Immunocytochemical Identification of Cells Containing Insulin, Glucagon, Somatostatin, and Pancreatic Polypeptide in the Islets of Langerhans of the Guinea Pig Pancreas With Light and Electron Microscopy

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ABSTRACT Cell types containing insulin, glucagon, somatostatin, and pancreatic polypeptide were identified in guinea pig islets with light and electron microscopic immunoperoxidase staining. Cells containing immunostainable insulin (B cells) are located throughout the islets, including the islet periphery, and contain irregularly shaped granules (350-550 nm). Granule contents are of variable opacity and are often fragmented but not crystalloid. Cells containing immunoreactive glucagon (A cells) are found in the interior of islets and contain numerous spheroid electron-opaque granules (250-350 nm). Cells containing immunoreactive somatostatin (D cells) have elongate, axonlike processes that end adjacent to islet capillaries. D cells, which are very numerous and distributed uniformly throughout the islet parenchyma, contain small spheroid granules (150-250 nm) of pale electron opacity. Cells with immunoreactive pancreatic polypeptide (F cells) are rare in islets but numerous among the exocrine parenchyma. F cells contain pale spheroid granules (100-200 nm). Morphological criteria are reliable indicators for A cells and B cells, but D cells and F cells require immunostaining for positive identification.

Evidence that the guinea pig exhibits a diabetic syndrome similar to human juvenile-type diabetes mellitus (Lang and Munger, 1976; Lang et al., 1977) and islet regeneration after alloxan treatment (Johnson, 1950a,b; Jacob, 1977) suggests that this species may provide a model for investigating islet cytophysiology and regeneration. In our studies on the effects of alloxan and streptozotocin on islet regeneration in the guinea pig (Baskin and Gorray, 1980; Baskin et al., 1981b; Gorray et al., 1981), we encountered uncertainties about identification of guinea pig islet endocrine cell types by both light and electron microscopy. The islets of Langerhans of the mammalian pancreas contain at least four cell types which secrete insulin (B cell), glucagon (A cell), somatostatin (D cell), and pancreatic polypeptide (F

cell) (Erlandsen, 1980; Larrson, 1980; Munger, 1981; Appel and Like, 1982). The morphological features of these cell types have been characterized using immunocytochemistry for their identification in most common laboratory animals. Although immunocytochemistry is now the method routinely used for the microscopic identification of islet cell types, the guinea pig is an exception, since relatively recent light microscopical studies on guinea pig islets have still relied on tinctoral staining criteria for identification of islet cell types (Berggren, et al., 1979; McGadey and Reid, 1981).

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Immunocytochemical identification of an islet cell type in the guinea pig has been demonstrated only for B cells (Baskin et al., 1981a). Although Larsson et al (1976) reported identifying pancreatic polypeptidelike immunoreactivity in guinea pig islets, no micrographs were shown. Ravazzola et al. (1979) reported that guinea pig islets contained cells which reacted with antiserum to glicentin but not to an antiglucagon serum which stained rat glucagon cells. Ultrastructural identification of guinea pig islet cell types has been based on morphological criteria (Lacy, 1957, 1959, 1961; Caramia et al., 1965; Munger and Lang, 1973) that were established before immunocytochemical techniques were widely used for ultrastructural identification of islet cell types. Therefore, we undertook an immunocytochemical investigation of guinea pig islet cell types with both light and electron microscopy in order to base identification of guinea pig islet cells on immunocytochemical criteria.

# MATERIALS AND METHODS Light Microscopy

The splenic portion of the pancreas of 400gm male guinea pigs (Hartley) was removed under anesthesia and placed in Bouin's fixative for 24 hours. Tissues were dehydrated in ethanol-toluene, embedded in paraffin, and sectioned at  $3-7 \mu m$ .

#### Electron Microscopy

Portions of the pancreatic splenic lobe were immersed in 1% paraformaldehyde, 1.25%glutaraldehyde in 0.1 M phosphate buffer (Sorensen's), pH 7.2, for 2 hours at 4°C, postfixed in 2% osmium tetroxide in the same buffer for 1 hour at 4°C, and embedded in Epon 812. Ultrathin sections were mounted on nickel grids for immunostaining and electron microscopy.

