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EXPERIMENTAL THYROIDITIS IN THE RHESUS MONKEY

V. ELECTRON MICROSCOPIC INVESTIGATIONS

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SUMMARY

Electron microscopic observations of sections from thyroid glands obtained from Rhesus monkeys immunized with monkey thyroid suspensions incorporated in complete Freund's adjuvant are described. Severe changes in the target organ as well as the appearance of circulating autoantibodies testify to the successful production of thyroiditis by this procedure in the experimental animals. The principle morphological changes previously observed by light microscopy could be confirmed. By means of the electron microscope, further insight into the actual changes of the ultrastructure of the thyroid cells were obtained. Characteristic features of the ultrastructure alterations were the vacuolization of the endoplasmic reticulum and the increase of colloid droplets, with evident signs of confluence. Apparently by the confluence of colloid droplets, and further by the accumulation of material derived from the autolytic processes within the cells, masses consisting of a homogeneous fine granular substance are formed, filling large areas within many thyroid cells. In the early stage of cell alteration there can be found an increased number of dense bodies. They disappear in cells with severe damage. A necrotic process in the follicular epithelium occurs in connection with the most intense inflammatory infiltration. These inflammatory cells lie either outside or within the follicle. In the early stage of alteration they are between the follicular cells but with the increase of the damage these cells can also be found intracellularly. Only with severe damage of the follicular cells does the basement membrane seem to be disrupted.

INTRODUCTION

Experimental autoimmune thyroiditis can be produced in the Rhesus monkey by injection of homologous thyroid extract incorporated in complete Freund adjuvant (Kite, Argue &

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Rose, 1966; Doebbler & Rose, 1966; Rose *et al.*, 1966). The animals develop antibodies to thyroid constituents and histological lesions which are characterized by a reduction in the size of the thyroid follicles and by infiltration by inflammatory cells of different types (Terplan *et al.*, 1960). A main feature of this infiltration, which at first is focal and then may spread over the gland, is the accumulation of lymphocytes and plasma cells. Starting in the perivascular zone and invading the adjacent follicles, its intensity does not necessarily correlate with the antibody titre in the serum of the animals.

Relatively complete information has been published on the ultrastructure of the normal human thyroid (Heimann, 1966) as well as on the thyroid of some experimental animals in normal and pathological conditions (Demsey & Peterson, 1955; Ekholm & Sjostrand, 1957; Wissig, 1960, 1961, 1963; Ekholm, Zelander & Agrell, 1963; Seljelid, 1967a, b). In thyroiditis some changes in the homogeneity of the colloid (Terplan *et al.*, 1960) and of cytochemical properties of the thyroid cells were reported (Sobel & Geller, 1964). In addition, there are accumulations of cellular debris within follicles.

In order to understand the pathogenetic mechanism of these alterations, an electron microscopic study was carried out on experimental thyroiditis in monkeys. Attention is here focused on the alterations of the thyroid epithelium and its relationship with the infiltrating elements and the intrafollicular colloid.

MATERIALS AND METHODS

Production of experimental thyroiditis

Seven Rhesus monkeys (*Macaca mulatta*), three females and four males, of approximately 3 years of age, were immunized by injection of thyroid suspensions incorporated into complete Freund's adjuvant, following the technique used by Kite *et al.* (1966). Thyroid biopsies were performed under pentobarbital anaesthesia; the neck was incised in the midline; by careful dissection the connective tissue and muscles were separated and the thyroid gland was exposed on both sides of the trachea. After ligation of inferior thyroid artery and dissection of the isthmus from trachea, approximately one-half of a lobe was removed. Specimens were fixed in 10% formalin for 24 hr, embedded in paraffin and sections were stained with haematoxylin and eosin.

For electron microscopic study, thyroid tissue was fixed for 1½ hr in 5% purified glutaraldehyde with either s-colloidin buffer or 0·1 M-phosphate buffer, pH 7·25. The tissues were rinsed several times in buffer alone for 12–24 hr. The post-fixation was done in 1·33% osmium tetroxide in 0·1 M-phosphate buffer (pH 7·25) for a period of 2 hr. After dehydration in graded alcohols, the tissues were treated with propylene oxide and embedded in Epon 812. Thin sections were obtained with a Porter–Blum ultramicrotome, stained with 1% uranyl acetate and lead citrate and studied in a Siemens Elmiscop 1. Simultaneously, thicker sections (0·5 or 1 μ) were made from the same block and stained with toluidine blue. As controls, the thyroids of six monkeys (three normal, one stimulated with TSH and two injected with monkey gastric mucosa extract with complete Freund's adjuvant) were also examined.

RESULTS

All monkeys injected with thyroid extract began to produce antibodies between the 2nd and 7th week. At various times they showed the three types of antibodies studied (haemagglutinating, complement fixing and cytotoxic antibodies). All seven monkeys had considerable

histological changes in their thyroid glands. The two with the highest titres of circulating antibodies also showed the most extensive changes in the thyroid gland. The serological findings have been published in detail (Kite *et al.*, 1966; Doebbler & Rose, 1966; Rose *et al.*, 1966; Andrada, Rose & Kite, 1968).

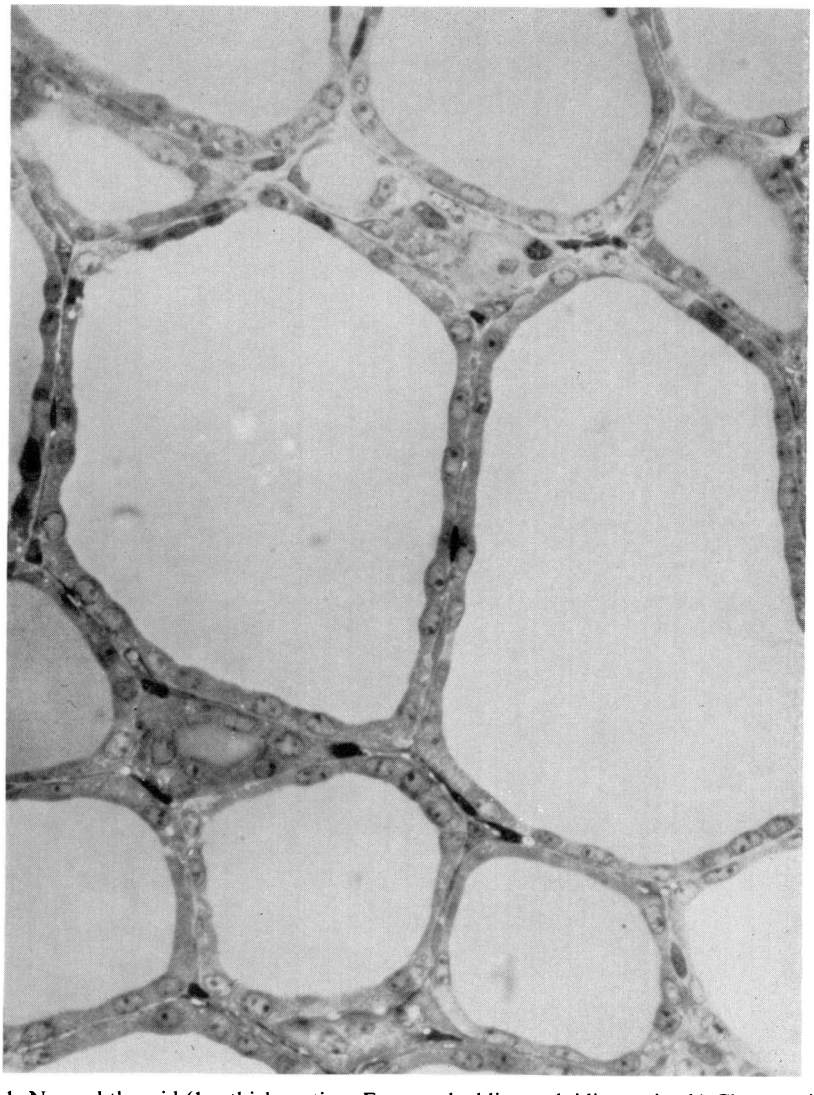

FIG. 1. Normal thyroid (1 μ thick section, Epon embedding, toluidine stained.) Characteristic of normal thyroid is the uniform structure of the thyroid follicular cells and the smooth edge of the lumina. The follicular cells are predominantly flat. $\times$ 400.

