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Ultrastructural Study of a Calcitonin-Secreting Tumor

Typology of the Tumor Cells and Origin of Amyloid

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Summary. The authors studied electron microscopically a calcitonin secreting tumor, giving special attention to the typology of tumor cells and the origin of amyloid. Two types of neoplastic cells were observed. The first type formed most of the tumor and corresponded to the thyroidal C-cells, in agreement with previous ultrastructural investigations on medullary carcinomas. The secretory granules accumulated at one pole of the cell facing intercellular microfollicular-like spaces filled with extruded granules. The second type of cells showed larger and denser granules which never exhibited patterns suggesting their extrusion. The relationship between these cells and the appearance of an ectopic Cushing's syndrome is discussed. Both hypotheses of the origin of amyloid, namely the tumoral and the stromal received some ultrastructural evidence. The peculiar type of stromal cell, already reported by Lietz and Donath, probably represents a modified fibroblast.

Zusammenfassung. Es wird eine ultrastrukturelle Untersuchung über einen Calcitonin-sezernierenden Tumor speziell im Hinblick auf die verschiedenen Zelltypen und den Bildungsort des Amyloid vorgelegt. Dabei werden zwei Tumorzellformen unterschieden. Die überwiegende Zellform stellen C-Zellen dar, wie sie in früheren elektronen-mikroskopischen Untersuchungen von medullären Schilddrüsencarcinomen beschrieben worden sind. Die Sekretgranula sind an einem Zellpol angeordnet. Die intercellulären Räume sind mit ausgeschleusten Granula angefüllt. Der zweite Zelltyp besteht aus Zellen mit größeren und dichteren Granula. Die Beziehungen zwischen diesen Zelltypen und dem Auftreten eines ektopischen Cushing-Syndroms werden diskutiert. Hinsichtlich der Entstehung des Amyloids ergeben sich aus den elektronenmikroskopischen Befunden sowohl Hinweise dafür, daß das Amyloid in den Tumorzellen gebildet wird, als auch dafür, daß hierfür die Stromazellen in Frage kommen. Die früher von Lietz und Donath beschriebene Stromazelle wird als modifizierter Fibroblast angesehen.

Medullary carcinoma of the thyroid, the calcitonin-secreting tumor first identified by Hazard *et al.* in 1959, has been extensively investigated (Alboores—Saavedra *et al.*, 1964; Williams *et al.*, 1966; Ibanez *et al.*, 1967; Bertoli *et al.*, 1967; Cunliffe *et al.*, 1968; Block *et al.*, 1967; Steiner *et al.*, 1968; Milhaud *et al.*, 1969). Despite the large number of studies, however, several questions have still to be fully explained (Bordi *et al.*, 1972).

The purpose of this communication is to present the ultrastructural findings from a case of a calcitonin-secreting carcinoma, with special reference to the typology of tumor cells and the mechanism of amyloid deposition. This report provides electron microscopic evidence of the occurrence of two types of secretory granules in the cells of medullary carcinoma.

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Materials and Methods

Case Report. The patient, a 12-year-old boy, was seen because of an enlargement of the right laterocervical lymph nodes which first was noted ten months before. Serum calcium and phosphorus as well as other routine studies were normal. At surgery several firm nodes grouped in a mass measuring about 3×5 cm were removed. Histology revealed a medullary carcinoma with abundant amounts of amyloid. The thyroid was carefully examined by means of a scan with ^{131}I and arteriography, but no findings suggesting primary tumor were found. During the next 9 months two relapses developed in the laterocervical or supraclavicular regions and were both removed by surgery. Histological patterns corresponded to previous ones, but the amount of amyloid was greatly decreased. The liver was enlarged and a massive metastatic involvement was disclosed by a laparotomy. Diarrhea, consisting of 5–6 daily liquid stools, appeared. After a further 7–8 months the patient developed a typical Cushing's syndrome including moon face, cutaneous striae rubrae, hypertension, high levels of urinary 17-chetosteroids (ranging from 13.4 to 14.8 mg/24 h; normal values: 3.8–5.8 mg/24 h) and 17-hydroxycorticosteroids (85–100 mg/24 h; normal values: 7–14 mg/24 h) and of plasma free 11-hydroxycorticosteroids (93–124 γ/100 cc; normal values: 12–18 γ/100 cc). The thyroid has been repeatedly examined up till today and another scintigram with ^{131}I has been performed, but symptoms of primary tumor were not found despite the three years period elapsing from the first recovery. No further recurrences in the laterocervical or supraclavicular regions have been seen. Diarrhea and Cushing's syndrome still persist. The plasma level of calcitonin is 11721 mU/l (95% confidence limits: 6761 and 23496); it corresponds to 40–60 times the normal value (154–300 mU/l, Mazzuoli *et al.*, 1971). Tests for pheochromocytoma gave negative results. No evidence of familial incidence was observed. This case was included in an earlier clinico-pathologic report (Bordi *et al.*, 1969) as case 3.

Light Microscopy. Specimens were fixed in 10% formalin. Paraffin sections were stained with hematoxylin and eosin, Congo Red, thioflavin T, Grimelius' silver nitrate method (1968) and Masson-Hamperl argentaffin reaction modified by Singh (1964). Congo Red stained slides were also examined under polarized light.

Electron Microscopy. Small specimens were taken from laterocervical neoplastic tissue during the third operation and were quickly fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4), post-fixed in buffered 1% osmium tetroxide (pH 7.4) and embedded in Durcupan Fluka. Thick sections were cut with a glass knife on a LKB 3 microtome and stained with 1% Toluidine blue. Selected thick sections were also stained with the argentaffin reaction; controls were obtained from sections of duodenum mucosa. Thin sections were stained with uranyl acetate and lead citrate and were examined in a Siemens Elmiskop I electron microscope.

Statistical Study of Secretory Granules. The measures were performed on electron micrographs taken at a magnification of 30000 (calibrated by a Fullam grating replica). The diameter of the granules was measured including the limiting membrane and the largest diameter was used for those granules that deviated from a spherical shape. The frequency distribution of the granule diameters was determined for three types of granules (see the Results) with class intervals of 17 mμ. In order to compare the means of diameter measures between different granule types the Student's two-sided test was used.

