# THYROID ULTRASTRUCTURAL CHANGES INDUCED BY HYPOTHERMIA

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By

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Investigations have shown that the hypothalamus and pituitary respond to decreases in body temperature by stimulating the thyroid gland to release $T_3$ and $T_4$ hormones. This study was designed to investigate ultrastructural changes of the thyroid gland induced by hypothermia. The ultrastructural changes produced by stimulation by Thyroid Stimulating Hormone were also examined as an adjunct to the hypothermic stimulation of the gland.

There was a significant increase in microvilli on the luminal border of follicle cells along with a remarkable increase in numbers of dense granules. The dense granules also demonstrated a polarity being found near the border of the colloid lumen. The glandular changes induced by Thyroid Stimulating Hormone were very similar to those of hypothermia.

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# CHAPTER 1

## INTRODUCTION

The history of the thyroid gland was summarized by Rolleston in 1936 (34) and by Galen (10) in his De Voce which described the gland briefly. Vesalius in 1543 (41) was really the first to give a full description of the gland followed by Wharton in 1656 (43), who suggested that the function of the gland was to round out and beautify the neck, by filling in the vacant spaces about the larnyx. Other suggestions were that the gland was a lymphatic gland, a receptacle for worms (41), or a lubricant organ for the larnyx (10)

Experimental thyroidectomies were performed as early as 1827 to study the relationship between the thyroid gland and other body functions. The concept of an internal secretory function was first introduced by King nine years later in 1836. Since the parathyroids were not recognized until Gley rediscovered then in 1891, death generally followed such operations. The final identification of the crucial role of the parathyroids, apart from the thyroid, came in 1898 with the removal of the parathyroids alone (42).

Baumann in 1836 was the first to associate iodine with the thyroid. Oswald in 1899, and Gley and Bourcet in 1900 identified the presence of organic bound iodine in plasma.

Gross and Pitt-Rivers in 1954 discovered the presence of a compound with only three iodine atoms, triiodothyronine ($T_3$), in the gland and in blood plasma. This was significant because $T_3$ was found to be more physiologically potent than the previously discovered tetraiodothyronine ($T_4$) which contains four atoms of iodine. Recent evidence indicates that a fraction of the $T_3$ in human plasma may be derived from deiodination of $T_4$ (42) in the circulation. This point will be discussed later.

The thyroid is now accepted as a major component of the endocrine system. It is integral to the general hormonal balance of the body, and especially affects growth, development, and metabolism. The gland itself is under the direct control of the anterior pituitary and its hormone. Under control from the anterior pituitary, the whole course of metabolism is regulated by the release of trophic hormones into the general circulation. These hormones regulate the endocrine glands, including the thyroid, with direct action of the special hormone: growth hormone. This is how the direct action of the thyroid controls overall processes such as growth and differentiation.

The thyroid is the chief organ of iodine metabolism and is found in all vertebrates. The thyroid has several functions in connection with the iodine metabolism in the

body: 1) it actively concentrates the iodine from the circulating blood and converts it into organically bound iodine; 2) it acts as a reservoir of the thyroid hormones, which it fixes as thyroglobulin and stores it in its follicles; 3) it regulates the liberation of this stored hormone under the constant and restraining control of the TSH of the pituitary; 4) it is a very efficient assimilator of iodine liberated during metabolism of the thyroid hormones, which it stores and later releases should an exogenous supply of iodine be deficient(40).

The hormone is stored in the thyroid itself as bound thyroglobulin. It is liberated then by the thyrotrophic hormone of the anterior pituitary, which controls proteolysis. The transport forms of the thyroid hormones consist of thyroxine and a small amount of 3,5,3'-triiodothyronine which circulate in the blood stream as plasma-bound proteins. A very small amount of hormone is in a free active state. Generally, thyroxine and $T_3$ are the hormones which actually penetrate through the cell membranes and eventually interact with receptor surfaces within the cell (40).

The purely basic function of the thyroid gland is to produce and secrete the thyroxine, triiodothyronine, and also the other iodinated amino acids. This takes place within the framework of the specific protein of the thyroid gland--thyroglobulin. This globulin itself contains large

amounts of organically bound iodine.

The thyroid hormone formation is based on two continuous fundamental processes: the circulation of iodine inside the thyroid gland and the biosynthesis of thyroglobulin. These both are very precise processes in perfect balance (40).

## General Morphology

In looking at the structure of the thyroid stroma, the gland has an expansive blood supply and a relatively simple lymphatic and nervous supply. The thyroid receives its blood principally via two or three pairs of thyroid arteries which are branches of the large arteries and veins of the deep cervical area. These are the superior and inferior thyroid arteries and veins, respectively The artery branches off the carotid artery just above the thyroid cartilage of the trachea. These radiate throughout the interior of the gland in the connective tissue septa around the gland's capsule. Gradually they carry blood to the capillary plexuses which surround each follicle near the follicular basal epithelial cells. Many of the perifollicular capillaries lie within grooves indenting the peripheral border of the follicle. This seems to allow more extensive contact between epithelium cells and its capillary connections. With this blood vessel network, the thyroid gland, in comparison to other tissues, has a very rich blood supply. Some examinations show that the inner portion of the gland gets a richer blood supply but there is nothing conclusive.

These perifollicular capillaries, as they are called, are similar in structure to those in the kidney, intestine, and other endocrine tissues; i.e. a single layer of endothelial cells which in many regions is extremly thin and fenestrated (30).

Reinhoff (32) studied the distribution of lymphatics in the thyroid gland of the dog. He observed that the terminations of the lymph vascular system formed large cisternae or bursellae in the spaces between follicles. These cisternae are then interconnected in a network by smaller lymph capillary channels. If examined in three dimensions, the bursellae are wedge-shaped or plaque-shaped sinuses filling the available free space between follicles. They are not easily recognizable in ordinary preparations of thyroid tissue. The lymph system network is situated further from the follicles than the blood capillary network. Reinhoff also points our that because of this distance, it is likely the blood capillary network, rather than the lymphatics, carries away the thyroid hormones after its release from the follicle.

The nervous supply to the thyroid gland has been most thoroughly studied by Nonidez in the dog (26,27,28). The dog's thyroid recieves innervation from the parasympathetic and sympathetic divisions of the ANS. It is likely that it has sensory innervation also. One distinct thyroid nerve enters the apical pole of each lateral lobe with the superior thyroid artery. It consists principally of

parasympathetic fibers from the superior laryngeal nerve, a branch of the vagus. Additional sympathetic fibers reach the gland as extensions of the carotid plexus entering with the thyroid arteries. After forming a rich plexus among the follicles, most nerves end in association with vessels of the arterial tree, but a few terminate among the follicles. Stimulation of the sympathetic component causes vasoconstriction of its vascular supply, whereas vasodilation results from stimulation of the parasympathetic component

There are two distinct types of cells which comprise the thyroid gland. These are follicular cells and parafollicular cells. Both cell types vary in ultrastructure and function.

## Follicle Cells

The ultrastructure of the follicular cells and its celluar organelles and inclusions deserves discussion at this point. The nucleus ususlly lies towards the basal pole of the follicle cell. It is spherical, but often invagination of the nuclear membrane occurs. It may contain nucleoli and, under electron microscopic examination, its matrix is composed of a relatively homogeneous dense dispersion of fine granules. The nuclear membrane consists of two parallel lamellae which are periodically interrupted by fenestrations. In salamanders that have repeatedly been injected with TSH, the volumn of the nucleus of the follicular cells doubles. At the same time, the nucleoli enlarge and

the amount of RNA in the nucleus increases (14).

The mitochondria of follicular cells are more or less uniformly distributed throughout the cytoplasm. They are of conventional structure; i.e., a double limiting membrane and internal cristae formed by infoldings of the inner membrane. The mitochondria may contain small dense granules which are usually more numerous in other tissues besides the thyroid (30). In a recent study (29), there were striking ultrastructural features noticed in thyroid follicular cells. This was the presence of prominant, large membrane-enclosed paracrystalloid bodies. These are derived from mitochondria and were formed by the reorganization of the cristae into parallel closely opposed membranes. In these studies of rats and bats, it was suggested that a rapid shutdown of active follicular cells appears to promote thyroid paracrystalloid formation from mitochondria. The paracrystalloids were never seen in parafollicular cells nor in other types of thyroid cells like the mast or inter-follicular cells.

The endoplasmic reticulum or ergastoplasm is perhaps the most prominant organelle in the cytoplasm. Unlike the exocrine cells of the pancreas, hepatic cells, or serous or mucus cells of the salivary gland, which consist of broad, highly flattened vesicles in parallel arrays, the ergastoplasm of follicle cells is polymorphic and dilated. They are randomly distributed throughout the cells but tend

to be more numerous around the nucleus on the apical side. Each vesicle is single, membrane-bound, with many ribosomes affixed to the outer surface. Free floating ribosomes are also distributed throughout the cell. The ergastoplasmic vesicles contain a finely fibrillar, low density material similar to the colloid. It is suggested that the vesicles are dilated and filled with substances which will eventually be incorporated into follicular colloid(30).

Several aggregations of Golgi bodies appear in the apex of the follicular cells. They are often closely associated with ergastoplasmic vesicles and small colloid droplets which will be discussed shortly. With stimulation, the Golgi increase in size and number and the colloid droplets are determined to arise from the Golgi vesicles and are secretory in nature(3).

The apical surface of the follicular cells is a structurally complex region which undergoes modifications and variations depending on the functional state of the gland. The plasma membrane bordering the colloid is approximately 80 $A^{o}$ thick and is three layered. A dense inner layer is separated by an intermediary pale layer from the less dense outer layer. Numerous finger like projections called microvilli can be present and project into the colloid lumen. These microvilli are not of uniform length or shape. They consist of a core of cytoplasm with a plasma membrane bordering them. There are always numerous small vesicles distributed

randomly throughout the apical area of follicular cells. A small number of these vesicles open into the lumen area and because of this they represent some form of pinocytosis (30). and will be termed in this study as secretory vesicles.

On the other end of the follicle cell, the basal membrane forms a closely apposed boundary at the base of each follicle cell and follicle. It is a fibrillar layer approximately 400 $A^{o}$ wide and rich in mucopolysaccharides. Small groups of reticular or collagenous fibers are often associated with its outer surface. Occasionally pseudopods may arise from the basal surface. These usually extend parallel to the basement membrane and undermine the parent cell or adjacent epithelial or interstitial cell. However, this occurs only occasionally in normal cells(30).

The remaining ultrastructural components of the follicular cells are the secretory droplets and other secretory inclusions of the cell. There has been much confusion on the terminology used to describe each type of varied structure. The following information will attempt to describe each type and clarify the nomenclature used in this study.

Under electron microscopy or light microscopy, the population of droplets within follicular cells is heterogeneous and very numerous. Among droplets of varied size and content, they may be grouped into a single class on the basis of characteristics they share in common. The first such class is the single membrane limited vesicles containing

homogeneous material of the same approximate denstiy as that of the colloid lumen. They are called the colloid droplets. When Biondi (1) first started the modern view of the thyroid secretory histology, he recognized the follicle colloid as a secretory product of the follicle cell. He described the intracelluar droplets as material similar to luminal colloid. Then Dempsey and Peteraon (5) described these membrane-limited structures which they named colloid droplets, but they interpreted these as part of the endoplasmic reticulum. Later, Wissig (44) described large, smooth-surfaced, membrane-limited structures with contents similar to the follicle colloid. He concluded these corresponded to the droplets seen in light microscopy. The colloid droplets are spherical in shape and usually lie near the center to middle of the follicle cell as well as on the apical area of the follicle. However, in the apical area the colloid droplets are much smaller and are termed "apical vesicles". It is often difficult to differentiate between small colloid droplets and Golgi material due to their close proximity within the cell (30). Nadler (25) conclusivly demonstrated that these colloid droplets could be broken down in the cytoplasm and the thyroxine released into circulation, however, he did not indicate if the material in the colloid droplet was iodinated thyroid hormone or not.

The "apical vesicles" of follicular cells have usually been interpreted as pinocytotic just like the colloid droplets.

However, apical vesicles may contain material which is a precursor of the colloid. This has been speculated but is not yet known. These vesicles seem to be the same, or very closely so, to the colloid droplets, but in a different region of the follicular cell--the apical area (46).

To summarize the terminology of the secretory vesicles and to avoid confusion, the following terms will be used in this study: 1) colloid droplets: are found in follicular cells only and are single-membraned, pinocytotic vesicles containing a material of the same density or nearly so, to that of the colloid in the lumen; 2) apical vesicles: are the very small vesicles in the apical region of the follicle cells and are accepted to be the same as colloid, i.e. contain material of the same density as the colloid; 3) dense granules: are found in both follicular and parafollicular cells, are very electron dense and are usually large and spherical in shape; 4) secretory vesicles: are of moderate electron density, are present only on luminal borders and frequently are seen breaking off into the follicular lumen; 5) multivesicular bodies: are vesicles that have more than one vesicle enclosed inside the outer membrane, are of varying density dependent on the vesicles enclosed and are infrequently observed in follicle cells; and finally 6) vacuoles: are used in the classic definition, are of varying shape, appear to contain little material, and have very little electron density.

In general terms, the follicular cells are truncated pyramids which are oriented in relation to the colloid lumen. Their bases are separated from perifollicular capillaries by a basement membrane. The cell apices have irregularly arranged microvilli which may protrude into the colloid in the follicular lumen. Terminal bars are also seen toward the apex of the cells and occur between the individual follicular cells on the luminal border. Because of this, the follicular cells seem to exhibit a definite polarity (46). The endoplasmic reticulum or ergastoplasm is found throughout the cytoplasm of the cell with its cisternae widely dilated and containing material of low electron dinsity. Ribosomes cover the surface of the membranes of the cisternae and are also free-floating throughout the cytoplasmic matrix. The Golgi zone contains groups of flattened vesicles with its sacs irregular in shape and dilated. There are also a class of large, smooth-membraned spheroids which are the colloid droplets and are of variable size. Identical vesicles are present in the apical zone of the cell which are the apical vesicles. Another type of dark electron dense vesicles are present and are called the zymogen-like or dense granules.

## Parafollicular Cells

The other type of thyroid gland cell is the parafollicular cell. The term "parafollicular" denotes those cells which originate in the follicular epithelium, enlarge markedly,

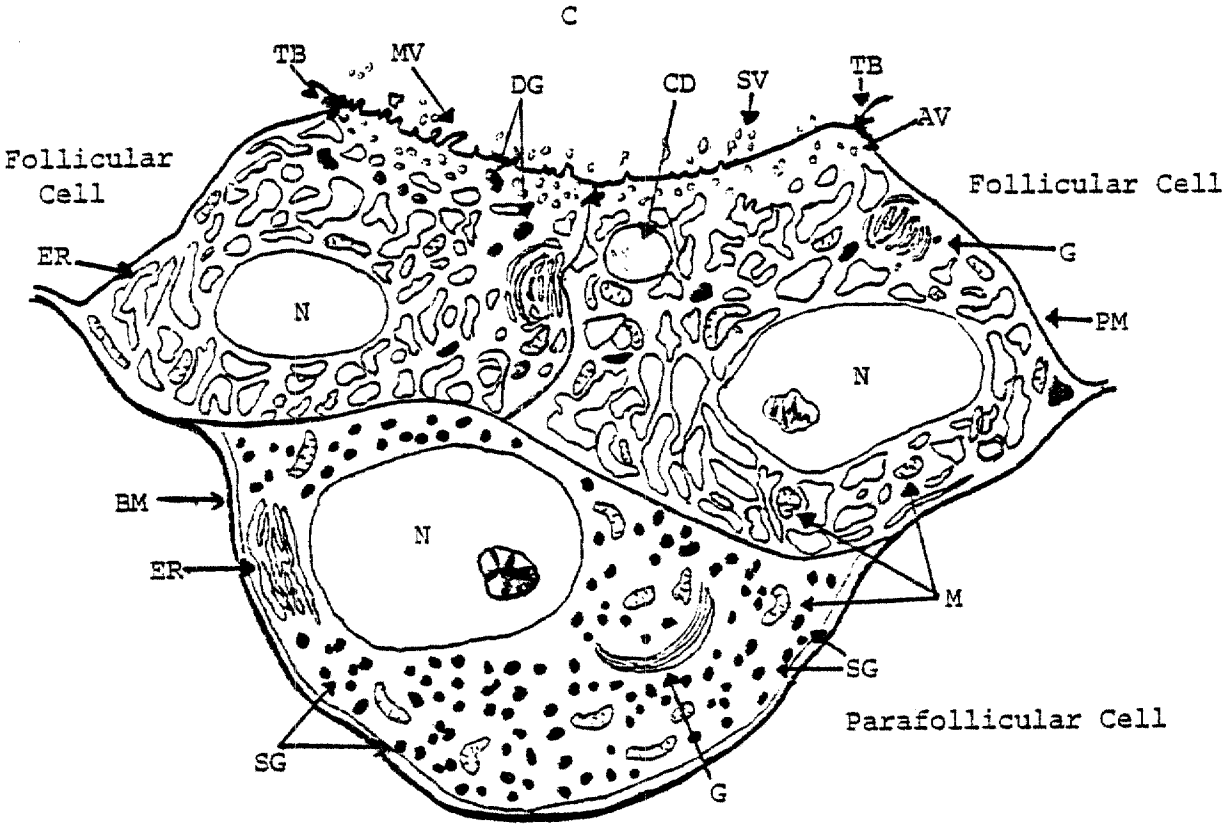


Fig. A. Drawing illustrating the relationship between follicular and parafollicular cells. They are contained within the follicular basement membrane (BM) but the parafollicular cells do not make contact with the colloid (C). Follicular cells may mave microvilli (MV) which protrude into the colloid lumen. The follicular cells are joined by terminal bars (TB) between cells along the plasma membrane (PM). Secretory vesicles (SV) are seen breaking off into the lumen. Dense granules (DG) are present in the follicular cells and the ergastoplasm (ER) is distributed throughout the cell with characteristically dilated cisternae. The Golgi zone (G) have partly distended sacs. One cell is shown with a colloid droplet (CD). The parafollicular cells have Golgi zones (G) with flattened sacs. The ergastoplasm (ER) seems to become collected in one area of the cell. The characteristic secretory granules (SG) are dark and numerous in parafollicular cells. The nucleus (N) and mitochondria (M) are similar in both cell types.

migrate from the epithelium and come to lie in close proximity to the follicle (31). Parafollicular cells in light microscopical studies are generally smaller than follicular cells. They have a paler cytoplasm and big, darkly staining vesicles. The cells are found either in the follicle wall or in the interfollicular space. Electron microscopic studies reveal an abundance of these dark granules of a diameter of about 2000 $A^{o}$ and these "secretory granules" are the main characteristic of the parafollicular cell (7).

