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Pancreatic Polypeptide – A Postulated New Hormone: Identification of Its Cellular Storage Site by Light and Electron Microscopic Immunocytochemistry*

L.-I. Larsson, F. Sundler and R. Håkanson

Departments of Histology and Pharmacology, University of Lund, Lund, Sweden

Summary. A peptide, referred to as pancreatic polypeptide (PP), has recently been isolated from the pancreas of chicken and of several mammals. PP is thought to be a pancreatic hormone. By the use of specific antisera we have demonstrated PP immunoreactivity in the pancreas of a number of mammals. The immunoreactivity was localized to a population of endocrine cells, distinct from the A, B and D cells. In most species the PP cells occurred in islets as well as in exocrine parenchyma; they often predominated in the pancreatic portion adjacent to the duodenum. In opossum and dog, PP cells were found also in the gastric mucosa. In opossum, the PP cells displayed formaldehyde- induced fluorescence typical of dopamine, whereas no formaldehyde-induced fluorescence was detected in the PP cells of mouse, rat and guinea-pig. Also in these latter species, however, PP cells appear to possess amine-handling properties, a feature common to many peptide hormone-producing cells. The ultrastructure of the PP cells was defined by combining immunohistochemistry of semi-thin plastic sections with electron microscopy of adjacent ultrathin sections. PP cells show the ultrastructural features of peptide hormone-secreting cells. The PP cells of cat and dog contain fairly large, rather electron-lucent granules, and are probably identical with the previously described F cells. The PP cells of rat, guinea-pig, chinchilla and man contain small, fairly electron-dense granules. In these latter species no F cells are found. By immunoperoxidase staining of ultrathin sections, the PP immunoreactivity was found to be localized to the cytoplasmic granules. These observations provide support for the view that PP is a true pancreatic hormone.

Key words: Pancreatic hormones, "pancreatic polypeptide", islet cells, gastrointestinal hormones, immunocytochemistry, fluorescence histochemistry.

While purifying chicken insulin Kimmel and coworkers detected a straight chain peptide of 36 amino acids which they named avian pancreatic polypeptide (APP) [1, 2]. By radioimmunoassay APP was detected in pancreatic extracts from a number of birds and reptiles, and was found to circulate in plasma where its level varied with the prandial state [3]. From mammalian pancreas Chance and colleagues isolated peptides that were very similar to APP [see 4]. The bovine pancreatic polypeptide (BPP) was found to have 16 of its 36 positions identical with APP. Only little information is available concerning the physiological role of the pancreatic polypeptides. APP has been found to stimulate gastric acid secretion and hepatic glycogenolysis in chickens [5]. The peptide did not raise the blood sugar level but lowered plasma glycerol, which probably indicates stimulated hepatic lipid synthesis [5]. Among the effects of BPP were inhibition of pentagastrin-stimulated gastric acid secretion and relaxation of the gall-bladder [4]. The alleged hormonal nature of these polypeptides [4, 5] is supported by immunohistochemical studies in which APP and human pancreatic polypeptide (HPP) were found to be stored in pancreatic endocrine-like cells distinct from the A, B and D cells [6, 7]. The APP cells were found to share many features with well-known peptide hormone-producing cells such as numerous cytoplasmic granules and the ability to take up and decarboxylate administered amine precursors [6].

In the present study we employed immunocytochemistry at the light and electron microscopic

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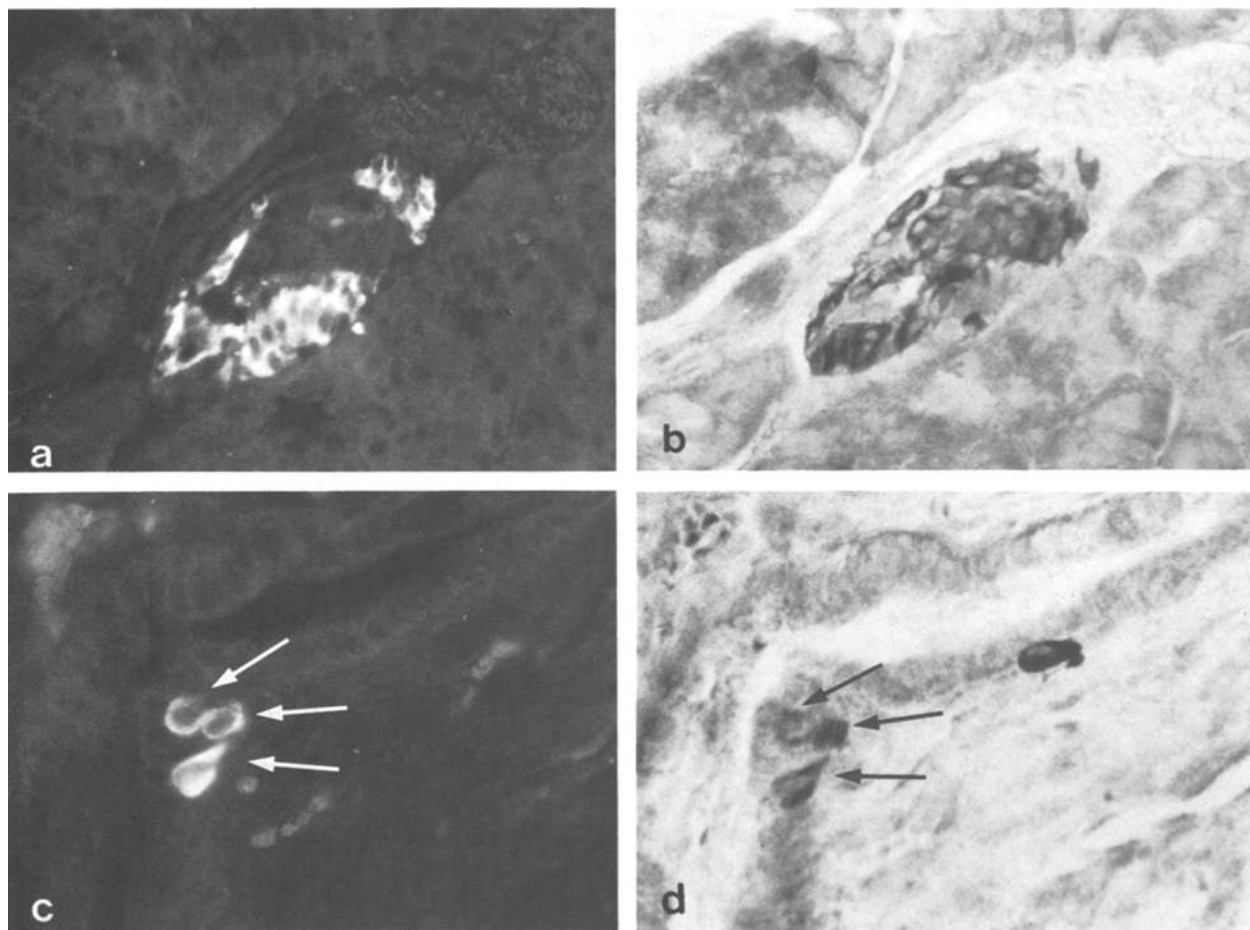

Fig. 1. Mouse pancreas, duodenal lobe. **a)** Immunofluorescent PP cells at the periphery of an islet. (HPP antiserum, diluted 1:20). **b)** Restaining with silver (Grimelius' technique) shows the argyrophilia of the PP cells not to exceed the background staining. Numerous glucagon cells are demonstrated by their strong argyrophilia. (× 300) **c)** Immunofluorescent PP cells in ductal epithelium (HPP antiserum, diluted 1:20). **d)** Restaining with silver (Grimelius' technique) shows the weak argyrophilia of the PP cells to contrast with the nearly unstained columnar cells. Note one single strongly argyrophil cell (unidentified). (× 400)

level to identify the cells that store the pancreatic polypeptide.

Materials and Methods

Antisera

Antisera against the human and bovine pancreatic polypeptides were generously supplied by Drs. R. E. Chance and Nancy Moon at Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Ind., U.S.A. The antisera were raised in rabbits, using both subcutaneous and intramuscular injections at multiple sites of highly purified HPP or BPP homogenized in complete Freund's adjuvant (1 mg HPP or BPP per rabbit). Subsequent injections were given with the antigen homogenized in incomplete Freund's adjuvant (O,5 mg HPP or BPP per rabbit). The HPP antiserum is used for radioimmunoassay at a working dilution of 1:75000. As tested by radioimmunoassay neither the HPP nor the BPP antiserum crossreacts with insulin, glucagon, gastrin I and II, secretin, cholecystokinin or APP. A glucagon antiserum (No. 4304), characterized in detail elsewhere [8], was kindly provided by Dr. Jens Holst, Department of Clinical Chemistry, Bispebjerg hospital, Copenhagen, Denmark. Fluorescein isothiocyanate-labelled and unlabelled sheep antiserum against rabbit IgG was obtained from Statens Bakteriologiska Laboratorium (Stockholm, Sweden). Peroxidase-antiperoxidase (PAP) complex was purchased from Cappel Laboratories, Downington, Pa., U.S.A.