Results

Light Microscopy

The histological examination of biopsy specimens revealed a typical pattern of medullary carcinoma of the thyroid (Fig. 1). Clusters, sheets and cords of polyhedral and rather uniform cells were separated by an irregular trabecular stroma of variable density. Mitoses were infrequent. Amyloid was both interspersed among the neoplastic cells or located within stromal septa. The intercellular amyloid was composed of small globoid masses which often coalesced; the stromal one was grouped in larger and irregularly rounded clumps. Both intercellular or stromal amyloids were markedly stained by Congo Red, showing a brilliant green to yellow-pink dichroism under polarized light (Fig. 2) and were fluorescent after thioflavin T. A moderate number of small argyrophilic granules were present in the cytoplasm of many

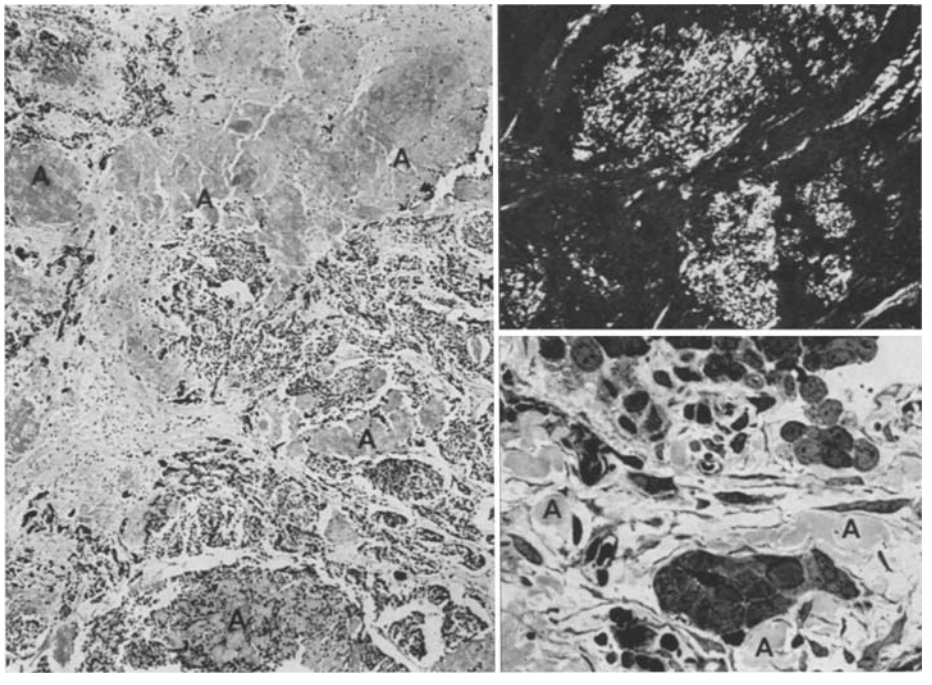


Fig. 1. Low power micrograph showing the histology of the tumor. Large masses of amyloid (*A*) are present both in stromal septa and in neoplastic cell clusters. Hematoxylin-Congo Red, × 50

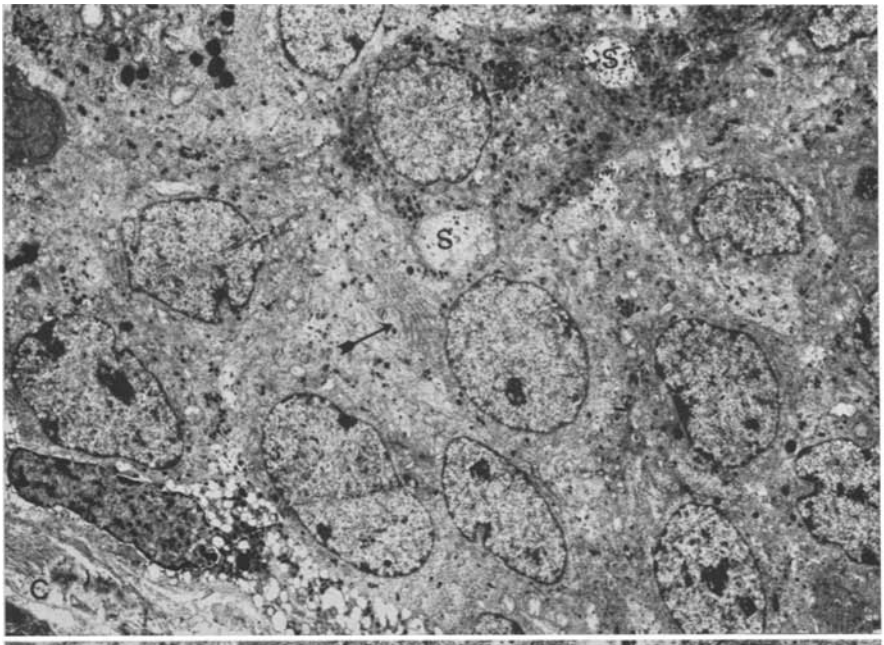
Fig. 2. Dichroism of amyloid substance under polarized light. Collagen fibers between neoplastic clusters also shown birefringence. Hematoxylin-Congo Red, × 75

Fig. 3. In the stroma surrounding the clusters of tumor cells amyloid substances (*A*) and numerous spindle stromal cells showing thin and long cytoplasmic processes are seen. Thick section, toluidine blue, × 450

but not all tumor cells following Grimelius' method. Cells showing positive argentaffin reaction were not seen.

Electron Microscopy

The ultrastructural study revealed two types of tumor cells (Figs. 4–6). The first type cells had moderate amounts of cytoplasm; the nuclei frequently showed a wavy outlines with small clumps of chromatin, against the nuclear membrane. The rough endoplasmic reticulum was abundant; in the perinuclear areas it was characterized by a parallel array of several (up to 10) cisternae (Figs. 4, 6–8, and 10) lying together with numerous free ribosomes and polysomes and rare secretory granules. Microfilaments and microtubules, were often seen in the ectoplasmic regions. The filaments were arranged in an intimate network and were prominent near the cell-to-cell contacts. The Golgi apparatus consisted of flattened sacs, vacuoles and vesicles and was not prominent. Mitocondria were short, round or navicular, with few cristae and dense granules; the matrix was sometimes clarified.