In the dog, extensive formation of parafollicular cells is found in early development. These cells can be easily differentiated from the ordinary epithelial cells lining the follicle because of these dark secretory granules in their cytoplasm (31).

The function of these cells is uncertain. Early literature suggests that they served as a regenerative center for the follicle. Light microscopists believe that these cells have a secretion, endocrine or neuroendocrine function. Most adopted the idea of a secretory function. The nature of the secretory product is not known, but observation points to calcitonin (7).

Parafollicular cells are always on the periphery of the follicle and never reach the lumen of the follicle. They often form a ring around a follicle's basement membrane. Any group of parafollicular cells is always bound by one communal basement membrane. Their shape is round,

oval or polyhedral. The polyhedral shape usually predominates (7). Raymond (31) concluded that the occurrence of parafollicular cells in a follicle is correlated with follicular activity. It seems that parafollicular cell formation is related to active colloid secretion. That is the reason there are very few parafollicular cells in very large or very small follicles, but rather in those cells which are in rapid growth and extreme activity.

The nucleus of the parafollicular cell is not remarkably different from the structure and function in follicular cells. However, it does have a smooth outline in contrast to the irregular outline of follicular cells (7).

The mitochondria, in sections cut from parafollicular cells, are generally randomly distributed throughout the cytoplasm as is in follicular cells. They very in size and shape but the long slender shape seems to predominate. Branched forms have also been observed. The inner membranes are more oblique or longitudinally oriented as opposed to the transverse arrangement in follicular cells. The finely granular mitochondrial matrix has a higher density than the cytoplasmic ground substance. Dense mitochondrial granules are very few in number, if present at all (7).

The ergastoplasm or endoplasmic reticulum is usually confined to an area of the cytoplasm which is practically free of granules. This area is generally towards the cell periphery away from the intercelluar space. The shape of

of the ergastoplasm is of flattened cisternae which may follow closely the shape of the plasma membrane. The cisternae have a very uniform width and are much narrower than those of the follicle cells, measuring 200-300 $A^{o}$ wide. The contents are fluffy and slightly denser than the cytoplasmic matrix. Their surface is loaded with ribosomes as is the follicular ergastoplasm. The ribosomes often gather in groups of three to five. There are also numerous free ribosomes in the cell matrix (7).

The Golgi apparatus is very extensive. It is located near the bulk of the ergastoplasm. The Golgi saccules are arranged in curved or circular rows. Most saccules are flattened or elongated or oval. The bounding membrane of the ordinary saccules are smooth and about 60 $A^{o}$ thick. The boundary membrane of widened saccules on the other hand often appear partially coated with a 100-200 $A^{o}$ thick bristle-like or spine-like substance. Bristle-coating is also observed on some of the granules which are filled with a dense substance. Another saccule type is also present on occasion. These multi-vesicular bodies are considerably less numerous than in follicular cells. Fibrils are also common in the Golgi area of parafollicular cells. They are of a diameter of about 50 $A^{o}$ and are generally in bundles of five or six fibriles in parallel array (7).

The plasma membrane of parafollicular cells is a single dense line of about 90 $A^{o}$ thickness. Upon very high magnification the membrane appears to be three layers and can

have invaginations. Desmasomes are usually present between parafollicular cells but not between parafollicular and follicular cells (7).

Specific granules are perhaps the most conspicuous feature of parafollicular cells. These are in a rounded formation with a mean diameter of approximately 1400 $A^{o}$. The granules are bound by a membrane 60-70 $A^{o}$ thick with three layers. The granules contain a substance with a moderate to extremly high density. These granules are irregularly distributed in the cytoplasm and usually on the opposite side of the cell from the Golgi areas. There are transitions between the moderate and intensly dense granules that are present. This seems to follow the hypothesis that the Golgi apparatus is responsible for concentration of the secretory products (7).

Dense bodies are also present in parafollicular cells as well as the follicular cells. They are thought to contain acid phosphatase. In contrast to the great number found on the follicle cells, parafollicular cells contain sparse amounts of dense bodies. The dense bodies are easily distinguished from the specific granules because they are larger, have thicker three layered membranes and consist of very dense particles (7).

Two hypotheses have been proposed on the origin of parafollicular cells. Godwin (11) proposed that these cells come from the ultimobranchial body and are embryologically

different from the follicular cells. Further investigation would be needed to prove this theory. The other hypothesis is that the parafollicular cells are derived from follicular cells that lose contact with the colloid (39). This theory was supported by Sarkin and Isler (35) who experimented with the hypothesis that the increase in parafollicular cells after growth hormone treatment is due to mitosis. They found that the mitotic rate of these cells is much too low after hormonal treatment to account for the increased number of parafollicular cells. So, they concluded that the cells must have arisen from the only other cells present--the follicular cells.

In summary, the parafollicular cells have a nucleus with an even outline and lack indentation that are characteristic of the follicular cell. The mitochondria of the parafollicular cells are more slender than those of the follicular cell and have a more longitudinally arranged inner membrane and the ergastoplasm has a more irregular arrangement with much narrower cisternae. The specific granules of the parafollicular cells are the most characterisitc thing in the cell and have no counterpart in the follicular cell. Lastly, the parafollicular cells never reach the follicular lumen.

## Hormone Synthesis

According to Turakulov (40), the modern view of synthesis of thyroid hormones occurs in at least two general processes: 1) the synthesis of iodotyrosines accompanied by the incidental iodination of histidine, and 2) the condensation of two

molecules of iodotyrosine with the formation of thyroxine and triiodotyrosines ($T_3$ and $T_4$ respectively).

Recent electron microscopic studies determined that the actual biosynthesis of thyroglobulin is a unique process occurring in different parts of the follicular epithelium cell. These steps are: 1) the biosynthesis of the protein, 2) the addition of the carbohydrate components to the the polypeptide chain and then, 3) the iodination itself. The biosynthesis of the polypeptide chain occurs in the ribosomes and polysomes of the follicular cell of the thyroid gland. The specific template of mRNA is used in the standard protein synthesis mechanism. It begins with a 6S polypeptide formation. Association leads to a formation of a 12S product which, by divalent dimerization, yeilds the 17S prethyroglobulin molecule. This is converted by halogenation into true thyroglobulin containing an average of 30 atoms of iodine and weighing 19S (40).

After the polypeptide chain of thyroglobulin is synthesized, the carbohydrate moiety is added. Intracelluar protein transport is found to be of great significance to the addition of the carbohydrate to the peptide chain. Rapid transport of a protein-bound label of ribosomes to coarse microsomes including bound membranes and contents of vesicles was established. However, no transfer from these fractions to the smooth microsomes was observed. The results of the experiments indicate slow secretion of protein from the

ergastoplasm into the colloid. This shows that glycosylation occurs within the membranes of the ergastoplasm rather than only within the Golgi formations (41).

Attention should be paid to this iodination of the thyroid precursor--thyroglobulin--and its export from the cell. The main component of the follicle itself is the glycoprotein thyroglobulin. It is also the important precursor of the main exportable thyroid hormones triiodothyronine ($T_3$) and thyroxine ($T_4$). This reserve state of glycoprotein is stored as colloid in the follicle themselves. These are also subunits of the thyroglobulin found in the epithelial cells where they may be in a state of migration towards the completed molecule (40).

Before the thyroglobulin is a complete molecule, it must be iodinated and this also brings up extensive research done to ascertain the site, method and mechanism of iodination. The iodine used is present in the blood in very small concentrations of approximately 0.1-0.5 ug/100ml blood. In order for this small amount of iodine to be used, there must be a very efficient mechanism for concentrating it. This is one basic function of the thyroid gland as a whole. This formation of blood iodine by the gland has two consecutive aspects: the accumulation of iodide and then its oxidation into elementary iodine ($I_2$). The iodine must be in this elementary form to react with thyroglobulin. The free iodine is in equilibrium with the circulating iodine in the

blood and the bound iodine which can pass through membranes. This free iodine in the blood is integrated into organic compounds immediately after it passes through the thyroid gland. Some of the iodides in the gland can come from dehalogenated iodotyrosines released from the thyroglobulin during proteolysis (40).

The kinetics of iodine uptake by the thyroid during an increase of serum iodide levels are equally compatable with two interpretations(30). First, is a diffusion and loose binding of the iodine and second, a diffusion and active transport of iodine. Experiments with $SCN^-$, which can dislodge large amounts of thyroidal iodine without itself being incorporated into the gland to any significant extent, have also been seen as evidence for transport of iodine. This displacement of iodine from binding sites by $SCN^-$ would require that it "washes out" the iodine from the binding sites. Iodine probably enters the thyroid involving a temporary complexing of iodine with a carrier situated in the thyroidal cell membrane. The nature of this mechanism is not yet known. Other experiments by Slingerland(37)years earlier determined that the transport of iodine requires oxygen, a temperature of very near $37^{\circ}$ C., and a pH of near to that of body fluids. He stopped iodine transport with an oxidative phosphorylation poison DNP, thus eliminating the energy component of the iodine movement mechanism.

The currently accepted view about thyroid iodine uptake is that it is an active process linked with the energy supplied by normal cell metabolism(9). In this paper Freinkel and Ingbar showed that an active metabolically linked process of oxidation is essential for iodine transport into the thyroid gland against the gradient. They also halted transport with the addition of inhibitors of oxidative respiration.

Several papers have investigated the actual site of iodination of thyroglobulin. It is accepted that this process occurs in the follicular lumen. Eckholm and Wollman (8) proved this using high-resolution autoradiography. The use of silver grains showed a concentration located in a zone overlying the periphery of the luminal colloid. This zone is the apical portion of the follicle.

Other experiments using radiolabeled iodine (8), showed that iodine is concentrated almost exclusively in the follicular lumen. There was no deposit in the cell cytoplasm. This iodination and concentration also occurs very rapidly. An approximate time given was within fifteen minutes, when the iodine had made its way into the thyroglobulin present in the follicular lumen. Only this completly iodinated thyroglobulin with a sedimentation coefficient of 19S can exist in the apical part of the follicular cell. This shows that the polymerization of the thyroglobulin precursors must take place continuously during their migration from the rough ergastoplasm to the apical zone of the cell(21). After it

reaches the apical area of the cell, Nadler et. al.(23) described this release of the thyroglobulin from the vesicles as a "bubble of soap coming up to the surface of the water where it explodes, leaving behind a soap film". So, membranes of the apical vesicles reach the cell surface and fuse with the plasma membranes where they break open and release their contents into the follicular colloid in the lumen.

Other investigation with thyroid follicular cells showed that the iodination of thyroglobulin involves the participation of enzymes obtained from the microsomes of the thyroid. Mono- and diiodotyrosines, in connection with thyroglobulin, are formed in a system generating water and peroxide. The peroxidase enzyme is known to be necessary for oxidation of iodide and iodinating tyrosine to monoiodotyrosine (MIT) and diiodotyrosine (DIT)(40). The series of reactions are most probably the following(40):

$$AH_2 + O_2 \longrightarrow H_2O_2 + A_2$$

$$H_2O_2 + 2I^- + 2H^+ \longrightarrow 2\,H_2O + I_2$$

$$I^2 + \text{Tyrosine} \longrightarrow \text{MIT} + I^+ + H^+$$

Deiodination also occurs. This process, mediated by a deiodinase enzyme, continuously removes excess MIT and DIT liberated during proteolysis of iodinated thyroglobulin. Absence or a very low concentration of MIT and DIT in the circulating blood, despite their high percentage in thyroglobulin and the liberation during hormonal secretion, is explained by their rapid deiodination is this way (40).

In looking at the way these synthesized thyroid hormones leave the follicle cells, a lot of theories arise as to the mechanism. In the modern view, the thyroglobulin synthesized on the cytoplasmic organelles, is in a continuous migration toward the apex of the epithelial cells and into the cavity of the follicle. It is there where the hormone attains its final form in a colloid mixture (40).

The thyroglobulin is a reserve product in the follicle lumen. It contains the ready made hormones in a bound form. When the thyroid hormone is needed by the body, the thyroglobulin is decomposed and the iodinated amino acids enter the blood stream (40).

It is accepted now that direct secretion into the blood is impossible. This is true because the iodination of thyroglobulin and the formation of thyroxine and triiodothyronine takes place in the follicle colloid(12). There is most probably a lysosome-colloid droplet fusion inside the follicular cell. This occurs after colloid is taken back into the cell from the lumen by pinocytosis. Upon fusion of these two celluar structures the thyroglobulin proteolysis takes place into its $T_3$ and $T_4$ products. Recent studies have shown that this proteolysis can occur not in the lumen but in a phagolysosome type structure formed after a colloid droplet and a lysosome fuse. After this point, the iodinated amino acids could enter the blood stream in their $T_3$ and $T_4$ product forms. However, this theory is

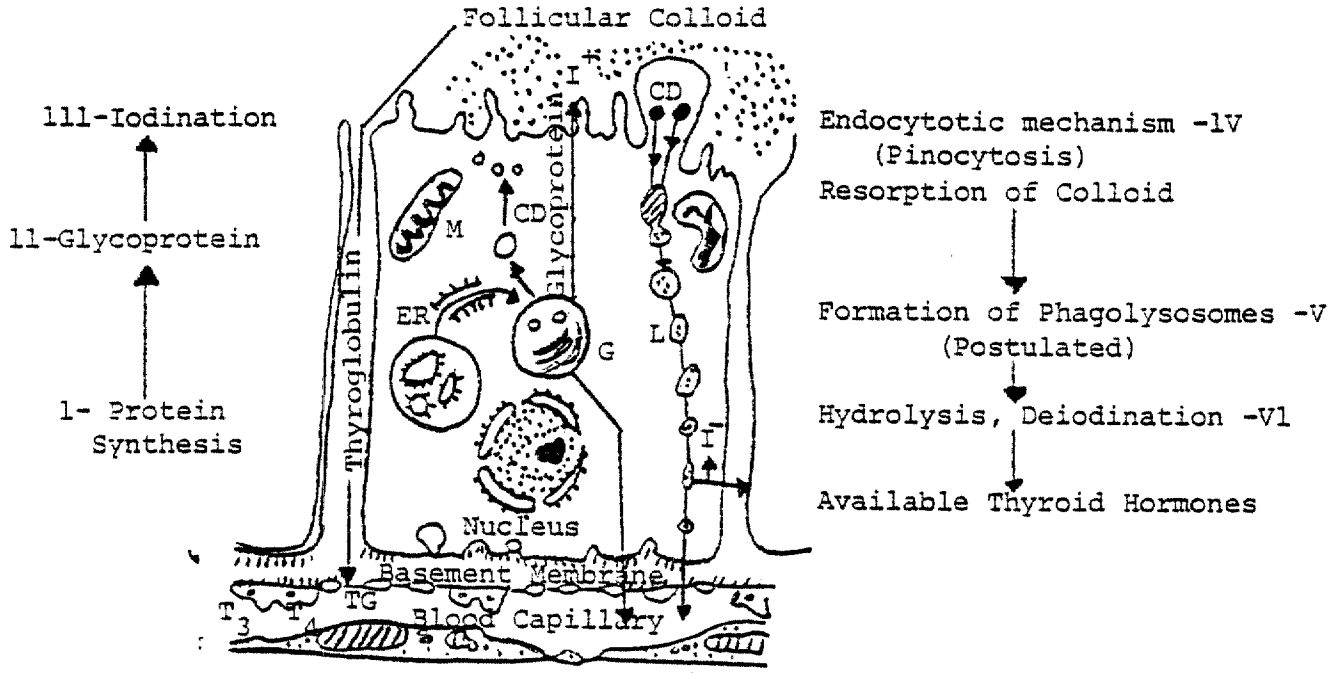


Figure B. Schematic diagram showing intracelluar migration and proteolysis of thyroglobulin. M- mitochondria, G- Golgi complex, L- lysosome, ER- ergastoplasm, CD- colloid droplet, TG- thyroglobulin, $T_3$- triiodothyronine, $T_4$- thyroxine.

uncertain (40).

Kawada et. al (18) studied the rate of hydrolysis of thyroglobulin in a lysosome system. They, too, suggest that lysosomes fuse with colloid droplets in the follicular cells, and this is a necessary step in the thyroid hormone secretion mechanism.

In summary, the movement of exportable protein from ergastoplasm to colloid corresponds to a maturation process of the thyroglobulin. First, in the ergastoplasm the protein moiety is synthesized, then it migrates to the Golgi zone where the carbohydrate moiety is added, and finally, in the follicular lumen, the glycoprotein thyroglobulin is iodinated with a formation of the full active thyroglobulin. The secretion then is a result of the breakdown of the thyroglobulin. This breakdown of thyroglobulin seems to take place within the cells after colloid droplets are pinocytosed from the lumen back into the cell itself. Then the free molecules of thyroxine ($T_4$) and triiodothyronine ($T_3$) are released for movement into the circulation system (see Fig. 2).

