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Immunhistochemical and Electron Microscope Analysis of Adenomas of the Thyroid Gland

II. Adenomas With Specific Cytological Differentiation

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Summary. Histological, immunhistochemical and electronmicroscopic studies of 12 human, scintigraphically "cold", thyroid adenomas with specific cytological differentiation identified four different cell types: oxiphil cell, clear cell, ergastoplasm-rich cell and mitochondrion-rich cell.

The oxiphil tumor cell can be recognized light-microscopically by its large size and its eosinophilic granular cytoplasm. Most of these cells do not produce thyroglobulin. The ultrastructural characteristics of oxyphil cells are principally mitochondria in great numbers and many large lysosomes. Clear cell adenomas show a trabecular growth pattern. The tumor cells have an abundance of cytoplasm which contains small acidophilic granules. Immunhistochemically we were able to demonstrate thyroglobulin in small amounts within cytoplasmic granules and more extensively within the follicle lumina. Electronmicroscopically we observed a large number of smooth surfaced vacuoles of varying size, extraordinary large lysosomes and occasional cisternae of rough endoplasmic reticulum, the latter probably corresponding to the immune-histochemically identified thyroglobulin granules. The ergastoplasm-rich-cell adenomas, which to the best of our knowledge have not been previously described, show a predominantly micro-to normofollicular architecture histologically without intrafollicular colloid. The cytoplasm of the ergastoplasm-rich cells reveales a strong positive thyroglobulin-staining reaction. The fine structure of these cells is characterized by the abundance of cisternae of the rough endoplasmic reticulum. The mitochondrion-rich-cell adenomas exhibited a microfollicular structure with an intensive acidophilic granular staining at the basal part of the tumor cells. Immunhistochemically and electronmicroscopically we found some morphologic and functional features which differentiate these cells from the oxyphil cell. Thyroglobulin was located predominantly in the apical portion of the cytoplasm in the mitochondrion-rich cells without sharp demarcation from the

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luminar thyroglobulin. Electron microscopically fewer basal and laterally located mitochondria were seen in mitochondrion-rich cells compared with oxyphil cells. As we could not find any sign of functional activity in the oxyphilic, clear cell and ergastoplasm-rich cell adenomas we analysed those aspects of the lysosomal system not concerned with the enzymatic digestion of thyroglobulin.

Key words: Thyroid – Adenoma – Ultrastructure – Immunhistochemistry – Thyroglobulin – Lysosomes.

Introduction

In a previous paper we examined cellular aspects of autonomous "hot" adenomas and scintigraphically "cold" adenomas (Dralle and Böcker, 1977). It was demonstrated that autonomous adenomas consisted of hypertrophic follicular cells while "cold" adenomas contain principle-(main-) cells that can not be distinguished morphologically from normal follicular cells. This publication presents histological, immunohistochemical and electronmicroscopic observations of 12 human thyroid adenomas with specific cytologic differentiation. In addition to the well known oxyphilic (Askanazy, 1898; Langhans, 1907; Wegelin, 1926; Lennox, 1948; Hamperl, 1950 and 1962; Tonietti et al., 1967; Klinck et al., 1970; Michel-Bechet et al., 1971; Kennedy and Thompson, 1974; Roediger, 1975) and clear cell adenoma (references see Stoll and Lietz, 1973), two further adenoma types presented: one type is composed of ergastoplasm-rich cells and the other of mitochondrion-rich cells.

The present study reveals new findings which might help to clarify some of the controversial questions regarding the cytogenesis and functional properties of these tumors. Lysosomes are directly related to the secretion of thyroid hormones. Therefore, the structural characteristics of the lysosomal system are described and discussed in terms of their relationship to the function of these adenomas.

Material and Methods

12 of 71 human thyroid adenomas included in this series were composed of cells which, based on lightmicroscopic, immunhistochemical and electron microscopic findings represented a specific cytological differentiation.

Scintigraphically all tumors were inactive on scan. For details of tissue preparation see Dralle and Böcker (1977).

Results

Specific differentitation was seen in the following types: 2 oxyphilic adenomas, 2 clear cell adenomas, 5 adenomas consisting of mitochondrion-rich cells and 3 consisting of ergastoplasm-rich cells. The tumor architecture varied considerably within each adenoma, nevertheless, the cytological features of each were sufficiently different to justify a distinction.

Light Microscopic Observations

In the two instances in which *clear cell adenomas* were examined microscopically the tumor cells were arranged in compact cords (Fig. 1 a). The hyperchromatic nuclei were small and round. There was an abundance of cytoplasm which contained small acidophilic granules. Small follicle lumina filled with colloid were seen occasionally.

Adenomas composed of *ergastoplasm-rich cells* showed a predominantly micro-to normofollicular architecture (Fig. 1 b). The follicle lumina did not contain colloid. The cytoplasm of the tumor cells was somewhat less abundant and had an acidophilc granular appearance. Nuclei were small or medium sized with moderate amount of chromatin.

Oxyphilic adenomas (Fig. 1 c) showed a predominantly trabecular pattern with the occasional formation of small follicles, the lumina of which contained only clumps of colloid. The polygonal cells were the largest in the whole series, with fine granular cytoplasm and distinct cell borders. A characteristic feature was a nuclei tendency to chromatin agglomeration with the formation of irregular configurations.

Adenomas of *mitochondrion-rich cells* (Fig. 1 d) exhibited a microfollicular pattern of growth, the tumor cells had a cuboidal shape. The cytoplasm showed an intensively acidophilic granular staining especially at the cell base. The nuclei were relatively large and hyperchromatic.

Immunhistochemical preparations showed typical thyroglobulin content and distribution patterns in the four adenoma types.

Nearly all *clear cells* contained small amounts of thyroglobulin located within small cytoplasmic granules (Fig. 2). The small follicle lumina usually showed a moderately intense staining.

The *ergastoplasm-rich cell* was characterized by the extraordinary abundance of cytoplasmic and the lack of extracellular thyroglobulin with empty follicle lumina (Fig. 3).

The *oxyphilic adenomas* contained a few cells which showed large amounts of thyroglobulin (Fig. 4). However, in most tumor cells no thyroglobulin was detectable.

