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Immunhistochemical and Electron Microscope Analysis of Adenomas of the Thyroid Gland

I. A Comparative Investigation of Hot and Cold Nodules

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Summary. Histologic, immunhistochemical and electron microscopic changes in 8 cases of scintigraphically proven autonomous thyroid adenomas are described and compared with non-functioning adenomas. Adenomas with a light microscopic appearance suggesting autonomy show follicles which are mainly small or normal sized and lined by columnar thyroid cells with a partly vacuolated and eosinophilic cytoplasm. Immunhistochemically a high content of thyroglobulin corresponds to the amount of rough endoplasmatic reticulum. Typical ultrastructural criteria are a well developed cytocavitary network, numerous mainly apically localized lysosomes, prominent Golgi fields with sprouting vesicles and autophagic vacuoles. The cell surface is, when compared to non-functioning adenomas, enlared apically by numerous long microvilli and basally by deep infoldings. On contrast to experimentally TSH-stimulated animal thyroids, colloid droplets and pseudopodia are rare. The morphological findings are compared with recent biochemical results and the diagnostic value of electron microscopy is discussed.

Key words: Thyroid gland – Thyroid adenoma – Ultrastructure – Immunhistochemistry – Thyroglobulin – Lysosomes.

Introduction

The autonomous thyroid adenoma is a benign, endocrine active tumor with normal or increased production of thyroid hormones. The clinical diagnosis of an autonomous adenoma is based on the scintigraphic analysis after exogenous suppression with T_3 and/or stimulation of the thyroid tissue with TSH.

A further subdivision of autonomous adenomata into compensated and decompensated types results from purely radiodiagnostic criteria (Börner et al., 1971; Heinze et al., 1975). Scintigraphically, autonomous adenomas are not

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always hot areas, but may represent warm or even cold nodules within the thyroid tissue. In the differential diagnosis of hot and warm nodules, mainly TSH-dependent and TSH-independent adenomas have to be differentiated (Miller and Hamburger, 1965; Silverstein et al., 1967). Cold nodules are either non-functioning adenomas (Horst et al., 1960; Meadows, 1961; Huber and Riccabona, 1962; Miller et al., 1965), haemorrhagic or other cysts (Uthgenannt and Weinreich, 1965; Pfannenstiel, 1974; Ma and Ong, 1975; Galvan and Pohl, 1976) or, as in 10 to 30 percent, malignant tumors (Groesbeck, 1959; Horst et al., 1960; Meadows, 1961; Zukschwerdt and Horst, 1962; Börner et al., 1965; Fuchsig and Keminger, 1967; Pörtener and Ungeheuer, 1967; Brown and Kantounis, 1975). Thus the morphologist has to help decide wether cold nodules represent malignant or benign processes.

In cases of scintigraphically hot nodules a lightmicroscopical judgement of the funtional activity of thyroid tumors is regarded as impracticable by most authors (Bay, 1965; Zukschwerdt, Bay and Horst, 1963; Miller and Hamburger, 1965; Horst et al., 1967; Thiemann, Bay and Jäniche, 1967; Fuchsig and Keminger, 1967; Zeidler et al., 1969; Schneider, Thiemann and Bay, 1970; Linder and Voigt, 1971; Pohl et al., 1973). However Fontolliet-Girardier (1972) was able to wolk out some characteristic lightmicroscopical criteria for the autonomous adenoma in a histologic analysis. These included an increased number of follicular cells, a distinct eosinophilia, cytoplasmic granulations and poorly defined limits between the adenoma and the extranodular tissue.

As lysosomes play an important role in the digestion of thyroglobulin and in the elaboration of the specific thyroid hormones it seems reasonable to assume that adenomas with different functional activity display ultrastructural differences in their lysosomal system. It is evident, therefore, that a study of autonomous adenomas must include a comparative fine structural analysis of the morphological alterations of the lysosomal systems of functioning and non-funtioning thyroid adenomas. Thus the aim of this study is to analyse the ultrastructural characteristics of hyperfunctioning and non-functioning thyroid cells and in this way to improve the differential diagnostic criteria for thyroid adenomas. Later, morphologic findings are compared with biochemical results to obtain more information concerning the cytopathology of these tumors.

Material and Methods

Between 1973 and 1976 71 thyroid adenomas were investigated both by light and electron microscopy. Specimens were obtained from the Surgical Department of the University of Hamburg (Chief: Prof. Dr. H.-W. Schreiber). In 42 of these cases a thyroid scintiscan was performed. 13 of them were hot nodules, 29 cold nodules. 8 thyroid adenomas could be diagnosed as autonomous adenomas by scintographic examination after suppression and/or stimulation.

For light microscope, the tissue was fixed in neutral formalin and embedded in paraffin. Paraffin sections were stained with the H-E, PAS and Giemsa reaction. For the immunhistochemical investigations, the same paraffin material was used. After deparaffinizing, the sections were incubated with a rabbit-anti-human-thyroglobulin, a goat-anti-rabbit-immunglobulin and finally with a peroxidase-anti-peroxidase-complex according to the method of Sternberger (Sternberger et al., 1970). After being stained by the diaminobenzidine-reaction (Graham and Karnowsky, 1960), these sections were examined in a normal light microscope.

Table 1a. Age of patients

Adenoma type	Mean	Range	*N*
Autonomous adenomas	42	32–56	8
"Morphologically active adenomas"	36	28–47	3
Cold normofollicular adenomas	41	13–66	22
Trabecular-microfollicular adenomas	48	30–64	9

Table 1b. Sex of patients

Adenoma type	Male	Female
Autonomous adenomas	3	5
"Morphologically active adenomas"	3	0
Cold normofollicular adenomas	2	20
Trabecular-microfollicular adenomas	1	9

Table 1c. Nodular weight of the operation sections

Adenoma type	Nodular weights (grams)
Autonomous adenomas	12–40
"Morphologically active adenomas"	5–20
Cold normofollicular adenomas	4–85
Trabecular-microfollicular adenomas	3–45

For electron microscopic investigation, thyroid tissue was postoperatively fixed in 2.5% glutaraldehyde immediately and postfixed with 1% osmium tetroxide solution and Epon 812. Ultrathin sections were contrasted with uranium acetate and lead citrate and examined with a Philips E 300 electron microscope. Quantitative analyses of the lysosomal system were performed on 8 autonomous adenomas, 3 "morphologically active adenomas", 31 cold adenomas of the normofollicular and trabecular-microfollicular type and 10 adenomas with special cytologic differentiation.