## TSH Effects

Direct action of thyroid stimulating hormone (TSH) to stimulate thyroidal hormone sedretion has been demonstrated in a variety of *in Vitro* studies (6,8,22,24,30,36,40). These studies have covered several areas such as the increased activation of adenyl cyclase production, increase in reabsorption of the colloid from follicular lumen stores, discharge

of the liberated iodothyronine hormones and hydrolysis of the resorbed thyroglobulin.

As described by Wollman(45)the complex of reactions in the response of TSH include the phagocytic uptake of colloid, the basal migration of intracelluar colloid droplets, fusion of colloid droplets with lysosome and/or dense granules, and the hydrolysis of the colloid to liberate iodotyrosines and iodothyronines. The TSH initiate a mechanism for colloid phagocytosis and iodination and release. After the initiation of the colloid phagocytosis phase of response, the rate of liberation of secretory products does not seem to be governed by the TSH level but by the availability of colloid from the intracelluar colloid droplets. However, it is possible that the intracelluar TSH level is the ultimate governor of the secretory products(22). Nadler et. al.(24) injected TSH in rats and found a great increase in colloid droplets at different stages. The droplets were first formed at the border with the lumen. Then, the droplets move into the cell body and predominate in the apical area of the cell. Finally, the droplets move further in and distribute themselves throughout the cell. They also determined that the increase in number of droplets was not due to splitting of preexisting ones, but from an increased production of new ones.

## Hypothermia

The effects of cold on the thyroid and hormone secretion have been studied only recently. In 1942 Ring (33) studied the basal metabolic rates of rats in relation to thyroid activity when exposed to cold. He saw the increased BMR upon cold exposure but could not really explain it. Leblond et. al. in 1943 (19) were in agreement regarding increased activity of the thyroid in animals exposed to cold. They did radioiodide studies on rate exposed to whole body cold. They too saw the increase in radioiodide in the thyroid after cold exposure. In another study (4) the rate of thyroid hormone secretion was said to approximately double when exposed to cold. Cold adaptation also was determined not to lead to reduction of thyroid hormone production (16). This same study concluded that thyroid hormones are essential for support of cell activity when exposed to cold. In other studies, the rate of release of radioactive thyroid hormone from the thyroid gland was determined to be a direct index of thyroid activity. This study obtained direct evidence for and increased rate of release of thyroid hormones in rats exposed to cold (2).

In a 1977 study (38), a rise in thyroid hormones and thyroid releasing factor was seen in rats exposed to cold, however, the mechanism still was not clear. An excellent study done by Haenke in 1977 (13) studied the effects of cold on thyroid hormone or $T_3$ and $T_4$ production in the isolated

thyroid exposed to hypothermia. He found that the thyroid showed a significant increase in $T_3$ and $T_4$ blood levels when exposed to temperatures of 25°C - 36°C. This showed that the thyroid is directly effected by decreased temperatures and that it may exert a direct control over the body temperature, independent of the hypothalamus and pituitary gland. Exposure to acute cold had no significant effect on thyroid gland function.

Ultrastructurally, and electron microscopic study was done on cold exposed rats (5). Complete gland observation was done with findings that backed up theories of earlier studies. The most obvious observation was a vast increase in colloid droplet formation upon cold exposure. This was also seen by Kajihara et. al. in guinea pigs (7). There are obvious large, empty looking sacs present upon cold exposure. The cytoplasm contains a great increase in very dark granular elements. They are electron dense, vary in size and are distributed throughout the cell. The most noteworthy change they found was an increase in follicular cell height. Inside the cell, the ergastoplasmic sacs become dilated. The lumen of the sacs contain a homogeneous colloid-like substance with moderate electron density. This was interpreted to be a colloid precursor about to be released (5).

All previous studies on the effects of hypothermia on the thyroid have been through whole body cooling. These

proceedures have ellicited the response of the hypophysis and its release of TSH as well as other hypothermic responses of the whole body. The purpose of this investigation was to observe and record ultrastructural changes in the thyroid gland during isolated hypothemic perfusion. This proceedure eliminated the whole body responses previously seen by other investigations.

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# CHAPTER II

## MATERIALS AND METHODS

Mongrel dogs were used as experimental animals in this study. No preference was given to the sex of the animal. All animals were maintained on Purina Dog Chow and water ad lib.

Each animal was anesthetized with an intravenous injection of sodium pentabarbital, 30 mg/kg. All animals had an I.V. started in the cephalic vein of the front leg, with an #18 Angiocath (Muuray-Hill, N.J.). The initial dose of approximately two thirds of the calculated dosage of anesthesia was injected rapidly. The remaining one third was injected slowly. All animals were maintained in plane two of stage three of surgical anesthesia(2) which is characterized by an absence of corneal reflexes. Immediately after proper anesthesia was obtained, an endotracheal cannula was inserted to assure a clear air passage. Throughout the procedure, the dog was ventilated on room air.

With the animal supine and ventral side up, an incision was made from the hyoid cartilage to the supra-sternal notch. Bleeding from the cutaneous vessels was controlled by cauterization. The subcutaneous connective tissue was separated along the midline by the cautry and blunt dissection. The trachea was exposed between the sternohyoid muscles.

All bleeders which could not be cauterized satisfactorily were tied off with 4-0 braided silk suture. The right section of the sternohyoid muscle as well as any connective tissue was retracted laterally with a nonfenestrated retractor. The animal's right carotid artery was located and isolated. Extreme care was taken not to disturb the nerve supply to the area. The inferior thyroid vein was isolated. The right thyroid gland, the right superior thyroid artery and the superior thyroid-common carotid artery intersections were isolated. A 2-0 silk ligature was placed around the common carotid artery immediately distal to the superior thyroid artery branch.

Prior to cannulation, sodium heparin, 3 mg/kg, was injected into the cephalic vein to prevent coagulation in the perfusion tubes. Heparin plasma concentrations were maintained with a repeated injection of approximately 1.5 mg/kg as needed. All cannulas were filled with a 1/100 dilution of sodium heparin in physiological saline proir to their insertion or use. All cannulas and tubing were made of standard polyethylene tubing (Clay Adams) of various sizes.

The right common carotid artery was bypass-cannulated proximal to the superior thyroid artery branch and the ligature around the common carotid was tied above the common carotid-superior thyroid artery branch. This produced the isolated circulation desired.

A variable temperature cryostat water bath was used as

the cooling unit. The water bath was maintained at $\pm$ 1.0°C. of the desired temperature. Blood, pumped by the systemic circulation, was allowed to flow from the common carotid artery through the P.E. tubing into a glass cooling coil which was immersed in the variable temperature water bath. The blood was returned to the thyroid gland through P.E. tubing encased by a water jacket filled with the cold water from the bath to minimize changes in blood temperature between the water bath and the perfusion point. A Yellow Springs Thermister probe was incorporated into the P.E. tubing systen to constantly moniter the blood temperature. Another thermister probe was wrapped around the cooling coil and this temperature was also constantly monitered. There was no significant loss of blood temperature between the cooling coil and the animal (Figure C).

Hypothermic perfusion was maintained for 30 minutes and the animals were terminated with an overdose of sodium pentabarbital. The thyroid gland was immediately removed from the animal, cut into small pieces and placed in a fixative mixture of 2% gluteraldehyde in sodium cacodylate. This fixative prepared the tissue for subsequent electron microscopic examination. The tissue was prepared and embedded following the proceedures adapted form several authors(1,3,4). The tissue was removed from the fixative and the inner and outer portions of the gland were seperated. These two seperate sections were then cut into approximately 1 $mm^3$

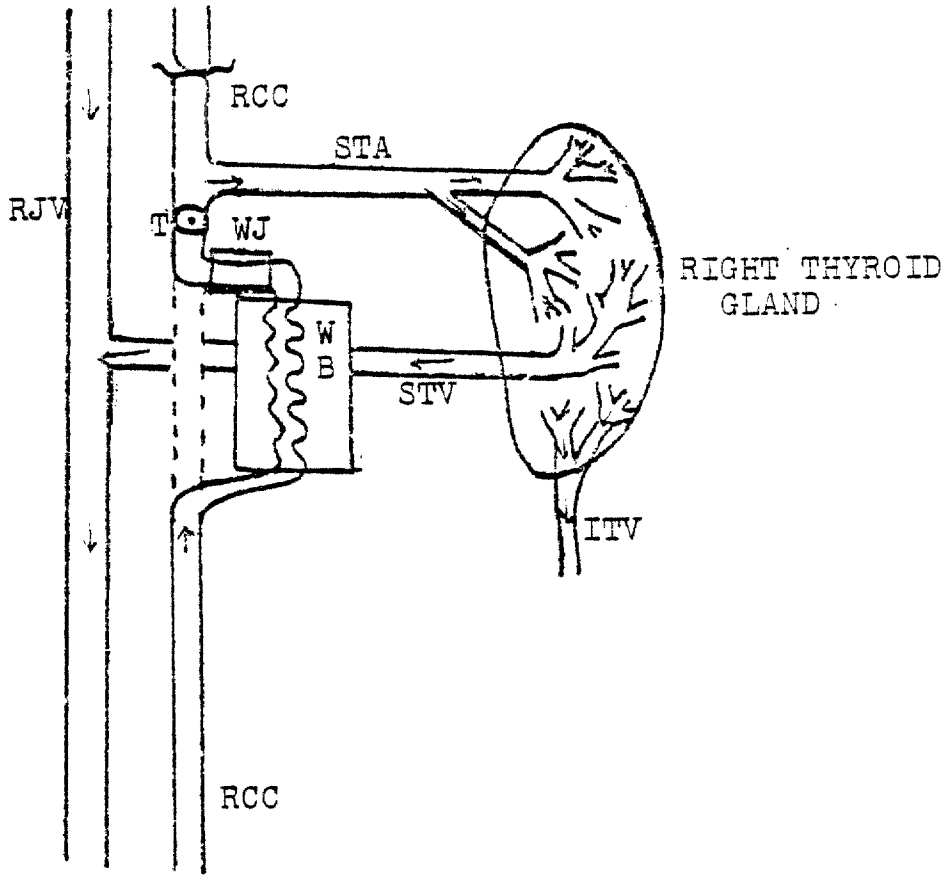


Fig. C. Thyroid perfusion system: RCC= right common carotid artery; RJV: right external jugular vein; STA= superior thyroid artery; ITV= inferior thyroid vein; STV= superior thyroid vein; RCC= right common carotid artery; WB= water bath; WJ= water jacket; T= thermister probe.

pieces of tissue. Both portions of the gland were treated the same in the embedding procedure. The $1mm^3$ pieces of tissue were then placed in a 50-50 mixture of sodium cacodylate and osmium tetroxide for 30 minutes. After this period of staining and fixing, the tissue was washed twice in a 2% sodium cacodylate solution. The tissue was dehydrated with successive washes in 50%, 70%, 85%, 95%, and 100% ethanol solutions with two quick washes of the alcohol and then one wash which lasted for 5 minutes. The washing prodedure was amended when using the 100% ethanol where the tissue was given ten quick washes and one for 5 minutes. The alcohol in the tissues was replaced with propylene oxide with 2 quick washes and one for 5 minutes. The proplyene oxide was replace with the epon resin (Ernest Fullem, N.Y.) in gradual 50-50, 25-75, and 0-100% mixtures of propylene oxide-epon, respectively. Following the staining and dehydrating the pieces of tissue were placed in standard Beem capsules (Ernest Fullem, N.Y.) filled with the Epon resin. The epon capsules were then polymerized overnight in an oven kept at a constant $60^{o}C$. temperature.

The polymerized epon capsules were removed from the plastic molds. The tip containing the tissue was trimmed under the microscope to approximately ½ $mm^2$. With trimming complete, the tissue was cut into approximately 60 to 90 mu thick slices by a Porter-Blum model MT-2 Ultramicrotome, and placed on a copper grid (Ernst Fullem, N.Y.). The

grids, with the tissue slices in place,were post-stained by placing then on drops of uranyl acetate-ethanol solution for 5-10 minutes. The grids were washed with ultrapure water and allowed to dry on filter paper. The grids were placed on drops of 2% soltion of lead citrate in a petri dish. Sodium hydroxide pellets were placed in the petri dish and a cover placed over the dish to absorb the $CO_2$. After 5-10 minutes, the grids were again washed with ultrapure water and placed on filter paper to dry.

The grids were examined on an RCA model EMU-3G electron microscope and pictures taken of appropriate areas. The negatives were developed and the pictures printed for examination.

Fifteen animals were used in this study. Five of the fifteen were control animals which were treated in exactly the same way as the experimantal animals except that the perfusion temperature was maintained at normal body temperature of 37°C. All other animals were maintained at a perfusion blood temperature of 26-31°C. The surgical procedure lasted approximately one and one half hours and a hypothermia period of 30 minutes was used.

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# CHAPTER III

## RESULTS

The results of this study are presented in three sections of electron photomicrographs. Section one consists of Figures 1-8, which are selected representative electron micrographs of normal control thyroid gland ultrastructure. Section two, Figures 9-14, consist of representative electron micrographs of hypothermic thyroid gland ultrastructure. The final section consists of Figures 15-20, which show thyroid gland ultrastructure under Thyroid Stimulating Hormone (TSH) stimulation.

Figure 1 (2500X): General overview of a portion of a control thyroid follicle. The relationship between the follicular and parafollicular cells can be seen. F= follicular cell; PC= parafollicular cell; L= lumen.

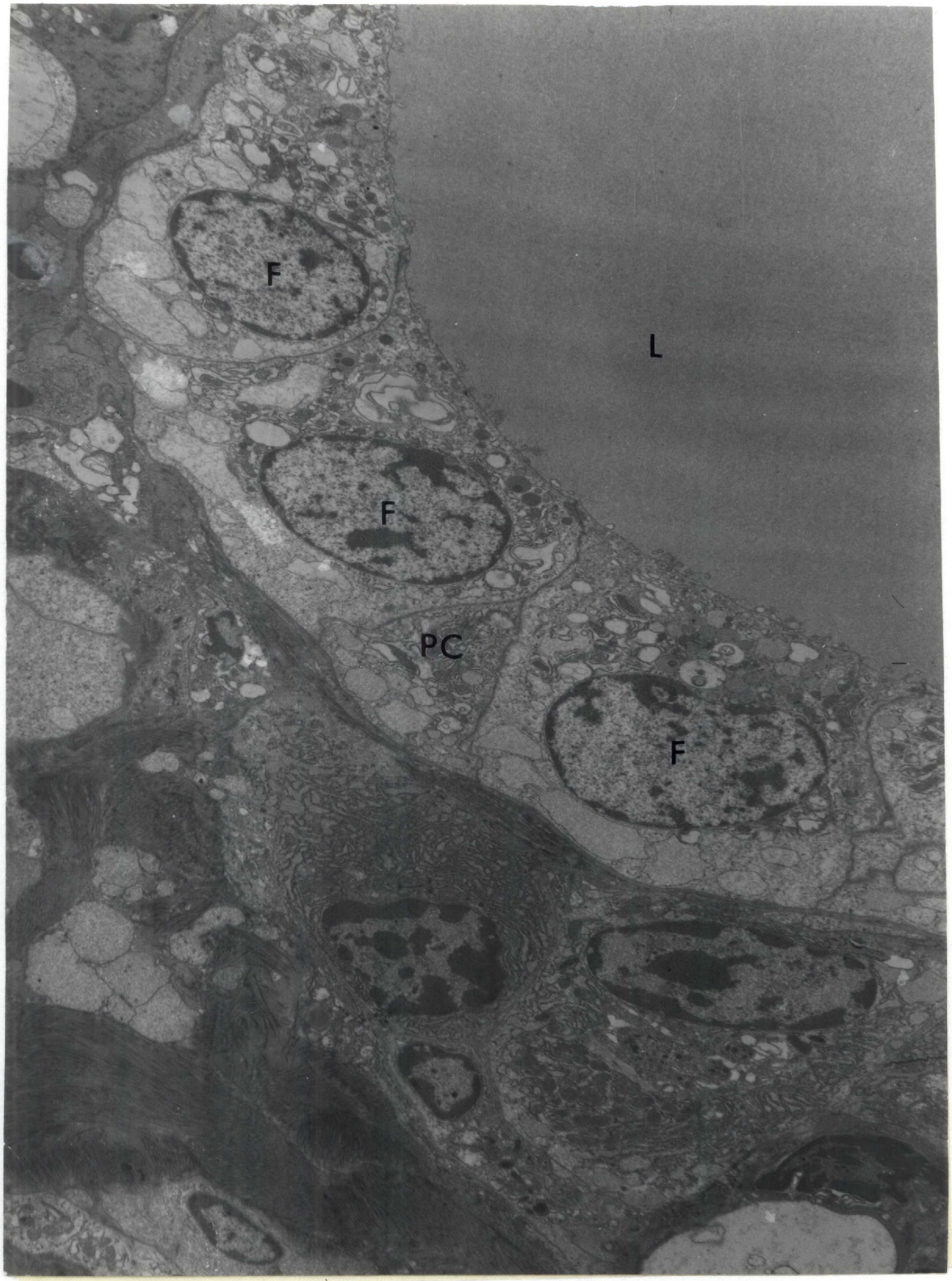
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Figure 2 (4250X): Two control follicular cells. M= mitochondria; ER= ergastoplasm; R= free ribosomes; SV= secretory vesicles; BM= basal membrane; PM= plasma membrane; CD= colloid droplet; AV= apical vesicles; DG= dense granules; MVB= multi-vesicular body; V= vacuole; TB= terminal bar; L= lumen.

