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The Endocrine Cells of the Pancreas and Related Tumours

Ultrastructural Study and Classification

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Summary. Up to seven endocrine cell types have been identified ultrastructurally in the pancreas, including glucagon A cells, insulin B cells, somatostatin D cells, pancreatic peptide F cells and 5-hydroxytryptamine EC cells. In addition, D_1 cells, which have been proposed as the cell type producing VIP and possible P cells of unknown function are seen. Various patterns of endocrine cell differentiation have been found in 20 endocrine pancreatic tumours. Well and poorly differentiated B cells have been identified in 6 insulinomas, diagnostic G cells in 3 out of 7 gastrinomas, D_1 and/or F cells in 7 diarrheogenic tumours. Moreover, cells apparently unrelated to the prevalent clinical syndrome have been noted in 8 of the 20 tumours. Granular non diagnostic cells (poorly diagnostic gastrin cells? D_1 cells?) were particularly frequent in gastrinomas; agranular or poorly granular cells, either of "active" or "stem cell" type, were present in nearly all tumours, particularly in diarrheogenic tumours, gastrinomas and malignant insulinomas. A cytological classification of pancreatic endocrine tumours is proposed.

Key words: Pancreatic endocrine cells – Insulinomas – Gastrinomas – WDHA tumours – Ultrastructure.

Endocrine cells differing ultrastructurally from well-known A, B and D cells have been repeatedly observed in the normal pancreas as well as in pancreatic tumours. "F" and "E" cells (Munger et al., 1965), "enterochromaffin type II" cells (Parrilla et al., 1969), "type 3" cells (Misugi et al., 1970), "presumptive gastrin-secreting" cells (Like and Orci, 1972), "type IV" cells (Deconinck et al., 1972; Munger, 1972), "D_1" cells (Vassallo et al., 1972), "S cells" (Forssman, 1976) and cells "with very small granules" (Wilander and Westermark, 1976) have been described in adult and/or foetal pancreas. "Gastrin" or "G" cells

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have been found in pancreatic gastrinomas (Creutzfeldt et al., 1971 and 1975; Solcia et al., 1975), "D_1" or "type IV" cells have been reported in gastrinomas, insulinomas and tumours causing the watery diarrhea, hypokaliemia and anachlorydria (W.D.H.A.) syndrome (Vassallo et al., 1972; Creutzfeldt, 1975; Bordi et al., 1975; Rambaud et al., 1975), "EC" cells have been noted occasionally in insulinomas and gastrinomas (Creutzfeldt et al., 1973 and 1975) as well as in rare argentaffin carcinoids arising in the pancreas (Patchefsky et al., 1974). Moreover, a relationship of D_1 (type IV) cells or tumour cells "with atypical granules" to endocrine stem cells has been postulated (Creutzfeldt et al., 1975).

In this paper we have reinvestigated the ultrastructure of the endocrine pancreas and related tumours to ascertain 1) which endocrine cell types occur in normal pancreas, 2) which cells are present in pancreatic tumours associated with endocrine hyperfunction syndromes, and 3) which patterns of cell differentiation from stem cells to mature endocrine cell types can be recognized. The results of this investigation provide the basis for a cytological classification of pancreatic endocrine tumours which at least in part can be correlated with their functional behaviour and prognosis.

Material and Methods

Samples from the pancreas of guinea-pig, dog, cat, pig and man (adult, neonatal and foetal pancreas) were fixed in 2.5% gluteraldehyde or in 2% paraformaldehyde + 2% glutaraldehyde in 0.1 M Soerensen's phosphate buffer pH 7.3. Part of the specimens were postfixed in osmium tetroxide and embedded in Epon-Araldite mixture; ultrathin sections were stained with uranyl acetate and lead citrate. Other aldehyde-fixed specimens were cut with the Smith-Farquhar tissue sectioner; 100–150 μm sections were stained with Masson's, Grimelius' and Sevier-Munger's silver techniques as reported previously (Vassallo et al., 1971a; Capella and Solcia, 1972). They were then dehydrated and embedded in resine; ultrathin sections were observed in a Zeiss EM10 electron microscope with and without slight uranyl counterstaining.

Specimens of 20 endocrine pancratic tumours were also processed as above. All the tumours had been classified functionally on the basis of the associated clinical syndrome, 6 as insulinomas, 7 as gastrinomas and 7 as WDHA tumours (diarrheogenic or Verner-Morrison's tumours). Increased serum levels of insulin, gastrin and vasoactive intestinal peptide (VIP), remission of clinical symptoms and biochemical changes after removal of tumour tissue, hormone assays on tumour extracts and/or immunohistochemical detection of related hormones in tumour cells, confirmed the clinical diagnoses.

For granule morphometry the diameter of all granules found in randomly selected micrographs of 3 to 7 cells for each cell type was measured. For comparison, granules of 5 pyloric G cells from previous work (Vassallo et al., 1971b) were also measured. The mean diameter (d) of such granules was calculated and corrected to account for the sectioning artifact with the formula: $D=(4/\pi)\cdot d$, where D is the corrected diameter (Baetens et al., 1976b).

Results

Normal Pancreas

In all species investigated, cells differing ultrastructurally from A, B and D cells were found to represent a resticted minority of the whole islet cell population, while accounting for the majority of endocrine cells scattered in the acinar tissue and ducts.

Table 1. Diameter (d) of granules in some endocrine cells of human Pancreas (adult + foetal) and pancreatic tumours

Cell types	No of granules counted	Mean d in nm	SD	$(4/\pi)\cdot d$
Pancreas:				
A cells	718	252	± 65	321
B cells	352	302	± 75	385
D cells	556	259	±148	329
D_1 cells	774	128	± 20	164
F cells (min ds)	460	118	± 28	150
F cells (max. ds)	460	186	± 49	237
Pyloric mucosa:				
G cells	652	290	± 46	372
Insulinomas:				
B cells, case 1	231	263	± 59	335
B cells, case 3	684	144	± 39	183
B cells, case 5	422	161	± 35	205
Gastrinomas:				
G cells, case 2	297	203	± 62	258
gnd cells, Case 3	365	126	± 30	161
P cells, case 6	407	115	± 20	146
Diarrheogenic tumours:				
D_1 cells, case 1	687	127	± 23	161
F cells, case 5 (min. ds)	622	147	± 34	188
F cells, case 5 (max. ds)	622	210	± 52	267

gnd cells = granular non-diagnostic cells

The following types of cells were identified:

(1) Small, irregularly shaped cells with relatively small (Table 1) round granules showing a moderately dense core either with closely applied membrane or with interposition of a very thin ring of less dense material (Fig. 1). The granules reacted slightly to fairly intensely with Sevier-Munger's silver (Fig. 14a) and Grimelius' silver while failing to react with Masson's argentaffin reaction. In agreement with our previous investigations (Capella et al., 1971; Capella and Solcia, 1972; Vassallo et al., 1972; Buffa et al. 1976b) we labelled this cell as a D_1 cell. It was present in both the islets and exocrine tissue, with marked changes in number and distribution according to the animal species considered. Our D_1 cell seems to correspond to "type 3" cell of Misugi et al. (1970), "type IV" cell of Deconinck et al. (1972), part of the "small granule cells" of Cavallero et al. (1974: see Fig. 13) and the "S cell" of Forssmann (1976).

(2) Medium-sized cells showing intensely osmiophilic granules of pleomorphic, peculiar shape and reacting heavily with both the argyrophil techniques of Grimelius' and Sevier-Munger and the argentaffin technique of Masson (Fig. 2). This cell was identified as the argentaffin or enterochromaffin EC cell storing 5-hydroxytryptamine (5HT), known to be well represented in the gut mucosa (Solcia et al., 1975). It occurred infrequently in the islets, while being frequently