Results

In the present study the results of our investigations on 8 autonomous adenomas and 31 nonfunctioning adenomas are described. Lightmicroscopically three of the follicular adenomas which were not examined scintigraphically turned out to be adenomas with morphological criteria of increased endocrine activity. Furtheron these adenomas will be called "morphologically active adenomas". The findings on adenomas with special cytologic differentiation are published elsewhere (Böcker and Dralle, 1977).

Clinic. The age and sex of the patients and weights of the tumors are recorded in Table 1. Table 1a shows the age distribution of our patients. In Table 1b (the sex of the patients) the relatively high portion of male patients with autonomous adenomas and "morphologically active adenomas" is striking. But the

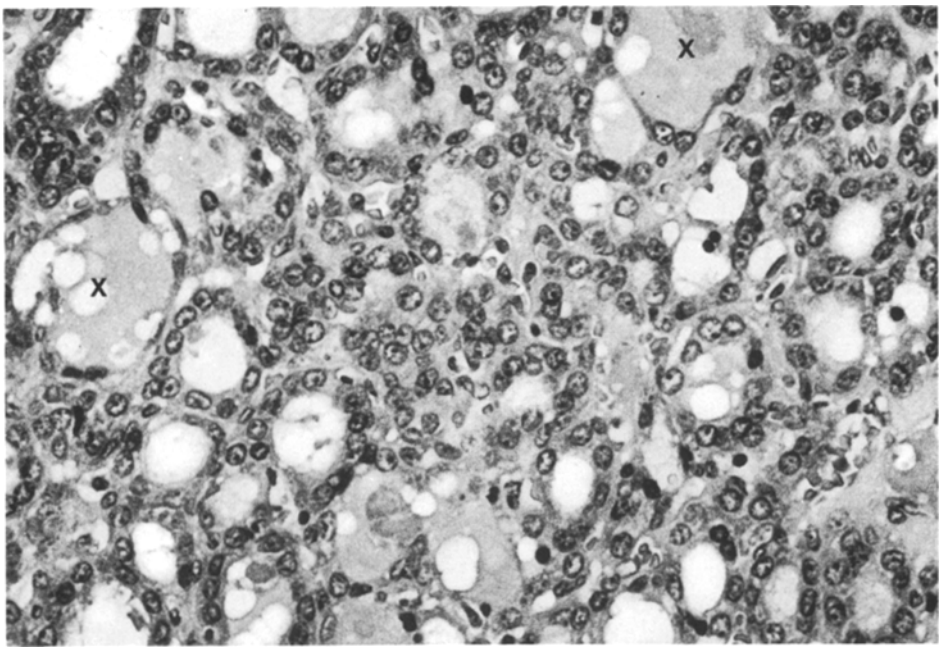

Fig. 1. Autonomous adenoma. Normofollicular organization with only few larger follicles lined by a flat epithelium (*x*). Thyrocytes are partly vacuolated. ×250

comparatively low number of adenomas in both groups does nor allow any conclusions to be drawn. The weights of tumors are listed in Table 1c.

Morphology. Lightmicroscopically follicular organization predominates among the autonomous adenomas. Only in one case was a trabecular-microfollicular pattern discovered. Usually the follicles are small to normofollicular, larger follicles are found only rarely and these are lined with a flat epithelium (Fig. 1). The cytoplasm of the thyrocytes is partly vacuolated and only slightly or moderately eosinophilic. This results in a faded appearance of the tumor compared to normal tissue. In addition, mainly foamy colloid predominates in larger follicles. Marginal vacuoles can be seen only in part of the adenomas. The 3 "morphologically active adenomas" have the same light microscopic appearance. Scintigraphically cold adenomas may show either a follicular or a trabecular-microfollicular pattern. Those of follicular structure consist of medium-sized to large follicles with cuboidal or flattened follicular cells. The colloid is intensely stained. The cell borders are usually more distinct than those of autonomous adenomata.

The trabecular-microfollicular adenomas are in contrast characterized by their greater structural immaturity with a growth pattern seen in embryonic thyroid gland. The tumor cells contain a large nucleus and have a narrow cytoplasmic ring. Only in few cases are genuine microfollicular areas seen.

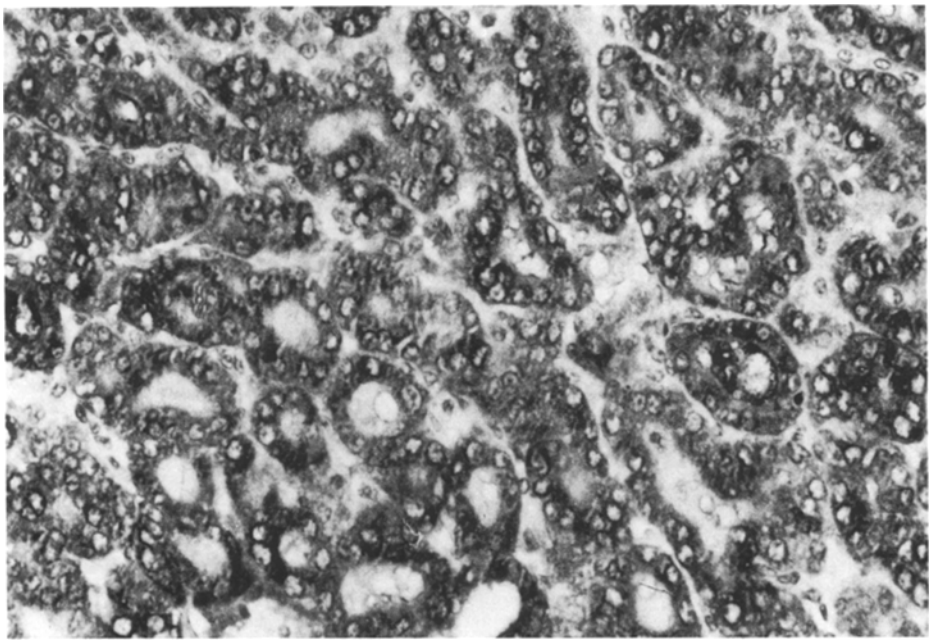

Fig. 2. Autonomous adenoma. Immunhistochemical stain of thyroglobulin. Note enhanced staining reaction of the cytoplasm of the tumor cells. ×250