In *mitochondrion-rich cell adenomas* thyroglobulin was predominantly located in the apical portion of the cytoplasm, without sharp demarcation from the thyroglobulin of the follicle lumina (Fig. 5).

Electron Microscopic Observations

The ultrastructure of the four cell types provided a rationale for the histological and the immunhistochemical observations. The fine structure of the *clear cell adenomas* has recently been described by Stoll and Lietz (1973). The cytoplasm contains many agranular empty vesicles of different sizes. Small areas of cytoplasmic matrix are occupied by oval or round cisternae of the rough endoplasmic reticulum (Fig. 6), mitochondria and extraordinary large lysosomes with a median diameter of 500 nm. These areas probably correspond to the immunhistochemically demonstrated thyroglobulin granules observed with the light microscope.

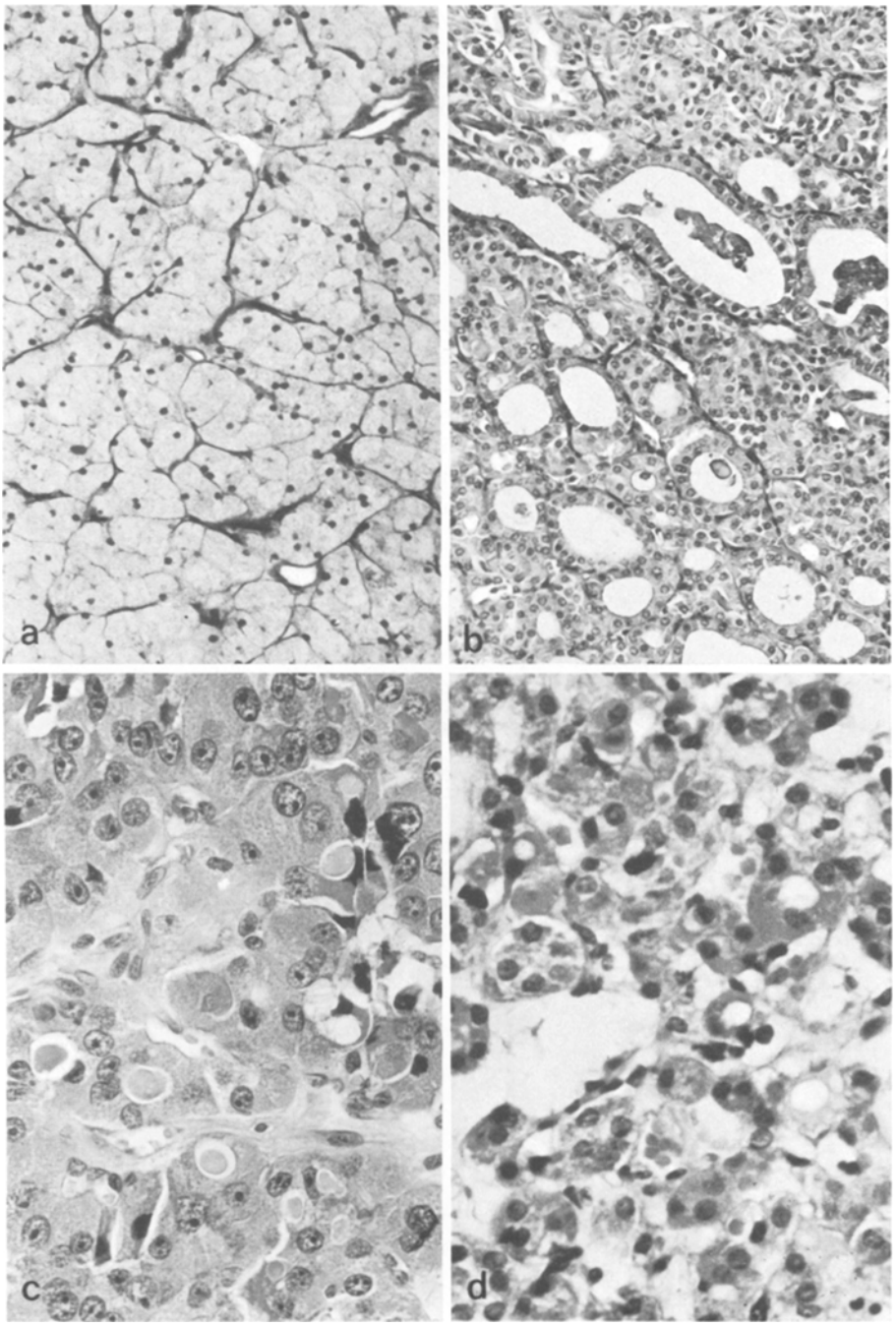
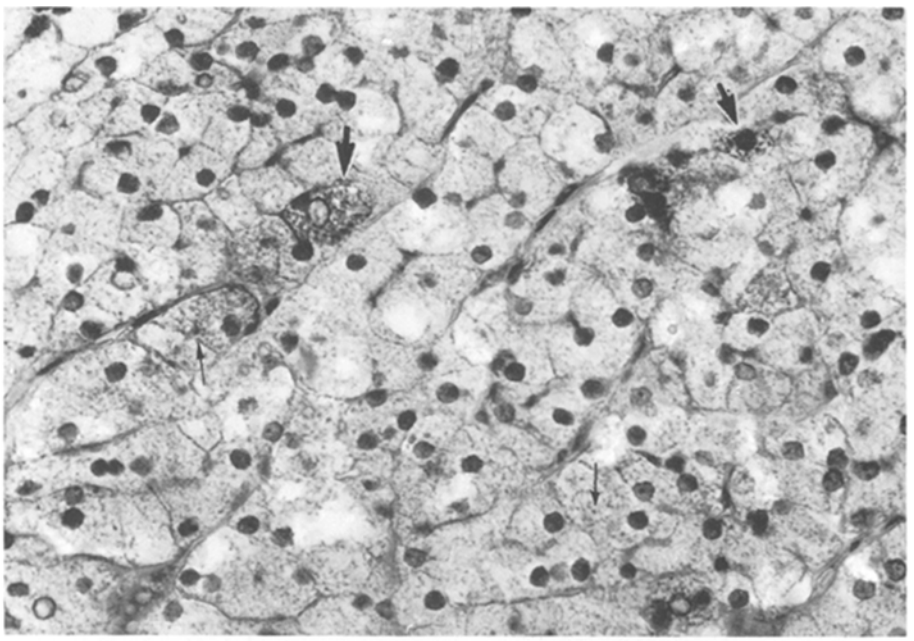


