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Immunocytochemistry and Electron Microscopy of an Argentaffin Endocrine Tumour of the Pancreas

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Summary. An endocrine pancreatic tumour that had not caused any endocrine symptoms was examined by histological, immunocytochemical and electron microscopic techniques. The majority of the tumour cells were argentaffin and contained secretory granules of the enterochromaffin cell type. Immunocytochemically a minority of tumour cells reacted to antisera against β-endorphin, met- and leu-enkephalin, gastrin, somatostatin and ACTH. The tumour was thus multihormonal, and appeared to be more closely related to the classic Carcinoid tumours of the mid-gut than to most pancreatic endocrine tumours.

Key words: Endocrine tumour – Pancreas – Argentaffin reaction – Immunocytochemistry – Electron microscopy

Introduction

The endocrine tumours of the pancreas mostly contain several different types of endocrine cells and are thus multihormonal. In spite of this, when clinical symptoms arise they are nearly always derived from hypersecretion of only one of the hormones (Larsson et al. 1975; Arnold et al. 1976; Larsson 1978; Heitz et al. 1979; Klöppel et al. 1979). Depending on the symptom-causing hormone, the tumour may be classified as an insulinoma, a gastrinoma, a Verner-Morrison tumour, a glucagonoma or a somatostatinoma. In addition to these tumours, there are rare reports of 5-hydroxytryptamine (5-HT) producing tumours of the pancreas (Patchetsky et al. 1972 and 1974). Most of these cases have been associated with the carcinoid syndrome. In the present report a "silent" argentaffin endocrine tumour of the pancreas is described. It was characterized immunocytochemically and electron microscopically.

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Case Report

The patient was a 78-year-old woman who at the age of 43 had undergone an antral resection (Billroth I) for duodenal ulcer but who otherwise had been healthy until December 1979, when she began to have epigastric pain. An X-ray examination of the stomach revealed displacement of the major curvature in the antral region. At gastroscopy elevation of the gastric mucosa was observed in a defined area and a biopsy from that area revealed atrophic gastritis but no tumour tissue. Laboratory tests gave normal results, except for a sedimentation rate of 47 mm. Preoperative hormone levels were not examined. An exploratory laparotomy was performed and a round mass was found in the tail of the pancreas. The tumour displaced the gastric wall but did not infiltrate into it. There were no metastatic nodules in the liver and no enlarged lymph nodes in the intestinal mesentery. No palpable mass was found in the stomach or in the small or large intestine. The tail of the pancreas was excised together with the tumour.

The specimen consisted of pancreatic tissue measuring $9 \times 2 \times 2$ cm, containing a round mass with a diameter of 2 cm. The cut surface of the tumour was yellow.

Material and Methods

Light Microscopy. Tumour tissue was fixed in 10% formalin, dehydrated in graded ethanols, cleared in xylene and embedded in paraffin. Sections 4 μm thick were stained with van Gieson stain, haematoxylin and eosin, the argentaffin technique of Masson-Hamperl as described by Singh (1964), and the argyrophil stains of Grimelius (1968) and Sevier-Munger (1965).

Immunocytochemistry. The antisera used, and their working dilutions, are given in Table 1. For the demonstration of immunoreactivity, the peroxidase-antiperoxidase (PAP) method was employed (Sternberger 1974). The controls used were: a) The first-layer antiserum was replaced by normal rabbit or guinea-pig serum; b) the antiserum was preincubated at 4° C for 24 h with rabbit anti-human Clq complement diluted 1:50 (Dako, Denmark, Lot No. 038B) and c) the antisera were incubated with the corresponding antigen or related antigen(s) (100 μg/ml diluted antiserum) at 4° C for 24 h.

Electron Microscopy. About 1 mm^3 blocks of the formalin-fixed tumour were cut and post-fixed in 1% OSO_4 in phosphate buffer, dehydrated in ethanol and embedded in Epon. After localization of the desired tumour tissue in approximately 1 μm thick toluidine-blue-stained sections, ultrathin sections were prepared with an LKB Ultrotome, contrasted with uranyl acetate-lead citrate and viewed in a Zeiss EM 9 electron microscope at 60 kV.

