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Histology and Histopathology

A multihormonal tumor of the pancreas producing neurotensin associated with the WDHA syndrome. Histology, histochemistry and origin

Tatiana Bani-Sacchi[1], Giancarlo Bartolini[2] and Giancarlo Biliotti[3]
[1]Institute of Histology and General Embryology, University of Florence, Florence, Italy;
[2]Service of Pathology, Hospital of Pistoia, Pistoia, Italy;
[3]Institute of I Surgical Clinic, University of Florence, Florence, Italy

Summary. A pancreatic tumor associated with severe WDHA syndrome has been studied histologically and immunohistochemically. Light microscopy revealed that the growth pattern of the tumor varied greatly from zone to zone but with prevailing solid arrangement of the tumoral cells. The majority of the endocrine cells showed numerous eosinophilic, PTAH-positive, and argyrophilic secretory granules, that were ultrastructurally similar to those of normal and tumoral neurotensin-containing cells. A minority of the endocrine cells had secretory granules ultrastructurally different from the aforementioned ones, but these were not diagnostic on purely morphological grounds.

Inside the tumor, immunohistochemistry demonstrated a majority of neurotensin-immunoreactive cells, sparse and small clusters of VIP-immunoreactive cells and few, dispersed pancreatic polypeptide-immunoreactive cells.

Some structural and ultrastructural aspects of the tumoral stroma have also been reported.

Ducts and solid masses of duct-like cells were also found, and small clusters and singly dispersed duct-like cells were seen invading the endocrine tissue and undergoing mitoses. Such features suggest that the tumor originated from precursors located in the medium-sized and small pancreatic ducts.

Because of the multihormonal nature of the tumor, the role of neurotensin and VIP in producing the patient's symptoms is discussed and a synergistic action of the two hormones is suggested in causing the particularly severe WDHA syndrome.

Offprint requests to: Prof. *T.* Bani-Sacchi, Istituto di Istologia e Embriologia Generale, Viale G. Pieraccini n.° 18, 1-50139, Firenze, Italy

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Introduction

In the watery diarrhoea, hypokalemia, achlorydria syndrome (WDHA), mono- or multihormonal tumors producing VIP have been repeatedly reported (Larsson et al., 1976; Polak et al., 1976; Barraclough and Bloom, 1979; Bani-Sacchi et al., 1980; Long et al., 1981a; Heitz et al., 1982; Unwin et al., 1982; Capella et al., 1983), and VIP has been regarded for a long time as the most likely causative agent of the clinical symptoms. This opinion was based upon the knowledge of the biological actions of the peptide and its elevated plasma levels in patients with diarrheogenic syndrome (Bloom et al., 1973; Blom and Polak, 1975; Said and Faloona, 1975; Bloom, 1978a; Bloom, 1978b; Cooperman et al., 1978; Ebeid et al., 1978; Gardner, 1978; Modlin et al., 1978; Bani-Sacchi et al., 1980; Krejis et al., 1980; Long et al., 1981b; Unwin et al., 1982; Kane et al., 1983).

However, many of the tumors in patients with the WDHA syndrome secrete more than one hormone and excessive amounts of VIP have been associated with high levels of other peptides (Larsson et al., 1976; Jaffe et al., 1977; Bloom and Gardner, 1978; Jaffe, 1979; Long et al., 1981b; Unwin et al., 1982), the action of which in causing the clinical symptoms was difficult to define and still remains to be determined. At present, the role of VIP as the only mediator of the WDHA syndrome seems to be questionable (Field and Chang, 1983), also because the disease has been recognized in patients with normal plasma levels of VIP (Holst, 1979) and in patients with increased levels of agents other than VIP (Schinitt et al., 1975; Jaffe and Condon, 1976).

In the last few years, multi- or monohormonal pancreatic neurotensinomas (Gutniak et al., 1980; Grube, 1984; Shulkes et al., 1984) and high plasma levels of neurotensin (Sundler et al., 1979: Blackburn et al., 1981) have been found in patients with the WDHA syndrome. Hence neurotensin has been regarded as a candidate peptide in giving rise to the characteristic clinical picture of the WDHA syndrome (Grube, 1984).

