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Histochemistry and Fine Structure of Bronchial Carcinoid Tumours

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Summary. The histological, histochemical and ultrastructural features of six carcinoid tumours of the larger bronchi are described. Electron microscopy and methods known to stain endocrine cell granules selectively allowed differentiation of three types of endocrine cells. Electron microscopy revealed that two of these cell types were similar to endocrine cells normally found in the pulmonary epithelium of human foetuses. These cells had small, round membrane-bound secretory granules of uniform size and shape, or much larger, round secretory granules, tightly surrounded by a membrane and almost homogeneous in appearance. The secretory granules of the third cell type were characterized by large polymorphic secretory granules, vesiculated or tightly surrounded by a membrane. These cells were reactive to staining with the argentaffin silver method and were quite similar to the enterochromaffin cell known from the digestive tract. Scattered mastocytes which reacted to some of the granule staining methods were easily identified by electron microscopy.

For long it has been known that carcinoid tumours of the intestinal tract are derived from endocrine cells in the intestinal mucosa (Gosset *et al.*, 1914). Feyrter (1954) suggested the origin of carcinoid tumours of the lung to be endocrine cells in the bronchial epithelium. This was supported by electron microscopic investigations of Bensch and his group (Bensch *et al.*, 1965a, b; Gmelich *et al.*, 1967). The present investigation was undertaken in order to ascertain whether there are cytochemical or ultrastructural resemblances between endocrine cells in the pulmonary epithelium of human foetuses (Hage, 1972, 1973 in press) and the cells of 6 bronchial carcinoid tumours.

Material and Methods

The material comprised surgical specimens of 5 bronchial carcinoid tumours and a biopsy from 1 bronchial carcinoid tumour; from the latter case material was obtained at autopsy, too.

The standard method of preparation was fixation in 10% formaldehyde followed by paraffin embedding. Additional material from all tumours was fixed in: 1) glutaraldehyde 6%, 2) Bouin's fluid, 3) glutaraldehyde-picric acid (Solcia *et al.*, 1968).

For routine examination sections were stained by haematoxyline-eosin. The following techniques for endocrine cell granules were applied: lead haematoxyline, Pb-H (Solcia *et al.*, 1969b); HCl-toluidine blue (Solcia *et al.*, 1968) 1 N HCl, 60° C, 3hr and toluidine blue at pH 2 and 5 was used; Grimelius' silver method (Grimelius, 1968); Masson-Hamperl's silver method (Singh, 1964); zanthydrol and diazonium reaction (Solcia *et al.*, 1969a). Human duodenum served as control-tissues. For identification of amyloid, congo red and methyl-violet were applied. In order to differentiate between various cell types sections were stained by one method, decolorated, and restained by another method; or the various methods were performed on alternative sections from serial sections of a single block.

Five bronchial carcinoid tumours were examined by electron microscopy. Blocks of tissues (about 1–2 mm^3) were fixed in ice-cold 3% glutaraldehyde in 0.2 M cacodylate buffered

sucrose, post-fixed in osmium tetroxid 1% for 2 hours and embedded in epon. The sections were cut on a Reichert OM U2 ultramicrotome. One μm thick sections were stained with toluidine blue and examined in light microscope in order to locate areas to be trimmed for thin sections. The latter were stained with Zn-uranylacetate 4% and Pb-citrate 0.4% prior to examination in a Hitachi HS-8 electron microscope. Argentaffin silver reaction for electron microscopy was performed (Håkanson *et al.*, 1971). After conventional fixation, embedding and sectioning ultra-thin sections were placed on nickel grids and were exposed to an ammoniacal silver nitrate solution in the dark for $^1/_2$ hour at 60° C (Singh, 1964). The grids were then rinsed in redistilled water and air dryed before examination.

Results

All the tumours were located in larger bronchi (Table 1). In case number I, II, IV and VI tumour protruded into the bronchial lumen causing obstruction and atelectasis. On cut surface they were all homogenous, pink or greyish. In case number IV and VI regional lymph nodes were invaded. By light microscopic examination all tumours were located to the submucous layer of the bronchi with a distinct separation from the bronchial surface epithelium by a layer of fibrous tissues. In case number IV squamous metaplastic changes of the surface epithelium were observed; ulceration was never seen. Invasion of the lung was noted in case number II, IV, V and VI.

The *histological features* of the tumour varied from case to case and even in different areas of the same case. Tumour cells might be arranged in solid sheets of varying shape and size, surrounded by fibrous tissue (alveolar pattern). The cells might be arranged in anastomosing cords with two or more rows of cells along thin bridges of vascular stroma (trabecular pattern). Rosette-like figures and nuclear palisading were often found in tumours with trabecular pattern.