Fig. 1a–d. Histological aspects of adenomas with specific cytologic differentiation. **a** Clear cell adenoma. **b** Ergastoplasm-rich cell adenoma. **c** Oxyphilic adenoma. **d** Mitochondrionrich-cell adenoma. ×250



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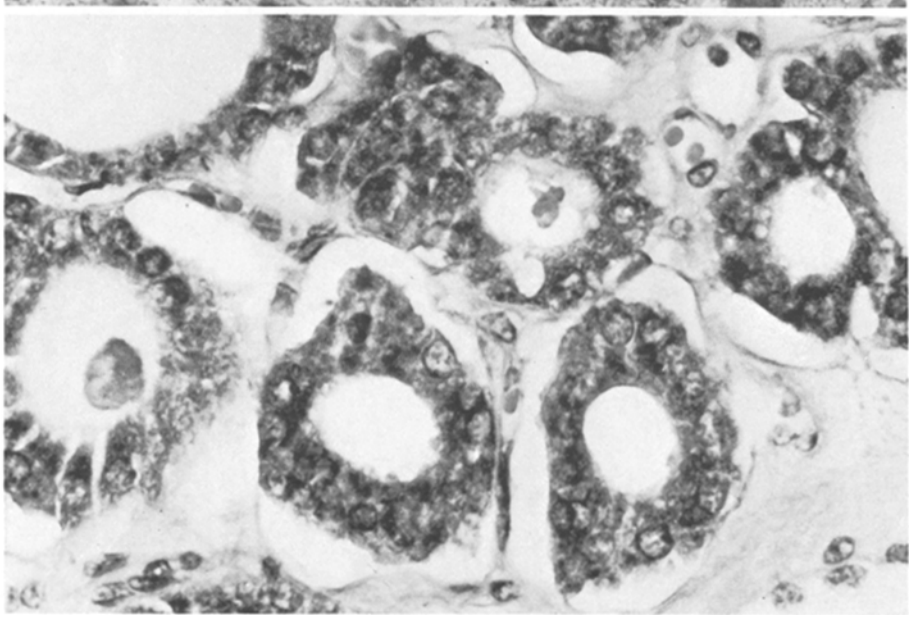

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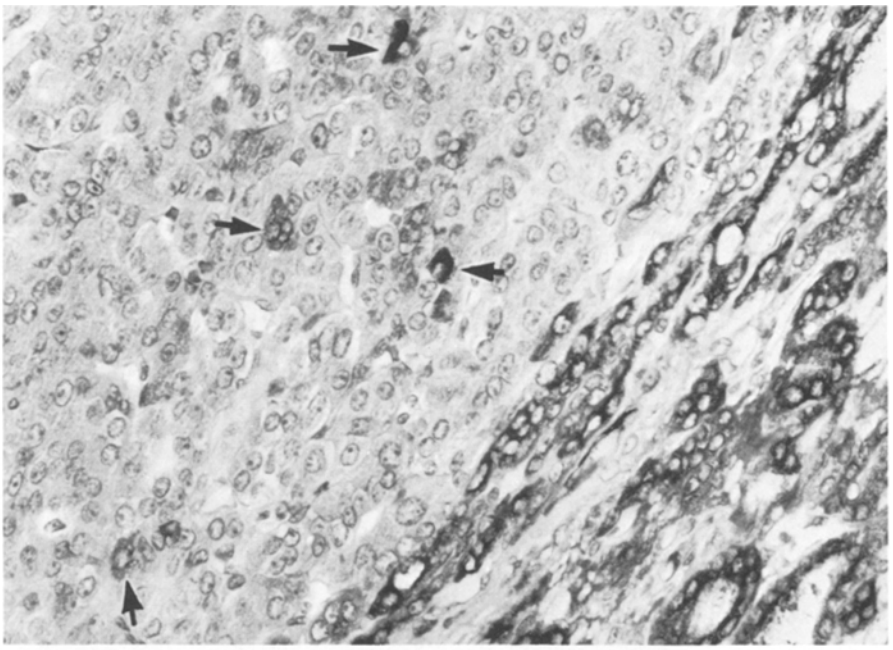
Fig. 2. Clear-cell adenoma. Immunhistochemical demonstration of thyroglobulin. Tumor cells reveal thyroglobulin in very small granules (*arrows*). ×250

Fig. 3. Ergastoplasm-rich-cell adenoma. Immunhistochemically the tumor shows a large amount of thyroglobulin which is almost completely confined to tumor cells. ×400



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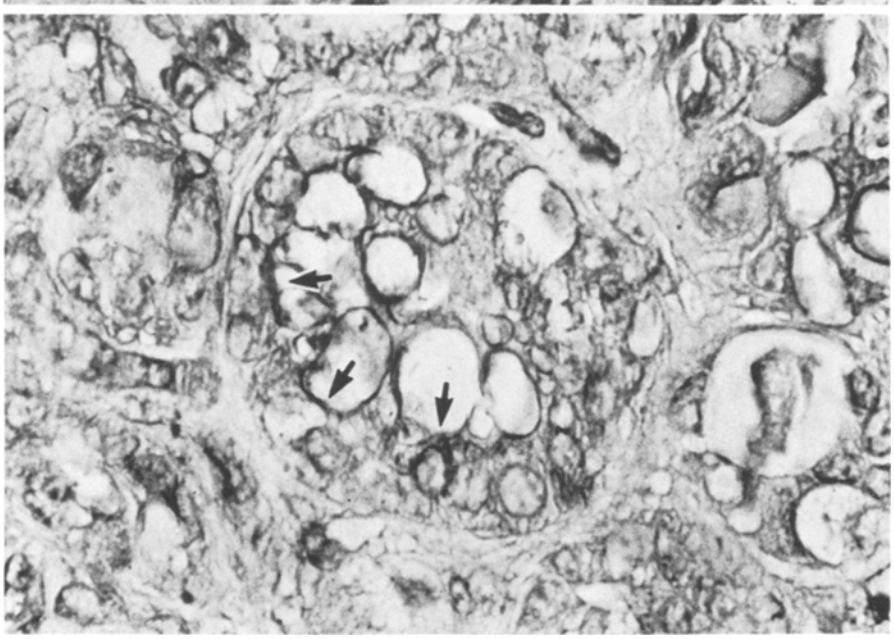

