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Ultrastructure of the Thyroid in De Quervain's Subacute Granulomatous Thyroiditis

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Summary. An electron microscope study of excised thyroid fragments from a case of De Quervain's subacute thyroiditis is reported. Deposit of material along the basement membrane making the latter apparently thickened, cytoplasmic alterations and stratification of thyroid cells seem to be induced by the thyroiditis process itself. The characteristic giant cells are syncytia resulting from fusion of inflammatory cells. The pathology is specific of a granuloma and is strikingly different from that of the other thyroidites.

Papers devoted to the ultrastructure of normal human thyroid are relatively rare (Noseda, 1954; Irvine *et al.*, 1963; Nève, 1965; Toujas *et al.*, 1969). In the last few years, several papers on the ultrastructure of the diseased human thyroid have also appeared (Binet *et al.*, 1963; Irvine *et al.*, 1963; Heimann, 1966; Seman *et al.*, 1968; Nève, 1969). In autoimmune thyroiditis, no matter what the clinical symptoms, an identical morphological pattern was observed under the electron microscope (Nève, 1969). De Quervain's disease is a relatively uncommon form of thyroiditis the etiology of which differs from that of autoimmune thyroiditis. The thyroid ultrastructure in this disorder has not been reported although Volpé *et al.* (1967) mentioned the absence of virus in electron microscopic studies of needle biopsy material in two cases of subacute thyroiditis (unpublished data).

Because of the rarity of the disease, it seemed worthwhile to report the observations made on a biopsy of thyroid from a single case of De Quervain's thyroiditis. The fine structure of subacute thyroiditis differs significantly from that of other forms of thyroiditis.

Clinical Report and Methods

A 37 year old woman (File 69951) entered the hospital because of a painful swelling in the neck of three weeks duration accompanied by fatigue, malaise and fever. Physical examination revealed a diffusely enlarged thyroid. A detailed clinical report will appear in another paper. An elevated erythrocyte sedimentation rate and leucocytosis were present. Studies of thyroid function included an elevated $PB^{127}I$ of 9.1 gamma per cent. The serum TSH level was not increased. The uptake of ^{131}I was uniformly suppressed over the thyroid gland. No thyroid antibodies or LATS (Bonnyns, 1968) was detected in the blood. A surgical exploration[1] with biopsy was carried out in order to eliminate the possibility of neoplasm. Each thyroid fragment excised was divided into two parts: one was fixed in Bouin's fluid, embedded in paraffin, sectioned at 7 μ and stained with haematoxylin-eosin and PAS for

1 Performed by Professor A. Brémer (Department of Surgery, Hôpital Saint-Pierre, Brussels).

histological studies; the other was used for electron microscopic study. These samples were immersed within one half minute of excision in a drop of fixative containing glutaraldehyde 4.2% in 0.1 M Millonig's buffer at pH 7.4 (Millonig, 1962). The specimens were then divided with a razor blade into small fragments of about 1 mm^3 which were placed in fresh chilled fixative at 4° C for 2 hours. They were rinsed overnight with 0.1 M phosphate buffer solution containing 0.54 g per cent glucose, and postfixed with osmium tetroxide 2 per cent in glucosed Millonig's buffer at pH 7.4 (1962). Dehydration took place in rising concentrations of ethanol or acetone. The specimens were embedded in Epon or in Vestopal. Ultrathin sections were made with a diamond knife on a LKB and a Sorvall "Porter-Blum" ultramicrotome. Sections were stained with both uranyl acetate and lead citrate (Reynolds, 1963). A Siemens Elmiskop 1 and an EM6B AE1 electron microscope were used. Semi-thin sections stained with toluidine blue were also examined.

Results

Light Microscopy (B 67/1249)

The histological sections were examined by Professor P. Dustin, who confirmed the diagnosis. Only a few areas of the thyroid were not affected by the thyroiditis. In the affected areas, the histological pattern was variable from field to field. Formative lesions of some follicles were characterized by a multilayering of the epithelial cells, ascertained on examination of serial sections. Many follicles showed a partial or complete disruption of their lining epithelium caused by intrafollicular infiltration. The interfollicular infiltration was made up principally of mononuclear inflammatory cells, sometimes associated with polymorphonuclear neutrophils. Multinucleate giant cells appeared either whithin affected follicles or mixed with granulomatous tissue. Some of the giant cells seemed to be formed by fusion of thyroid cells. In most of the giant cells, the PAS reaction was weak or absent.

The interfollicular fibrosis was prominent in areas containing irregular groups of abnormally small follicles which had an intact epithelial lining and which were free from intrafollicular infiltration.

Electron Microscopy

The observations interest first the epithelial changes undergone by the thyroid cells; the description of the inflammatory process will follow.