Results

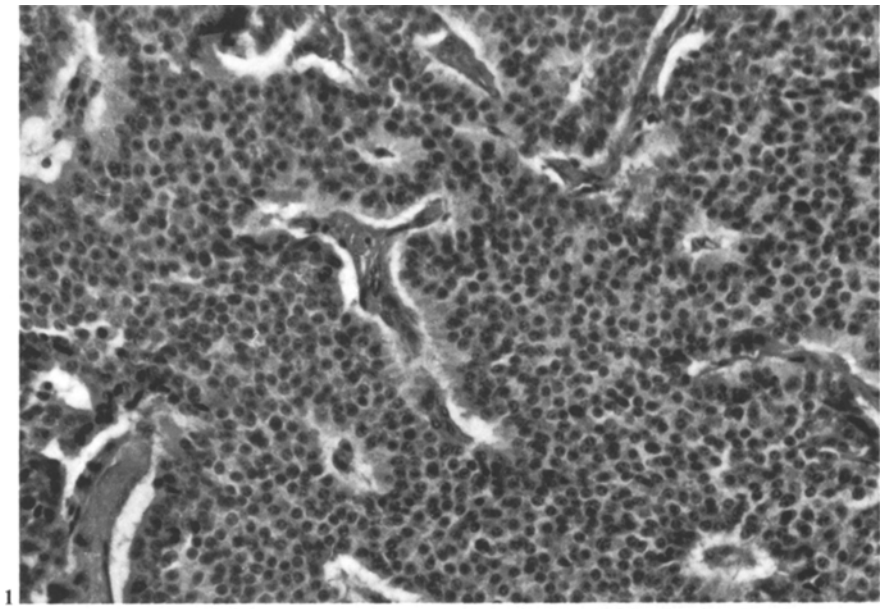
Light Microscopy. The tumour consisted of fairly uniform cells growing in buds or anastomosing cords. The tumour cell nuclei were round and regular and contained a fine central nucleolus. Around the tumour cell bundles a delicate fibrovascular stroma was seen (Fig. 1). In some areas there was a relatively sharp border between the pancreatic tissue and the tumour tissue, but in other areas the tumour was seen to infiltrate the pancreatic parenchyma.

Argentaffin and Argyrophil Reaction. With the argentaffin reaction of Masson most but not all tumour cells were stained. The number of silver grains in individual silver positive tumour cells varied (Fig. 2). The argyrophil reactions of Grimelius and Sevier-Munger were also positive in the majority of tumour cells.

Table 1. Survey of the antisera used and the immunoreactions of the endocrine pancreatic tumour. All the antisera were raised in rabbits except those against insulin and synthetic ACTH (1–24), which were raised in guinea-pigs

Antisera raised against	Obtained from	Code No.	Working dilution	Immunocytochemistry of the tumour
Porcine insulin	L. Wide, Dept. Clin. Chem., Univ. Hosp., Uppsala, Sweden		1:1,000	–
Synthetic ovine somatostatin	J.F. Rehfeld, Dept. Med. Biochem., Univ. Aarhus, Denmark	213/3	1:400	(+)
Porcine glucagon	G. Lundqvist, Dept. Clin. Chem., Univ. Hosp., Uppsala, Sweden		1:500	–
Bovine pancreatic polypeptide	R.E. Chance, Eli Lilly, Indianapolis, Ind., USA		1:1,600	–
Synthetic human gastrin-17	J.F. Rehfeld, Dept. Med. Biochem., Univ. Aarhus, Denmark	4562	1:600	(+)
Synthetic human substance P	P.C. Emson, MRC Neurochem. Unit, Univ. Cambridge, UK	SP 8	1:100	–
Synthetic human substance P	S.E. Leeman, Lab. Hum. Reprod., Harvard Med. Sch., Boston, USA	F2SPAb	1:100	–
Porcine motilin	G. Lundqvist, Dept. Clin. chem., Univ. Hosp, Uppsala, Sweden	46A2	1:100	–
Mixture of synthetic human Leu-and met-enkephalin	L. Terenius, Dept. Pharmacol., Biomedicum, Univ. Uppsala, Uppsala, Sweden	336 K	1:400	(+)
Synthetic human α-endorphin	L. Terenius, Dept. Pharmacol., Biomedicum, Univ. Uppsala, Uppsala, Sweden	11 B	1:100	–
Synthetic human β-endorphin	L. Terenius, Dept. Pharmacol., Biomedicum, Univ. Uppsala, Uppsala, Sweden	372	1:800	(+)
Bovine ACTH	Ferring Azermittel, Germany	K8770601	1:600	–
Synthetic ACTH (1–24)	L.I. Larsson, Dept. Med. Biochem., Univ. Aarhus, Denmark	GPL54	1:250	(+)
Synthetic ACTH (18–39)	L.I. Larsson, Dept. Med. Biochem., Univ. Aarhus, Denmark	CLIP	1:250	(+)

– = indicate a negative immunocytochemical reaction; (+) = indicate a positive immunocytochemical reaction in a minority of tumor cells