Immunhistochemically the autonomous adenoma independent of its structural type shows a pronounced specific staining reaction indicating a high concentration of thyroglobulin in this tumor. Thyroglobulin is mainly demonstrable in the cytoplasm of the thyrocytes and to a lesser extent in the colloid. Neither the nucleus nor the interstitium show thyroglobulin staining (Fig. 2). The "morphologically active adenomas" react similary to autonomous adenomas.

Cold normofollicular adenomas on the whole show a lower intensity in immunhistochemical reactions than autonomous adenomas.

Despite their structural immaturity trabecular-microfollicular adenomas give a specific staining reaction. This hints at established thyroglobulin synthesis in the cells of these tumors.

Electron Microscopy. In the normofollicular or, rarely, the trabecular-microfollicular type of autonomous adenomas, the cell is mostly cuboidal to columnar (Fig. 3). The surface is enlarged apically by numerous long microvilli (Fig. 4) and basally by deep infoldings (Fig. 5). The most striking characteristic of these cells is the extensive development of the cytocavitary-lysosomal system.

1. The rough endoplasmic reticulum is well developed, often dilated and bordered with ribosomes (Figs. 3 and 4). While the apical cytoplasm usually contains round to oval profiles of rough endoplasmic reticulum ("vesicular

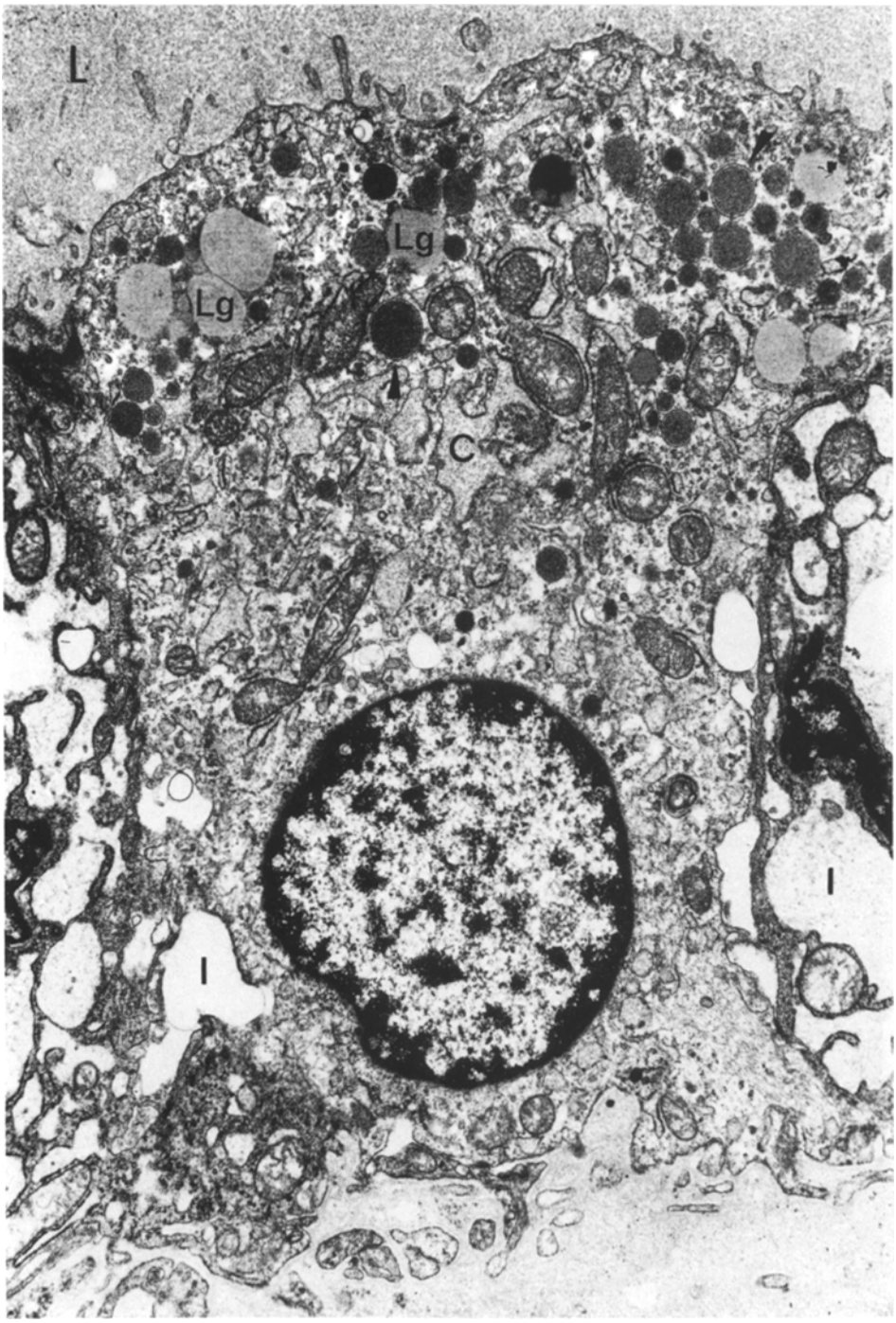

Fig. 3. Autonomous adenoma. Cylindric cell shape. Apically a well developed cytocavitary-lysosomal system with quite large lysosomes (arrows), lipofuscin granules (*Lg*) and dilated cisternae (*C*) of the rough endoplasmic reticulum. Basally the interstitium (*I*) is widened and contain fine flockular material (thyroglobulin? serum proteins?) L = follicular lumen. × 8100