Epithelial Changes. The areas free of thyroiditis showed normal follicle cells. In the cytoplasm of a large number of these cells, membrane-limited "lipid inclusions" were observed: these were made up of gathered globules presumably of lipid nature, frequently associated with dense and very osmiophilic material.

In the areas with inflammatory infiltration, the follicular structure was either disrupted or maintained. Except at the place of disruption by the inflammatory invasion, the follicles were always surrounded by a basement membrane which characteristically appeared as though thickened. This thickening appeared to result from the deposition of material along the basement membrane. This material, often winding upon itself, was closely coupled to the normally thick basement membrane and was of similar density. The total thickness was as great as 2 μ (Figs. 1 and 2).

In some follicles, the unilayered limiting thyroid cells were cylindrical (Fig. 1). The intercellular spaces appeared normal and were closed at the apex by a

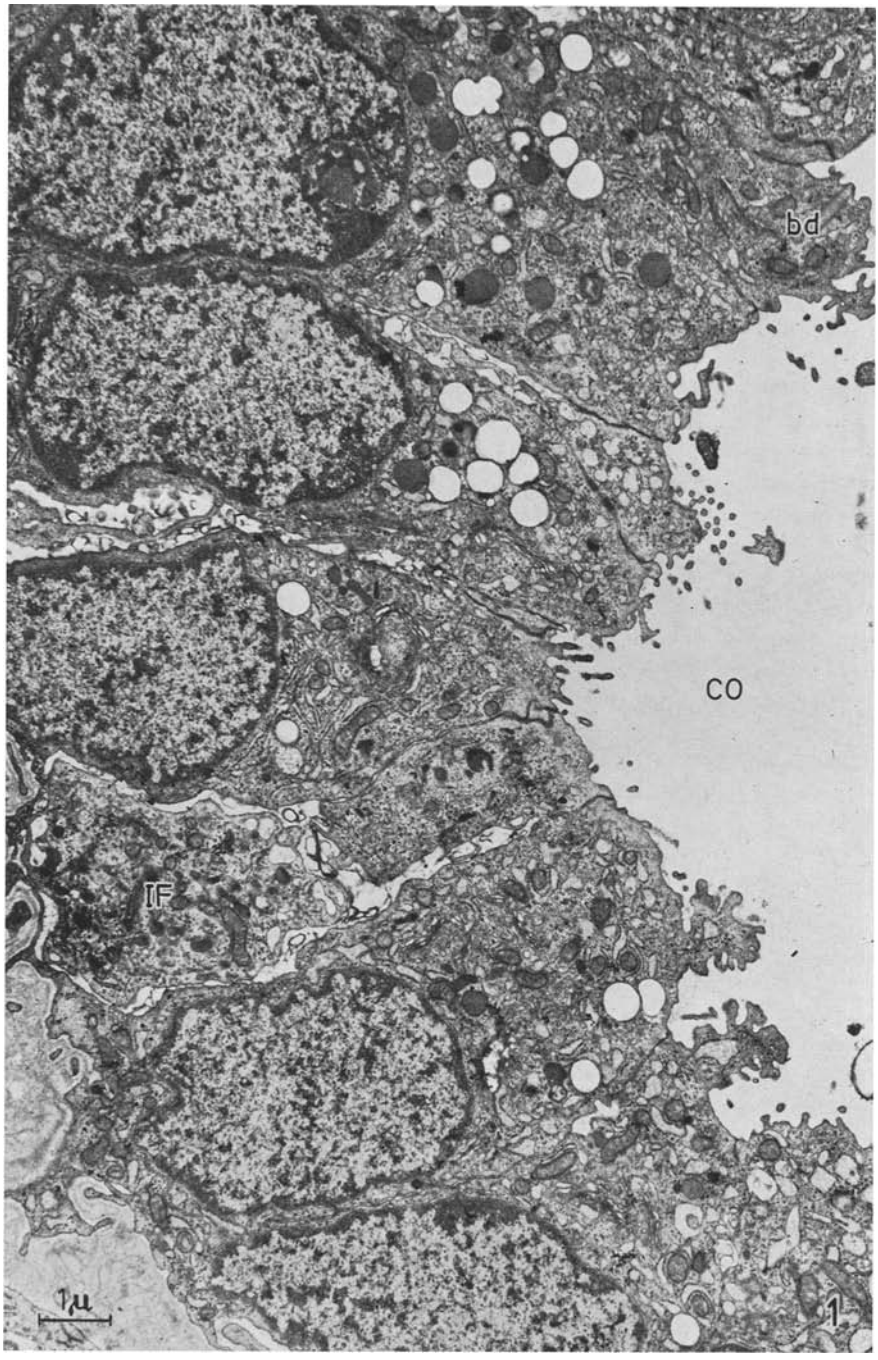

Fig. 1. Cylindrical thyroid cells bordering the colloid lumen (*CO*) of a follicle. The cells display at their apex cytoplasmic protrusions without colloid droplets. An inflammatory cell (*IF*) is located between the follicle cells. At the top of the upper cell, the basal body (*bd*) of a flagella can be observed. ×9,500