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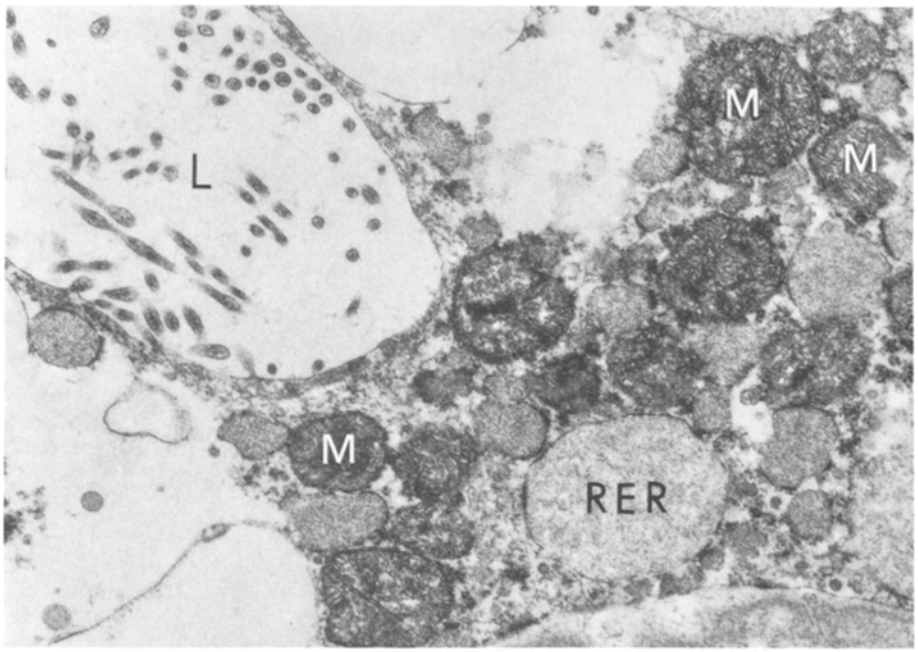
Fig. 4. Oxyphilic adenoma. Only few cells within the tumor contain thyroglobulin (*arrows*). On the right side normal, slightly compressed thyroid tissue is seen, the follicle cells of which contain a large amount of thyroglobulin. PAP-method. ×250

Fig. 5. Mitochondrion-rich cell adenoma. Immunhistochemically a moderate amount of thyroglobulin (*arrows*) can be localized within tumor cells and in the follicle lumen. ×540



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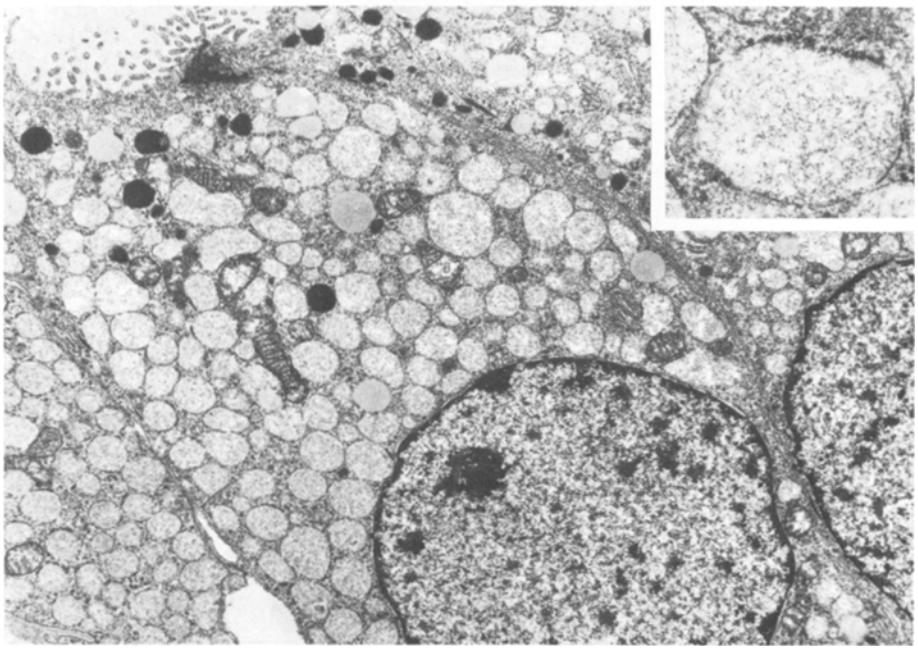

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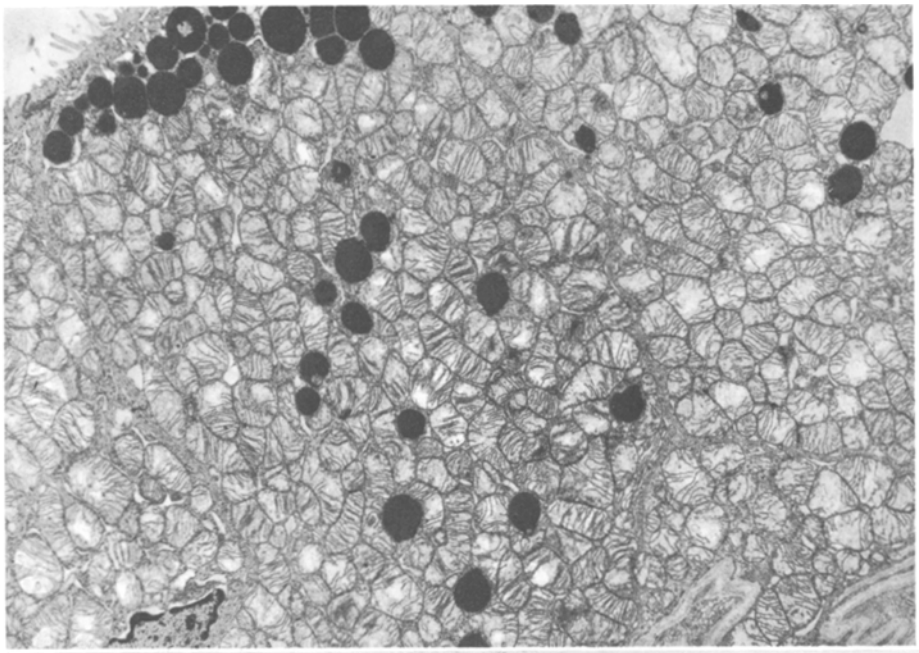
Fig. 6. Clear-cell adenoma. Apical portion of tumor cell with dilated cisternae of rough endoplasmic reticulum (*RER*) and mitochondria (*M*). *L* follicle lumen. ×21,000