Tissue Materials

Optical Microscopy. Adult mice, rats, hamsters, guinea-pigs, rabbits, opossums, chinchillas, cats and

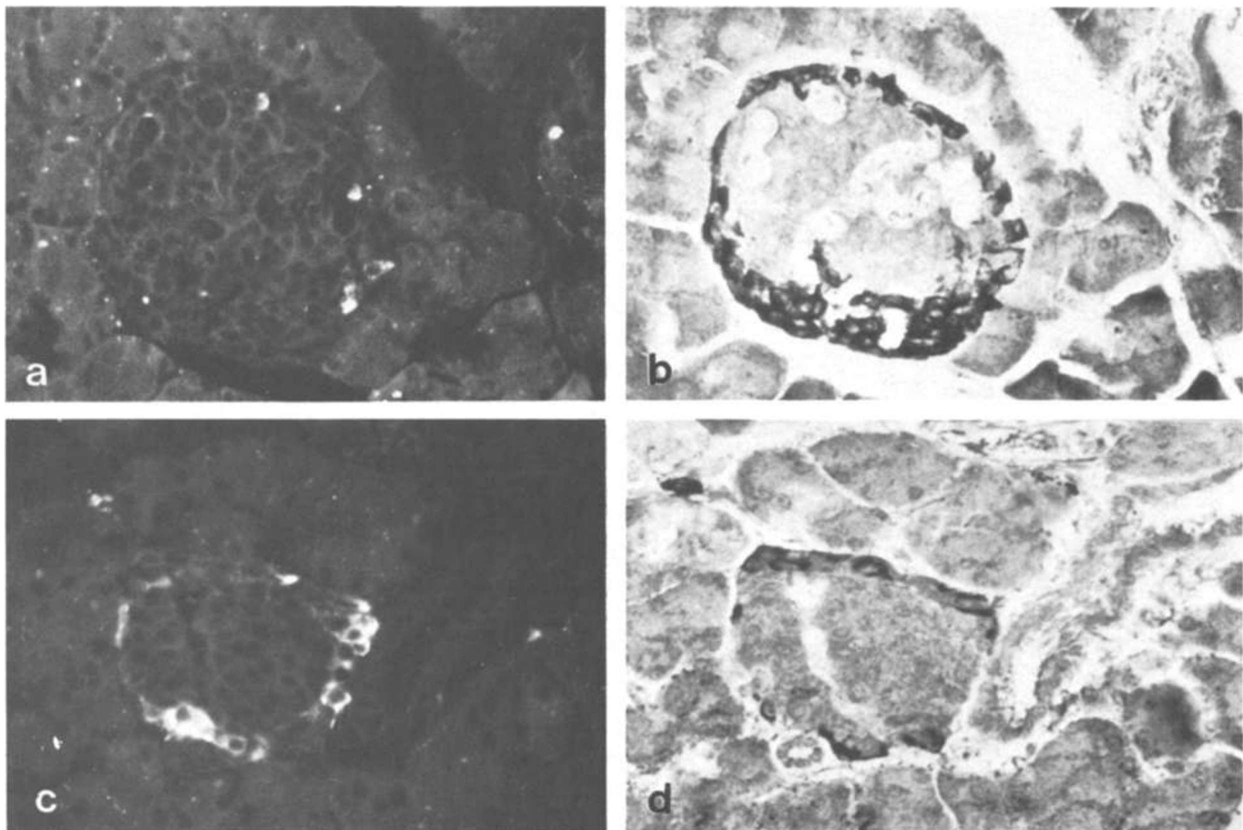

Fig. 2. Hamster pancreas. **a)** Pancreatic tail. A few immunofluorescent PP cells are seen at the islet periphery (HPP antiserum, diluted 1 : 20). **b)** Restaining with silver (Grimelius' technique). PP cells show no or very weak argyrophilia. **c)** Duodenal lobe. Islet containing fairly numerous PP cells. **d)** Restaining with silver as in b. (× 200)

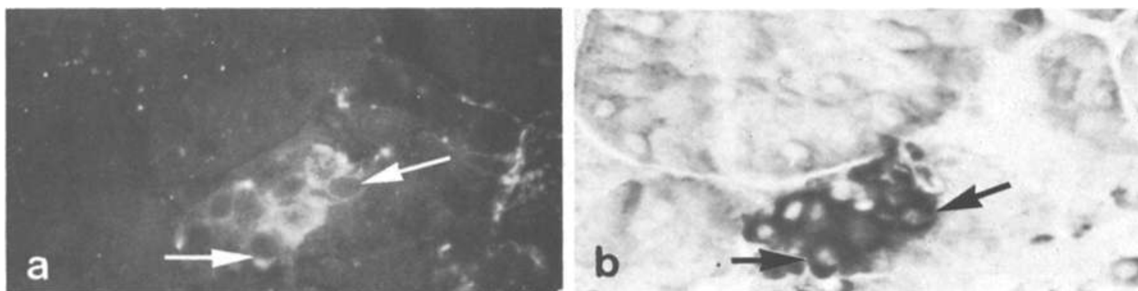

Fig. 3. Opossum, pancreatic body. **a)** Section from freeze-dried, formaldehyde gas-treated specimen. A cluster of islet cells display formaldehyde-induced fluorescence characteristic of dopamine. **b)** Staining the same section with HPP antiserum (diluted 1:80, PAP method) shows the fluorescent cells to be PP cells (× 450). Arrows inserted for orientation

dogs of both sexes were killed by exsanguination under diethyl ether anaesthesia (mice, rats, hamsters, guinea-pigs and opossums), by intravenous injection of air (rabbits), or by intraperitoneal injection of Nembutal® or urethane (chinchillas, cats and dogs). Pancreas of sheep, horse and cow was obtained from a local slaughterhouse. Human pancreas was obtained at surgery for gastric carcinoma or peptic ulcer (10 patients; courtesy of Dr. G. Liedberg, Department of Surgery, University of Lund, Lund, Sweden).

Tissue specimens were taken from the body and tail of the pancreas. Whenever possible we included specimens from pancreatic tissue adherent to the descending duodenum. In dogs this portion is prominent and has been referred to as the uncinate process or the duodenal lobe [9]. The latter term will be used subse-

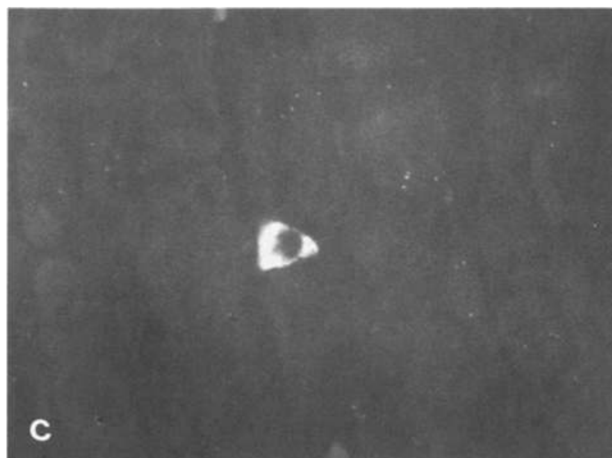

Fig. 4. Opossum stomach, oxyntic gland area. Single immunofluorescent PP cell in the glandular epithelium. (HPP antiserum, diluted 1:20) (× 600)

Table 1. Distribution of PP cells in the pancreas and gastrointestinal tract of different mammals

		Pancreas		Gastro-intestinal tract
		Extra insular location	Insular location	
Mouse	Duodenal lobe	(+)	+ +	–
	Tail	(+)	+	
Rat	Duodenal lobe	–	+	–
	Tail	–	+	
Hamster	Duodenal lobe	+	+ +	n. t.
	Tail	(+)	(+)	
Guinea-pig		+ +	+ +	–
Chinchilla	Duodenal lobe	+ +	+	n. t.
	Tail	+ +	+	
Rabbit	Duodenal lobe	+ + +	(+)	–
	Tail	(+)	(+)	
Opossum	Duodenal lobe	+	+ +	+ (stomach)
	Tail	+	(+)	
Cat	Duodenal lobe	+ + +	(+)	n. t.
	Tail	+ +	+	
Dog	Duodenal lobe	+ + +	(+)	+ (stomach)
	Tail	+	+	
Sheep		(+)	+ + +	n. t.
Cow		(+)	+ +	n. t.
Horse		+ +	+ +	n. t.
Man		(+) to +	+	n. t.