The thyroid gland of normal Rhesus monkeys

Each follicle of the normal thyroid gland consists of an outer layer of epithelial cells which surrounds the colloid. In the light microscope, the cells vary in height and arrangement but are predominantly flat (Fig. 1). The follicles are closely related to the blood and lymph

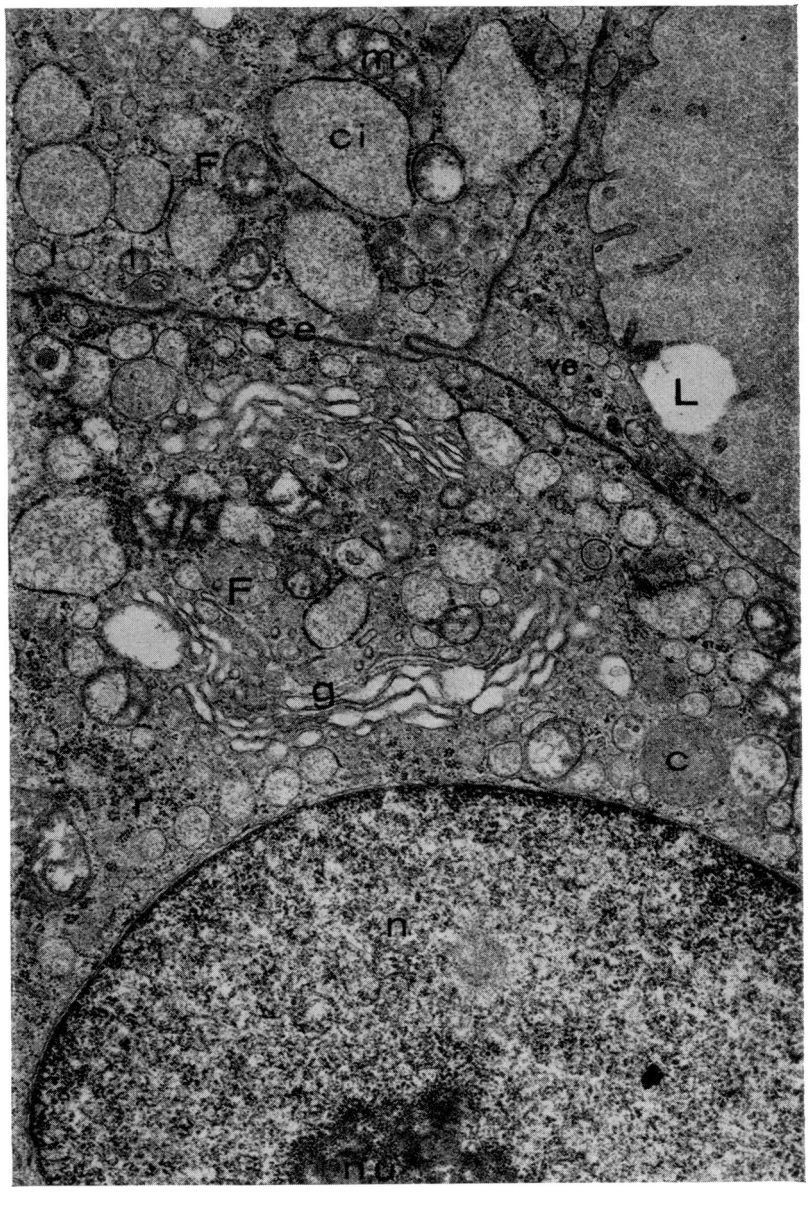

FIG. 2. Normal thyroid (for fixation and embedding technique, see 'Material and methods'). The apical cell zone is shown with several microvilli. Beneath the plasma membrane there are several small vesicles (ve). The endoplasmic reticulum forms cisterna (ci). The membranes of the endoplasmic reticulum have attached ribosomes (r), but on some parts they are missing(↑). The lateral cell membranes (ce) are quite straight. There are only few mitochondria (m); the cytoplasm shows few granules; one of them seems to be a colloid droplet (c). The Golgi apparatus (g) is prominent. n, Nucleus; nu, nucleolus; L, lumen; F, follicular cell. ×11,200.

capillaries. The endothelium of the blood capillaries is flat and of the fenestrated type. The whole follicle is enclosed by a basement membrane (basal lamina) that has a thickness of about 500 Å.

The cell membranes of the epithelial cells can easily be demonstrated electron microscopically. They have a thickness of 70–80 Å. Microvilli are present at the luminal surface. These microvilli are irregularly implanted and vary in width and length (Fig. 2). The basal portion of the cell membrane runs more or less in an undulated line and there are infoldings at irregular distances. The lateral cell borders, which are predominantly straight show loop-

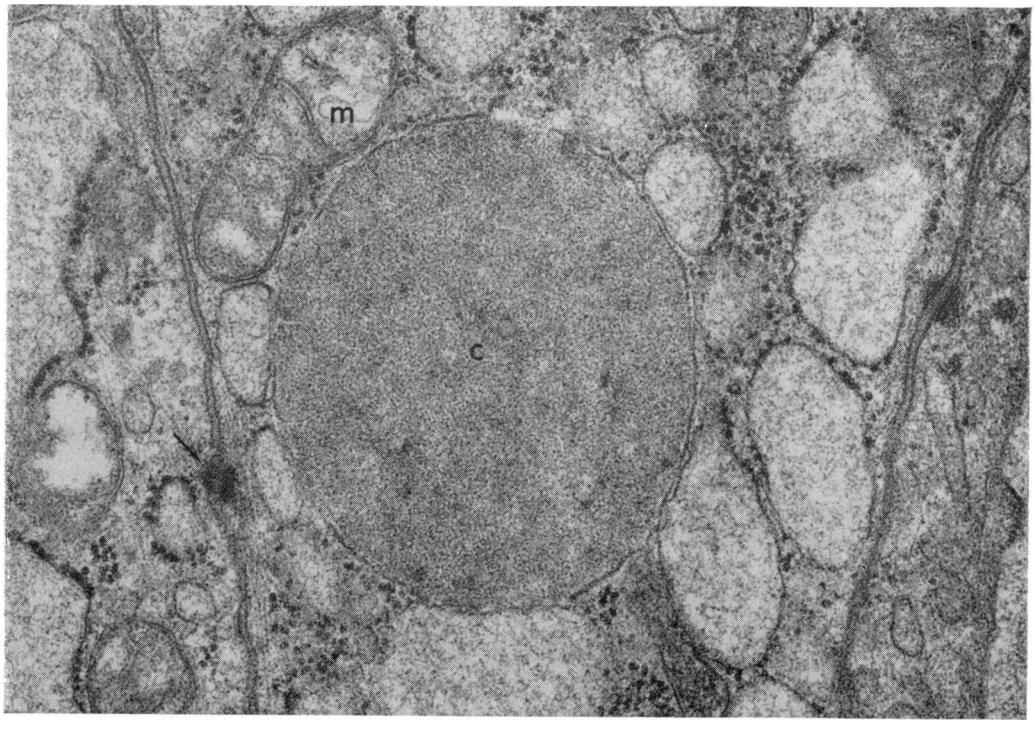

FIG. 3. Normal thyroid. Higher magnification of a colloid droplet (c). Notice the surrounding membranes. The tight junction (desmosome) between the epithelial cells is very electron dense. m, Mitochondria. ×40,000.

like infoldings (Fig. 2). The intercellular space measures 140–150 Å at the upper part of the cells, but is wider at the base. Tight junctions can be found on the lateral sides of the cells. They are very electron dense and have the same structure as in human thyroids as described by Heimann (1966), (Fig. 3). The nuclei of the epithelial cells, which fill a major part of the cell, are located centrally or toward the base. They contain one or more nucleoli, and are poor in chromatin.

