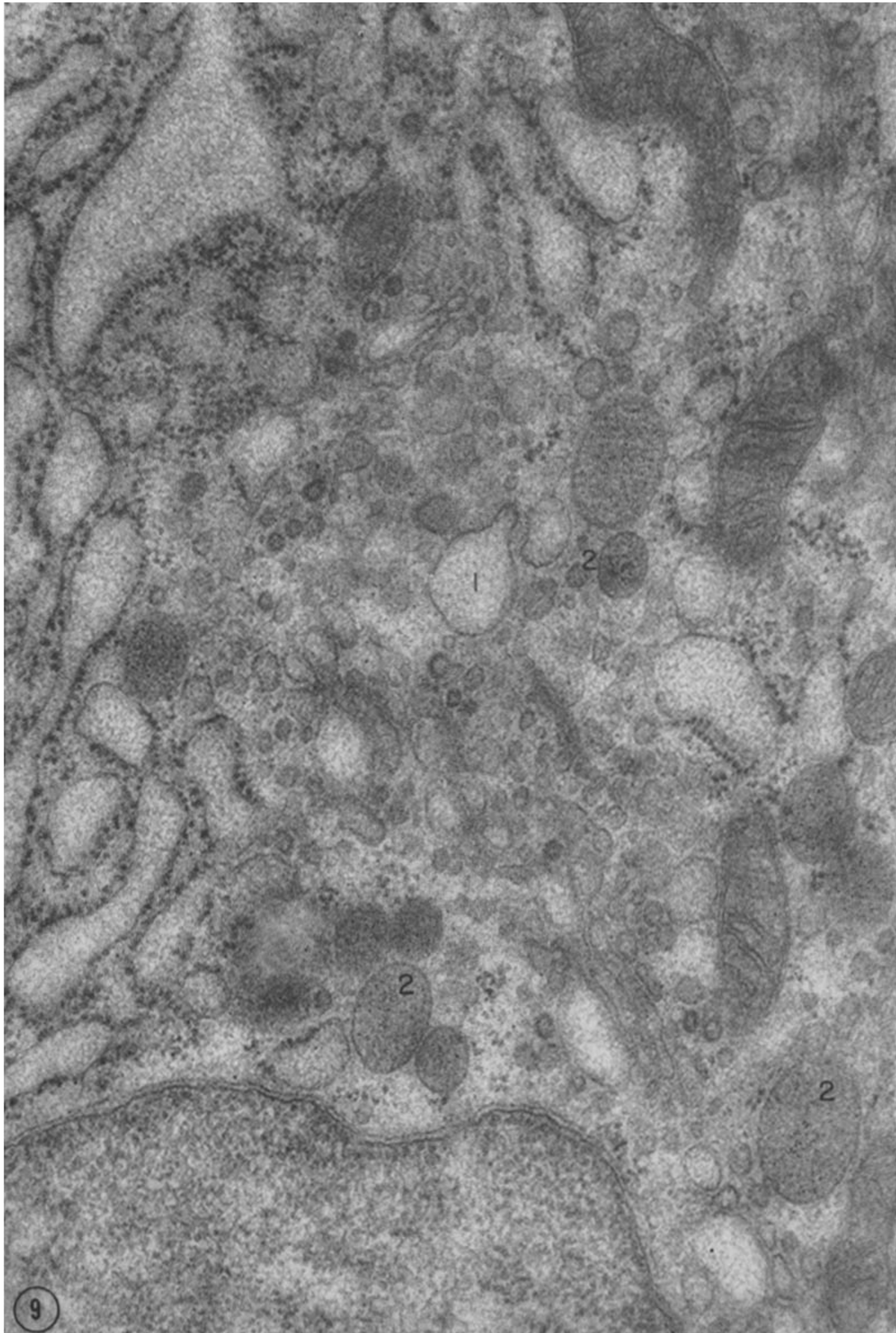
The degree of secretory stimulation is far from uniform in follicular cells of the 1- and 2-hour specimens, but the most responsive cells have a characteristic appearance. Their apical cytoplasm is crowded with secretory droplets. The largest tend to lie near the apical border (Fig. 6), and some may be enclosed within protoplasmic processes which extend from this border into the colloid. Droplets of medium size are generally found deeper in the cytoplasm, and their range of distribution extends to the level of the nucleus. Individual collections of Golgi vesicles cluster along the apical and lateral surfaces of the nucleus, and these are surrounded by small numbers of the smallest colloid droplets. The latter blend morphologically with Golgi vesicles so that they cannot always be distinguished from one another. The distribution of the colloid droplets within the follicular cells suggests that they are formed initially in the Golgi region, and as they enlarge, they move towards the apex of the cell from which they are discharged into the follicular lumen.

In normal follicular cells the Golgi apparatus consists of small clusters of vesicles and flattened cisternae. The cytoplasm contains few colloid

FIGURE 9

Thyroid gland of a rat that received 0.5 USP unit thyrotrophic hormone 2 hours before autopsy.

A portion of the nucleus of a follicular cell appears at the lower border of the figure, and vesicles of the Golgi apparatus occupy the center of the figure. A small Golgi vesicle appears to be uniting with a larger vesicle at *1*. Larger vesicles which are filled with a substance of substantially greater density and contain many fine dense particles (*2*) are scattered at the periphery of the Golgi apparatus. These are believed to be newly formed colloid droplets. × 61,000.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droplets, and those present do not appear preferentially localized in the vicinity of the Golgi elements. The small size of the apparatus and scarcity of colloid droplets suggest that the secretory activity of the follicular cells is quiescent. By 1 and 2 hours after injection of TSH, the apparatus has expanded in volume as the result of proliferation of its vesicular components, and small colloid droplets are found at its periphery (Fig. 9). Although proliferation of Golgi vesicles takes place within a short span of time, no obvious clues revealing the mode by which new vesicles form were detected.