–, absent or only occasionally seen; (+), rare, but regularly seen; +, moderate number; + +, fairly numerous; + + +, abundant; n. t., not tested.

quently. Specimens were also taken from the stomach wall (along the major curvature) and from the small and large intestines of mice, rats, guinea-pigs, rabbits, opossums and dogs.

In one experiment 3 guinea-pigs were injected with L-3,4-dihydroxyphenylalanine (L-dopa) (100 mg/kg ip). They were sacrificed 2–3 h later.

Some specimens were fixed in 10% neutral formalin or in Bouin's fluid for 3–24 h, dehydrated in graded ethanol solutions and embedded in paraffin. Other specimens were frozen to the temperature of liquid nitrogen in a propane-propylene mixture and freeze-dried. They were then exposed to formaldehyde gas at 80° C for 1 h [10] and embedded in paraffin or Araldite. Paraffin sections (5 μ) were deparaffinized in xylene whereas Araldite sections (1 μ) were deplasticized according to Mayor, Hampton and Rosario [11].

Electron Microscopy. Rats, guinea-pigs, chinchillas and cats were perfused for 5 to 10 min via the ascending aorta with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, or with a mixture of 4% formaldehyde and 0.5% glutaraldehyde in 0.1 M sodium phosphate buffer pH 7.4, under diethyl ether or urethane anaesthesia. Small pieces of tissue from the tail of the pancreas (rats, guinea-pigs and chinchillas) or from the duodenal lobe (cats) were rapidly dissected out and immersed in the fixation fluid for 1 to 4 h.

Specimens from human and canine pancreas were fixed by immersion in glutaraldehyde or in the formaldehyde-glutaraldehyde mixture for 1 to 4 h.

Some specimens were post-fixed in 1% OsO_4 for 2 h, dehydrated in graded ethanol solutions, contrasted *en bloc* for ½ h in a mixture of 1% phosphotungstic acid and 0.5% uranyl acetate in ethanol and embedded in Araldite. Other specimens were processed as above, except that postfixation in OsO_4 was omitted.

Granule Morphometry. Material from pancreas of rat and man was subjected to quantitative electron microscopy. At least 10 islets of each species were examined and photographed. The photographs were reproduced in magnification 20000 × or 45000 ×. For each type of endocrine cell the granule size was established by measuring the diameter of all granules in 3–10 cells.

Immunocytochemistry

Immunofluorescence. Sections were carried down to water through graded ethanol solutions and exposed to antisera against HPP or BPP, usually in dilution 1:20 and 1:160, respectively. Other sections were exposed to the glucagon antiserum in dilution 1:20.

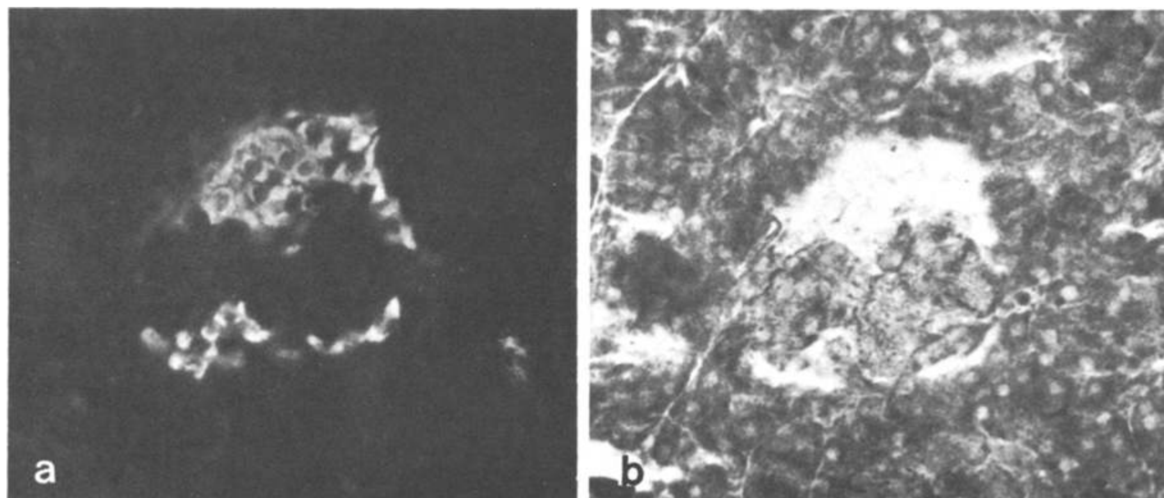

Fig. 5. Sheep pancreas. **a)** Immunofluorescent PP cells (BPP antiserum, diluted 1:160) occurring in clusters at the periphery of an islet. **b)** Restaining with silver (Hellerström-Hellman method) shows PP cells to be argyrophobic (i. e. less stained than the background). (× 200)

Following rinsing in phosphate buffered saline, pH 7.2 (PBS), the sections were exposed to fluorescein-labelled sheep anti-rabbit IgG (SBL, Sweden) in dilution 1:20. The sections were rinsed, mounted in phosphate-buffered glycerine (pH 7.2) and examined in a Leitz Orthoplan fluorescence microscope equipped with an epi-illumination system (filter setting No. 3; peak excitation at 490 nm). Controls were those recommended by Goldman [12] and included the application of antigen-inactivated antiserum (100 μg HPP or BPP per ml antiserum diluted 1:20).

EM Identification of Immunoreactive Cells. Sections (1 μ) from non-osmium fixed specimens were mounted on glass slides, deplasticized according to Mayor, Hampton and Rosario [11], postfixed for 2 h in Bouin and then processed for demonstration of HPP or BPP immunoreactivity (as described under "immunofluorescence"). The ultrastructural identity of the immunoreactive cells was established by examination in the electron microscope of the immediately adjacent ultrathin sections, contrasted *en grid* with lead citrate and uranyl acetate. The major difficulty with this type of identification is to combine acceptable ultramorphology with preserved immunoreactivity. For this purpose we tested glutaraldehyde, formaldehyde and mixtures thereof as fixatives (see "electron microscopy").

Peroxidase-Antiperoxidase (PAP) Immunocytochemistry. Deparaffinized sections or ultra-thin plastic sections, etched in 5% hydrogen peroxide, were stained according to the peroxidase-antiperoxidase method [see 13]. The sections were incubated sequentially with normal sheep serum (diluted 1:30), antiserum to HPP (diluted 1:80 (light microscopy) or 1:200 (electron microscopy) antibody against rabbit IgG raised in sheep (SBL, Sweden; diluted 1:30), and rabbit antiperoxidase bound to horseradish peroxidase, i. e. the peroxidase-antiperoxidase (PAP) complex, (diluted 1:80). All antisera were diluted with 0.05 M Tris buffer pH 7.6 (TBS) containing 0.25% human serum albumin (Behringwerke, FRG). Washing of the grids were carried out with TBS containing 1% serum albumin [13]. The sections were exposed to a solution of 3,3'-diaminobenzidine (Sigma, USA) and hydrogen peroxide in TBS [13]. In this solution the bound peroxidase catalyses the formation of a brown reaction product. Ultra-thin sections were post-treated with OsO_4 which renders the brown precipitate highly electron dense. For controls we used HPP antiserum inactivated by excess HPP (100 μg HPP per ml antiserum diluted 1:80).

Formaldehyde-Induced Fluorescence

Sections from freeze-dried, formaldehyde-treated specimens were mounted in Entellan (Merck) and examined in the fluorescence microscope (standard filter setting No. 2; peak excitation at 405 nm). We examined pancreatic specimens of untreated mice, rats, guinea-pigs, opossums and L-dopa-injected guinea-pigs in this way.

