Virchows Arch [Pathol Anat] (1985) 408:163–172

Virchows Archiv A

Ultrastructural, immunohistochemical and biochemical studies on amylase and ACTH producing lung cancer

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Summary. Tumour tissue from a lung cancer patient who showed elevated serum amylase and adrenocorticotropin (ACTH) was studied ultrastructurally, immunohistochemically and biochemically. Histologically the tumour was a small cell carcinoma. On electron microscopic examination the tumour cells contained large zymogen-like granules within the cytoplasm. Furthermore, cells which possessed many small dense core granules of the endocrine type were also observed. It was of interest that the large zymogen-like granule-containing tumour cells had microvilli at the apical border, connected by desmosomes and forming lumina showing adenocarcinomatous differentiation. Electrophoretic analysis of the serum revealed that the major elevated amylase was of the salivary type with minor components. Immunostaining clearly demonstrated that most of the tumour cells possessed immunoreactive ACTH, whereas salivary amylase was only found in occasional clusters of the tumour cells. The results seem to indicate that the tumour showed both endocrine and exocrine characteristics – an amphicrine carcinoma, expressing amylase and ACTH simultaneously.

Key words: Small cell carcinoma – Amylase – ACTH – Ultrastructure – Immunohistochemistry

Introduction

A hormone-producing tumours which originate from an organ that normally does not show hormone production has been called an "ectopic hormone-producing tumour" (Liddle et al. 1965; Odell and Wolfsen 1975; Baylin and Mendelsohn 1980). Most of these tumours are lung tumors such as small cell carcinoma or bronchial carcinoid, oesophageal cancer, breast cancer and prostastic carcinoma (Reyes et al. 1980; Vuitch and Mendelsohn

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1981; Woodard et al. 1981). The hormonal substances produced include ACTH, MSH, PTH, calcitonin, human chorionic gonadotropin and others. There have been several case reports of tumours producing more than one hormone (Hammer and Sale 1975; Abe et al. 1977; Heitz et al. 1982).

There have been very few cases with elevated amylase content in serum, urine and ascitic fluid without defined lesions in either the salivary gland or pancreas (Weiss et al. 1951; Ende 1961; Amman et al. 1973). Case reports on lung cancer producing salivary type amylase have been made (Sirsat et al. 1976; Gomi et al. 1976; Otsuki et al. 1977; Yokoyama et al. 1977; Flood et al. 1978; Morohoshi et al. 1980; Katayama et al. 1981). However, few reports have demonstrated the simultaneous production of amylase and the hormone. Only two cases have been reported where both salivary amylase and ACTH were produced in addition to MSH (Gomi et al. 1976).

We have investigated ultrastructurally, immunohistochemically and biochemically a lung tumour from a patient who showed elevated serum amylase and ACTH. The results obtained indicate that the tumour showed both endocrine and exocrine characteristics, that is to say, it was an amphicrine carcinoma.

Materials and methods

Case report. A twenty-six year-old male complained of anterior chest pain and a dry cough. Chest X-ray revealed an abnormal shadow in the right upper lung. On admission, physical examination disclosed diminished respiratory sound in the right chest anteriorly, lymph node swelling in the right supraclavicular region and systolic hypertension. Laboratory data showed hypokalaemia, hyperglycaemia, hyperamaylasaemia and elevation of cortisol and luteinizing hormone (LH) (Table 1). Further chest X-rays disclosed an elliptical tumour shadow of 10×8 cm in the right middle lobe. Bronchofiberscopic findings showed narrowing and stenosis of the right eparterial bronchus and the right middle lobar bronchus by the tumour. A biopsy was obtained from both the bronchial lesion and the lymph node. The patient died of pneumonia a month after admission.

An autopsy was performed. Tumour tissue from the biopsy, the lung tumour, metastatic tumour in the liver, pancreas, salivary glands, bilateral adrenal glands, and pituitary gland were subjected to a morphological investigation.

Light Microscopy

The tumour tissue was fixed with 10% formalin. Paraffin sections were stained by haematoxylin and eosin (H & E), Grimelius and periodic acid-Schiff-alcian blue.