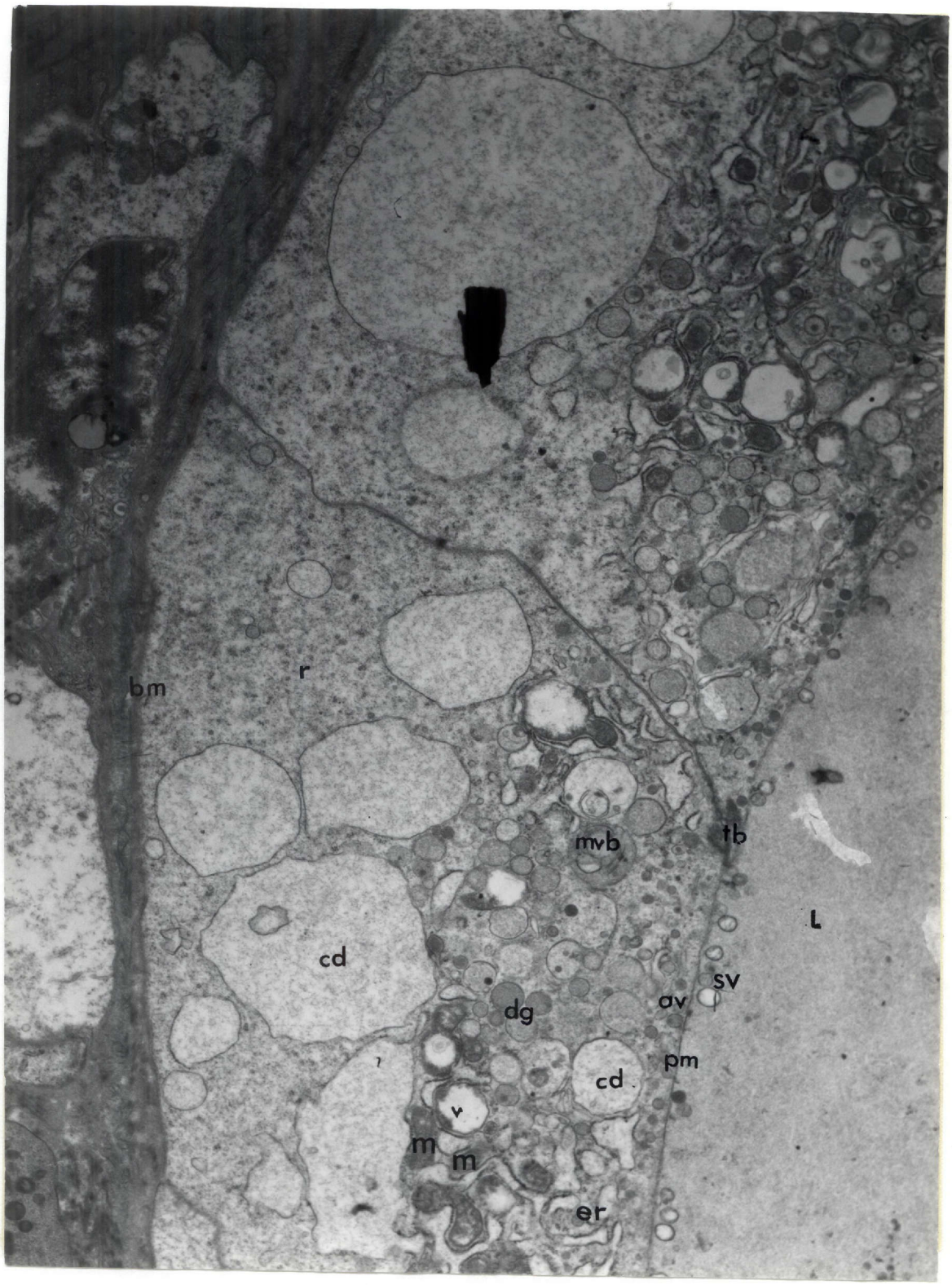
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Figure 3 (7700X): Control follicular and parafollicular cells. Nucleus is not visable in parafollicular cell due to the plane of section. Note irregularly shaped branched mitochondria. N= nucleus; M= mitochondria; ER= ergastoplasm; R= ribosomes; G= Golgi bodies; MV= microvilli; SV= secretory vesicles; BM= basal membrane; PM= plasma membrane; CD= colloid droplet; AV= apical vesicles; DG= dense granules; MVB= multivesicular body; V= vacuole; SG= specific granules (not stained very darkly); F= follicular cell; PC= parafollicular cell.

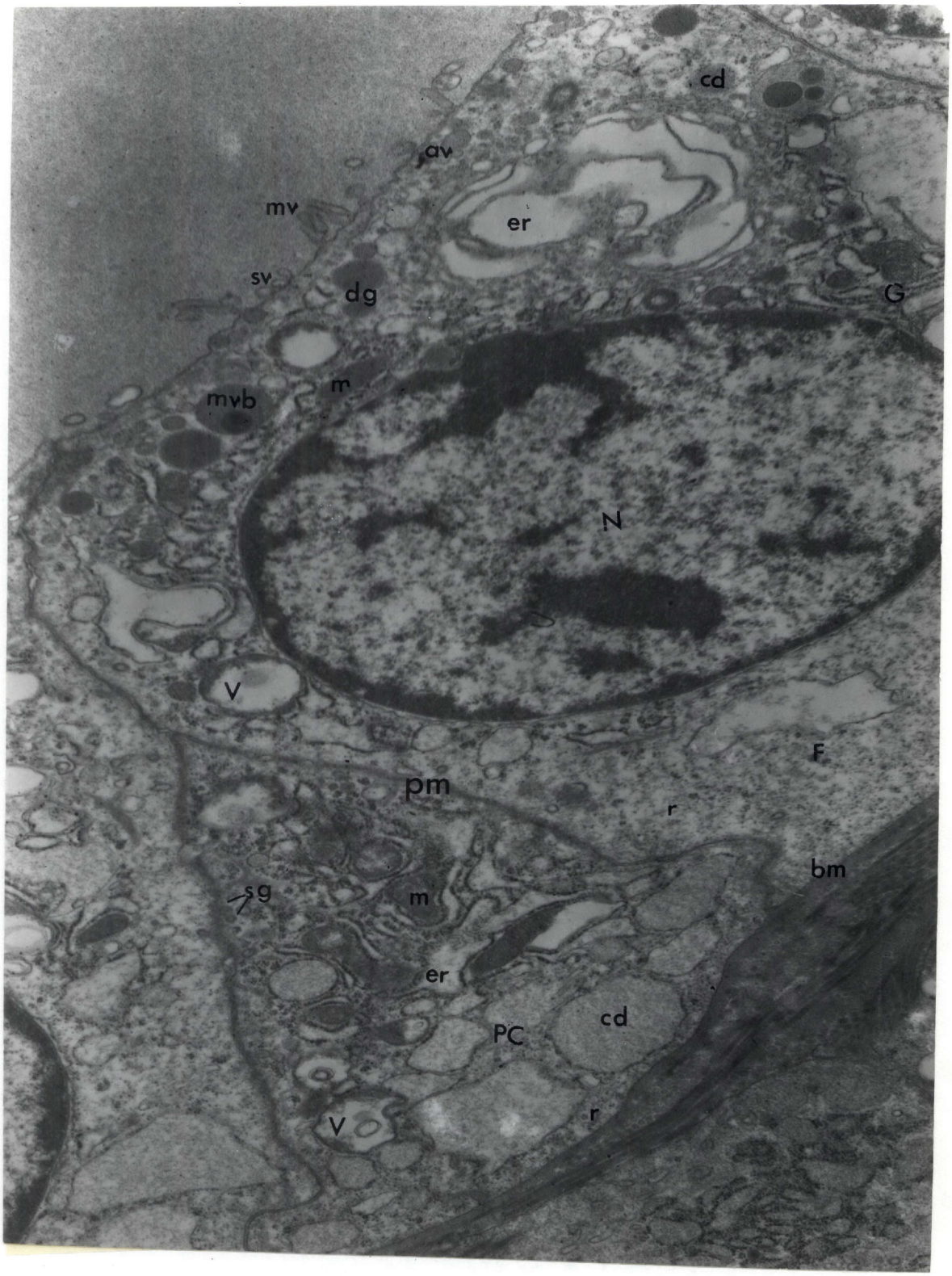
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Figure 4 (11,500X): High magnification view of control follicular cell. N= nucleus; M= mitochondria; ER= ergastoplasm; R= fibosomes; G= Golgi bodies; SV= secretory vesicles; PM= plasma membrane; CD= colloid droplets; AV= apical vesicles; DG= dense granules; L= lumen.

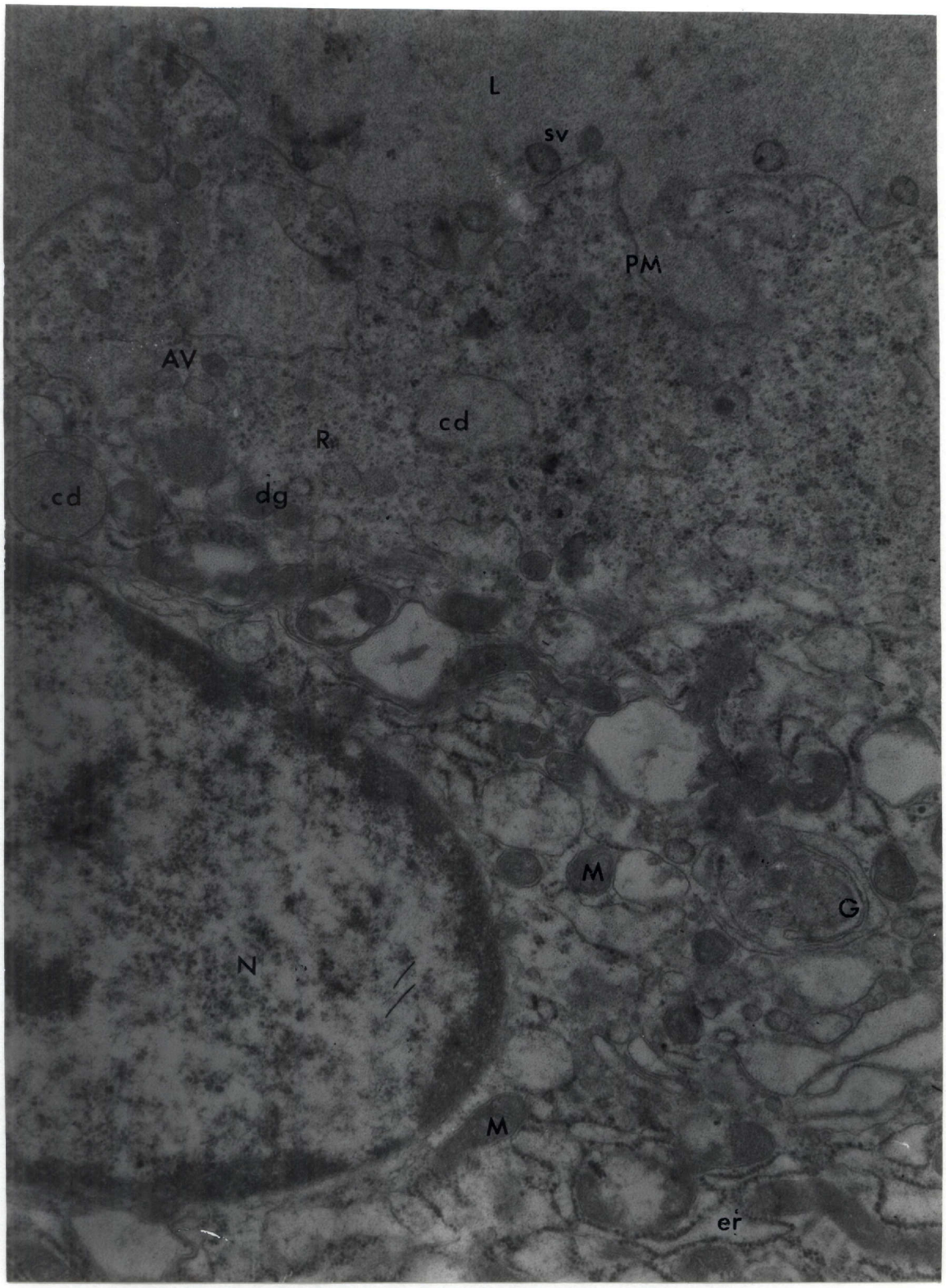
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Figure 5 (11,500X): High magnification of luminal border of control follicular cell. Note numerous secretory vesicles breaking off of plasma membrane into the lumen. The mitochondrial cristae are easily visible. N= nucleus; M= mitochondria; G= Golgi bodies; SV= secretory vesicles: CD= colloid droplets; AV= apical vesicles; DG= dense granules; L= lumen.

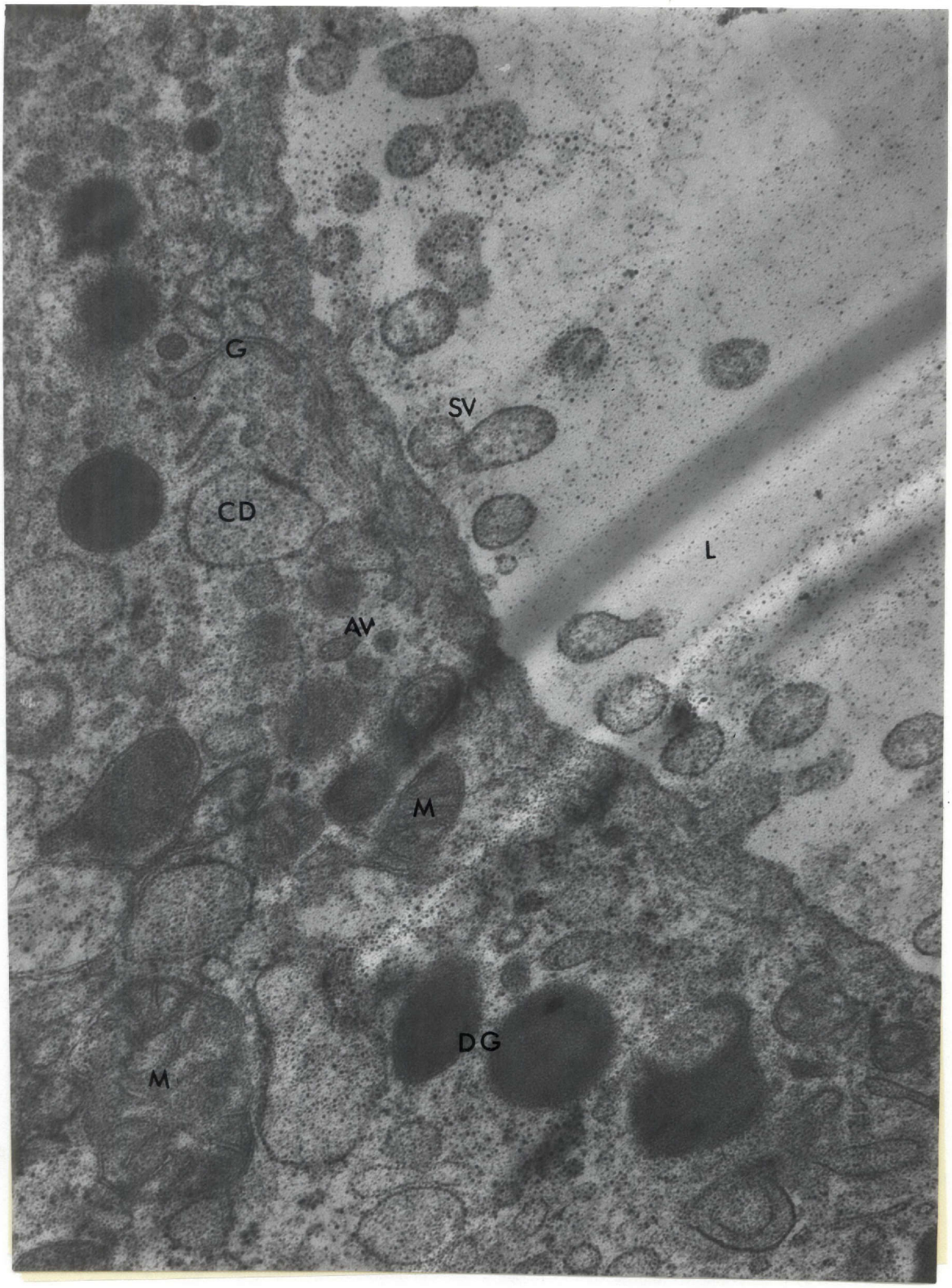
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Figure 6 (32,200X): Very high magnification of plasma membrane-luminal border of a control follicular cell. Secretory vesicles breaking off from plasma membrane into luminal border (see arrow). Apical vesicles and a dense granule are visible. DG= dense granule; AV= apical vesicle; SV= secretory vesicle; L= lumen; PM= plasma membrane.

