Morphologic Patterns and Diagnostic Criteria of VIP-Producing Endocrine Tumors

A Histologic, Histochemical, Ultrastructural, and Biochemical Study of 32 Cases

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Thirty-two tumors (31 pancreatic and one jejunal) all associated with severe watery diarrhea, increased VIP levels in blood and most with hypokalemia, were investigated. The VIP content of tumor tissue ranged from 23 to 15,000 pmol/g. VIP immunoreactive cells were detected histochemically in 24 of 28 tumors investigated, PP immunoreactive cells in 11 of 28 tumors, hCG (α chain) immunoreactive cells in 12 of 25 tumors, and neuron specific enolase (NSE) immunoreactive cells in 24 of 26 tumors (the 2 negative results were due to inadequate fixation). All cases showed light microscopic features of epithelial endocrine tumors. Electron microscopy demonstrated a prevalence of agranular, poorly granulated and a minority of well granulated cells. Most secretory granules were round, small (150 ± 30 nm diameter) and of moderate electron density, resembling those of the so-called D1 cells. By electron immunocytochemistry, PP was directly localized in a subpopulation of relatively larger granules (154 ± 22 nm core diameter) showing closely applied membranes. VIP-storing granules, directly identified only in the jejunal tumor, appear to correspond to a subpopulation of slightly smaller P-type granules (126 ± 37 nm core diameter) showing a narrow, clear halo. The origin, behavior, and diagnostic criteria of VIPomas are discussed.

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PANCREATIC and extrapancreatic tumors have been reported in association with the watery diarrhea, hypokalemia and achlorhydria (WDHA) syndrome.[1–5] Among extrapancreatic tumors, some ganglioneuromas and ganglioneuroblastomas have been shown to produce large amounts of vasoactive intestinal polypeptide (VIP) which is the most likely mediator of the diarrheogenic syndrome.[2–6]

The nature of pancreatic VIP-producing tumors (VIPomas) is somewhat less obvious, mainly due to a dispute about the cellular origin of such tumors. Their epithelial endocrine nature was generally accepted by pathologists[1,7] at the time when VIP was universally considered to be a gut hormone. Subsequent immunohistochemical investigations of human and other mammalian tissues, including pancreas, have failed to provide conclusive evidence for the presence of VIP in endocrine cells, while showing, undisputedly, its localization in nervous structures.[8] Consequently, some authors have suggested that pancreatic VIPomas originate from a neurogenic stem cell.[9]

Previous reports concerning the morphology of pancreatic diarrheogenic tumors have dealt only with single or a few cases.[10–13] In this study, the clinical, biochemical, histologic, histochemical, and ultrastructural findings in 32 diarrheogenic tumors (31 from the pancreas and 1 from the jejunum) are summarized and discussed. The epithelial endocrine nature of VIP-producing pancreatic and gut tumors is reaffirmed and pertinent diagnostic criteria are outlined.

Material and Methods

Thirty-two tumors (including metastatic tissue in 20 cases) were investigated. Thirty-one were pancreatic

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TABLE 1. Clinical and Pathologic Features

Case	Sex/age	Tumor VIP (pmol/g)	Predominant clinical manifestation	Location of tumor	Recognized metastatic sites
1	F/NK	303.0	WDHA	Pancreas (tail)	Liver, lymph nodes
2	F/38	ND	WDHA	Pancreas	
3	F/NK	787.9	WDHA	Pancreas	Liver
4	F/NK	1450.0	Watery diarrhea	Pancreas	
5	M/NK	1363.6	Watery diarrhea	Pancreas	Liver
6	M/45	1787.0	Watery diarrhea	Pancreas	Kidney
7	M/NK	ND	Watery diarrhea	Pancreas	Liver
8	F/66	1450.0	WDHA	Pancreas (tail)	Liver
9	M/57	303.0	WDHA	Pancreas	
10	M/28	1250.0	Watery diarrhea	Pancreas	
11	F/NK	272.7	WDHA	Pancreas	
12	M/NK	796.0	WDHA	Pancreas	
13	F/69	ND	WDHA	Pancreas	
14	M/38	251.0	Watery diarrhea	Pancreas	Liver
15	F/60	115.0	Watery diarrhea	Pancreas	Liver
16	M/52	33.0	Watery diarrhea hypokalemia	Pancreas (tail)	Liver
17	M/NK	23.0	Watery diarrhea	Pancreas	Liver
18	M/36	384.0	Watery diarrhea	Pancreas (head)	Liver
19	M/50	ND	WDHA	Pancreas	
20	F/39	ND	WDHA	Pancreas (head)	
21	M/60	ND	Watery diarrhea hypokalemia	Pancreas (tail)	
22	F/48	869.0	Watery diarrhea hypokalemia	Pancreas (tail)	
23	F/43	ND	WDHA	Pancreas (head)	Liver
24	M/79	ND	WDHA	Pancreas (tail)	
25	M/51	ND	WDHA	Pancreas (tail)	Lymph nodes
26	M/55	ND	WDHA	Pancreas (tail)	Liver
27	M/NK	ND	WDHA	Pancreas (head)	Lymph nodes
28	F/47	ND	Watery diarrhea	Jejunum	Liver
29	M/42	ND	WDHA	Pancreas (tail)	Liver
30	F/61	ND	WDHA	Pancreas (head)	Liver
31	F/55	ND	WDHA	Pancreas (tail)	Liver
32	F/32	15000.0	WDHA	Pancreas	Liver

NK: not known; ND: not determined.

VIPomas and one was a VIP-producing jejunal tumor. The relevant clinical information and other data are presented in Table 1.

Tissue Preparation

Light microscopy: Surgical specimens were fixed in routine histologic fixatives (Bouin's fluid or formalin) or were promptly frozen in Freon 22, freeze-dried, and fixed in benzoquinone, diethylpyrocarbonate (DEPC) or formaldehyde vapor.[14] All specimens were embedded in paraffin.

Histochemistry: For light microscopy the sections were stained with hematoxylin eosin, periodic acid-Schiff's reagent (PAS), Congo red, the Grimelius argyrophil reaction,[15] the Masson argentaffin reaction, and the diazonium test for 5-hydroxytryptamine.[16]

Immunocytochemistry: Sections (4 μm thick) were cut and immunostained by the immunofluorescence technique[17] and the unlabeled antibody peroxidase–antiperoxidase (PAP) method.[18]

Controls for specificity of reactions were performed as follows: (1) using nonimmune 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 respective antigen (1–40 μg/ml); (3) omitting the first layer; and (4) using complement-deprived, aggregate-free antihormone sera.[19]

Electron microscopy (EM): Specimens suitable for ultrastructural study were available in 21 cases. Most of the tumors were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, postfixed in 1% OsO_4, dehydrated in graded alcohols and embedded in Epon or Araldite. The polymerization of the resin was done at 60°C.

The maximum diameter of all the granules found in randomly selected electron micrographs (enlarged ×28,000) of five cells from each tumor was measured. The mean and standard deviation (SD) of these diameters was calculated.