Table 1. Serological analysis of the case

	Normal range	Feb. 2	Feb. 14	Feb. 25
ACTH	(15–85 pg/dl)	800	800	
Cortisol	(4.5–20.0 μg/dl)	80	80	
Aldosterone	(47–131 pg/dl)	87.1		
LH	(5.0–9.0 mIU/ml)	15.9		
FSH	(up to 10 mIU/ml)	6.8		
GH	(up to 5 ng/ml)	0.3		
Amylase	(25–140 IU/l)	3,418	1,942	2,376

Electron microscopy

For electron microscopy a part of the tumour was fixed with 1% glutaraldehyde, 4% formalin in 0.1 M cacodylate buffer at pH 7.2, postosmicated and embedded into epon 812. Ultrathin sections were examined with a JEOL 100B electron microscope.

Immunohistochemistry

Paraffin sections were stained for both salivary amylase and ACTH. Tissue blocks of good preservation and tumour tissue free from necrotic change were chosen for the immunostaining. Prior to the application of the primary antibody, 0.6% H_2O_2 in methanol solution for 30 min was applied in order to inhibit the endogenous peroxidase activity in the sections. For amylase staining, antihuman salivary amylase rabbit serum (Nordic) was applied for 30 min. After rinsing the specimens, a biotynyl antirabbit Ig G sheep serum and avidin-biotin-peroxidase complex was used successively for 30 min. Finally, specimens were incubated in a solution containing 0.05% DAB, 0.01% H_2O_2 in 0.05 M Tris-HCl buffer, pH 7.6. Parotid gland from the patient was stained similarly as a positive control and the nonimmune rabbit serum was used as a negative control. The antiamylase antibody obtained commercially was cross reactive with pancratic amylase and was therefore preabsorbed by the freeze-dried human muscle and kidney tissue prior to use. For the immunostaining of ACTH, anti-ACTH swine serum (Dakopatts) was used. Specimens were stained with the peroxidase-antiperoxidase (PAP) method by Sternberger et al. (1970). Some of the deparaffinized sections were subjected to proteolytic enzyme treatment according to the method by Mepham et al. (1979). Briefly, solutions of pronase (protease type VII, Sigma) were prepared immediately before use at concentrations of 0.025% in 0.05 M Tris-HCl buffer, pH 7.6. The solutions and sections were allowed to equilibrate at 37° C and the enzyme treatment was carried out at this temperature for 10 min.

Results

Tumour cells have small nuclei that are rich in chromatin, and the cytoplasm is scanty showing an inconspicuous cell border. The cells show no particular structures but show a trabecular arrangement (Fig. 1). The nuclei of the tumour cells facing the connective tissue stroma are compact and arranged rectangularly giving a palisading appearance (Fig. 1). The mucin staining is negative. There are few positive cells in the Grimelius stain. The pancreas, salivary glands and pituitary gland are histologically normal. The right and left adrenal glands weigh 14 and 16 g respectively. The cortex of each gland is thick showing cortical hyperplasia.

Fine structurally, the nuclei of the tumour cells are round or oval and have occasional nucleoli. The cells possess large zymogen-like granules of 0,5–1,5 μm in diameter which have electron dense central cores delimited with a limiting membrane (Fig. 2). These large granule-containing cells connect with desmosomes and possess microvilli at the apical border resulting in the formation of lumina (Fig. 2). The cells forming a lumen possess junctional complexes. In addition, cells containing small endocrine-like granules are frequently observed. These granules have variable electron dense cores delimited with a limiting membrane and are 100–300 nm in size (Fig. 3). These small granule-containing cells do not show any particular structure like a duct. Occasionally cells possessing both zymogen-like granules and endocrine-like granules are noticed (Fig. 4).

A densitometric scanning of the cellulose acetate membrane electrophoretogram of the patient's serum was performed. The main amylase elevated