Examination of formaldehyde-induced fluorescence was followed by staining with HPP antiserum (PAP method). Two major difficulties arose with this type of correlation. First, the formaldehyde-induced fluorophores were easily diffusible in water and could thus not be observed in sections affixed to slides with conventional methods. However, with Szombathy's

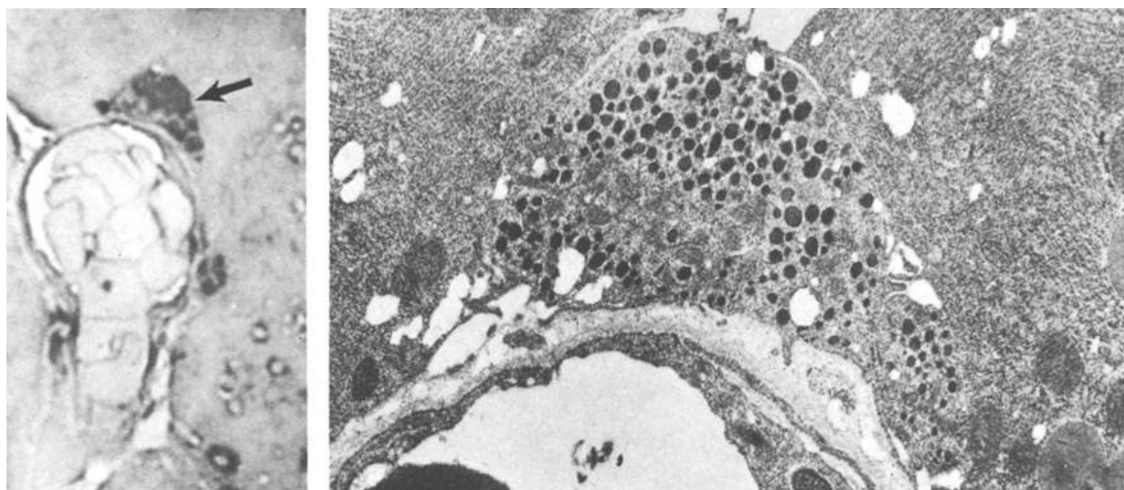

Fig. 6. Chinchilla pancreas. Formaldehyde-glutaraldehyde fixation. Left. Semithin section. Prominent PP cell process (arrow) close to a thin-walled vessel (HPP-antiserum diluted 1:80, PAP method) (× 800). Right. Adjacent ultrathin section. The PP cell process contains numerous small electron-dense granules (× 13800)

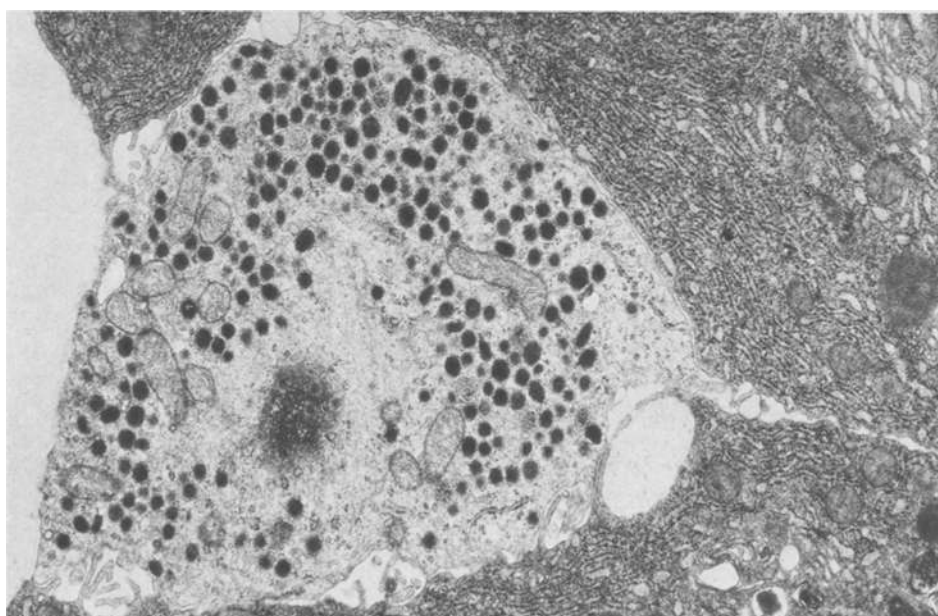

Fig. 7. Guinea-pig pancreas. glutaraldehyde-fixed, osmicated specimen. PP cell situated in between exocrine cells (× 14000)

fluid [14] (containing only 20% of the recommended water content) the sections were firmly adherent to the slides with minimal diffusion of the fluorophores. Second prolonged exposure of the sections to UV-light reduced the immunoreactivity of the pancreatic polypeptide. By using rapid film (Kodak Plus-X) for documenting the formaldehyde-induced fluorescence, the time of exposure to UV-light could be reduced.

The spectral properties of the formaldehyde-induced fluorophores were analyzed in a Leitz microspectrograph [15]. For recording of excitation spectra, sections were carried on quartz slides and the exciting light was passed through an optical system consisting of quartz components. All curves were corrected for instrumental errors as described elsewhere [15]. Differentiation between the fluorophores of norepinephrine and dopamine was performed by stepwise acidification of the sections in the fumes of concentrated hydrochloric acid [15]. By this procedure the excitation peak of the dopamine fluorophore is shifted from 410 nm to approximately 370 nm while that of

the norepinephrine fluorophore is shifted to a much shorter wave-length (approximately 330 nm).

Histological Stains for Islet Cells

Sections were stained with aldehyde fuchsin [16] or with silver according to Hellerström-Hellman [17] or Grimelius [18] for the demonstration of islet B, A_1 (D) and A_2 (A) cells, respectively.

Results and Comments

Occurrence and Distribution of Cells Storing Pancreatic Polypeptide

Cells displaying immunoreactivity with the BPP and HPP antisera were found in the pancreata of all species studied and in the gastrointestinal tract of some species. Optimal immunoreactivity was obtained with Bouin-fixed sections or with formaldehyde-fixed sections that were briefly refixed (1–2 h) in Bouin's fluid. All controls were negative. Henceforth the immunoreactive cells will be referred to as PP cells. BPP as well as HPP antisera demonstrated PP cells in all species studied. However, in certain species (hamster, rabbit, opossum and sheep) the HPP antiserum gave more intense staining than did the BPP antiserum, whereas the reverse was true in other species (rat, cow and horse). In mouse, guinea-pig, chinchilla, cat, dog and man the two antisera produced equivalent results.

The frequency of PP cells varied greatly between different species and also between different regions of the pancreas. A detailed description of the differences is given below (see also Table 1). As PP cells occur both in islets and scattered in the exocrine parenchyma a comparison between the number of PP cells relative to other pancreatic endocrine cell types is very difficult, especially since the PP cells may be abundant in certain parts of the pancreas and rare in other parts. Nevertheless it can be said that in mouse, rat and hamster the number of PP cells is low. On the other extreme is the sheep where the PP cells are abundant, sometimes even exceeding the insulin cells in number. In guinea-pig, chinchilla, opossum, cow and horse, a rough estimate shows that the PP cells are about as numerous as the glucagon cells. In rabbit, cat and dog the PP cells are numerous in the duodenal lobe where their number averages that of the insulin cells (see Table 2). By radioimmunoassay, the canine duodenal lobe was found to contain 51 μg insulin per g and 55 μg pancreatic polypeptide per g (Ronald E. Chance; personal communication), a figure which agrees well with the immunohistochemical results. In the tail of the pancreas the PP cells are considerably fewer – well below the number of insulin cells; in this location radioimmunoassay shows 61 μg insulin per g and 13 μg pancreatic polypeptide per g (Ronald E. Chance; personal communication), again in good agreement with the immunohistochemical results.

Table 2. Relative frequency of PP cells as compared to other islet cell types[a]

Species	Duodenal lobe	Tail
Mouse	+ +	(+)
Rat	+	+
Hamster	+ +	(+)
Guinea-pig	+ +	+ +
Chinchilla	+ +	+ +
Rabbit	+ + +	(+)
Opossum	+ +	+
Cat	+ + +	+
Dog	+ + +	+
Sheep	n. t.	+ + +
Cow	n. t.	+ to + +
Horse	n. t.	+ +
Man	n. t.	+

[a] It should be noted that this comparison may not be entirely justified because of the different distribution (insular versus extrainsular) of the various cell types.
(+); rare
+; somewhat below or averaging the frequency of A_1 cells
+ +; averaging the frequency of glucagon cells
+ + +; exceeding or averaging the frequency of insulin cells

Since the distribution and properties of PP cells differed in the various species investigated, the results are presented separately for each species.

Mouse. Very few PP cells were detected in the body and tail of the pancreas, whereas they were more numerous in the duodenal lobe (Fig. 1). The immunoreactive cells were located mainly at the periphery of the islets – only occasionally were PP cells detected in exocrine parenchyma or in ductal epithelium (Fig. 1). No PP cells were found in the gastrointestinal tract.

Rat. The PP cells were about equally numerous in the tail of the pancreas and in the duodenal lobe. They were less numerous in the pancreatic body. The PP cells were almost exclusively located at the periphery of the islets, where they occurred intermingled with the glucagon cells. In some islets PP cells formed a continuous peripheral lining. From comparisons of adjacent semithin (1 μ) sections, stained with glucagon antiserum and BPP antiserum, respectively, it could be established that the pancreatic polypeptide did not reside in the glucagon cells. No PP cells were found in the gastrointestinal tract.

