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Ultrastructural and Histological Study of 11 Bronchial Carcinoids

Evidence for Different Types

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Summary. Seven of eleven bronchial carcinoids investigated showed cells with small granules resembling P cells which have already been described in human fetal and adult lung; two of these P cell tumours showed distinctive paraganglioid features. One tumour showed peculiar ultrastructural findings resembling closely those previously reported by Black (1969) in a so called "pulmonary oncocytoma". Three remaining cases showed large secretory granules resembling those of type 3 cells already described by Hage (1973b) in bronchial carcinoids; one of these tumours produced large amounts of 5-hydroxytryptamine (5HT). It is concluded that, on cytological grounds, at least two types of tumours can be distinguished among bronchial carcinoids, i.e. P cell and type 3 cell tumours. Moreover, two varieties of P cell carcinoids have been recognized, showing either the less frequent and more distinctive paraganglioid structure or the more common trabecular structure.

Key words: Bronchial carcinoids – Different types – Cells of origin – Ultrastructure – Histology.

Introduction

Carcinoid tumours of the lung have been reported to produce various hormone-like substances, including 5-hydroxytryptamine (5HT), 5-hydroxytryptophan (5HTP), ACTH, MSH, corticotrophin releasing factor (CRF), vasopressin (ADH), GH, prolactin, parathormone, calcitonin, insulin, glucagon and vasoactive intestinal peptide (VIP) (Pariente et al., 1967; Upton and Amatruda, 1971; George et al., 1972; Said, 1974; Imura et al., 1975). Hormone-like substances

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have been also detected in non-tumour lung tissue, among which biogenic amines (Lauweryns and Peuskens, 1972; Hage, 1976), VIP-like peptides (Said and Mutt, 1969 and 1977), angiotensin-like activity (Berkov, 1974; Allison and Clay, 1976), a bombesin-like peptide (Wharton et al., 1978), big ACTH (Gewirtz and Yalow, 1974) and ADH-like peptides (Vorherr et al., 1970), have all been described. As possible sources for these compounds three cell types have been identified ultrastructurally in human fetal lung; type 1 or P_1 *cells*, with very small vesicular and haloed granules, type 2 or P_2 *cells* with small cored granules and *type 3* cells with relatively large homogenous granules (Hage, 1973a; Capella et al., 1978b).

Despite these heterogeneous cytological and biochemical findings, data concerning the occurrence of cytologically distinct bronchial carcinoids are controversial: multiple cell types have been described by Hage (1973b) but subsequently denied by Bensch and his associates (Bonikos et al., 1976b).

In this paper, based on 11 bronchial carcinoids, we present histological and ultrastructural evidence supporting the characterization of different types of tumours. In addition, tumour cells have been compared with the endocrine cells of non-pathological lung described in a previous paper (Capella et al., 1978b).

Material and Methods

Eleven bronchial tumours diagnosed as bronchial carcinoids on histological grounds have been investigated:

Case 1 (2385PR76): female, 25 year-old. Iceberg-like tumour of the right middle lobar bronchus, 5 cm large. No aggressive pattern or metastases. Alive and well 2 years after lobectomy.

Case 2 (2717PV76 and 2950PV76): male, 39 year-old. Polypoid tumour filling the left inferior lobar bronchus, 3 cm large, without local infiltration or metastases. Alive and well 2 years after lobectomy.

Case 3 (104315PR): female, 49 year-old. Polypoid tumour, 2 cm large, filling the right inferior lobar bronchus. No signs of aggressive behaviour or metastases. Alive and well 3 years after lobectomy.

Case 4 (1930PV78): female, 37 year-old. A 2 cm, iceberg-like tumour of the right superior lobar bronchus, infiltrating the surrounding lung tissue and the adjacent main right bronchus. Moderate nuclear hyperchromatism and atypia, rare mitoses, histological signs of local and vascular infiltration (atypical carcinoid), without metastases. Alive and well 5 months after lobectomy and partial resection of the main right bronchus.

Case 5 (3775VA78): female, 34 year-old. A 3.5 cm well demarcated tumour of the left superior lobar bronchus, partly protruding in the lumen, partly extrabronchial; without local infiltration or metastases. Alive and well 2 months after lobectomy.

Case 6 (36411PV): female, 21 year-old. Small (1.5 cm) tumour of the right inferior lobar bronchus, mostly extrabronchial, well circumscribed and without metastases. Alive and well 7 years after lobectomy.

Case 7 (S72/2280): female, 41 year-old. A 2.5×1.5 cm tumour filling the upper lobar bronchus of the left lung, with extrabronchial component; well circumscribed and without metastases. Alive and well 6 years after lobectomy.

Case 8 (109451PR): male, 50 year-old. Iceberg-like tumour of the right inferior lobar bronchus, 7–8 cm large, with extensive osseous metaplasia of the stroma, signs of local infiltration and metastasis in a hilar lymph node. Alive and well 3 years after lobectomy.

Case 9 (S70/3549): male, 65 year-old. Iceberg-like tumour filling the left lower lobar bronchus, removed by lobectomy. Multiple liver metastases appeared 5 years later, when flushing and increased urinary (3000 μmol; normal <37) and plasma (1.58 μg/ml; normal=undetectable) levels of 5-hydroxyindolacetic acid (5HIAA) and plasma levels of 5HT (2.6 μg/ml; normal=0–0.002) were found. Despite the metastases the patient is still alive and relatively well 8 years after the first diagnosis.

Case 10 (Specimens kindly given by Dr. M. Canepa, see case 1 of Canepa et al., 1969): male, 65 year-old. Iceberg-like, large tumour obstructing the main right bronchus and infiltrating widely the surrounding tissues. It was reputed inoperable and repeatedly biopsed endoscopically in the subsequent thee years, to remove the obstruction. Then the patient was lost to follow up.

Case 11 (8142PV76): male, 55 year-old. A 3 cm tumour of a large bronchus in the inferior right lobe, mostly extrabronchial and infiltrating the surrounding parenchima; no metastases. Alive and well 2 years after lobectomy.

