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Islet cell carcinomas in dogs

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Summary. Seven cases of dog islet cell carcinomas were studied by conventional and immunohistochemical light- and electron-microscopy. Antisera to insulin, pancreatic polypeptide, somatostatin and glucagon were used. In 6 tumours several hormones were demonstrated. Glucagon never occurred. Insulin was the only hormone present in every tumour, thus it seems to be a good marker for these neoplasmas.

Liver metastases contained less immunoreactive cells than primary tumours and cell types found in primary carcinomas were sometimes not present in liver metastases.

In two cases a degenerative neuropathy occurred.

Key words: Islet cells – Carcinoma – Dog – Morphology – Immunohistochemistry

Introduction

Islet cell tumours are not rare in man, and cause severe clinical symptoms because of their hormonal secretions (Woodtly and Hedinger 1977a and b; Klöppel et al. 1979; Klöppel 1981; Heitz et al. 1982; Alumets et al. 1983; Heitz et al. 1983).

Adenomas and carcinomas of islet cells have also been reported frequently in dogs but few cases have been observed in cattle, cats and swine (Kircher and Nielsen 1976; Capen 1978).

Since dogs live in the same environmental conditions as man, studies on endocrine tumours of dog pancreas may contribute to comparative pathology. Tumour-bearing dogs are adult animals, aged between 5 and 12 years, boxers and terriers being the most affected breeds, without a sex predisposition (Kircher and Nielsen 1976; Capen 1978).

Although endocrine neoplasms of dog pancreas produce hormones in

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most cases, to our knowledge, their endocrine characteristics have not yet been described immunohistochemically. Thus we present 7 canine islet cell carcinomas studied by conventional and immunohistochemical light-microscopy and, in 2 cases by electron-microscopy.

Material and methods

The breed, sex and age of the dogs are given in Table 1. In cases 1 to 5 necropsies were performed a few minutes (e.g. case 1) up to 12 h (e.g. case 4) after euthanasia. Pancreatic regions were anatomically defined according to Miller et al. (1964).

Tissues were fixed in a 4% paraformaldehyde solution, phosphate buffered at pH 7.4, for at least 24 h. For the electron-microscopic study of cases 1 and 4 the tissue was postfixed in 2% OsO4 solution (pH 7.4) for 2 h. Tissue blocks for ultrastructural immunohistochemistry were not postfixed. In cases 6 and 7 surgical samples of neoplasms were fixed in 10% formalin solution.

For conventional light-microscopic study tissues were embedded in paraffin, sectioned and stained with haematoxylin-eosin. Nerves were stained also with the Holmes' silver method. Other tissue portions were infiltrated and embedded in Spurr's low viscosity medium, semithin sectioned and stained with toluidine blue. Subsequently, thin sections were cut from selected areas of the same blocks, stained for contrast with uranyl acetate and lead citrate and studied under the electron-microscope.

Pancreatic hormones were localized immunohistochemically in paraffin or semithin sections for light- and on thin sections for electron-microscopy. Commercially purchased primary antibodies were: 1) rabbit anti-glucagon (UCB-Bioproducts. Bruxelles, Belgium), 2) guinea-pig anti-insulin (Immuno Nuclear Corporation, Stillwater, MN, USA), 3) rabbit anti-pancreatic polypeptide (UCB-Bioproducts), and 4) rabbit anti-somatostatin (UCB-Bioproducts).

Semithin and thin sections were pretreated with Mayor's reagent to partially remove the polymerized resin. For light-microscopy antisera were diluted 1:2000 and for electron-microscopy 1:500 (anti-somatostatin 1:1000). Conjugation with peroxidase anti-peroxidase (PAP) complex (Miles Laboratories, Elkhart, IN, USA) or with anti-guinea-pig peroxidase (Dako Corporation, Santa Barbara, CA, USA) for insulin, was conventionally performed. For specificity control, primary antisera were substituted with normal homologous sera.

Light-microscopic immunohistochemical semithin sections were observed and photographed by differential interference contrast optics.