Electron immunocytochemistry: Material for electron immunocytochemistry was processed as for conventional EM, except that postfixation with OsO_4 was omitted, and in some cases, polymerization was carried out at 18°C (using ultraviolet light),[22] since VIP antigenicity is lost following the polymerization of resin at higher temperatures.[23] Sections showing silver to light gold interference colors (60–100 nm) were collected on nickel grids and immunostained as described elsewhere.[24]

TABLE 2. Antibodies

Antibodies to:	Code no.	Region directed	Working dilution IF	Working dilution PAP
1. Vasoactive intestinal polypeptide	VIP 89N	Whole molecule	1/500	1/5000
	VIP 98P	C-terminal	1/400	1/4000
	VIP 5603	C-terminal	1/1000	1/4000
	VIP 652	Whole molecule	1/2000	1/20000
	VIP 324	Whole molecule	1/2000	1/20000
	VIP 369	Whole molecule	1/400	1/4000
2. Adrenocorticotrophic hormone	ACTH 133	Unknown	1/200	1/2000
3. Bombesin	BN 627	C-terminal	1/400	1/2000
4. Calcitonin	CT 5	Mid/N-terminal	1/200	1/2000
5. Glucagon	GLUC 499	N-terminal	1/1000	1/5000
6. Endorphin	END 388	Mid/N-terminal	1/800	1/1600
7. Gastrin	GAS 655	C-terminal	1/400	1/4000
8. Gastric inhibitory polypeptide	GIP 378	Mid	1/600	1/15000
9. Growth hormone	GH 134	Unknown	1/1000	1/7500
10. Methionine-enkephalin	m-ENK 507	C-terminal	1/400	1/4000
Leucine-enkephalin	l-ENK 493	C-terminal	1/400	1/2000
11. Motilin	MOT 407	C-terminal	1/200	1/600
12. Neurotensin	NT 357	Whole molecule	1/800	1/40000
13. Neuron-specific enolase	NSE 553	Unknown	1/800	1/4000
14. Bovine pancreatic polypeptide	BPP 146-6	Unknown	1/3000	1/12000
Human pancreatic polypeptide	HPP 248-4	Unknown	1/3000	1/12000
15. Secretin	SEC 53	Unknown	1/2000	1/50000
16. Somatostatin	SOM 624	Mid	1/400	1/4000
17. Substance P	SP 479	C-terminal	1/2000	1/20000
18. Insulin	INS 413	Unknown	1/800	1/4000
19. Glicentin	GLI R64	N-terminal	1/1000	1/10000
20. α-human chorionic gonadotropin	α-hCG 364	Unknown	1/800	1/1600

A two-step technique using a colloidal gold-labeled goat antirabbit IgG was used following the basic approach of Faulk and Taylor,[25] as modified by Romano *et al.*[26]

Colloidal gold particles (12 nm and 20 nm in diameter) were prepared using trisodium citrate as a reducing agent.[27] The labeling of purified IgG with colloidal gold was carried out using a modification[28] of existing methods.[26,29,30]

The controls for specificity were identical to those used in light microscopy. The dilutions of the antisera employed were in the range between the high dilutions used for the light microscopic PAP method and the lower dilutions used for immunofluorescence (Table 2).

Antisera

All primary peptide antibodies (Table 2) were raised in rabbits except for anti-insulin, which was raised in guinea pigs. Anti-VIP sera 89N and 98P were kindly donated by Dr. S. I. Said, Dallas, Texas; and anti-VIP 5603 by Dr. J. Fahrenkrug, Copenhagen, Denmark. Antisera to corticotropin, calcitonin, growth hormone, insulin and α-chain hCG (human chorionic gonadotropin) were obtained from Wellcome Laboratories; antibovine and antihuman pancreatic polypeptide sera (BPP 146/6 and HPP 248/4) from Dr. R. E. Chance, Lilly Laboratories, Indianapolis; and antiglicentin R64 serum from A. J. Moody, Novo Research Laboratories, Copenhagen, Denmark. Antiserum to neuron specific enolase (NSE) was kindly donated by Dr. P. J. Marangos, National Institute of Mental Health, Bethesda, Maryland. All other peptide antisera, including anti-VIP 324, 369 and 652, were raised at the Royal Postgraduate Medical School.

Radioimmunoassay for VIP

Tissue extracts were prepared following the acid/alcohol extraction method of Kenny[20] and also by direct extraction in boiling water. High avidity, high titer antibodies were produced in rabbits by immunization with carbodiimide-coupled VIP with bovine serum albumin carrier.[21] Antibodies showed no cross-reactivity with secretin, glucagon or gastric inhibitory polypeptide. The antibodies used for radioimmunoassay were found to react with the VIP molecule and not with synthetic C- or N-terminal fragments.

Pure monoiodinated VIP label for radioimmunoassay was prepared by trace labeling of VIP with ^{125}I using the lactoperoxidase technique. The product was purified on a high-resolution ion-exchange chromatography column.

Results

Conventional Light Microscopy

The histologic structure of the tumors was assessed according to the criteria advocated by Martin and Potet[12]

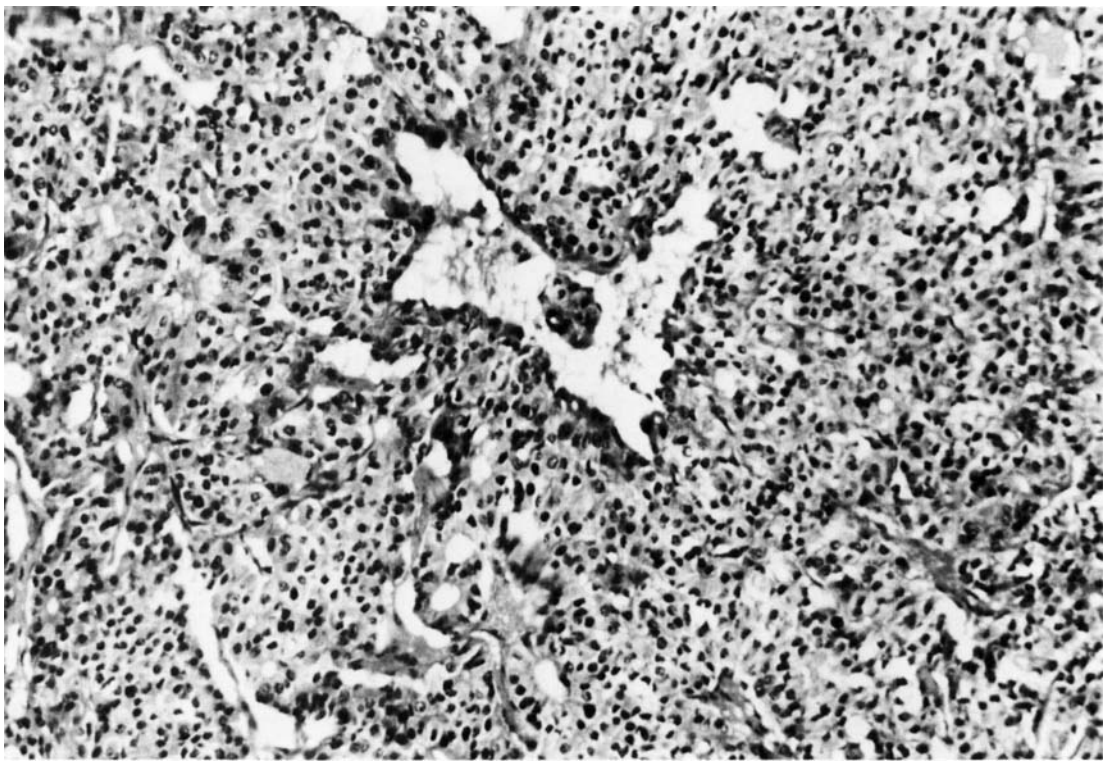

FIG. 1. Sheets of medium-sized epithelial cells interrupted by lacunar spaces. Notice well developed ductules (bottom centre) (Case 17) (H & E, ×180).

and Niewenhuijzen Kruseman *et al.*[31] Five tumors consisted of broad trabeculae of tumor cells with an anastomosing pattern (type IIb structure). Eleven tumors (including the jejunal VIPoma) had a solid pattern of growth (type IV structure), which was sometimes interrupted by irregular cystic spaces filled with weakly eosinophilic material (Fig. 1). Eight tumors displayed a mixture of type IIb and type IV patterns, the latter pattern often predominated. A tubuloacinar pattern (type III) was only seen admixed with other patterns, type IIb in four cases and type IV in four cases. Tumors with solid nests of cells (type I) or with a ribbon-like, gyriform pattern (type IIa), frequently found in well-differentiated glucagonomas, were not seen. Individual tumor cells had either granular eosinophilic or clear, relatively abundant, cytoplasm. They were polygonal in shape or, within acini tubules and trabeculae, cylindric or cuboidal. The nuclei were hyperchromatic and polymorphic in 28 cases: the polymorphism was slight in 10 cases and was clearly evident in 18 tumors. In addition, frequent, often atypical, mitoses were observed in four tumors (Cases 6, 9, 22, and 23).