We have investigated a patient with the WDHA syndrome associated with a multihormonal tumor of the pancreas producing neurotensin, VIP and PP. The aim of the study is to give a histological and immunohistochemical description of the tumor, an ultrastructural characterization of the endosecretory granules, and to provide morphological data for elucidating its cellular origin.

Materials and methods

Case report. A twenty six year old man had complained for fifteen days prior to hospitalization of repeated episodes of dyspepsia, vomiting and watery diarrhoea (up to 20 stools daily). On hospitalization, the patient suffered from weakness, tachycardia (110 beats/min) and severe hypotension (85/40 mm Hg). Biochemical investigations demonstrated: azotemia 1.87 g % (n.v. 0.25-0.45 g %), glycemia 0.87 g % (n.v. 0.90-1.10 g %), natremia 115 mEq/l (n.v. 130-143 mEq/l), kalemia 1.3 mEq/l (n.v. 3.5-5.1 mEq/l). ECG showed abnormalities of the ventricular repolarization phase and PR elongation (0.22). Despite the efforts to relieve symptoms by administration of appropriate fluids and electrolytes, steroids and antidiarrhoeal drugs, the patient died from heart failure on the third day after admission.

At autopsy a 3 x 3 cm encapsulated tumor was found embedded in the surface of the tail of the pancreas. The histopathological examination led to the diagnosis of pancreatic adenoma. The tumor was well-defined against the surroundings and no local infiltration could be demonstrated, nor was there evidence of metastases.

Histology. Pieces of the tumoral tissue were removed at autopsy 24 hours after death. They were fixed in 10% formalin in sodium phosphate buffer, pH 7.2, for 48 hours at room temperature. Some pieces were also rinsed in the same buffer and re-fixed in Bouin's fluid for 24 hours at room temperature. All the specimens were dehydrated in graded ethanol, passed through xylol and embedded in paraffin. Sections 5 µm thick were cut and stained with hematoxylin and eosin, toluidine blue, PAS and hematoxylin, PAS and orange G, Azan method, Tibor-Papp method, Grimelius' silver (Grimelius, 1968) and phosphotungstic acid hematoxylin (PTAH).

Small fragments of formalin-fixed tissue were rinsed in sodium phosphate buffer 0.1 M, pH 7.4, and postfixed in 1% OsO_4 in the same phosphate buffer. These specimens were then dehydrated in graded ethanol series, passed through propylene-oxide and embedded in Epon 812. Both semithin and ultrathin sections were cut and stained with toluidine blue - Na tetraborate and uranyl acetate plus alkaline bismuth subnitrate, respectively. The ultrathin sections were examined with a Siemens Elmiskop 102 electron microscope at 80 kV. Because of the inadequate preservation of the autopsy tissue for electron microscopy, a detailed ultrastructural study of the tumor could obviously not be performed.

Immunohistochemistry. Sections 5 µm thick of formalin-fixed, paraffin-embedded tissue were deparaffinized, rehydrated and immunostained by the unlabeled antibody peroxidase-antiperoxidase (PAP) method (Sternberger, 1979). The following antisera were used:

1. Anti-neurotensin (NT 357, directed against the whole molecule; working dilution 1:40,000).
2. Anti-VIP (VIP 89 N, directed aginst the whole molecule; working dilution 1:5,000).
3. Anti-bovine pancreatic polypeptide (BPP 146-6, directed against the C-terminal portion; working dilution 1:12,000).
4. Anti-calcitonin (CT 5, directed against the Mid/N-terminal portion; working dilution 1:2,000).
5. Anti-glucagon (GLUC 499, directed against the C-terminal portion; working dilution 1:5,000).
6. Anti-alpha human chorionic gonadotropin (alpha HCG 364, directed against an unknown molecular region; working dilution 1:1,600).

All primary peptide antibodies were raised in rabbits. Concerning the characterization of the antisera, see Capella et al. (1983).

Controls for specificity of reactions were performed as follows:

1. Using non-immune rabbit serum as first layer.
2. Incubating adjacent serial sections with the same diluted antiserum, but on one section of the pair using the antiserum absorbed with an excess of the relevant antigen (1-40 µg/ml).
3. Omitting the first layer.
4. Using complement-deprived, aggregate-free antihormone sera (Buffa et al., 1979).