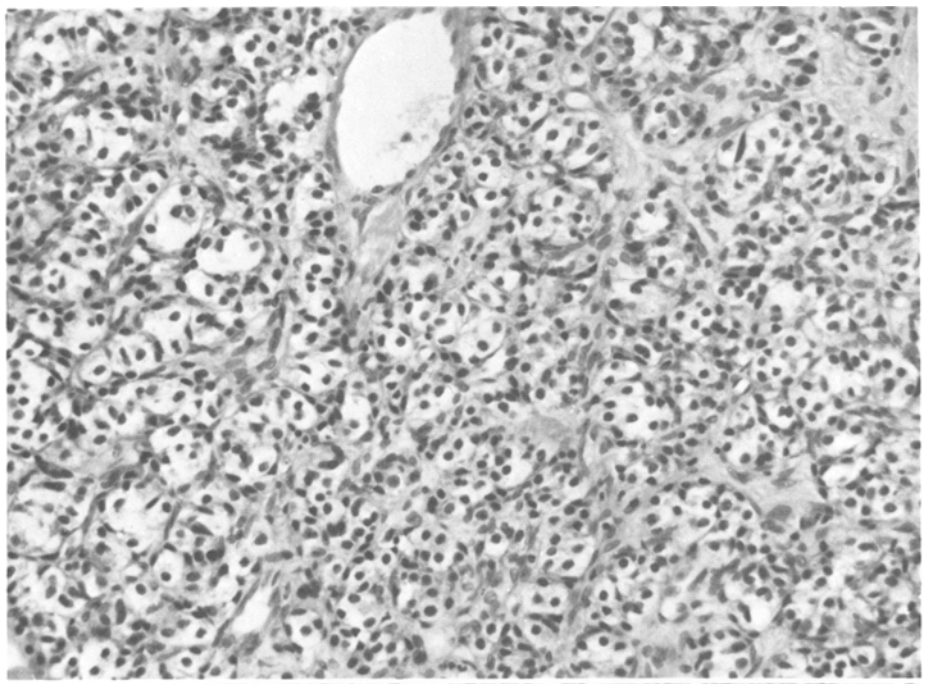
Specimens of tumour tissue were fixed in 4% formaldehyde or Bouin's fluid. Paraffin sections were stained with Grimelius' (1968) and Sevier-Munger's (1965) silver techniques, lead haematoxylin (Solcia et al., 1969a), and diazonium, argentaffin and fluorescence techniques for 5HT (Solcia et al., 1969b). Small samples of tumour 9 were freeze-dried; paraffin sections were treated with formaldehyde vapour and observed by fluorescence microscopy to detect biogenic amines (Pearse, 1968).

Fresh samples of tumours 1, 2, 3, 6, 7, 8, 9 and 10 as well as formalin-fixed samples of tumours 4, 5 and 11 were fixed with 2.5% glutaraldehyde or 2% paraformaldehyde +2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 and embedded in Araldite or Epon-Araldite mixture. Sections were stained with uranyl acetate and lead citrate. Some aldehyde-fixed samples were stained with Grimelius' and Sevier-Munger's silver techniques before embedding (Vassallo et al., 1971). The diameters of all granules found in 4 to 8 cells from each tumour were measured and their mean and standard deviation (SD) were calculated.

Results

Light Microscopy

Solid sheets of uniform tumour cells separated by thin fibrovascular septa were found in all tumours. This common pattern was associated either with (a) extensive areas characterized by a microlobular, distinctly paraganglioid structure (cases 1 and 2) (Fig. 1) or with (b) a more or less prominent trabecular pattern (all remaining cases), often with thin cords and convoluted festoons, sometimes (cases 3, 5 and 6) with microacini showing true lumen (Fig. 2). In tumours with paraganglioid features cells were small, with clear to faintly acidophilic cytoplasm, poorly reactive with argyrophil techniques, unreactive with lead haematoxylin and Masson's argentaffin reaction. In tumours with a trabecular pattern cells were polygonal in solid sheets or broad trabeculae, or columnar in thin cords, with fairly aboundant, acidophilic cytoplasm. Relatively large, oncocytoid cells with abundant, granular and acidophilic cytoplasm were observed in case 8. Argyrophilia was poor in cases 3, 4 and 5, slight in cases 6, 7 and 8, rather intense in cases 9, 10 and 11. Staining with lead haematoxylin was evident in the latter three tumours and in some cells of



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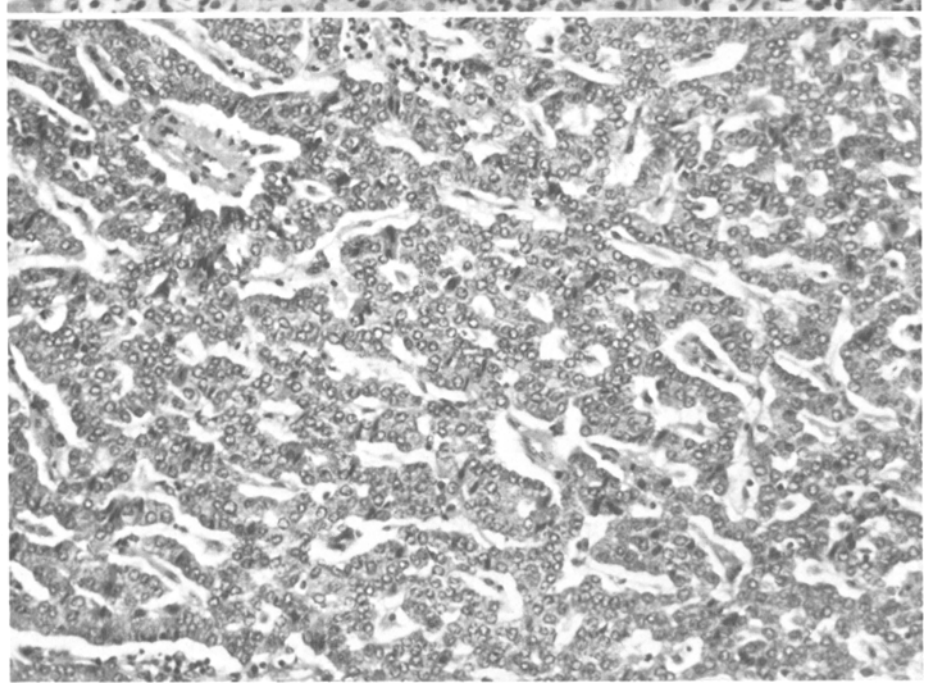