Fig. 7. Ergastoplasm-rich cell adenoma. Tumor cell with numerous round cisternae of rough endoplasmic reticulum and large apically located lysosomes. ×7500. *Inset*: higher magnification of a cisterna. ×23,000



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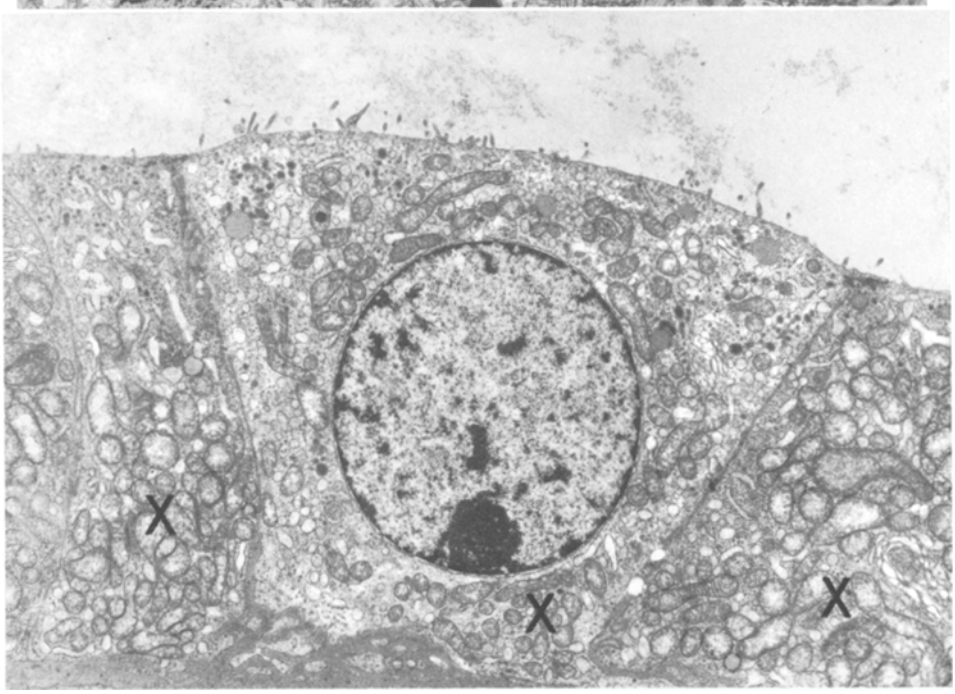

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Fig. 8. Oxyphil-cell adenoma. Tumor cell with numerous mitochondria and large lysosomes. No other cell organelles. × 5900

Fig. 9. Mitochondrion-rich cell adenoma. In contrary to oxyphilic adenoma cells, these tumor cells contain only small lysosomes and many cytoplasmic vesicles. Rough endoplasmic reticulum is well developed. In many tumor cells the mitochondria are basally located. (*X*). × 5800

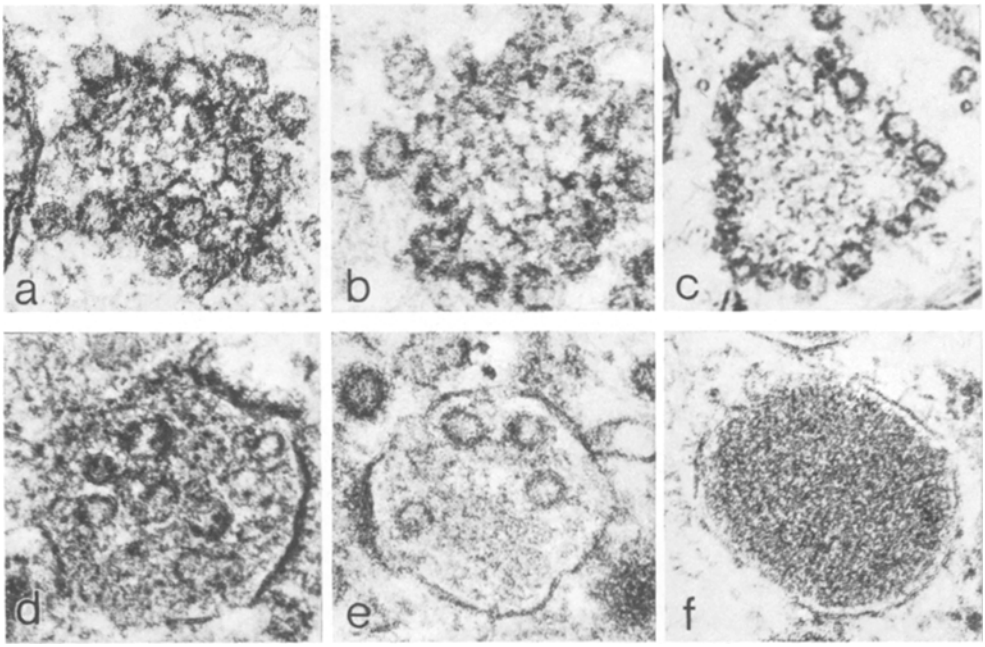

Fig. 10a–f. Stages in the formation of lysosomes from small Golgi vesicles. **a** Small cytoplasmic vesicles. **b** and **c** The membranes seem to dissolve and form a new common membrane (**d**). By inclusion of small vesicles, a multivesicular body results (**e**). **f** Lysosome. ×75,000

The ergastoplasm-rich cell (Fig. 7) contains cytoplasm full of ergastoplasmic cisternae with a finely granular colloid-like material. Golgi apparatus and cytoplasmic vesicles are barely developed. Lysosomes, which occur in moderate number in the apical cytoplasm, are large and correspond in size to the lysosomes of oxyphil and clear cells.