Results

Histology

The tumoral parenchyma consisted of round or polyhedral cells arranged to form irregular cords (Fig. 1), trabecules, or solid masses (Fig. 2). The architectural pattern varied from zone to zone, with a prevalence of the solid arrangement. The cytoplasm of the tumoral cells appeared finely granular and sharply stained with eosin, fairly basophilic with toluidine blue, pale red with the Azan method, weakly positive with the PAS reaction and argyrophilic with Grimelius' silver (Fig. 3). The argyrophilia was due to the cytoplasmic granules, which were also made easily evident by PTAH, appearing deeply stained in blue-violet against a very clear cytoplasmic matrix. Sometimes the tumoral cells were binuclear, but mostly they had only one round or oval nucleus. Occasionally tumoral cells with usual amounts of

cytoplasmic granules were combined to form pseudocrypts and showed a cuticular border at their lumenal poles (Fig. 4). In semithin sections almost all tumoral cells exhibited abundant cytoplasm filled with tightly packed, pale blue granules, and clear nuclei with small chromatin clusters and large nucleoli. In some instances giant and pleomorphic nuclei also occurred (Fig. 5). A minority of the tumoral cells were flattened or with angular profiles and showed more deeply stained cytoplasm and denser nuclei (Fig. 6). These cells were often located at the periphery of solid nests and masses or associated to form small clusters.

Under electron microscope the great majority of tumoral cells displayed numerous secretory granules. They were round or ovoid-shaped, measured approximately 200-250nm in diameter and showed a homogeneous content of variable density, but most often very osmiophilic, adhering to the limiting membrane or separated from it by a narrow electron-lucent cleft (Fig. 7). Some tumoral cells were encountered which contained granules different from the aforementioned ones. This second type of granule measured about 250-300 nm in diameter and had a very dense round core, eccentrically placed and separated from the limiting membrane by a wide, clear halo (Fig. 8).

The tumoral stroma consisted of fine septa of connective tissue interposed between the cords and surrounding small tumoral cell clusters in the solid masses. In most of the zones examined, the stroma showed basement membranes, characteristically PAS-positive and reticular sheats (Fig. 9). Small blood vessels ran through the interstitium. In some areas the blood capillaries were close to the parenchymal structures; in other areas there were wide perivascular spaces filled with abundant, colorless, hydropic substance. Neither fibrosis nor signs of amyloid deposition were recognized in the stroma. On the other hand, numerous strands and irregular deposits of PAS-positive material were found in the interstitium (Fig. 10). Similarly shaped homogeneous and osmiophilic masses were seen in semithin sections. An accurate exploration of serial sections showed that such deposits were adjacent to the blood capillaries or to their remnants.

Electron microscopy revealed that such deposits consist of tightly clustered basal laminae intermingled with collagen-like material (Fig. 11), sometimes embedding damaged endothelial cells and connective tissue cells.

In a limited region of the tumor, cells were found which were different from those forming the endocrine parenchyma. Such cells in fact were lacking in cytoplasmic granules and had peculiar staining properties. They were especially evident in sections stained with PTAH, in which they showed a pink cytoplasm instead of blue-violet and granular as the endocrine tumoral cells. These agranular cells were combined in epithelial fashion to form nodules of various sizes, incompletely wrapped in connective tissue envelopes. Some of the nodules were solid, and others showed solid epithelial masses continuous with duct-like structures. These duct-like structures were formed by columnar cells bearing an apical cuticular border abutting into the lumen, which often contained a deeply stained and flocculent material. Some isolated ducts were also found near the largest nodules (Fig. 12) and small clusters or singly dispersed duct-like cells were seen invading the surroundings of the nodules and intermingled with the endocrine cells (Fig. 13). Inside the nodules, cells in mitosis were also present with some frequency (Fig. 14).