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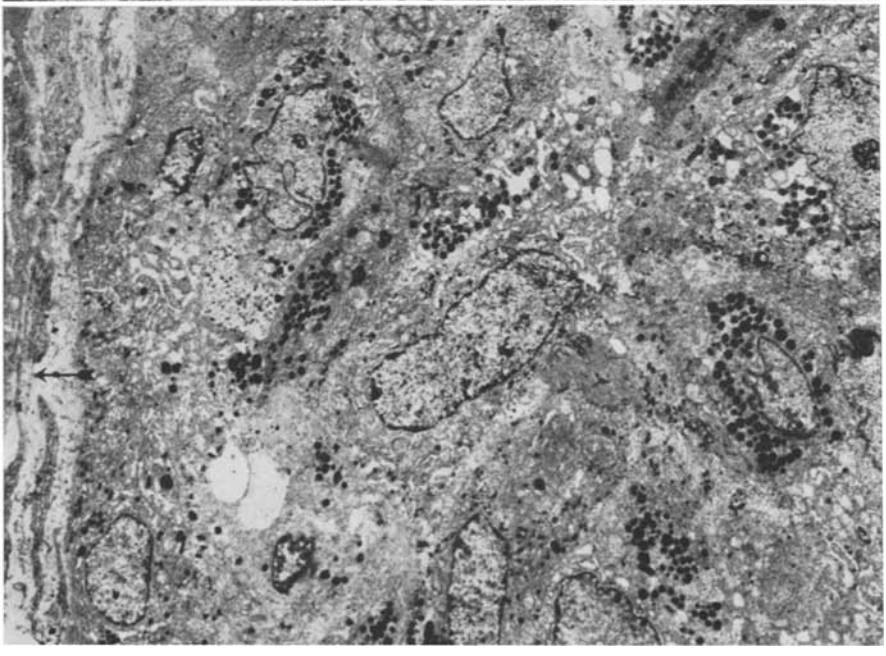

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Fig. 4. Cluster of tumor C-cells appearing "light" and "dark"; the rough endoplasmic reticulum is arranged in a lamellar fashion (arrow). Micro-follicular like spaces (*S*) are present between adjacent cells and are surrounded by patterns of granule accumulaiton. A second type of cell, with large dense granules, appears in the upper left corner. A vacuolated tumor cell is present in the peripheral layer of the cluster (lower left corner). *C* collagen fibers. × 4000

Fig. 5. Unusual high number of second—type cells intermingled with the C-type ones. The cells adjacent to the stroma show marked dilatation of the endoplasmic reticulum. In the stroma long and thin cytoplasmic processes arising from fibroblastic-type cells lie parallel with the neoplastic cluster (arrow). × 4000

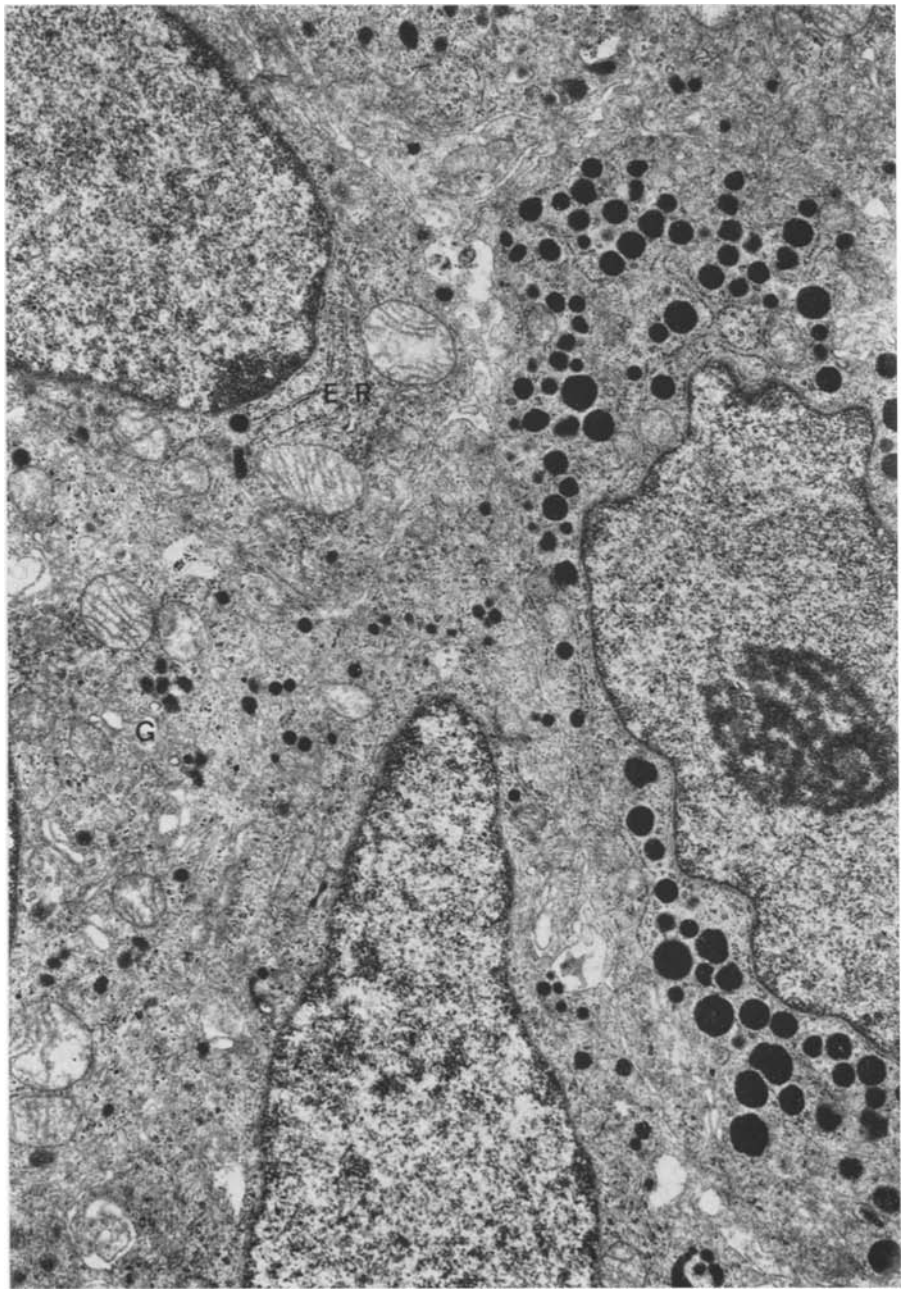
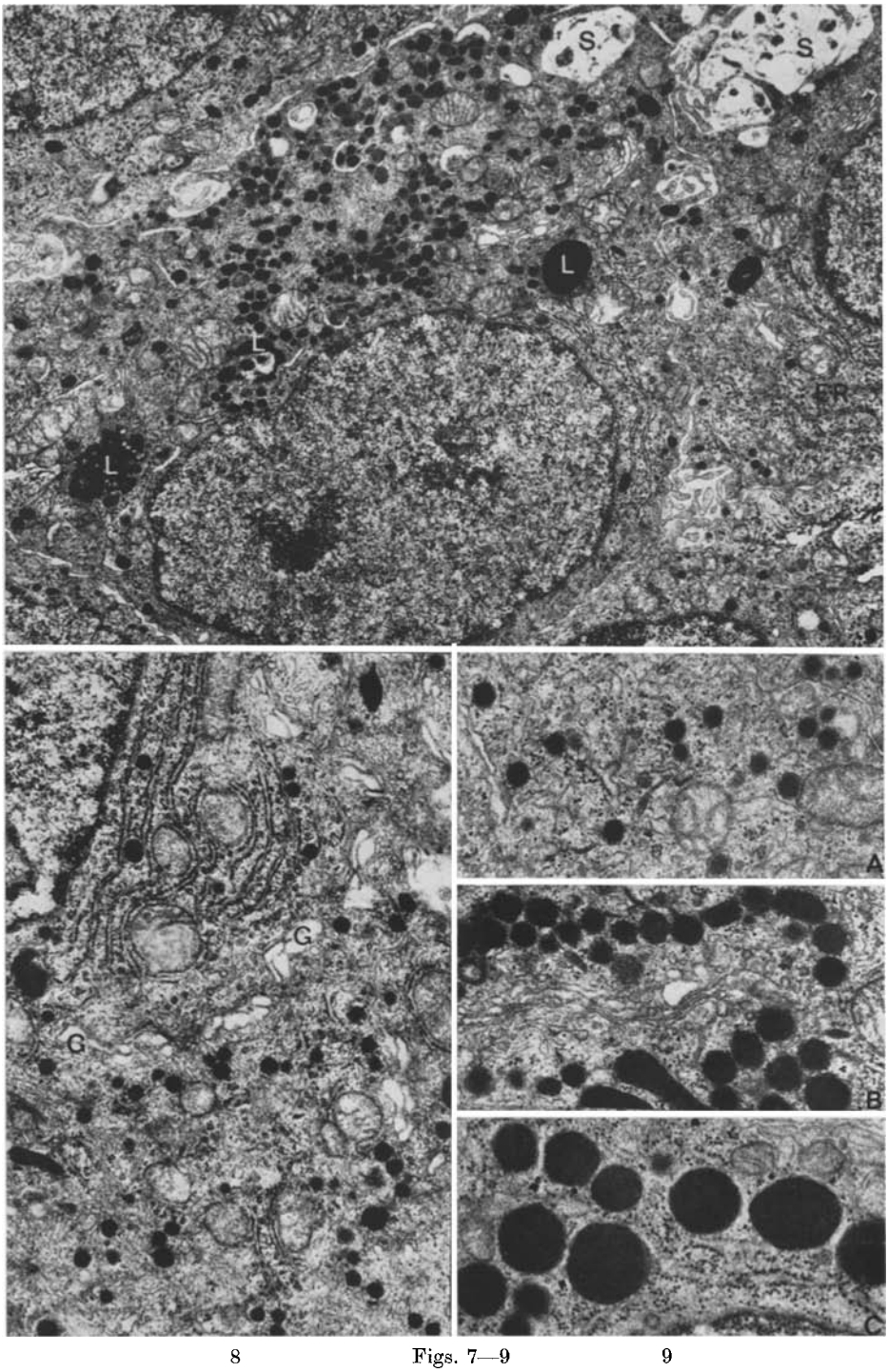