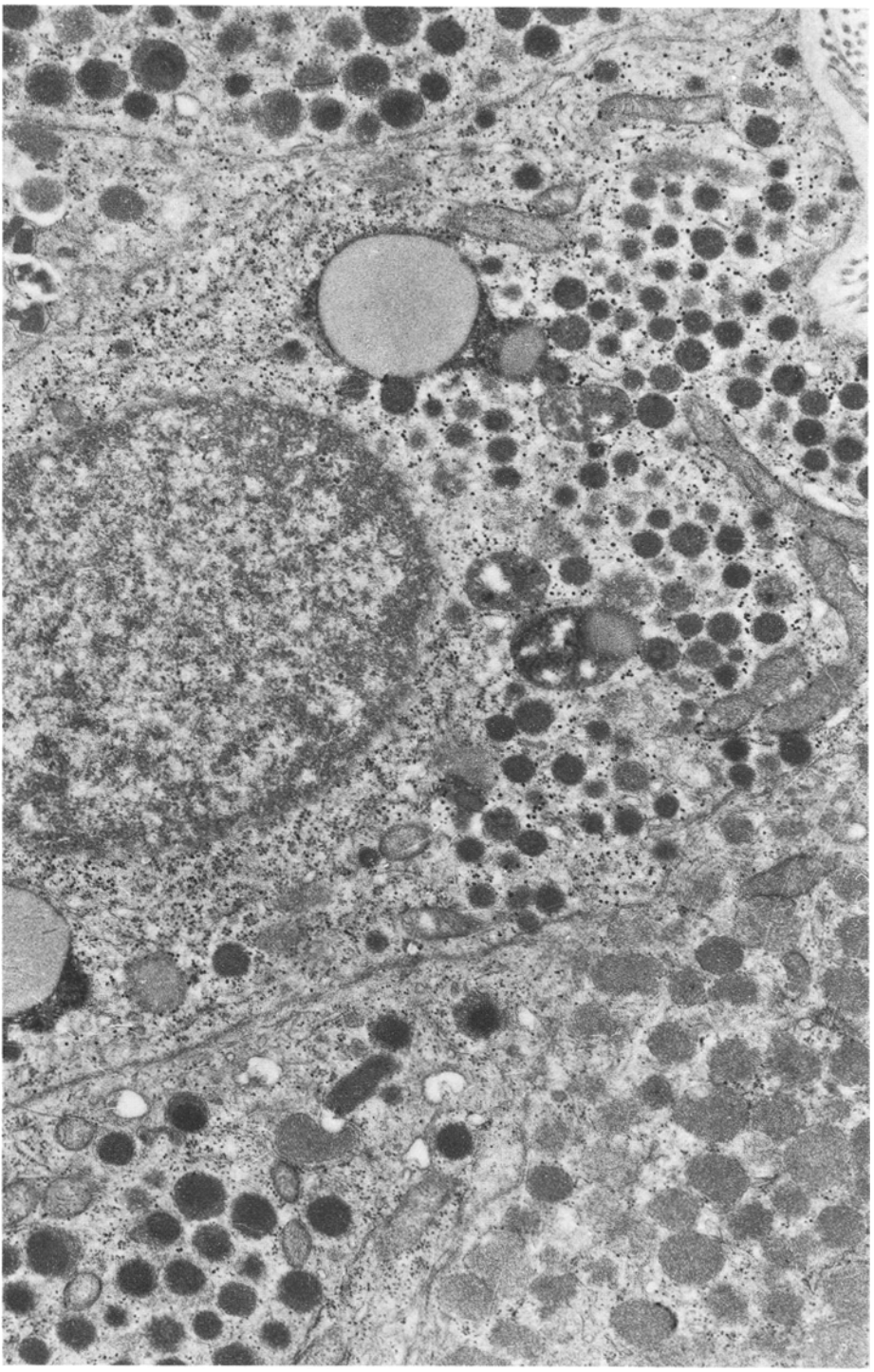


Fig. 1. D_1 cell and part of A, B and D cells in a human pancreatic islet. ×28,000

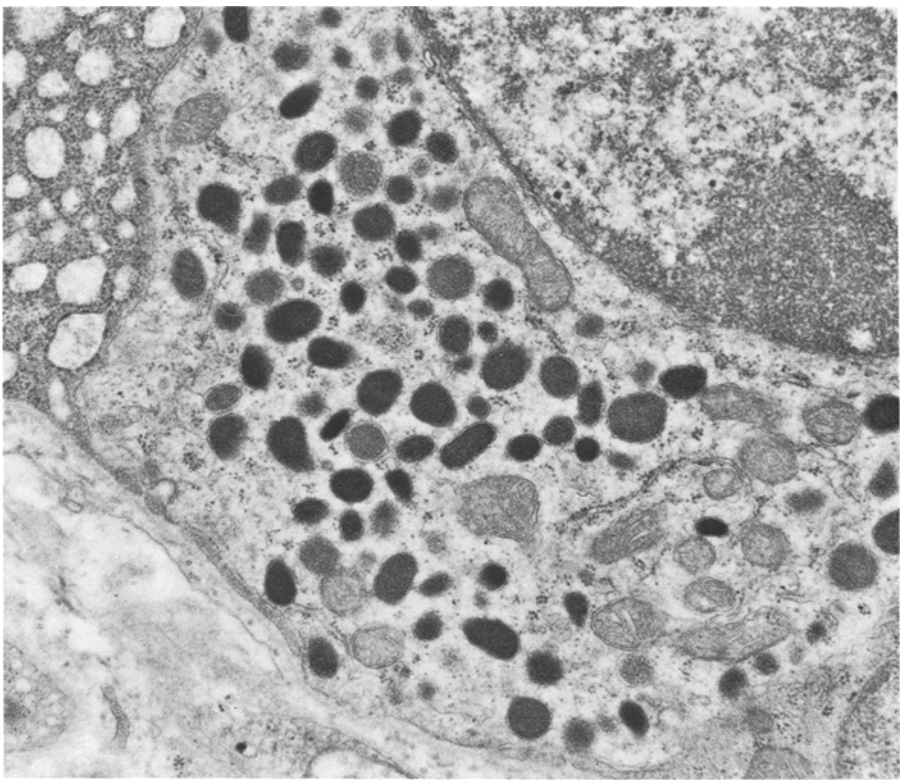
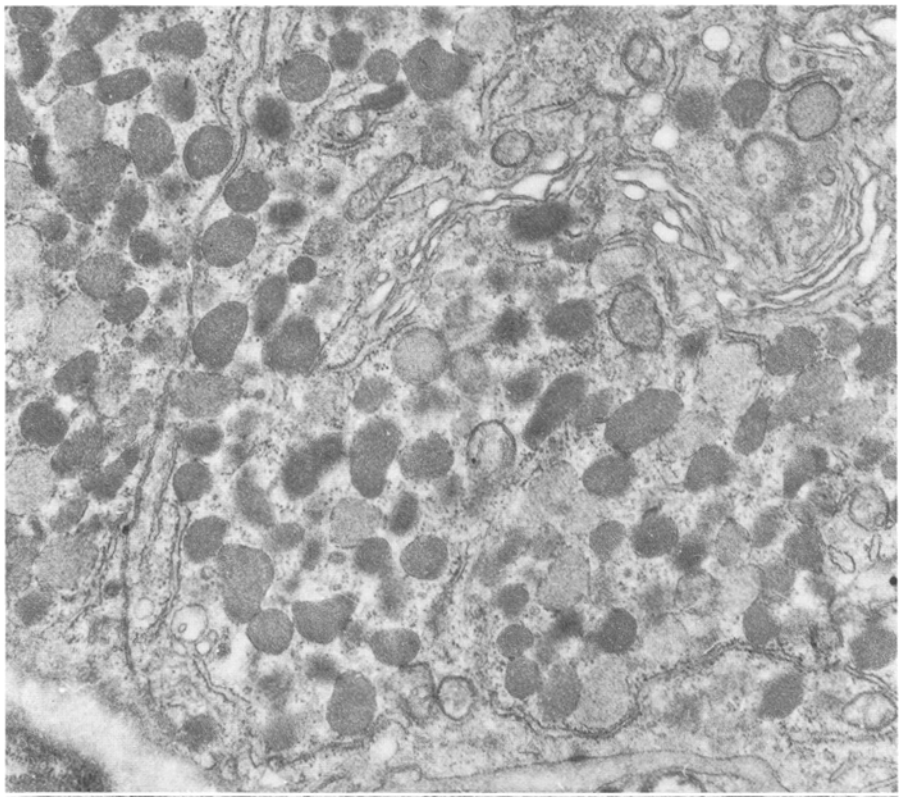


Fig. 2. EC cell of the guinea-pig pancreas. ×23,000

scattered in the ducts and acinar tissue of the guinea-pig and cat; in the human pancreas it was found only very rarely. Most 5HT-storing heavily argyrophil cells of the dog exocrine pancreas proved to be mast cells. EC cells are obviously identical with the enterochromaffin cells already reported in the pancreas by Parrilla et al. (1969), Capella and Solcia (1972), Lechago and Bencosme (1973), Solcia et al. (1974) and Cavallero et al. (1974).

(3) Medium-sized, often angular or elongated cells showing angular or round granules – ovoid, rod-, spindle-, wedge-, or pear-shaped or even quite irregular granules are seen, of very variable size, density and inner texture, from compact and fairly osmiophilic to very loose and electron lucent (Figs. 3 and 4). The cells with a predominance of electron lucent, vesicular granules reproduced the ultrastructural patterns of "F cells" and "X cells" as decribed by Munger et al. (1965) and Lazarus and Shapiro (1971). In the dog, they were numerous in the exocrine tissue – where they were scattered as single cells or were grouped in small clusters – and in the small islets of the uncinate process, but were found in small numbers in the remaining islets. Few of these cells were found in the pancreas of the cat or some other species. However, cells with prevalence of compact, round to angular granules reproducing the ultrastructure of "E



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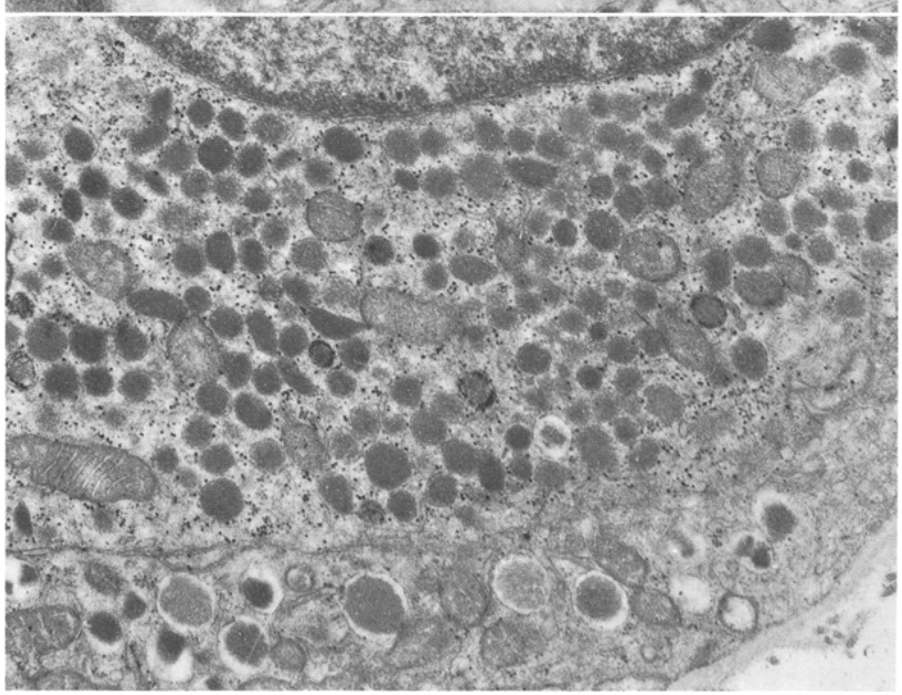