Table 1. Breed, sex and age of the animals

Case Nr.	Breed	Sex	Age (in years)	Clinical presentation
1. (GB 484/80 305)	collie	male	7.5	hyperinsulinism hypoglycaemic crises
2. (IP 4309/82 2699)	Irish setter	male	8	hypoglycaemic crises
3. (Ae 3947/80 2714)	collie × groendale	female	10	mild diabetes mellitus
4. (GB 415/84 347)	German shepherd crossbred	female	8	hyperinsulinism hypoglycaemic crises peripheral polyneuropathy
5. (15711 D4371 C)	collie	male	6	hypoglycaemic crises peripheral polyneuropathy
6. (P 635/84)	dachshund	male	11	hypoglycaemic crises
7. (P 907/84)	boxer	male	8	hypoglycaemic crises

Table 2. Site, histological and immunohistochemical patterns of the primary tumours and their metastases

Case Nr.	Primary tumour						Metastasis					
	Pancreatic site	Histological patterns[a]	Cell types				Localisation	Histological patterns[a]	Cell types			
			alpha	beta	PP	delta			alpha	beta	PP	delta
1.	angle	lobular		+++	++	+/−	liver	trabecular, lobular		+++	+	
2.	right lobe	lobular		+++	++		liver	lobular		++		
3.	left lobe	trabecular, lobular		++	++	++	liver	trabecular		+/++	+/−	+
4.	left lobe	tubular, lobular, trabecular		++		++	liver pancr. lymph.	lobular		+		+
5.	right lobe	lobular		++	+/−		liver	lobular		+		
6.[b]	left lobe	lobular, trabecular		+++								
7.[b]	angle	lobular, trabecular		+++	+	+						

[a] In order of frequency
[b] Only pancreas biopsy available

+ rare, ++ numerous, +++ very numerous

Results

Three of tumour-bearing dogs were collies, the remaining 4 of various breeds. The mean age was 7.9 ± 1.4 years; male animals were overrepresented (Table 1). No pancreatic region was particularly affected (Table 2). Tumours were round or oval solitary nodules, 1.5 to 2.5 cm in diameter, firmer than, and sometimes completely embedded in the surrounding exocrine pancreas and thus not visible from the serosal surface. The boundary between the grey neoplastic tissue and the normal tissue was sharp.

Liver metastases were seen in the 5 necropsied dogs as numerous, round, white, quite firm nodules, 0.5 to 1.5 cm in diameter, clear-cut from the normal parenchyma. In the two animals which underwent surgery liver metastases were seen at laparotomy.

1. Light-microscopy

A. Conventional (Table 2). Primary tumours were partly encapsulated by connective tissue frequently infiltrated by neoplastic cells, in part spreading into the exocrine pancreas. Connective tissue septa in turn penetrated into the epithelial component leading to a lobular (Fig. 1a), trabecular (Fig. 1b) and tubular (Fig. 1c) pattern. The lobular pattern, associated with one or both of other types, was most frequent.

Tumour cells had a pale, finely granular, slight basophilic cytoplasm. Their nuclei were small, vesicular, irregularly shaped with prominent nucleoli. Nuclei were round in areas of tubular pattern. Mitoses were infrequent, but single necrotic cells were often seen, fused into large foci in case 3 only. A pronounced intratumoral amyloidosis was seen in case 7 (Fig. 2a). Intrahepatic metastases consisted of non-encapsulated round foci of neoplastic cells, surrounded by glycogen loaded hepatocytes. Both trabecular and lobular patterns were present, but not at the same time. Cytological characteristics were identical to the primary neoplasms. Metastases of case 4 contained necrotic foci. Intravenous neoplastic emboli scattered throughout the liver parenchyma were common.

In case 4 tumour cells invaded a pancreatic lymph-node. In case 3 pronounced ballooning degeneration was present in islet cells of non-tumour parenchyma.

A clinically diagnosed peripheral neuropathy in cases 4 and 5 was caused by degenerative lesions of the radial and ischial nerves, characterized by axonal swelling, breakdown and macrophage infiltration of axoplasm and endoneurial lymphocytic infiltration (Fig. 3a–c).