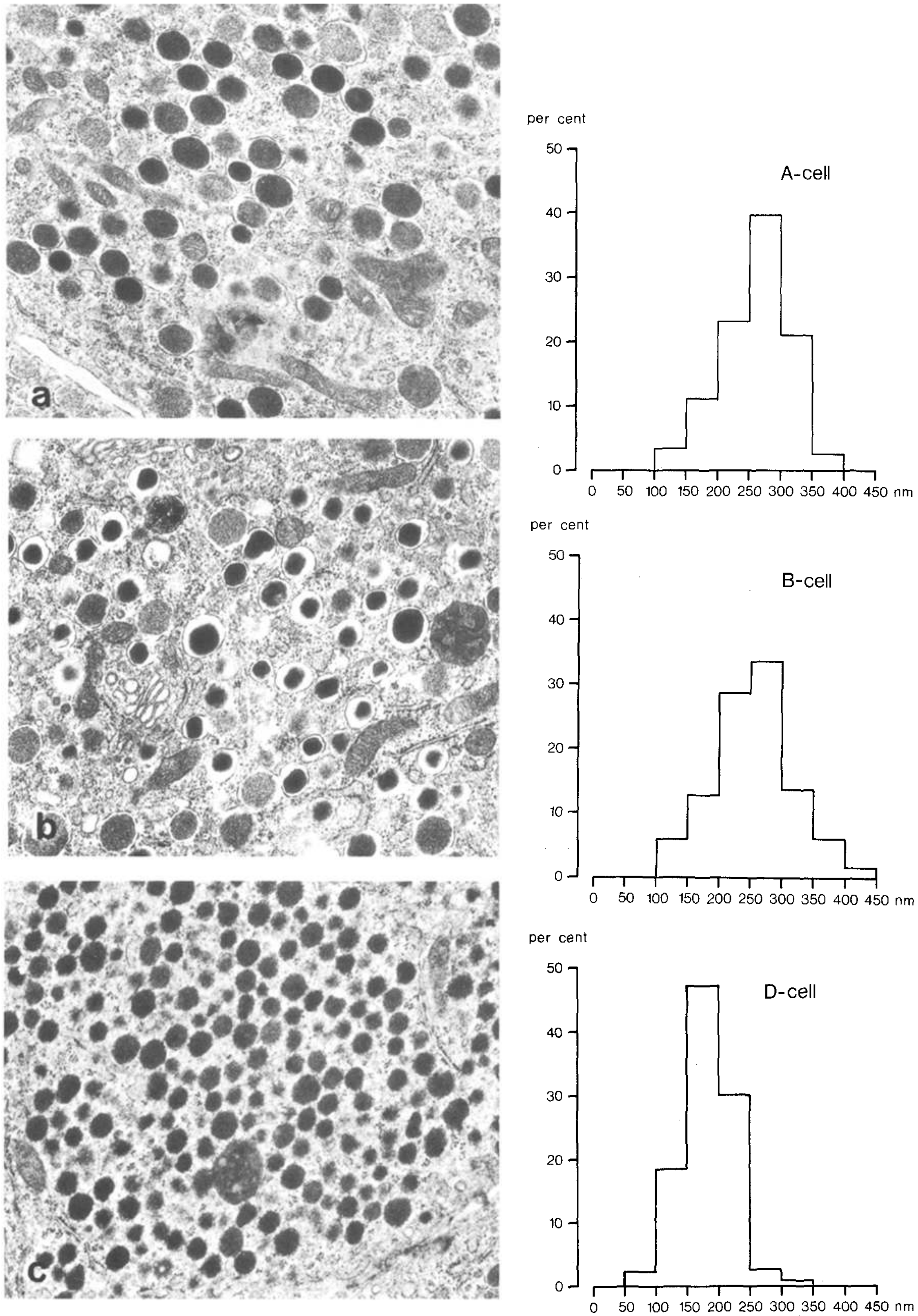

Fig. 8. I

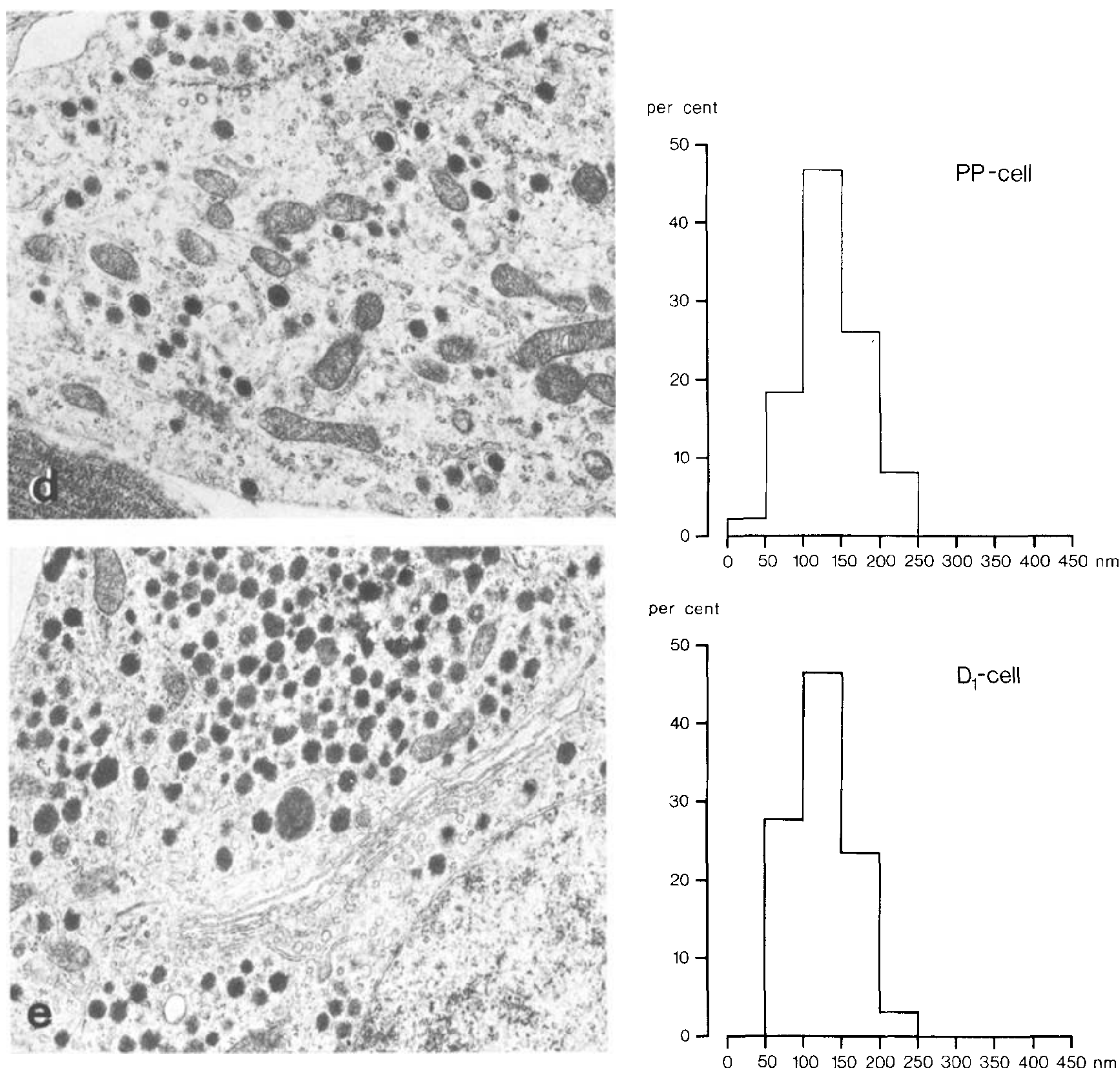

Fig. 8. II Rat pancreas. Glutaraldehyde-fixed, osmicated specimen. Ultrastructure of cytoplasmic granules of the different islet cell types (left panel, × 19000) together with granular size distribution histograms (right panel). **a)** A cell, mean granule diameter in nm 270 ± 61 (S. D.), n = 248 (n denotes number of granules measured). **b)** B cell, 245 ± 68 (S. D.), n = 383. **c)** D cell, 175 ± 42 (S. D.), n = 448. **d)** PP cell, 140 ± 42 (S. D.). n = 179, and **e)** D_1 cell, 130 ± 33 (S. D.), n = 870

Hamster. The distribution and number of PP cells in the body and tail of the pancreas were very similar to that in the mouse. Thus, in the tail we observed only an occasional PP cell in the islets (Fig. 2 a) in addition to a few cells in extrainsular locations. In the duodenal lobe the PP cells were much more numerous (Fig. 2 c). Here, they occurred mainly in islets, where they occasionally formed a continuous peripheral lining. Quite a few PP cells were found in the exocrine parenchyma, where they were squeezed in between neighbouring acinar cells. Occasionally PP cells were found also in the epithelium of large and medium-sized ducts.

Guinea-Pig. The PP cells were fairly uniformly distributed in the pancreas, although they appeared to be somewhat more numerous in the portion adjacent to the duodenum. They were about as numerous in islets as in exocrine parenchyma, but were rarely seen in ductal epithelium. The uniform distribution of PP cells made estimation of their number fairly easy. In 200 g guinea-pigs the number of PP cells per visual field (Leitz standard 10 × immersion objective, eye-piece 12,5 ×) was 50–70. No PP cells were found in the gastrointestinal tract.