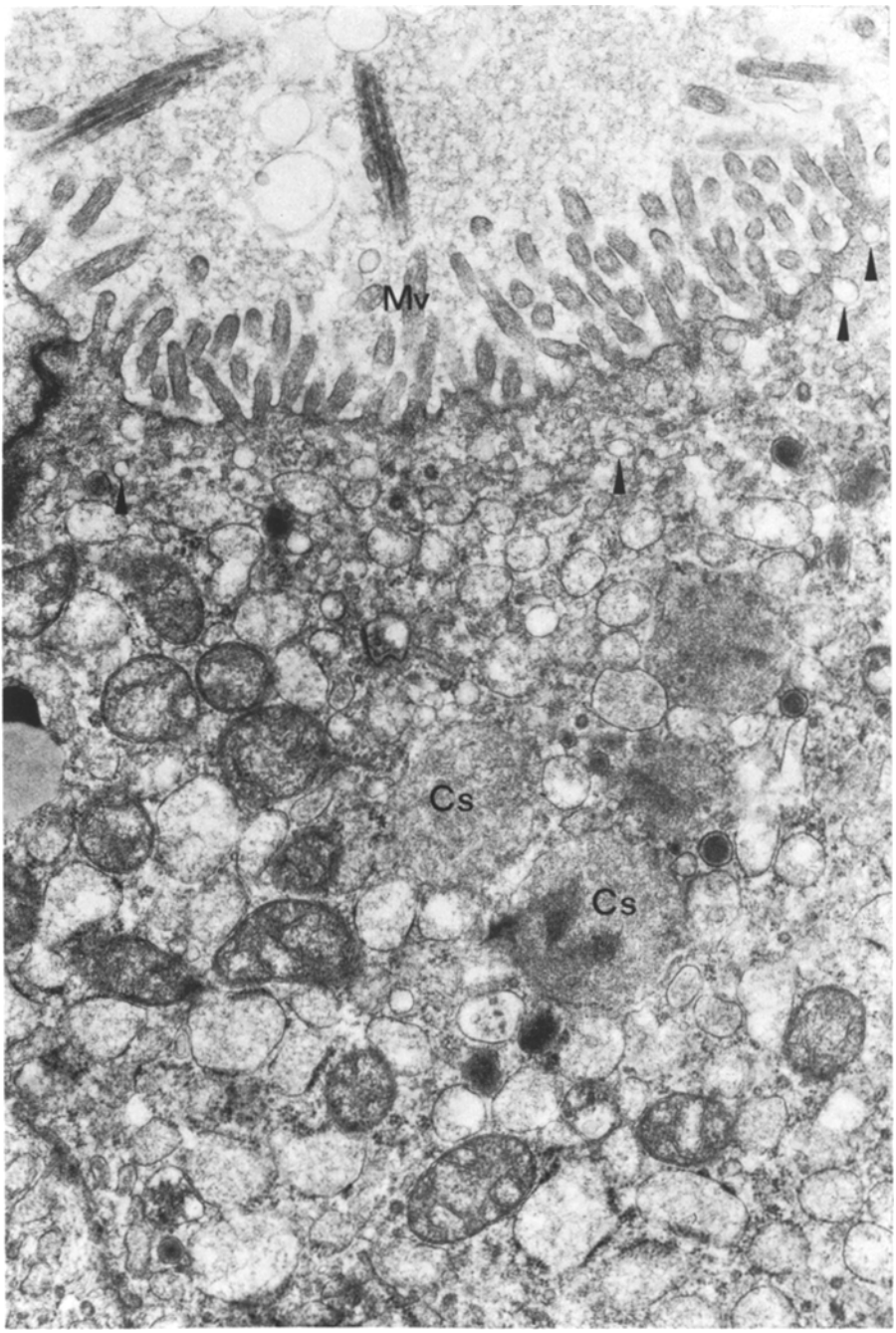

Fig. 4. Autonomous adenoma. Apical cytoplasm with numerous microvilli (*Mv*) and subapical vesicles (arrows) and enlarged round to oval cisternae of the rough endoplasmic reticulum. Four cytosomes (*Cs*) with less intensively stained interior are seen (colloid droplets? phagolysosomes?). ×13,300

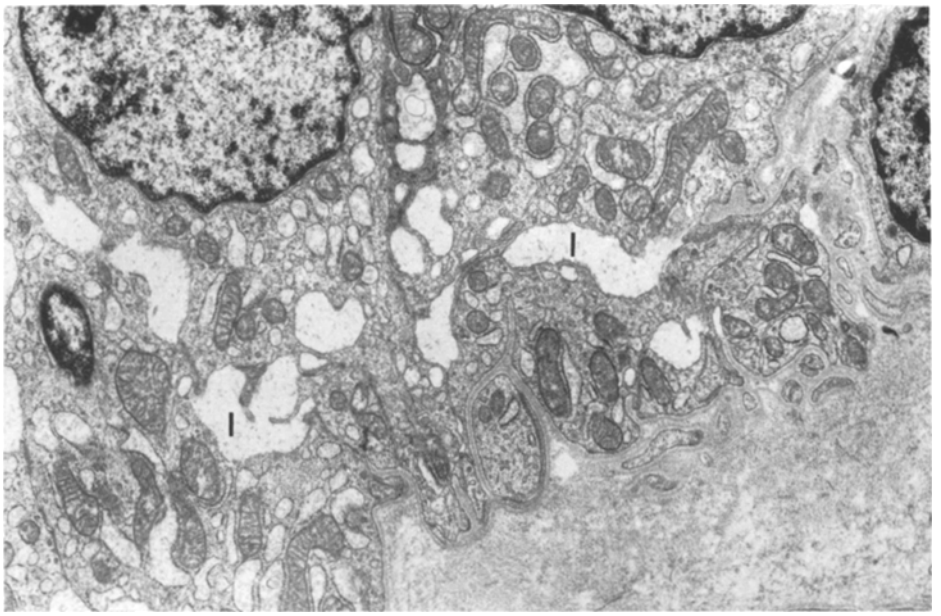

Fig. 5. Autonomous adenoma. Basal cytoplasm with deep infoldings. The intercellular spaces (*I*) are dilated and contain a flockular material. ×10,200