Vascular and/or perineural invasion at the periphery of the tumor, was present in 11 (Cases 1, 4, 5, 6, 12, 13, 17, 23, 25, 29, and 31) of 21 cases investigated in detail. Eight of these 11 cases were with metastases.

The amount of stroma varied from case to case, but it was rather abundant and richly vascularized in most of the cases. Amyloid deposits were not identified in any of the tumors.

Most of the cases (12 of 17 tested) showed some Grimelius-reactive argyrophil cells. The argyrophil cells were more numerous and intensely stained in tissue fixed with Bouin's fluid or formalin, than in DEPC-fixed tumors, where they were weakly stained or apparently absent.

Immunocytochemistry

The results are summarized in Table 3. Immunoreactivity to all the anti-VIP sera employed was found in 24 of the 28 tumors investigated, and was usually restricted to a minority (1 to 20%) of the tumor cell population (Fig. 2). In 11 tumors HPP- and BPP-immunoreactive (IR) cells were found (Fig. 3). Examination of serial sections stained respectively with anti-VIP and anti-PP sera revealed that PP-immunoreactivity was present in separate tumor cells from those containing VIP-immunoreactivity. A small number of glucagon-IR cells were observed in Cases 1 and 32 and a few somatostatin- and insulin-IR cells were present in Cases 9, 17, 20 and 22. A few scattered neurotensin-IR cells were present in Cases 18 and 32 and met/leu-enkephalin-IR cells in Case 14.

Twelve tumors of 25 investigated contained α-hCG-

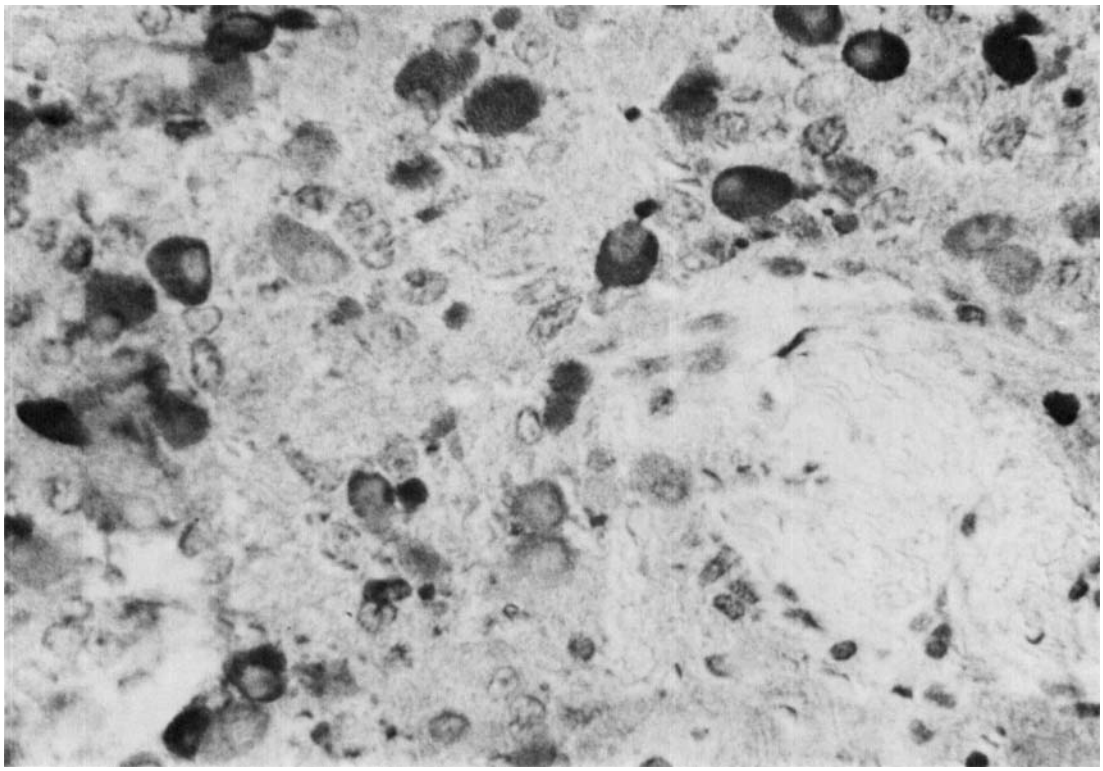

FIG. 2. Sparse VIP-immunoreactive cells in a pancreatic diarrheogenic tumor (Case 3) (PAP method, ×2000).

IR cells, which varied in number and distribution in different tumours and showed no obvious correlation with the frequency and distribution of VIP- or PP-IR cells.

Neuron-specific enolase immunoreactivity was investigated in 26 tumors. Of these, 24 contained NSE-IR cells (Fig. 4), while the remaining 2 did not show any immunoreactivity to the NSE antiserum.

The latter result is very likely to be due to inadequate fixation since immunoreactivity for various peptides, including VIP, was also absent or poor (Table 3). Tissue for electron microscopy was not available for these two cases. However, they were included because clinical information and detection of a high level of tissue VIP showed that they were VIPomas. The NSE immunostain-

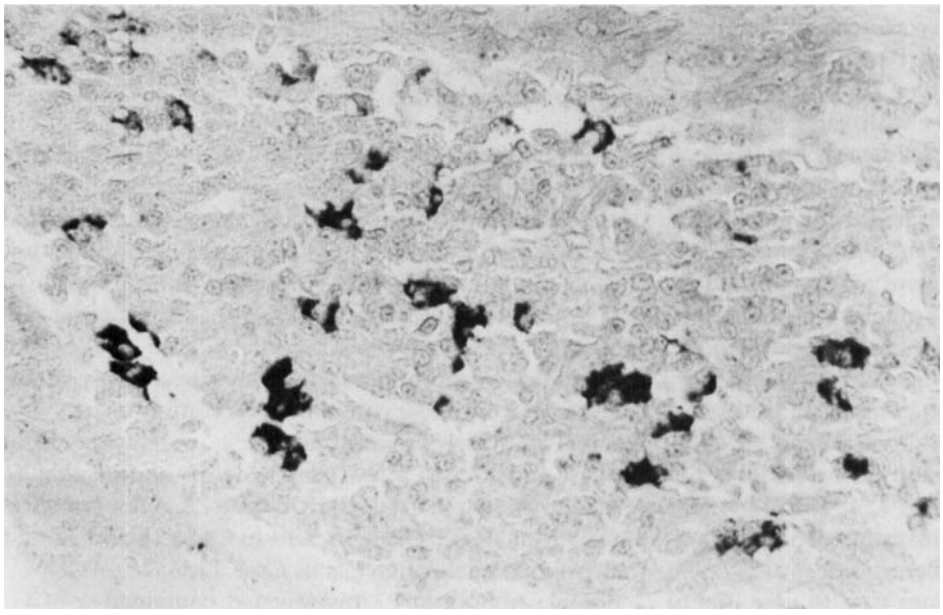

FIG. 3. Sparse PP-immunoreactive cells in a pancreatic diarrheogenic tumor (Case 5) (PAP method, ×333).