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Fig. 1. Paraganglioid structure of tumour N. 1. Hematoxylineosin. ×200

Fig. 2. Trabecular pattern with thin, convoluted cords in tumour N. 9. ×180

Table 1. Light and electron microscopic findings in 11 bronchial carcinoids

Case No.	Light microscopy	Electron microscopy			
		Secretory granules			Relevant findings
		Type	Size (nm ± SD)	Number counted	
1	Paraganglioid areas	P	112 ± 30	327	Neuroid patterns, fibrous bodies, few granules
2	Paraganglioid areas	P	120 ± 21	368	Fibrous bodies
3	Trabecular pattern, microacini	P	about 100		Very few granules
4	Trabecular pattern	P	about 110		Very few granules
5	Trabecular pattern, microacini	P	about 110		Very few granules
6	Trabecular pattern, microacini	P	138 ± 22	444	Fairly granular
7	Trabecular pattern	P	131 ± 22	516	Numerous granules
8	Trabecular pattern,	P	134 ± 24	796	Numerous granules
	"oncocytoid" cells	?	217 ± 67	635	Target granules
9	Trabecular pattern, 5HT	Type 3	209 ± 43	539	Numerous granules
10	Trabecular pattern	Type 3	212 ± 54	495	Numerous granules
11	Trabecular pattern	Type 3	205 ± 47	483	Numerous granules

case 8, was poor in case 7 and absent in the remaining cases. All tumours failed to react with 5HT tests in routinely fixed sections; however in freeze-dryed sections tumour cells of case 9 showed slight formaldehyde-induced yellow fluorescence.

Electron Microscopy

Ultrastructural findings allowed us to distinguish two groups of tumours, chiefly on the basis of secretory granules morphology. In addition, one case (N. 8) showed somewhat peculiar features.

Tumour cells of the first group (cases 1–7) showed round, small granules (mean diameters below 140 nm) resembling those of pulmonary P cells (Table 1 and Figs. 1–6). Within this group, tumours characterized by paraganglioid pattern at the optical level (cases 1 and 2) showed remarkable ultrastructural similarities. Their cells showed thin, long cell processes and complex interdigitations (Fig. 3). The granules were few, small, often with a clear space in between the membrane and a central or eccentric core of variable osmiophilia (Fig. 4a). Several empty vesicles, from 50 to 200 nm, were also found. Granules and vesicles were often concentrated in the cell processes or were marginated; sometimes they were grouped at one side of focal thickenings and fusions of the cytoplasmic membranes of adjacent cells. This pattern, mimicking synaptic-like junctions to some extent, has already been described in carotid body cells (McDonald and Mitchell, 1975). Microfilaments, microtubules and smooth en-

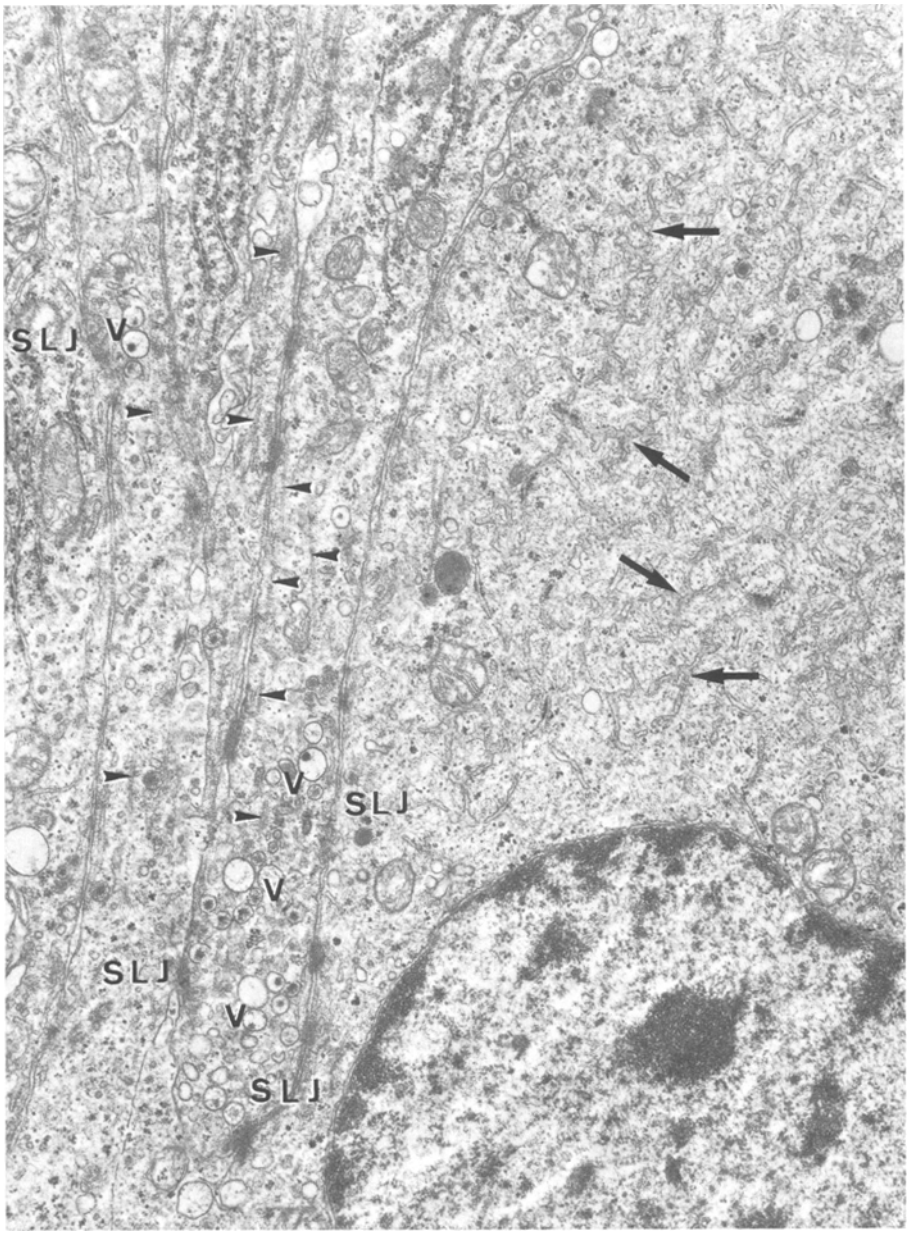

Fig. 3. Ultrastructure of the same tumour as Fig. 1. A large aggregate of branching smooth walled tubules (*arrows*) and microfilaments occupies the paranuclear region of the cell on the right. Elongated cell processes (*left*) contain microtubules (*arrowheads*), granules and vesicles (*V*); these often accumulate on one side of focal synaptic-like junctions (*SLJ*). ×14,340