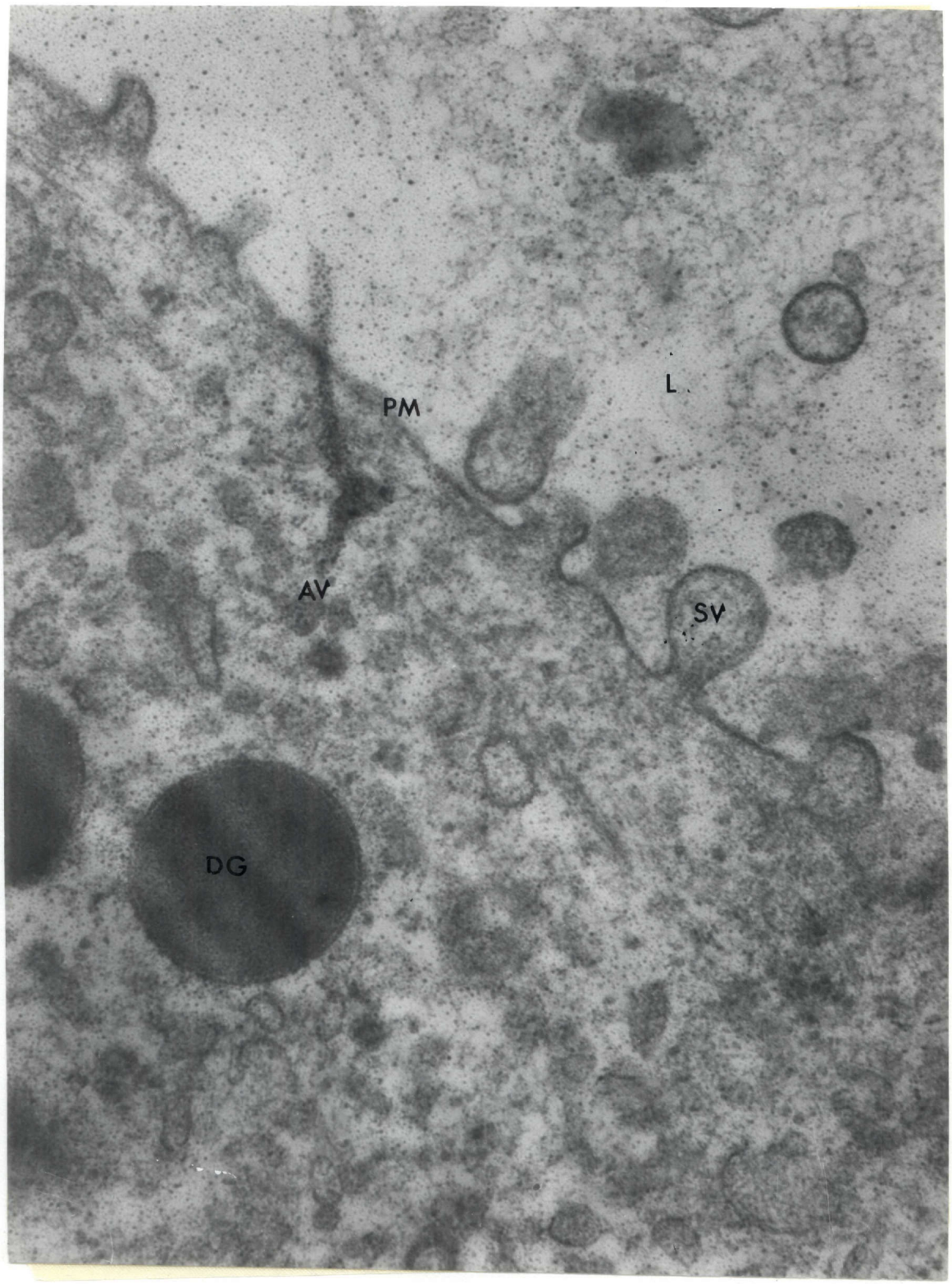
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Figure 7 (11,500X): Very prominant dense granules can be seen in this control follicle cell. Large colloid droplets are present with the same density as the luminal material. Secretory vesicles can be seen breaking off into follicular lumen. Two large multivesicular bodies are visible. SV= secretory vesicle; AV= apical vesicle; CD= colloid droplet; DG= dense granule; M= mitochondria; N= nucleus; MVB= multi-vesicular body.

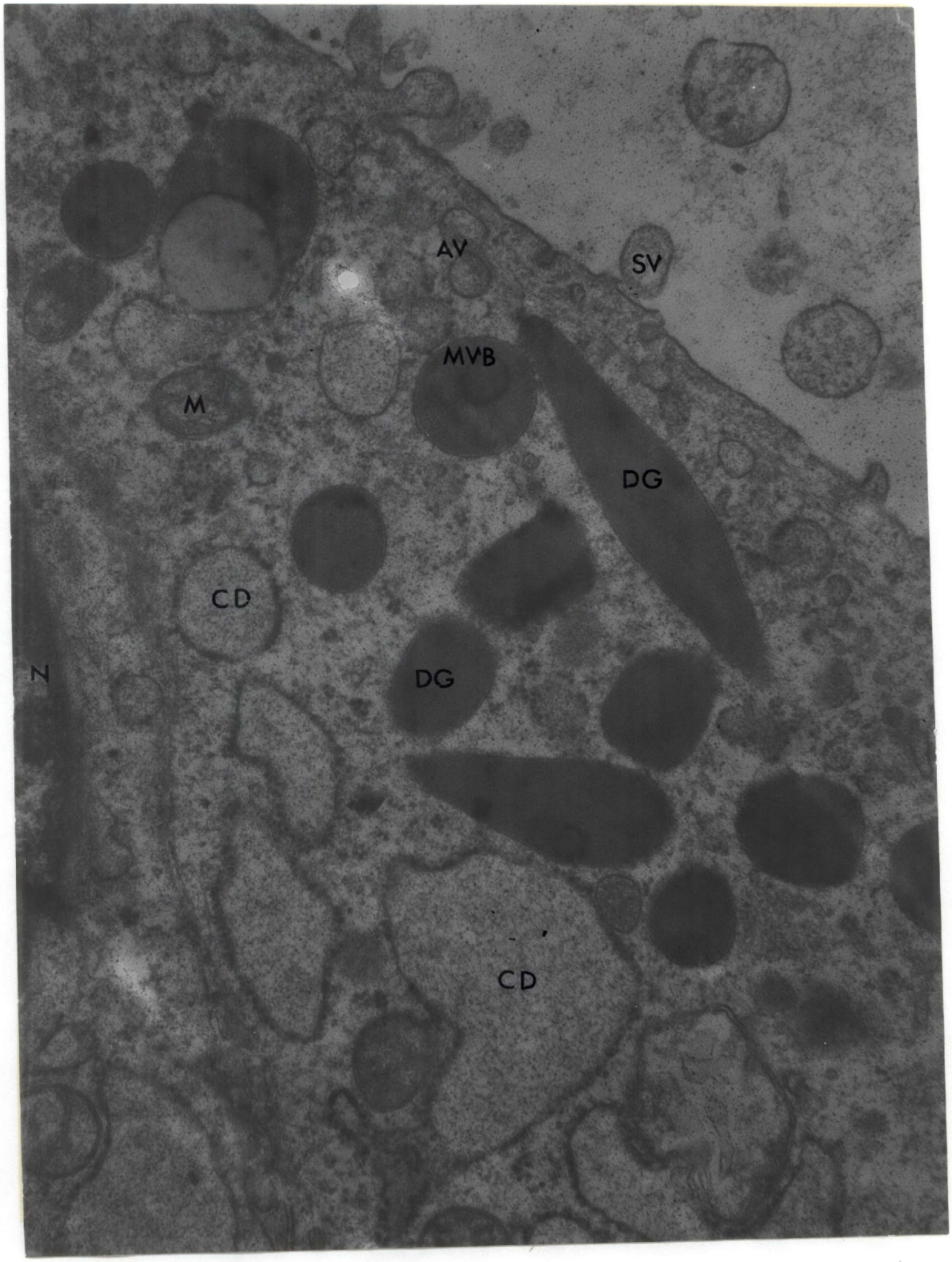
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Figure 8 (4250X): Low magnification of two control follicular cells with one parafollicular cell present around a blood filled perifollicular capillary. The thin endothelial lining of the capillary is visible. The characteristic very dense secretory granules in the parafollicular cell are easy identification of cell type. F= follicular cell; PC= parafollicular cell; CAP= perifollicular capillary; PM= plasma membrane; BM= basal membrane; N= nucleus; G= Golgi bodies; SV= secretory vesicles; CD= colloid droplet; AV= apical vesicle; DG= dense granule; SG= secretory granule.

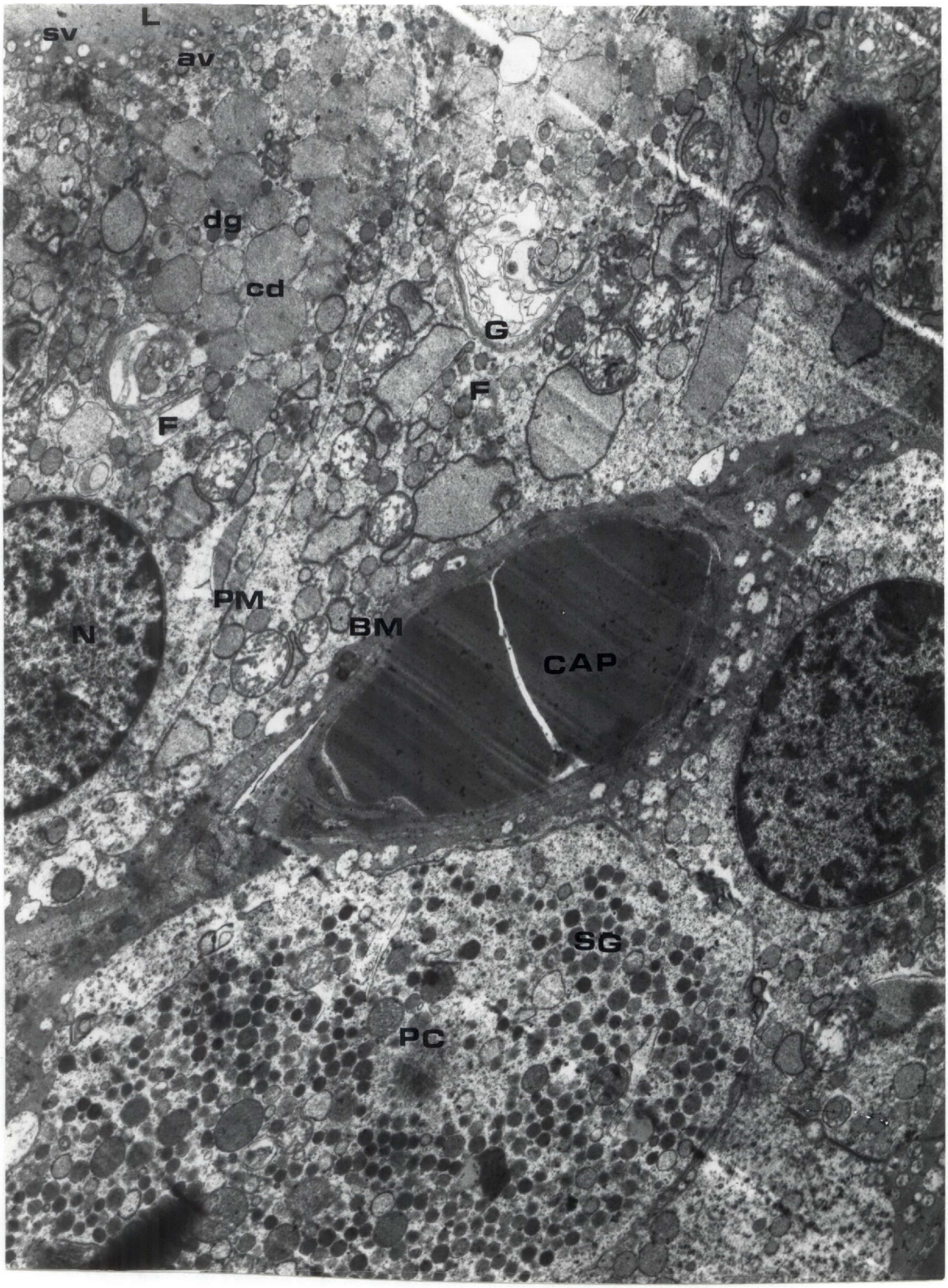
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Figure 9 (2500X): Overview of three hypothermic follicles and a central perifollicular capillary. Note the extensive amounts of microvilli present on the luminal borders of all follicles indicating extreme stimulation of celluar activity and secretion. L= lumen; CAP= perifollicular capillary.

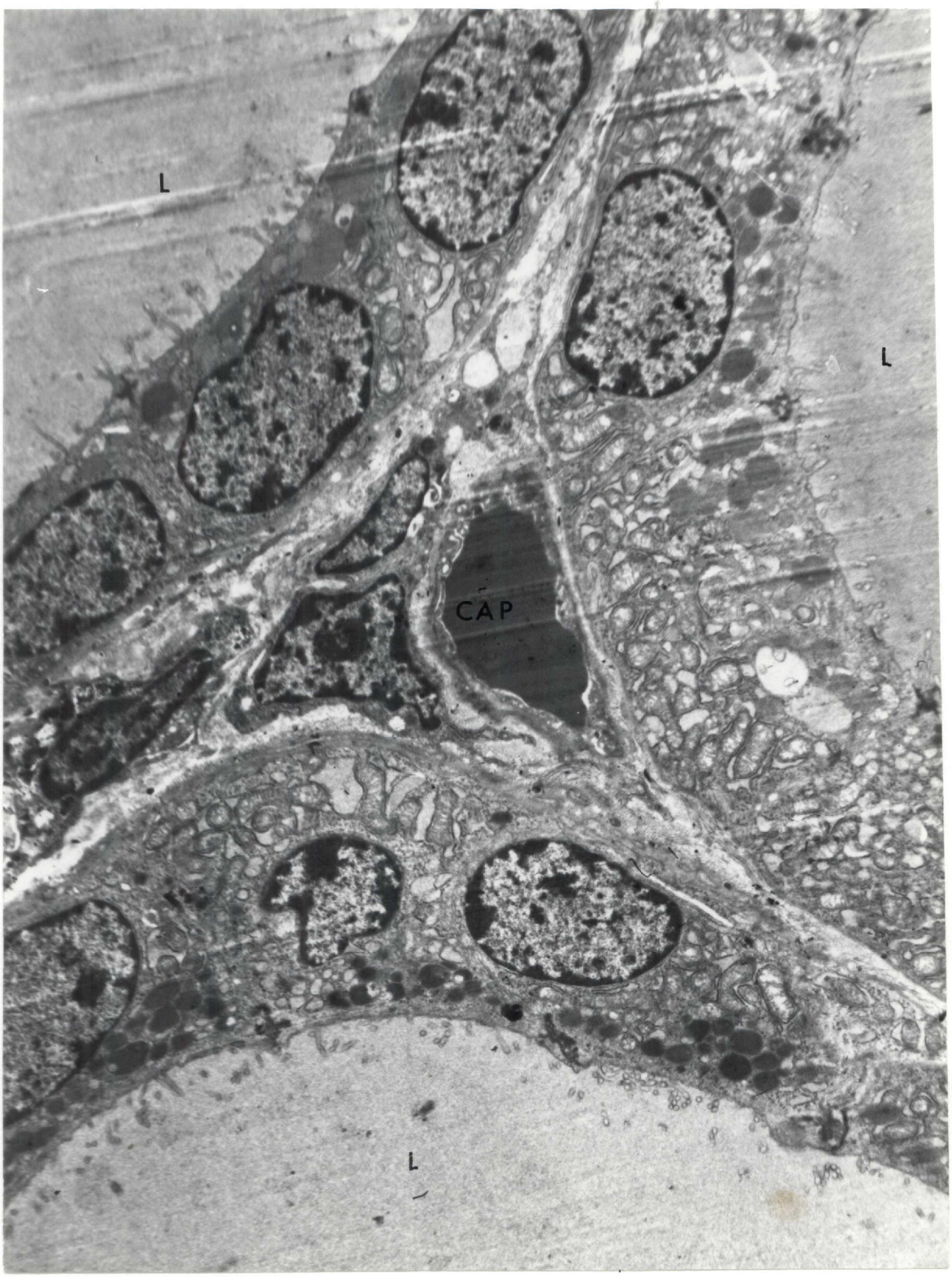
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Figure 10 (5500X): The edge of two hypothermic follicles and the perifollicular capillary inbetween. Numerous microvilli are present on the luminal border. Several large dense granules are present along apical area of the cell. There are few colloid droplets present--mostly dense granules. N= nucleus; CAP= perifollicular capillary; BM= basement membrane; PM= plasma membrane; ER= ergastoplasm; DG= dense granules; CD= colloid deoplets; MVB= multi-vesicular body; MV= microvilli; TB= terminal bar.