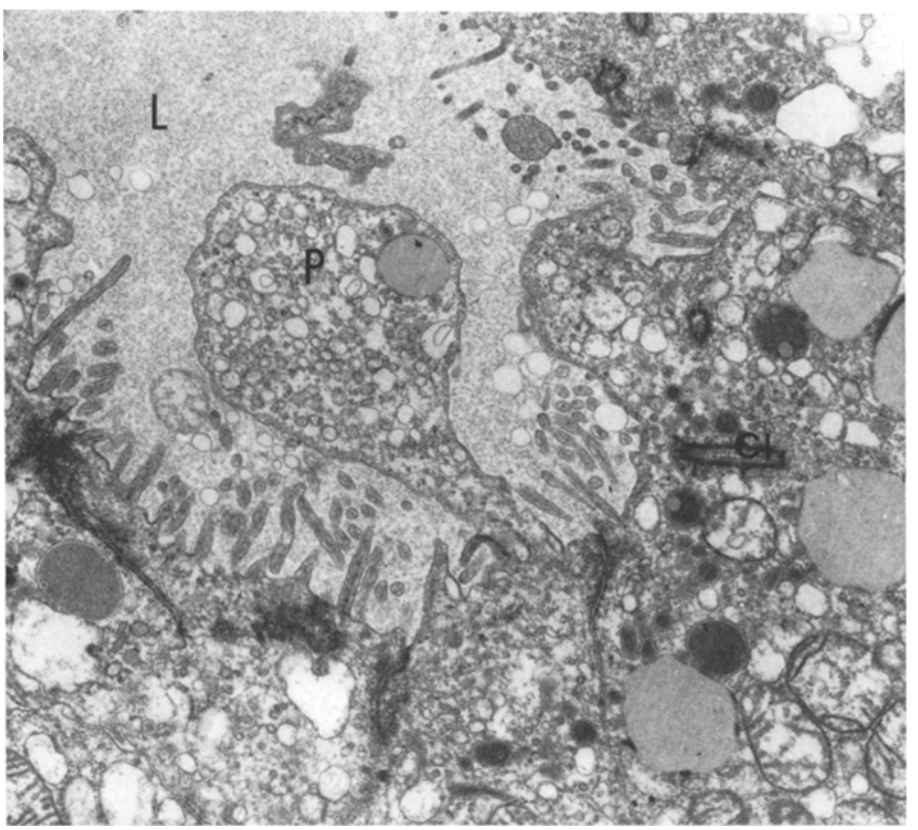

Fig. 6. Autonomous adenoma. Apical cytoplasm with a tennis racket formed pseudopodium (*P*). It contains one lipid droplet and numerous vesicles (approximately 50–250 nm in diameter) lined by a single membrane. Their electron density is less intensive than that of the follicular lumen (*L*). The apical cytoplasm contains a basal body of a cilium (*Cl*). ×15,300

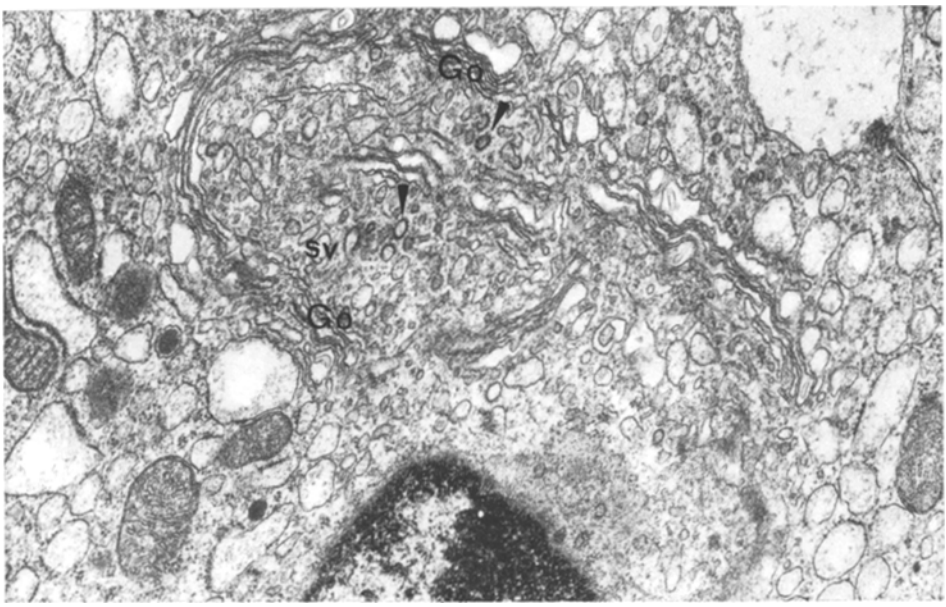

Fig. 7. Autonomous adenoma. Golgi system (*Go*) with numerous cisternae and sprouting vesicles (*sv*), some (arrows), whose membrane resembles a toothed wheel ("coated vesicles"). ×20,300

ergastoplasm", Herman, 1960), in the lateral and basal cytoplasm partly communicating cisternae are dilated ("lamellated ergastoplasm", Herman, 1960) (Figs. 5 and 7).

2. The Golgi system (Fig. 7) is extended and shows numerous vesicles of approximately 50 to 100 nm in size, that sprout from the cisternae of the Golgi fields. The material in some of these vesicles condenses, starting from the centre, to form small lysosome like bodies. Others develop a membrane whose outline resembles a toothed wheel ("coated vesicles", Fig. 7). As a rule, the sprouting of the vesicles takes place on the concave side of the Golgi system.