The ultrastructural features of *oncocytes* (Fig. 8) are well known from previous publications (Hamperl, 1950, 1962; Heimann et al., 1973; Valenta et al., 1974). Mitochondria are present in large number. The large lysosomes, mainly located apically, have a median diameter of 370 nm. All other cell organells are present in small numbers.

Harcourt-Webster (1968) and Tremblay and Pearse (1960) in a histochemical study were able to distinguish a particular cell from the Hürthle-cell. It was characterized by an abundance of mitochondria and by being smaller than oxyphil cells and was called the *mitochondrion-rich cell* (Fig. 9). The present immunhistochemical and electron microscopical analysis of adenomas confirms the existence of this cell type. When compared with the typical oxyphil cell the mitochondrion-rich cell is characterized by a smaller number of usually basally and laterally localized mitochondria, and the presence of many small lysosomes with a diameter of 160 nm and few lamellae of the rough endoplasmic reticulum in the cytoplasm. In contrast to other adenomata, numerous small cytoplasmic vesicles are seen throughout the cytoplasm.

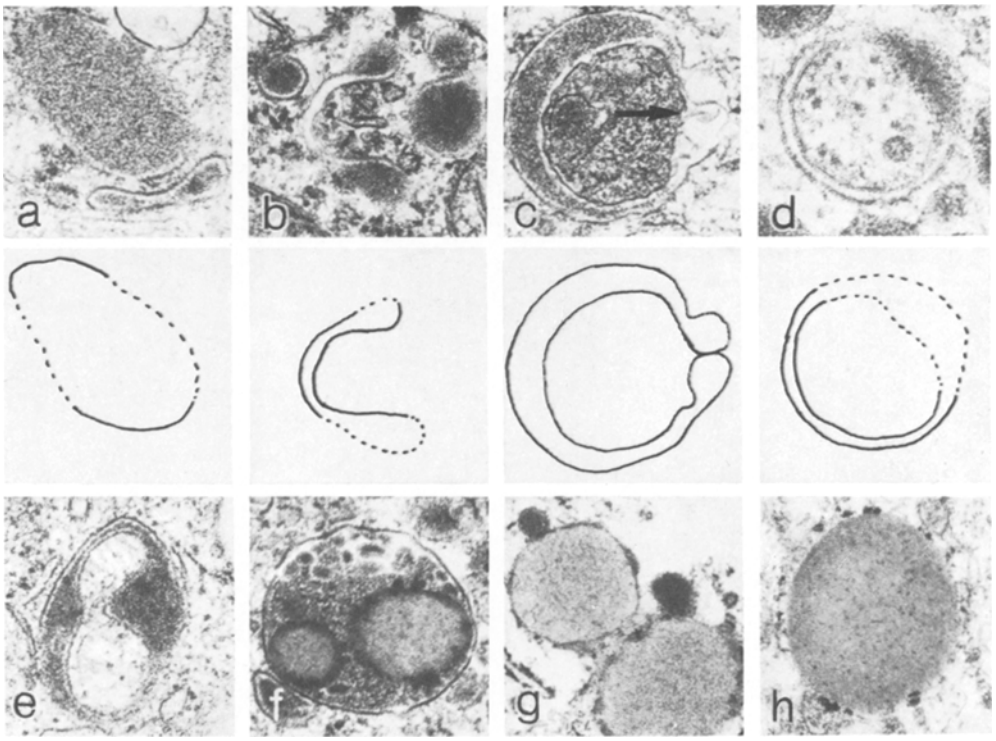

Fig. 11. Cellular autophagy. Suggested steps in the formation of autophagic vacuoles. **a** normal lysosome. **b** and **c** increasing invagination of the lysosomal membrane with segregation of a cytoplasmic area. **d** and **e** By fusion of the touching ends (arrows) an autophagic vacuole results. **f** Transformation to secondary lysosomes by dissolution of the inner membrane. **f, g, h** Lipofuscine granules. × 49,000

The Lysosomal System

In the thyroid gland the lysosomal enzymes play a part in the elaboration of hormones (Wollman et al., 1964; Seljelid, 1975) and also with autophagic processes (Nunez and Becker, 1970). From their general morphological aspects, it is suggested that the oxyphilic, the clear cell and the ergastoplasm-rich cell adenomas are functionally inactive: for this reason these cells seem suitable for a study of those aspects of the lysosomal system that are not concerned with the enzymatic digestion of thyroglobulin.

According to our ultrastructural and cytochemical studies the lytic system of these cells can be devided into the following components (Fig. 10): 1. Small cytoplasmic vesicles with contents of low electron density, which probably originate from cisternae of the Golgi field. 2. Typical lysosomal dense bodies of varying size. 3. Multivesicular bodies and 4. autophagic vacuoles. Clusters of small smooth surfaced vesicles are frequently seen at the ends of the Golgi cisternae. Some have contents of high electron density and seem to represent small primary lysosomes with diameters of 60–110 nm. Another type of small

vesicle with contents of low electron density is also observed. From these vesicles, multivesicular bodies seem to evolve in several steps (a) small vesicles, which probably originate from Golgi cisternae, are first localized in small groups. (b) The membranes of the outer vesicles apparently fuse and form a large vesicle, the newly formed membrane of which has irregular outlines with patchy thickening. As this larger body usually includes a few of the original small vesicles, a mulitivesicular body appears. Acid phosphatase preparations clearly reveal a number of small vesicles within the multivesicular bodies. These show a positive staining reaction, indicating that they belong to the lytic system of the cells.

Autophagosomes and/or autophagolysosomes are rarely encountered in adenomas. The membrane kinetics of autophagic vacuoles are unknown (Ericsson, 1969; Pfeifer, 1976). During the present study images were found (Fig. 11) which resemble those found by Nunez and Becker (1970) in the bat thyroid and which suggest that autophagic vacuole seems to result from an invagination of the lysosomal membrane itself, with lysosomal enzymes, located between the two membranes, forming the autophagic vacuole. Transformation to the autophagolysosomes seems to result from dissolution of the inner membrane.