terminal bar (Robertson, 1959). Many irregular cytoplasmic processes arising from the apex protruded into the colloid lumen. These protrusions resembled the classical pseudopods observed in normal thyroids after thyrotropin administration. However they differed by the fact that they contained ergastoplasmic cisternae and never contained droplets. A well-developed Golgi apparatus with frequent stretched cisternae associated with rounded vacuoles was observed above the basally-located nucleus. The remaining cytoplasm was occupied by normal-looking mitochondria associated with rough endoplasmic cisternae which very often were dilated. Dense bodies, lipid inclusions and many polyribosomes were scattered throughout the cytoplasmic matrix.

A frequently encountered pattern in both intact and ruptured follicles was the superposition of epithelial cells. Around the colloid lumen or some of its remnants, polyhedrical or cylindrical thyroid cells were multilayered. The boundaries between those cells were difficult to discern but became more evident near the terminal bars. The distinct apical-basal polarity was frequently lost as shown by the random position of the centrioles in the cytoplasm.

Sometimes, only large clusters of epithelial cells were seen, with only a suggestion of a colloid surrounded by four or five cells of the cluster. In many of these cells, rough endoplasmic cisternae were frequently dilated; the mitochondria and the Golgi complexes appeared normal. The cytoplasm also contained many free polyribosomes and some "lipid inclusions".

Inflammatory Infiltration and Giant Cells. The inflammatory infiltration was extensive and very destructive. It was principally formed by mononuclear cells with some polynuclears. Among the inflammatory cells, histiocytes or epithelioid cells predominated (Gusek, 1959). Lymphocytes, plasmocytes, monocytes and macrophages (Bernhard *et al.*, 1965) were recognized less frequently. When the follicle was disrupted, the invading cells very often occupied the whole colloid lumen (Fig. 2). Inflammatory cells could also be found inside the basement membrane between the thyroid cells (Figs. 1 and 2). Occasionally, the inflammatory cells formed large clusters of cells of one type. Such clusters of epithelioid cells were not always easily distinguished from clusters of thyroid cells. However, close examination revealed many differences. In the epithelioid cells, there were no terminal bars and no microvilli; the Golgi apparatus and the ergastoplasmic cisternae were not so prominent; the latter were not associated with the mitochondria in such an obvious manner as in the thyroid cells.

A large amount of connective tissue was present in the inflammatory reaction. Where very small follicles were prominent, bundles of collagen fibers became the major feature of the zones involved by thyroiditis. Fibers considered to be fibrin (Peach, 1962; Hovig *et al.*, 1968) and easily recognizable because of their high electron opacity, were sometimes mixed with the collagen and with microfibrils or perhaps reticulin fibers (Clark, 1962). No emperipolesis phenomenon was encountered.

"Giant cells" constitute the specific diagnostic criterion of De Quervain's thyroiditis. Cells diagnosed as giant on semi-thin sections corresponded to several different structural organizations when observed with the electron microscope. Their aspects varied from a closely associated cluster of epithelioid cells (Fig. 3) to an authentic syncytium (Figs. 4 and 5): all the intermediate stages could be