Immunohistochemistry

The most frequent type of tumoral cells displayed immunoreactivity to neurotensin. They were round or ovoid and had abundant cytoplasm with finely granular immunostaining. Strongly immunoreactive cytoplasmic areas were seen on the side facing the interstitium (Fig. 15). A minority of the tumoral cells were VIP-immunoreactive. These cells formed sparse clusters, were angular or elongated, had rather scarce cytoplasm and showed homogeneous immunostaining (Fig. 16). A few PP-immunoreactive cells were also seen, singly dispersed in the tumoral mass or forming small clusters of two to three units (Fig. 17). None of the other antisera tested (i.e. anti-calcitonin, anti-glucagon, and anti-alpha human chorionic gonadotropin) revealed any immunoreactive cells.

Fig. 1. An area of the tumoral parenchyma with cells arranged in cords. Hematoxylin and eosin. × 250

Fig. 2. An area of the tumoral parenchyma with cells in solid arrangement. Hematoxylin and eosin. × 250

Fig. 3. The argyrophilia of the tumoral cells after Grimelius' silver stain is evident at the level of the secretory granules. x 1,500

Fig. 4. Pseudocrypt formed by cells with granules positive to Grimelius' silver stain. x 1,500

Fig. 5. A binuclear tumoral cell (left) and some cells with giant nuclei. Semithin section stained with toluidine blue-Na tetraborate. × 1,500

Fig. 6. The most frequent type of tumoral cells are round or oval and have clear nuclei and abundant cyotplasm. A minority of the tumoral cells are angular or flattened and have denser nuclei and a more deeply stained cytoplasm. Semithin section stained with toluidine blue-Na tetraborate. x 1,500

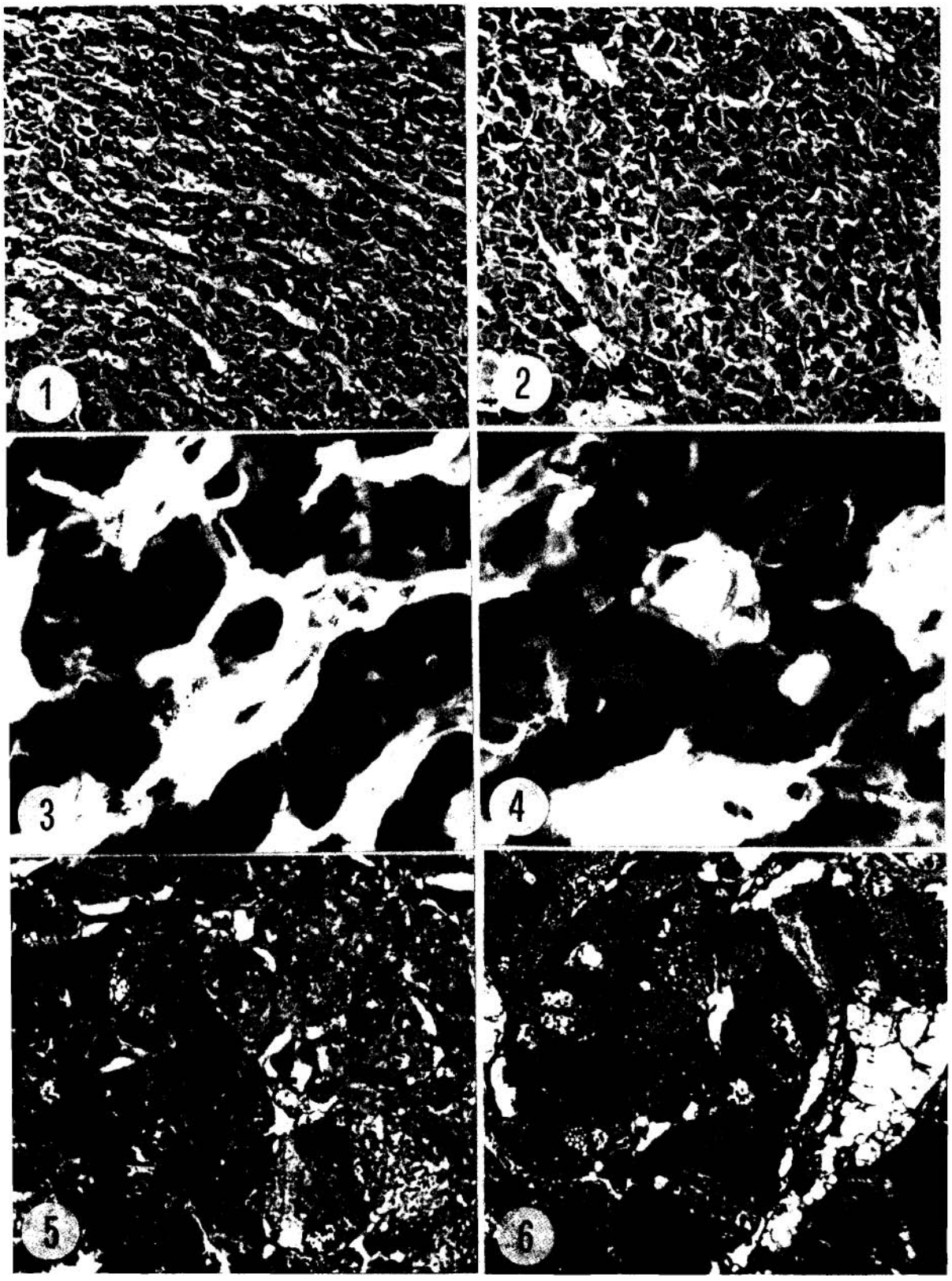
Fig. 7. Electron micrograph of the secretory granules of the most frequent tumoral cell type. × 55,000