Fig. 6. The two cell types of the neoplasm are illustrated. C-type cells (on the left) show small and sparse secretory granules, parallel lamellae of rough endoplasmic reticulum (*ER*) and well developed Golgi apparatus (*G*). — The second type cell is characterized by large, numerous, uniformly dense round granules. The rough endoplasmic reticulum profiles are scattered. × 13000



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Secretory granules were observed in all cells. They usually exhibited round, uniform, electro-dense cores surrounded by a definite double membrane closely applied or separated by a thin clear halo (Fig. 6 and 9a). The diameter (including the membranes) varied between 85 and 250 mμ, (mean 134 ± 26 mμ). These granules were spread out through the cytoplasm but were more abundant in the ectoplasmic regions, without a discernible polarity. Furthermore in each cell a large portion of cytoplasm, facing intercellular follicular-like spaces, was filled by a large amount of secretory granules, which differed from the ones previously described because of their shape, density and volume (Fig. 4, 7, and 9b). The shape was often oval or elongated with a slightly irregular profile; the matrix of cores varied from strong and uniform to moderate and granular density; the diameters increased, ranging from 85 to 390 mμ (mean 183 ± 49 mμ). The difference between the diameter means of scattered and accumulated granules was significant ($P < 0.001$). Near the accumulated granules the Golgi apparatus was often seen (Fig. 7 and 9b); irregular lysosome-like structures were more large and numerous than in other portions of the cytoplasm (Fig. 7). The membrane of both types of granules sometimes appeared to fuse with the plasma membrane.

The plasma membrane was slightly undulating and showed small rare junctional complexes. The intercellular space, 250 Å thick, was often enlarged to form micro-follicular-like cavities (Fig. 4, 7, and 10), such as described by Meyer (1968). These cavities were defined by several (up to 4) adjacent cells and their lumen was filled with extruded and fragmented granules. In the large peripheral pseudofollicular spaces collagen fibers and networks of amyloid fibrils were intermingled with the extruded granules (Fig. 11).