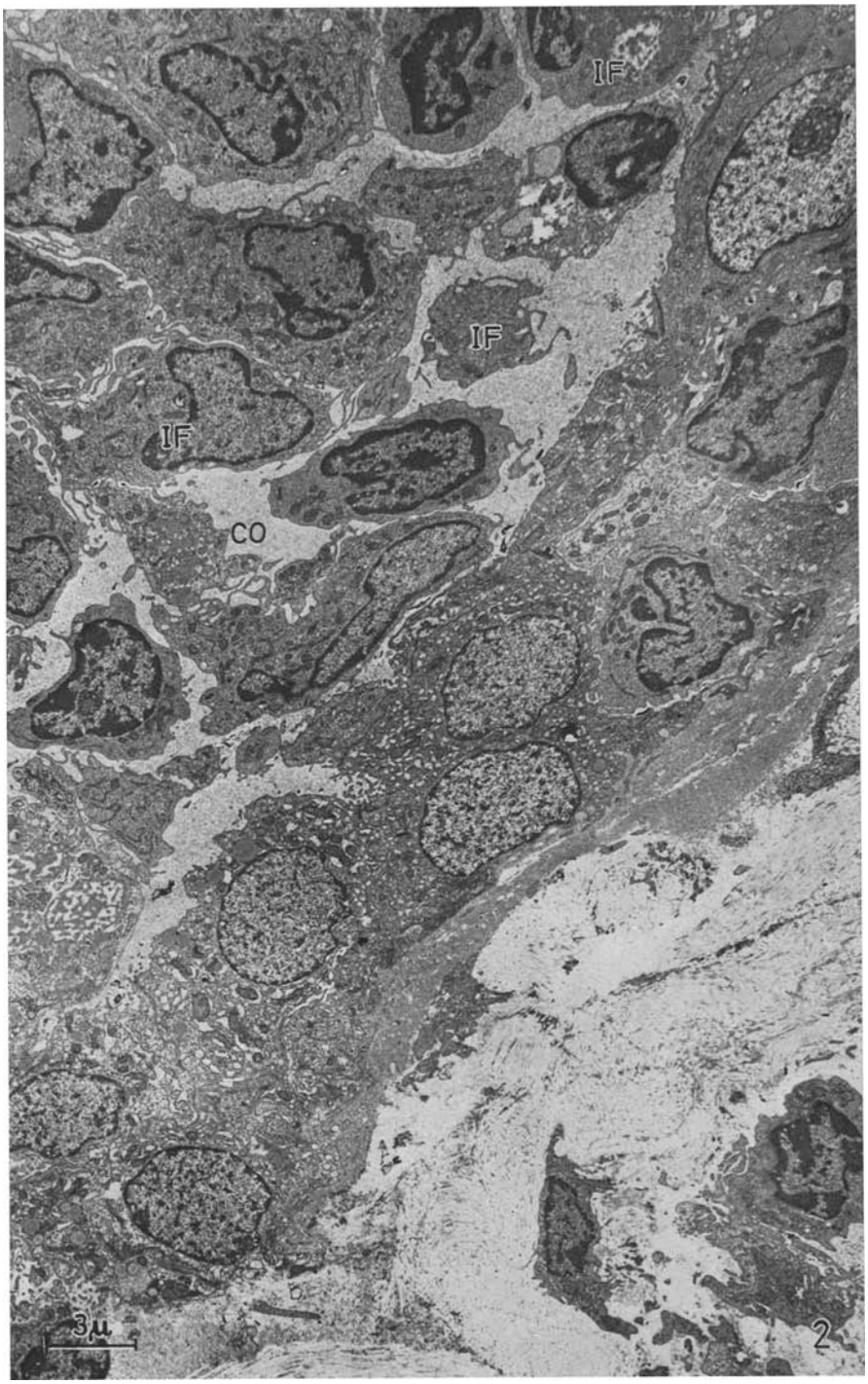

Fig. 2. Part of a ruptured follicle. Inflammatory cells (*IF*) are present in the colloid (*CO*) and between the thyroid cells. Basement membrane is falsely thickened and connective tissue is prominent outside of the follicle. ×4,000