Elongated mitochondria are seen in small number in the cytoplasm (Figs. 2 and 3). Quantitatively the endoplasmic reticulum of the rough type varies considerably. In flattened cells there are predominantly small elongated cysternae, whereas in cuboidal cells the endoplasmic reticulum is better developed, consisting of flattened and dilated cysternae. In

the apical part of the cells the cysternae of the endoplasmic reticulum are in general smaller than in the basal part of the cells. It should be mentioned that in normal follicular epithelial cells the membranes of the endoplasmic reticulum appear to a large extent without ribosomes. Free ribosomes and polysomes are frequent (Figs. 2, 3 and 4).

The Golgi apparatus usually is located in the apical part of the cell near the nucleus (Fig. 2). It is composed of parallel, flattened saccules, vacuoles, and small vesicles. The Golgi apparatus is variable in size from cell to cell, probably depending on the plane of

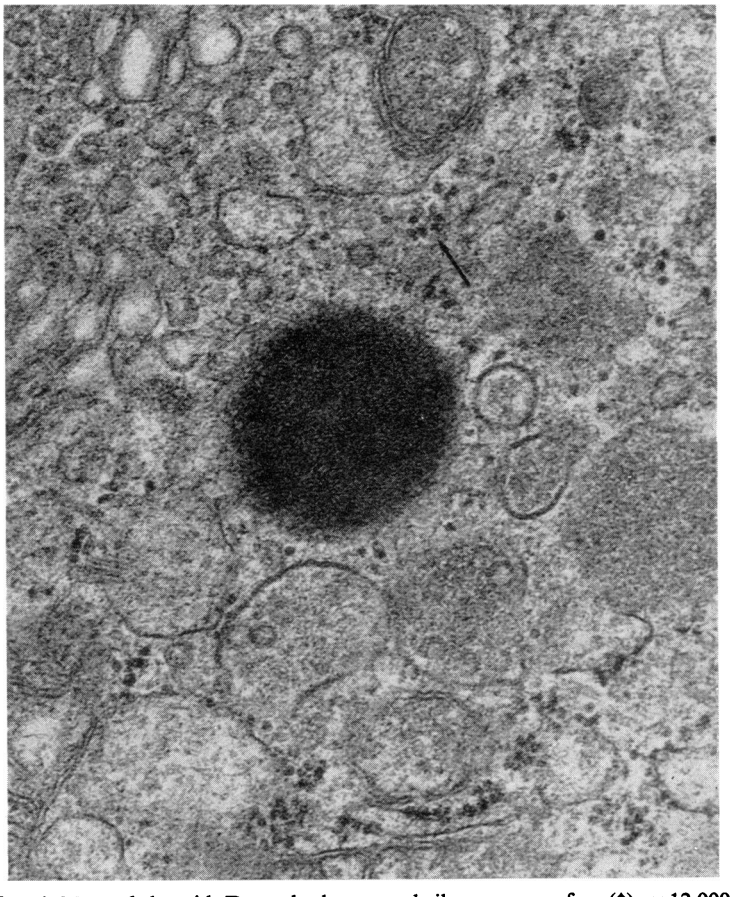

FIG. 4. Normal thyroid. Dense body; several ribosomes are free (↑). × 12,000.

sectioning. Vesicles similar in size to the Golgi vesicles can also be demonstrated in the vicinity of apical and basal portions of the plasma membrane.

Different kinds of cytoplasmic droplets are present in epithelial cells of the thyroid gland. They vary in size as well as in electron density (Figs. 2, 3 and 4). On this basis colloid droplets can be differentiated from other, more dense, bodies and from lipid globules. The colloid droplets, 0·5–1 μ in size, are surrounded by a membrane and lie predominantly in the apical part of the cell (Figs. 2, 3 and 4). Rarely could more than five colloid droplets be counted in one cell in these sections. The dense bodies measure 0·3–0·8 μ, and are predominantly found in the apical part of the cell. The number of dense bodies in the follicular

epithelial cells is usually greater than the number of colloid droplets. In normal thyroid glands a few globules of other types are present, probably representing lipid droplets.

The thyroid gland of Rhesus monkeys with experimental thyroiditis

The morphology of thyroid glands in experimental autoimmune thyroiditis differs markedly from the normal. All seven monkeys in this particular experimental series

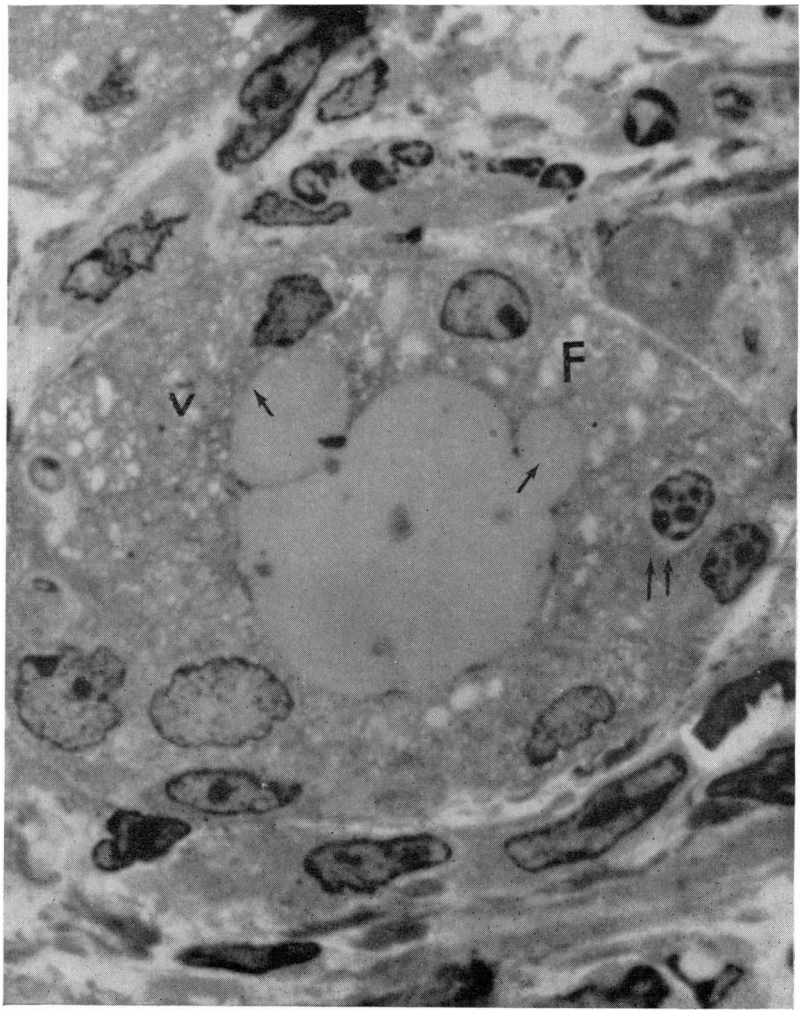

FIG. 5. Thyroiditis (1 μ thick section, Epon embedding, toluidine stained). The characteristic feature is the irregular shape of both the follicular lumen (↑) and the follicular cells (F). Notice the vacuolization (v) of some follicular cells. In the follicle can be seen infiltrating (↑↑) mononuclear cells. × 1000.