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cells" as described by Munger et al. (1965) or "F cells" as described by De Hoyos-Guevara (1969) and Watari (1973), were found to be numerous in the exocrine tissue of the cat, guinea-pig, and pig but were less numerous in the human exocrine pancreas. Within the islets they were usually found at the periphery and were more numerous in the juxtaduodenal pancreas than in its splenic part. The size of granules, although variable from cell to cell and even in the same cell, was smaller in the guinea-pig and man than in the cat and pig. The frequent occurrence of both vesicular and compact granules inside the same cell suggests that such pleomorphic cells probably belong to a single cell type, likely corresponding to the pancreatic polypeptide (PP) cell of Larsson et al. (1976b). We prefer to call all these cells "F cells" whether showing E-type or F-type features according to the original description of Munger et al. (1965). F cells were unreactive or only very slightly reactive with Sevier-Munger's and Grimelius' methods; they were unreactive with Masson's argentaffin reaction. In general F cells were more numerous than any other endocrine cell type in the exocrine pancreas, with special reference to the acinar tissue.

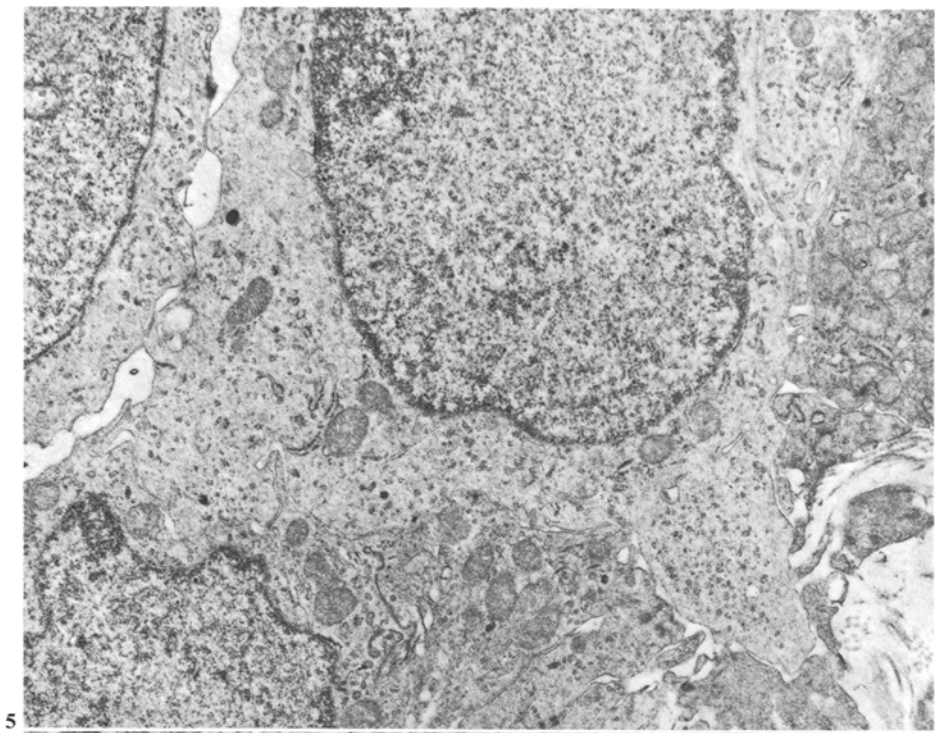
(4) Cells with very small round, moderately argyrophil granules resembling bronchial and gut P cells (Capella et al., 1977a) were found very infrequently in the human foetal pancreas. These cells might correspond to the "fourth type" of islet cell described by Munger (1972), and to the "cell with very small granules" found by Wilander and Westermark (1976) in the human foetal pancreas.

(5) Cells fully reproducing the ultrastructural features of pyloric gastrin (G) cells have not been found. Evidence for epithelial agranular "C cells" as described by Caramia et al. (1965) was rather inconsistent in adult islets; most of the apparently agranular cells were found to store granules on serial sectioning. Schwann cells, as described by Kobayashi and Fujita (1969) have been regularly found in dog islets, their long thin processes, enveloping both nerves and endocrine cells, resembled the sustentacular cells of the carotid body.

Most endocrine cells of human foetal pancreas reproduced the ultrastructural features of cell types found in adult pancreas; however, a minority of cells showed smaller granules, either with the usual structure or with less diagnostic features due to partial lack of halo (A cell granules) and of crystalloid pattern (B cell granules). Evidence for a fully granular cell to be interpreted as a stem cell common to all or part of mature endocrine cell types was not obtained. A few cells with poorly developed organelles and scattered ribosomes showed few, small, non-diagnostic granules resembling "progranules" usually found in the Golgi area of mature endocrine cells. Such cells were interpreted as the first step of development of the agranular stem cell into endocrine cells of whatever type. Somewhat similar cells have been found in the islets of lower vertebrates (Falkmer and Östberg, 1976).

Fig. 3. Two F cells in the pig pancreas. Note the variable shape and osmiophilia of their secretory granules. ×18,000

Fig. 4. F cell and part of a B cell (below) in a human islet. ×21,000



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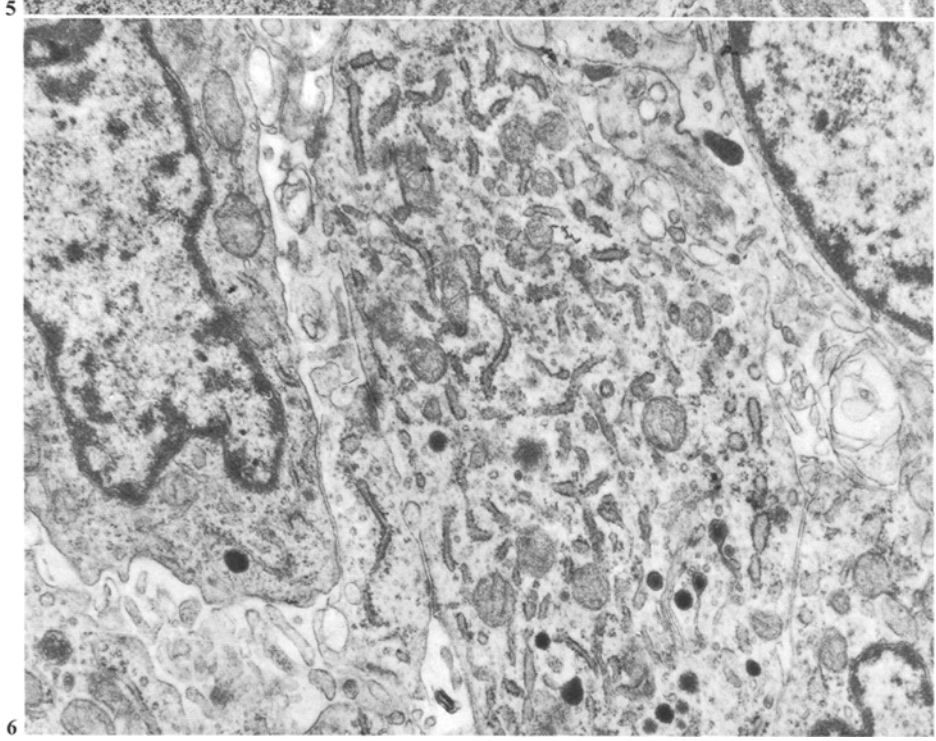

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Fig. 5. Agranular "stem" cell with poor organules and scattered ribosomes in gastrinoma case 2. $\times 14{,}000$

Fig. 6. Agranular and poorly granular "active" cells in diarrheogenic tumour case 2. Note well developed reticulum showing dilated cisternae filled with secretory product. $\times 14{,}000$

Pancreatic Endocrine Tumors

Various patterns of endocrine cell differentiation were observed in the pancreatic tumours under study. Besides well differentiated tumour cells reproducing the ultrastructure of normal cell types, cells with less diagnostic granules, poorly granulated cells of questionable or no diagnostic help and agranular cells were found. Various amounts of endoplasmic reticulum and Golgi structures were observed in agranular and poorly granular cells, ranging from cells with highly developed organelles suggesting active secretion to cells with poorly developed organelles and few scattered ribosomes suggesting undifferentiated "stem" cells (Figs. 5 and 6). Agranular cells sometimes resembled undifferentiated ductular cells of foetal pancreas. The interpretation of cells with less diagnostic granules was helped by 1) careful comparison with endocrine cells of non-tumour pancreas or gut, with special reference to foetal tissues, 2) the application to tumour specimens of silver stains at the ultrastrutural level, and 3) comparison with results of histochemical tests applied to paraffin sections of the same or adjacent