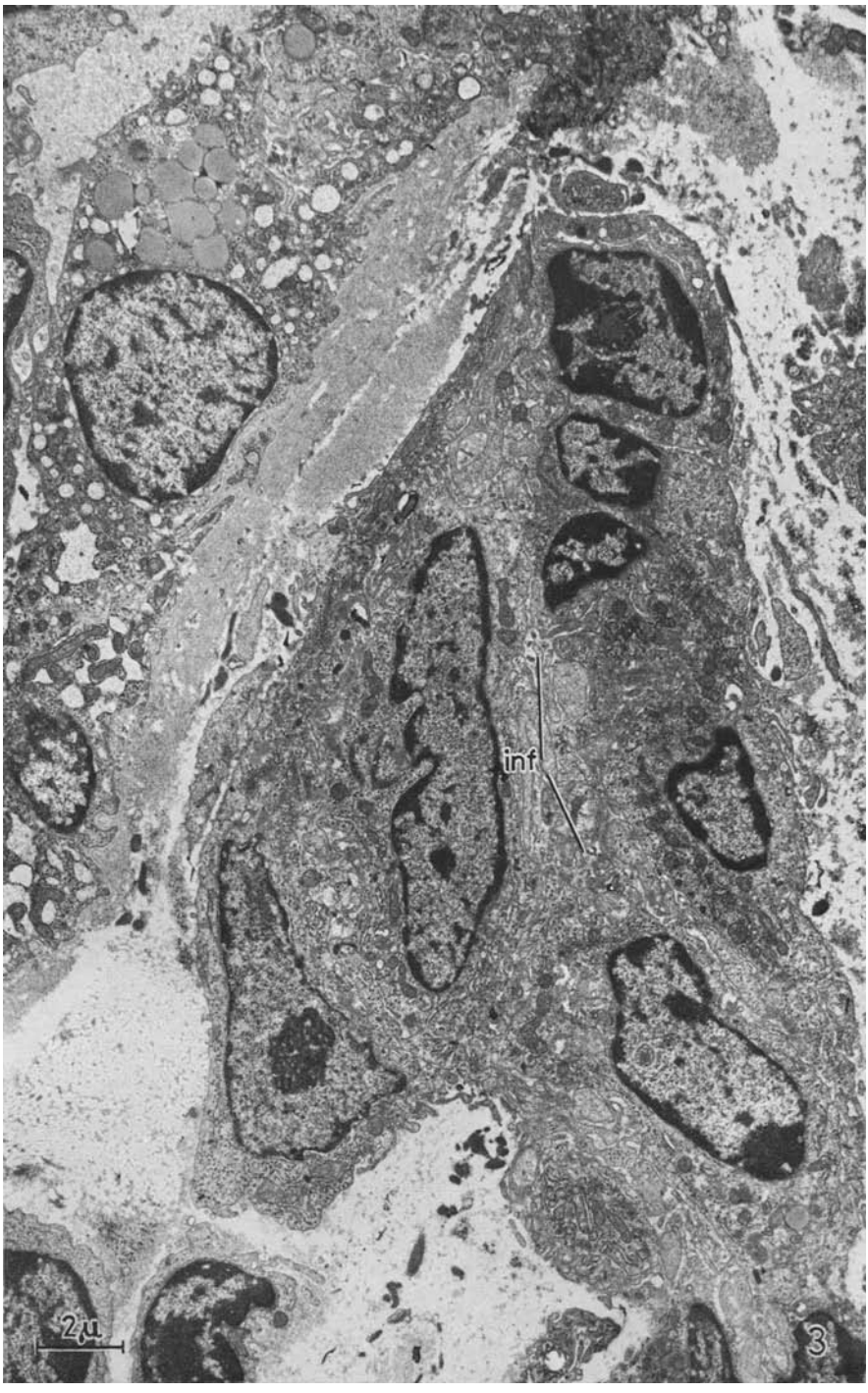

Fig. 3. Electron micrograph of closely clustered inflammatory cells corresponding to what was considered as a giant cell under the light microscope. Notice the infoldings (*inf*) connecting the cells. In the upper left corner, the follicle wall is made up of cells which contain dilated rough endoplasmic cisternae and "lipid inclusions". ×5,400