showed severe thyroiditis. In the light microscope our observations were basically in accord with previously reported findings (Terplan *et al.*, 1960). In the areas with pronounced inflammatory infiltration the pathological changes of the follicular epithelial cells are

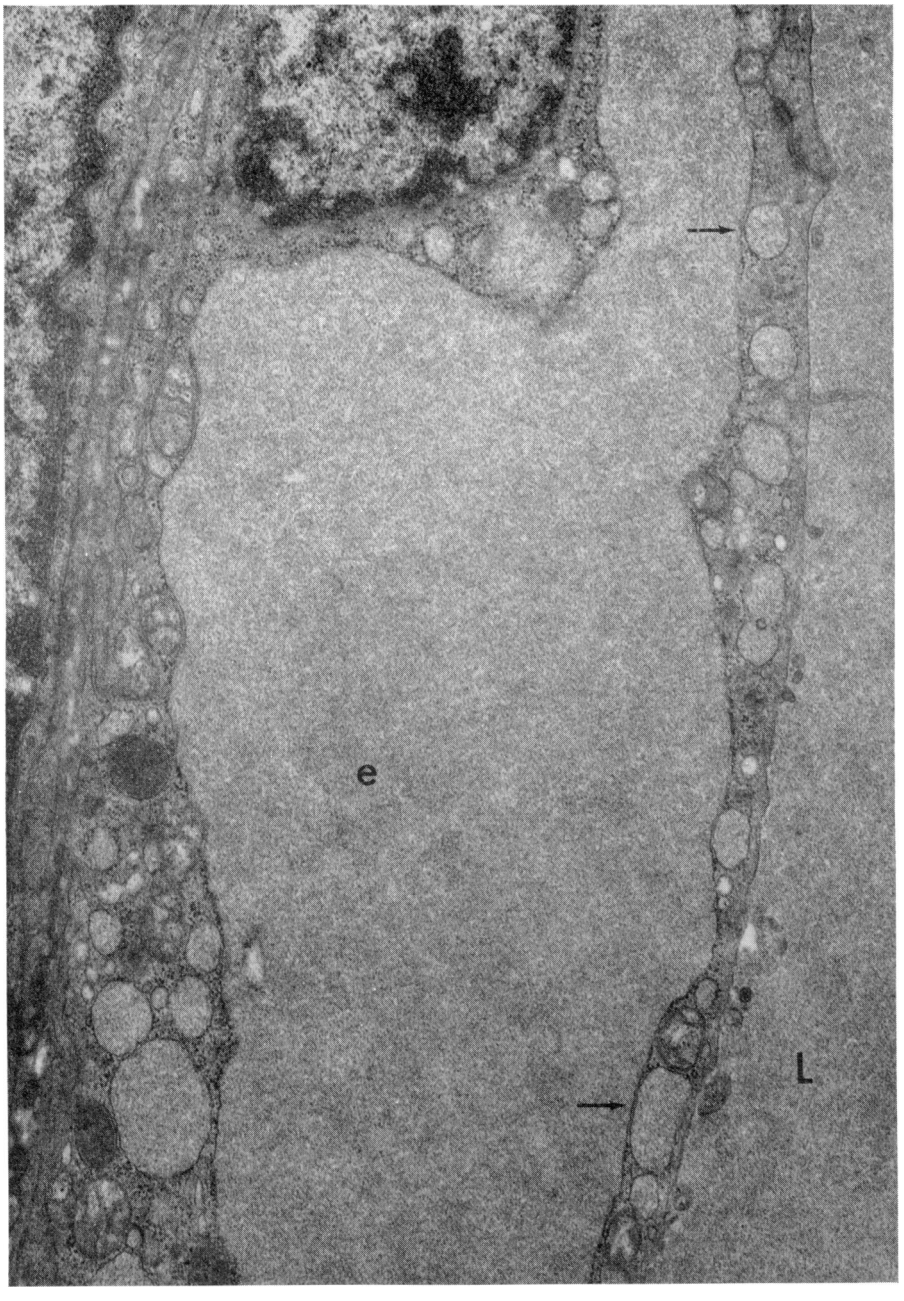

FIG. 6. Thyroiditis; epithelial cell with an enormously dilated strand of endoplasmic reticulum (e). Notice the absence of ribosomes in some parts of the endoplasmic reticulum (↑). There is no significant change in the structure of the other cell organelles, but the microvilli seem to be diminished. ×40,000.

especially severe. Their arrangement is altered considerably and there is also a significant increase in the size of the follicular cells. In the light microscope vacuolization is frequent (Fig. 5). The outline of the follicular lumina is rather irregular. Mononuclear cells can be found in the lumen of some follicles.

The electron microscopic findings parallel those of the light microscope. First, in areas in

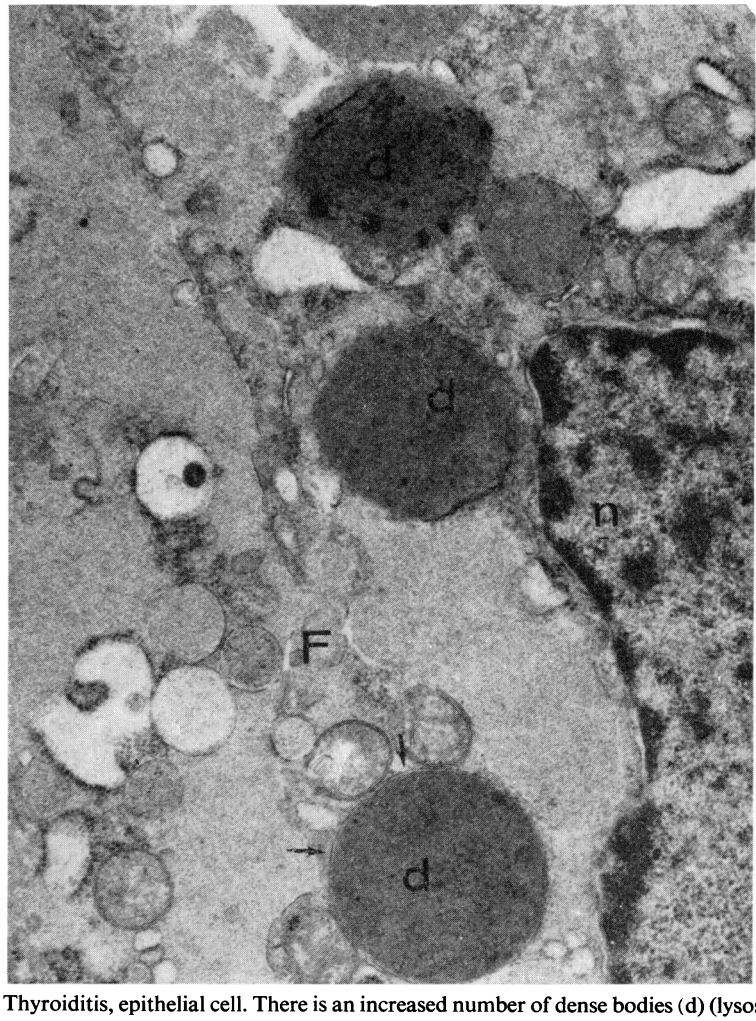

FIG. 7. Thyroiditis, epithelial cell. There is an increased number of dense bodies (d) (lysosomes). They are mostly located in the apical part of the cell and are surrounded by a membrane. The epithlial cell shows signs of destruction. n, Nucleus; F, follicular cell. × 18,000.

which no inflammatory reactions are present (as observed in semi-thin sections), normal fine structures prevail. The changes in the ultrastructure of the epithelial cells seem to occur predominantly in those areas in which inflammatory infiltrates are demonstrable.