The tumor cells displayed pronounced changes in the density of their cytoplasmic matrix, so that they could be divided into "light" and "dark" ones (Fig. 4). Moreover the cells located at the periphery of the neoplastic clusters often underwent marked modifications of their structure. The matrix of the cytoplasm became clarified, the mitochondria appeared swollen and degenerate, the nucleus was deeply indented with marked clumpings of chromatin (Fig. 4 and 5). The secretory granules exhibited changes similar to, but more pronounced than, those observed when they accumulated. The most important finding was represented by the marked dilatation of the endoplasmic reticulum. Cellular limits were poorly distinguishable and the cells often displayed a syncithium-like appearance. The basement membrane, which was well preserved elsewhere,

Fig. 7. C-type cells: the secretory granules accumulate at one pole of the cells adjacent to intercellular pseudofollicular spaces (*S*). The size, shape and density of these granules differ from those sparse ones. Near the accumulated granules large lysosome-like structures (*L*) and the Golgi apparatus (*G*) are present. *ER* parallel lamellae of rough endoplasmic reticulum. × 11000

Fig. 8. Perinuclear area showing the typical lamellar fashion of the rough endoplasmic reticulum. Numerous vesicles of the Golgi apparatus (*G*) and scattered small granules are present. × 14000

Fig. 9A—C. Comparison among different patterns of secretory granules. A Sparse granules of C cells; B accumulated granules of C cells; C granules of the second type cells. × 25000

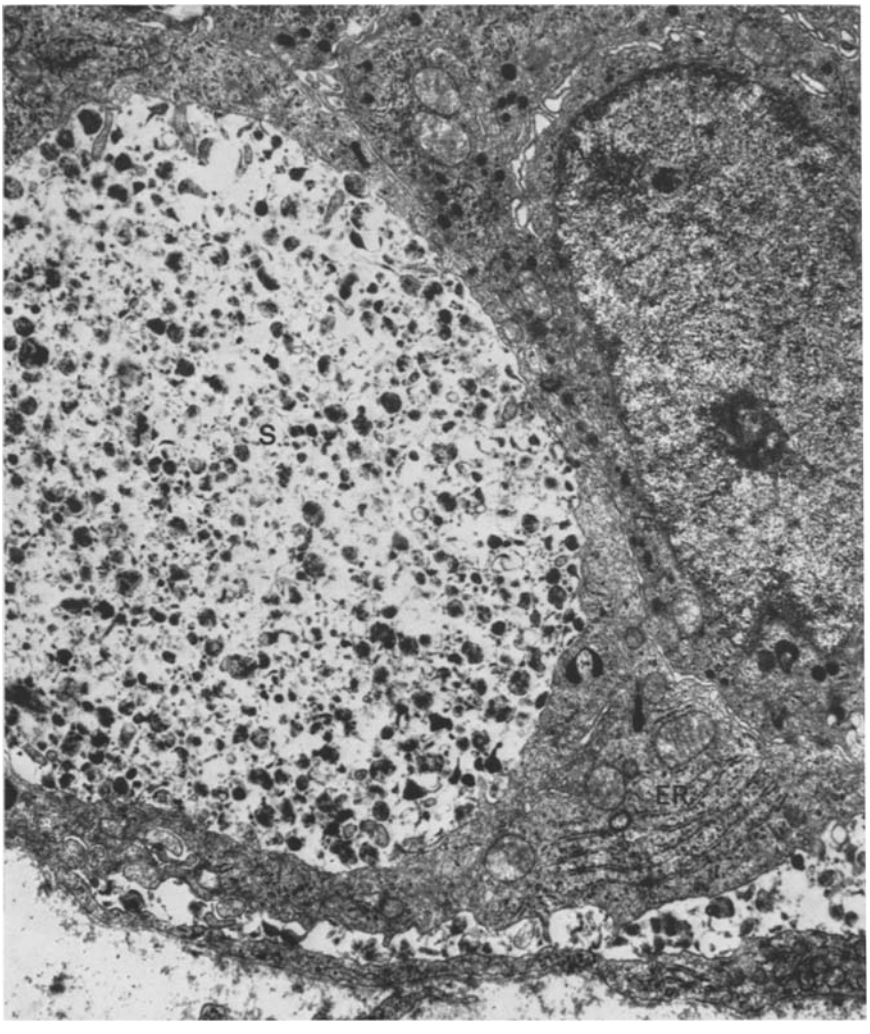
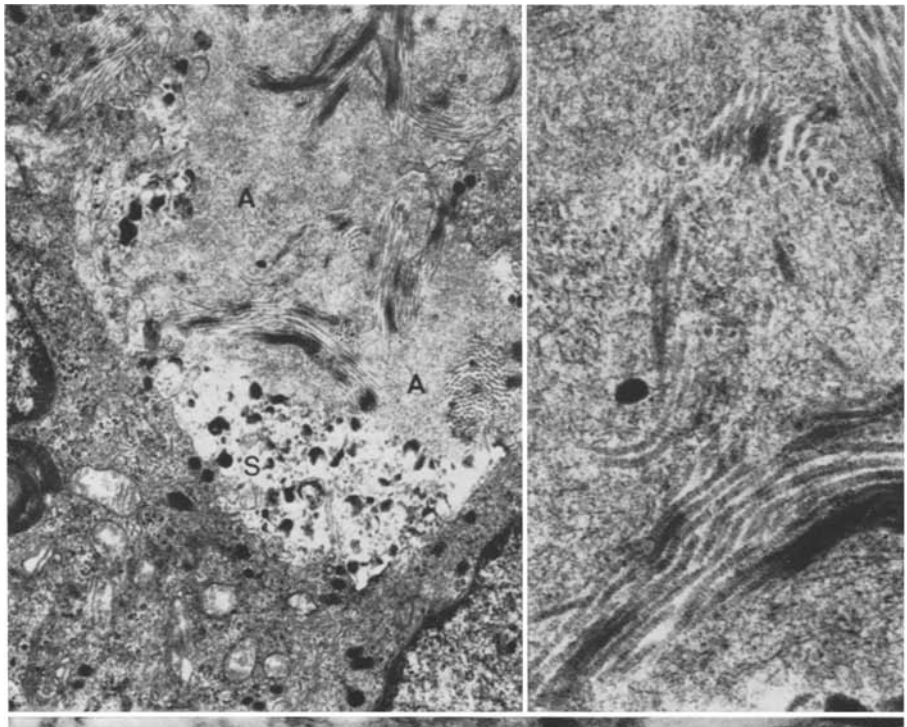


Fig. 10. Large microfollicular like space filled with extruded and fragmented granules. The long and narrow cytoplasmic fragment of a tumor cell interposed between an enlarged intercellular space and the basement membrane mimics the cytoplasmic processes of the stromal cells. *ER* parallel lamellae of rough endoplasmic reticulum. × 13000

disappeared in these areas so that the vacuolized cells were kept in touch with the stroma and cytoplasmic fragments were sometimes observed among the bundles of collagen fibers and the amyloid clumps (Fig. 13).

The second type cells, which were interposed between the first type ones, were infrequently seen; only in one block did they appear to be very numerous (Fig. 5). The shape of these cells was regular and oval. The nucleus appeared to be large and indented with finely distributed chromatin. The cytoplasm exhibited a dense matrix with a large number of microfilaments, microtubules.