The diameter of the smallest colloid droplets clustered around the Golgi apparatus falls within the same size range as the largest spherical Golgi vesicles. In most instances, the two can be distinguished from one another because the content of the droplets is generally of slightly greater density and includes a small number of fine dense particles (Figs. 8 and 9). The particles are of varied size, but the largest do not exceed 75 A in diameter. The occurrence of fine particulate material in droplets of follicular cells has been noted previously in the mouse (Ekholm and Sjöstrand, 1957 *a*; 1957 *b*) and rat (Wissig, 1960). At present its chemical nature is unknown, but it resembles the fine particles associated with hemosiderin and inclusions believed to contain iron (Richter, 1957; Bessis and Breton-Gorius, 1959). The density of the particles appears to be the same in unstained sections and in sections stained with lead salts. They do not display the internal structure characteristic of ferritin molecules. A small number of droplets which contain a very few fine dense particles and which have a content only as dense as that of Golgi vesicles (Fig. 8) appear to represent transitional stages between Golgi vesicles and colloid droplets.

Colloid droplets throughout the cytoplasm of follicular cells have common morphologic features by which they can be singled out. They are generally spherical; they are enclosed by a smooth membrane 70 to 80 A thick; and their content, although different in density from droplet to droplet, is homogeneous and often includes a fine dense particulate component. Despite this fundamental similarity, individual droplets display differences which appear to depend upon their size and location.

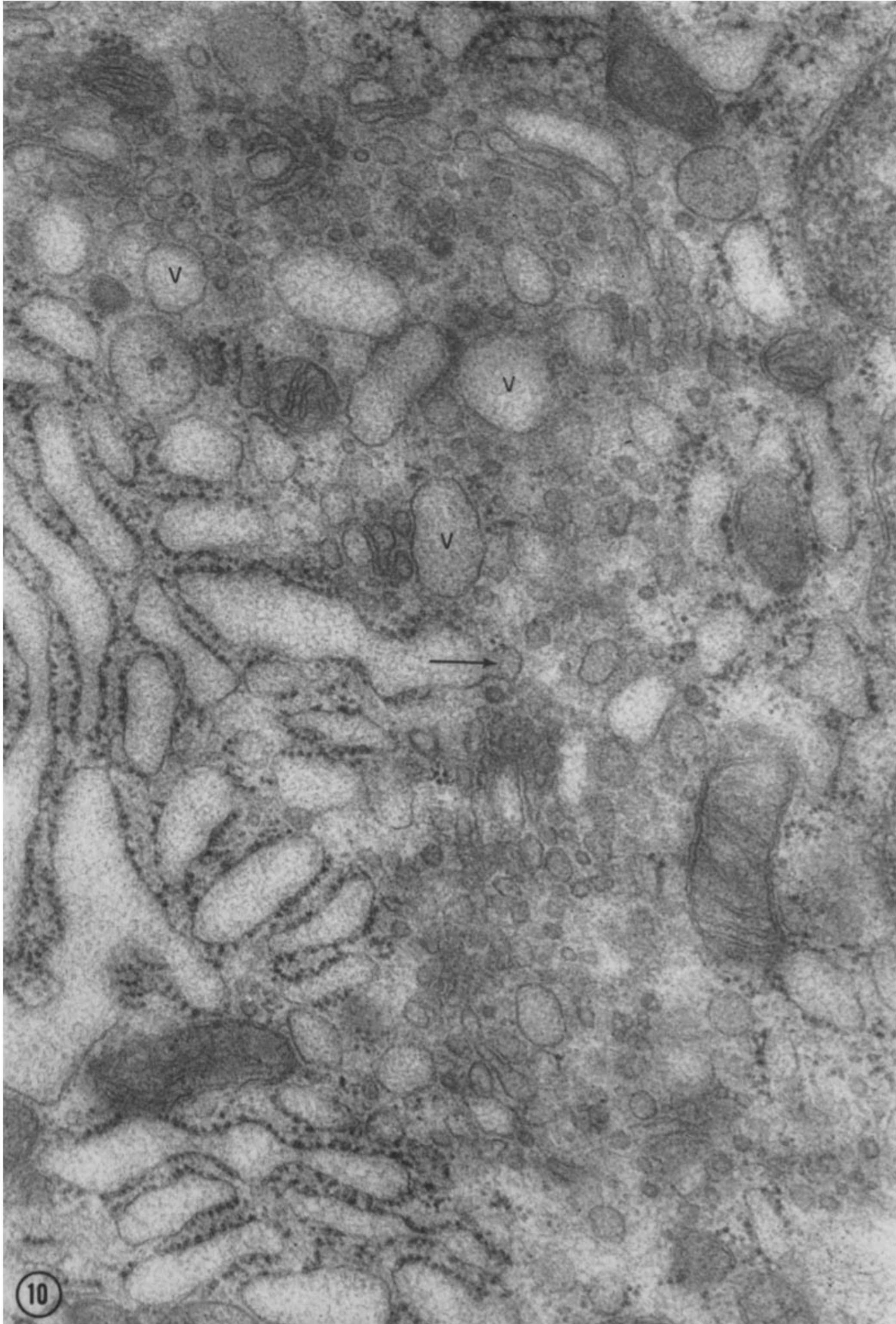
The content of the small- and medium-sized droplets is relatively adielectronic and richly interspersed with fine dense particles (Figs. 7 and 9). Although particle counts were not carried out, it seems that, as droplets progress from small to medium size, the concentration of particles and the density of their content do not change markedly (Fig. 7). However, if large droplets are examined, it is clear that their content is more dielectronic than that of smaller droplets and that they contain fewer fine dense particles (Fig. 7). Colloid visualized at high magnification by electron microscopy is comprised of a meshwork of very fine fibrils (Figs. 11 and 12). The content of the largest colloid droplets has a similar appearance but its fibrils are more closely packed together (Fig. 12). Neither follicular colloid nor the largest colloid droplets contains particulate material. It appears, therefore, that as the droplets grow from medium to maximum size their content decreases progressively in density, indicating perhaps that it is becoming more dilute, and the fine dense particles disappear.