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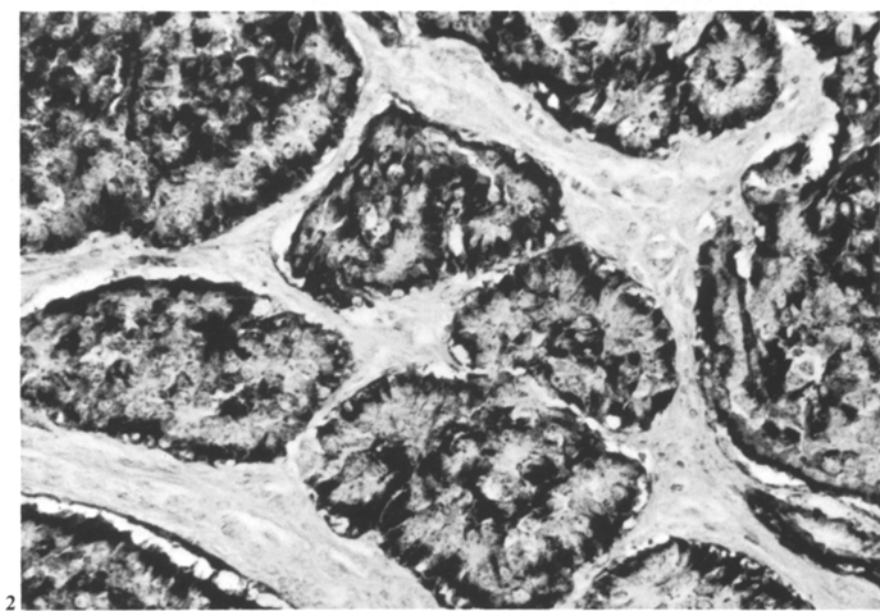

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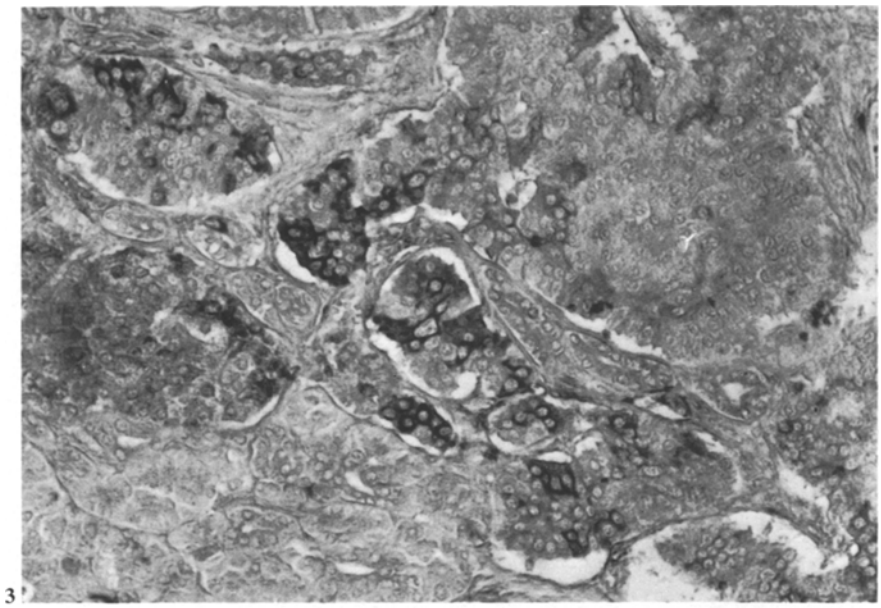
Fig. 1. Light-microscopic photograph of the endocrine pancreatic tumour, showing monomorphic tumour cells growing in buds. Haematoxylin and eosin. ×250

Fig. 2. Tumour tissue displaying strong silver granulation in the majority of its cells. Masson-Hamperl. ×250