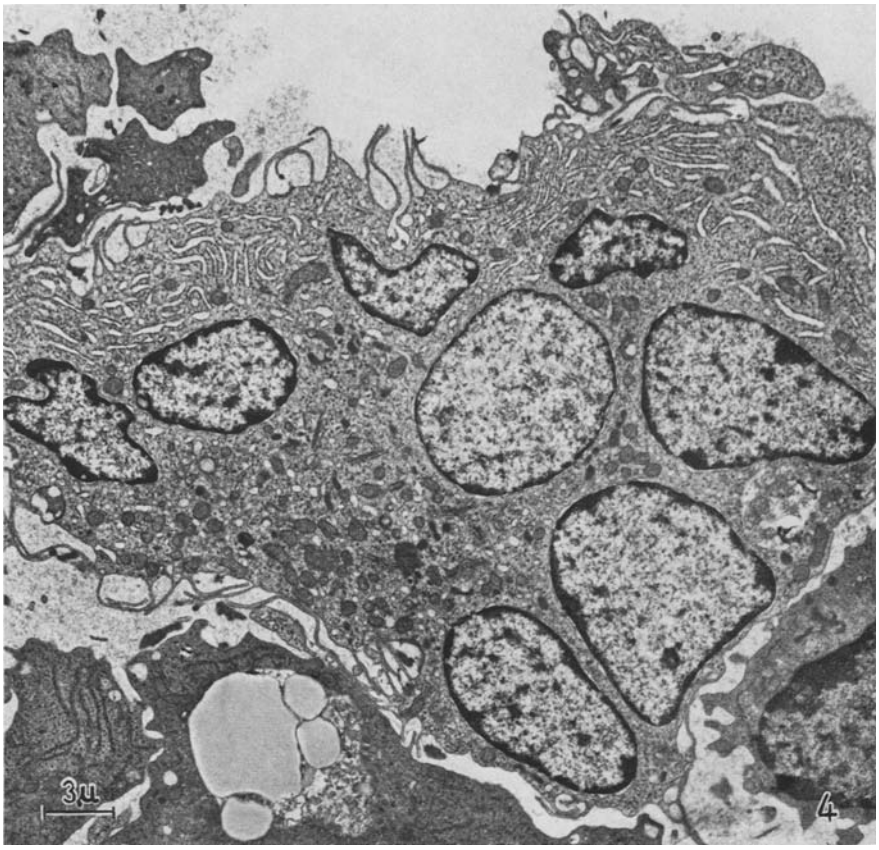

Fig. 4. Syncytial giant cell with peripheral cytoplasmic infoldings. ×3,000

encountered. Sometimes, the giant cells were made up of a single cytoplasm in which several nuclei, often with prominent nucleoli, were scattered. Parallel arrays of rough endoplasmic cisternae were not especially associated with the mitochondria and often occupied a peripheral position within the cytoplasm. Several groups of flattened Golgi cisternae, microfibrillar material, microtubules, dense bodies and "lipid inclusions" were seen throughout the cytoplasm. The amount of the "lipid inclusions" varied from one cell to another. Long and thin cytoplasmic infoldings surrounded with an amorphous substance and some supposed fibrin strands bristled the limits of the giant cells (Fig. 6). Sometimes, individual epithelioid cells were intimately fastened to an authentic syncytium by cytoplasmic infoldings. Alternatively, the peripheral parts of the giant cells were composed either by individual and completely limited cells or by cells the limits of which were visible at the exterior of the giant cell but became more and more difficult to distinguish toward the center of the giant cell and finally vanished (Fig. 6). In some cases, the center of the giant cell was completely different from its periphery, the center being occupied by a collection of osmiophilic hetero-