Fig. 8. Electron micrograph of the secretory granules of a less frequent tumoral cell type. × 55,000

Fig. 9. Reticular fibers in the tumoral stroma. Tibor-Papp method. x 600

Fig. 10. Interstitial deposits of PAS-positive material. × 1,500.

Fig. 11. Ultrastructural appearance of an interstitial deposit formed by tightly packed basal laminae intermingled with collagen-like material. A damaged endothelial cell (right) and a mast cell (left) are ambedded into the deposit. x 22,000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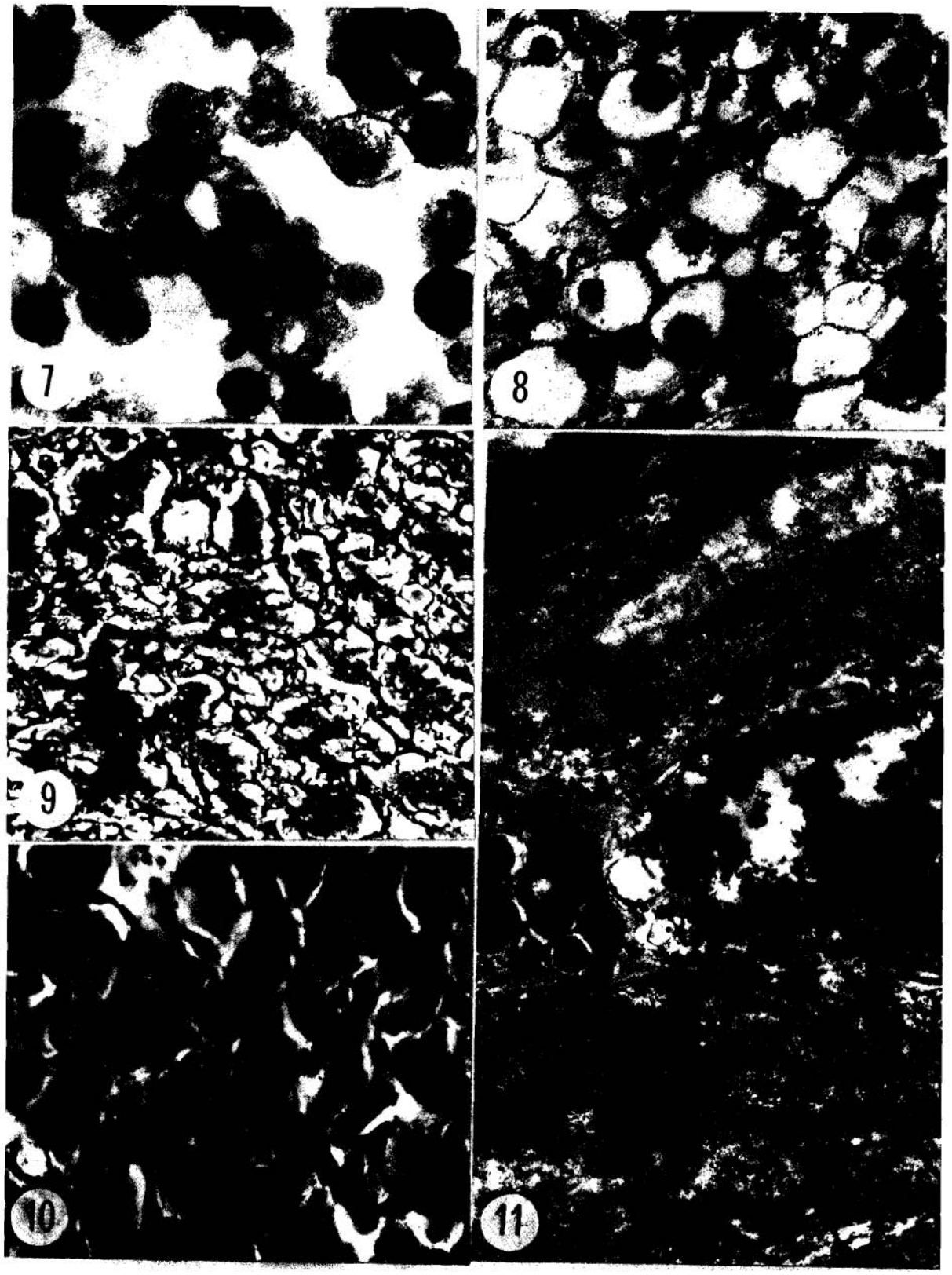