Table 3. Ultrastructural descriptions of human (non-tumourous) islet cell types[a]

Authors	Year	Fixation	Cell type, diameter in nm			
			A	B	D	Small-granulated cells
Shibasaki and Ito (26)	1969	5% glutaraldehyde + OsO_4	450–650 small ~ 450	300–370	450–650	–
Misugi et al. [27]	1970	OsO_4	250–400 mean 300	200–400	"type IV" ~ 450 (less osmiophilic than "type III")	"type III," ~ 150 (frequent in cases of neonatal hypoglycemia, rare in controls)
Deconinck et al. [28]	1971	2.5% glutaraldehyde + OsO_4	350–450	350–500	"type III" 450–800	"type IV" 350–500[b] "type V" 150–300
Deconinck et al. [29]	1972		150–350 and 300–450	350–500	"type III" 450–800	"type IV" 150–300
Jirásek and Kubeš [30]	1972	5% glutaraldehyde + OsO_4	–	–	290–620	"type VI" 120–240
Vassallo et al. [31]	1972	2.5% glutaraldehyde (or 2% formaldehyde + 2.5% glutaraldehyde) + OsO_4	200–400 mean 330	200–400 mean 300	–	"small granule cell" 150–180
Munger [32]	1972	not specified	200–300	–	250–300	"fourth cell type" ~ 150
Greider et al. [33], [34]	1970, 1974	2.5% glutaraldehyde + OsO_4	225–425	225–350	150–250 250–450	
Klöppel et al. [35]	1974	3% glutaraldehyde + OsO_4	150–400	350–500	300–700	"type IV" 150–400
Own results[c]	1976	a) 2% glutaraldehyde + 4% formaldehyde, or b) 0.5% glutaraldehyde + 4% formaldehyde + OsO_4	a) 200±124 (378) b) 235± 47 (162)	250±59 (262) 300±61 (153)	220±48 (148) 230±50 (175)	D_1: 135±28 (169) 155±39 (388) PP: 110±23 (299) 125±36 (213)

[a] The cells are classified as proposed by the respective authors
[b] In material from neonates the type IV cell is small-granulated
[c] Mean ± S. D. (n)

Chinchilla. The PP cells occurred mainly in the exocrine parenchyma, where they tended to form small groups, often characteristically situated around small thin-walled vessels. PP cells were also seen peripherally in islets. The PP cells were somewhat more numerous in the duodenal lobe than in the other parts of the pancreas.

Rabbit. In the tail and body of the pancreas immunoreactive cells were exceedingly rare. They occurred either scattered in the exocrine parenchyma or peripherally in the islets. In the duodenal lobe, however, PP cells were very numerous, forming small extrainsular clusters. No immunoreactive cells were found in the gastrointestinal tract.

Opossum. The PP cells were most numerous in the duodenal lobe and body of the pancreas, where they occurred predominantly in islets (Fig. 3). Some islets contained numerous PP cells whereas others contained only few. The PP cells were mostly peripherally located. In some specimens extrainsular PP cells occurred singly or in small clusters. PP cells were much less numerous in the tail of the pancreas, where they occurred mainly in the exocrine parenchyma. In the oxyntic mucosa of the stomach a few scattered immunoreactive cells were seen (Fig. 4). PP cells were very rare in the antropyloric mucosa and in the remainder of the gastrointestinal tract no such cells were found.

Cat. In the body and tail of the pancreas the PP cells were fairly numerous and occurred mainly in extrainsular locations although a few were seen peripherally in islets. In the duodenal lobe the number of immunoreactive cells was much greater than in the other parts of the pancreas. The PP cells of the duodenal lobe occurred singly or in small groups in the exocrine parenchyma.

Dog. As with rabbit and cat the PP cells were very numerous in the duodenal lobe and fewer in the rest of the pancreas. In the duodenal lobe the PP cells were extra-insular and occurred either singly or, more often, in small groups. In the body and tail they were less numerous and occurred both in islets and disseminated in exocrine parenchyma and ductal epithelium. PP cells were also found in the oxyntic and antropyloric mucosa of the stomach. The cells were about equally numerous in both parts and were mainly

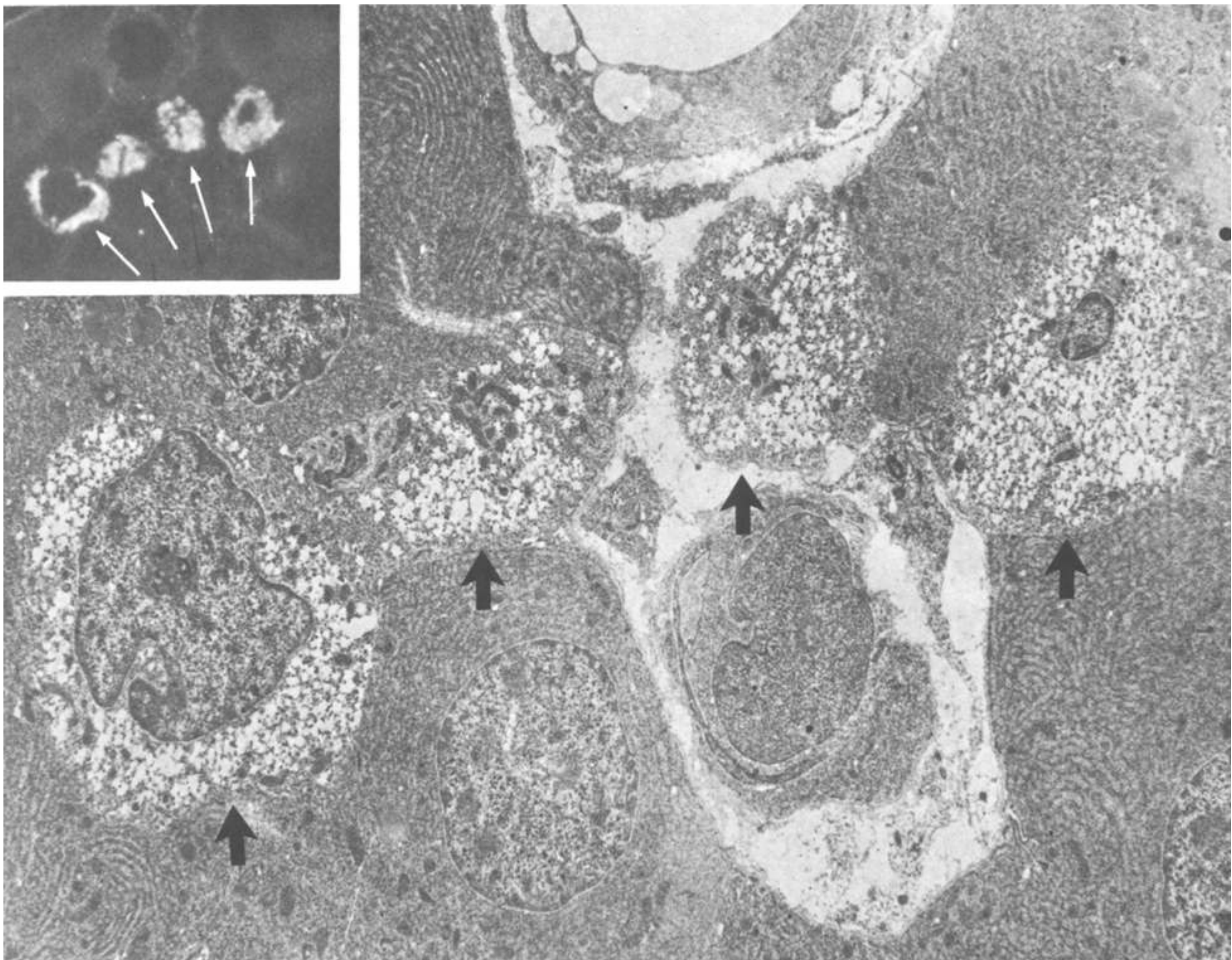

Fig. 9. Cat pancreas, duodenal lobe. Glutaraldehyde-fixed, non-osmicated specimen. Four cells with densely packed, electron-lucent granules. In some granules a small electron-dense core is visible (× 8000). The cells were identified as PP cells by staining the adjacent semithin sections with HPP antiserum (diluted 1:20, immunofluorescence) (insert, × 600). Arrows inserted for identification

situated in the basal half of the glands. No PP cells were found in the small intestines.

Sheep. PP cells were very numerous and occurred scattered in some islets whereas in others they were concentrated at one pole (Fig. 5a). Extra-insular PP cells were few.