3. The lysosomes which are mainly localized in the apical cytoplasm are quite large (approximately 250 nm) and mainly round to oval, but few are also shoe or pear shaped.

4. In the subapical cytoplasm, numerous vesicles of approximately 100 to 150 nm in diameter are developed (Fig. 4).

5. In only a few cases can pseudopodia and colloid droplets be observed (Fig. 6). The pseudopodia contain numerous vesicles of approximately 50 to 250 nm in diameter. Compared with micropinocytotic vesicles colloid droplets are rare.

The ultrastructure of "morphologically active adenomas" differs only slightly from that of the autonomous adenomas just described.

Cold adenomas of predominantly normofollicular structure are usually composed of cuboidal follicular cells (Fig. 8). Functionally reduced activity is associated with limited development of those organelles that participate in thyroglob-

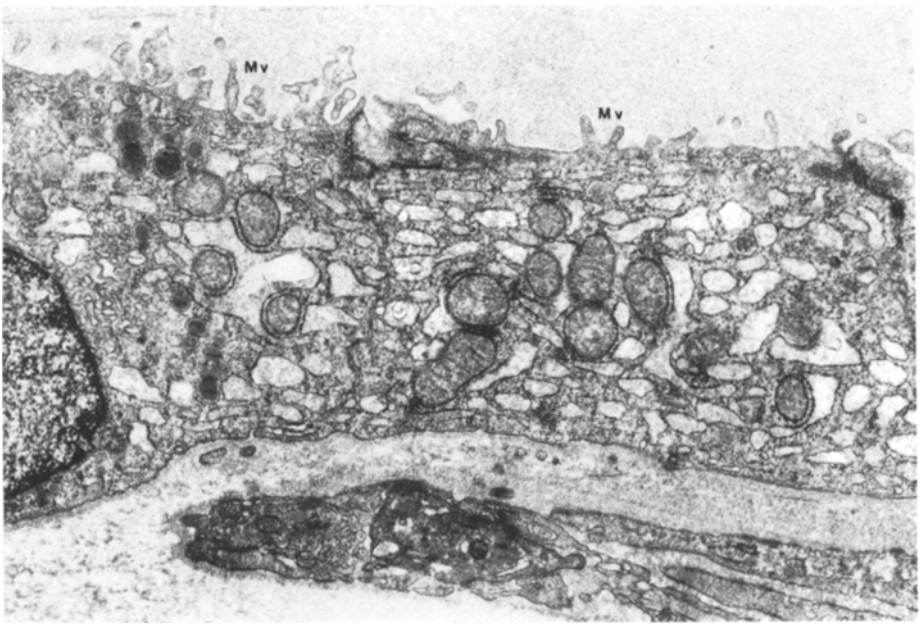

Fig. 8. Normofollicular cold adenoma. The cubic cells with few apical microvilli (*Mv*) are poor of organelles. The basal cytoplasm is only slightly infolded. ×13,300

ulin metabolism. In the Golgi apparatus only few shell shaped tubes with some sprouting granules are formed. The lysosomes have a smaller diameter (approximately 190 nm) and are less abundant when compared with the autonomous adenomas. The rough endoplasmic reticulum occasionaly shows cavitary formations. Lipofuscin granules may appear in great number and may form clusters. The trabecular-microfollicular adenomas consist of cord-shaped cell aggregations (Fig. 9). The developmental sequence of follicle formation with transformation of its constituent sheets of cells into colloid-filled spheres (Fig. 10) can easily be followed in these tumors. The internal organization of the cells usually parallels the degree of development of the follicles showing poorly differentiated cells with only few organelles or highly organized tumor cells typical of the normal thyroid gland. The lysosomes are on average somewhat smaller (approximately 180 nm) than the lysosomes of follicular adenomas. In more highly differentiated cells they may appear in great number (Fig. 10).

Lysosomes in Different Thyroid Adenomas. (For formal pathogenesis and function of lysosomes compare Part II). In Tables 2 and 3, the lysosomal systems of different types of adenomas are evaluated statistically. Apart from the above described autonomous, "morphologically active adenomas", follicular and trabecular-microfollicular adenomas, adenomas with a special cytological differentiation are analysed. The latter include the oxyphile adenoma, the ergastoplasm-

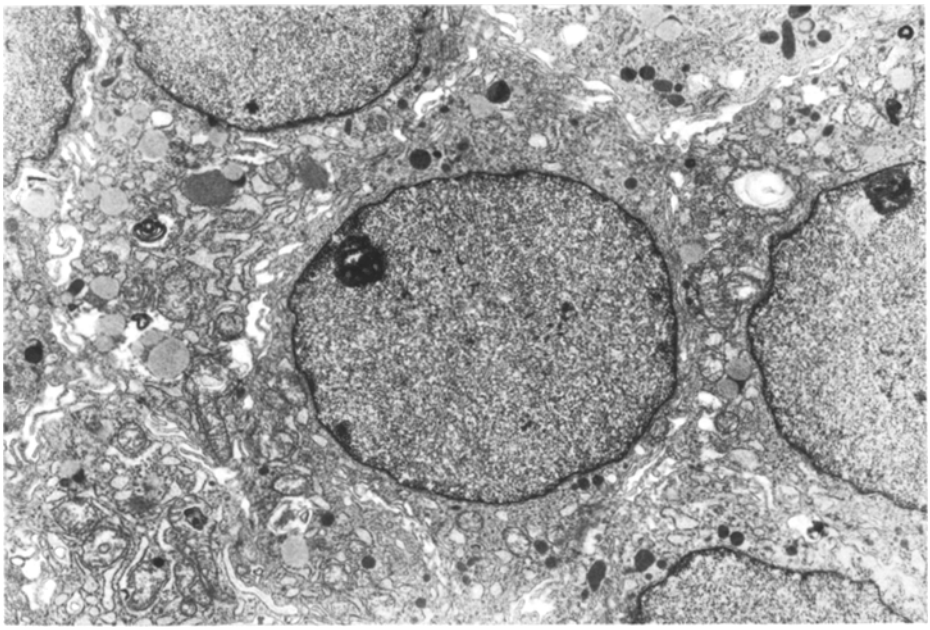

Fig. 9. Trabecular-microfollicular adenoma. Undifferentiated cell type. The large nucleus is surrounded by a small cytoplasm with only few lysosomes, some tubes of the rough endoplasmic reticulum and some mitochondria. ×8,200