An interesting feature of the Golgi region is that fine particles similar to those contained within the small- and medium-sized droplets are dispersed throughout the cytoplasmic matrix between Golgi vesicles (Inset, Fig. 8). On the one hand, it is

FIGURE 10

Thyroid gland of a rat that received 0.5 USP unit thyrotrophic hormone 2 hours before autopsy.

Dilated vesicles of the ergastoplasm characteristic of thyroid follicular cells lie to the left in the figure. A peripheral segment of the nucleus appears at the right border of the figure. Golgi vesicles and cisternae lie in the cytoplasm between these organelles. Large vesicles (*v*) are scattered at the edge of the apparatus. Despite their large size they are not identified as colloid droplets because their content is relatively dielectronic and they do not contain fine dense particles. An arrow indicates a small Golgi vesicle which appears to be on the point of budding off from the end of an ergastoplasmic cisterna. The limiting membrane of the cisterna in this region is free of ribosomes. × 59,000.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possible that these particles may have been released from small colloid droplets in the vicinity. Or, on the other hand, they may accumulate in the Golgi region prior to being incorporated into newly forming colloid droplets. In view of our ignorance of the chemical nature of the particles at this stage, we can only note their existence and say nothing of their importance in the physiology of the follicular cell.

A feature of the morphologic relationship between the ergastoplasm and the Golgi apparatus during heightened secretory activity is worthy of note. The vesicles of the Golgi apparatus are often surrounded by masses of ergastoplasm (Figs. 9 and 10). Ribonucleoprotein particles are distributed more or less evenly over the surface of the ergastoplasmic membranes. However, small areas of the membrane may lack a coating of particles, and occasionally these appear to give rise to small, smooth surfaced vesicles by a budding process (Fig. 10). This is the only structural feature thus far observed which might be interpreted as evidence for the direct transfer of material in bulk between the ergastoplasm and the Golgi apparatus.

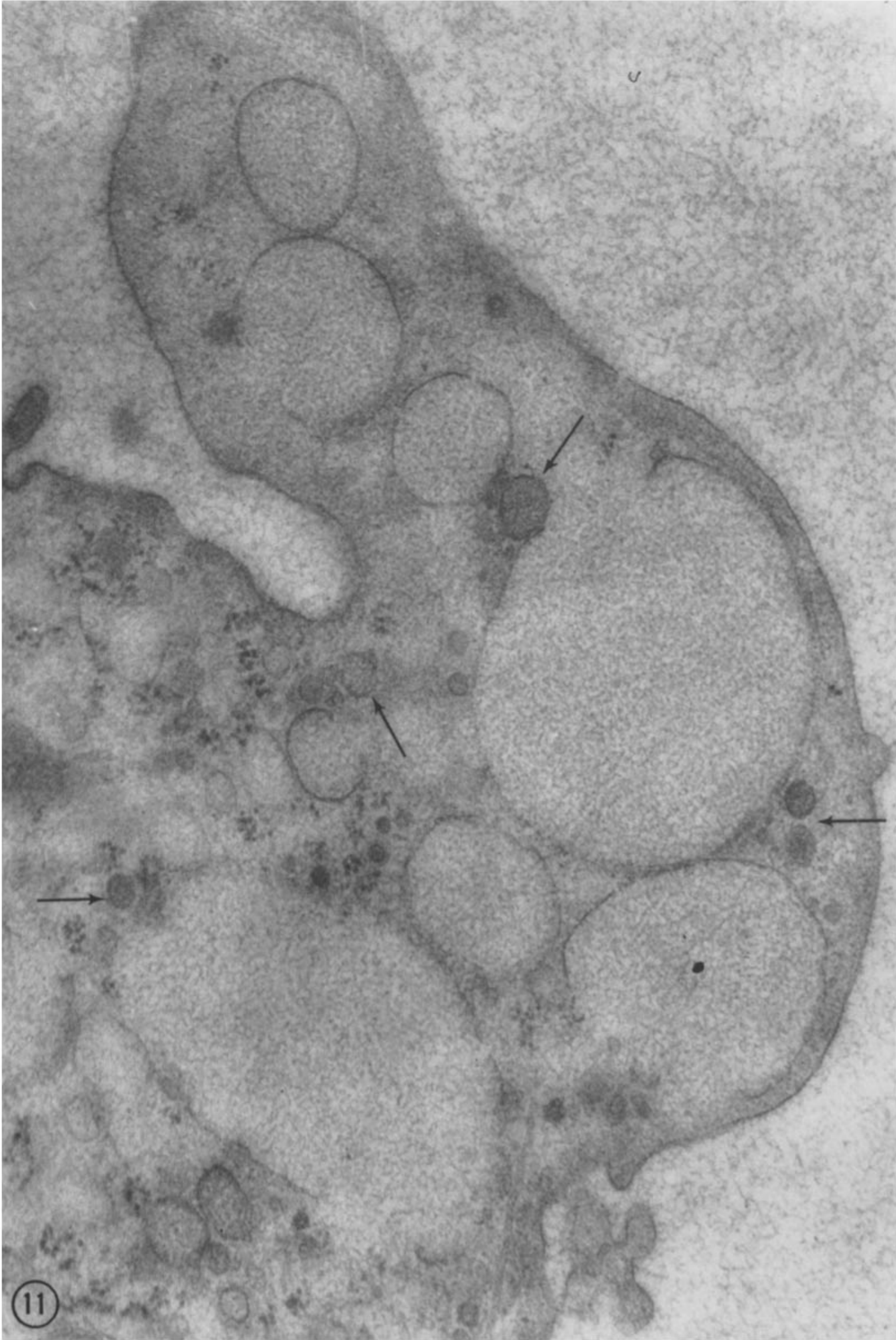
In the follicular cell of the normal thyroid gland the ergastoplasm consists typically of large, dilated pleomorphic vesicles which display only a limited tendency to be oriented in parallel arrays (Wissig, 1960). Their limiting membrane and coating of ribosomes resembles that of other glandular cells. The content of the ergastoplasmic sacs, which in the thyroid gland constitutes a relatively voluminous component of the ergastoplasm, is slightly denser than the surrounding cytoplasmic matrix. After fixation with osmium tetroxide, its finely fibrillar texture, similar to that of blood plasma and follicular colloid, suggests that in the living animal it exists in the form of a dilute protein solution. Indeed, if the secretory apparatus of the thyroid gland is comparable with that of the exocrine pancreas (Siekevitz and Palade, 1958), one would expect the content of the ergastoplasmic sacs to be newly synthesized protein which, after being packaged in secretory droplets in the Golgi apparatus (Caro, 1961; Palade, Siekevitz, and Caro, 1962), will ultimately be contributed to the follicular colloid. Although the accelerated secretion of colloid instigated by TSH is reflected in the proliferation of secretory droplets and elements of the Golgi apparatus, no comparable structural change in the ergastoplasm has been detected. Since the ergastoplasm is presumably the initial source of newly synthesized secretory protein (see review by Haguenau, 1958), its increased activity seems to require only more rapid utilization of already existing structural elements.