Tumour cells were of uniform cylindro-cuboidal, polygonal or spindle-shape with round, oval or triangulated nuclei. In sections stained by haematoxyline-eosin the cytoplasm was bright acidophilic or water-clear. A few multinucleated giant cells were observed in case number II. A moderate cellular polymorphism was observed in case number IV, V and VI. Mitotic figures were few except in case number V.

The histochemical results are summarized in Table 1. It must be added that only some of the tumour cells were reactive to the various methods applied. All tumours without case number VI contained more than one endocrine cell type. In case number I and IV glutaraldehyde fixed sections were stained by Pb-H, subsequently decolorated in dilute HCl and restained by diazonium technique. Some of the Pb-H reactive cells were not stained by diazonium technique, most in case number I. By using serial sections of a single block fixed in formaldehyde alternative sections were stained by diazonium and argentaffin (Masson-Hamperl) techniques, by diazonium and argyrophil (Grimelius) techniques or by argentaffin and argyrophil techniques. It was observed that all cells reactive to staining with argentaffin and diazonium techniques were argyrophilic, too, but most cells were argyrophilic only. Furthermore some argentaffin cells were not reactive to staining with diazonium technique. Attempts to use Pb-H and argyrophil technique on the same or alternative sections of blocks fixed by glutaraldehyde, formaldehyde or Bouin's fluid were not successful. Staining with toluidine blue after acid hydrolysis revealed a small number of cells some of which were red-violet, some blue-

Table 1. Age, sex and macroscopically descriptions of six cases of bronchial carcinoid tumours

Case number	Age in years ♀	Age in years ♂	Site of tumour	Size of tumour	Gross appearance
I	25		right middle lobar bronchus	$2 \times 1^1/_2 \times 2$	globoid lobulated firm greyish
II		85	left main bronchus		polypodoid soft pink
III		65	left lower lobe, segmental bronchus	$2^1/_2 \times 2 \times 2$	globoid lobulated firm greyish
IV		62	right middle lobar bronchus	$4^1/_2 \times 4 \times 4$	globoid smooth firm pink
V	35		right lower lobe apical segmental brochus	$1 \times 2 \times 2$	infiltrating growth firm greyish
VI		28	right lower lobar bronchus	$2 \times 2 \times 2^1/_2$	globoid lobulated soft pink

violet. At pH 2 of the toluidine blue solution only few metachromatic cells were observed probably mastocytes. The HCl-toluidine blue method could not in my hand tolerate association with the other techniques but using diazonium reaction and HCl-toluidine blue on alternative sections of glutaraldehyde fixed blocks the number and distribution of blue-violet cells corresponded to the number and distribution of cells reactive to diazonium technique.

In case number II, III and V endocrine cells were stained only by the argyrophil silver method and Pb-H. In case number VI argyrophilic but otherwise unreactive cells were demonstrated.

By *electron microscopic examination* the tumours were found to be made up of polygonal cells some of which with several long cytoplasmic processes (Fig. 1). Plasmamembranes of neighbouring cells were separated from one another by narrow clefts and were only occasionally interdigitating. Desmosomes were never observed. A basement membrane paralleled the cell membranes adjacent to connective tissue (Fig. 2). Microvilli-like projections from the cell surface were occasionally observed (Fig. 3). In most cases light and dark cells were observed (Fig. 4). The dark appearance was not caused by organelles but seemingly by a quality of the hyaloplasm. The cytoplasm contained round or elongated mitochondria, often a prominent Golgi complex, stacks of rough endoplasmic reticulum and free ribosomes. In many cells lysosome structures were observed, in particular residual bodies with ultrastructural features suggesting lipofuscin could be recognized (Fig. 4).

Most tumour cells contained *secretory granules*. The number and appearance of these granules was variable between and within tumours, but within single cells all granules had the same morphology. Only few granules were found in tumour cells of case number VI. On basis of the morphology three types of secretory granules could be distinquished (Table 2). In some cells the secretory granules were characterized by their small size, almost uniform, round shape and the homogenous appearance of their central dense core. These granules were tightly surrounded by a membrane, although sometimes a narrow clear space could be