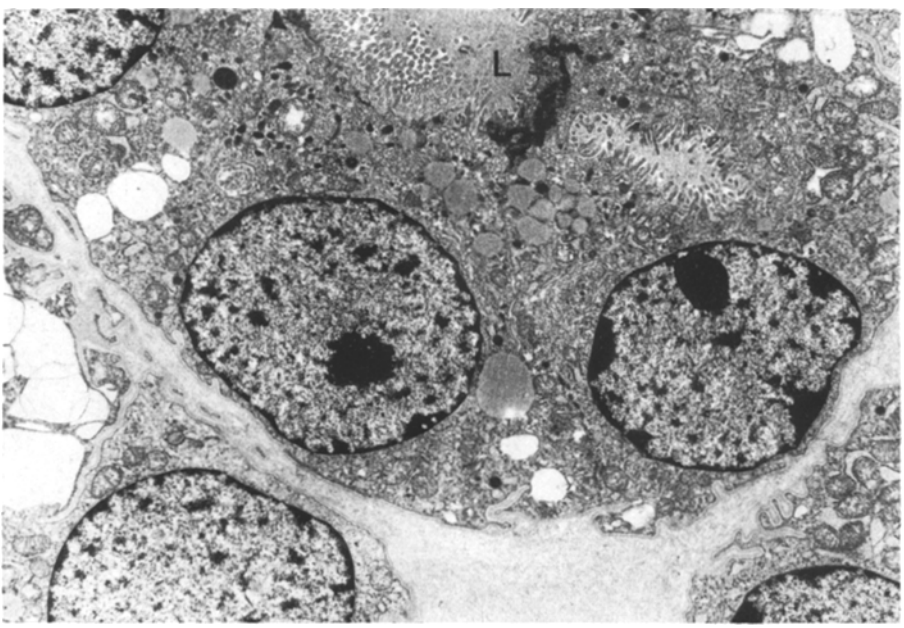

Fig. 10. Trabecular-microfollicular adenoma. Microfollicular organization. Cubic cells with numerous apically localized lysosomes and autophagic vacuoles forming clusters. *L* follicular lumen. ×6100

Table 2. Diameters of lysosomes in autonomous adenomas, "morphologically active adenomas", cold normofollicular and trabecular-microfollicular adenomas (group A) and in adenomas of a special cytological differentiation as oxyphile adenomas, ergastoplasm-rich-cell adenomas, mitochondrion-rich-cell adenomas and clear-cell adenomas (group B)

Adenoma type	Mean	Standard deviation	*N*
A. Autonomous adenomas	248	±159	947
"Morphologically active adenomas"	195	±127	36
Cold normofollicular adenomas	189	±78	117
Trabecular-microfollicular adenomas	178	±79	404
B. Oxyphile adenomas	364	±159	161
Ergastoplasm-rich-cell adenomas	263	±144	231
Mitochondrion-rich-cell adenomas	160	±61	190
Clear-cell adenomas	832	±261	10

Table 3. Unifactorial variance analysis of diameters of the lysosomes of autonomous adenomas, "morphologically active adenomas", cold normofollicular and trabecular-microfollicular adenomas and of adenomas with special differentiations (oxyphile adenomas, ergastoplasm-rich-cell adenomas, mitochondrion-rich-cell adenomas and clear-cell adenomas).

Adenoma types	P=0.01 probability
1. Autonomous adenomas—"morphologically active adenomas"	no significance
Autonomous adenomas—cold normofollicular adenomas	significance
Autonomous adenomas—ergastoplasm-rich-cell adenomas	significance
2. Cold normofollicular adenomas—trabecular-microfollicular adenomas	no significance
Trabecular-microfollicular adenomas—mitochondrion-rich-cell adenomas	significance
3. Mitochondrion-rich-cell adenomas—oxyphile adenomas	significance
Mitochondrion-rich-cell adenomas—ergastoplasm-rich-cell adenomas	significance

rich-cell adenoma[1], the mitochondrion-rich and clear cell adenoma. Table 2 records the average lysosomal diameter of the groups of adenomas mentioned. All adenomas with a special cytologic differentiation were nonfunctioning. In Table 3 the results of an unifactorial variance analysis are shown (indicating significant values relate to the 1% level). A statistical comparison between the lysosomal system of autonomous adenomas with that of other types of adenomas reveals the following:

1. There is no significant difference in number and size between the lysosomal population of the autonomous adenomas and that of the "morphologically avtive adenomas". On the other hand the lysosomal system of all other adenomas differs significantly from that of autonomous adenomas in regard to the size of lysosomes. For instance the cold follicular and the ergastoplasm-rich-cell adenomas have quite a different population to the autonomous adenomas.

[1] Adenomas, consisting of cells whose cytoplasm is filled with profiles of rough endoplasmic reticulum (see also Thyroid adenomas, Part II)

Table 4. Differential diagnosis of autonomous adenomas, cold normofollicular and trabecular-microfollicular adenomas.

Differential diagnostical criterias	Autonomous adenomas	Cold normofollicular adenomas	Trabecular-microfollicular adenomas
Scintigram	(cold), warm or hot nodule	cold nodule	cold nodule
Thyroglobulin synthesis	intensive	moderate	negative or slighty positive
Endocrine activity	increased	decreased	decreased
Ultrastructure	appr. 250 nm	appr. 190 nm	appr. 180 nm
Lysosomes diameter number	+++	(+), ++	+, ++, +++
rough endoplasmic reticulum	+++	++	+−++
mitochondria	++	++	+−++
Golgi system	+++	+−++	+
lipofuscin granules	++	++−+++	+−++

\+ = decreased development
++ = normal development
+++ = increased development

2. The lysosomal diameters of cold follicular and trabecular-microfollicular adenomas do not differ significantly from one another.