Cow. A fair number of PP cells occurred at the periphery of most islets. Occasionally extra-insular cells were also found.

Horse. PP cells were found in the exocrine parenchyma as well as peripherally in the islets. Extra-insular cells occurred mostly in clusters of 2–5 cells. PP cells were regularly observed in the epithelium of large and medium-sized ducts.

Man. The tissue material was from the body and tail of the pancreas. The PP cells occurred in about equal number in these two regions. A varying number of PP cells were found, usually at the islet periphery. Rather few PP cells were found scattered in the exocrine parenchyma and in the epithelium of small and medium-sized ducts. In two out of the ten patients (both suffering from gastric carcinoma) peculiar extra-insular accumulations of PP cells were found. Here, the PP cells assumed a cylindrical shape and formed discoid bodies, one to two cells thick. Small ducts were often encountered in the immediate vicinity of these bodies. No connections with ductal epithelium could be found. Whether this represents a normal arrangement of PP cells is unknown.

Histological and Histochemical Properties

The PP cells did not stain with aldehyde fuchsin and they were nonargyrophil with the Hellerström-Hell-

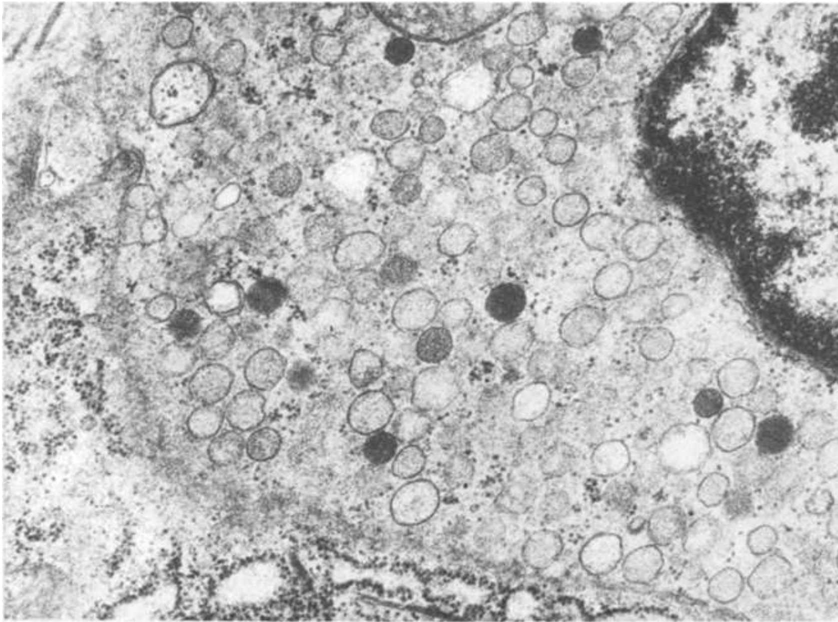

Fig. 10. Cat pancreas, duodenal lobe. glutaraldehyde-fixed, osmicated specimen. Cytoplasmic granules of a PP cell situated in exocrine parenchyma. The granules display varying electron-density. This cell type has previously been labelled F (orX) cell. (x 20000)

man silver staining method. In sheep pancreas the PP cells were actually argyrophobe, their staining intensity being far weaker than the background coloration (Fig. 5b). The PP cells stained very weakly with silver in the Grimelius procedure. In islets, as well as in exocrine parenchyma, the staining intensity of the PP cells did not exceed that of the background (Figs. 1b and 2d). However, in PP cells present in ductal epithelium the weak argyrophilia stood out against the nearly unstained columnar cells (Fig. 1d).

Only pancreas of mouse, rat, guinea-pig and opossum was examined for formaldehyde-induced fluorescence. No fluorescence was observed in the PP cells of mouse, rat or guinea-pig pancreas. In guinea-pig pancreas, yellow fluorescence was displayed by the insulin cells and by the enterochromaffin cells, known to contain 5-HT (cf. 19). In opossum pancreas, strong green formaldehyde-induced fluorescence was observed in cells mostly located at the periphery of the islets. Staining with HPP antiserum revealed that the green fluorescent cells were PP cells (Fig. 3). Microspectrofluorometry showed the fluorescence to be indistinguishable from that of dopamine. Green fluorescence typical of dopamine was also displayed by mast cells in the exocrine parenchyma.

Following adminstration of L-dopa to guinea-pigs strong green, formaldehyde-induced fluorescence was displayed by most islet cells and by numerous endocrine-like extra-insular cells. The enterochromaffin cells retained their endogenous yellow fluorescence. Restaining with HPP antiserum enabled us to show that the PP cells were among the green fluorescent cells in insular as well as extrainsular locations. It should be noted, however, that a significant proportion of the green fluorescent cells – insular as well as extra-insular – were distinct from the PP cells. Conceivably, this indicates the presence in the exocrine parenchyma of a third endocrine cell population besides the PP cells and the enterochromaffin cells.

Ultrastructural Properties

PP cells were identified at the ultrastructural level by comparing semithin sections stained with HPP or BPP antiserum with adjacent ultrathin sections (Fig. 6). Following fixation in formaldehyde-glutaraldehyde mixture, the PP cells of rat, guinea-pig, chinchilla and man exhibited moderate immunoreactivity. No immunoreactive cells were observed in sections from material fixed in 2.5% glutaraldehyde. In cat and dog PP cells, however, the immunoreactivity was preserved with both methods of fixation.

In rat, guinea-pig and chinchilla the PP cells contained small membrane-bound granules. The dense core was separated from the surrounding membrane by a narrow, but well defined, electron-lucent halo (Figs. 7 and 8). The PP cells contained a fair number of slender mitochondria and free ribosomes as well as areas of rough endoplasmic reticulum. The granular morphology seems to be the only ultrastructural feature which is useful in distinguishing the PP cells from

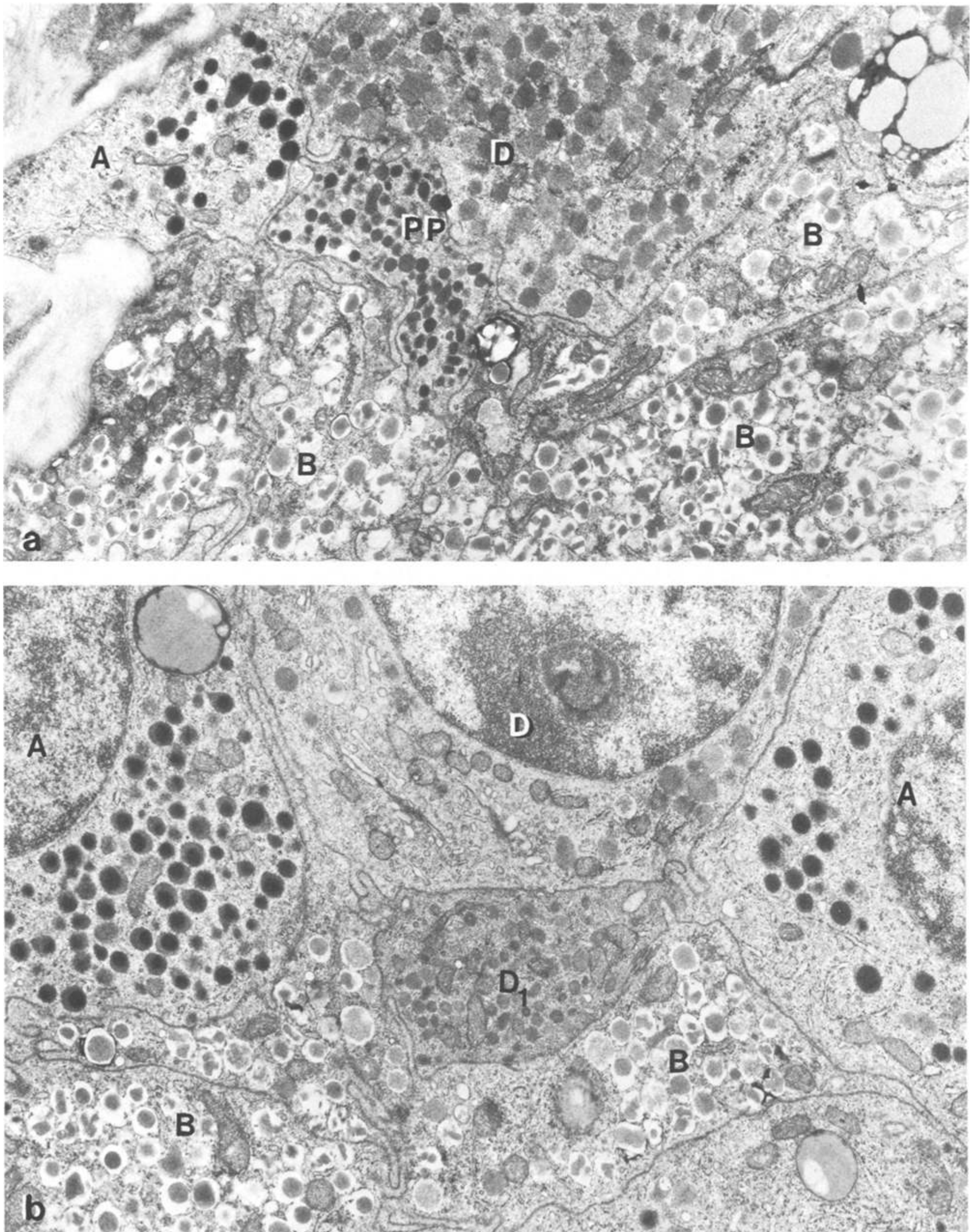

Fig. 11. Human pancreas. Formaldehyde-glutaraldehyde-fixed, osmicated specimen. **a)** Islet area containing A, B, D and PP cells. **b)** Islet area containing A, B, D and D_1 cells (× 15600)