Fig. 14. A nodule of duct-like cells, some of which are in mitosis. PTAH. x 1,500

Fig. 15. NT-immunoreactive cells (PAP). x 850

Fig. 16. VIP-immunoreactive cells (PAP). x 850

of other agents (Schmitt et al., 1975; Jaffe and Condon, 1976), thus suggesting that peptides other than VIP can also give rise to the WDHA syndrome.

We believe that neurotensin should not be excluded from the list of possible etiological agents of the syndrome

information exists in the literature that patients with diarrheogenic tumors had plasma levels of neurotensin greater than normal (Blackburn et al., 1981; Maier et al., 1982), and plasma levels of the peptide were very high in a patient with a mixed tumor containing neurotensin-, VIP- and PP-producing cells (Shulkes et al., 1984), quite similar to that studied here, thus suggesting that neurotensin is secreted by the tumors into the blood.

Many of the symptoms in our patient, in fact, could be explained by the known biological actions of neurotensin (see Reinecke, 1985): dyspepsia and vomiting may be due to the action of neurotensin in inhibiting gastric acid secretion and in affecting the motor activity of the digestive tract; the severe hypotension and cardiac disturbances may depend, at least partly, upon the influence of the peptide on systemic blood pressure and cardiac function, and the watery diarrhoea may be accounted for by the action of neurotensin in enhancing intestinal secretion (Reasbeck et al., 1982). A possible synergistic action of neurotensin and VIP may account for the great severity of the symptoms and the precipitous clinical course of our patient.