Discussion

In our analysis of 71 human thyroid adenomas the following five cell types were distinguished:

1) Main (principle) cell (including "undifferentiated" cells in trabecular adenomas, atrophic cells in macrofollicular and hypertrophic cells in autonomous adenomas).

2) Oxyphil (oncocytic) cell = Askanazy cell (Fig. 12a).

3) Clear cell (Fig. 12b).

4) Ergastoplasm-rich cell (Fig. 12c).

5) Mitochondrion-rich cell (Fig. 12d).

87% of the tumors studied were main cell adenomas, as already described (Dralle and Böcker, 1977). 17% of the adenomas (cell types 2–5) analysed in this study revealed architectural and cytologic features which were easily distinguished by light microscopic, immunhistochemical and by electron microscopic features. These were regarded as adenomas with specific cytological differentiation.

Cell types 2–5 were characterized by a selective increase of *one* cell organelle with a decrease in number and/or in size of all or most other cell organelles. The organelles involved were the mitochondria in the oxyphil cell and mitochondrion-rich cell, the smooth endoplasmic reticulum in the clear cell and the rough endoplasmic reticulum in the ergastoplasm-rich cell.

The best studied example is the *oxyphil cell.* This cell type can easly be recognized light-microscopically by its large size, eosinophilic granular cytoplasm (see recent review by Roediger, 1975), its high content of oxydative mitochondrial enzymes, basophilic granularity of the cytoplasm after phosphotuncsic-acid-hematoxyline staining, and by reduction and/or lack of thyroglobulin synthesis.

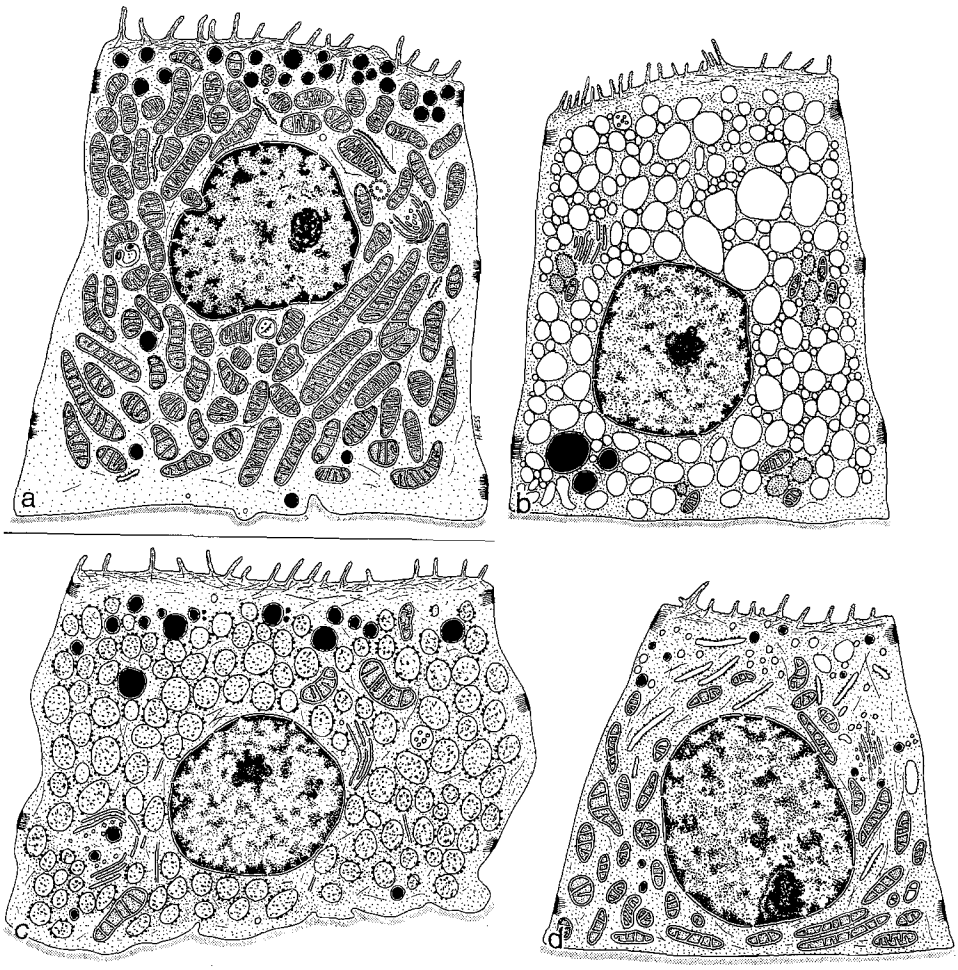

Fig. 12a–d. Schematic diagram of the different cell types. **a** Oxyphil cell, **b** clear cell, **c** ergastoplasm-rich cell, **d** mitochondrion-rich cell

Our immun-histochemical findings clearly indicate that most of the cells of oxyphilic adenomas do not produce any thyroglobulin. But there are few cells which showed a very intensive reaction and which were considered to be main cells. The nature of the process leading to oxyphil cell change is unknown. Recently it has been suggested that the oxyphil cell represents a metaplastic follicle cell, in contrast to the earlier views of Hamperl (1950).

In an ultrastructural analysis Stoll and Lietz (1973) have analyzed the *clear cell*. It is characterized by an abundance of smooth surfaced vacuoles of varying size which either develop from the Golgi field or from endocytotic vesicles. Whereas earlier authors (Langhans, 1907) have suggested an origin of the clear cell from the parathyroid, most authors now (Hedinger et al., 1967) accept that this cell is derived from the follicle cell. The present study revealed cytologic

features supporting the latter hypothesis. These features are the immunhistochemically demonstrated thyroglobulin and the electronmicroscopically observed profiles of rough endoplasmic reticulum, Golgi complexes and lysosomes in most of the tumor cells.