In the initial stage most of the epithelial cells in thyroiditis are cuboidal in shape. Microvilli of the epithelial cells are very rare (Fig. 6). The endoplasmic reticulum in some instances shows an extensive vacuolar dilatation. In this early stage no other ultrastructural

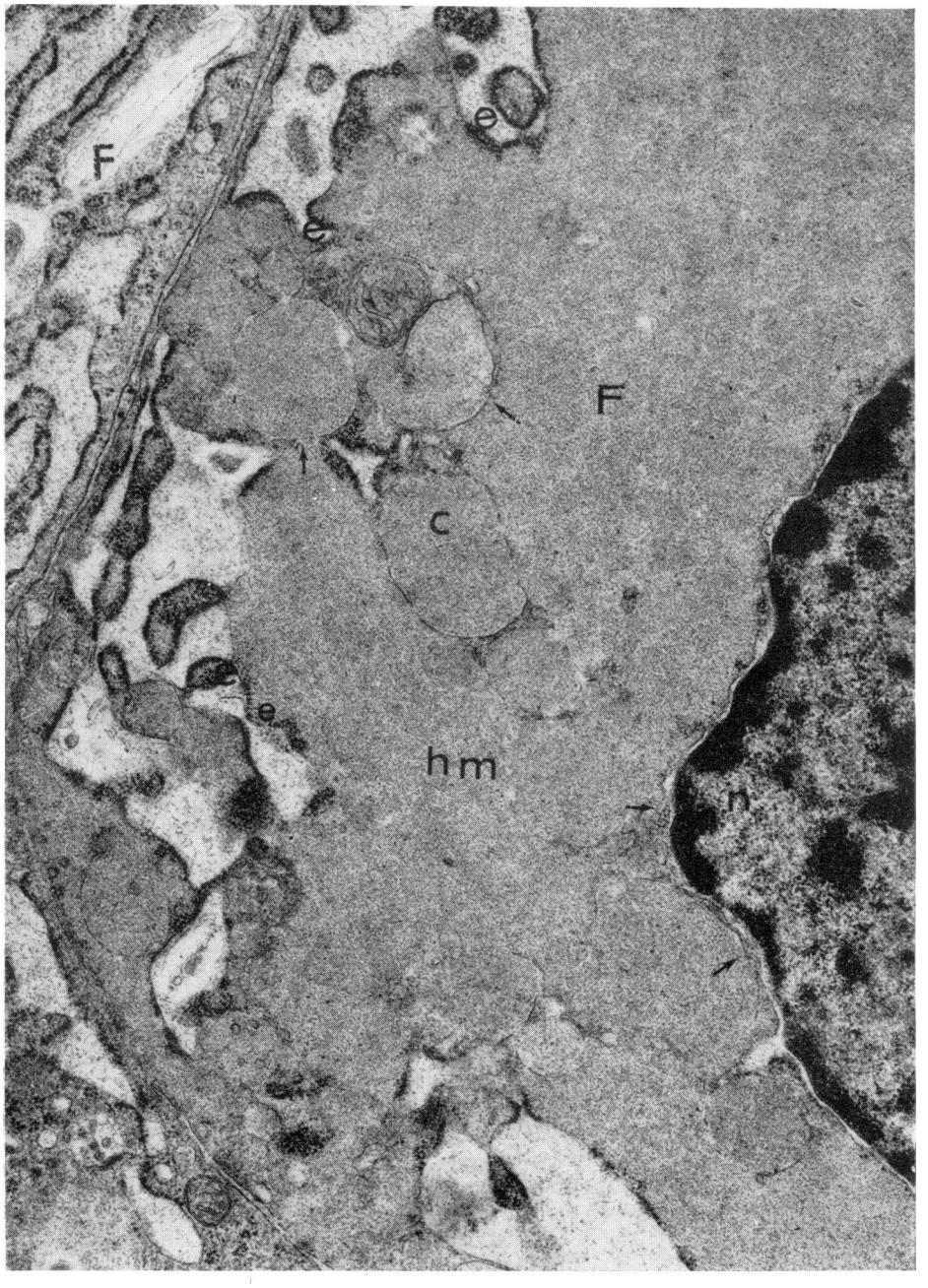

FIG. 8. Thyroiditis, epithelial cell. Note the mass of homogeneous material (hm) in which sometimes the outline of a membrane (c) can be recognized. The endoplasmic reticulum (e) lies mainly at the edge of this mass and shows signs of dissolution. The nucleus (n) is very electron-dense and the outer nuclear membrane is separated from the inner membrane. At the upper left there is another epithelial cell with well developed endoplasmic reticulum (F). × 32,400.