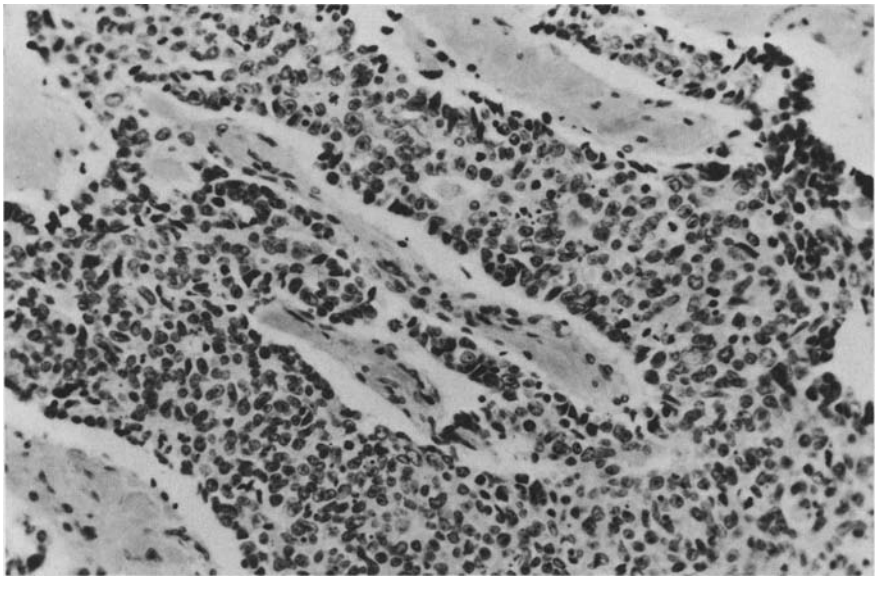

Fig. 1. Light microscopic photograph of the tumour. The tumour cells are proliferating with a trabecular arrangement. H & E. × 180

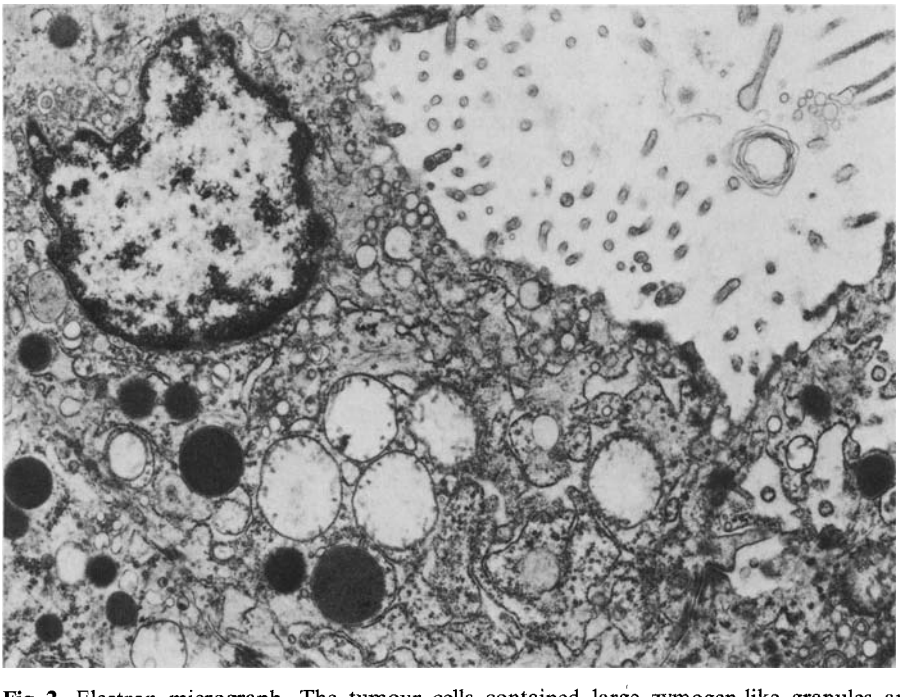

Fig. 2. Electron micrograph. The tumour cells contained large zymogen-like granules and formed the lumen. × 10,000