The means by which the content of colloid droplets is released into the follicular lumen can only be surmised. It is a common occurrence in actively secreting glands for the follicular cells to emit large protoplasmic tabs into the colloid. The protoplasmic tabs lack microvilli on their surface, and, apart from cytoplasmic matrix, the principal structures they contain are relatively large colloid droplets (Figs. 11 and 12). In some instances, the droplet's content, because of its greater density, is obviously dissimilar to colloid (Fig. 11). This is not the case for all droplets within apical tabs, for some have a content quite similar to colloid (Fig. 12). The apical tabs may be of considerable size and display bizarre contours. Because of this combination of circumstances, it is difficult to identify

FIGURE 11

Thyroid gland of a rat that received 0.5 USP unit thyrotrophic hormone 2 hours before autopsy.

A large tab of cytoplasm extends from the apex of a follicular cell into the follicular colloid. The follicular colloid clearly consists of a meshwork of fine branching filaments. The surface of the tab is covered by the apical plasma membrane and lacks microvilli. Numerous droplets of varied size and density lie in the matrix of the tab and in the cytoplasm immediately adjacent to it. The small dense droplets (*arrows*) probably correspond to the vesicles of the superficial cytoplasmic layer observed in follicular cells of the unstimulated gland (see Wissig, 1960). The large droplets appear to be typical colloid droplets. Although their content is noticeably more adielectronic than the follicular colloid, the fact that they do not contain any fine dense particles is interpreted as evidence that they have reached the final stages of development and that they are ready to be extruded. Interruptions in the limiting membrane of the colloid droplets in this and other figures are presumably artifacts. × 57,000.


11

droplets at the moment of their release with any degree of assurance. Large invaginations in the surface of apical pseudopods could represent secretory droplets whose content is in the process of merging with the follicular colloid, or they could represent a section through a haphazard infolding of the surface of an apical process (Figs. 13 and 14). During the release of protein secretory material in merocrine glands, such as the exocrine pancreas (Palade, 1959), mammary gland (Wellings *et al.*, 1960; Bargmann *et al.*, 1961), and anterior pituitary (Farquhar, 1961), the secretory droplet's membrane joins with the apical plasmalemma and thus avoids being shed by the cell with the secretory material. Since membranous material has not been observed in the follicular colloid, secretion is probably released in a similar fashion by the follicular cell. However, because of the difficulty in identifying droplets at the moment of their release, stronger evidence to support this assumption cannot be offered at this time.

The droplets which have been described up to this point can be fitted into a continuous series of forms ranging in size from small Golgi vesicles to large colloid droplets on the point of being extruded into the lumen of the follicle. Presumably, the series represents the structural transformations through which secretory material passes from the moment it is first gathered in packets until its release by the follicular cell. Other droplet-like inclusions which will be described below can be identified in the cytoplasm of the follicular cells, but, on the basis of their structure, these do not fit into the developmental sequence of colloid droplets. Their importance in the follicular cell has not yet been discovered.

Aberrant forms of medium-sized droplets were infrequently observed (Fig. 8). Their matrix substance is for the most part homogeneous but much more adielectronic than that of the more common type of droplet. Their content of fine particles is also far more concentrated. At first glance, it appears as though they might represent the end product of a syneretic or dehydrating process imposed upon ordinary colloid droplets. However, if shrinkage of the droplet actually occurs, it must take place in an orderly manner for the extremely dense droplet preserves a smooth spherical outline.

Droplets belonging to another, more populous class of inclusions are limited by a single membrane. Their content can resemble that of colloid droplets to a certain degree, but it is more heterogeneous and can include a conglomeration of granules and membranous residues (d_1 in Fig. 7). Following thyrotrophic hormone stimulation, the number and distribution of these droplets remains essentially unchanged in sharp contrast to the proliferation of the colloid droplets. Their lack of response appears to indicate that they are not typical secretory droplets, and for this reason they have not been included in the category of colloid droplets. Although their composition and function are unknown, they resemble organelles that have been designated as lysosomes in other tissues (de Duve, 1959).