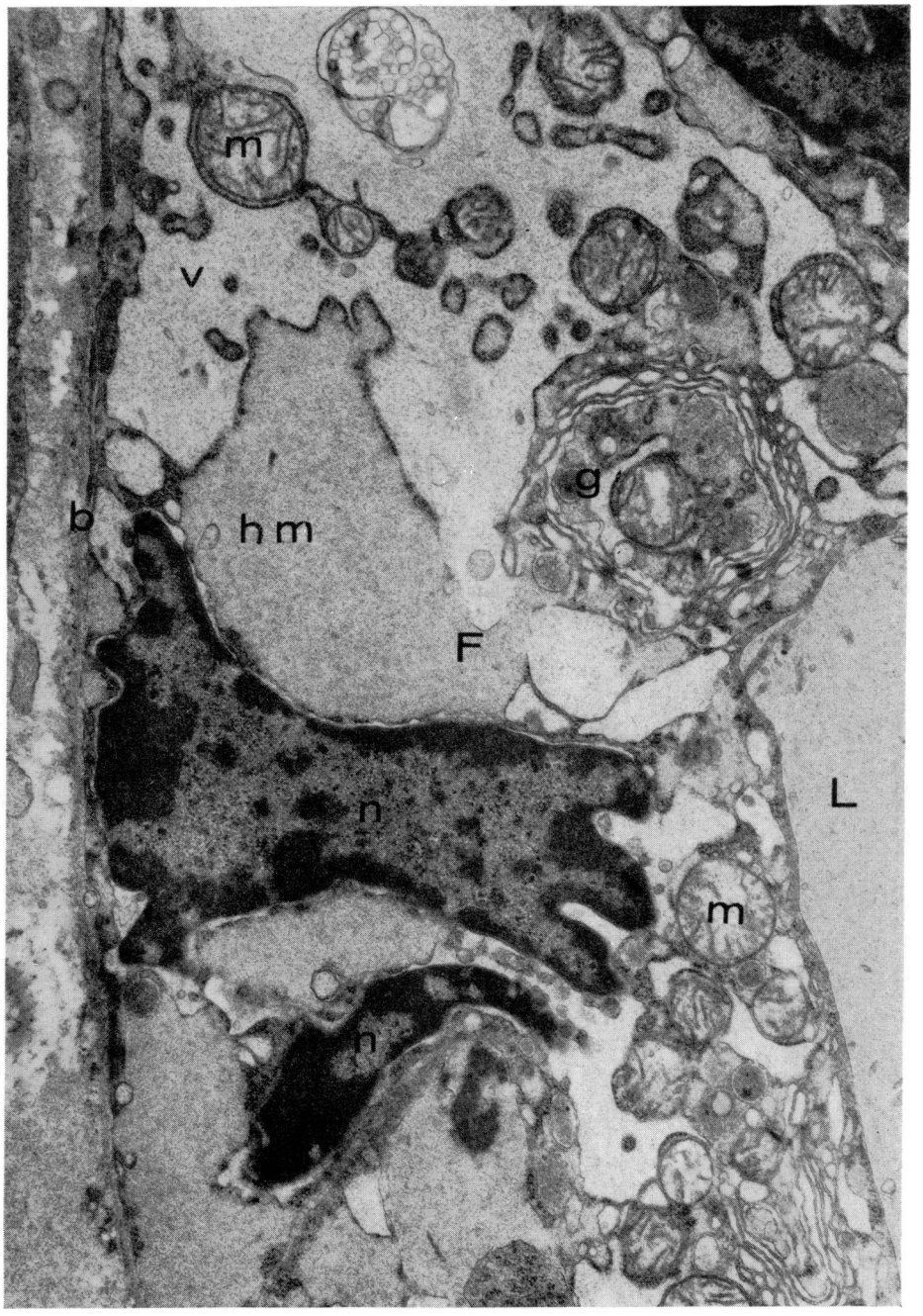

FIG. 9. Thyroiditis, epithelial cell. The figure shows a typical epithelial cell with signs of focal necrosis. The nucleus (n) is very electron-dense and picnotic and is surrounded by fine granular material. Notice also the vacuolization of the cytoplasm (v). The mitochondria (m) are swollen. The Golgi apparatus (g) seems to be preserved. At the basal part of the cell are seen the outlines of the basement membrane (b). hm, Homogeneous material; F, follicular cell; L, follicular lumen × 13,500.

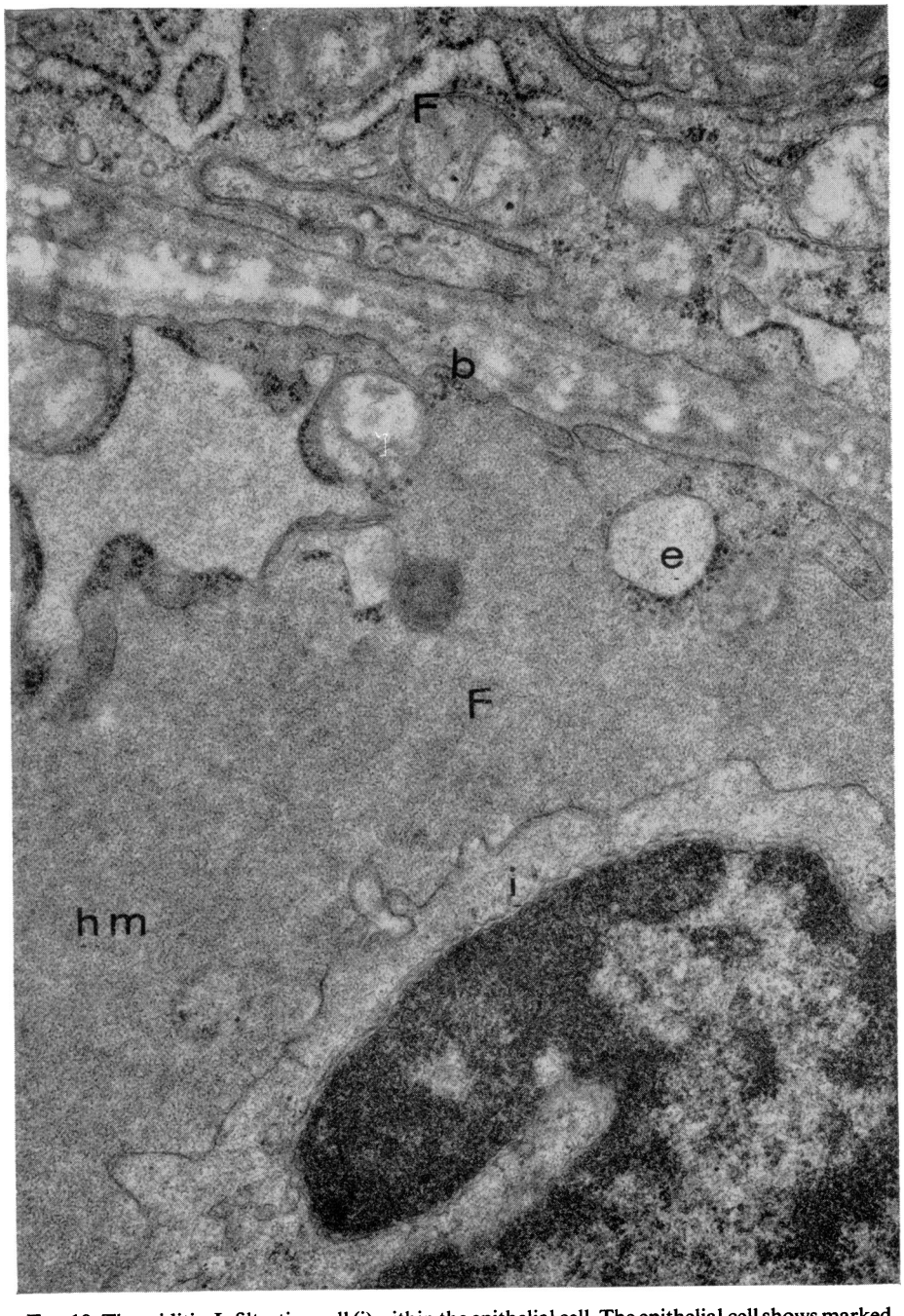

FIG. 10. Thyroiditis. Infiltrating cell (i) within the epithelial cell. The epithelial cell shows marked degeneration. Noteworthy is the position of the infiltrating cell within the extended fine granulated material (hm). F, Follicular cell; b, basement membrane; e, endoplasmic reticulum. ×31,500.