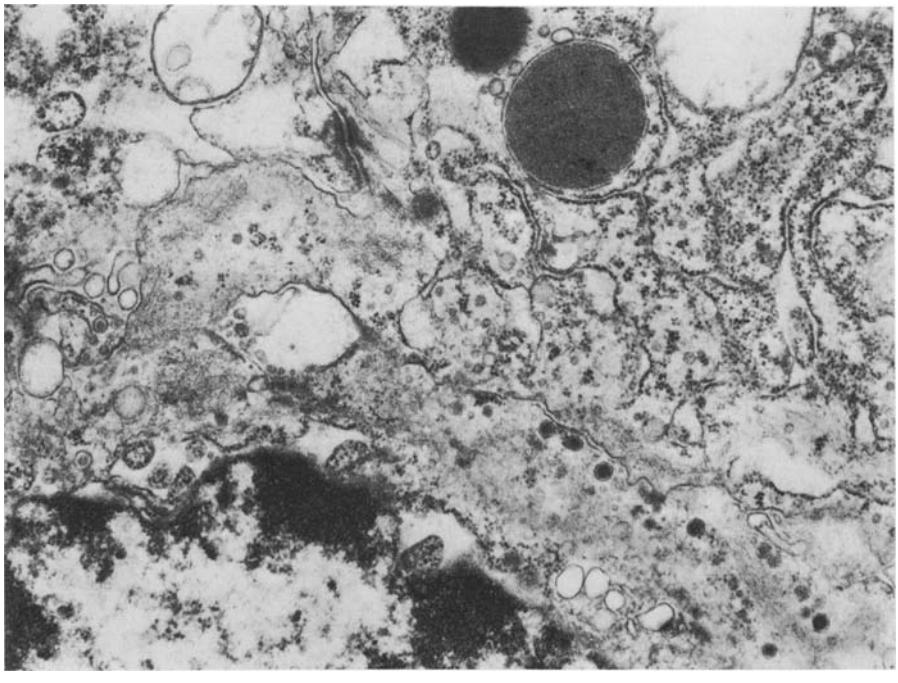

Fig. 3. Electron micrograph. The tumour cells also contained small dense core granules. Note that the upper cell has large zymogen-like granules. × 20,000

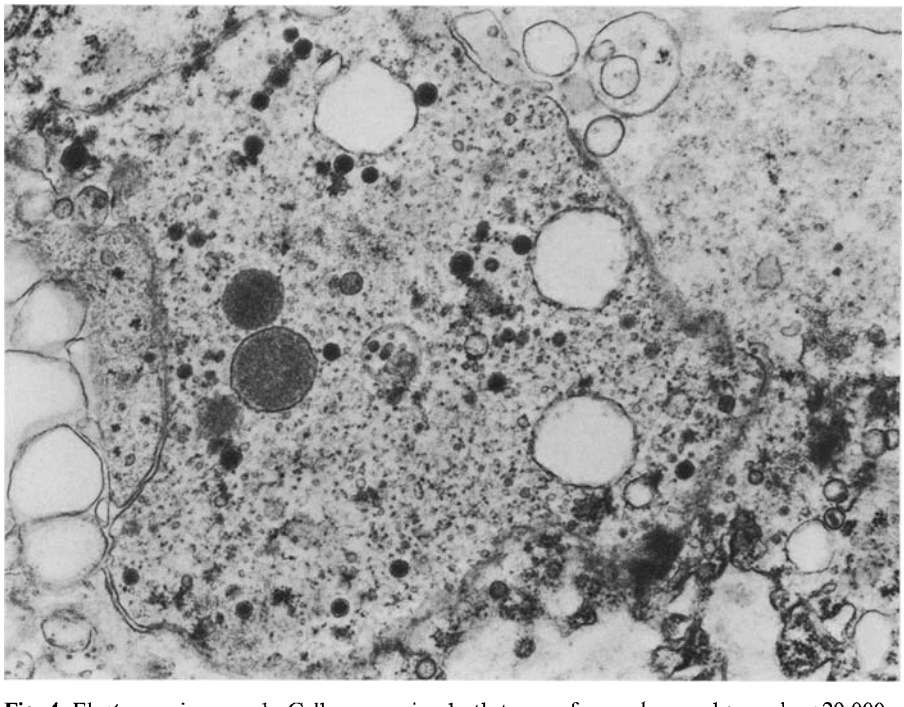

Fig. 4. Electron micrograph. Cells possessing both types of granule are observed. × 20,000