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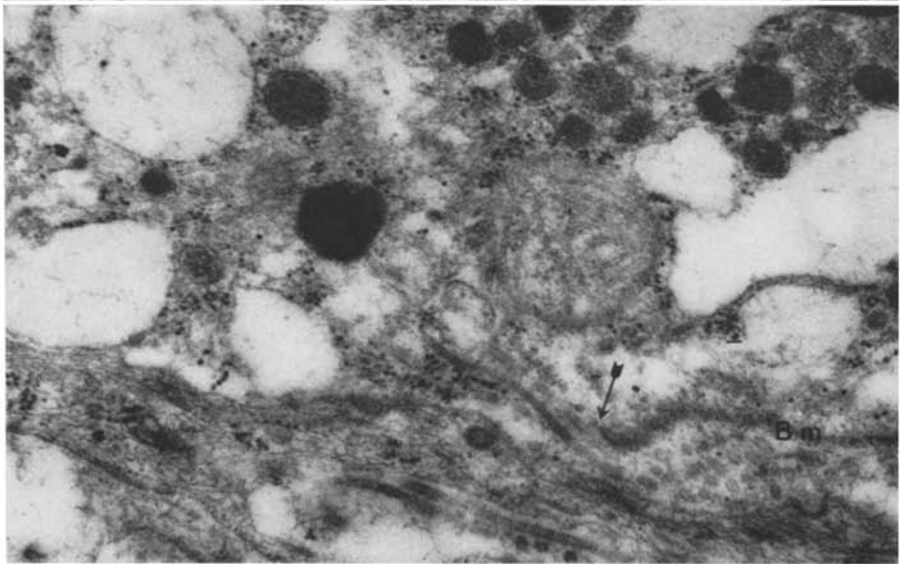

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Fig. 11. Pseudofollicular space (*S*) in which amyloid fibrils (*A*) are present together with collagen fibers and extruded granules. × 12000

Fig. 12. Particular of Fig. 11 showing the intimate network of amyloid fibrils interspersed with the collagen fibers. × 39000

Fig. 13. Vacuolated tumor cell (above) keeping in touch with the collagen fibers mixed with amyloid fibrils (below). The basement membrane (*Bm*) is interrupted (arrow). × 34000

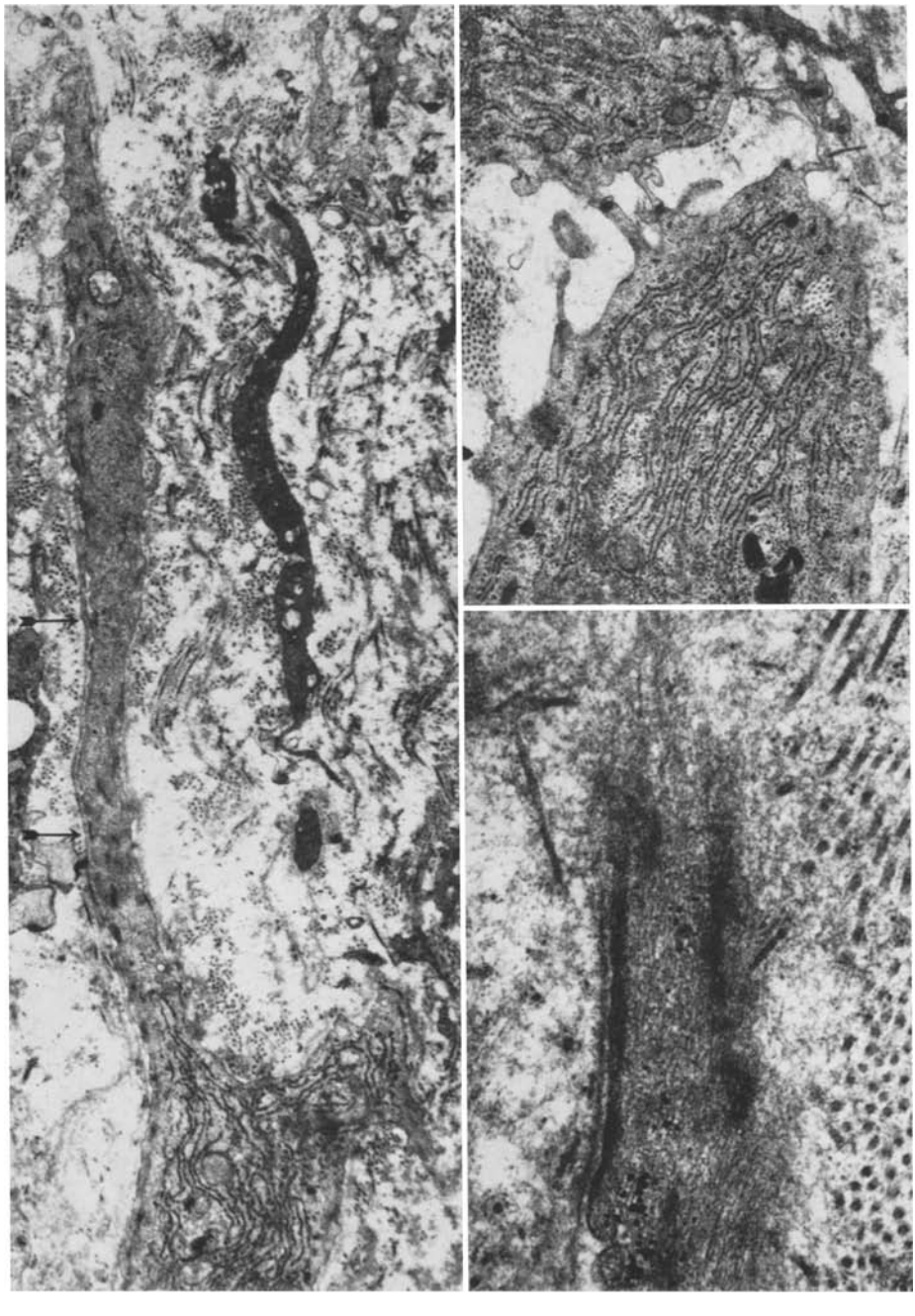

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Fig. 14. Peculiar type of stromal cell. In the body the rough endoplasmic reticulum is abundant. The long cytoplasmic process is filled with sheaths of microfilaments and intercalated dense bodies. A portion of another more dense process is present on the right. Thickening of extracellular fibrillar material resembling basement membrane can be seen (arrows). × 6500

free ribosomes and polysomes (Fig. 6). The endoplasmic reticulum was usually rough and consisted of vesicles and tubules: a lamellar fashion was never seen. The Golgi apparatus was small and the lysosomes were rare. The characteristic feature was represented by the abundance and appearance of secretory granules, which were round and large with diameters ranging from 85 to 485 mμ (mean 222 ± 85 mμ). This mean significantly differed from those of both types of granules observed in the first cell-type ($P < 0.001$).