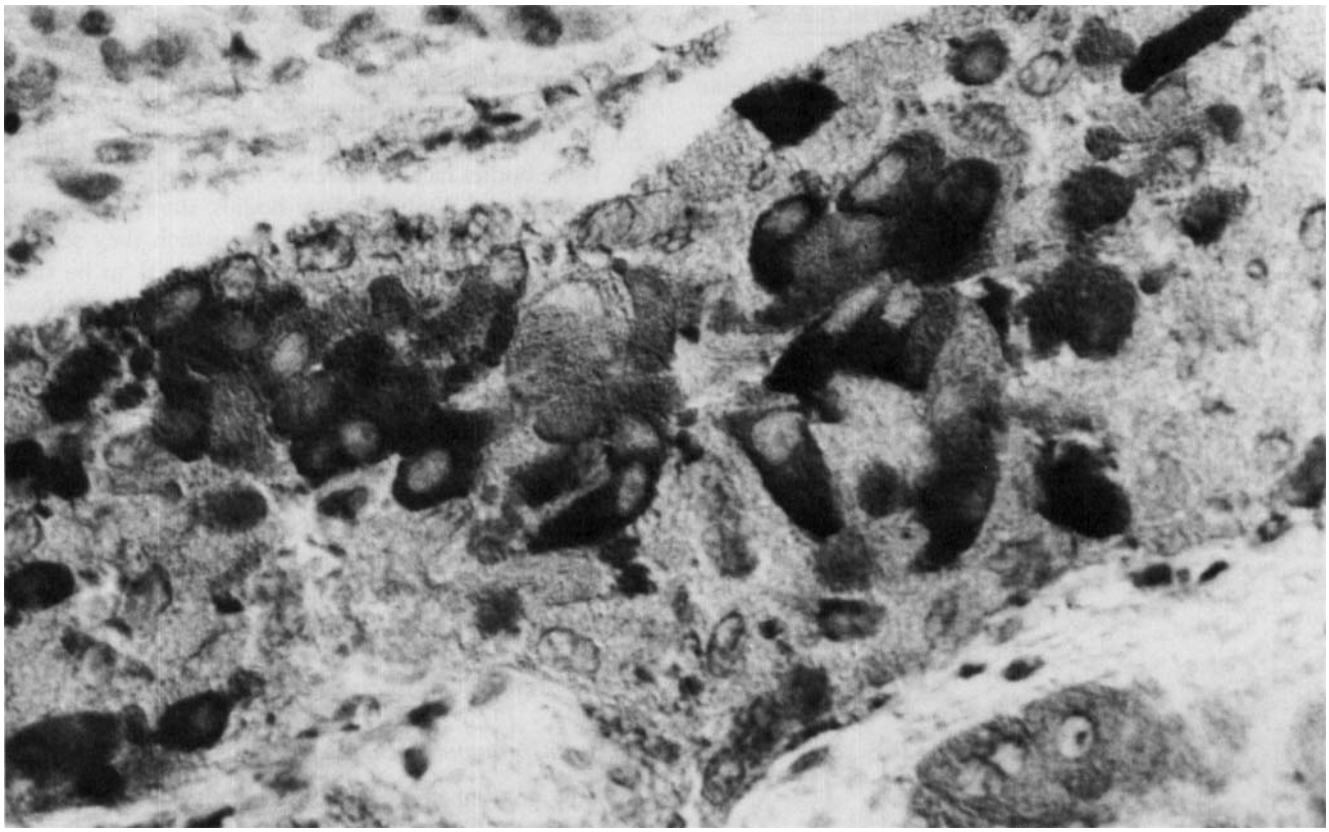

FIG. 4. Neuron specific enolase (NSE) immunostaining in a pancreatic diarrheogenic tumors (Case 4) (PAP method, ×2750).

ing in the positive cases was strong, regardless of the primary or metastatic nature of the tumors. There appears to be some parallelism between immunostaining for VIP and NSE, although NSE-IR cells were often more numerous than VIP-IR cells.

No differences in the extent and degree of the immunostain were observed between the conventional liquid fixed and the freeze-dried vapor-phase-fixed material.

Electron Microscopy

All of the neoplasms investigated (21 cases) were composed of epithelial cells which contained endocrine secretory granules. Cell contours varied from round to oval or polygonal, and there was no constant relationship of cells to each other. In six cases, the cells were closely apposed while in the remaining tumors the cells were often separated by interfacial canals into which varying numbers of microvilli or filopodia protruded. Duct-like profiles packed with well-developed microvilli were observed in 11 cases. Cell junctions of macula adherens type were encountered in all cases. Desmosomes were also observed in 14 cases and tight junctions in 9 cases, which also showed duct-like profiles. All of these features are consistent with the tumor cells being of epithelial nature (Fig. 5).

In 19 of 21 tumors, most cells showed an irregular nucleus, abundant and often dilated ergastoplasmic cisternae, well-developed Golgi zones, numerous mitochondria and a few scattered, inconspicuous secretory granules (*poorly granulated "active" cells*). A few, small virtually agranular cells with poorly developed organelles (*stem cells*) were observed in 17 of the 19 cases, while scattered cells containing more regular nuclei and abundant secretory granules, together with fairly well developed cell organelles (*well differentiated endocrine cells*), were consistently represented in 10 of the 19 cases, and were occasionally found in the remaining tumors.

The granules in both the well differentiated and the poorly granulated cells were mostly small (120–180 nm diameter) and spherical, with a uniform core of moderate electron density (Figs. 5, 6, and 7) somewhat resembling those of the so-called D1 and P cells in the normal human pancreas and gut.[32,33] Sometimes, cells with larger granules (140–180 nm) showing a tightly applied membrane (Fig. 5, *Inset*) and resembling closely the granules found in some human PP cells,[34] were distinguishable from cells with smaller (120–150 nm) granules showing a narrow clear space between the core and the membrane (Figs. 6 and 7) and somewhat resembling P cells of the human fetal pancreas.[33]

TABLE 3. Immunoreactive Cells Present in Tumors

Case no.	VIP cells	PP cells	α-hCG cells	NSE cells	Other IR cells
1	++	–	++	++	GLUC+
2	+	–	–	+	–
3	+	+	–	+	–
4	+	–	–	++	–
5	+	+	+	+	–
6	+	+	+	+	–
7	+	+	+	++	–
8	–	+	–	–	INS+
9	+	+	–	+	INS+, SOM+
10	+	–	–	+	–
11	–	–	–	–	–
12	+	+	++	+	–
13	+	–	+	+	–
14	+	–	–	+	m-ENK+
15	++	–	ND	+++	–
16	ND	ND	ND	ND	ND
17	+	+	++	+	INS+, SOM+
18	+	–	–	+	NT+
19	+	–	–	+	–
20	+	+	+	++	INS+, SOM+
21	–	–	+	+	–
22	+	+	–	+	INS+, SOM+
23	++	–	+	+	–
24	+	–	–	+	–
25	+	–	+	++	–
26	ND	ND	ND	ND	ND
27	ND	ND	ND	ND	ND
28	+	–	ND	++	–
29	ND	ND	ND	ND	ND
30	–	–	+	ND	–
31	+	–	–	ND	–
32	++	+	ND	++	GLUC+, NT+

ND: not done; +, ++, +++: increasing number of immunostained cells; –: no immunostained cells.

However, this distinction was often impossible to make due to the wide range of granule size and structure. In the prevalent population of sparsely granulated cells, small, haloed granules were observed more frequently than the larger variety.