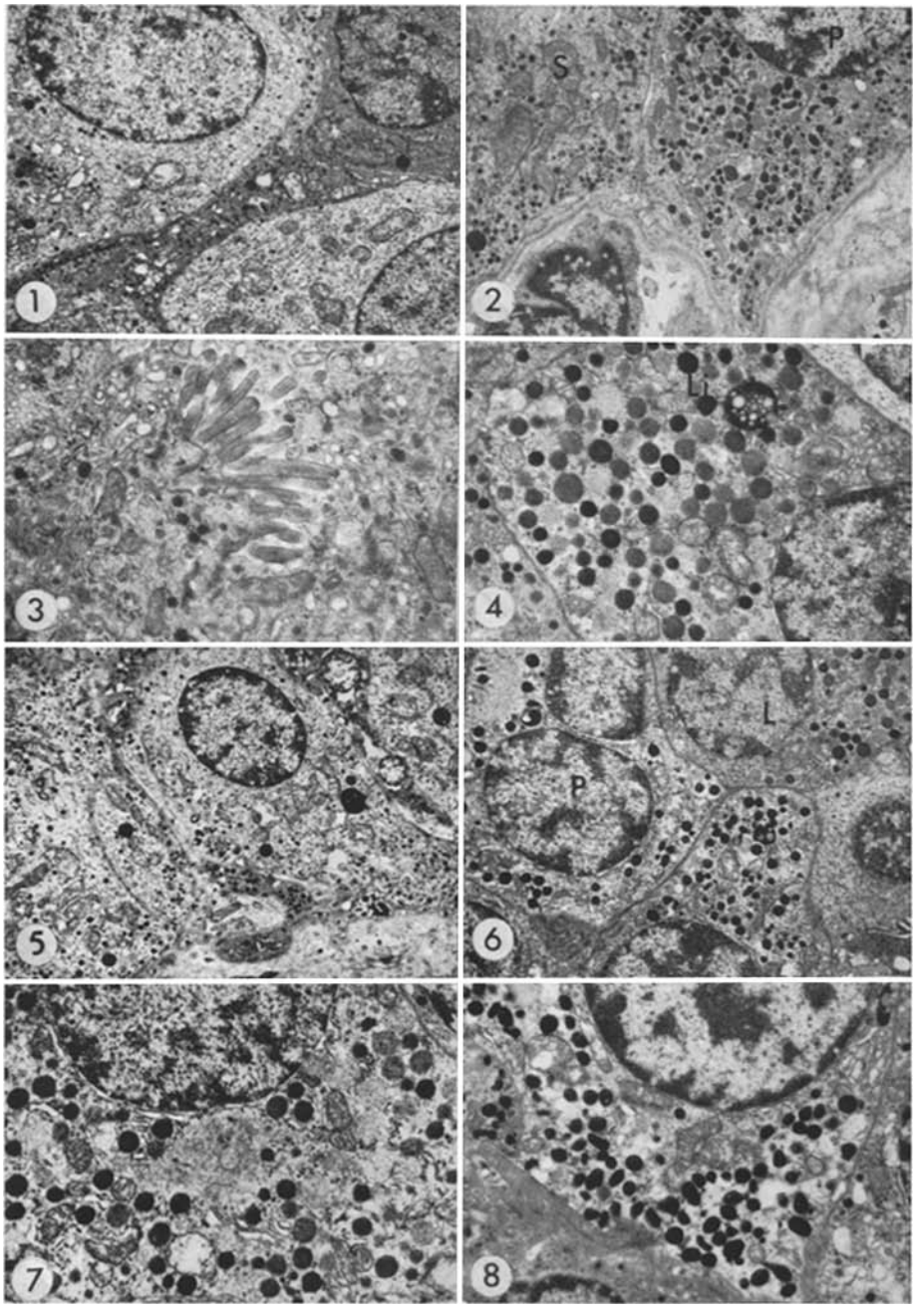

Fig. 1. Electron micrograph of bronchial carcinoid tumour case number I (× 3150). Light and dark cells with small, round secretory granules and cytoplasmic extensions

Fig. 2. Electron micrograph of bronchial carcinoid tumour case number V (× 4350). Cell with small, round secretory granules (*S*), and polymorphic secretory granules *P*. A basement membrane paralleled the cell membranes adjacent to connective tissues