Table 2. Cell types of pancreatic insulinomas, gastrinomas and diarrheogenic tumours

Tumours	Cell types[a]									
	B	A	D	D_1	F	P	EC	G	gnd	apg
Insulinomas:										
Case 1, benign	W5									1
Case 2, benign	WP3								2	1
Case 3, benign	WP3	P1		R	R	1	R			
Case 4, uncertain	P1	P1			R				1	3
Case 5, malignant	P3									3
Case 6, malignant	P2			P1	?				1	2
Gastrinomas:										
Case 1								WP5		1
Case 2		R			W1			WP2		3
Case 3				?				?	4	2
Case 4		P1		?				?	2	3
Case 5	?			?				?	4	2
Case 6						W2		R	1	3
Case 7				?				?	1	5
Diarrheogenic Tumours:										
Case 1				W3						3
Case 2				W2		R				4
Case 3				P2						4
Case 4				P2						4
Case 5				W1	W3				1	1
Case 6			P1	P1	P1				2	1
Case 7				?					1	5

gnd = granular non-diagnostic; apg = agranular or poorly granular
W = well differentiated; P = poorly differentiated; R = rare; ? = possible nature of granular non-diagnostic cells

[a] The number of cells of each type has been roughly estimated, from 1 (about 10%) to 6 (100%)

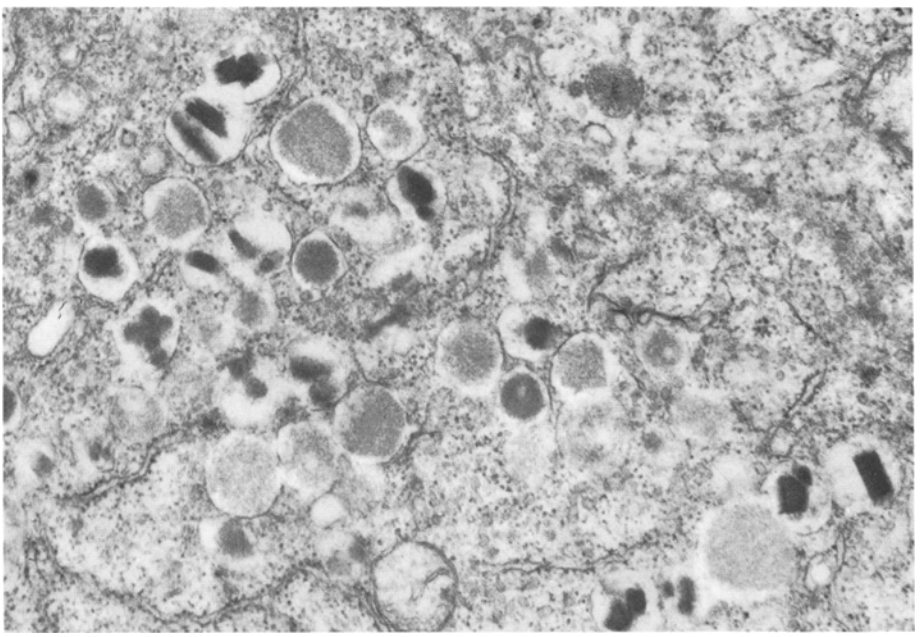


Fig. 7. Highly diagnostic crystalline-like granules in a well differentiated B cell adenoma (insulinoma case 1). Note also non-crystalline round clear granules. ×28,000

samples of tumour tissue, as suggested by Bordi et al. (1975). Results are summarized in Table 2.

B cells with more or less diagnostic granules were prominent in the majority of insulinomas (Fig. 7). Often, the granules of tumour B cells were smaller (Table 1), sometimes with single crystalline bodies instead of the multiplicity found in granules of normal cells; in some tumour cells, interpreted as poorly diagnostic B cells, most of the granules showed an amorphous core of various density, with only occasional attempts at crystal formation (Fig. 8). Some cells showing small round or fairly irregular granules with a homogeneous non-diagnostic core were also tentatively identified as B cells because of their close similarity to adjacent cells bearing some diagnostic crystalline granules. The non-reactivity of comparable granules in specimens treated with argyrophil methods helped in distinguishing such cells from D_1 cells or P cells (Fig. 14c). Agranular cells were more often found in malignant than in benign insulinomas.

Diagnostic G cells were well represented only in 2 out of 7 gastrinomas; often tumour cells showed small granules with rather compact core of little diagnostic help (Tables 1 and 2; Figs. 9 and 10). Frequently, it was difficult to assess wether we were dealing with poorly diagnostic G cells or with D_1 cells. Poorly granulated and agranular cells were found more often in gastrinomas than in insulinomas.

Scattered, poorly diagnostic A cells were noted in two insulinomas and two gastrinomas. Their granules were smaller than those of normal A cells

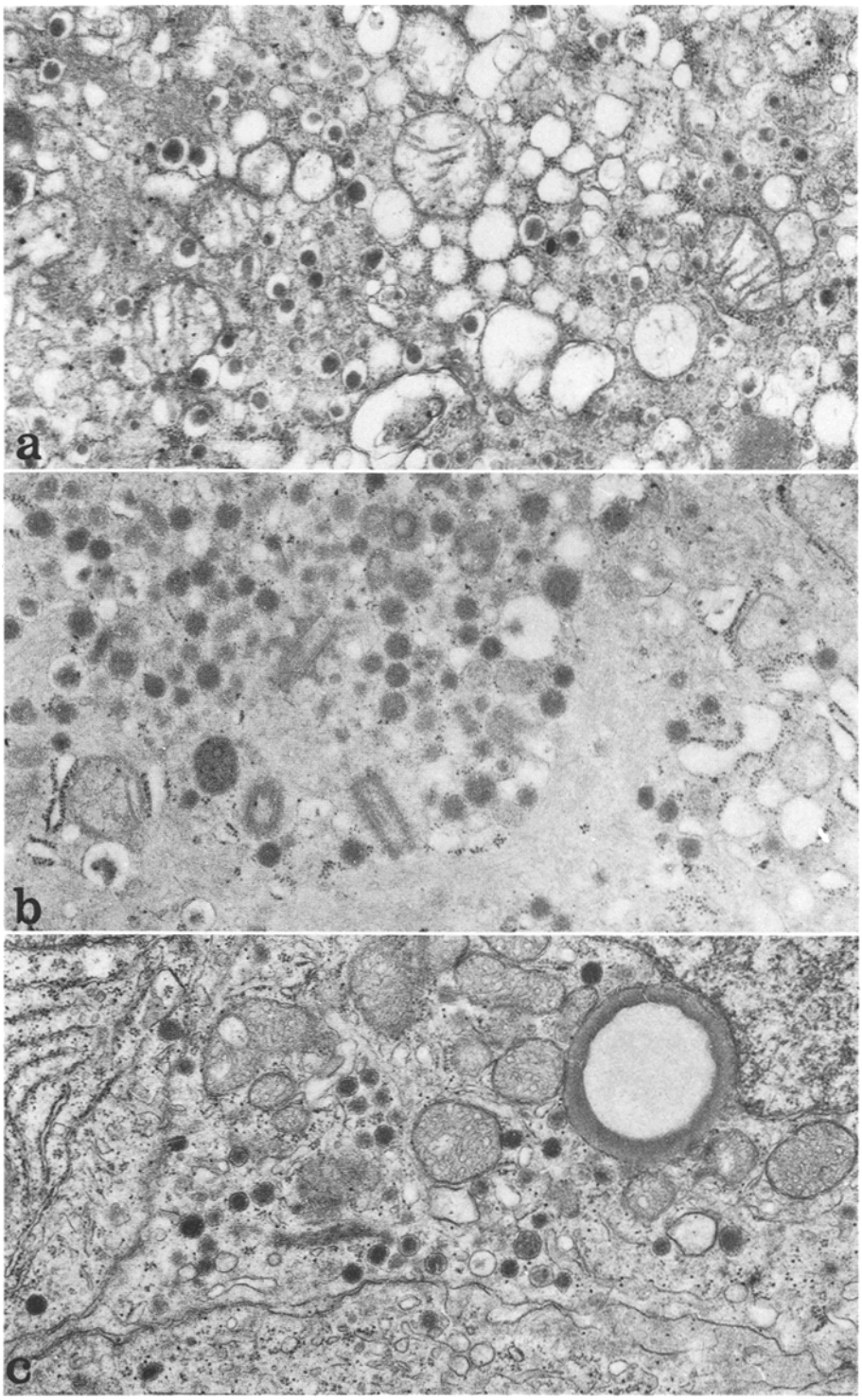
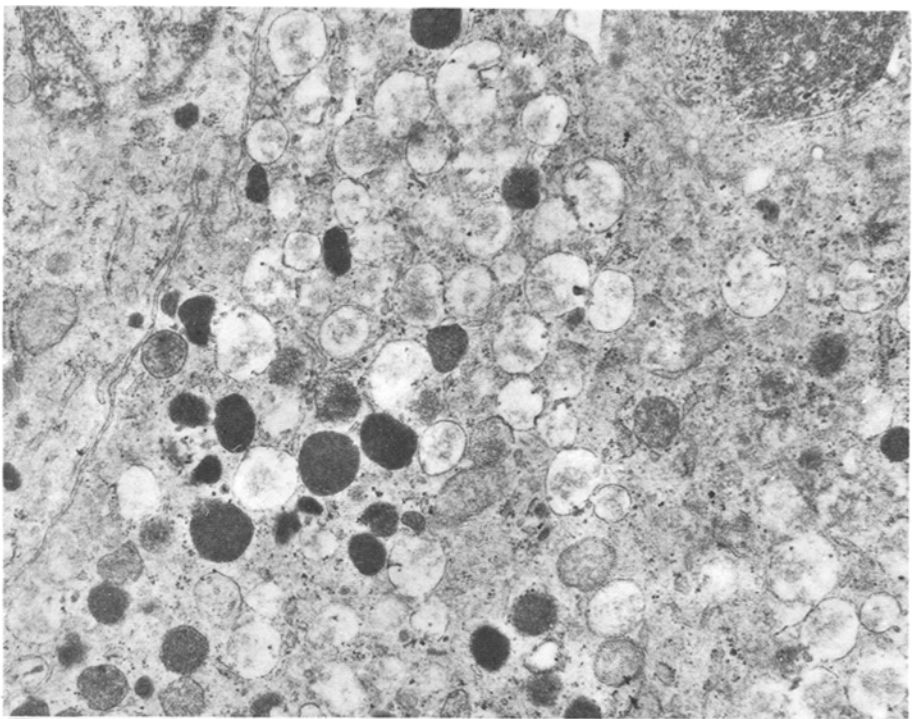