DISCUSSION

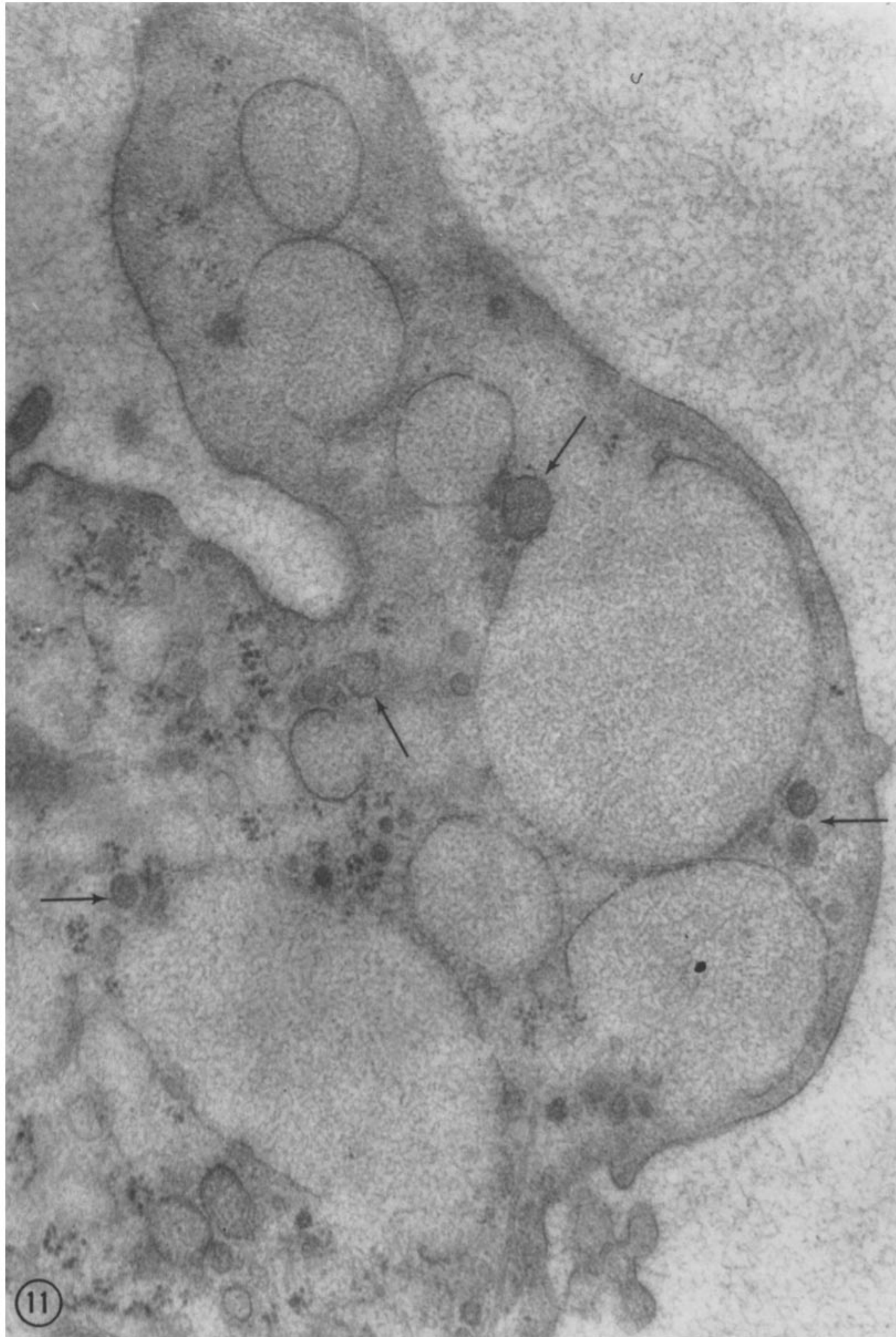
Significance of Colloid Droplets

The experiments reported in this paper were carried out in order to study the fine structure of the protein-secreting apparatus of the thyroid follicular cell during activation induced by thyrotrophic hormone. In general, our findings suggest that as far as the formation and extrusion of secretory droplets are concerned, the thyroid gland behaves in a manner similar to that of the acinar tissue of the pancreas, the exocrine gland most carefully studied in this respect thus far (Sjöstrand and Hanzon, 1953; Palay, 1958;

FIGURE 12

Thyroid gland of a rat that received 0.5 USP unit thyrotrophic hormone 2 hours before autopsy.

The large apical process in the field contains two droplets. The droplets, although of different size, are similar in that their content is of homogeneous texture without particulate inclusions. The colloid and the contents of the droplets appear as meshworks of fine branching filaments. The filaments seem to be more tightly packed together in the droplets than in the colloid. Small vesicles with dense contents are distributed in the cytoplasmic matrix adjacent to the large droplets as well as in the microvillus in the lower left corner of the figure. × 59,000.


11

Palade, 1959). It appears that colloid droplets develop initially from Golgi vesicles and that ultimately the contents of the mature droplets are released from the apex of the follicular cell without loss of apical cytoplasm and without rupture or loss of either the membrane surrounding the droplet or the apical plasmalemma. However, our observations tell us little concerning the original site of synthesis of the protein content of the droplet, but, by analogy with the pancreas (Siekevitz and Palade, 1958), it is, nonetheless, assumed to be the ergastoplasm.

It must be admitted, however, that the structures we have described as components of a secretory apparatus might operate in the reverse direction and serve as an apparatus for the resorption and proteolysis of colloid. Undoubtedly a majority of cytologists would agree, however, that the changes occurring in stimulated follicular cells, *e.g.*, hypertrophy of the Golgi apparatus, appearance of transitional forms between Golgi vesicles and small colloid droplets, increased numbers of colloid droplets in the apical cytoplasm, and alterations of the apical surface suggestive of the release of droplets into the follicular lumen, are typical signs that a secretory apparatus has stepped up its function; but, in reality, acceptance of this hypothesis is based on circumstantial evidence and has been questioned by some investigators.