In Case 14, target-like vesicular granules were observed; such granules resembled those of so-called enterocatecholamine cells described in the rat,[35] rabbit, and human stomach,[36] and possibly related to P and/or D1 cells. In addition to small granule cells, a few cells with large dense granules were also observed in Cases 3 and 16, and cells with both large dense granules and small granules were found in Cases 10 and 28.

As shown in Table 4, well granulated cells were prevalent only in two cases (Cases 1 and 27). In both of these tumors, most cells contained granules which, although partly resembling those of D1 cells,[32] were very variable in shape, resembling in this respect F-type PP granules found in some mammalian species.[32,37] In addition, a minority of D cells with low electron density granules (220–240 nm diameter) were observed. A few A cells containing typical alpha granules were also seen in Case 1.

In some epithelial tumor cells, elongated osmiophilic bodies, resembling morphologically the Weibel-Palade bodies of the vascular endothelium, were observed (Fig. 8). These bodies lacked however, the fibrillar and tubular substructure of Weibel-Palade bodies; they were closely related to the Golgi cisternae, from which they seemed to originate, and they sometimes appeared to be marginated along the cell membrane. Their distinction from lysosomes was sometimes difficult.

A number of regressive changes were observed in scattered cells of some tumors, including an increased number of mitochondria (up to oncocytoid pattern), swelling and loss of mitochondrial cristae, dilatation of endoplasmic reticulum and an increased number of lysosomes, occasionally showing granulophagia.

As a whole, the jejunal tumor (Case 28) was ultrastructurally indistinguishable from most pancreatic VIPomas. In particular, its main population of secretory granules closely resembled the smaller, haloed granules of pancreatic tumors (Compare Fig. 7 with Fig. 6).

Electron Immunocytochemistry

Using gold-labeled antisera, we were able to find PP immunoreactivity in tumor cells of the D1 type; the immunostaining was clearly seen in the spherical secretory granules of these cells (154 ± 22 nm in diameter) (Figs. 9A and 9B). We were able to localize VIP (in Case 28) to secretory granules of tumor cells using the immunogold technique on ultraviolet-cured (at 18°C) resin sections (Fig. 7).

A statistical analysis of the granule populations (both those immunoreactive for PP and those nonreactive for PP and, therefore, presumably containing VIP) was carried out using an unpaired Student's *t* test. With mean ± SD values of 145 ± 26 nm and 126 ± 37 nm in diameter for PP and VIP secretory granules, respectively, the granule populations were shown to be significantly different ($0.05 > P > 0.02$).

Radioimmunoassay Results

The mean (±SEM) tissue VIP concentration in the 17 cases studied (Table 1, column 4) was 1555.2 ± 851.

Discussion

All of the pancreatic diarrheogenic tumors we have investigated showed the light and electron microscopic features of epithelial endocrine tumors. They reproduced the histologic patterns already reported in well-known epithelial endocrine tumors of the pancreas,[12,31,38] including trabecular and tubuloacinar structures highly dis-

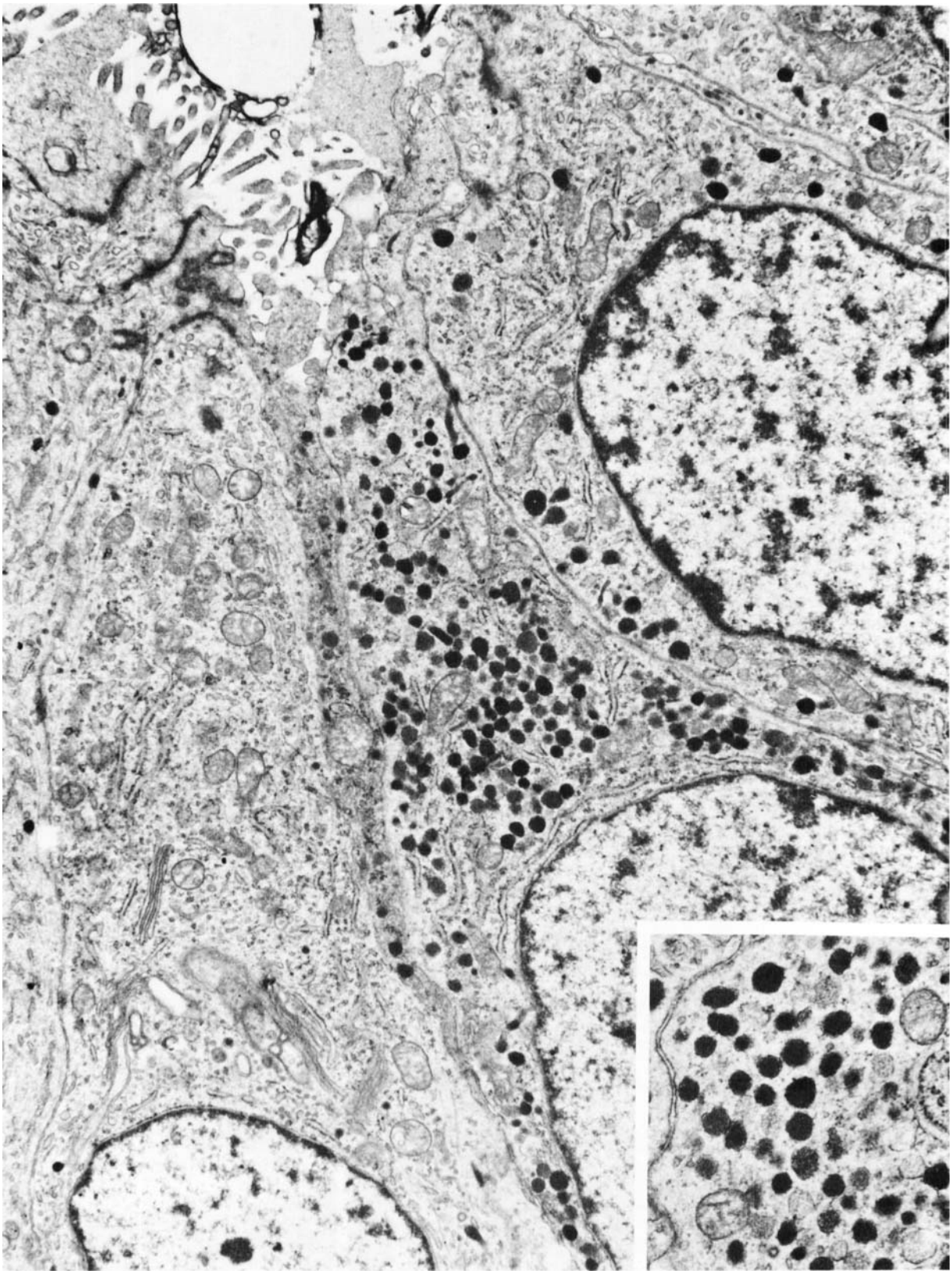

FIG. 5. Epithelial endocrine cells with well developed organelles forming a ductule, the lumen of which (upper left) is filled with microvilli. Notice juxtaluminal junctional complexes (×15,000). *Inset:* Granules with closely applied membranes (Case 27) (×30,000).