Fig. 8a–c. Different patterns of poorly diagnostic B cell granules in insulinomas (**a** case 3; **b** case 6; **c** case 5). ×18,000



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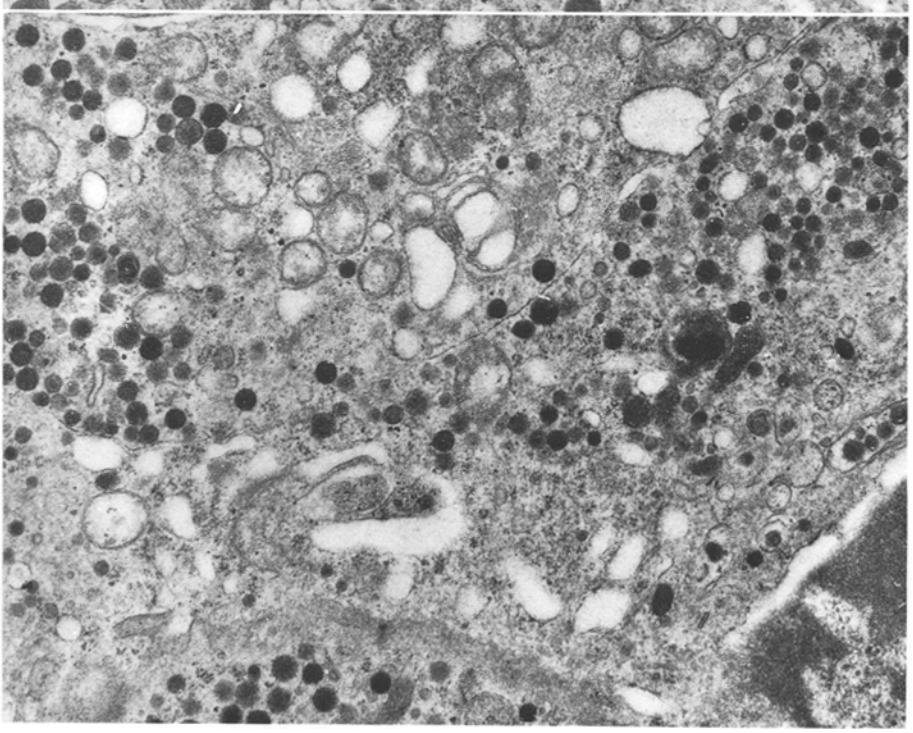

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Fig. 9. Highly diagnostic G cell granules in gastrinoma case 2. × 23,000

Fig. 10. Round small granules in gastrinoma case 3: poorly diagnostic G cell granules or D_1 cell granules? × 18,000

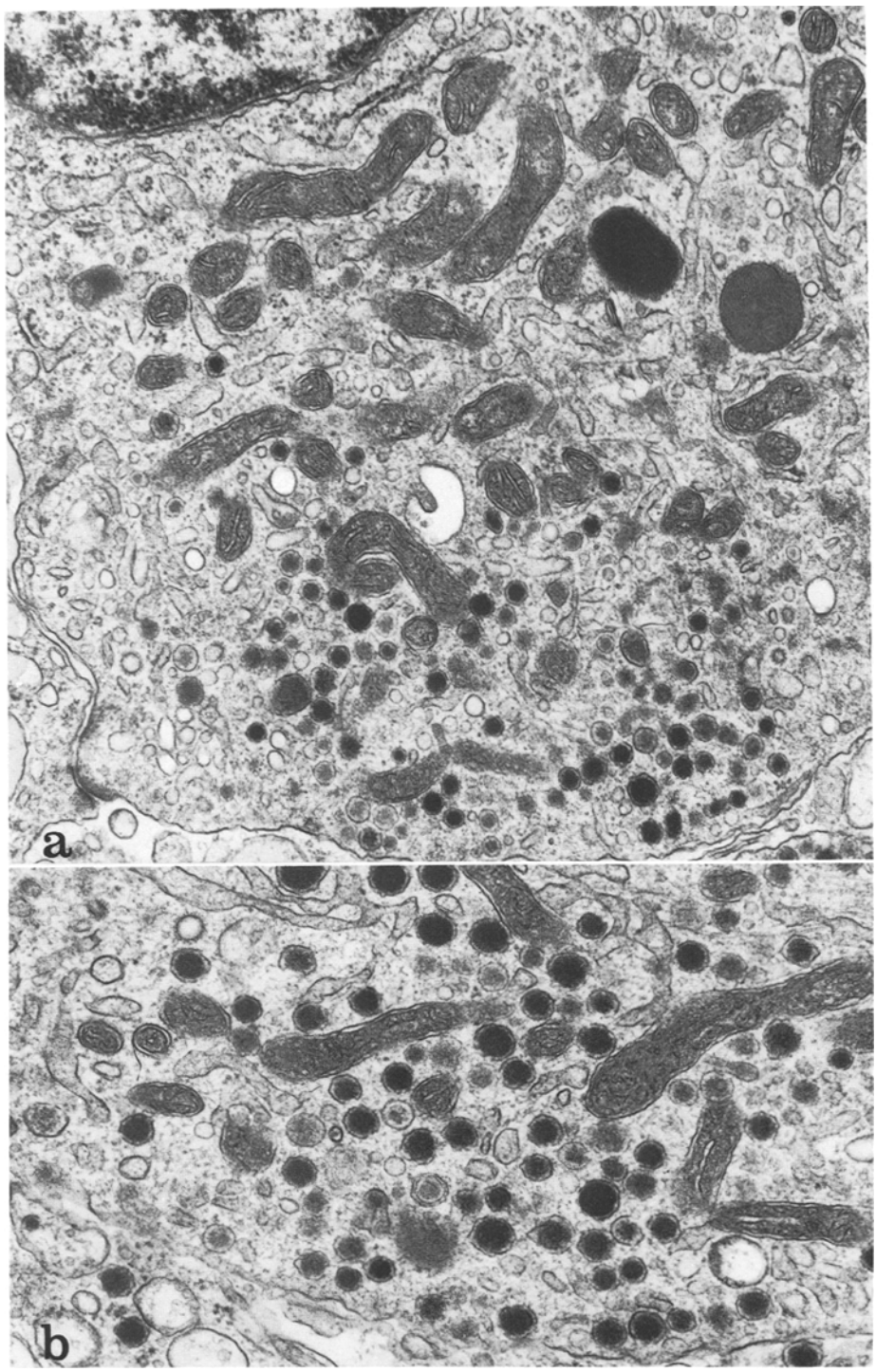

Fig. 11 a and b. P cells in gastrinoma case 6. Note the evident halo of cored granules and some small clear vesicles. **a** ×28,000; **b** ×36,000