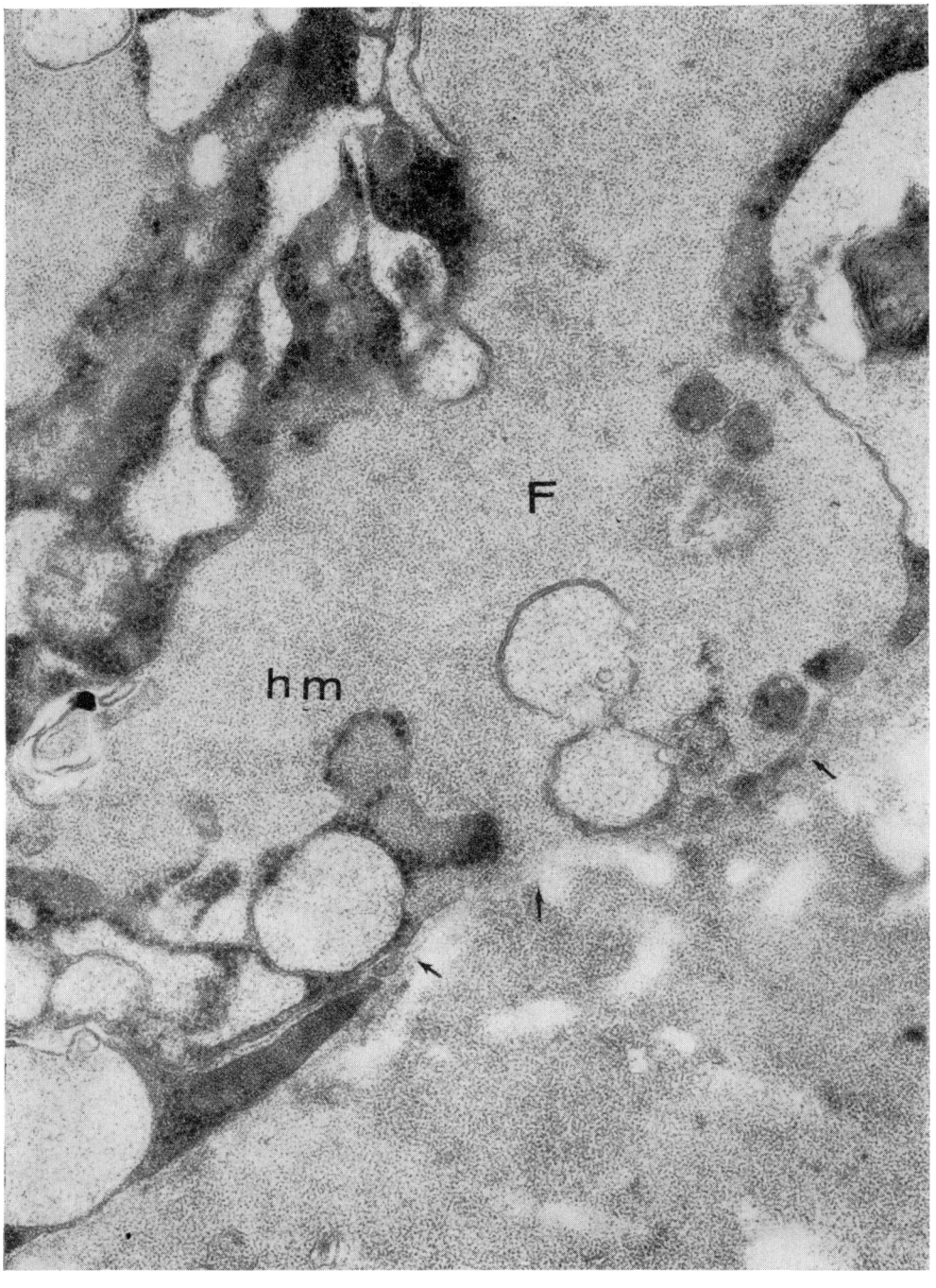

FIG. 11. Thyroiditis. Basal epithelial zone. There is no basement membrane (↑) to be seen underneath a markedly necrotic epithelial cell. Fine granulated material probably originated from the epithelial cell (hm). F, Follicular cell. × 24,000.

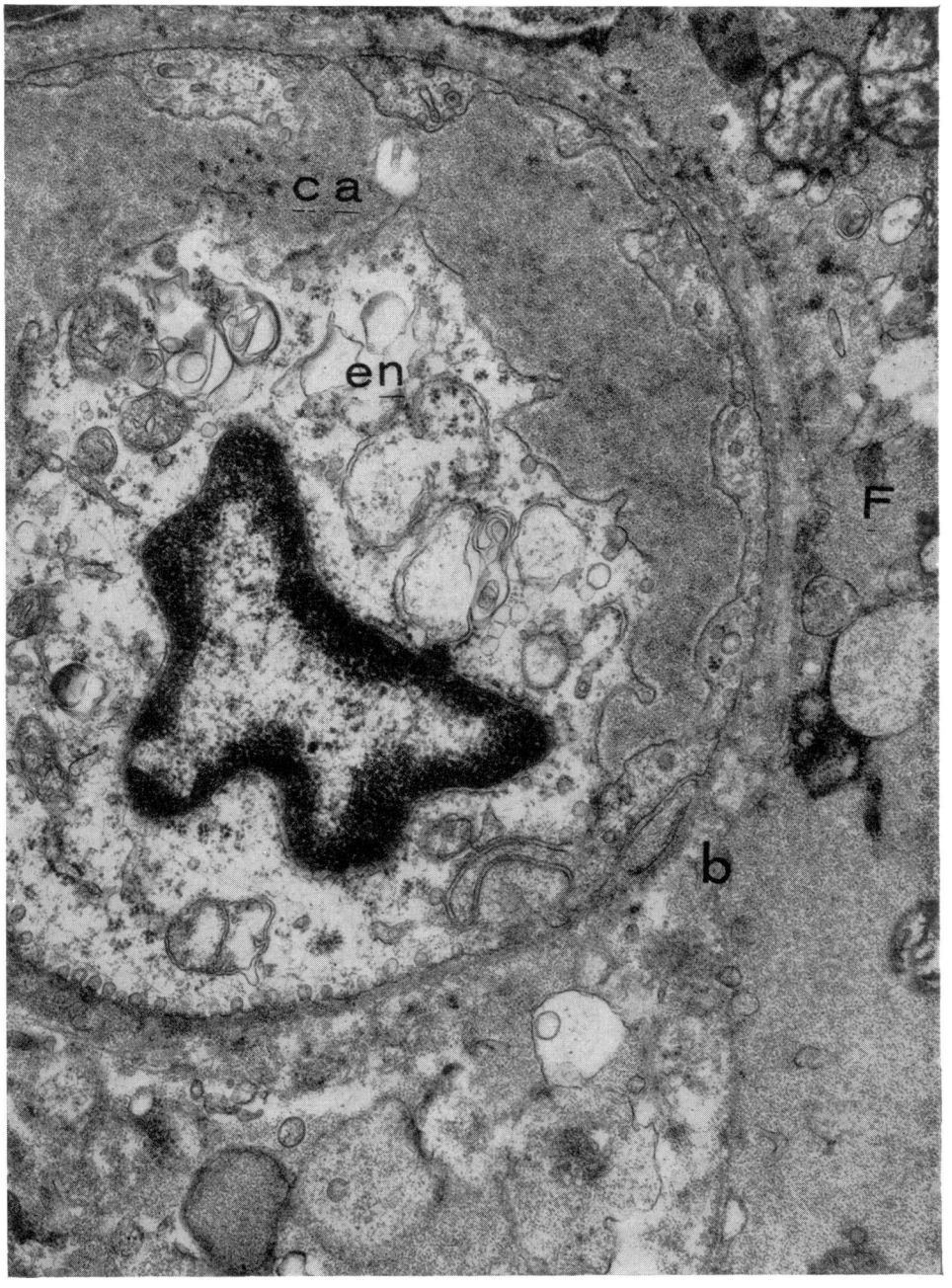

FIG. 12. Thyroiditis, capillary (ca). Its endothelial cell (en) is enlarged and shows a vacuoled cytoplasm. The follicular cell (F) next to the capillary is necrotic. b, Basement membrane. × 22,000.

changes are apparent. In the same follicle, however, other abnormal epithelial cells can be found which are characterized by the pronounced enrichment of granules having the morphological characteristics of colloid droplets. They vary markedly in their size, and the membranes which surround the colloid droplets show irregular disruptions (Figs. 7 and 8). There are certain areas in which the membrane of the colloid droplets is completely absent and a confluence of these droplets seems to occur. At the same time, the dense bodies in