For instance, Ponse (1951) concluded that the presence of chromophil droplets in protoplasmic streamers extending into the follicular lumen and in the apical cytoplasm indicates that follicular cells are actively phagocytizing colloid. In this way, the large thyroglobulin molecule can presumably enter the follicular cell where it is hydrolyzed in order to liberate thyroid hormone. Nadler, Sarkar, and Leblond (1962)[1] examined follicular cells at a series of short intervals after stimulation by TSH and noted that droplets appear first in apical pseudopods and subsequently in the apical cytoplasm of follicular cells. Thus, the droplets seem to move in a direction away from the follicular lumen, and for this and other reasons (see below) they are inclined to support Ponse's views. Although cytologists generally agree on the structural changes observed in follicular cells shortly after administration of TSH, opinion is divided as to whether they signify that colloid is entering or leaving follicular cells.

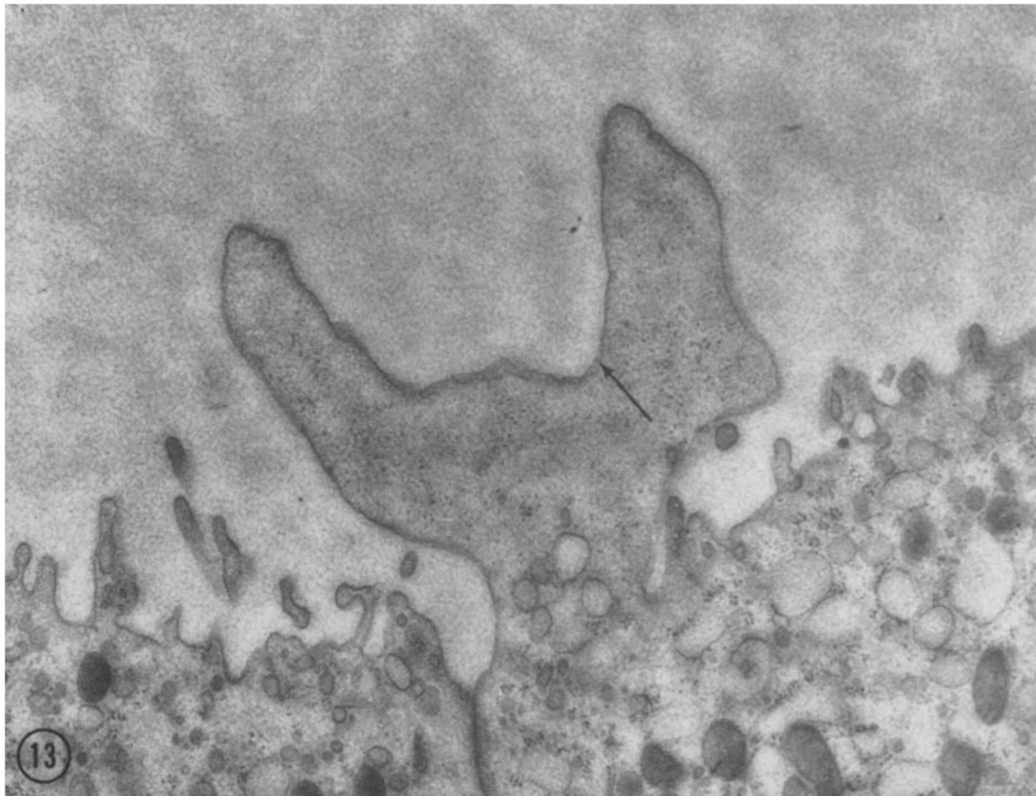
The secretory apparatus of the exocrine pancreas is structurally similar to that of the follicular cell. Autoradiographic studies with labeled amino acid have shown that zymogen granules of acinar cells contain newly synthesized protein which was formed into droplets within the Golgi apparatus (Caro, 1961; Caro and Palade, 1961; Palade *et al.*, 1962). Nadler *et al.* (1962) carried out a similar experiment with the thyroid gland. They injected rats intraperitoneally with leucine-H^3, and collected thyroid specimens for autoradiography ½ hour and 5½ hours later. In addition, shortly before autopsy the rats received an injection of TSH to stimulate production of colloid droplets. In the ½-hour specimens, abundant droplets were observed in apical pseudopodia, but none contained radioactivity, whereas, in the 5½-hour specimens, radioactivity was detected both in follicular colloid and cytoplasmic colloid droplets. The authors deduced that radioactive label appears in colloid droplets only after follicular

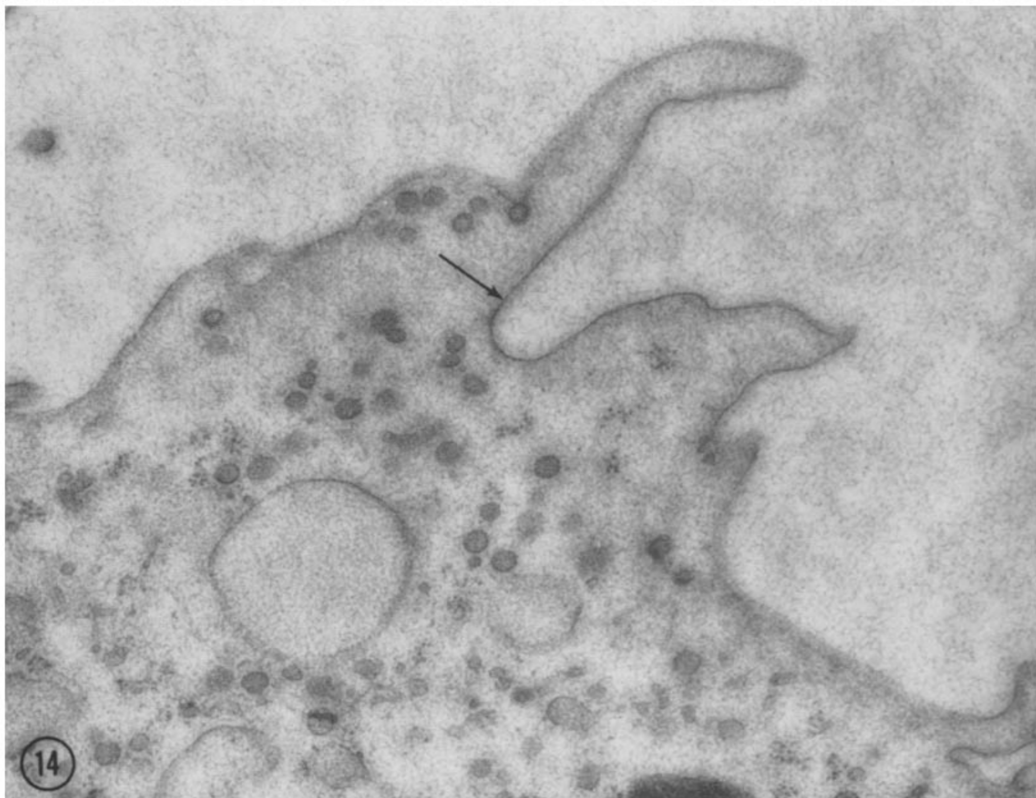
[1] The author sincerely appreciates Dr. Leblond's generosity in providing him with a copy of this paper while it was still in press.