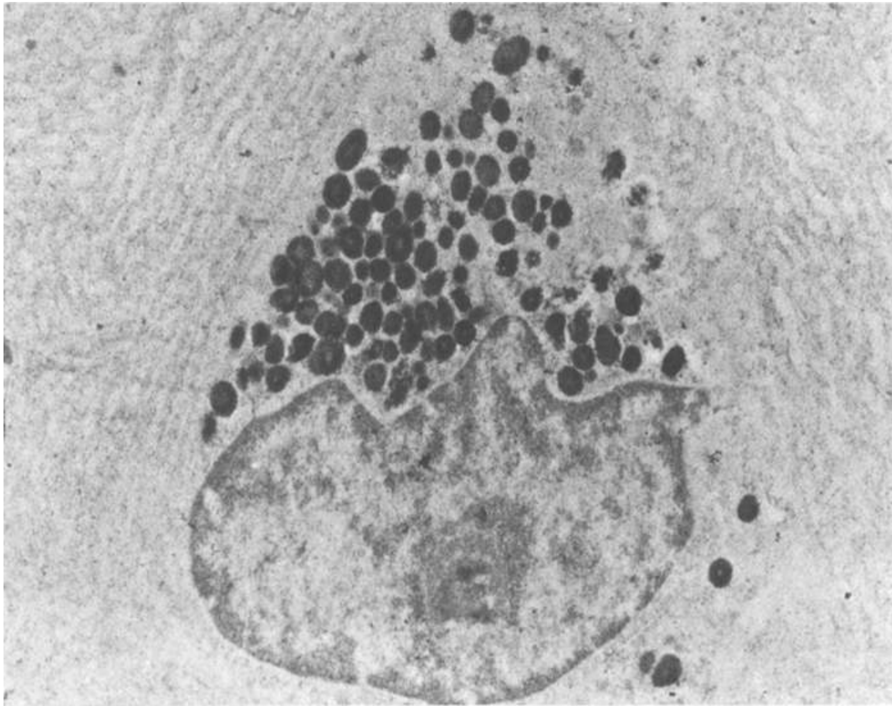

Fig. 12. Cat pancreas, duodenal lobe. PAP staining of section from glutaraldehyde-fixed, non-osmicated specimen (HPP antiserum, diluted 1:200). The cytoplasmic granules are heavily loaded with electron-dense material (particularly at the granular periphery), indicating the presence of pancreatic polypeptide (× 11000)

the other islet cells of the pancreas. Granule diameter and ultrastructure in the different islet cell types of the rat pancreas is illustrated in fig. 8. B cell granules were characterized by a wide electron-lucent halo between the dense core and the surrounding membrane. The A cell granules were slightly larger than those of the B cells and had a narrower halo separating the dense core from the membrane. D cell granules had a weakly stained surrounding membrane, closely applied to the dense core. In addition, cells having small granules, which in other respects resembled the D cell granules, were regularly observed. We refer to these cells as D_1 cells. The granules of these cells resembled the PP cell granules in size but not in morphology (see above). They were also more frequent in the centre of the islets than were the PP cells.

The PP cell granules in the cat and dog pancreas (non-osmicated specimens) had a wide electron-lucent halo surrounding a small, irregular, moderately electron-dense core (Fig. 9). In osmicated specimens the granules contained material of varying electron density, evenly distributed within the limiting membrane (Fig. 10). The diameter of the granules ranged between 200 and 250 nm. The nucleus of the PP cells in cat and dog often showed characteristic deep indentations, a feature which assisted in recognizing these cells.

In man the PP cells contained small cytoplasmic granules of moderate to high electron density (Fig. 11). In osmicated specimens the mean diameter of the PP cell granules was 125 nm. The corresponding value for A cell granules was 235 nm, for B cell granules 300 nm and for D cell granules 230 nm (see Table 3). In addition to the PP cells another small-granulated islet cell type was found. The granules of this cell type had a mean diameter of 155 nm and were of fairly low electron density, in this respect resembling D cell granules. These cells are referred to as D_1 cells[1].

After PAP-staining the cytoplasmic granules of the cat PP cells were heavily loaded with electron-dense precipitates (Fig. 12).

Discussion

A biologically active peptide was recently isolated from the pancreas of birds (avian pancreatic polypeptide) [3] and of mammals (mammalian pancreatic polypeptide) [4]. The avian pancreatic polypeptide (APP) has been localized to a system of extra-insular

[1] As the D_1 cells differed in the electron density of their granules it cannot be excluded that the D_1 cells may comprise several small-granulated cell types.

endocrine-like cells of the chicken pancreas [6]. Subsequently, the cells storing human pancreatic polypeptide (HPP) were demonstrated in the pancreas of adult and foetal man [7]. The APP as well as the HPP cells were found to be distinct from the A, B and D cells. The purpose of the present investigation was to identify the cells storing the pancreatic polypeptide by immunocytochemical, ultrastructural and conventional histological and histochemical techniques in different mammals.

In all species studied the pancreatic polypeptide is stored in endocrine-like cells (PP cells) distinct from the A, B and D cells. The PP cells occur in islets as well as in exocrine parenchyma. The relative frequency of PP cells in these two locations varies markedly from one species to another. In opossum and dog PP cells occur also in gastric mucosa. Like most peptide hormone-producing cells the PP cells possess amine handling properties [cf. 19, 20, 21]. Thus, in opossum the PP cells appear to store dopamine, whereas they are devoid of histochemically demonstrable arylethylamines in the other species studied (mouse, rat and guinea-pig). However, as exemplified in the guinea-pig, the PP cells are able to take up exogenous amine precursors (such as L-dopa) and convert them to their corresponding amine.

The PP cells were identified at the electron microscopic level by immunofluorescent staining of semi-thin sections, combined with electron microscopy of adjacent ultra-thin sections and occasionally by peroxidase-antiperoxidase immunocytochemistry on ultra-thin sections. The ultrastructure of the PP cells is subject to species variation. In all species studied the PP cells have an endocrine-like appearance with characteristic cytoplasmic granules. In cat and dog the PP cells are most probably identical with the previously described F (or X) cells [22, 23]. In the other mammals studied (rat, guinea-pig, chinchilla and man), cells with the ultrastructural characteristics of F cells are not found. In rat, guinea-pig and chinchilla the PP cell granules are small and have an electron-lucent halo between the dense core and the surrounding membrane. Another small-granulated cell type (by us referred to as the D_1 cell) has granules devoid of such a halo. But for their size the D_1 cell granules are very similar to the D cell granules. The human PP cell contains small, moderately electron-dense granules, with the membrane closely applied to the dense core. Previous electron microscopic studies on human pancreatic islets have revealed the presence of cells having granules of smaller size than those of the A, B and D cells[2]. In the literature such cells have been given various names (see Table 3). It should be noted, however, that the human pancreas contains at least two distinct small-granulated islet cell types – the one containing the smallest granules being the PP cell. Of the islet cell types previously described in the human pancreas the type V cell of Deconinck et al. [28] seems to correspond best with the PP cell.

By applying immunohistochemistry at the ultrastructural level we could show that the pancreatic polypeptide is contained in the PP cell granules. This provides further support for the assumption that the pancreatic polypeptide represents a hormone. Its physiological importance has yet to be determined.

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[2] By now, at least two distinct small-granulated islet cell types can be recognized: PP cells and D_1 cells. We use the term D_1 cells to include all small-granulated islet cells except PP cells. Small-granulated cells have been reported to make up or be a constituent of endocrine pancreatic tumours [see 24]; insulomas often contain several peptide hormone-producing cell types [25]. Hence, some of the small-granulated cells observed in such tumours may be PP cells.

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Dr. L.-I. Larsson
Institute of Medical Biochemistry
University of Aarhus
DK-8000 Aarhus C
Denmark