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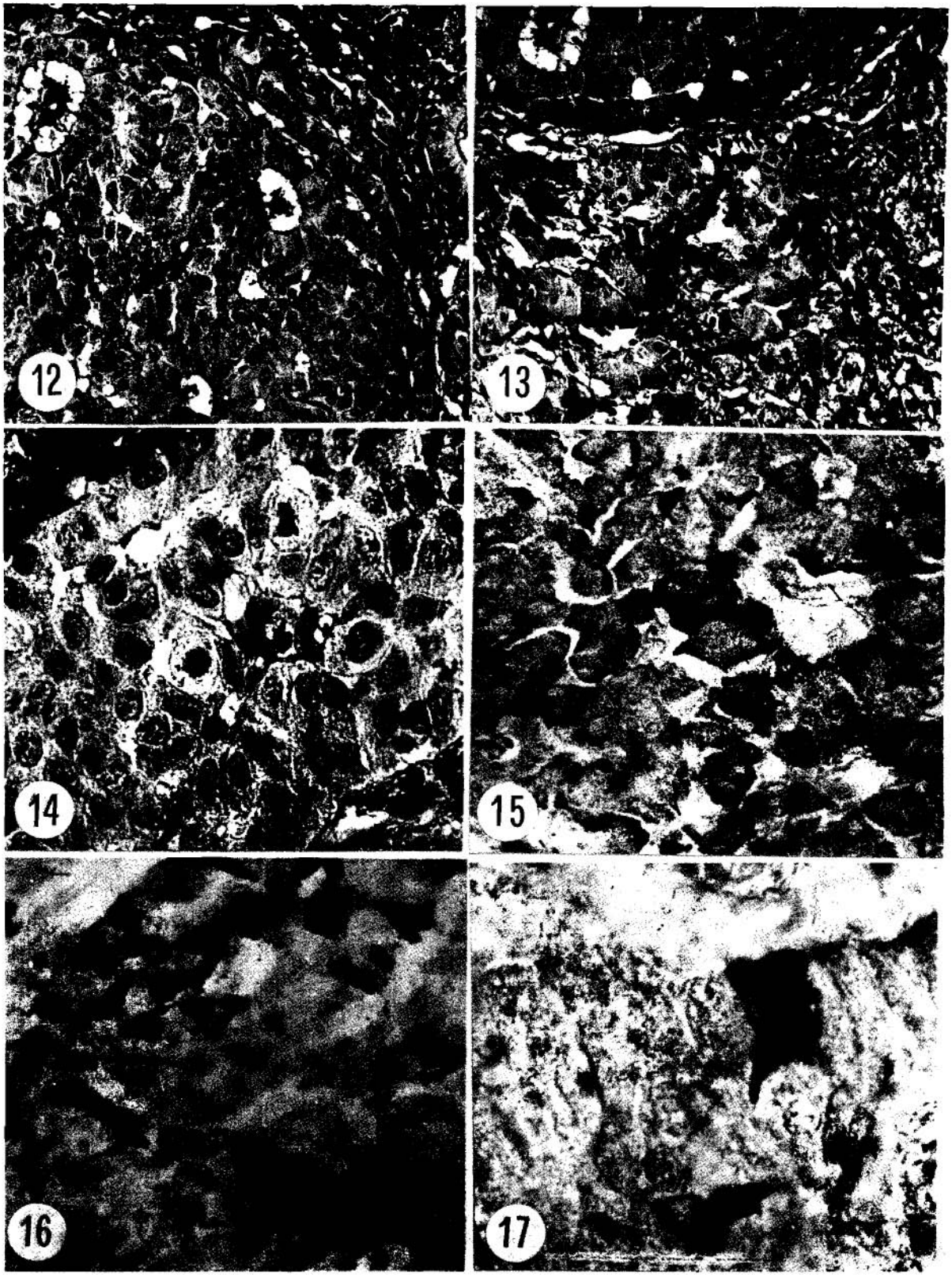
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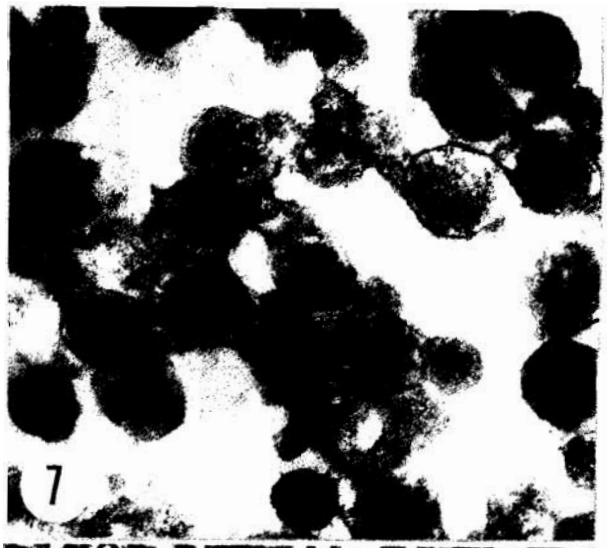
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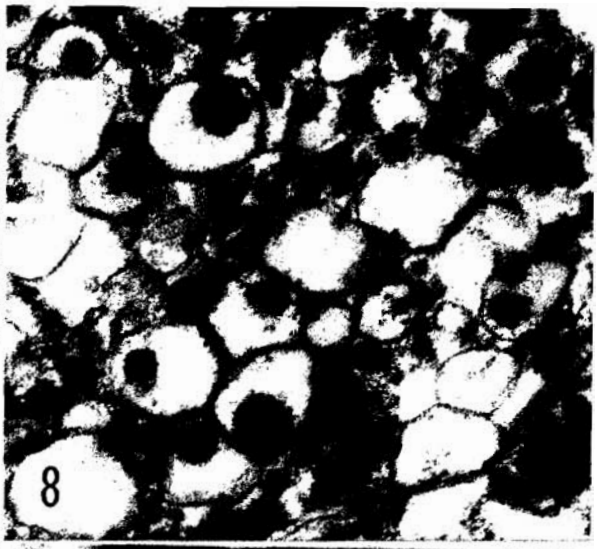