FIGURES 13 AND 14

Thyroid glands of rats that received 0.5 USP unit thyrotrophic hormone 1 hour (Fig. 13) and 2 hours (Fig. 14) prior to autopsy.

Both figures show apical regions of follicular cells in glands that are actively secreting colloid. In each figure a follicular cell extends a large pseudopod into the follicular lumen. The tip of each pseudopod is indented to form a cup-shaped depression. The depressions may be nothing more than transitory invaginations in the surface of these pleomorphic structures. Alternatively, the depressions may represent colloid droplets in the process of emptying their contents into the follicular lumen. If the latter is the case, the apical plasmalemma lining the depressions (*arrows*) would formerly have served as the limiting membrane of the droplets. × 25,000 (Fig. 13); × 38,000 (Fig. 14).


13


14

colloid is labeled, and concluded that colloid droplets contain material phagocytized from the follicular lumen.

Some questions can be raised concerning this interpretation. The lack of label in colloid droplets after the short time interval, the crucial one in this case, may only indicate that the interval was not long enough to permit absorption of radioactive amino acid from the peritoneal cavity and its incorporation into the protein of colloid droplets. In the actively secreting exocrine pancreas, for example, radioactive label is just entering newly formed zymogen granules near the Golgi apparatus 20 minutes after *intravenous* injection of labeled amino acid (Caro, 1961). It is also possible that the earliest droplets formed contain protein synthesized some time earlier by the follicular cells and stored temporarily in their cytoplasm, perhaps within ergastoplasmic sacs, before being packaged in secretory droplets. Thus, despite the recent application of radioactive tracers to this problem, the question whether colloid droplets contain newly resorbed or newly synthesized colloid still remains undecided.

Golgi Apparatus

In the follicular cells of the unstimulated rat thyroid gland the Golgi apparatus is inconspicuous, consisting of relatively few spherical vesicles and small arrays of parallel flattened cisternae (Dempsey and Peterson, 1955; Wissig, 1960). It appears autonomous and gives little evidence of any relationship to other organelles suggestive of an interchange of either its membranous components or of the substances they enclose. The results of this and an earlier study (Herman and Fitzgerald, 1961) disclose that dramatic changes are induced in the apparatus within a short period of time by thyrotrophic hormone. Its unit elements rapidly increase in number, and their development into mature colloid droplets is accelerated. The organization and composition of the apparatus should, therefore, be considered plastic, capable of being modified by the controlling influence of the anterior pituitary hormone. Whether the response of the organelle is directly controlled by the trophic hormone or is secondary to some other responses of the follicular cell is a moot point.

Although during stimulation the Golgi apparatus undergoes changes which emphasize its role in the formation of colloid droplets, it is difficult to transpose this behavior into physicochemical events that might enable us to predict, for example, the complement of enzymes it should contain. It seems likely that data from fractionation and histochemical studies will prove more valuable than purely morphologic studies in this respect, and progress towards this end is beginning to be made (Kuff and Dalton, 1959; Novikoff and Goldfischer, 1961).

Colloid Droplets

The colloid droplets undergo structural changes during their evolution which deserve comment, for the changes imply that the contents of the droplets are quantitatively and qualitatively modified over a period of time. To begin with, the smallest vesicles in the Golgi region do not contain fine dense particles. Particles are, however, scattered in the cytoplasmic matrix near them. Slightly larger vesicles with a somewhat more adielectronic content usually contain a few fine particles and have been identified in this study as colloid droplets in an early stage of development. This finding suggests that colloid droplets begin to acquire fine particles as they emerge from the Golgi apparatus. Later, as the droplets approach maximum size, their content of particles diminishes. As particles do not reappear in the cytoplasmic matrix around them, it seems likely that the particles undergo dissolution within the maturing droplet.

The morphology of the fine dense particles offers little clue to their chemical composition apart from their resemblance to various iron-containing proteins that have been observed by electron microscopy; *e. g.*, hemosiderin (Richter, 1957) and "micelles ferrugineuses" (Bessis and Breton-Gorius, 1959). Reports in the literature of the distribution of iron in the thyroid gland are rare. Grafflin (1939) examined the thyroid of a deer by microincineration techniques and discovered iron-containing granules in the apices of follicular cells. He did not identify the granules as colloid droplets and was unable to detect any iron in follicular colloid.

A second element should also be considered as a possible constituent of the fine dense particles. The thyroid gland incorporates iodine into its hormones. Iodine, like iron, has a high atomic number, and in sites of high concentration would be expected to be extremely adielectronic. It is tempting to speculate that the fine particles

consist of iodine which will be incorporated into the amino acid precursors of the thyroid hormones in the proteins of the colloid droplet. Once dispersed in protein in this fashion, the iodine would probably not scatter sufficient electrons to be detected by electron microscopy, and the particles would disappear.