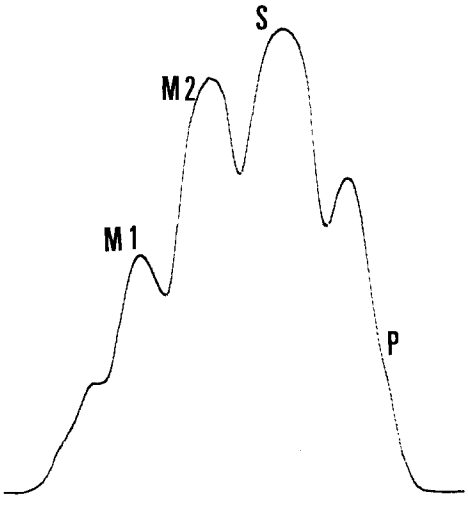

Fig. 5. Densitometric pattern of the serum amylase isozymes of the patient. The major elevated amylase was of the salivary type (*S*). *M1, M2*; minor component, *P*; pancreatic amylase

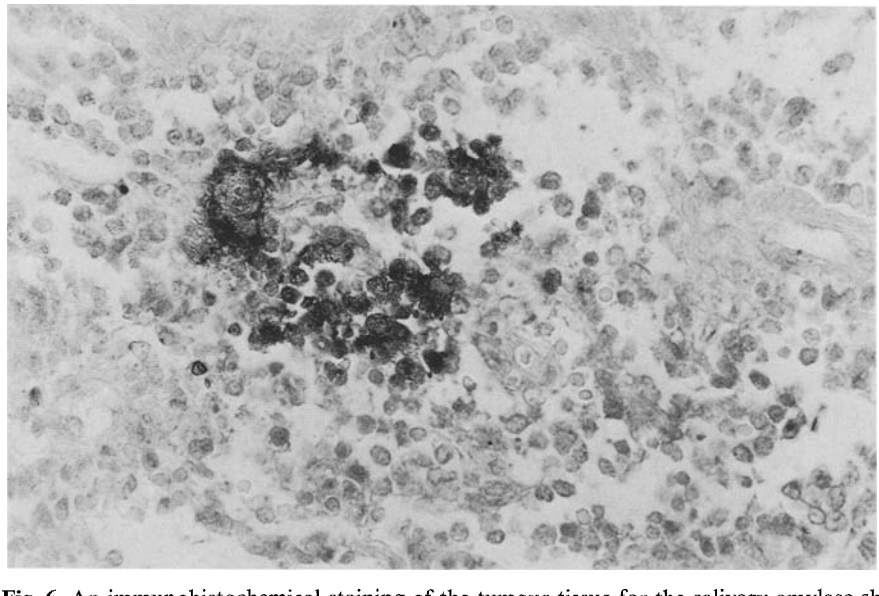

Fig. 6. An immunohistochemical staining of the tumour tissue for the salivary amylase shows that the various portions of the tumour tissue were positive. × 320

in the serum was of the salivary type associated with minor components (Fig. 5).

Immunohistochemical staining for the salivary amylase in the tumour tissue disclosed that various parts contained the protein (Fig. 6). The staining pattern was almost the same in the metastatic tumour in the liver, showing various clusters of positive cells within the tumour tissue. The parotid gland from the patient was also positive and the nonimmune serum

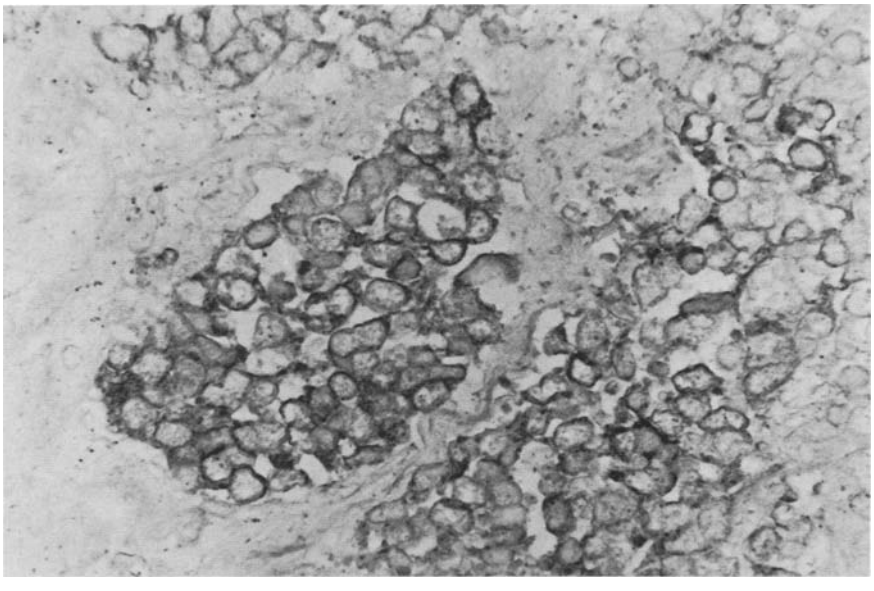

Fig. 7. An immunohistochemical staining of the tumour tissue for ACTH shows that most of the tumour cells were positive. × 400