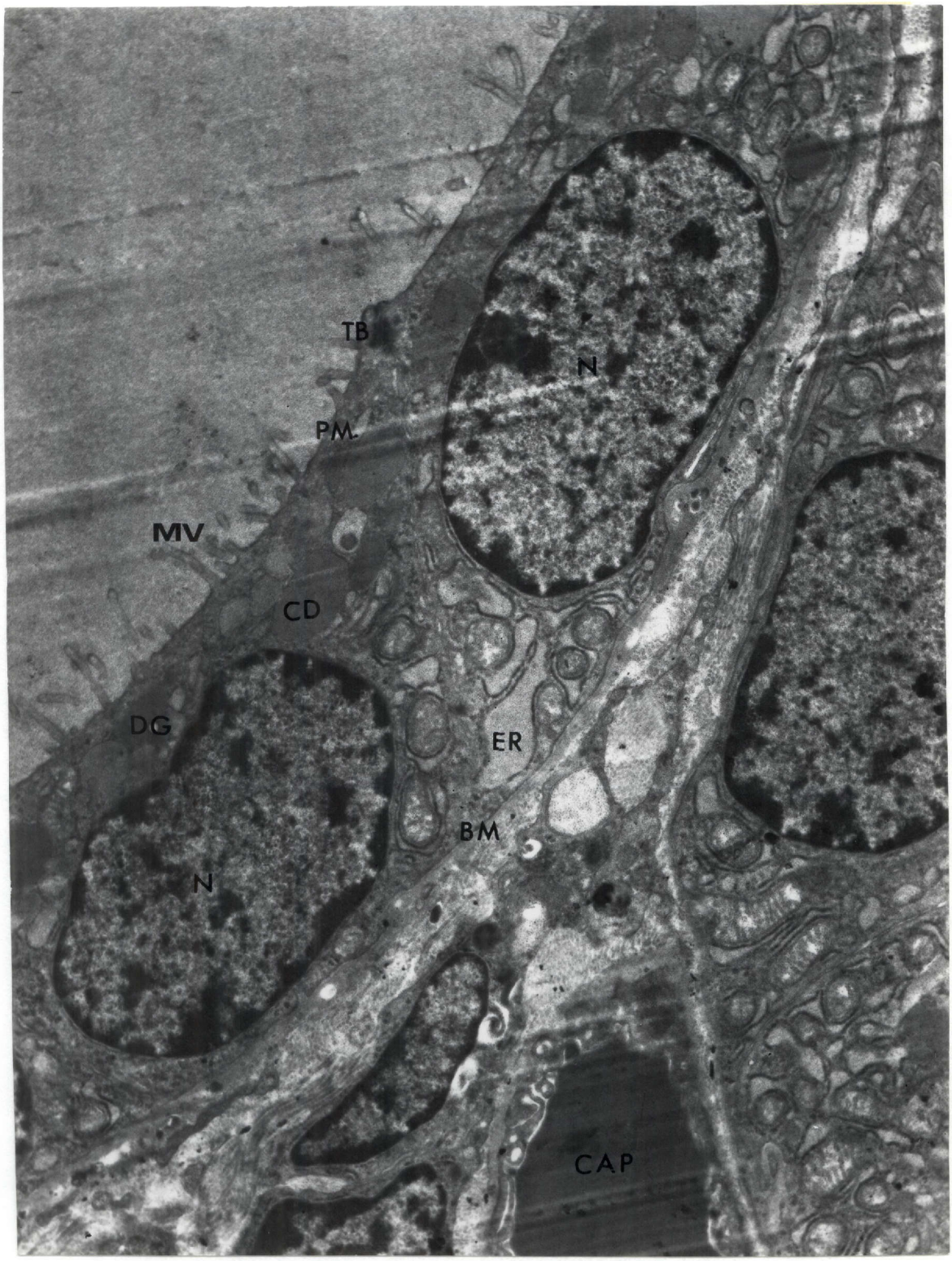
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Figure 11 (7700X): Higher magnification of hypothermic follicle cell. The polarity of the dense granules is shown also. Many microvilli protrude into the lumen. The dense granules vary in degrees of density indicating a possible migration from cytoplasm to lumen and back into the cell. L= lumen; TB= terminal bar; N= nucleus; MV= microvilli; M= mitochondria; G= Golgi bodies; ER= ergastoplasm; DG= dense granules; AV= apical vesicles; BM= basal membrane; PM= plasma membrane.

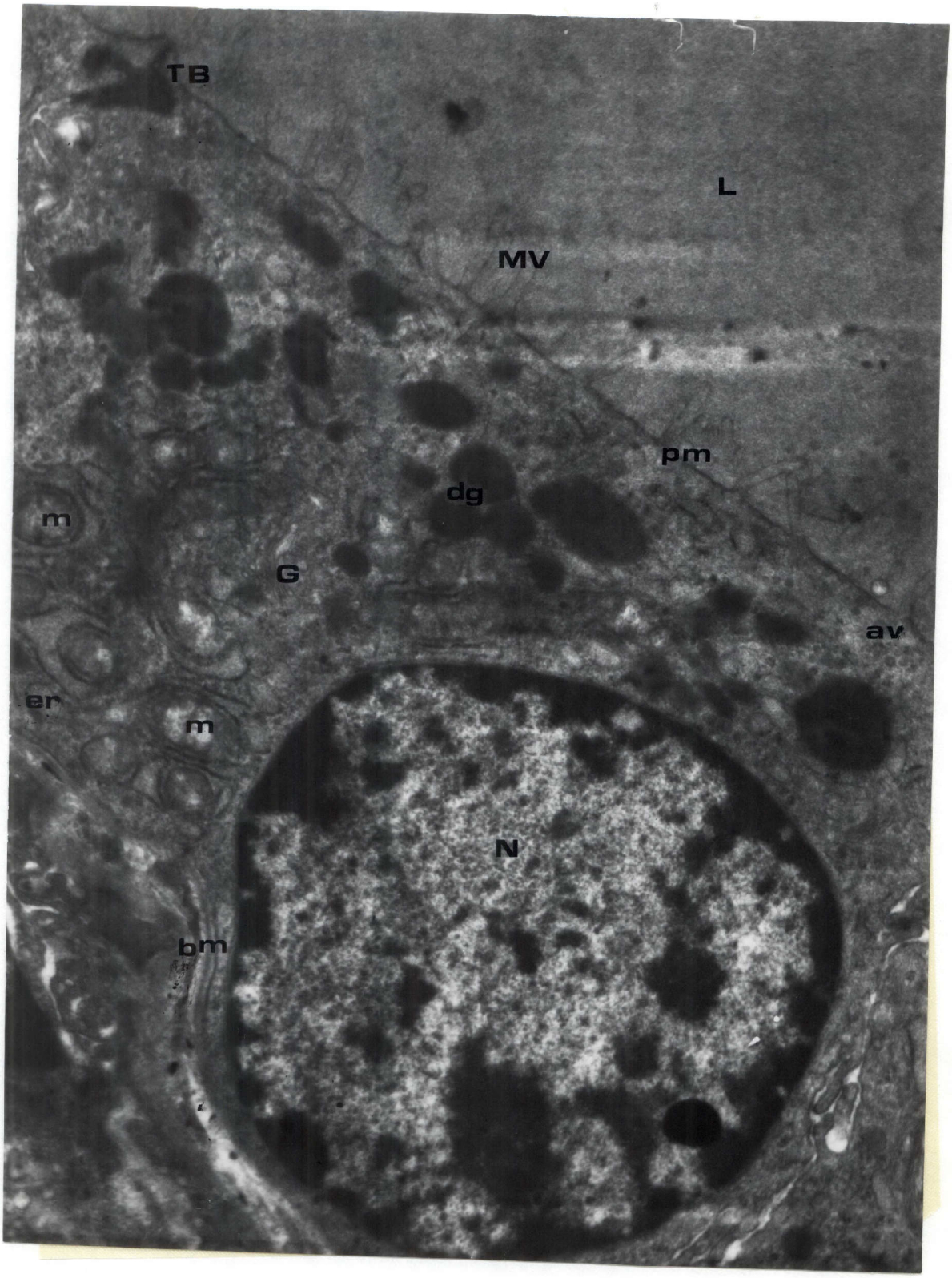
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Figure 12 (5500X): Two hypothermic follicle cells. Numerous microvilli extend into the lumen indicative of increased celluar activity and secretion. The numerous dense granules seem to orient themselves towards the apical area of the cells. Numerous mitochondria are also present. N= nucleus; BM= basal membrane; PM= plasma membrane; CAP= perifollicular capillary; MV= microvilli; DG= dense granules; M= mitochondria; CD= colloid droplets.


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Figure 13 (11,500X): Higher magnification of a few microvilli as produced by hypothermia. The edge of the nucleus can be seen. The microvilli are extentions of the plasma membrane and contain cytoplasmic material. MV= microvilli; L= lumen; PM= plasma membrane; N= nucleus.

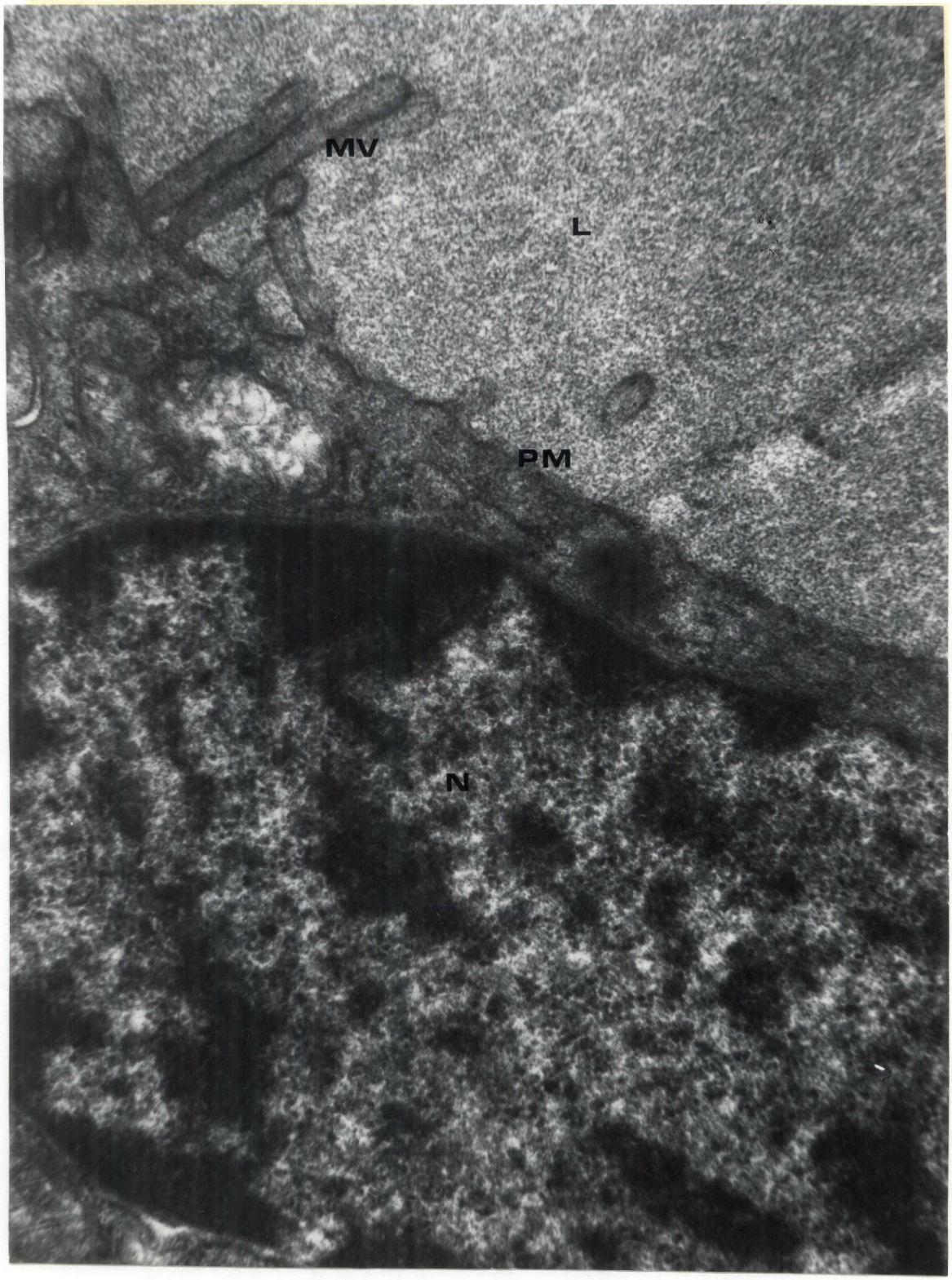
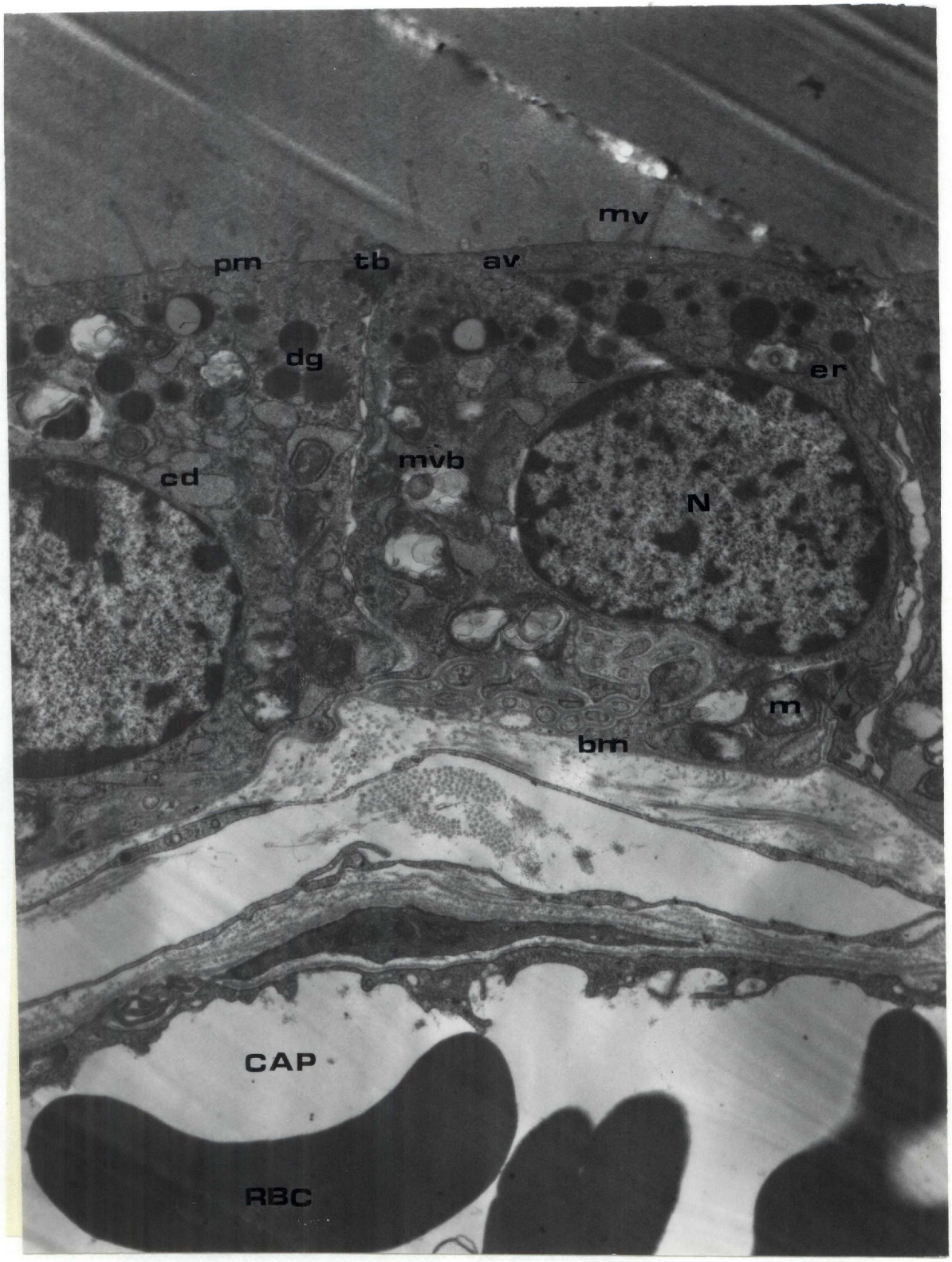

MV
L
PM
N

Figure 14 (5500X): Overview of a hypothermic follicle. The lumen as well as the interstitial tissue can be seen. Note the excellent view of a perifollicular capillary with the thin fenestrated endothelium and red blood cells. N= nucleus; M= mitochondria; ER= ergastoplasm; MV= microvilli; BM= basal membrane; PM= plasma membrane; G= Golgi bodies; CD= colloid droplets; DG= dense granules; AV= apical vesicles; MVB= multi-vesicular body; TB= terminal bar; CAP= perifollicular capillary; RBC= red blood cell.


mv
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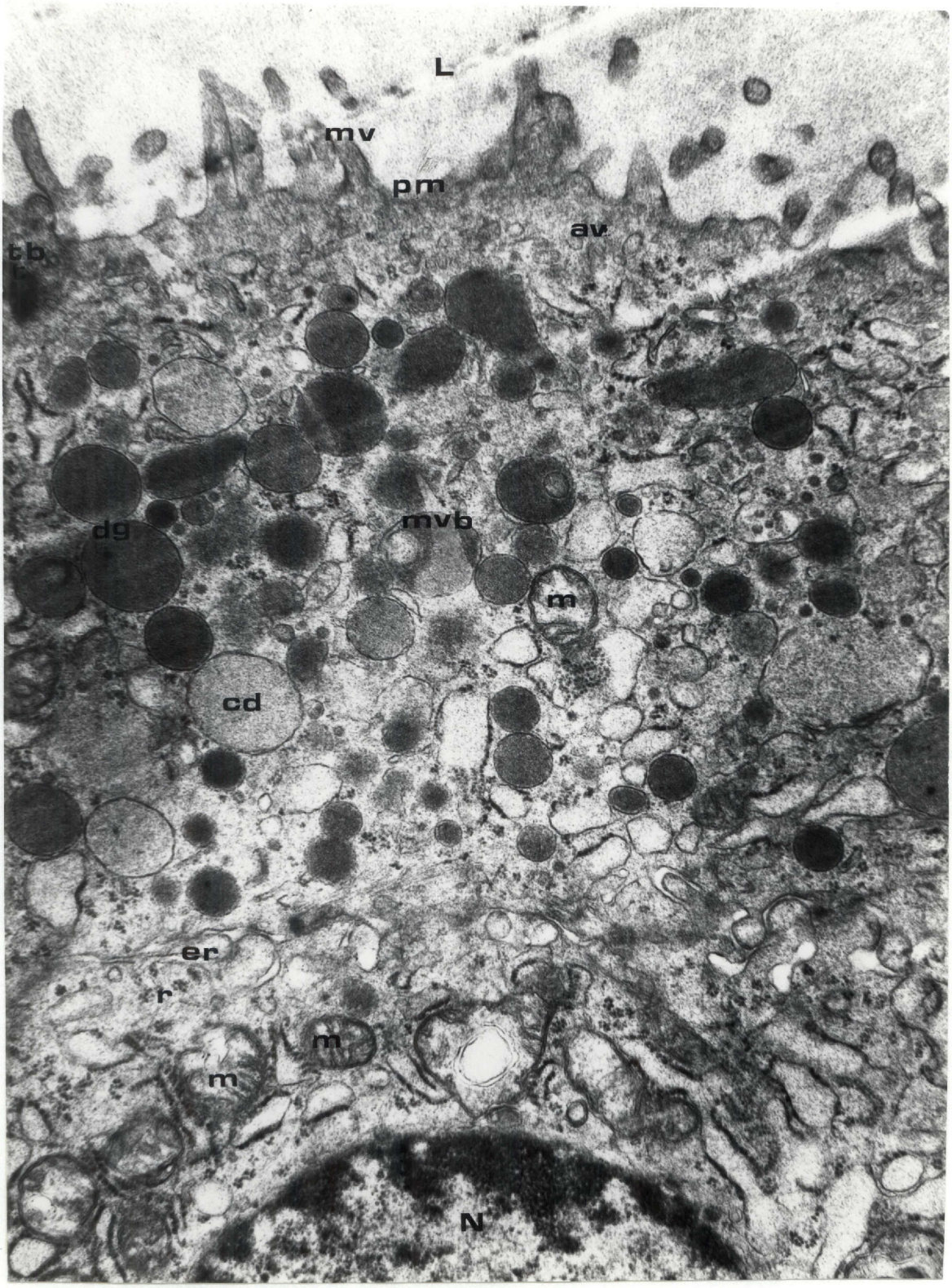
m
bm
CAP
RBC

Figure 15 (11,500X): Luminal border of one TSH stimulated follicular cell. Note large number of microvilli on luminal border protruding into the colloid in the lumen. M= mitochondria; N= nucleus; ER= ergastoplasm; R= free ribosomes; SV= secretory vesicles; MV= microvilli; PM= plasma membrane; CD= colloid droplet; AV= apical vesicles; DG= dense granules; MVB= multi-vesicular vody; TB= terminal bars; L= lumen.