B. Immunohistochemistry (Table 2). Six of the 7 primary tumours contained several hormone producing cell types. Insulin occurred in every neoplasm, pancreatic polypeptide (PP) in 5 and somatostatin in 4 cases. No glucagon positive cells were present. Insulin (Fig. 2d; Fig. 4a, b) and PP (Fig. 2e; Fig. 4c, d) containing cells were ovoid or elongated; somatostatin cells (Fig. 4e, f) round or ovoid, filled by immunoreactive hormone granules. Metastases contained less cell types and hormones than primary tumours.

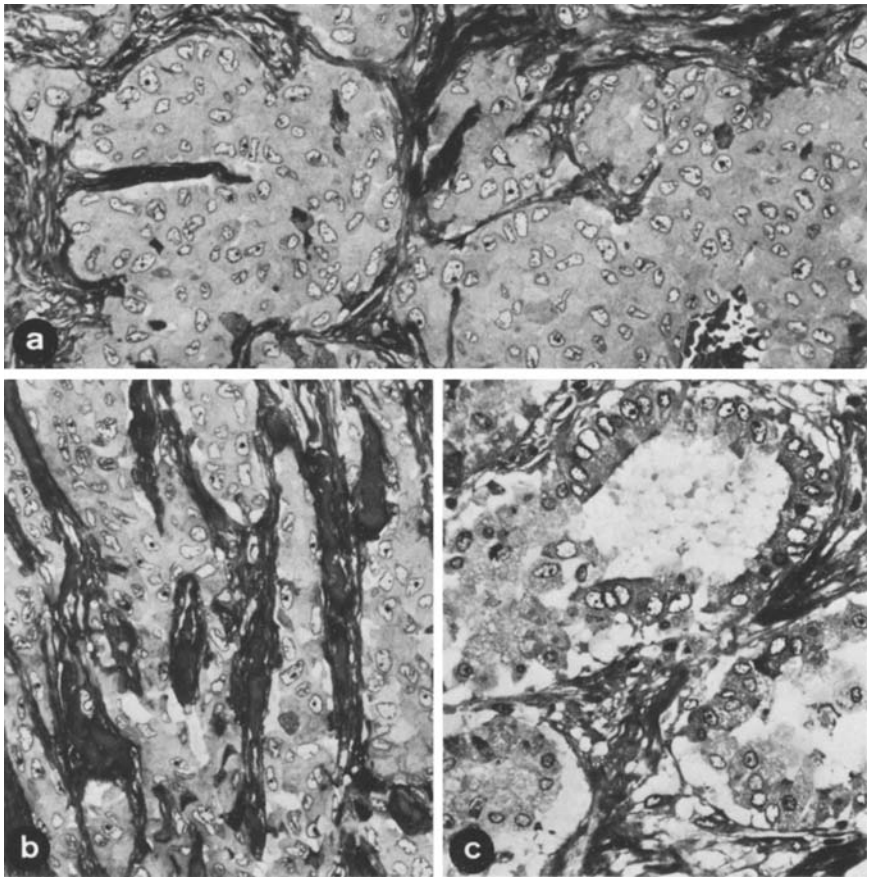

Fig. 1a–c. Lobular (**a**), trabecular (**b**) and tubular (**c**) patterns of tumour cells. Semithin sections, toluidine blue staining. ×330

A relationship between histological patterns and cell types did not exist in either primary tumours or in metastases.

In case 3 islet cells were only slightly stained by anti-insulin. In case 7 the amyloid was negative for insulin (Fig. 2b, c).

2. Electron-microscopy

In both cases 1 and 4 most cells were characterized by various amounts of round or ovoid intracytoplasmic granules ranging from 350 to 850 nm in diameter (Fig. 5a). They were surrounded by an unit membrane, contained round or elliptic dense cores, encircled by a clear halo (Fig. 5b), and were positive for anti-insulin (Fig. 6a, b).

In case 1 some cells contained small dense granules, 150–250 nm in diameter, located at the periphery of their cytoplasm (Fig. 5d, e) and positive for anti-PP (Fig. 6c, d).