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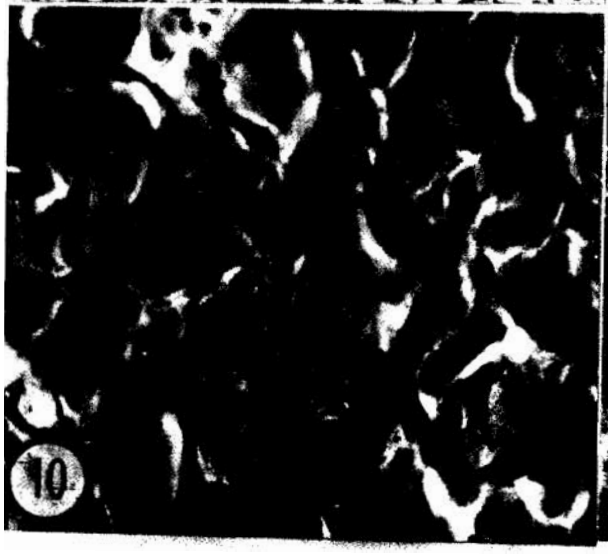
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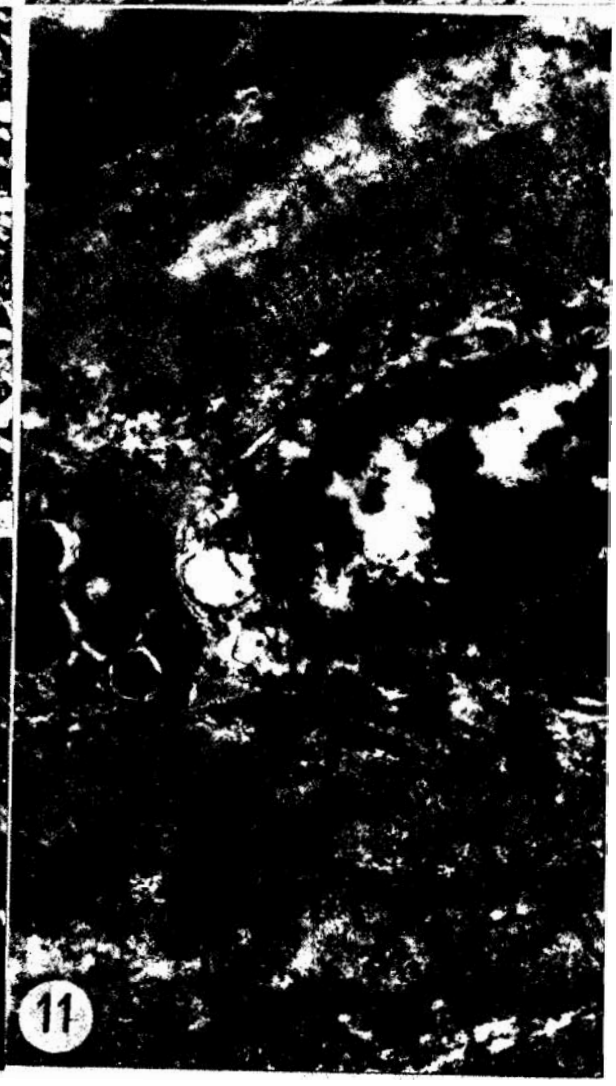
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