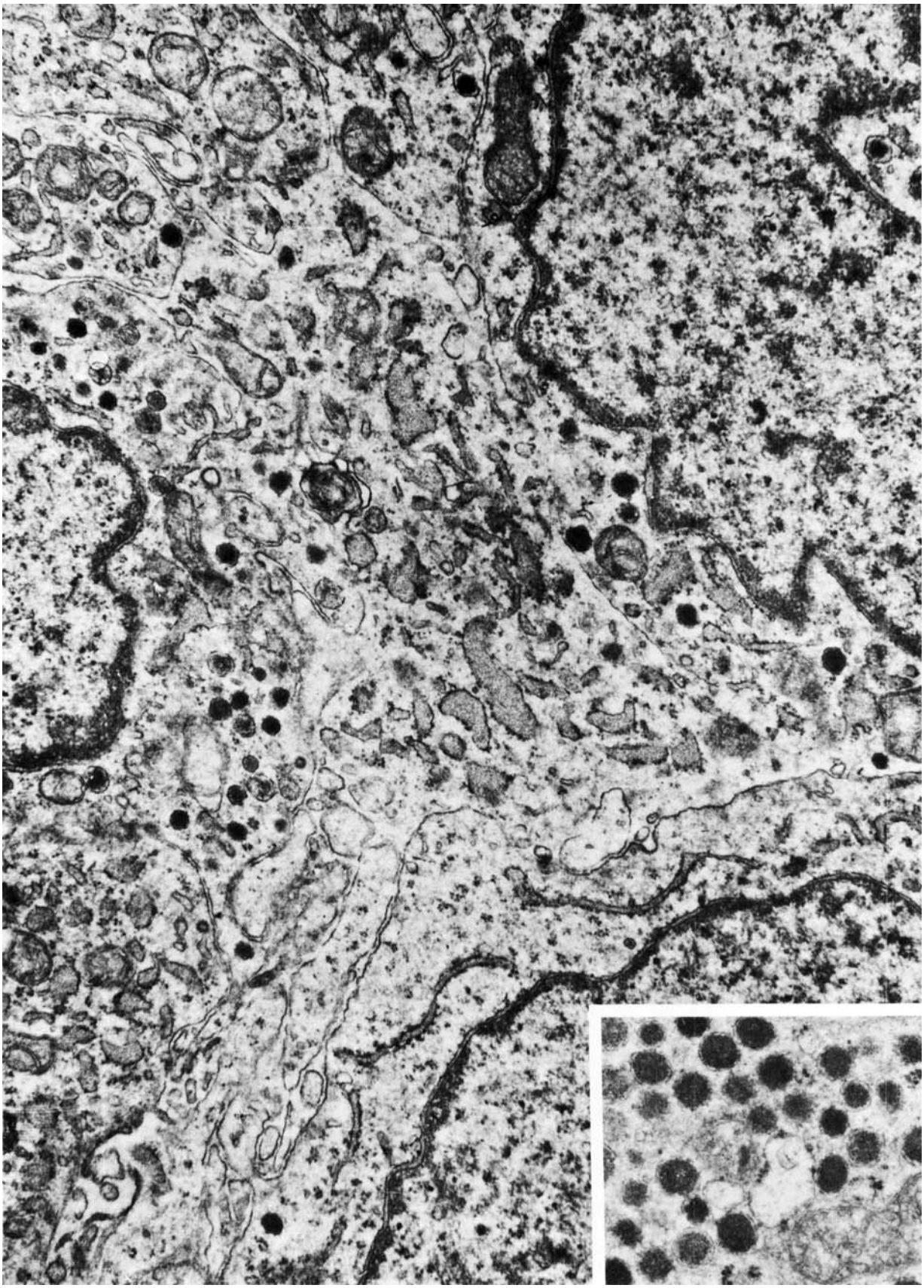

FIG. 6. Poorly and sparsely granulated cells in a pancreatic diarrheogenic tumor (Case 3) (×30,000). *Inset:* Haloed granules (Case 10) (×44,000).

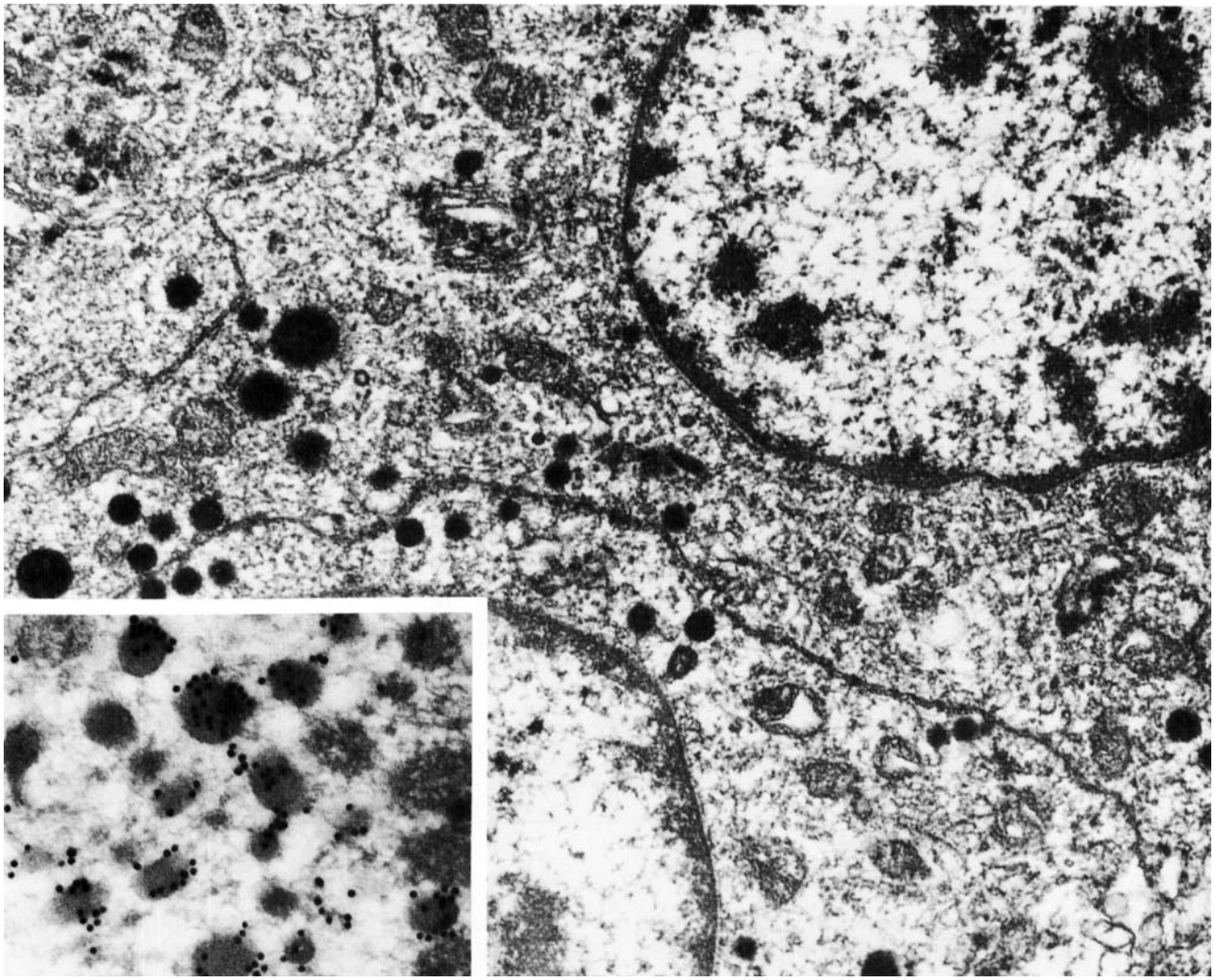

FIG. 7. Poorly granulated cells in the jejunal diarrheogenic tumor (Case 28) (×33,000). *Inset:* VIP granules stained with the immunogold technique (Case 28) (×62,000).