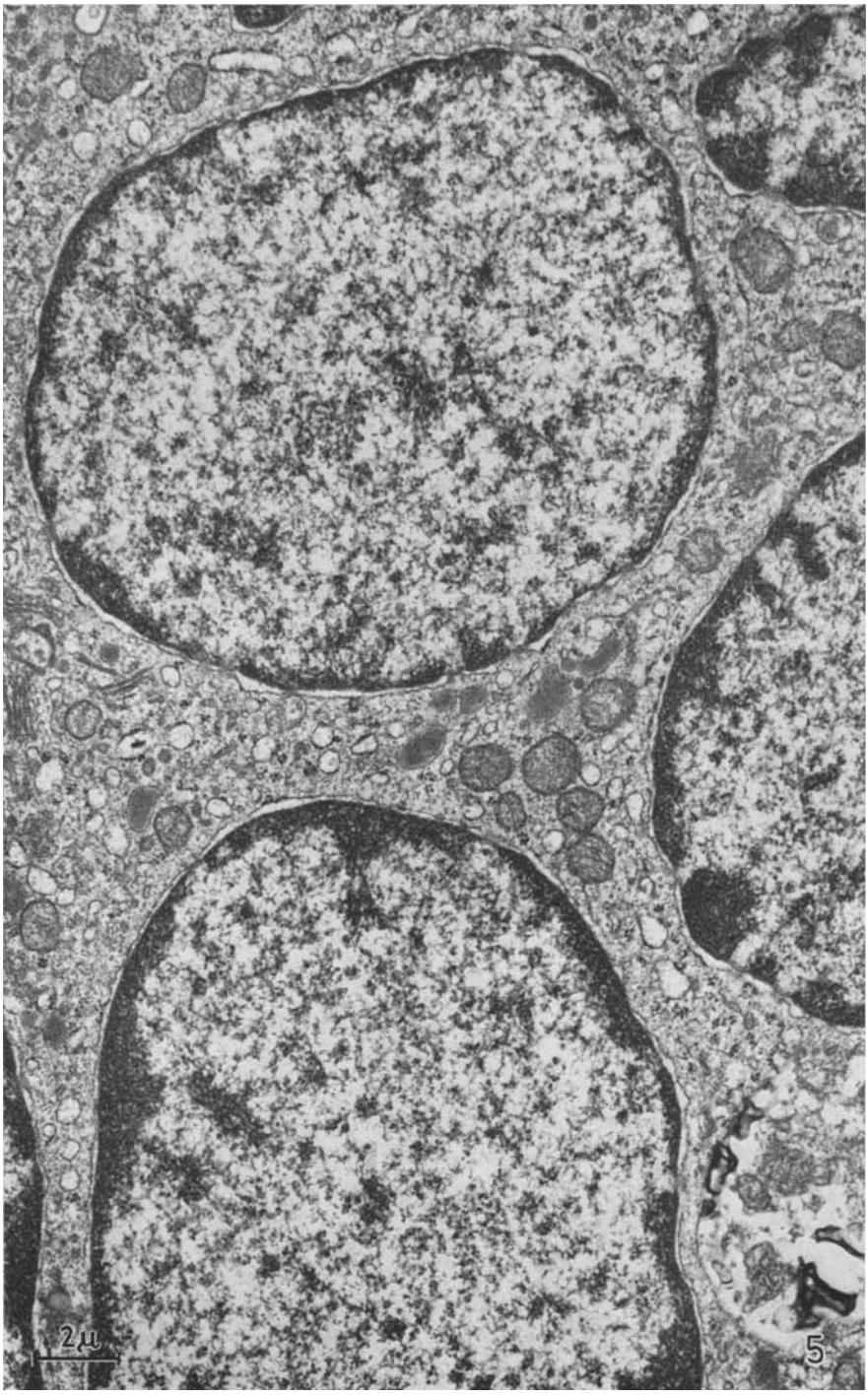

Fig. 5. Details of the former micrograph. No separating membranes exist between the different nuclei which are scattered throughout a single syncytial cytoplasm. × 6,000

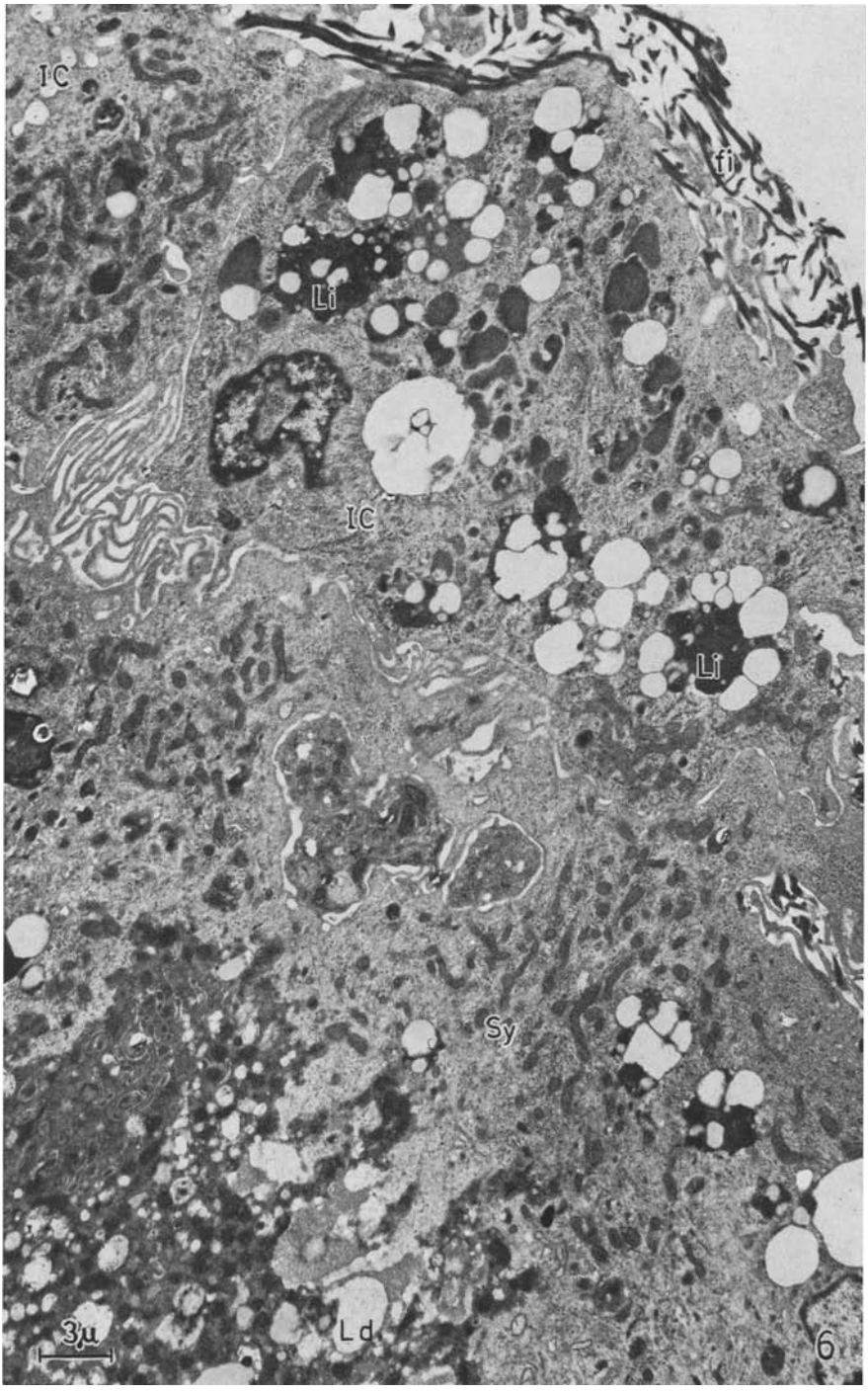

Fig. 6. Formation of giant cells. Peripheral individual cells (*IC*) with many "lipid inclusions" (*Li*) have not yet fused with the central syncytium (*Sy*). Strands of fibrin (*Fi*) are visible in the upper right corner. ×3,000

geneous dense material mixed with some remnants of nuclei and pale homogeneous droplets presumably of lipid nature (Fig. 6).