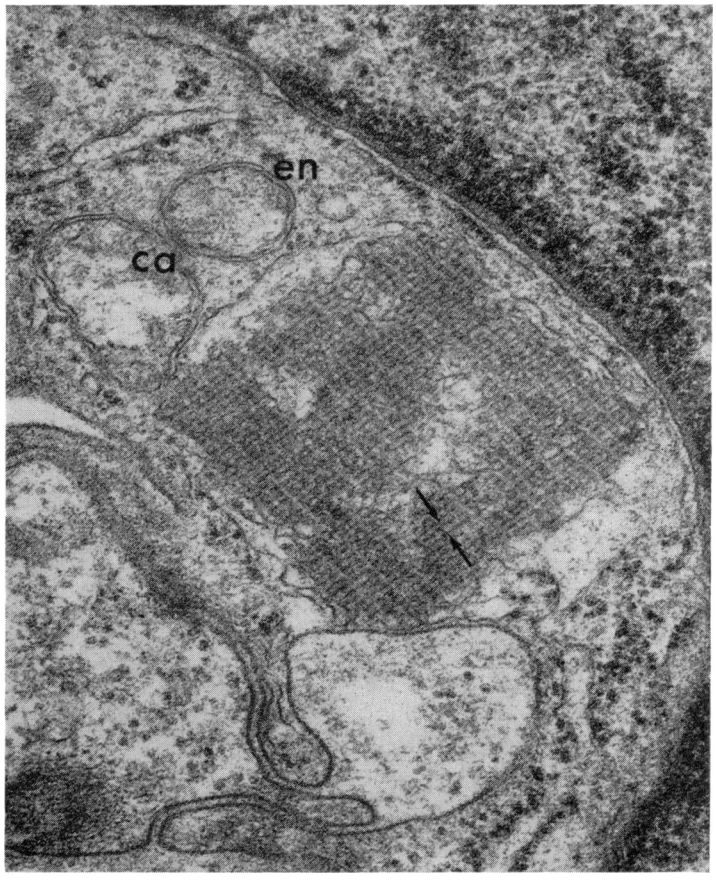

FIG. 13. Thyroiditis, capillary (ca). In the endothelial cell (en) there is a structure exhibiting a crystalline pattern. The distance between the parallel lines is 210 A (↕). ×47,520.

these cells are increased in number (Fig. 7). Frequently epithelial cells with many dense bodies show signs of necrosis in the cytoplasm (Fig. 7). An accumulation of colloid droplets is found in the apical as well as in the basal part of the cells. With the increase in the number of granules, the number of mitochondria is clearly diminished. The endoplasmic reticulum is scarcely present (Fig. 8).

These minor alterations lead into more severe damage with the following ultrastructural characteristics. The cytoplasm of the cells is extensively packed with fine granular material.

In these areas confluence of droplets can be observed forming homogeneous, finely granular masses, in which the shape of droplets can still be recognized. The membranes of the droplets seem to be in the stage of dissolution (Fig. 8). The endoplasmic reticulum in these cells containing the homogenous masses has almost disappeared. We could observe that these masses appear primarily in the basal part of the cells (Fig. 9). The mitochondria, if present at all, are very much enlarged, and badly preserved.

Another typical feature is picnosis of the nuclei (Fig. 9). The space between the outer and inner membrane becomes wider and the density of the nucleoplasm is much increased. The organelles of the cytoplasm show a remarkable degree of alteration. Besides the fine granular deposits (Figs. 9, 10 and 11), there is a very widely dilated endoplasmic reticulum. Mono- and polymorphonuclear cells are frequently found outside the follicles as well as between the follicular epithelial cells and also inside the follicular lumina (Figs. 5 and 10). Follicles even after invasion by inflammatory cells seem to have normal basement membranes. However, a large number of epithelial cells show severe changes as described above, in association with the infiltrates of mononuclear cells into the follicles. In the areas where the infiltration into the follicle is intense, monocytes and granulocytes are sometimes present within the follicular epithelial cells (Fig. 10). The cell membrane separating the infiltrating cells from the thyroid cells is no longer demonstrable. The infiltrating cells are embedded in a fine granular material forming the homogeneous masses.

In even more heavily infiltrated areas destruction of many follicular cells can be observed. This final stage of follicular destruction is characterized by the disruption of the apical and basal cell borders of epithelial cells. In these areas the basement membrane disappears and fine granular material can now also be found outside the follicular cell proper (Fig. 11).

Alterations in the ultrastructure of the capillaries are frequent (Fig. 12). A characteristic increase in the size of the endothelial cells is seen. In many endothelial cells a crystalline structure with periodicity of 210 Å is present (Fig. 13).

DISCUSSION

Experimentally produced autoimmune thyroiditis is characterized by a multitude of considerable changes in the ultrastructure of the thyroid gland. Among the more impressive are the dilatation of the endoplasmic reticulum and the accumulation of droplets within numerous follicular cells. These droplets have electron microscopical properties similar to the ones described in normal thyroid and seem to be identical with thyroglobulin reabsorbed from the colloid lumen (Bauer & Meyer, 1965). A confluence of colloid droplets accompanied by a dissolution of the membrane surrounding the droplets seems to occur. A characteristic feature is the appearance of widespread, fine granular material within the thyroid cells. In the final stage of necrosis the entire cell, with the exception of the partially autolysed cell nucleus, seems to be filled with this kind of material. There is an increase in the number of dense bodies (probably representing lysosomes) (Novikoff, 1963) in those cells which are not as yet badly damaged; however, with the increase of the damage, these organelles are reduced in number and are less and less prominent as cell necrosis progresses.

The necrotic process of the follicular cells is mainly observed in close proximity to infiltration of lymphocytes, plasma cells, leucocytes and macrophages. It is noteworthy that the

infiltrating cells seem to appear first between the individual follicular cells, and later within the follicular cells with the greatest degree of damage.

Sobel & Geller (1965) in an electron microscopic study of experimental thyroiditis in the guinea-pig suggested the non-specific nature of the several ultrastructural changes in the thyroid cell. We mostly agree with Sobel and Geller's findings and interpretation of their observations. However, in addition to these, we have observed striking differences which we consider rather characteristic for experimental thyroiditis in the monkey; among these are: dilatation of the endoplasmic reticulum, increased number of colloid droplets, and confluence of droplets resulting in a distinctive pattern of an homogeneous material. A very striking feature associated with severe infiltration by mono- and polynuclear leucocytes is the destruction of many intracellular organelles as well as the cell membrane of the endothelial cells. These differences might be partly due to the fact that our observations were made in monkeys. The degree of pathological change in these animals is extensive.

In keeping with the earlier electron microscopic investigations by Irvine & Muir (1963) on the ultrastructural changes in Hashimoto's disease we observed in the present study that the thyroid basement membrane was remarkably continuous. In particular we were able to show that an infiltration into the follicular cell or lumen is not necessarily connected with a prominent lesion of the basement membrane. A disrupted basement membrane was observed only in areas where the necrotic process in the follicular cell was very severe (Fig. 11).

As far as the droplets found in the follicular cells in thyroiditis are concerned, they seem, from the morphological point of view, to be identical with the colloid droplets of normal thyroid cells. The increased number of droplets may be indicative of thyrotropin stimulation (Seljelid, 1967a, b).

The question arises as to the nature of the fine granular material present within the follicular cells. Possibly this material is formed by the confluence of the colloid droplets, perhaps with additional material derived from autolytic breakdown. Whether this accumulated material is the cause or the result of the degenerating process cannot be determined. Obviously the degeneration of follicular cells occurs in connection with the invasion of inflammatory cells into the thyroid follicles. In some instances these inflammatory cells are found within the epithelial cells. Occasionally, however, degeneration of the epithelial follicular cells might start before the inflammatory cells penetrate directly into the epithelial cells. The capillaries of the affected areas are also involved in the pathological process, exhibiting hypertrophy as well as vacuolation of the endothelial cells.

The number of dense bodies is increased in early stages of cellular alteration. In this connection it is of interest to note that from the histochemical point of view the dense bodies are associated or identified with lysosomes (Novikoff, 1963). Because of the role lysosomes may play in cellular damage and eventual destruction of the cells (Slater, Greenbaum & Whang, 1963; Brandes & Anton, 1966) the decrease in the number of dense bodies in the latter stage of cellular destruction seems to be significant.

Regarding pathogenesis, several possible decisive factors may be involved—the action of the lysosomes originating either from the infiltrating cells or from the epithelial cells themselves, the physical accumulation in the cytoplasm of thyroglobulin-like substances which could not be metabolized, or some other mechanism. It should not be forgotten, however, that the entire process is initiated by the active immunization of the experimental animal with thyroglobulin, and that the severe changes in the thyroid gland occur as a sequela of this immunization.

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