tinctive of epithelial tumors and reactive with Grimelius' silver stain, the well established method for endocrine cell granules.[39] In addition, they were immunoreactive for NSE which is a good marker for neuroendocrine tumors.[40] Their epithelial nature was also supported by ultrastructural findings such as well developed and specialized cell junctions, duct-like profiles, and interfacial canals filled with microvilli. The endocrine nature of these tumors was confirmed by identification of secretory granules with size, structure, and staining patterns resembling those usually found in granules of established endocrine cells,[41] and by the detection of hormonal peptides in tumor tissue. In addition, the tumors we have investigated lacked any sign of neural differentiation, including neuronal cell bodies, nerve fibres or axons, rosette-forming immature nerve cells, intracellular neurotubules and synaptic vesicles of cholinergic, adrenergic or peptidergic type. Many of these features were prominent in a previously investigated VIP-secreting ganglioneuroma with the diarrhoegenic syndrome.[42] Besides VIP-immunoreactive cells, cells storing insulin, glucagon, somatostatin, neurotensin or, more frequently, pancreatic polypeptide, were observed. The presence of more than one peptide in pancreatic endocrine tumors seems to be consistent with other reports,[32,43,44,45] the clinical characteristics of the syndrome being determined by the predominant peptide.[46] The prevalence in 30 of the 32 tumors investigated of poorly granulated, nonimmunoreactive cells with well developed organelles together with the severe diarrheogenic syndrome and increased VIP (and often PP) levels in the circulation, found in association with these tumors suggest that, despite the impairment of the granule-linked hormone storage mechanism in most cells, hormone biosynthesis and secretion were preserved. As in previous investigations,[13,32,45,47] many of the granulated cells showed small, round secretory granules resembling the

TABLE 4. Ultrastructural Features

Case no.	Interfacial canals	Duct-like profiles	Poorly granulated cells	Well differentiated cells	Secretory granules: Type	Secretory granules: Size (nm ± SD)	Elongated osmiophilic bodies
1	+	+	+	+++	1. D1-F 2. D 3. A	166 ± 55 241 ± 59 205 ± 69	+
2	−	−	++++	−	D1	152 ± 42	++
3	+	+	++++	+	1. D1 2. Large, dense	148 ± 34 217 ± 50	+ +
5	−	−	++++	−	D1	178 ± 78	+
6	+	−	++++	−	D1	133 ± 56	+
7	+	−	+++	+	D1	148 ± 34	−
10	+	+	+++	+	D1	184 ± 44	+
14	−	−	+++	+	Target-like	200 ± 71	−
15	+	−	++++	−	D1	125 ± 25	+
16	+	+	+++	+	1. D1 2. Large, dense	162 ± 53 222 ± 71	+
17	+	+	+++	+	D1	153 ± 30	+
18	+	−	++++	−	D1	180 ± 46	+
19	+	−	++++	−	D1	134 ± 31	+
25	+	−	++++	−	D1	138 ± 38	+
26	+	−	++++	−	D1	145 ± 35	−
27	+	−	+	+++	1. D1-F 2. D	161 ± 32 210 ± 60	+
28	+	−	+++	+	D1	125 ± 32	−
29	+	+	+++	+	D1	132 ± 21	+
30	+	+	++++	−	D1	120 ± 17	+
31	+	+	++++	−	D1	155 ± 37	++
32	+	+	++++	−	1. D1 2. Large, dense	150 ± 42 nm 210 ± 47 nm	+

+–++++: Increasing number of structures.

typical D1/P type, known to be functionally heterogenous.[41,48] This functional heterogeneity was further demonstrated with the immunogold technique at the electron microscopic level, which enabled us to localize PP and VIP in distinct populations of D1 and P secretory granules of separate cell types.

The existence of an endocrine cell type responsible for the production of genuine VIP, with the 28 aminoacids originally extracted and characterized,[49,50] has not been demonstrated in mammals. The production of VIP by epithelial tumors of the human pancreas might, therefore, be due to a deviation in the differentiation process of some tumor stem cell, leading to the production of a peptide which, at least in its fully immunoreactive molecular form, seems inappropriate for a human pancreatic endocrine cell, although appropriate for human pancreatic nerves[51] and possibly for nonmammalian intestinal endocrine cells.[52] The relatively poor morphologic differentiation of most VIPoma cells seems in keeping with this interpretation of tumor cells as the product of abnormal differentiation along ancestral and/or inappropriate cell lines.

The occurrence in the jejunum of an epithelial VIPoma histologically, histochemically, and ultrastructurally indistinguishable from pancreatic VIPomas (see Case 28 of the current series), seems interesting in light of the apparent existence of specific epithelial endocrine cells producing VIP in the intestine of nonmammalian vertebrates[52] and of endocrine cells producing VIP-related peptides—different from authentic VIP—in the mammalian intestine.[53,54]

The role of VIP as a key mediator of watery diarrhea in the watery diarrhea, hypokalemia, achlorhydria (WDHA), or Verner-Morrison syndrome, had been reported before.[2,4,5] However, other diarrheogenic substances have also been implicated, including PP,[55] prostaglandins,[56] and calcitonin.[57,58] The diarrheogenic potential of PP seems questionable,[59] while calcitonin has been found only in a restricted number of pancreatic diarrheogenic tumors as reported in the literature[60,61] and in none of the current series. Hypersecretion of prostaglandin seems to occur frequently in patients bearing these tumors, and the diarrheogenic potential of this substance is well known.[56]