The *ergastoplasm-rich cell* has not been previously described. Morphologically the cell is characterized by its abundance of cisternae of the rough endoplasmic reticulum which are arranged in round profiles. They contain a large amount of thyroglobulin, which can be demonstrated immunhistochemically. Lightmicroscopically the cell body is smaller than the oxyphil cell, the cytoplasm is eosinophilic and granular so that our original histological diagnosis was oxyphilic adenoma. On PAS-staining these tumors show a slightly positive cytoplasmic reaction but even in areas with well formed follicles there is characteristically no or very little colloid. On the basis of the morphological features, it is suggested that this cell type, like the oxyphil and clear cell, represents a well defined and easily recognized metaplastic follicle cell.

In contrast to the cells described above, the *mitochondrion-rich cell* remains somewhat enigmatic in its functional activity and cytogenesis. From immunohistochemical findings it is evident that this cell synthesizes and secretes thyroglobulin into the follicle lumen. In addition, it is characterized by its large number of mitochondria, its many small lysosomes (in contrast to oxyphil cells), some stacks of rough endoplasmic reticulum and a moderately prominent Golgi field. It is our contention that this cell type corresponds to the mitochondrion-rich cell which according to the histochemical studies (DPN-diaphorase, succinate-dehydrogenase, TPN-diaphorase, TPN-linked isocitrate dehydrogenase activity) of Tremblay and Pearse (1960), Harcourt-Webster and Scott (1966) and Valenta et al. (1974) is found in a variety of thyroid disorders. To the best of our knowledge it is the first time that this cell has been found to be the predominant cell type in a human thyroid adenoma.

The morphological findings obtained suggest that cell types 2–4 are completely inactive hormonally and, furthermore that they do not even degrade thyroglobulin. Direct evidence supporting hormonal inactivity is provided by lack of thyroglobulin in oxyphilic adenoma cells and decrease in the number of those organelles involved in thyroglobulin-transport, i. e. the Golgi complex and the small cytoplasmic vesicles (exocytic and endocytic). An interesting finding is that in cell types 2–4 the lysosomes when compared with hormonally active cells (autonomous adenomas and thyroids in thyrotoxicosis) are very large (360 nm in diameter compared with 250 nm in hormonally active cells).

As the oxyphil cell is generally a non-thyroglobulin synthesizing and degrading cell, and as the lysosomes in this cell are very numerous, it may be hypothesized that large lysosomes represent the morphological equivalent of inactivity and possibly represent "physiological" enzyme storage. In the normal cell this is transformed to smaller lysosomes if required. It is suggested that the lysosomes are derived from the Golgi cisternae (Sobel, 1962; Seljelid, 1967; Coleman et al., 1968) but another mode of formation of the typical thyroid lysosomes was suggested by Nunez and Becker (1970). These authors are of the opinion that they develop from the multivesicular body, which in turn derives from the Golgi field (see Fig. 13). From our own electron microscopic findings it

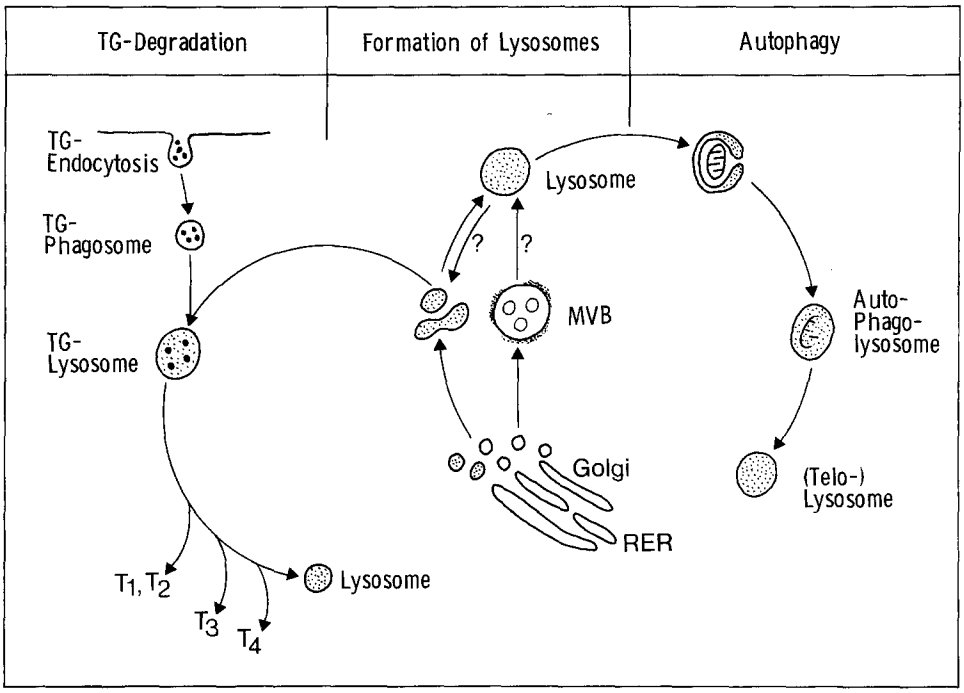

Fig. 13. Schematic diagram of the lysosomal system with thyroglobulin-degradation, formation of lysosomes and cellular autophagia

is suggested that both pathways are possible in thyroid adenomas. Some of the images obtained seem to suggest that the outer membrane of the multivesicular body develops from the small vesicles by a very complex process which is not clearly understood and deserves further study. Nevertheless, histochemical studies (Böcker, 1978) clearly indicate that multivesicular bodies belong to the lytic system of the adenoma cell.

The mode of formation of *autophagic vacuoles* has been seen in a number of different cell types (ref. see Pfeifer, 1976). Most hypothesis assume that the membranes surrounding autophagolysosomes developed from the endoplasmic reticulum (Hugon and Borgers, 1965; Glinsman and Ericsson, 1966; Cole et al., 1971; Ericsson et al., 1975) and/or from Golgi membranes (de Duve and Wattaux, 1966), lysosomal membranes (Novikoff et al., 1964; Seljelid, 1966; Seljelid and Ericsson, 1966; Bartok et al., 1967; Dixon, 1967) or de novo synthesis (Ashford and Porter, 1962; Napolitano, 1963). Nunez and Becker (1970) in their study of the bat thyroid suggest that autophagic vacuoles may also develop from the lysosomes themselves. From our own observations the same mechanism seems to occur in human thyroid adenomas.

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