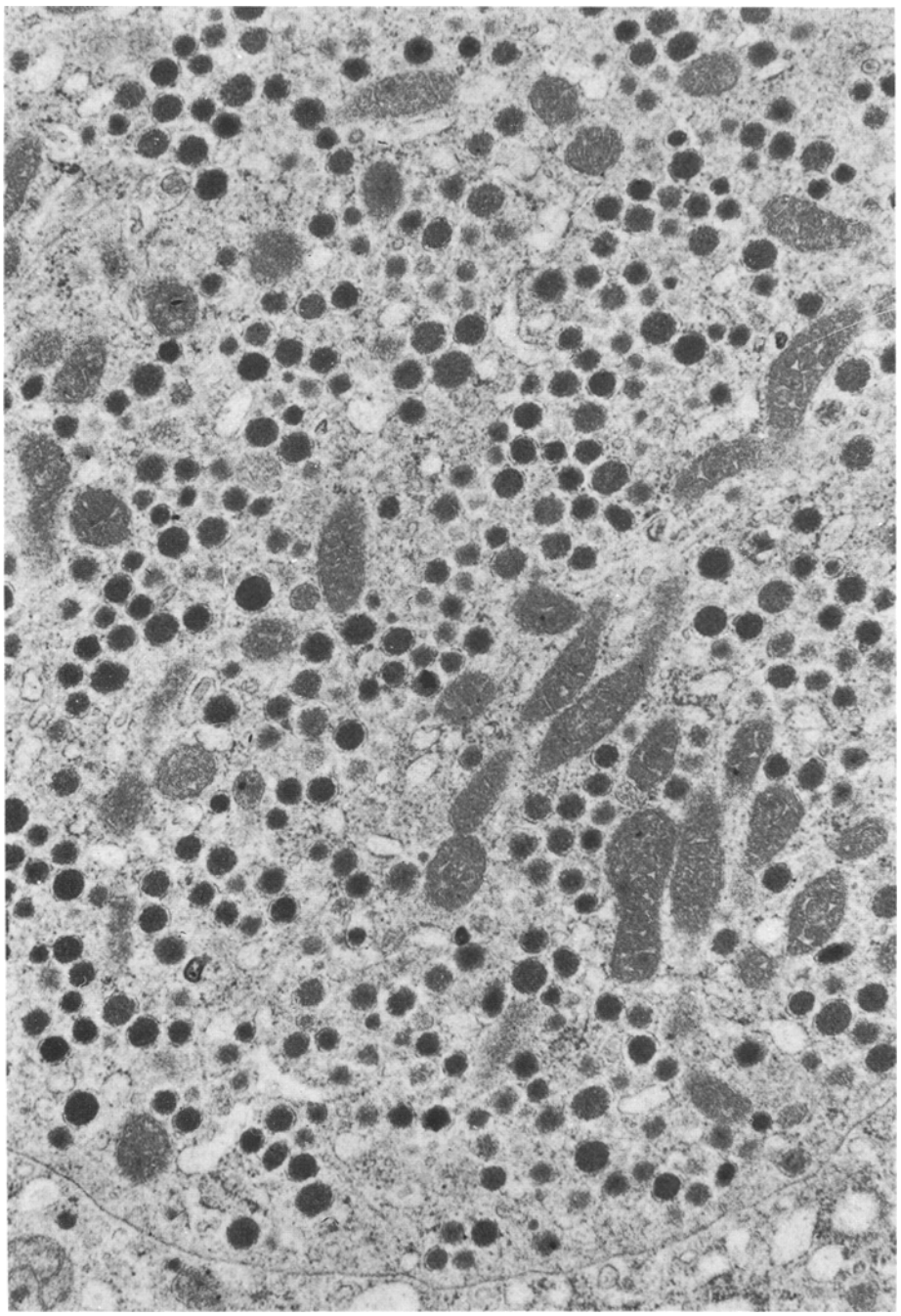

Fig. 12. D_1 cell in diarrheogenic tumour (vipoma) case 1. Compare with non-tumour D_1 cell of Fig. 1. ×28,000

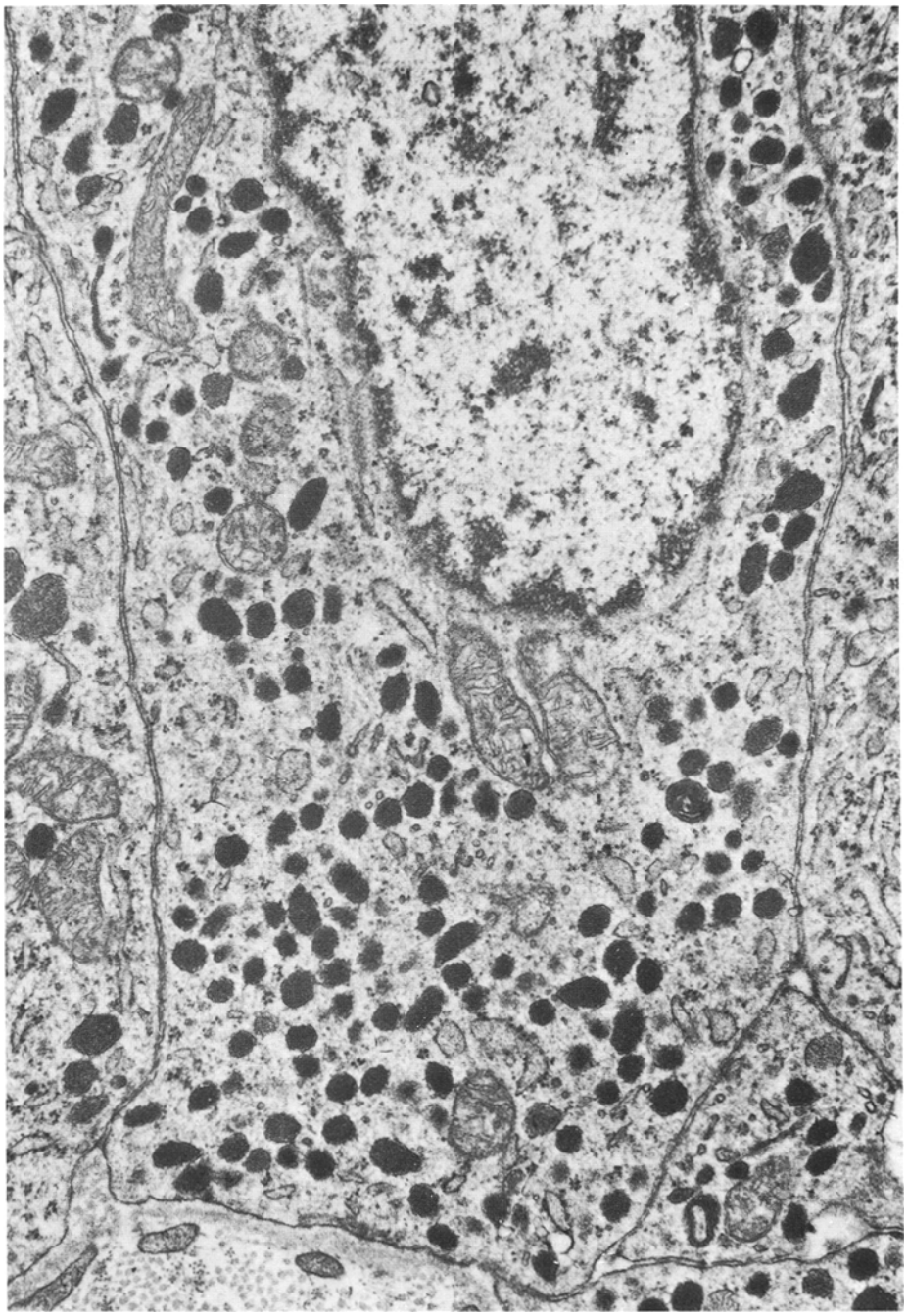

Fig. 13. F cells in diarrheogenic tumour case 5. Compare with non-tumour F cell of Fig. 4. ×28,000