The very dense and homogenous core was defined by a closely applied membrane (Fig. 9c). These granules were concentrated in the perinuclear region; findings suggesting their extrusion out of the cell were not observed.

The stroma consisted of bundles of collagen fibers, clumps of amyloid fibrils, fragments of degenerated tumor cells and several types of connective tissue cells. Stromal cells usually belonged to a peculiar cell-type, already described by Lietz and Donath (1970). In the toulidine blue-stained thick sections these cells were deeply basophilic and appeared to lie around the cluster of tumor cells and near the amyloid clumps (Fig. 3). They were composed of a body, and by two or more thin cytoplasmic processes which extend very far from it (Fig. 14). In the body abundant rough endoplasmic reticulum composed of cisternae filled with amorphous and slightly dense material, well developed Golgi apparatus and several navicular mitochondria with few cristae were present (Fig. 15). The nucleus exibited a round elongated shape with an irregular outline, finely distributed chromatin and a large nucleolus. The cytoplasmic processes appeared to be more heavily dense than the body and were composed of sheaths of closely packed microfilaments, rare mitochondria, smooth vesicles and glycogen granules. The microfilament sheaths with intercalated dense bodies were disposed along the longitudinal axis of the processes and were often more abundant beneath the plasma membrane. In the distal part of the processes the plasma membrane was often discontinued and the microfilaments spread out of the cell mixing with the amyloid fibrils (Fig. 16). On the outer side of the plasma membrane occasional apposition of a basement membrane-like material and collagen fibers were seen (Fig. 14). Because of their considerable length and irregular pathway the connection of the cytoplasmic processes with the body of the cells was lacking on a single section. If present the limit between body and processes was sharply defined and was characterized by the disappearance of the rough endoplasmic reticulum and by a strong increase and sheat disposition of microfilaments. Microfilaments were often present in the cellular body as well, particularly in the ectoplasmic region. When they became numerous the nucleus showed clumping of the chromatin, and the endoplasmic reticulum decreased. Stromal cells were sometimes joined together by means of small desmosomes (Fig. 15) which were observed both in the cellular body or in the cytoplasmic processes. Other types

Fig. 15. Cells of the same type as in Fig. 14 joined together by means of small desmosomal junctions. × 12500

Fig. 16. Apparent origin of amyloid fibrils from the peripheral process of a stromal cell: the cytoplasmic microfilaments spread out through discontinuities of cellular membrane, mixing with the amyloid fibrils interspersed among the collagen fibers. × 38000

of stromal cells were occasionally encountered. They were represented by mast cells, plasma cells and macrophages and were usually located around the blood capillaries. The amyloid consisted of fibrils 100 Å thick, usually single and mixed to the collagen fibers (Fig. 11–13, and 16); more seldom they were grouped in short sheaths or networks. Amyloid fibrils appeared to be abundant around the cytoplasmic processes of the elongated stromal cells, whilst no relationship to other stromal cells was observed.

Discussion

The first question in our study concerns the origin of the tumor we have investigated. In the present case no thyroidal involvement was seen despite a three year follow-up after the first removal of neoplastic tissue. However the diagnosis of medullary carcinoma was substantiated by several lines of evidence. The histological patterns was extremely close to that of medullary carcinomas arising in the thyroid, especially because of the large amyloid masses. The very high level of calcitoninemia (20 to 40 times the normal values) is known to occurr only in medullary carcinoma patients (Sturtridge *et al.*, 1969; Tashjian *et al.*, 1970). Some aspects of the clinical behaviour of the tumor, represented by the occurrence of hepatic metastases, diarrhea and Cushing's syndrome, is characteristic of calcitonin-producing tumors (Williams *et al.*, 1966; Williams, 1969). We are not able presently to state if this tumor originated from extrathyroidal C-cells or if it is a metastasis from an occult primary thyroid neoplasm. Medullary carcinomas of the thyroid are sometimes silent for some years after the discover of lymph nodes metastases (Tubiana *et al.*, 1968).

Typology of Tumor Cells

The ultrastructural findings of the greater number of tumor cells were consistent with those of parafollicular (or C) cells in the normal human thyroid (Teitelbaum *et al.*, 1971). This was in agreement with previous reports of medullary carcinoma (Meyer, 1968; Braunstein *et al.*, 1968; Gonzales-Licea *et al.*, 1968; Grimley *et al.*, 1969; Lietz and Donath, 1970). Neoplastic C-cells in our investigation exhibited marked polarity of secretory granules. These usually accumulated in the portion of the cytoplasm facing the pseudo-follicular spaces occupied by extruded granules. It is interesting to note that the granules displayed important changes in their morphology when they were grouped at the secretory pole of the cells. These changes were probably related to the mechanism of secretion. Furthermore, an important role may be played by lysosomes as suggested by their number, volume and close apposition to the accumulated granules.

Between the cells showing the aforementioned findings variations occured with sufficient regularity to receive notice. A difference in the density of cytoplasmic matrix resulted in cells appearing "dark" and "light". According to previous reports (Mc Dermott and Hart, 1970; Hachmeister and Zimmerman, 1971), this finding could be related to the different functional states of the cells. Moreover the appearance of the rough endoplasmic reticulum underwent important changes from cell to cell. The cisternal lumens, which were usually narrow,

often became markedly dilated in the cells located at the periphery of the neoplastic clusters. This change, previously reported by Braunstein *et al.* (1968) and by Benatre *et al.* (1971), was accompanied by margination of nuclear chromatin and by modifications of secretory granules similar to, but more pronounced than, those seen during the accumulation phase. According to Benatre *et al.* (1971), these findings represent regressive changes, probably related to the formation of amyloid.

Besides the different patterns of neoplastic C-cells a second type of tumor cell was found. These cells differed from the others because of the morphology of their secretory granules, which were larger, uniformly dense and round and appeared to lie in the perinuclear region without pattern suggesting their extrusion out of the cell. Furthermore the shape of the cells was oval and regular, the cytoplasm was less abundant and the rough endoplasmic reticulum never showed lamellar fashion.