The mitochondrion-rich-cell adenoma has a different lysosomal population to follicular and trabecular-microfollicular adenomas, but in regard to its thyroglobulin content and degree of cell differentiation it mainly resembles the latter group.

3. Ergastoplasm-rich-cell adenomas as well as oxyphile adenomas show a significantly different lysosomal type to the mitochondrion-rich-cell adenomas.

Discussion

The diagnosis of autonomous adenomas is based on the scintigraphically proved autonomy of the adenoma. The main clues to the microscopic diagnosis of autonomous adenomas are cuboidal to columnar cell shape, vacuolated eosinophilic cytoplasm, foamy colloid and on the whole a somewhat washed out appearance in the H-E-slide (Fontolliet-Girardier, 1972; Lietz, 1974). In spite of these morphologic criteria in some cases the diagnosis cannot be made with certainty. The diagnosis of autonomous adenomata remains a clinico-pathological exercise (Fontolliet-Girardier, 1972).

Ultrastructural investigations of nonfunctioning and autonomous adenomas further emphazise the difference in the fine structural characteristics of both tumor types. We are referring in particular to the distinctly developed cytoplas-

mic cytocavitary network, with enlarged tubes of the endoplasmic reticulum, an extended Golgi system and an increased number of subapical secretory droplets. Numerous lysosomes of about 250 nm in diameter which are localized mainly in the apical cytoplasm, characteristic enlargement of the cell surface because of increased and enlarged microvilli, basal infoldings and moderate increase of mitochondria are also found.

The cytocavitary-lysosomal system of autonomous adenomas can in particular be used as differential diagnostic criterium from non-endocrine active tumors. In our experiance the only adenoma likely to be confused ultrastructurally with autonomous adenomas is the trabecular-microfollicular lesion as the latter may contain considerable amounts of apical vesicles, lysosomes and organelles responsible for protein sythesis. In this case looking at large areas of the tumor may be necessary. Trabecular-microfollicular adenomas always contain undifferentiated cells poor in organelles. A comparison of the ultrastructure of experimentally stimulated animal thyroids (Herman, 1960; Wissig, 1963; Lupulescu and Petrovici, 1964 and 1968; Wetzel et al., 1965; Ekholm and Smeds, 1966; Olen, 1969; Itikawa and Kawada, 1974; Tice et al., 1974; Velicky and Titlbach, 1974; Pelletier et al., 1976; Ekholm et al., 1975) with that of human autonomous adenomas shows many structural similarities between both conditions of endocrine active tissue. The ultrastructural features of cylindric cells with enlarged ergastoplasmic cisternae and Golgi fields and numerous vesicles, provides suggestive of endocrine activity. However the following notable differences should be mentioned. Among the 8 autonomous adenomas examined in only rare cases could the formation of pseudopodia and some colloid droplets be observed. The pseudopodia contained no, or very small colloid droplets; numerous relatively small vesicles of 50–250 nm were be found whose electron density was distinctly lower than that of the follicular lumen. There were no hints of fusion between colloid droplets and lysosomes, an observation which was also made by Klinck et al. (1970) on normal human thyroids. These results point out that the reabsorption of thyroglobulin in autonomous adenomas is a matter of micropinocytosis rather than of phagocytosis, with development of pseudopodia and colloid droplets. The phagocytosis of colloid may thus signify a overwhelming reaction after acute experimental TSH-application.

These different types of reabsorption may be a consequence of the different mechanism of stimulation which work on autonomous adenomas and on TSH-stimulated thyroid tissue. At the present time the pathogenesis of autonomous adenomata is unknown. On the basis of morphological investigations of human nodular goiters Taylor et al. (1953) supposed that the development of solitary hyperfunctioning nodules from thyroid cells in TSH-independent from the beginning.

Thyroid hormone biosynthesis occurs in a sequence of steps, each of which may be the site of a defect. De Rubertis (1972) in an analysis of cold adenomas found a deficient iodine uptake. This results in desturbed organification of iodine and finally in reduced hormone systhesis. The author suggested a defect in the transfer of c-AMP to iodine metabolism. Van den Hove-Vandenbroucke et al. (1973) in another study on cold adenomas described altered thyroglobulin systhesis with insufficient coupling of the carbohydrate moiety to the protein

core of thyroglobulin to the site of iodination. In addition thyroglobulin endocytosis and hydrolysis of the reabsorbed substance were found to be impaired. Intralysosomal accumulation of hydrolysed products are said to occur within the enlarged and structurally changed phagolysosomes (Lupulescu and Boyd, 1972).

Our own results show somewhat smaller lysosomes in cold follicular adenomas when compared with autonomous adenomas. From these findings we conclude 1) that in both types of adenoma lysosomes are involved in thyroglobulin degradation in contrast to the lysosomes of adenomata with special cytoplasmic differentiation, 2) that the larger lysosomal bodies of autonomous adenomas when compared with cold adenomas may be the result of an increased number of fusions between lysosomes and thyroglobulin endocytosis vesicles.

In recent years the autonomy of autonomous adenomas has been doubted more and more. From the clinical investigations of Mahlstedt et al. (1973) and Ingrisch et al. (1974), it appears that the autonomous adenoma can be stimulated in vivo with TSH. In vivo and in vitro investigations of Burke and Szabo (1972) have shown that the basal cyclic-AMP – levels of autonomous adenomas do not differ from those of paranodular tissue. The stimulation capacity of TSH on c-AMP formation and glucose oxidation, however, is two to four times higher in autonomous adenomas when compared with paranodular tissue. A special hyperresponsivity to TSH could be a very early phase in the development of endocrine active tumors, according to Burke and Szabo (1972), a phenomenon that cannot be demonstrated by morphological methods alone.

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