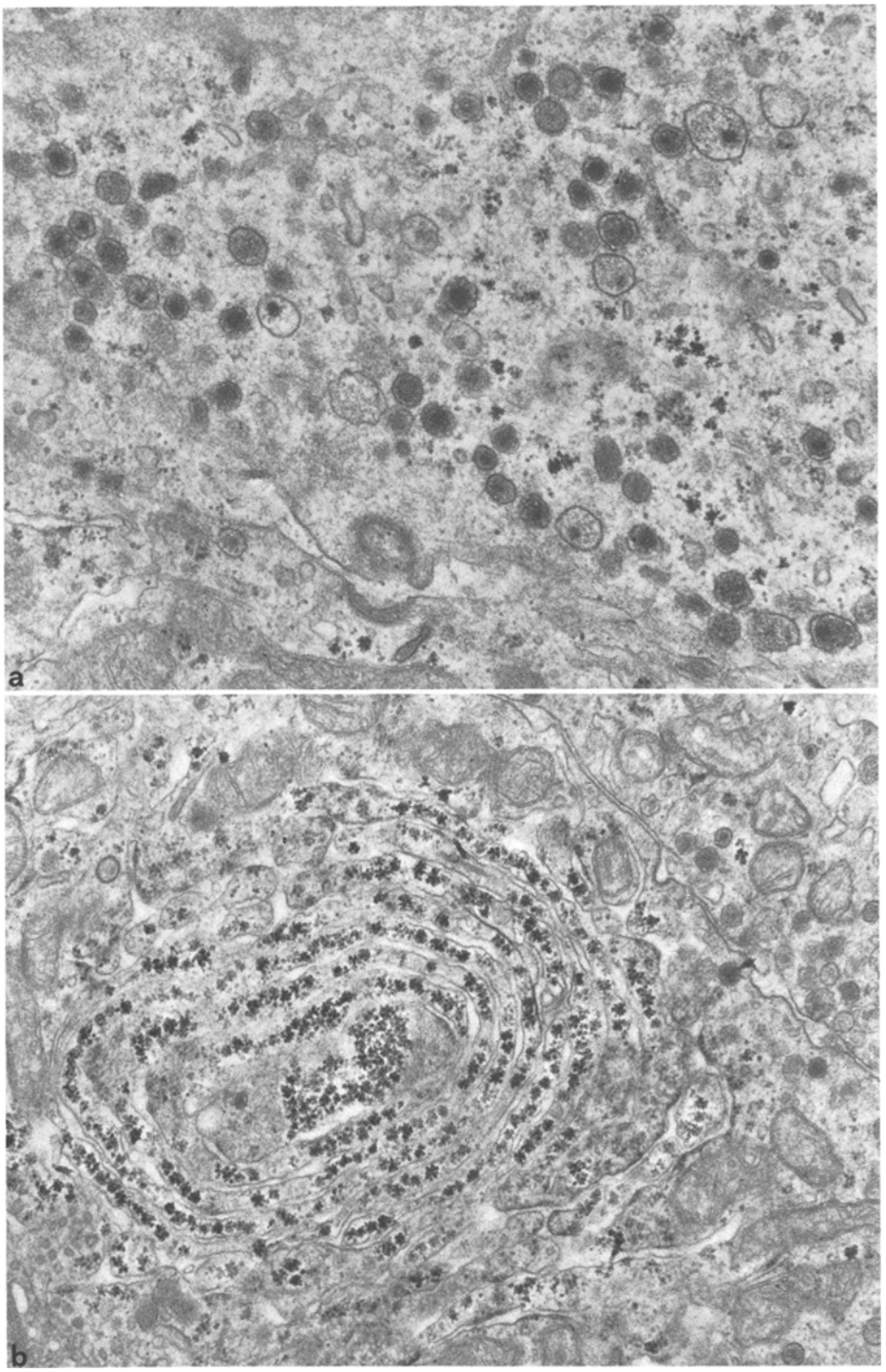

Fig. 4a and b. Paraganglioid tumour N. 2 showing P type secretory granules, some of which with eccentric core (**a**), and a finger-print (**b**). **a** ×35,000; **b** ×30,000