The occurrence of cells containing different types of secretory granules is now a well known characteristic of several endocrine neoplasms, including bronchial (Hosoda *et al.*, 1970) and duodenal (Weichert *et al.*, 1971) carcinoids, insulomas (Tardini and Bordi, 1968; Heitz *et al.*, 1971) and carotid body-glomus jugulare tumors (Capella and Solcia, 1971). In the case of medullary carcinoma the study of different cell-types appears to be very important in view of the unusual number of humoral agents other than calcitonin secreted by this tumor. These agents include 5-hydroxytryptamine (Moertel *et al.*, 1965), prostaglandins and callicrein (Williams *et al.*, 1968), ACTH (Donahower *et al.*, 1968) and histaminase (Baylin *et al.*, 1970). As far as we are aware, the occurrence of two types of tumor cells has been previously reported only by Ljungberg (1970) on the basis of an histochemical study performed on a series of familial medullary carcinomas of the thyroid. The second type of cell, which were mixed with the calcitonin producing ones, was characterized by positive argentaffin, chromaffin and iodate and negative diazo-coupling and ninhydrin reactions. These results suggested that the argentaffin-chromaffin cells contained noradrenaline or its precursors (DOPA, dopamine). Several lines of evidence point to the fact that the second cell-type we observed with the electron microscope did not correspond to the second cell-type demonstrated by Ljungberg (1970) on light microscopy. In our study argentaffin reaction gave negative results both in paraffin sections and in thick sections from resin-embedded tissue. The cells observed by us exhibited an ultrastructural pattern strongly different from that of cells containing noradrenaline or other monoamines[1] and never showed cytoplasmic processes such as the Ljungberg's cells did. We were not able to perform biochemical or immunohistochemical investigation on neoplastic tissue. Therefore we cannot draw any conclusion about the nature of the second cell-type. However, it can be recalled that the patient developed a typical Cushing's syndrome. The appearance and distribution of secretory granules in our second type-cells closely resembled those seen in a pituitary ACTH-secreting tumor (Bergland and Torack, 1970) and are in agreement with the present knowledge of the cytology of the pituitary ACTH cell (Azzali, 1971). Cushing's syndrome

1 (Kobayashi *et al.*, 1970; Brown *et al.*, 1971; Toner *et al.*, 1971).

was not evident when specimens of tumor tissue were removed for ultrastructural study; it became manifest only several months later. This may account for the small number of cells of the second type we found. Any specific role ascribed to these cells must await confirmation by the fine-structure localization in the granules of appropriately labelled antibody to ACTH.

Origin of Amyloid

The mechanism of amyloid deposition in medullary carcinoma of the thyroid is still unknown. The difficulty lies in determining whether this substance originates from neoplastic rather than from stromal cells.

Albores-Saavedra *et al.* (1964) and Beskid (1964), on the basis of histochemical studies, suggested that the formation of amyloid results from the production of a precursor substance, mainly consisting of mucopolysaccharides and glycoproteins, within the tumor cells. Ibanez *et al.* (1967) pointed out also the plasmacytoid appearance of the neoplastic cells which leads one to suspect that the entire cytoplasm is converted into amyloid. Ultrastructural findings supporting an origin of amyloid from tumor cells have been also reported. They include the close relationship between the extruded secretory granules and amyloid (Huang and McLeish, 1968; Meyer, 1968), the very rare fine fibrils similar to the amyloid ones detected within the cytoplasm of neoplastic cells (Huang and McLeish, 1968; Gonzales-Licea *et al.*, 1968; Meyer, 1968; Amouroux *et al.*, 1970) and the abnormalities in limits between tumor cells and stromal amyloid, consisting of absence of the basement membrane and interruption and ill-definition of the cytoplasmic membrane (Huang and McLeish, 1968; Meyer, 1968; Amouroux *et al.*, 1970).

However recent studies had drawn attention to the role of the tumor stroma in the production of amyloid (Grimley *et al.*, 1969; Koslowsky *et al.*, 1969; Lietz and Donath, 1970). Particular interest was paid to the occurrence of a peculiar type of stromal spindle cell, which was found to contain large amounts of fine fibrils similar to the amyloid ones (Grimley *et al.*, 1969; Lietz and Donath, 1970). These cells appeared to be closely applied to the amyloid as well as to the tumor cell clusters and were regarded as responsible for amyloid production (Lietz and Donath, 1970).

In our study both hypotheses received some ultrastructural evidences. Arguments supporting the origin of amyloid from the neoplastic cells were as follows: 1. the occurrence of amyloid fibrils near the degenerated peripheral tumor cells where the basement membrane was absent and 2. the occurrence of amyloid in the pseudofollicular cavities filled with extruded secretory granules, even though this finding was evident only in the presence of collagen fibers. Conversely, ultrastructural data suggesting the amyloid nature of the intercellular fibrils were not available. These fibrils probably belonged to the microfilament system which is a normal component of several types of cells (Buckley and Porter, 1967).

The role of stromal cells in the genesis of amyloid was emphasized by the large amount of fibrils filling the long cytoplasmic processes. These fibrils were ultrastructurally similar to the amyloid ones, in agreement with the results of Lietz and Donath (1970), and were often observed to spread out of the cells,

mixing with the surrounding extracellular amyloid. The nature of this peculiar type of stromal cell is controversial. In our opinion it represents a modified fibroblast. This assumption is based upon the spindle shape, the close relationship with collagen fibers without any perivascular arrangement and the abundance of the rough endoplasmic reticulum. The occurrence of microfilament sheaths, dense bodies and defective basement membrane were all reminiscent of mio-epithelioid or smooth muscle cells, (Lietz and Donath, 1970). However these findings were also described in modified fibroblasts such as the ones involved in the granulation tissue (Gabbiani *et al.*, 1971) or in Dupuytren's disease (Gabbiani and Majno, 1972).

Finally, ultrastructural similarities were noted between the long, narrow cytoplasmic fragments of neoplastic cells lying on the inner side of the basement membrane and the stromal cells (Fig. 10). However neoplastic cells in transition to stromal cells, such as observed by Grimley *et al.* (1969) in tissue culture, were never seen.

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Note Added in Proof. Since the manuscript was submitted for publication, the patient died, 3 years and 4 months after his first admission to the hospital. At post-mortem examination the final cause of death was purulent peritonitis subsequent to severe perforating diverticulities of the right colon. A small tumor, weighing 8 grams, was found adjacent to the inferior surface of the thyroid without infiltrating the gland. Metastases were present in the liver, lungs, mediastinal and peripancreatic lymphnodes.

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