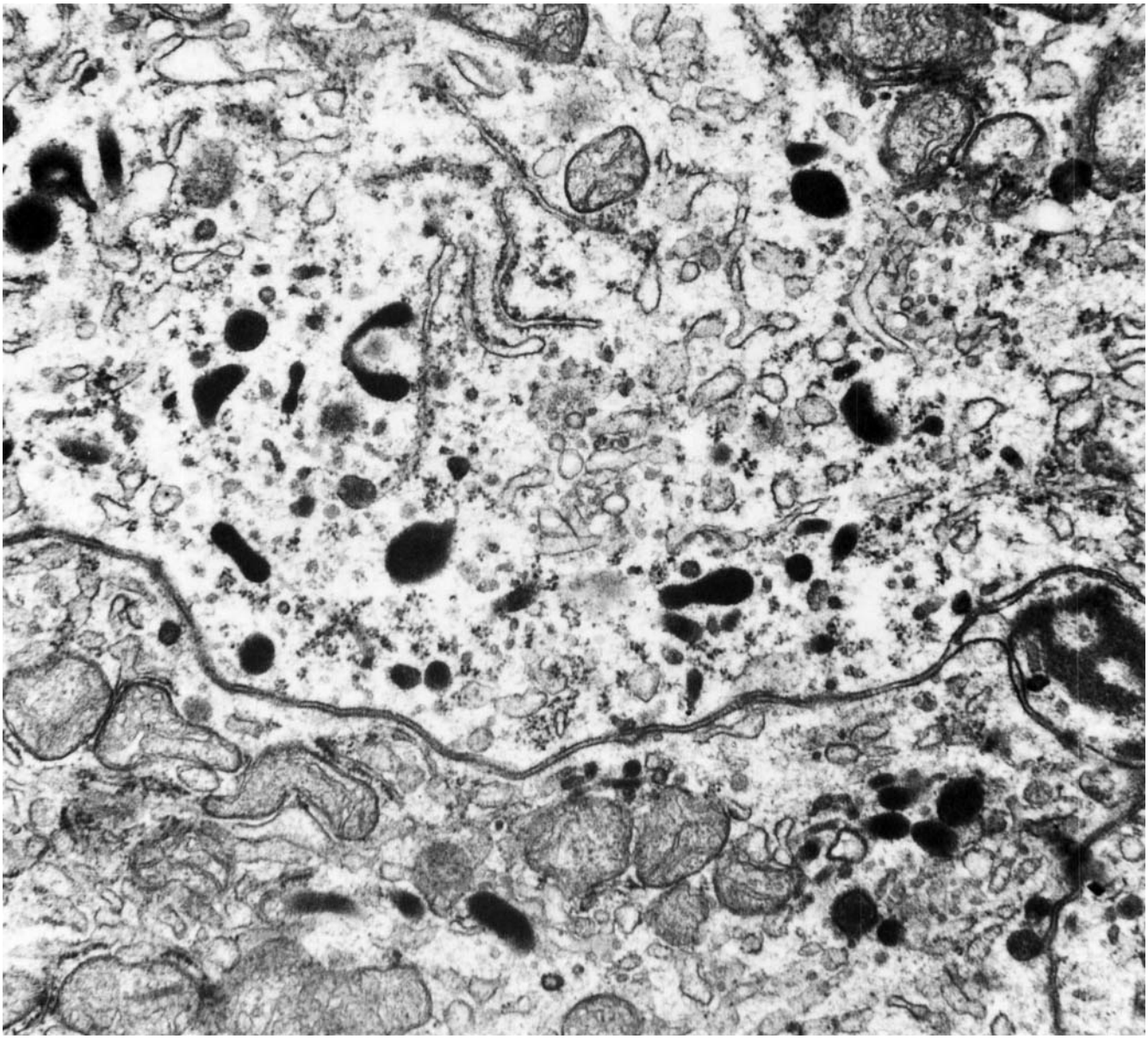

FIG. 8. Elongated osmiophilic bodies in tumor (Case 2) (×30,000).

In previous reports, about 50% of the diarrheogenic tumors were found to be malignant.[7,62] In our current series of VIP-producing tumors characterized using four separate diagnostic criteria—conventional histologic, immunochemistry, electron microscopy, and biochemistry—20 of 32 cases (62.5%) presented metastases (19 of 31 pancreatic cases = 61.3%). This behavior, with a high incidence of relatively low-grade malignancy, can be compared with that of pancreatic gastrinomas (63% to 90% malignancy rate according to various investigations)[45] and clinically active, "functioning" glucagonomas or somatostatinomas, while differing from that of insulinomas (15% malignancy[63]) and clinically silent nonfunctioning, endocrine cell tumors of the pancreas.[64] This is also in keeping with the high incidence in diarrheogenic tumors (in 11 of 23 cases tested) of reactivity with antisera directed against the α-chain of hCG, reputed to be a marker for malignancy among pancreatic endocrine tumors.[65] Of course, for the diagnosis of diarrheogenic endocrine tumors of the pancreas, the clinical syndrome of watery diarrhea due to intestinal hypersecretion is essential. For the more specific diagnosis of VIPoma, increased VIP levels in blood (above 60 pmol/l) and the radioimmunologic or immunohistochemical detection of VIP in the

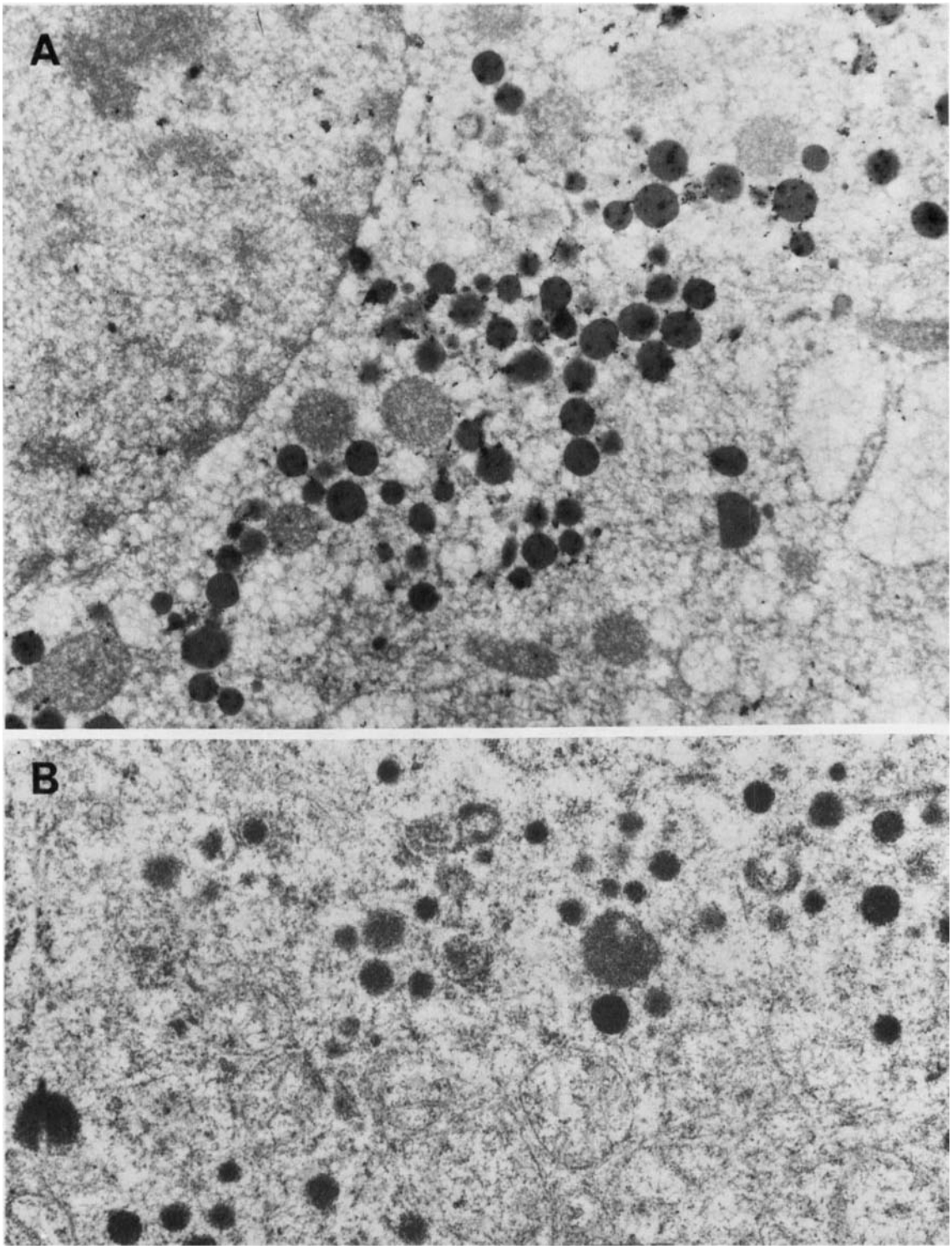

FIG. 9A AND 9B. (A) Secretory granules in a pancreatic tumor (Case 7) immunostained for BPP using the gold technique (×30,000). (B) Secretory granules, nonimmunoreactive with BPP antibodies in a different tumor cell, possibly corresponding to the VIP-immunoreactive cells shown by light microscopy (×30,000).

tumor, must be added to the clinical syndrome. The contributions of morphology to the diagnosis of VIPomas may be of various types: First, the recognition of the epithelial endocrine nature of a pancreatic tumor found in patients bearing the diarrheogenic syndrome, which can be obtained in any laboratory by conventional his-

tology plus some selective stains such as Grimelius' silver impregnation. In addition, the immunocytochemical detection of NSE can be useful. Second, the ultrastructural confirmation of the endocrine nature of the tumor, with special reference to the presence of small granuled D1 type or P-type cells and the prevalence of poorly granulated "active" cells. These features differ from those of well differentiated islet cell tumors, including most insulinomas, showing granules of distinctive structure resembling those of islet A, B, D, or PP cell granules, but fail to separate VIPomas from other poorly differentiated endocrine tumors; for instance, most gastrinomas showing only a few small spherical, nondistinctive granules.[32,45,65,66] Finally, the immunohistochemical detection of VIP and other hormone-like substances in tumor cells, which is unquestionably the step of highest diagnostic value, provided that care is taken to use specific anti-VIP sera under appropriate immunohistologic control.

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