L
mv
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r
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m
N

Figure 16 (22,800X): Enlarged view of luminal border of TSH stimulated follicular cell. Note slight variety in colloid droplet density indicative of migration of the droplets. M= mitochondria; AV= apical vesicles; R= free ribosomes; DG= dense granules; CD= colloid droplets; MVB= multi-vesicular body; PM= plasma membrane; L= lumen.

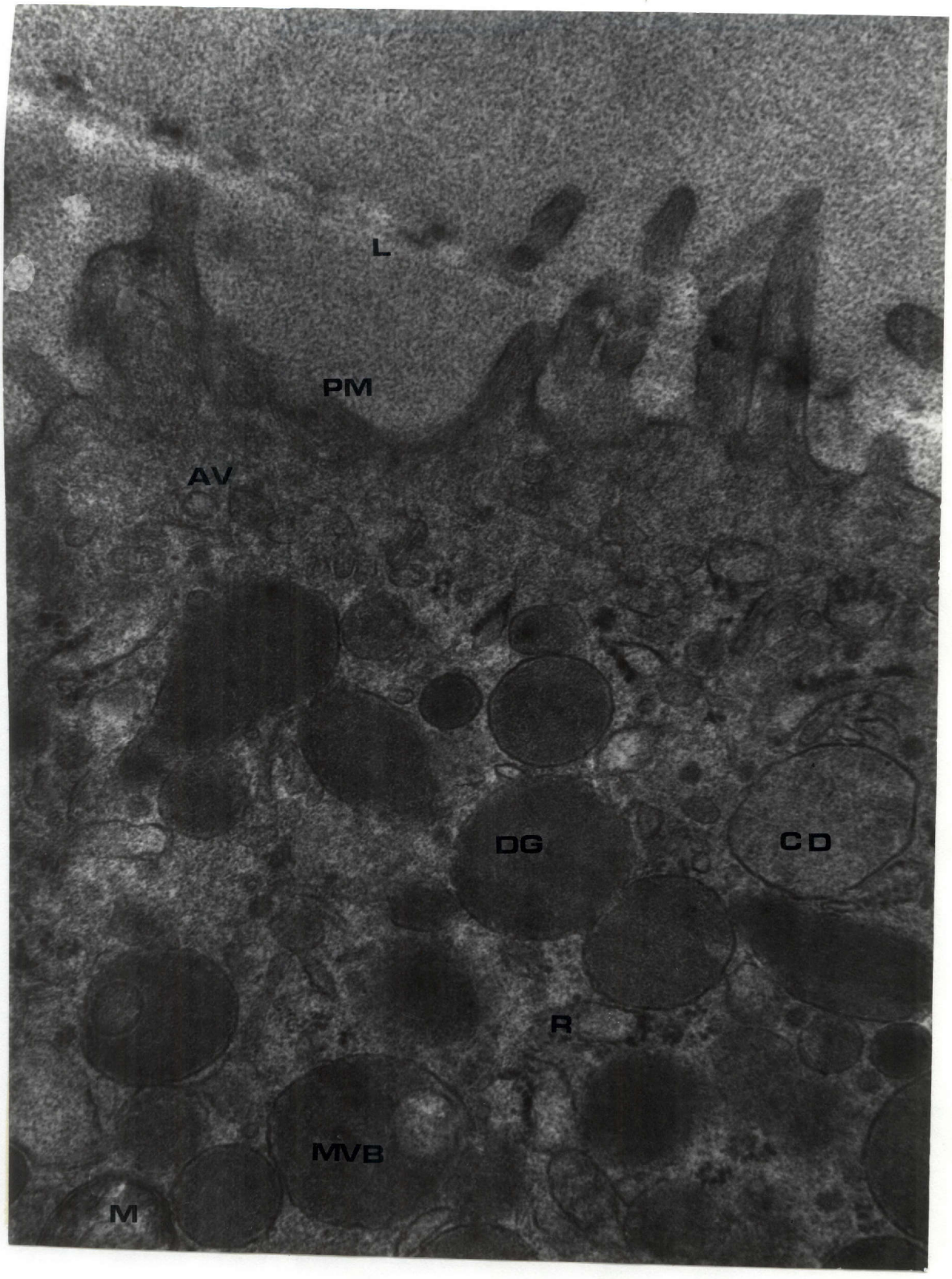
L
PM
AV
DG
CD
R
MVB
M

Figure 17 (7700X): Follicular cell after TSH stimulation. Note dark dense granules present throughout the cell possibly in migration through follicle cell to interstitium for glandular export. Also in lower cell there are ergastoplasmic saccules very full of a colloid-like material. The follicular lumen can not be seen but is located towards the top of the picture. M= mitochondria; N= nucleus; ER= ergastoplasm; R= free ribosomes; PM= plasma membrane; CD= colloid droplets; DG= dense granules.

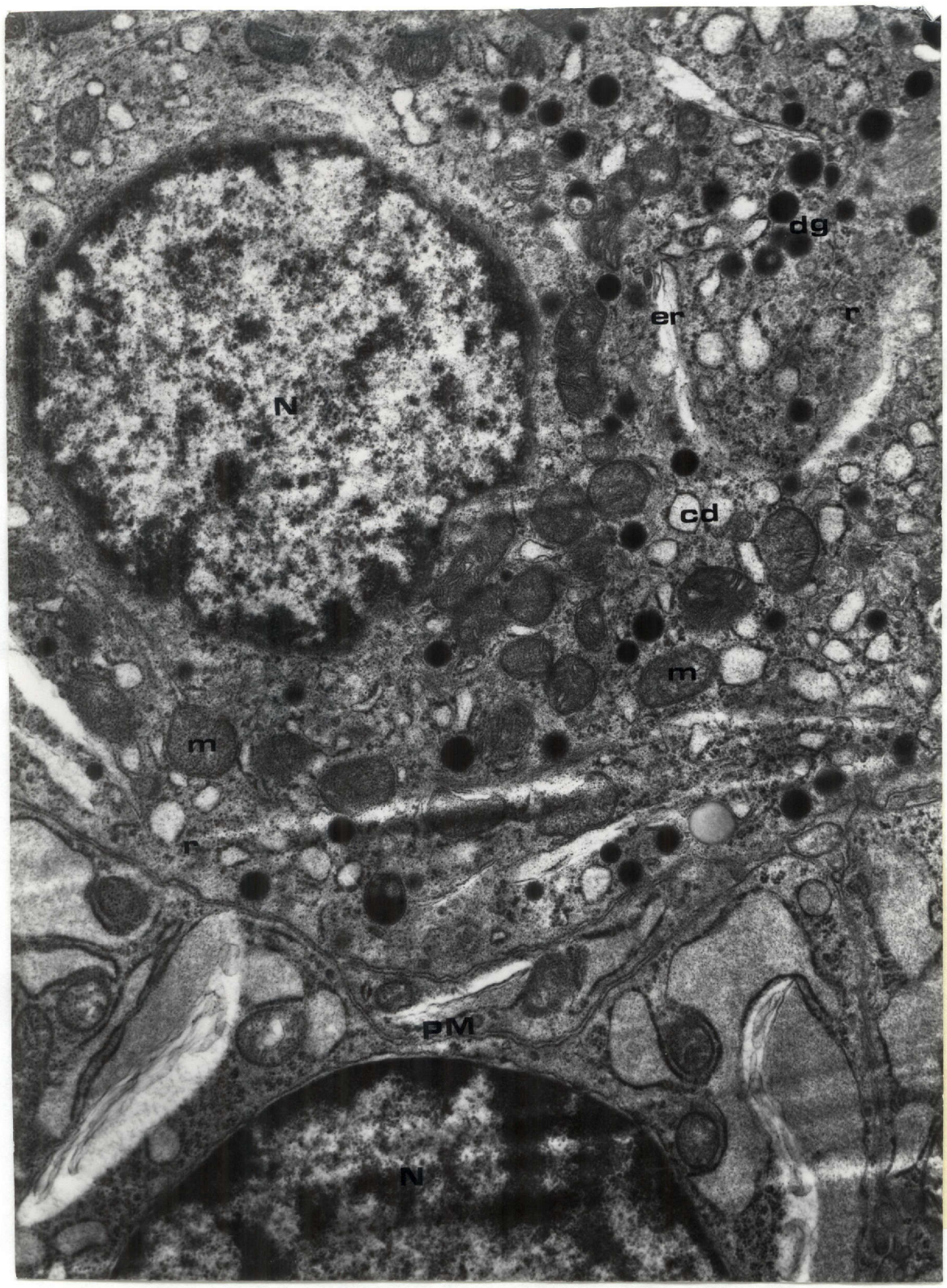
dg
er
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Figure 18 (2500X): The relationship between follicular and parafollicular cells of a TSH stimulated gland can be seen. Note the presence of dark dense granule-like structures in the interstitium of the follicle near the perifollicular capillary (see arrows). F= follicular cell; PC= parafollicular cell; CAP= perifollicular capillary.

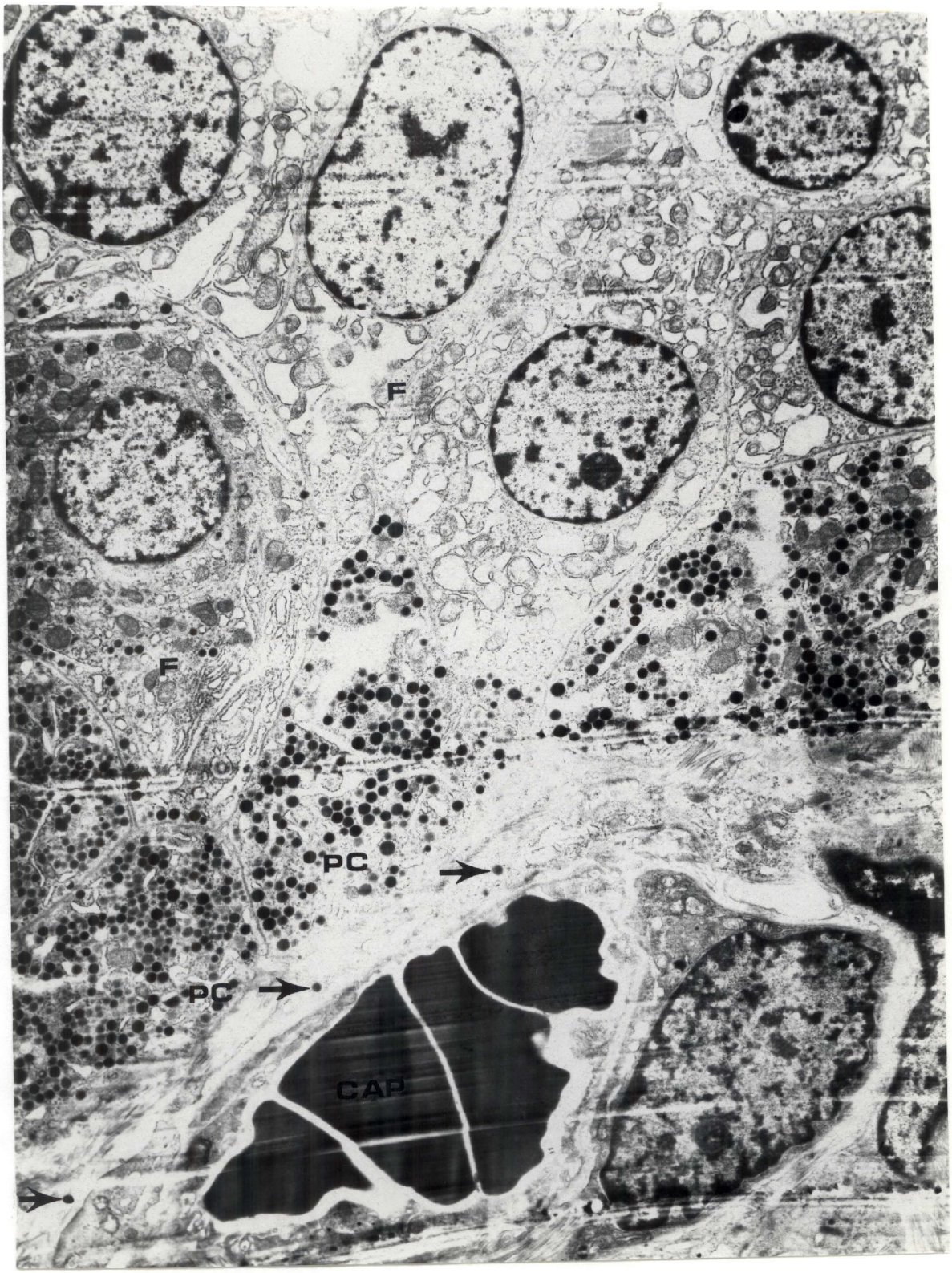

F
F
PC
PC
CAP

Figure 19 (4250X): Enlargement of same section of TSH stimulated follicle as Figure 19. Note the dark dense granule-like structures present in the interstitium near perifollicular capillary (see arrows). F= follicular cell; PC= parafollicular cell; CAP= perifollicular capillary.