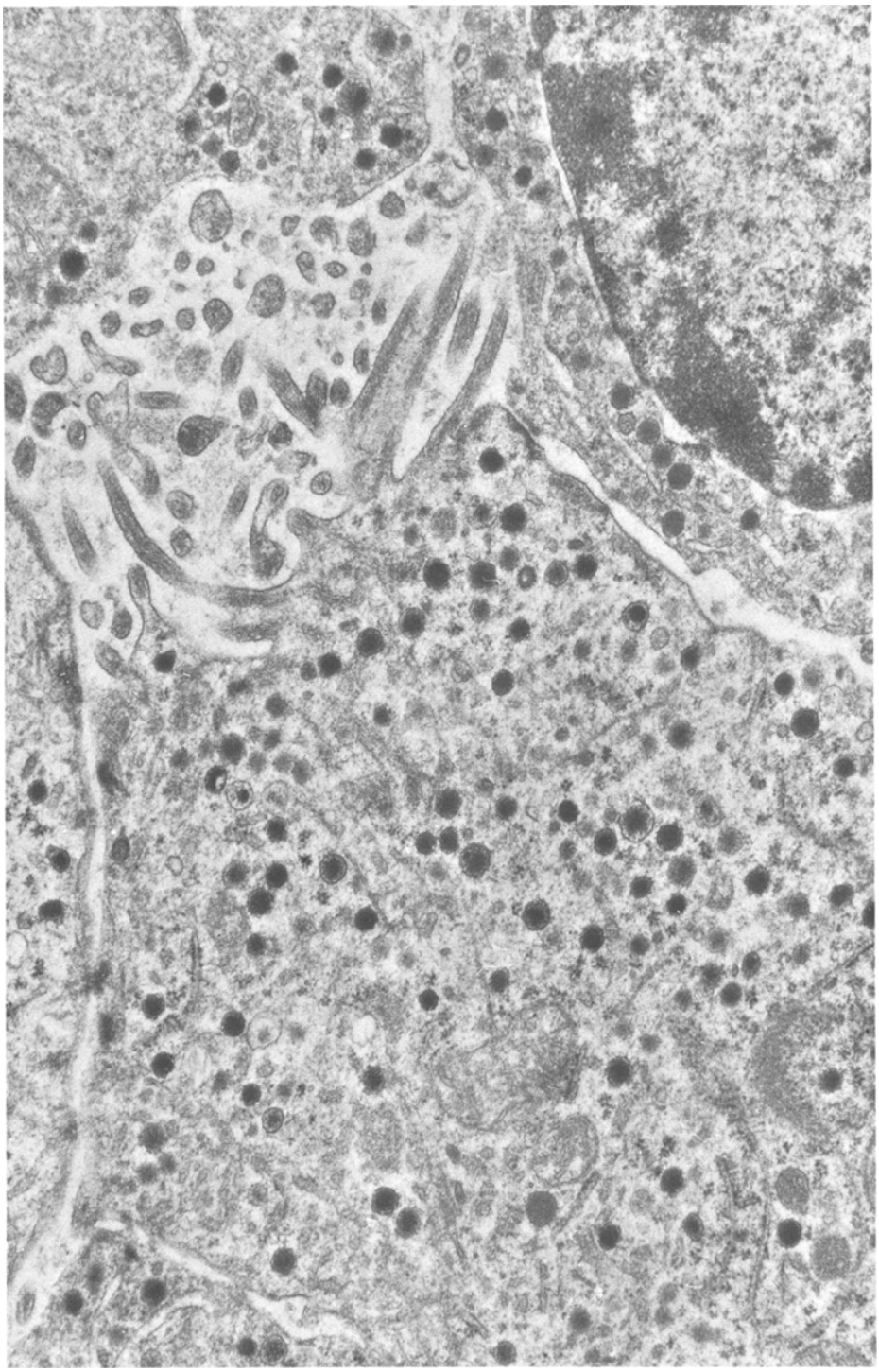

Fig. 5. Trabecular P cell carcinoid (case 7) showing small haloed granules and a lumen filled with microvilli. ×28,000

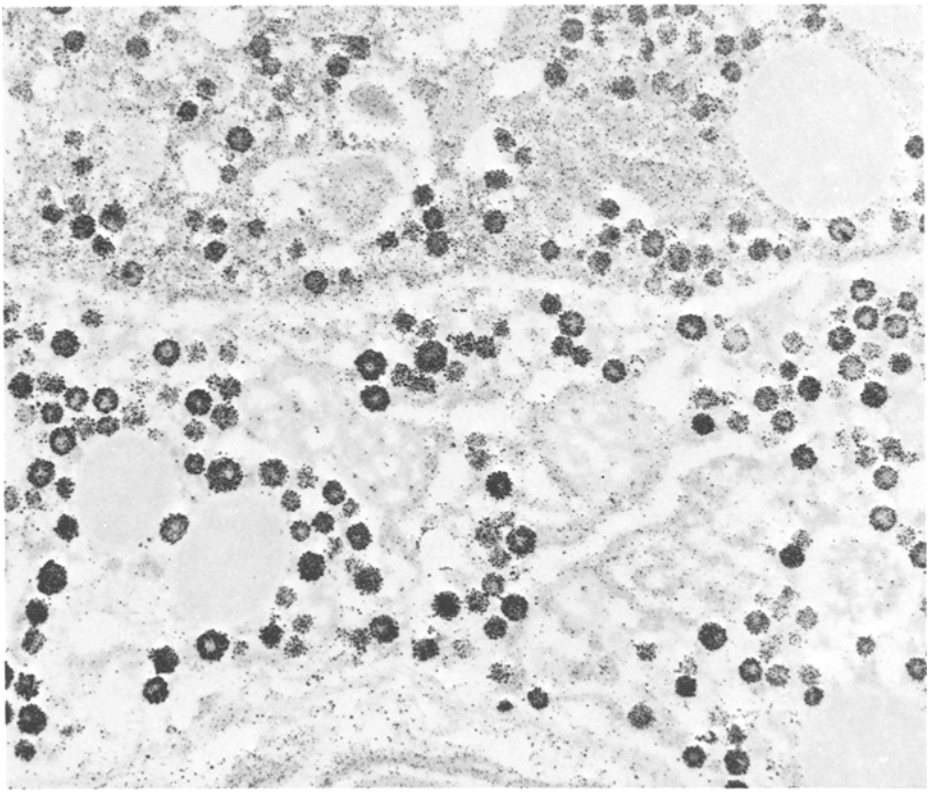

Fig. 6. Granules of two tumour cells of case 6, stained with Sevier-Munger's silver. Note the argyrophil halo surrounding the unreactive core. × 28,000

doplasmic reticulum were abundant. Frequently, aggregates of branching smooth walled tubules and microfilaments were found in the region between the nucleus and the Golgi apparatus (Fig. 3); they were similar to the "fibrous bodies" observed in pituitary adenomas (Horvath and Kovacs, 1978). Concentric whorls of smooth reticulum (so called "fingerprints"), with interposed glycogen particles, were also a prominent finding (Fig. 4b). The mitochondria were rather numerous, ovoid or elongated, while the Golgi complex was small. Scanty, irregular microvilli protruded in poorly developed intercellular spaces or at the base of the cells in direct contact with fibrovascular stroma. Tumour cells of cases 1 and 2 resembled in several aspects P_1 cells of fetal lung forming interdigitated groups (Capella et al., 1978 b). Only very few cells of case 2 showed larger solid granules resembling those of D_1 cells (See Discussion).