Unfortunately, this hypothesis is not compatible with what has already been discovered concerning the utilization of iodine for hormone synthesis by the thyroid gland. Initially iodine as iodide is "trapped" by the gland by being selectively sequestered from the circulation. Under normal circumstances, trapped iodide is rapidly used for hormone synthesis. However, if utilization of the trapped iodide is blocked by agents such as propylthiouracil, the gland will, nevertheless, store substantial quantities of iodide. The site of the stored iodide has been localized by autoradiography of thyroid specimens removed from animals injected with I^{131} and fixed by quick freezing. Under these conditions, the bulk of the iodide is stored within the follicular lumen and not within the follicular cells (Pitt-Rivers and Trotter, 1953; Doniach and Logothetopoulos, 1955). Furthermore, if I^{131} is administered to an untreated animal, the gland incorporates small amounts of radioactivity into protein within a matter of seconds. By autoradiography, the protein-bound radioactivity present under these conditions has been localized in the follicular colloid and not in the follicular cells (Wollman and Wodinsky, 1955). It is doubtful that follicular cells would be able to iodinate protein and secrete substantial amounts of it into the follicular lumen within such a short interval. These observations point to the follicular lumen rather than the epithelial cells as the site of the iodination reactions. It seems unlikely, therefore, that the fine dense particles would contain iodine to be used for this purpose. In spite of our lack of knowledge concerning the composition of the particles, it is of perhaps greater importance for our present purposes that their transient existence may serve as a sign of a transformation taking place in the chemical composition of the colloid droplet during its maturation.

The pattern of development of secretory droplets from Golgi vesicles in the thyroid gland differs in certain respects from that seen in other glands. In the anterior pituitary (Farquhar and Wellings, 1957; Farquhar, 1961), exocrine pancreas (Farquhar and Wellings, 1957; Palay, 1958; Palade, 1959), and mammary gland (Wellings *et al.*, 1960), the protein material within the secretory droplets is adielectronic and for that reason easily discernible. The material first appears in small quantities within large Golgi cisternae of irregular outline. At this point, the droplet is only partially filled and a clear space separates the content from the limiting membrane. Thereafter, the amount of dense material progressively increases until the clear space adjacent to the membrane is completely or nearly completely obliterated, and the droplet acquires a round silhouette. Although in these glands the droplet's content is sufficiently dense to be easily visible in the electron microscope, morphologic examination fails to uncover the means by which the content is collected.

In contrast, even in the thyroid gland of the rat in which secretion has been stimulated by injection of thyrotrophic hormone, large irregularly outlined Golgi cisternae which gradually fill with secretory material do not occur. In their evolution from spherical Golgi vesicles and throughout subsequent stages in their development, colloid droplets always appear completely filled with secretion and retain a round outline as they increase in diameter. As in the other glands, it has not been possible to discover how the colloid droplets collect their secretory content in order to increase in size. Since in all their recognizable forms they appear spherical and never dumbbell-shaped, it is not likely that growth occurs by fusion, unless fusion takes place relatively instantaneously. An alternative explanation stems from a hypothesis concerning the function of the Golgi apparatus advanced during the early part of this century. The morphologic data concerning the behavior of the apparatus in secretory cells was interpreted as signifying that this organelle serves as a center for condensing material synthesized elsewhere in the cell into secretory droplets (see the review by Kirkman and Severinghaus, 1938). More recently, Palay (1958) finds the hypothesis still generally valid in the light of data gathered by electron microscopy. If this hypothesis is applied to the thyroid gland in terms of what is currently known of the fine structure of the Golgi apparatus and colloid droplets, the limiting membranes of these organelles would be presumed to be capable of sequestering within their lumens thyroglobulin originally synthesized

by the ergastoplasm and thereafter released in soluble form into the cytoplasmic matrix. The constituents of the fine dense particles may also be incorporated into colloid droplets in a similar manner.

In the thyroid gland of the salamander stimulated by repeated injections of thyrotrophic hormone (Herman and Fitzgerald, 1961), the mode of formation of colloid droplets more closely parallels droplet formation in other glands. The difference between the thyroids of the rat and salamander in this respect may represent a true species difference or reflect differences in experimental procedure.

Other Cytoplasmic Droplets

The second class of droplets within follicular cells is a diverse group from the standpoint of structure and is not affected by the administration of thyrotrophic hormone. They do not possess common distinguishing morphologic characteristics which would allow them to be related to one another, and a major consideration in placing them under one heading at this point is convenience. Some of the bizarre forms resemble lysosomes (Novikoff *et al.*, 1956), but they cannot be identified as lysosomes with more certainty without some knowledge of their enzymatic properties. A number of the less complex droplets of this class are bounded by a single membrane and contain a relatively adielectronic homogeneous matrix substance in which fine dense particles identical with those of the colloid droplets as well as a few granules or vesicles are dispersed. These strongly resemble colloid droplets and the resemblance suggests that some members of the second class of droplets may be related to definitive colloid droplets by being derived from a common antecedent, in this case presumably a small colloid droplet. As we know little of the chemical properties and function of the droplets of this class, it is difficult to speculate upon what function could be served by sidetracking a number of immature colloid droplets into this line of development.

Apical Pseudopodia

The apical surface of the follicular cell undergoes drastic modulation during the release of colloid droplets into the follicular lumen. Its usual coat of microvilli disappears and is replaced by large, blunt pseudopodia whose appearance suggests that they are motile in the living cell. Although the pseudopodia consist principally of plasmalemma and proteins of the fluid cytoplasmic matrix, they are capable of advancing into the viscous colloid. Their movements presumably are accomplished by molecular forces akin to those which maintain the integrity of the microvillus border in the unstimulated follicular cell and which can operate without the aid of a visible structural framework. Unfortunately, little light has been shed up to the present time upon such provocative riddles as the molecular basis for this form of cellular activity, the roles played by the plasmalemma and the cytoplasmic matrix in it, and the manner by which their individual adjustments are coordinated to achieve an integrated response.

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