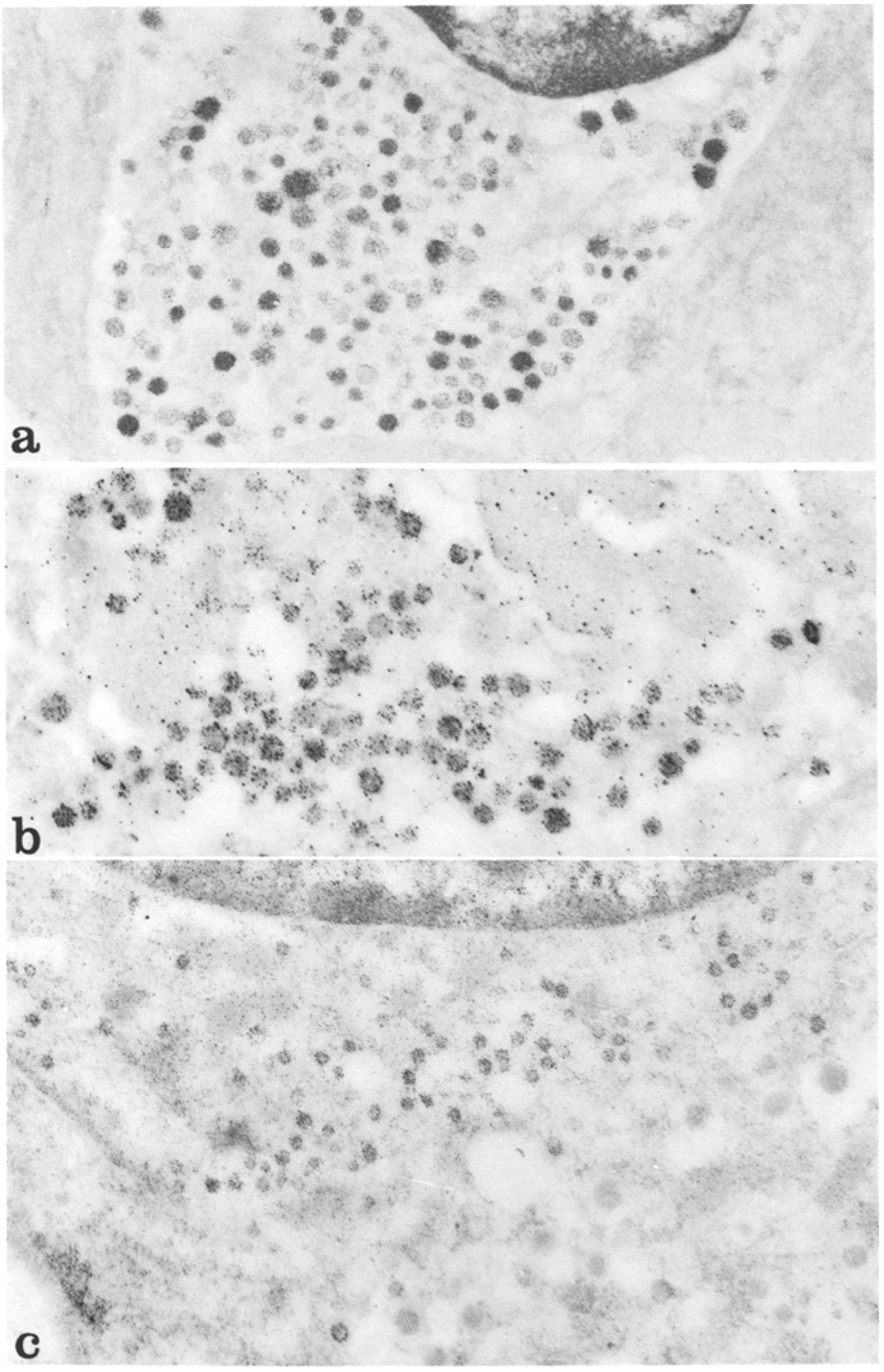

Fig. 14a–c. Aldehyde-fixed specimens stained with Sevier-Munger's (a, c) or Grimelius' silver (b). ×21,000. **a** reactive granules in a D_1 cell of the guinea-pig pancreas. **b** reactive granules of a D_1 cell in insulinoma case 3. **c** reactive halo of granules in a P cell (above) and unreactive poorly diagnostic granules in a B cell (below) of insulinoma case 3

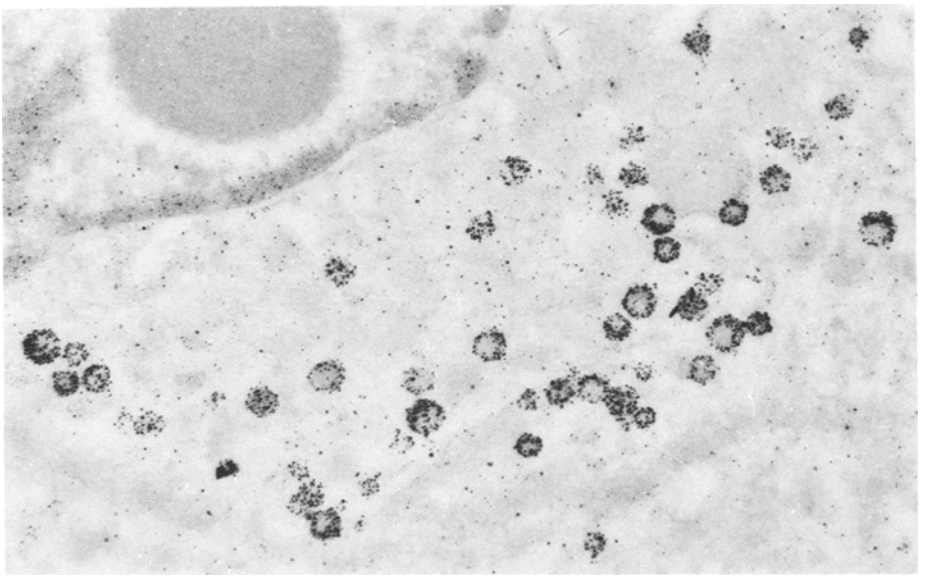

Fig. 15. Granules with heavily argyrophil halo in an A cell of gastrinoma case 4. Grimelius' silver. ×21,000

and showed a less prominent peripheral halo (more evident in some cells with Grimelius' silver (Fig. 15)). They resembled the A cell granules found in some glucagonomas (McGavran et al., 1966; Munger, 1972; Grimelius et al., 1971) as well as granules of some A cells in the human foetal pancreas. Although occasional tumour cells resembled normal D cells more or less closely, these have never been detected unequivocally in insulinomas or gastrinomas.

Cells somewhat resembling D_1 cells were found rather frequently in gastrinomas, less frequently in insulinomas. Cells with ultrastructural features fully diagnostic for D_1 cells were found more rarely in the same tumours. Grimelius' silver, which was helpful in distinguishing D_1 cells from B cells with poorly diagnostic or non-diagnostic granules, was of little help in distinguishing D_1 cells from poorly diagnostic G cells, both cells being reactive.

Few F cells have been found in several insulinomas and gastrinomas. Cells reproducing the ultrastructure of P cells were fairly numerous in a gastrinoma – also showing more or less diagnostic G cells and agranular cells – and scanty in an insulinoma (Fig. 11). Some tumour P cells showed thin dendritic-like processes with plenty of microtubules and cored granules, sometimes clear vesicles of about 500 Å in size were seen. A few EC cells with heavily argyrophil and argentaffin granules were found in one insulinoma (Fig. 16).

Results dealing with diarrheogenic tumours will be reported in details elsewhere (Capella et al., 1977b). The proportion of agranular, poorly granular and granular non-diagnostic cells was at least as high as in gastrinomas. The majority of granular cells resembled rather closely D_1 cells in 4 out of 7 WDHA tumours

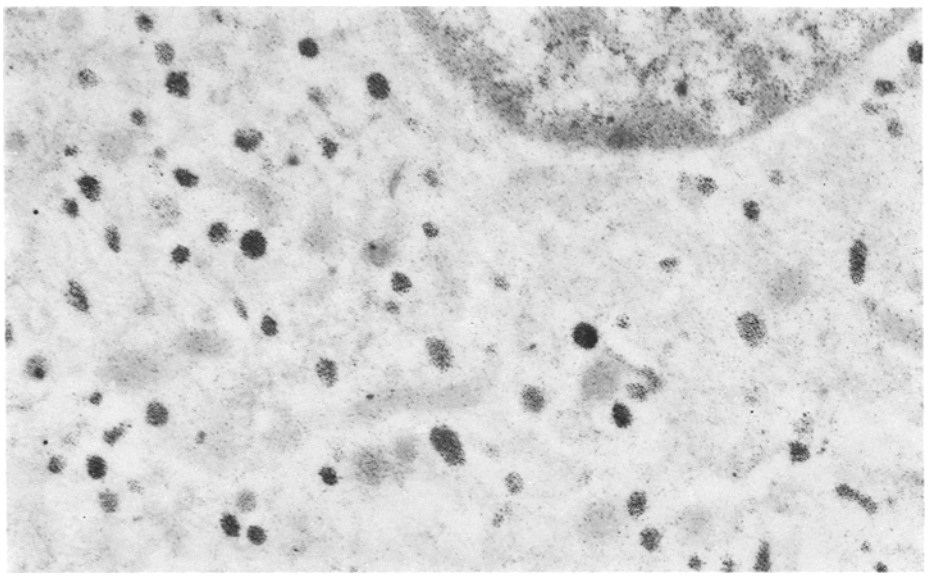

Fig. 16. Argentaffin granules in an EC cell of insulinoma case 3. Masson's reaction. ×21,000

(Fig. 12 and Table 1); a minority of granular cells resembled D_1 cells in two additional cases. In one case, the majority of tumour cells closely resembled F cells (Fig. 13); some F cells were found in another WDHA tumour. Apart from D_1 and F cells, only rare D cells in one case and rare P cells in another case were identified.

Some tumours, including gastrinoma case 7 and diarrheogenic tumour case 7, were mostly made up of agranular and poorly granular non-diagnostic cells. Although functionally these tumours were quite active and easily classified, ultrastructurally they were unclassifiable.

Discussion

Up to seven endocrine cell types can be identified ultrastructurally in the pancreas. Of these, A, B and D cells are well known; they produce glucagon, insulin and somatostatin respectively, and account for most of the cells occurring in the islets. EC cells with argentaffin, pleomorphic, highly osmiophilic granules are very scarce in the human pancreas although well represented in the cat and guinea-pig pancreas; most of them are scattered in the epithelium lining the ducts or in the acinar tissue. D_1 cells showing small, round granules are fairly represented in the islets and exocrine tissue. They probably account for most of the "C cells" described by Thomas (1937: see Fig. 1) in the guinea-pig; evidence for their possible involvement in the production of VIP or a VIP-like peptide has been recently offered on immunohistochemical grounds (Buffa et al.,

1976a, b). Cells with very small haloed granules resembling bronchial P cells (Capella et al., 1976) occur only seldom in the pancreas. In most species the majority of endocrine cells scattered in the exocrine tissue show ultrastructural patterns of "E cells", first identified in the opossum by Thomas (1937) and described ultrastructurally by Munger et al. (1965), or of "F cells", first reported by Bencosme and Liepa (1955) under the name of "X cells" and described ultrastructurally by Munger et al. (1965). Both ultrastructural patterns seem to belong to a single cell type – which we call F cell – likely corresponding to pancreatic peptide (PP) immunoreactive cells (Larsson et al., 1976b; Baetens et al. 1976a; Buffa et al., 1976b). These cells are to be distinguished from X cells described in the gastric mucosa (Capella et al., 1969; Solcia et al., 1975).