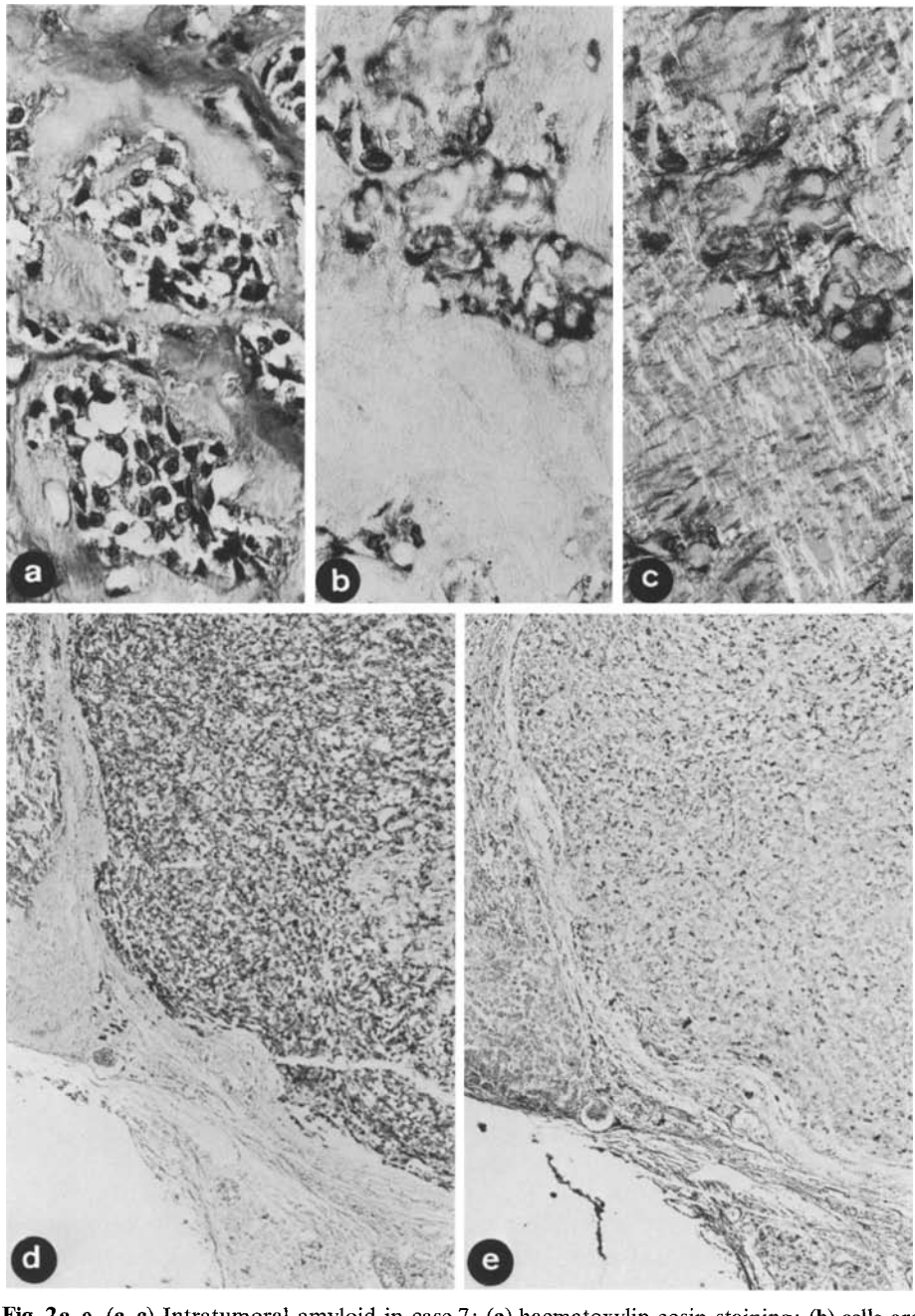

Fig. 2a–e. (**a–c**) Intratumoral amyloid in case 7: (**a**) haematoxylin-eosin staining; (**b**) cells are insulin positive and, (c) amyloid negative; immunoperoxidase indirect technique, birefringence of amyloid; × 330. Insulin containing cells are predominant (**d**) and PP cells numerous (**e**) in case 1; immunoperoxidase indirect (**d**) and PAP (**e**) technique, × 53