gave no reaction. ACTH was detected in the tumour cells by PAP staining (Fig. 7). The positive cells for ACTH were rather diffuse in tumour tissue compared with the amylase positive cells. The pituitary gland of the patient was positively stained and the nonimmune swine serum failed to stain a specimen. The sections without prior treatment by the enzyme gave no reaction at all.

Discussion

It is apparent from a histological point of view that the present tumour is a small cell carcinoma of the lung. According to previous reports (Liddle et al. 1965; Gould 1977; Gould et al. 1983) most lung tumours producing ACTH ectopically are small cell carcinomas as in the present case, except for bronchial carcinoids.

At the initial attempt of immunostaining for ACTH without pretreatment of specimens by the proteolytic enzyme we failed to obtain any reaction product at all. It seemed that the ACTH immunoreactivity in the tissue had been lost during the processing. However, the pretreatment of a specimen by the method of Mepham et al. (1979) recovered the antigenecity of ACTH successfully and specifically. Therefore it is suggested that proteolytic enzyme pretreatment of a tissue section is worth trying whenever the immunoreactivity of an antigen seems to be lost or diminished.

In the present case, immunostaining for ACTH in the tumour cells was clearly demonstrated. Since no particular lesion was found in the pituitary gland of the patient, it seems reasonable to state that the elevated serum

ACTH was due to the production and secretion of the substance by the tumour cells.

Electron micrographs of the tumour cells revealed that they possessed large zymogen-like granules, had microvilli at the apical surface, connected with desmosomes and formed lumina. These ultrastructural findings are the features of adenocarcinomatous differentiation of the tumour cells. In this connection, it is of interest that most of the lung cancers producing amylase have been reported to be well differentiated adenocarcinomas (Gomi et al. 1976; Yokoyama et al. 1977; Morohoshi et al. 1980; Katayama et al. 1981).

Immunohistochemical staining pattern for salivary amylase in the tumour tissue was focal and clusters of tumour cells were positive for the protein. This pattern may correlate to those parts of the tissue where the cells showed adenocarcinomatous differentiation, as seen in electron micrographs.

In the present case, both the salivary gland and pancreas were shown to be intact. The results obtained by immunohistochemistry and the electron microscopy indicate that the salivary amylase elevation in the serum of the patient originated from the tumour cells.

Feyrter (1938) had already established a remarkable concept of a diffuse epithelial endocrine system ("diffuse epithelial endocrine organ") by recognizing the clear cells (Helle Zellen) in many organs throughout the body, including the lung. He speculated that bronchial carcinoids may arise from these clear cells. Pearse (1969) placed cells capable of producing amine in the APUD system and assumed them to be of neural crest origin, as they are also able to produce polypeptide hormone. Kultchitzky cells have been considered to be potential members of the APUD series in the lung and have been assumed to be putative cells from which small cell carcinoma or bronchial carcinoid arise. However, accumulated evidence has indicated that the endocrine cells in the lung and the gastrointestinal tract seem to be endodermal in origin (Andrew 1974; Cheng and Leblond 1974; Cox and Pierce 1982; DeLellis et al. 1984). The findings that tumour cells in the present case showed both exocrine and endocrine nature by expressing amylase and ACTH may support the stem cell theory with multidirectional differentiation as proposed by DeLellis et al. (1984). The theory seems to be able to explain similar amphicrine tumours which have been seen in other organs, such as the thyroid, gastrointestinal tract and pancreas (Abt and Carter 1976; Isaacson 1981; Zaatari et al. 1983; Chejfec et al. 1984). The presence of the tumour cell possessing both types of granule simultaneously may indicate a transitional state of differentiation of the tumour cells.

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Accepted August 16, 1985