Whatever the structure of the giant cells, their component parts showed no resemblance to thyroid cells. Indeed, they had no border of microvilli, no terminal bars, no colloid droplets and no characteristic association of the rough endoplasmic cisternae with the mitochondria. Rather, they were similar to epithelioid cells (Gusek, 1959).

Giant cells which under the light microscope appeared to be formed by thyroid cells always corresponded to closely clustered individual thyroid cells without syncytium in electron microscopy.

No identifiable viral particles could be found either in the thyroid cells or in the inflammatory cells.

Discussion

As indicated in the first description of the disease (De Quervain, 1904), De Quervain's thyroiditis induces many changes in the thyroid and destroys the normal thyroid architecture.

The "lipid inclusions" observed frequently under the electron microscope presumably correspond to the "paravacuolar granules" observed under the light microscope by other authors (Söderström, 1952; Persson, 1968). Those inclusions have been considered by some as phagolysosomes (Heimann, 1966) or lipopigments (Toujas *et al.*, 1969). They have been described in normal human thyroids but in restricted number (Nève, 1965; Toujas *et al.*, 1969).

False thickening of the basement membrane and palisading of thyroid cells with cytoplasmic modifications were frequent findings in the present material. Previous histological studies (Stuart *et al.*, 1958; Meachim *et al.*, 1963) have already described a considerable "duplication" or thickening of the basement membrane.

The stratified aspect of some follicles suggested an increased number of cells per follicle, although no mitoses were seen. Increased mitoses could have occurred at an earlier time in the pathologic process. The stratification of cells seemed to involve a loss of cellular polarity as demonstrated by the lack of a border with microvilli and the random position of the centrioles. Apical plasma membrane protrusions, dilated cisternae, abundance of polyribosomes and well-developed Golgi zones were thyroid epithelial features which suggested enhancement of cellular activity (Nadler *et al.*, 1964). However, these morphological changes are not specific. No pseudopods or colloid droplets were seen. Thus the specific ultrastructural changes associated with increased thyroid activity, i.e. following the administration of thyrotropin (Wetzel *et al.*, 1965) were absent. The normal thyrotropin level and the absence of LATS in the serum suggest that the epithelial changes may be induced by the thyroiditis process itself, possibly by the neighboring inflammatory reaction, or by viral action or by any other unsuspected manner.

The inflammatory infiltration was strikingly different from that of autoimmune thyroiditis (Nève, 1969). The abundance of epithelioid cells and the aspect of the giant cells in De Quervain's disease resembled rather the granulomas of sarcoidosis (Wanstrup *et al.*, 1966) or of tuberculosis (Gusek *et al.*, 1959; Gusek, 1962).

As in those granulomas, fusion of mononuclear epithelioid cells seemed to be the origin of the syncytial giant cells. This is suggested by the existence of intermediate stages between a cluster of cells and a true syncytium. The heterogeneous material in the center of some giant cells seems to correspond to necrosis of cells and might be the expression of some senescence of giant cells.

The giant cells which under light microscope seemed to be formed by thyroid cells were shown to be only clusters of individual cells by electron microscopy. This explains the previously advanced hypothesis (Goetsch, 1940; Batolo *et al.*, 1967) of a thyroidal origin of the giant cells.

The present work does not support the idea that colloid eating by inflammatory cells underlies the pathogenesis of the disorder (Lindsay *et al.*, 1954; Meachim *et al.*, 1963). Neither the giant cells, nor the inflammatory cells showed colloid droplets. The latter were also exceptional in the thyroid cells.

In respect to the pathogenesis of the giant cells, the present observations recalled the process of fusion in ascitis cells infected by Sendai virus (Okada, 1969). This suggests that the disease might be induced by a virus. However, the present study failed to demonstrate virus particles and thus does not lend any positive support to this hypothesis.

In conclusion, the morphology of De Quervain's thyroiditis is specific and strikingly different from that of the other thyroidites.

A large part of this investigation was performed while the author was a "guest worker" in the laboratory of Physiology, National Cancer Institute, N.I.H., Bethesda, Maryland, U.S.A. The author would like to thank Dr. S. H. Wollman for his help, and Prof. P. A. Bastenie and Prof. P. Dustin for their advice and criticism of this manuscript.

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