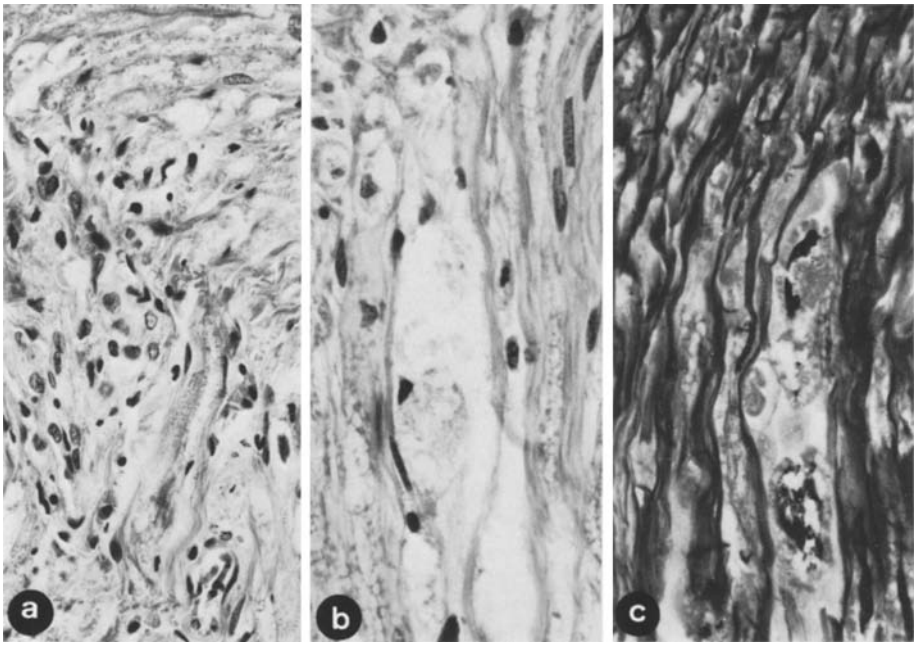

Fig. 3a–c. Lymphocytic infiltration (**a**), swelling, phagocytosis and fragmentation of axoplasm (**b, c**) in peripheral neuropathy. Haematoxylin-eosin (**a, b**) and Holmes' (**c**) staining. ×330

Other cells of case 4 contained few granules, 300–400 nm in diameter, without a halo and of slight electrondensity (Fig. 5a, c) which stained by anti-somatostatin (Fig. 6e, f).

In every cell type the most frequent organelles were mitochondria and polyribosomes. The Golgi apparatus was well developed. Dark cells corresponding to pyknotic ones seen by light-microscopy were also frequent.

Discussion

The age of the animals, the macroscopic, histological and metastatic patterns of the tumours resemble those in previous reports (Kircher and Nielsen 1976; Capen 1978). As to sex and breed, male animals and collies are overrepresented in our material.

No relationship appeared to exist between pancreatic localisation, histological pattern and cell types in primary tumours. Neoplasms with multiple hormones were predominant in our material, and should therefore be defined as multiple islet cell carcinomas (Williams et al. 1980). Insulin was always present, and thus is a good marker for dog pancreatic endocrine tumours. PP, as already observed in man (Heitz et al. 1976; Polak et al. 1976; Klöppel 1981), and somatostatin were also frequent whereas glucagon was absent. Contrary to previous reports on human cases (Schneider et al. 1980), intratumoral amyloid was negative to anti-insulin.