The remaining tumours with small granulated cells (cases 3–7) were characterized by prominent intercellular spaces and microacinar lumina, often filled with tufts of long, thin microvilli and with occasional cilia (Fig. 5). They showed abundant, round mitochondria, numerous lysosomes and well developed Golgi complex and rough endoplasmic reticulum, often dilated and sometimes forming parallel arrays. Smooth reticulum, microfilaments, microtubules and cell

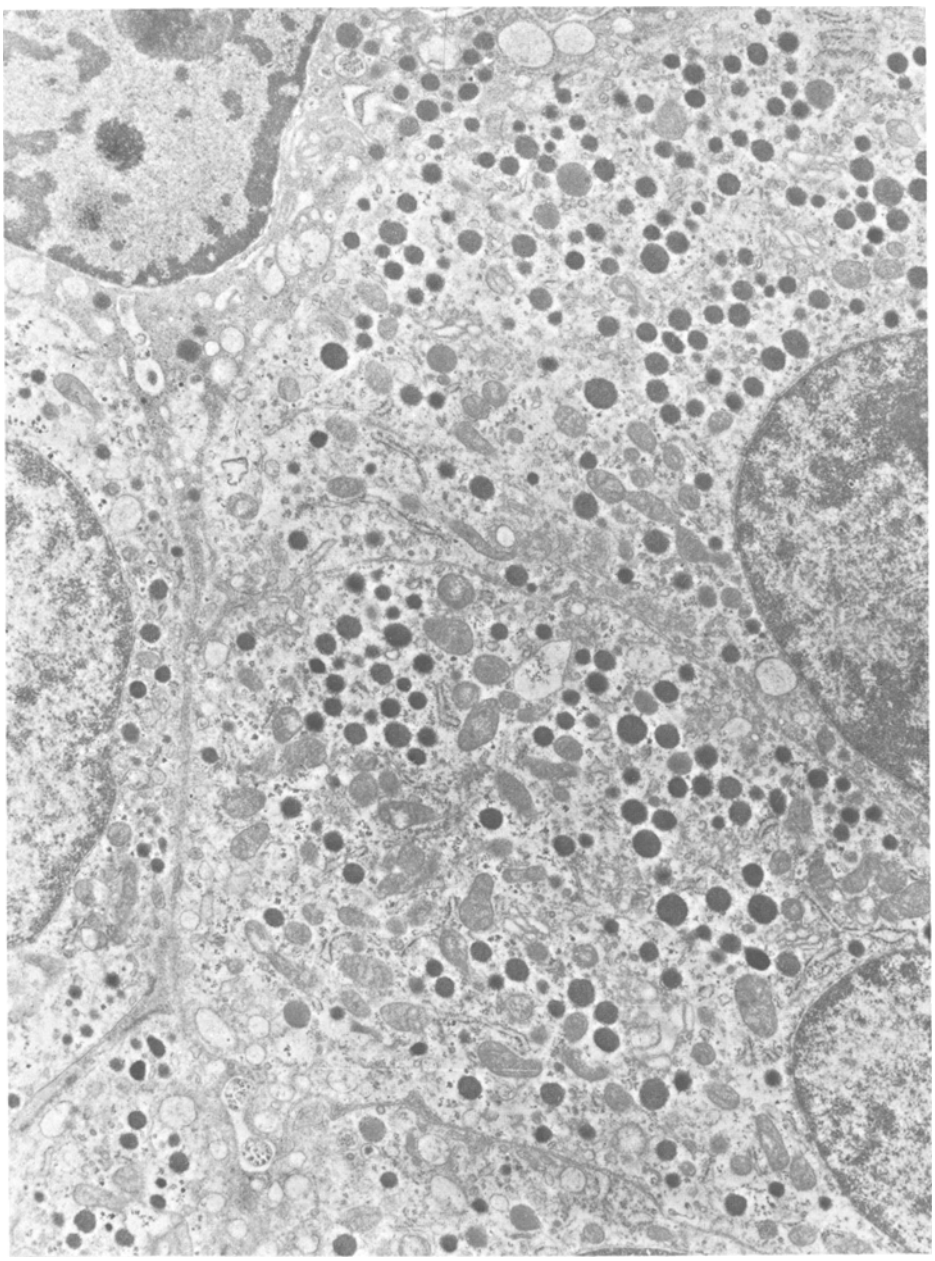

Fig. 7. Type 3 cell carcinoid (case 9). × 11,250

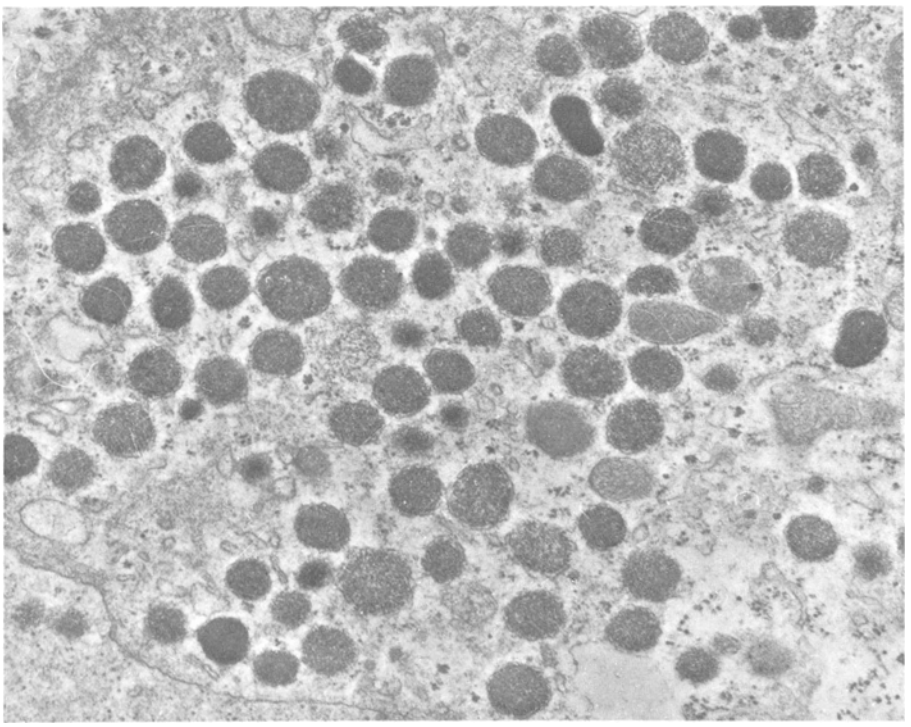

Fig. 8. Granules of a tumour cell in the type 3 cell carcinoid of Fig. 7. ×28,000