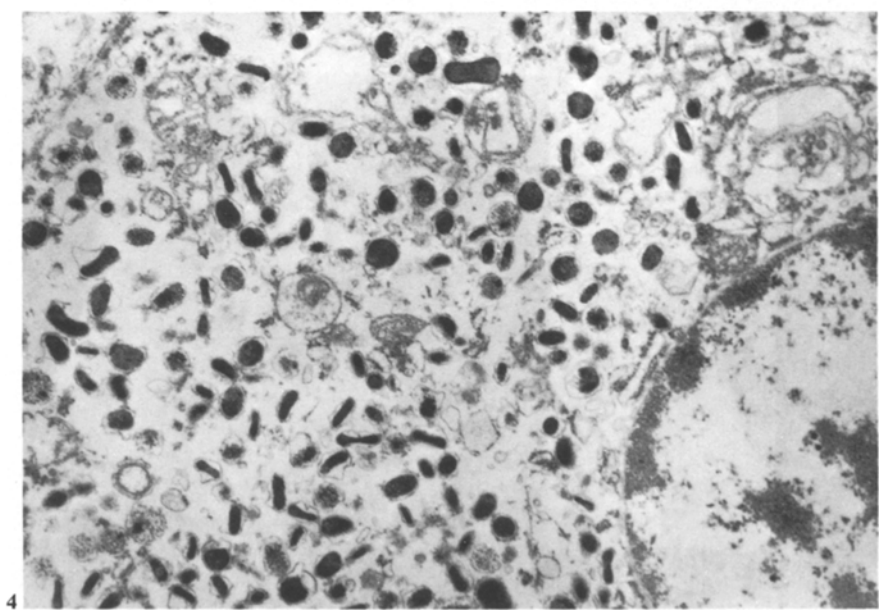


Fig. 3. Part of the tumour, with scattered cells immunoreactive to β-endorphin antibodies. PAP stain. $\times 250$

Fig. 4. Electron micrograph of a tumour cell. The cytoplasm contains granules with a pleomorphic central core surrounded by a narrow clear space and a limiting membrane. $\times 15{,}100$

Immunocytochemistry. A relatively large number of tumour cells reacted to antisera against β-endorphin and met- and leu-enkephalin (Fig. 3). However, the stained cells did not constitute the majority cell population of the tumour tissue. A few tumour cells showed immunoreactivity to gastrin and somatostatin antisera and to antisera against synthetic fragments of ACTH (1–24 and 18–39), but not against bovine ACTH. The rest of the antisera listed in the table gave negative results. No immunostaining was obtained when the first-layer antiserum was replaced by normal rabbit or guinea-pig serum. Moreover, the preincubation of the antisera with anti-Clq complement had no effect on the positive results obtained. All the antisera that gave positive results were inactivated after preincubation with the corresponding peptide.

Electron Microscopy. Although the electron micrographs were suboptimal in quality, a large number of relatively well preserved endocrine granules were observed in the cytoplasm of the tumour cells. These granules were pleomorphic and were surrounded by a limiting membrane. There was a narrow clear space between the limiting membrane and the granular core. The mean diameter of the granules was about 200 nm. Endocrine granules of other types were not found in the small pieces of tumour tissue studied electron microscopically.

Discussion

This tumour appeared to be quite similar to the typical carcinoid tumours occurring in the "mid-gut" segment of the intestine (Williams and Sandler 1963; Black 1968; Wilander et al. 1977). Thus, in common with these tumours, it consisted of uniform cells growing in buds or anastomosing cords, its cells were argentaffin, and plemorphic granules characteristic of enterochromaffin cells were observed on electron microscopy.

The argentaffin reaction in the majority of the tumour cells and the morphology of the intracytoplasmic granules indicate that 5-HT was the main hormone constituent of the tumour cells (Barter and Pearse 1953; Black 1968; Alumets et al. 1977). However, peptide hormone immunoreactivity (β-endorphin, met- and leu-encephalin, gastrin, somatostatin and ACTH) was also observed in a minority of the tumour cells, and thus the tumour may be said to belong to the group of multihormonal endocrine tumours of the pancreas (Larsson et al. 1975; Arnold et al. 1976; Larsson 1978). There were no clinical signs of endocrine activity. This may be explained by the fact that clinical symptoms in endocrine tumours are nearly always caused by hypersecretion of only one of the hormones present in the tumour, and 5-HT, which was probably the main hormone constituent of the present tumor, does not as a rule give rise to symptoms (carcinoid syndrome) before metastases to the liver have occurred. It may be presumed that cells in endocrine tumours do not always produce normal hormonal products, but fragments of or incomplete hormone peptides. This might explain, for instance, the differences in immunoreactivity observed with the various ACTH antibodies in the present case.

There are very few reports on argentaffin endocrine tumours of the pancreas (Patahy et al. 1959; Gloor et al. 1965; Patchefsky et al. 1974). This type of tumour seems to be more easily recognised if it shows endocrine activity and

gives rise to a carcinoid(-like) syndrome (Dollinger et al. 1967; Gordon et al. 1971; Patchetsky et al. 1972).

The histogenesis of argentaffin endocrine tumours of the pancreas is unclear. They may be derived from the enterochromaffin cells in the ducts of normal pancreata or from multipotent stem cells of the duct epithelium, with the ability to proliferate and differentiate into monoamine and/or various peptide hormone producing cells.

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