Cell granules resembled those occurring in normal dog islet cells (Lacy

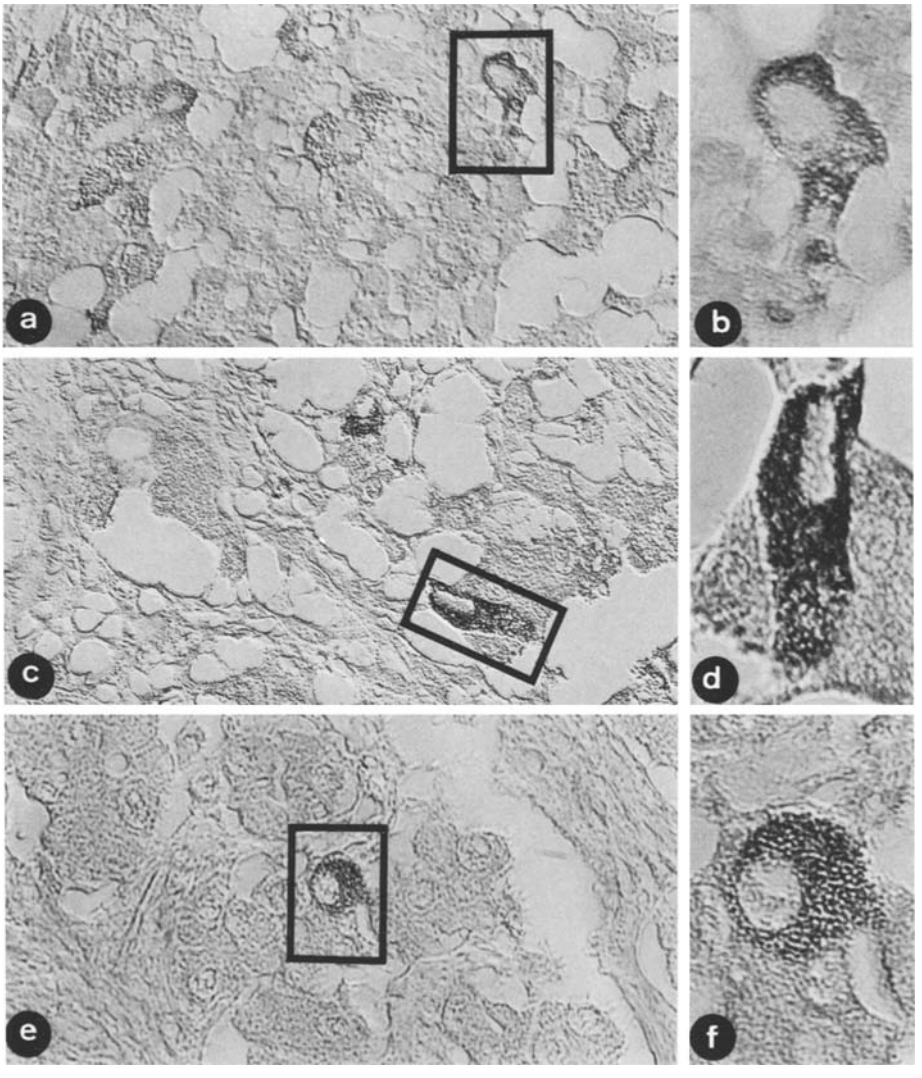

Fig. 4a–f. Beta (**a, b**), PP (**c, d**), delta (**e, f**) cells. Semithin sections, immunoperoxidase indirect (**a, b**) and PAP technique, differential interference contrast optics. **a, c, e** ×530; **b, d, f** ×1,320

1957; Munger et al. 1965; Sato et al. 1966; Larsson et al. 1976) except for insulin granules, which did not have the V or rod-shaped cores.

Liver metastases were observed in all necropsied dogs. There was no relationship between their histological patterns and cell types or between these and the histological patterns and localisation of the primary tumour. Semiquantitatively (Table 2) metastases seemed to have a lower hormonal content than primary neoplasms. Moreover, not all cell types found in primary carcinomas were always present in liver metastases.