processes were not prominent. Fibrous bodies were seen only in case 3. Secretory granules of cases 3 and 4 and of most cells in case 5 were extremely few, very small and more often scattered around the Golgi zone; they were interpreted as progranules. Granules of cases 6 and 7 and of a few cells in case 5 were small, fairly numerous, round, with moderately osmiophilic core and an evident, argyrophilic halo (Figs. 5 and 6). Tumour cells from such cases resembled human P cells, especially those cells scattered in adult bronchi and bronchioli or fetal P_2 cells (Terzakis et al., 1972; Hage et al., 1977; Capella et al., 1978b).

In the second group (cases 9, 10 and 11) tumour cells showed large, round to irregular granules of various density and inner texture (Figs. 7 and 8). The mitochondria were small and elongated, the rough endoplasmic reticulum, ribosomes and Golgi complex were fairly well developed; microtubuli and microfilaments were poorly represented. These tumour cells resembled "type 3 cells" found by Hage (1973b) to be scattered in a few bronchial carcinoids. No lumina, cilia, microvilli or complex interdigitations were seen in tumours 2 and 10; occasional microacini with microvilli were observed in tumour 11.

In case 8 some tumour cells reproduced the ultrastructure of the cells described in heavily granulated tumours of the first group (cases 6 and 7), including their granule size and structure. Other cells showed larger granules of double, often target-like structure, with argyrophil, poorly osmiophilic material surrounding an osmiophilic core. A few cells with large, moderately osmiophilic,

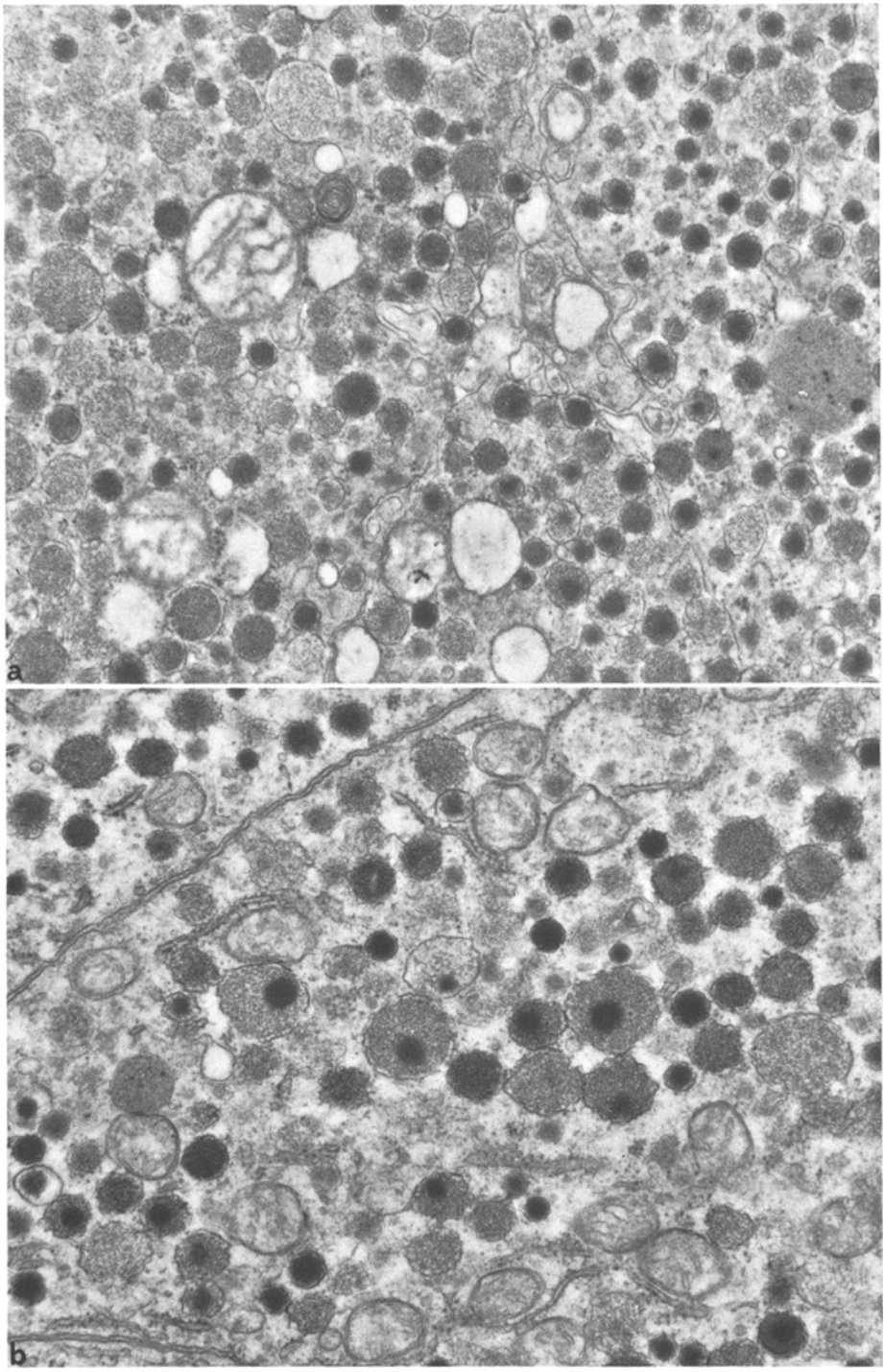

Fig. 9a and b. Case 8. Parts of tumour cells filled with secretory granules of various size, density and inner structure (homogeneous texture, central or eccentric core, target-like pattern). **a** and **b** ×28,000

homogeneous and argyrophobe granules, somewhat resembling those of gut D cells, were also observed. Finally, cells storing both haloed or target-like, argyrophil granules and solid, argyrophobe granules were found (Fig. 9).