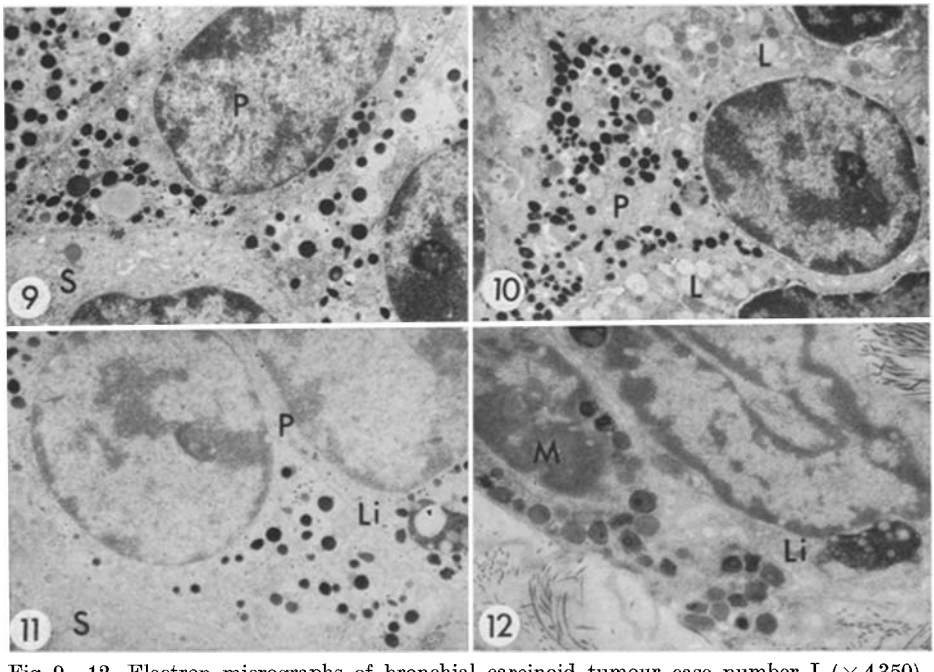

Fig. 9—12. Electron micrographs of bronchial carcinoid tumour case number I (× 4350). Masson-Hamperl argentaffin silver technique performed directly on ultrathin sections. Cells with polymorphic *P*, small, round *S* and large, round *L* secretory granules. Mast cell *M*. Lipofuscin bodies *Li*

observed between the central dense core and the membrane (Figs. 1, 5). The granules seemed to be concentrated in the cytoplasmic extensions. In the cell body some displacement toward the periphery could be noted. Other cells contained larger round granules of uniform size and bound by a discontinuous membrane. Their content varied between light granular and homogenous electron opaque. The granules of these cells were evenly distributed in the cell body (Figs. 4, 6, 7). Finally some cells contained polymorphic granules, polygonal, ovoid- or kidney shaped, vesiculated or tightly surrounded by a membrane (Figs. 2, 6, 8). These

Fig. 3. Electron micrograph of bronchial carcinoid tumour case number VI (× 11150). Microvilli-like structures

Fig. 4. Electron micrograph of bronchial carcinoid tumour case number I (× 5850). Cell with large, round secretory granules and lipofuscin bodies *Li*

Fig. 5. Electron micrograph of bronchial carcinoid tumour case number I (× 3150). Cells with small, round secretory granules

Fig. 6. Electron micrograph of bronchial carcinoid tumour case number I (× 3150). Cells with polymorphic *P*, and large, round *L* secretory granules

Fig. 7. Electron micrograph of bronchial carcinoid tumour case number I (× 5850). Cell with large, round secretory granules

Fig. 8. Electron micrograph of bronchial carcinoid tumour case number IV (× 5850). Cell with polymorphic secretory granules

Table 2. Morphology and staining characteristics of endocrine cell granules of six bronchial carcinoids

Case number	Morphology of granules	Argentaffin silver; thin-sections	Argentaffin silver a	Diazonium b	Argyrophil silver c	Lead haematoxyline b	HCl-HCluidine blue d
I	polymorphic	+	+	+	+	+	blue-violet
	large round	—	—	—	+ ?	+ ?	red-violet ?
	small round	—	—	—	+	—	—
II			—	—	+	+	—
III	large round	—	—	—	+ ?	+ ?	red-violet ?
	small round	—	—	—	+	—	—
IV	polymorphic	+	+	+	+	+	blue-violet
	small round	—	—	—	+	—	—
V	polymorphic	+	—	—	+	+	blue-violet
	small round	—	—	—	+	—	—
VI	small round	—	—	—	+	—	—

Fixative of choice: a = formaldehyde; b = glutaraldehyde; c = Bouin's fluid; d = glutaraldehyde-picric acid.

granules were reactive by application of ammoniacal silver (argentaffin reaction) to glutaraldehyde fixed specimens while granules of the other cell types were unreactive (Figs. 9–11). Besides these granules, lipofuscin bodies (Fig. 11) and granules of scattered mastocytes (Fig. 12) were reactive to the argentaffin silver method.