A peripheral neuropathy, similar to that observed in cases 4 and 5,

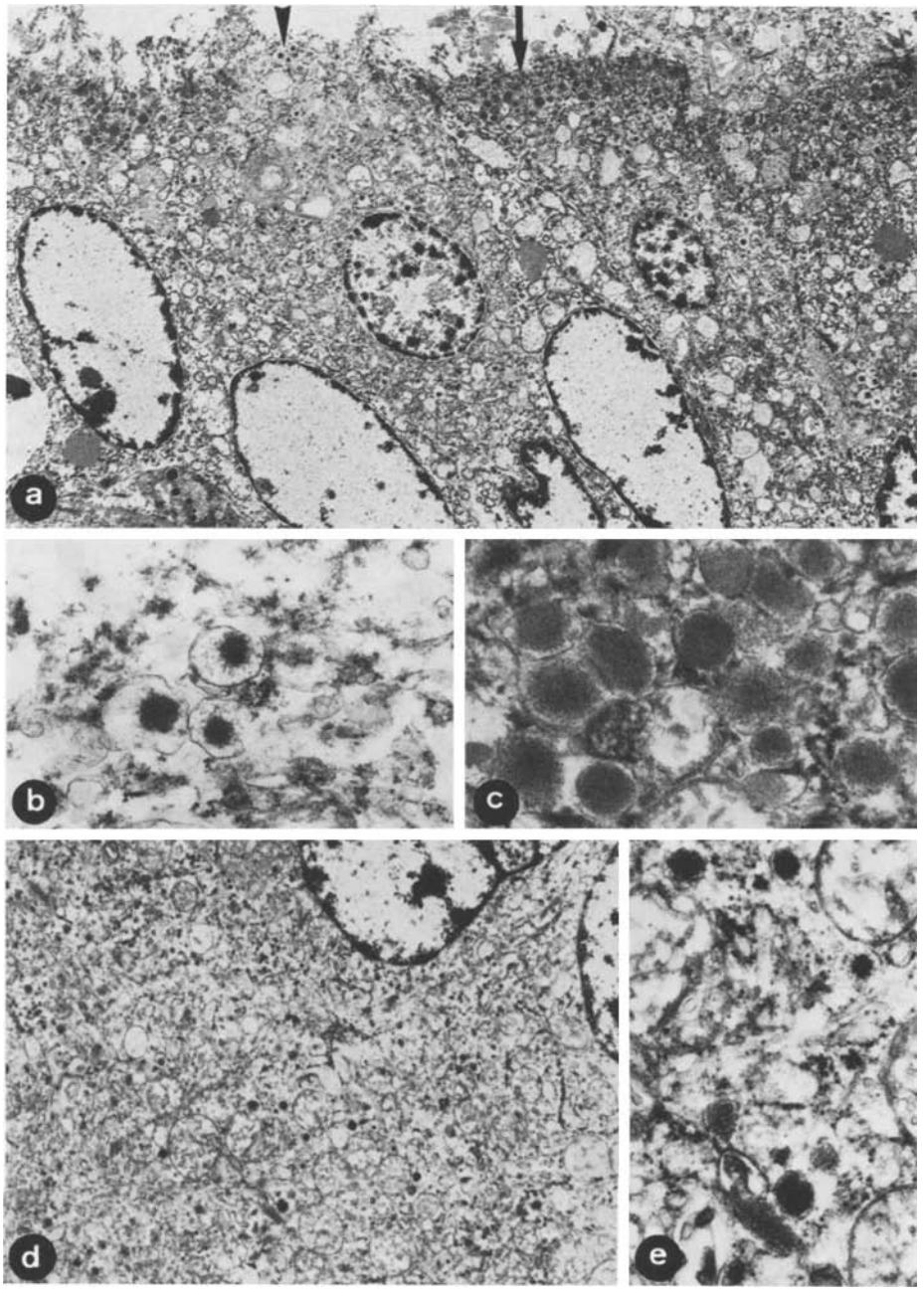

Fig. 5a–e. (**a**) Beta (⮝) and delta (⮙) cells (×3,900); detail of beta (**b**) and delta (**c**) cell granules (×27,000); PP cell (**d**, ×8,400) and detail of PP granules (**e**, ×27,000). Conventional electron-microscopy