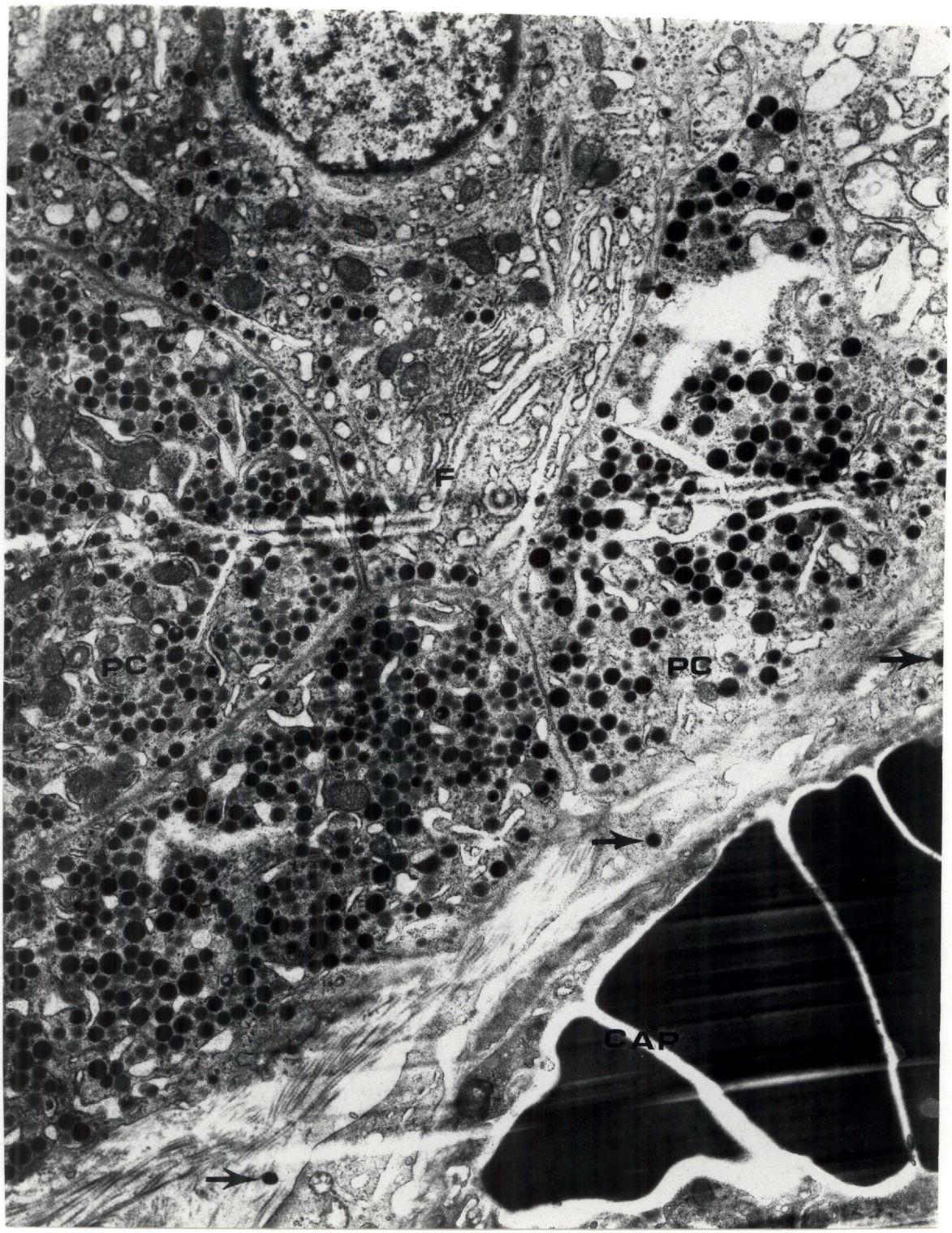
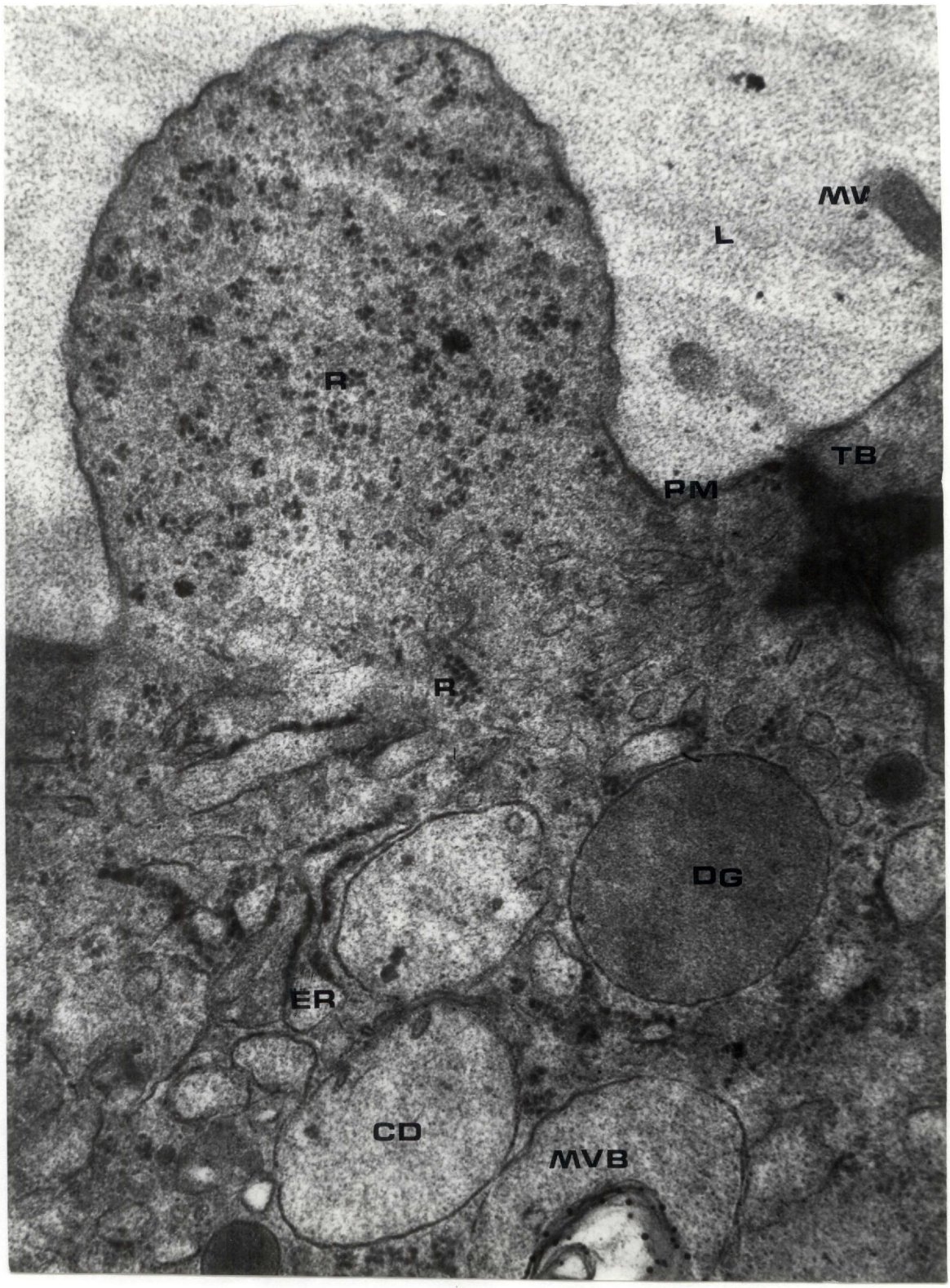

F
PC
PC
CAP

Figure 20 (22,800X): High magnification of TSH stimulated follicular lumen border. The celluar process called zeiosis can be seen with a knob or bleb developing on the luminal border and indicating extreme celluar activity. The bleb of knob developing contains cytoplasm with many free ribosomes. R= free ribosomes; PM= plasma membrane; MV= microvilli; TB= terminal bar; DG= dense granule; CD= colloid droplet; MVB= multi-vesicular body; ER= ergastoplasm; L= luman.


MV
L
R
TB
PM
R
DG
ER
CD
MVB

# CHAPTER IV

# CONCLUSIONS

## Follicular Cell Ultrastructure

The thyroid follicle is a ring shaped structure with the follicular cells lying in a single layer surrounding a central colloid containing lumen. Occasional parafollicular cells are inserted among the follicular cells at irregular intervals. Figure 1, an electron microscope section of a control normothermic gland, illustrates the overall relationship of the cells in part of a follicle. Several follicular cells which can be seen with an interspaced parafollicular cell, which was never observed to touch the colloid lumen. This agrees with the observations of Ekholm and Ericson(2).The shape of the parafollicular cell also follows the generally accepted polyhedral shape with its contents always within the follicles' basement membrane[2]. Parafollicular cells were also observed to be present in an interstitial area.

The celluar constituents of a normothermic gland are shown at a higher magnification in Figure 2. The normal cellular organelles of one follicle cell are labeled. The nucleus, which cannot be seen in this section, was normally spherical. The mitochondria were randomly

distributed throughout the cell and the cristae were usually visible (Fig. 2,5). The structure of the double limiting membrane and internal cristae, which are formed by infoldings of the inner membrane, was also seen by Pitt-Rivers and Trotter(7). The plasma membrane was easily visible with numerous secretory vesicles breaking away from the follicular cells and entering the lumen. In Figure 6, a higher magnification of this membrane phenomenon can be seen. The plasma membrane appears to swell, pinch off and release these secretory vesicles into the colloid lumen. How and when these vesicles are lysed or broken down in the lumen is unknown at this time and could not be determined in this study.

The basal membrane is next to the interstitial area of the follicle. Large colloid droplets (Fig. 2,7,8) are seen containing a material of the same density, or nearly so, to that of the colloid lumen. This similarity is what Wissig(9) concluded when he described these smooth-surfaced, membrane-limited structures. The colloid droplets are due to a pinocytotic mechanism which engulfs small portions of the colloid material. These colloid droplets are then thought to be broken down in the cytoplasm and its thyroxine released into circulation. Wissig did not indicate whether the dense material in the colloid droplets was iodinated thyroid hormones. The prominant ergastoplasm can be seen readily and is distributed throughout the cell (Fig. 2,3,4). The density

of the ergastoplasm is very light or approaches the density of the colloid. Pitt-Rivers and Trotter(7) suggested this density similarity was due to the fact that the vesicles of the ergastoplasm are filled with a substance which would eventually be incorporated into the follicular colloid. The density of these vesicles was always observed to be equal to or less than the colloid lumen. Colloid droplets can be smaller and in the apical area of the follicle cell, where they are termed "apical vesicles". Young and Leblond(10) reported similar results and interpreted the apical vesicles as pinocytotic. They also stated that the apical vesicles might contain a material which was a precursor to colloid material but this has not been proven. These small vesicles are described to be the same as the colloid droplets, but in a different area of the follicular cell (Fig. 2-7).

Dense granules can also be seen in follicle cells (Figs. 3,4,5,7). These vesicles are very electron dense, are present in the middle to apical area of a follicle cell, and are usually spherical in shape. Multi-vesicular bodies can occasionally be seen in follicle cells (Figs. 2,3,7). These are vesicles containing one or more other vesicles within the common outer membrane. Their density varies, as can be seen in the electron micrographs, and depends on the density of the vesicles enclosed. A vacuole is occasionally seen (Figs. 2,3). They have little or no electron density and are thought to function in the classical

definition--a storage vessicle. However, not much is known of their function in thyroid follicle cells (8).

Free-floating ribosomes and rough ergastoplasm are easily seen in Figure 3. There was an abundance of free ribosomes throughout all follicular and parafollicular cells. Pitt-Rivers and Trotter (7) pointed out that fingerlike projections called microvilli can be present in follicular cells and protrude into the colloid lumen. Figure 3 illustrates a few of these microvilli present in a control, normothermia animal.

Figure 8 is another overview showing several follicular cells and a parafollicular cell, with its characteristic dense granules around a perifollicular capillary. These perifollicular capillaries are of the usual structure like those in the kidney, intestine, and other endocrine tissues; i.e. a single layer of epithelial cells which may be extremly thin and fenestrated in certain areas (7).

## Parafollicular Cell Ultrastructure

The most characteristic organelle of a parafollicular cell is illustrated in Figure 8. These secretory granules were always the most prominant and easily identifiable organelle in a parafollicular cell (2), as compared to a follicular cell which only had the large, dark, electron-dense granules. The nucleus (Which cannot be seen in Figure 8) was not remarkabley different from the follicular cell nucleus. The randomly distributed mitochondria in parafollicular cells

vary in shape and size compared to follicular cells. The ergastoplasm was generally narrower with elongated cisternae as was reported by Ekholm (2) in his study. Any dense granules which were present in parafollicular cells, although few in number, were not easily seen due to the preponderance of the secretory granules. The parafollicular cell ultrastructure was generally observed to be similar to the follicular cell with previously mentioned exceptions.

## Hypothermia Ultrastructure

Under hypothermic conditions, there were remarkable changes in the thyroid follicular ultrastructure. Figure 9 illustrates the presence of extensive numbers of microvilli on the luminal border: one of the most striking changes present in the follicle cells.

Figures 10-13 presents enlargements of follicular cells. The microvilli protrude further into the lumen at certain areas over other areas of the luminal border. Dense granules, colloid droplets and occasionally a multi-vesicular body can be seen. A polarity is illustrated in Figure 11. The droplets aggregated in the luminal half of the cell near the microvilli. Young and Leblond (10) also observed this celluar polarity in their studies. There are two follicle cells in Figure 12 with the polar dense granules and a capillary in the interstitium. The dense granules were never observed to be present

in the outer portion of the follicle cells adjacent to the interstitium and blood capillaries. The relatively large microvilli can also be seen in the figure.

Figure 13 is a high magnification of a few microvilli present in hypothermic tissue. They contain material of the same approximate density and composition as the cytoplasm. This would appear to indicate that the microvilli are outreachings of the plasma membrane which are utilized to bring material into or away from the cells. This study was not able to ascertain whether the movement was in or out of the follicular cells.

Turakulov (8) believed that the thyroglobulin was synthesized in the cytoplasm and moved toward the cell lumen in a constant cycle. This can be seen in Figures 11 and 12 with varying density granules present throughout the cytoplasm. Gross et. al.(4) confirmed that the iodination of the thyroglobulin occurred in the follicle colloid and the iodinated thyroglobulin was then taken back into the cell by pinocytotic mechanisms. Since, Dempsey and Astwood (1) concluded the rate of thyroid hormone secretion approximately doubles when exposed to cold, this correlated with the significant increase in dense granules present in the hypothermic thyroid tissues as seen in Figures 9-12 and 14. The electron micrographs (Figures 9-14) show a great increase in dense colloid droplets as well as extensive microvilli formation indicative of increased celluar hormone production and overall increased

celluar activity. There was an obvious significant increase in the hypothermic tissue's microvilli over the normothermia tissue, as seen in all the electron micrographs (Figures 9-14). The colloid droplets and the dense granules both seem to show a definite polarity toward the follicular lumen and were almost exclusively located in the middle to luminal part of the follicular cells. The microvilli, colloid droplets and dense granules are indicators of extreme activity in the cell. Haenke, in 1977(5) found a significant increase in $T_3$ and $T_4$ blood levels in the isolated hypothermic thyroid gland. This electron microscopic study of ultrastructural changes indicate an increase in activity following hypothermia, which supports Haenke's findings. There was no attempt to determine the iodine content of the dense granules. However, since iodine is extremly electron dense, based on the electron micrographs in this study, it could logically be assumed, that the dense granules were most probably iodinated thyroglobulin back in the cell after its iodination in the colloid lumen.

In summary, three definite changes were observed in hypothermic thyroid cells when compared to normothermic: 1) there was a vast increase in dense granules under hypothermia; 2) there was a definite polarity developing between the granules in the follicular cells and the lumen; 3) there was extensive formation of microvilli indicative of a great increase in celluar activity and secretion by the follicular

cells. Some of these findings are unique and some are corroborative to previous researchers' findings concerning the differences between the thyroid follicular ultrastructure under normal and hypothermic temperatures. All three of these phenomena can be seen in Figure 14 which is an overview of two follicle cells, the colloid lumen and a perifollicular capillary with its endothelial border.

## TSH Stimulation

The three consistant findings in thyroid hypothermia ultrastructure were indicative of increased celluar activity and secretory rate. These observations led to an assumption that, if this conclusion was correct, Thyroid Stimulating Hormone (TSH) should produce the same ultrastructural changes.

Figures 15 and 16 are sections through TSH treated glands showing the luminal border of one follicular cell under different magnifications. There are a great number of microvilli protruding into the lumen. The dark dense granules can be seen concentrating in the portion of the cell closest to the lumen. Again, the varying density of the dense granules is indicative of some form of synthesis and movement away from the lumen(8). Their number is greatly increased over normothermia follicular cells. These three observations were seen in all hypothermic tissue electron micrographs. Figure 17 also shows one follicular cell with numerous dense granules distributed within the cell, but polarity of these granules has not developed. One possible

explaination would be the movement of the dark granules towards the cell interstitium for glandular export. Also present in one cell are large ergastoplasmic saccules full of a material which appears similar to, if not the same as, the colloid in the lumen.

Figure 18 and 19 show the relationship between follicular and parafollicular cells in a TSH stimulated gland. The characteristic secretory granules can be seen in these two electron micrographs. The presence of several dark secretory granules or dense granule-like structures in the interstitium near the perifollicular capillary can be seen. This may be a very important observation since it is not known when or how the thyroglobulin is exported or broken down. These dark secretory granules seem to be outside the follicle and possible on the way to the perifollicular capillary. The granules are intact and have not yet been broken down. Since the animal was stimulated by TSH, there is definitely increased activity produced as was the case with the hypothermic stimulation. This is evident by the increased number of dense granules and secretory granules present in the cells (Figures 15-19).

Figure 20 exhibits a celluar phenomen, zeiosis, indicatave of greatly increased activity. The term, zeiosis, was explained by Godman et.al (3).In normal untreated or stimulated cells, the microfilament web of the cortex is more or less continuous and serves as a barrier between the cytoplasm and the inner

face of the plasma membrane. Under certain conditions, increased celluar activity for example, the microfilaments become compacted into accumulations in the cortex. This discontinuity allows endoplasm to flow up the unprotected inner part of the membrane. These plugs or capsules can then protrude into early zeiotic knobs or blebs. The large zeiotic knobs and blebs may contain an expulsion of ergastoplasm, mitochondria, rough ergastoplasm, lysosomes and even nuclei. In Figure 20, however, only cytoplasm and free ribosomes were contained in the zeiotic knob. Godman stated that the protrusion of zeiotic knobs or blebs was temperature dependent. He found that at slightly decreased temperatures there was an overall increase in cells with zeiotic clusters. At much lower temperatures (approximately 25°C.) the process was slowed down much more than at normal cellular temperatures or temperatures slightly lower than normal body temperature. Jones et. al. (6) found celluar zeiosis or blebing to be present, caused by increased celluar hydrostatic pressure. They stated that the membrane covering, or excess surface membrane of celluar blebs or zeiotic knobs, was probably derived from the microvilli present. This is in partial disagreement with Godman et. al. (3) who made no mention of a microvilli-zeiotic knob relationship.

Two studies (3,6) have mentioned that the zeiotic process is indicative of extreme cellular activity and is temperature

dependent. No zeiosis was seen in the hypothermia-stimulated tissue, only the TSH stimulated tissue. One speculation for this descrepency is the possibility of greater stimulation by the TSH than hypothermia.

In comparing the hypothermia-stimulated thyroid follicular cells and the TSH-stimulated follicular cells, both situations showed similar changes. They both exhibited the increase in dense granule formation, the polarity developing between dense granules and the lumen, and the great preponderance of microvilli present. Since TSH stimulated thyroid cells to secrete their hormones, and the ultrastructural changes observed with TSH and hypothermia were the same, one must assume that hypothermia, in the temperature levels studied, must also stimulate the cells to secrete. This conclusion is in perfect agreement with the findings of Haenke(5) who reported an increase in $T_3$ and $T_4$ release from the thyroid during isolated hypothermic perfusion.

## Future Studies

The object of this study was to explore both the normal and hypothermia stimulated ultrastructure of thyroid cells. This investigation has shed some light on hypothermia and the thyroid, but there are several possibilities for future work to aid in further unraveling the mystery of the thyroid gland ultrastructure and function. Freeze fractioning and scanning electron microscopy studies could be undertaken to determine direction of movement.

Autoradiography, with labeled iodine in thyroid cells, is a logical step to back up this study's postulation of dense granules being iodinated prior to their abundant appearance under hypothermic and TSH stimulation. With radiolabeling, a scanning mechanism could be devised to scan the tissue slices to determine if the radioiodine is taken up in the dense granules. The development of a method to determine direction of movement of celluar inclusions would answer a lot of questions raised in the current study.

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