Most of the above endocrine cell types also occur outside the pancreas, as shown in Table 3, which has been drafted according to the most recent investigations (Hage, 1973a; Hakanson et al., 1973; Casanova et al., 1974; Buffa et al., 1975 and 1976a, b; Polak et al., 1975; Rufener et al., 1975; Solcia et al., 1975; Baetens et al., 1976b; Grimelius et al., 1976; Larsson et al., 1976b; Orci et al., 1976; Capella et al., 1977a; personal unpublished data). In fact, only B cells (and A cells in man) appear to be exclusive to the pancreatic, islets. Hence, the name islet cells seems appropriate for these cells, and related tumours should be called *islet cell tumours*. Some cells, as P, EC, X, F and D_1 cells

Table 3. Endocrine cells of the pancreas, gut and diffuse endocrine system

Lung Trachea Larynx	Thymus	Pancreas	Stomach		Intestine		Urethra prostate	Function proposed or ascertained
			Fundus	Antrum	Small	Large		
P	(P)	(P)	P	P	P	?	P	Some neurosecretory product?
(EC)	EC	EC	EC	EC	EC	EC	EC	5-hydroxytryptamine (5HT) + peptides
(X)	(X)	?	X	X	X	X		unknown peptide
		F	F	F	F	F		pancreatic peptide (PP)
		D_1	D_1	D_1	D_1	(D_1)		vasoactive intestinal peptide (VIP)?
		D	D	D	D			somatostatin
		B						insulin
		A	A		(A)			glucagon
			ECL					histamine[a] + unknown peptide
				G	G			gastrin
					S			secretin
					I			cholecystokinin (CCK)
					K			gastric inhibitory peptide (GIP)
					N			neurotensin?
					L	L		glucagon-like immunoreactivity (GLI-1)

( ) further studies required
[a] only in murines

are widely distributed in endodermal epithelia; for these cells Feyrter's concept of a "diffuse endocrine system" possibly with "paracrine" function (Feyrter, 1953) seems pertinent. Most tumours now known to involve these cells have been called *carcinoids* in the literature. D cells and related tumours should also be considered in this group; however, as far as the pancreas is concerned, D cells are mostly grouped in the islets, where they represent a regular component in all the species so far investigated. In the pancreas therefore, D cell tumours are perhaps better classified among islet cell tumours.

There remains a group of cells, including ECL, G, S, I, K, N and L cells, which are scattered in more or less restricted areas of gastrointestinal mucosa, where they display some peculiar endocrine and/or paracrine function. These cells and related tumours are somewhat in between those of the diffuse endocrine system and those of organised endocrine structures like the islets. They should be called gastric or intestinal endocrine-like cells; for brevity, *gastrointestinal endocrine* cells or *tumours*.

From these results and concepts, endocrine tumours of the pancreas can be classified as follows:

Islet Cell Tumours

Adenomas:
A cell type (glucagonoma)
B cell type (insulinoma)

Carcinomas:
A cell type (malignant glucagonoma)
B cell type (malignant insulinoma)

Carcinoids

EC cell tumour (argentaffinoma)
D_1 cell tumour (vipoma)? } WDHA syndrome
F cell tumour (PP-oma)? }

Gut-Related Endocrine Tumours

G cell tumour (gastrinoma)

Non Diagnostic and Poorly Differentiated Endocrine Tumours

(various endocrine syndromes)

The majority of islet cell tumours are well differentiated benign tumours mimicking more or less clearly the microlobular, ribbon-like and gyriform arrangement of normal islet cells (Porter and Frantz, 1956; Sieracki et al., 1960; Greider et al., 1974). Many A cell adenomas are asymptomatic or poorly symptomatic tumours discovered only at autopsy (Grimelius et al., 1975); however, clinically active tumours giving the peculiar "glucagonoma syndrome" are often malignant (Mallison et al., 1974; Recant et al., 1976). Malignant tumours ac-

count for only 10 to 20% of B cell tumours (Sieracki et al., 1960; Marks and Rose, 1965). A D cell component has been reported in several A cell tumours (Grimelius et al., 1975; Orci, 1976; Creutzfeldt, 1976). No pure D cell tumour has been reported so far.

In the carcinoid group malignant tumours are certainly more frequent (about 50% – Burkhardt and Mitschke, 1974; Martin et al., 1974) than in the islet cell group; however, prediction of their benign or malignant behaviour is quite difficult using present histologic criteria. Tumour cells are often arranged in solid nests and cords or show basaloid, trabecular and pseudoglandular structures. Proved argentaffin EC cell carcinoids producing 5HT are very rare in the pancreas (Patchefsky et al., 1974), although EC cells have been observed occasionally in B and G cell tumours. In our experience, P cells are better represented in pancreatic tumours than in normal pancreas. No significant correlation could be established between these cells and any functional syndrome. Similar cells have been found in lung carcinoids (Bensch et al., 1965; Hage, 1973b; personal unpublished findings) as well as in bronchogenic carcinomas, several of which have been shown to produce ACTH, ADH or CRF peptides (Upton and Amatruda, 1971; George et al., 1972; Hattori et al., 1972).

D_1 cells occur frequently in pancreatic tumours, including B cell tumours (Creutzfeldt et al., 1973) and G cell tumours (Vassallo et al., 1972; Creutzfeldt et al., 1975), where they may be difficult to distinguish from poorly diagnostic B or G cells. As reported above, we found D_1 cells in most WDHA tumours; in some cases D_1 cells accounted for the majority of tumour cells. The presence in WDHA tumours of cells with small granules probably corresponding to our D_1 cells, has also been noted by Greider et al. (1974) Creutzfeldt (1975) and Rambaud et al. (1975). Considering that many WDHA tumours have been shown to produce VIP (Bloom et al., 1973; Said and Faloona, 1975) and that D_1 cells have been recently suggested to produce VIP (Buffa et al., 1976a, b) it seems that the association of D_1 cells with WDHA tumours is functionally significant and more relevant than their association with insulinomas or gastrinomas. PP has been recently suggested to account for the diarrheogenic syndrome of some WDHA tumours (Schwartz et al., 1976). Our finding of predominant F cells – probably corresponding to PP producing cells – in two of such tumours support this suggestion.

Although the presence of gastrin – together with somatostatin – in the D cells of human islets has been recently confirmed (Erlandsen et al., 1976), the absence of pyloric-type gastrin (G) cells from normal adult pancreas is now agreed by most authors (Creutzfeldt et al., 1971; Lotstra et al., 1974; Solcia et al., 1975) and their presence in foetal pancreas is still disputed (Like and Orci, 1972; van Assche et al., 1976; Braaten et al., 1976; Larsson et al., 1976a). In pancreatic gastrinomas, somatostatin-storing cells or ultrastructurally identified D cells seem to occur only very seldom, if any, while more or less diagnostic G cells are frequently found. Certainly, gastrinomas quite often arise in the human pancreas, more often than in the pyloric or duodenal mucosa where G cells normally occur (Creutzfeldt et al., 1975; Solcia et al., 1975). However, gastrin production by tumours outside the pancreas and gastrointestinal mucosa is uncommon. They are exceptional in the lung, where the occurrence of ectopic

hormone producing tumours is more frequently reported. Thus, gastrinomas of the pancreas are perhaps to be considered as a very special kind of "ectopic" tumour, whose frequent occurrence might be explained on the basis of the well known common embryogenesis of the pancreas and the G cell area of gastroduodenal mucosa. The high rate of malignant (more than 60% – Ellison and Wilson, 1964) and poorly characterised tumours might well be related to their origin from immature endocrine cells undergoing inappropriate differentiation during neoplastic growth. As with carcinoids, attempts to discriminate benign from malignant gastrinomas are usually unsuccessful. It seems preferable to consider all gastrin producing tumours of the pancreas as potentially malignant.

Tumours entirely made up of agranular or poorly granular non-diagnostic cells should be grouped apart, as unclassifiable endocrine tumours. Immunohistochemistry may still be able to identify the hormone(s) produced by such tumours. They may develop severe functional syndromes, including multihormonal syndromes; these agranular or poorly granular active tumours, usually showing well developed organelles are to be considered as endocrine tumours with defective hormone storage mechanism rather than as stem cell tumours. The 2 tumours we classified in this group proved to be malignant; however, their histology was still distinctive in respect to undifferentiated pancreatic carcinomas.

In agreement with other investigators (Bordi and Bussolati, 1974; Creutzfeldt et al., 1975; Larsson et al. 1975) we found multiple cell types quite frequently in pancreatic endocrine tumours. In these cases, one population of cells often predominates, hence providing a reasonable base for tumour classification. The prevailing endocrine cell in parallel immunohistochemical tests or the clinically dominant endocrine syndrome may also be of help when two or more cell types are equally represented. Otherwise, more general diagnostic terms as carcinoid, islet cell tumour, or just pancreatic endocrine tumour should be used, with subsequent specification of cell types occurring in it.

The functional classification of pancreatic endocrine tumours, mostly based on clinical syndromes and hormone assays, and morphologic classifications, mostly based on ultrastructure and histochemistry, outline the clinical and pathological approach to the study of such tumours. Both approaches seem valid and both of them are recommended. However, when used alone, they describe only one aspect of tumour disease. The functional classification seems more convenient for poorly diagnostic, highly active tumours, while the morphologic classification seems more useful for well differentiated, silent tumours. In any case morphologic criteria must be used to gain more information on prognosis and to discriminate between neural and epithelial "vipomas", and among the latter, between pancreatic (D_1 cell tumours?) and non-pancreatic tumours (phoecromocytomas, bronchial carcinoids and carcinomas, etc.).

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