Discussion

The cells of origin of carcinoid tumours are known to be scattered endocrine cells in the epithelial lining of the digestive and respiratory tract. It may be supposed that a carcinoid tumour found in a given place reflects the morphological features of the endocrine cells normally found in that region. The similarity between the tumour cells and their parent cells is best reflected by the cytochemical and fine-structural characteristics of their secretory granules.

The cytochemical characteristics of endocrine cells in the pulmonary epithelium of human foetuses were described by Hage (1972). Using Grimelius' silver nitrate method argyrophilic cells were observed singly or in groups of two or more cells. By application of Pb-H and HCl-toluidine blue only few, often singly appearing cells were reactive. Later (Hage, 1973 in press) the fine structure of at least 3 types of endocrine cells, each of which possessed characteristic secretory granules, were observed. Cells described as type 1 contained small round membrane-bound granules with a dense core separated from the surrounding membrane by a narrow clear space and larger bubble-shaped granules containing small eccentric accumulations of high electron density reactive to argentaffin silver method performed directly on ultrathin sections. Cells described as type 2 contained small, round, membrane-bound granules of uniform shape and size, and cells described as type 3 contained larger granules of round shape and homo-

genous appearance, tightly surrounded by a membrane. All three cell types were reactive to argyrophil silver method performed directly on ultra-thin sections. Most cells were of type 1 and 2, often found together in groups, whereas cells of type 3 were few, similar in number and distribution to those reactive to Pb-H and HCl-toluidine blue.

Cells with fine-structural characteristics of type 1 cells in foetal bronchial epithelium were not found in the present tumour material. However, in a study of Black (1969) comparing the fine-structure of one pulmonary oncocytoma with the fine-structure of one typical bronchial carcinoid, he described cells in the oncocytoma not present in the carcinoid. The cells were characterized by large vesiculated granules admixed with smaller, round granules almost tightly bound by a membrane. Hamperl (1937) described two bronchial carcinoid tumours containing cells which he termed oncocytes. He described by light microscopy these cells as large cells containing abundant eosinophilic, granular cytoplasm and small pyknotic nuclei. Electron microscopic studies of oncocytomas in other tissues disclosed large mitochondrie packed cells without specific secretory granules. Supposing pulmonary oncocytomas are variants of bronchial carcinoid tumours their progenitor cell could be cell type 1 observed in the pulmonary epithelium of human foetuses.

Cells with fine-structural characteristics of type 2 cells in foetal bronchial epithelium could be observed in all carcinoid tumours examined by me. In case number VI this cell type was the only granulated cell type observed. That part of tumour used to cytochemistry was unreactive to all methods applied except argyrophil silver technique. Unfortunately argyrophil silver method could not be used directly on ultra-thin sections because of improper fixation. Cells with fine-structural characteristics of type 3 cells in foetal bronchial epithelium could be observed in two cases, although a wider range in size of the granules and a greater variation in electron density of the granules was noted. These cells were in case number I and III scattered among great numbers of the other granulated cell types described and are supposed to be identical with the red-violet cells observed in case number I and III after acid hydrolysis and staining by toluidine blue pH 5.

A cell type not observed in the bronchial epithelium of human foetuses was found in case number I, IV and V. These cells contained polymorphic granules of varying size reactive to argentaffin silver method applied directly on ultra-thin sections. In parts of these tumours preserved for argentaffin and diazo techniques, reactive cells were observed in case number I and IV but not in case number V. It was shown that these cells were argyrophilic too, and that they were stained by Pb-H. They are supposed to be identical with the blue-violet cells in case number I and IV by use of HCl-toluidine blue technique. The origin of the relatively large number of these cells in some bronchial carcinoids requires explanation. Gloor, Campiche and Baumann (1972) pointed out the importance of distinguishing between silver reducing meta- or orthochromatic mastocytes and silver reducing endocrine cells. In the present study scattered mastocytes reactive to argentaffin silver method were observed in all cases but they were easily distinguished from a much greater number of argentaffin, polymorph-granulated endocrine cells in case number I, IV and V. However, the fine-structure and the

staining pattern of these polymorph-granulated endocrine cells are quite similar to those of enterochromaffin cells of the human gastrointestinal tract (Pearse *et al.*, 1970). The occurrence of this cell type in bronchial carcinoids might be explained in two ways: they might arise from single preexisting endocrine cells in the bronchial epithelium or parabronchial glands not yet identified, or they could arise by modification of those endocrine cells which are normally present.

Transformation of one type of endocrine cells into another type has not been observed but is discussed by Polak *et al.* (1971) related to the occurrence of gastrin-cells in the fundic mucosal glands in pernicious anaemia.

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