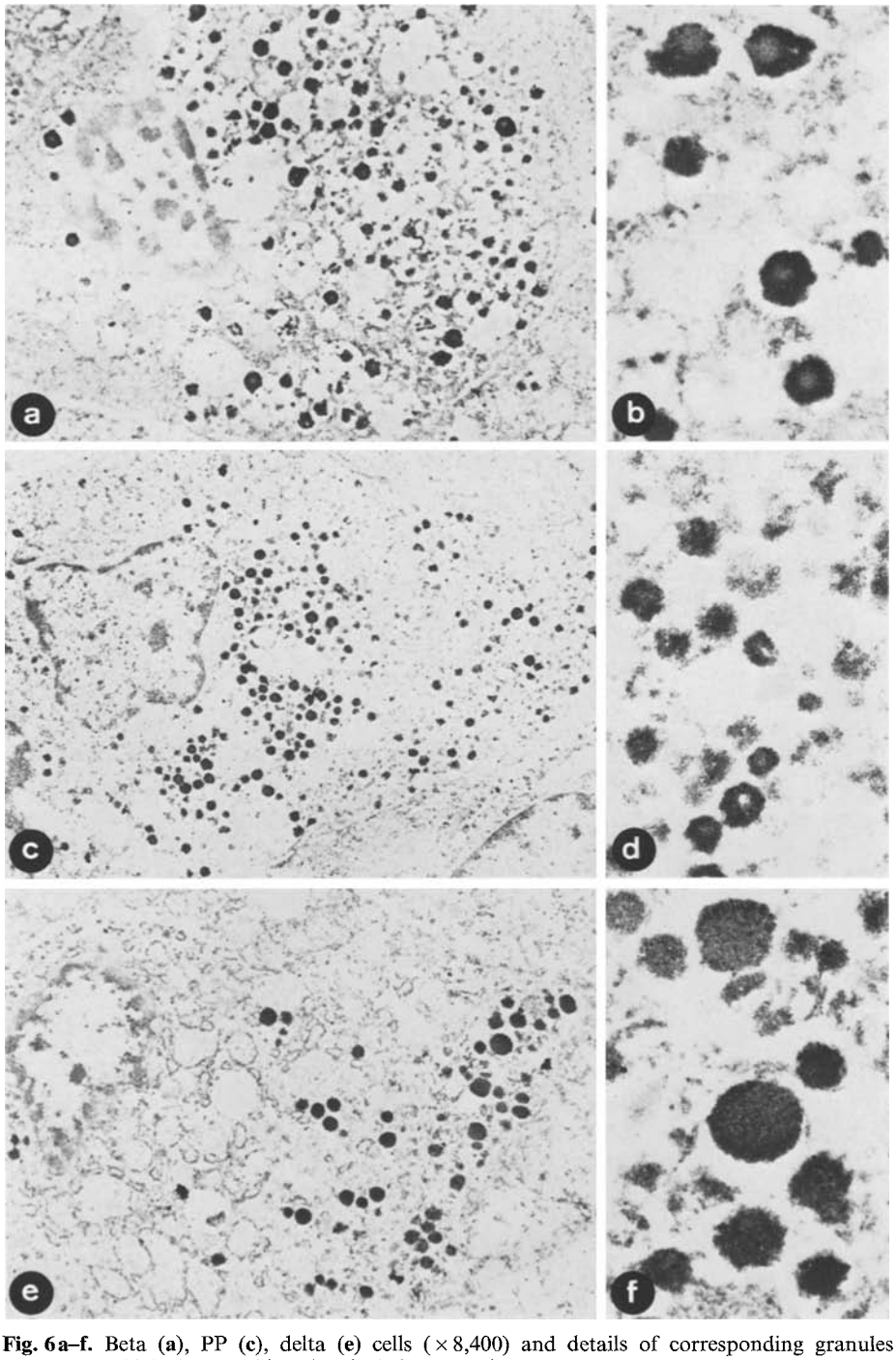

Fig. 6a–f. Beta (**a**), PP (**c**), delta (**e**) cells (×8,400) and details of corresponding granules (**b, d, f** ×27,000). Immunohistochemical electron-microscopy

was described in man concomitant with insulin producing tumours, yet with an unknown pathogenesis (Jaspan et al. 1982; Jayasinghe et al. 1983).

In conclusion, our cases indicate that endocrine pancreatic tumors of dogs frequently represent multiple islet cell carcinomas. Because neither localisation in pancreas nor the histological patterns give reliable information on the hormonal activity of tumours, immunohistochemical determinations are indispensable for classification of the cell types according to their function. Insulin seems to be the best marker for endocrine pancreatic neoplasms of dogs.

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