Discussion

At least two types of tumours were identified by electron microscopy among the eleven bronchial carcinoids investigated. The first type, composed of 7 cases, showed cells with small granules identified as pulmonary P cells (Capella et al., 1978b). The second type, comprising 3 cases, showed cells with large granules resembling the "type 3" tumour cells reported by Hage (1973b). The remaining tumour showed somewhat peculiar findings resembling closely those of the so-called pulmonary "oncocytoma" reported by Black (1969), although differing from true oncocytomas with mitochondria-laden cells.

In the light of previous results on bronchial type 3 cells (Hage, 1973a and 1976), the intense reactivity of tumours 9, 10 and 11 with Grimelius' silver and lead haematoxylin should support their identification as type 3 cell growths. However, the majority of bronchial type 3 cells showed granules smaller than those found in the tumours; they resembled gastric D_1 cells (Capella et al., 1978b), possibly storing bombesin-like peptides (Wharton et al., 1978). Only a few bronchial cells showed granules larger than 190 nm (Capella et al., 1978b), thus fitting in the range of tumour granules. Certainly the tumours are unrelated to D_1 cells; they are called here "type 3 cell tumours" to emphasise their similarity to the cells already described in bronchial carcinoids (Hage, 1973b) rather than to those of fetal bronchi.

The production of large amounts of 5HT by our tumour 9 is interesting in the light of some previously reported bronchial carcinoids. Case 28 of Hattori et al. (1972) and that of Brune et al. (1973) showed cells with large, dense granules, high 5TH content in tumour extracts, increased 5HT levels in serum, increased 5HIAA in urine and typical flushing and diarrhoea. Case D of Bensch et al. (1965b) showed – besides P type cells – scattered cells with larger, homogeneous granules as well as increased levels of 5HT in blood and 5HIAA in urines. A tumour with relatively large, round granules reacting with the Masson-Fontana argentaffin reaction has been reported by Bensch et al. (1968; see figs. 4, 5 and 9). Similar 5HT storing cells have been also found in two medullary carcinomas of the thyroid (Capella et al., 1978a). The relationship of such cells to gut EC cells also storing 5HT (Solcia et al., 1975), remains doubtful. Cells with pleomorphic granules resembling those of EC cells have already been reported in bronchial carcinoids in association with type 3 and/or P cells (Hosoda et al., 1970; Hage, 1973b). To our knowledge, only one ultrastructurally unquestionable EC cell carcinoid has been reported in the lung (Patchefsky et al., 1974). EC cells are lacking in adult or fetal human lung (Capella et al., 1978b).

The majority of lung carcinoids studied in this paper or reported in the literature (Bensch et al., 1965b; Verley, 1965; Picardi et al., 1972; Hage, 1973b), including peripheral carcinoids (Gmelich et al., 1967; Bonikos et al., 1977b) and so-called tumorlets (Bonikos et al., 1976a; Churg and Warnock, 1976)

were exclusively or largely made up of small granule cells resembling ultrastructurally the P cells of human (Bensch et al., 1965a; Basset et al., 1971; Terzakis et al., 1972; Hage et al., 1977; Capella et al., 1978a) and animal lung (Ericsson et al., 1972; Cutz et al., 1974). Similar tumours have also been reported outside the lung, as for instance in the thymus (Rosai and Levine, 1976), gut and pancreas (Capella et al., 1977).

Based on our light and electron microscopy studies a further subdivision of pulmonary P cell carcinoids into trabecular and paragangliodid tumours seems possible. The latter sometimes showed a neuroid pattern; they might be related to fetal P_1 cells forming interdigitated groups (Capella et al., 1978b) and to the so-called "neuroepithelial bodies or NEB", known to be made up of interdigitated, untraepithelial cells producing serotonin and synaptically joined to nerve endings (Lauweryns and Peuskens, 1972; Lauweryns et al., 1972 and 1973). Tumours resembling our paragangliod carcinoids histologically (Bonikos et al., 1976b: cases 1 and 5) or ultrastructurally (Gmelich et al., 1967) have already been described. From both our findings and those reported in the literature it seems that trabecular tumours predominate among P cell carcinoids.

Paraganglioid carcinoids are to be distinguished from true pulmonary paragangliomas with small P-type granules, like the case recently described by Singh et al. (1977). The close association with a pulmonary artery is a distinctive feature of true paragangliomas, while the presence of long microvilli protruding into intercellular spaces seems more suggestive of carcinoids. Trabecular carcinoids, especially those forming acini, are easily distinguished from paragangliomas on simple histological grounds.

No specific secretory products have been identified so far in P cell tumours. A bronchial P cell carcinoid of trabecular variety has been reported in association with acromegaly due to the production of a GH-releasing peptide by the tumour (Sönksen et al., 1976). Vasopressin (George et al., 1972), CRF (Upton and Amatruda, 1971), ACTH and MSH related peptides (Ratchliffe et al., 1973; Gewirtz and Yalow, 1974; Imura et al., 1975) and 5HT (Pariente et al., 1967; Hattori et al., 1972) have been repeatedly associated with lung carcinoids or oat cell carcinomas which, when studied ultrastructurally, often showed features suggesting more or less pronounced P cell differentiation. The similarity of granules of pulmonary P cells to those of nerve endings in the external zone of human median eminence has been already noted (Capella et al., 1978b).

In conclusion, despite the occurrence of tumours with mixed cell populations (Hage, 1973b; Bearzi et al., 1974), cytologically different lung carcinoids can be identified. At least two tumour types are to be distinguished, i.e. P and type 3 cell carcinoids, while it remains uncertain whether further subdivision of P cell tumours into trabecular and paraganglioid carcinoids on both histological and cytological grounds really separates two distinct tumour entities. Differencies of endocrine activity, natural history or behaviour may be expected among different tumour types. A possibly higher malignant potential of type 3 cell carcinoids, as suggested by the behaviour of two of our three cases, deserves further study.

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