#### *Immunocytochemistry*

Sections for light microscopy were immunostained with the peroxidase-antiperoxidase (PAP) method using diaminobenzidine (DAB) as the chromogen, as described in detail elsewhere (Baskin et al., 1981a). For electron microscopic immunostaining, a PAP method using 4-chloro-1-naphthol (CN) as the chromogen was employed (Baskin et al., 1982) with ultrathin sections of pancreases that had been fixed first in aldehyde and then postfixed in osmium tetroxide. Ultrathin immunostained sections were poststained with uranyl acetate and lead citrate (osmium tetroxide was not used after the CN). Antiserum to the following peptides were used (with species used to generate antiserum): (1) porcine insulin, pIN (guinea pig). This antiserum reacts with guinea pig B cells (Baskin et al., 1981a); (2) guinea pig insulin, gpIN (rabbit) from Dr. Cecil Yip (Gorray and Fujimoto, 1980); (3) porcine glucagon, pGL (rabbit), from Dr. Thomas Paquette (Asplind et al., 1981); (4) porcine glucagon, pGL (rabbit) from Dr. Robert Mc-Evoy (McEvoy et al., 1977); (5) synthetic somatostatin, SO (rabbit) from Dr. Jennifer Stewart (Penman et al., 1979); (6) porcine pancreatic polypeptide, pPP (rabbit) from Dr. Thomas Paquette (Paquette et al., 1981); (7) bovine pancreatic polypeptide, bPP (rabbit) from Dr. Ronald Chance (Eli Lilly Co.). Primary antisera were used at the following maximum dilutions for light microscopy (LM) and electron microscopy (EM): (1) pIN, 1:100 (LM, EM); (2) gpIN, 1:4,000 (LM), 1:200,000 (EM); (3) pGL, 1:1,000 (LM), 1:4,000 (EM); (4) SO, 1:1,000 (LM), 1:5,000 (EM); (5) pPP, 1:1,000 (LM), 1:4,000 (EM); (6) bPP, 1:1,000 (LM), 1:4,000 (EM). Immunocytochemical controls for each antiserum included absorbing diluted primary antisera with its specific homologous antigen and with each of the other islet peptides (Petrusz et al., 1976). Peptides for absorptions were (1) porcine insulin (Lilly); (2) glucagon (Sigma); (3) somatostatin (Beckman); (4) porcine pancreatic polypeptide (from Dr. Thomas Paquette and Novo Laboratories); (5) bovine pancreatic polypeptide (Dr. Ronald Chance, Lilly). All peptides were added to diluted primary antisera at 50  $\mu$ g/ml, allowed to incubate at 4°C overnight, and centrifuged prior to use. Additional controls included substituting normal guinea pig serum (NGPS) or normal rabbit serum (NRS) (depending upon species from which primary antiserum was obtained) for primary antiserum at the same dilution, diluting primary antisera from 1:50 to 1:10,000, omitting second antibody (sheep antirabbit lgG) or rabbit PAP complex, and staining control sections of rat pancreas. In preliminary studies, methanol-hydrogen peroxide treatment of representative sections (Straus, 1976) was used to block endogenous peroxidase reactivity in islet cells.

# RESULTS

## Light Microscopy

The majority of islet cells stained with antiserum to pIN or gpIN (Fig. 1). The insu-



Fig. 1. Guinea pig islets immunostained for insulin. a) Large islet and portions of two smaller islets. B cells are distributed throughout islet.  $\times 85$ . b) Higher magnification of an islet. Note B cells (B) surrounding nonstaining cells (mostly A cells), and absence of stroma separating islet from exocrine parenchyma (e).  $\times 340$ .



Fig. 2. Guinea pig islets immunostained for glucagon. a) Islet (delineated by dotted line) with A cells distributed throughout islet parenchyma.  $\times 85$ . b) Clusters of A cells (some indicated by arrowpoints) at islet periphery and within islet interior.  $\times 340$ .

lin-positive cells (B cells) were distributed in sheets and cords throughout the islet, with many located at the peripheral margin where the islet contacts exocrine tissue. The B cells were organized into cords or sheets which surrounded A cells. Cells which stained with GL antiserum (A cells) were organized into clusters located throughout the islet (Fig. 2). The A cells were slightly larger and more angular in profile than B cells and were generally separated from exocrine cells by B cells. Antiserum to SO revealed large numbers of angular or crescent-shaped islet cells with elongate processes (Fig. 3). The SO-positive cells (D cells) were located throughout the islet. They possessed elongate processes that appeared to contact islet blood vessels. Antisera to pPP and bPP stained numerous cells (F cells) in the pancreas, but most of the F cells were located outside the islets (Fig. 4). Islet F cells that stained for pPP or bPP were located at or near the periphery of the



Fig. 3. Guinea pig islet immunostained for somatostatin. a) Islet and portion of another (delineated by dotted lines). Note that numerous immunostained D cells are located mainly in islet interior.  $\times 85$ . b) Higher mag-

nification of an islet with immunostained D cells. D cells have elongate processes which contact capillaries (some indicated by arrowpoints). Note lack of stroma separating islet cells from exocrine parenchyma (e).  $\times 340$ .



Fig. 4. Immunocytochemical staining of pancreatic polypeptide in guinea pig pancreas. a) Cells containing pancreatic polypeptidelike immunoreactivity (some indicated by arrowpoints) in islets (delineated by dotted

line) and nonislet parenchyma.  $\times 85$ . b) Higher magnification of an islet with immunostained F cells. Note lack of stroma separating islet cells from exocrine (e) parenchyma.  $\times 340$ .

islets. The F cells usually were elongated, with one end rounded or blunt and the other end a tapering process.

No staining resulted in guinea pig pancreas when antisera were mixed with their respective specific hormones. Extensive absorptions of the pIN antiserum with guinea pig or porcine insulin are described elsewhere (Baskin et al., 1981a). Likewise, staining was abolished when second antibody or PAP was omitted from the immunostaining protocol and when NGPS or NRS was substituted for primary antisera. Dilution series for light microscopy showed that specific staining for all peptides could be detected at dilutions of 1:4,000, whereas background staining disappeared at dilutions of about 1:200. Staining of A cells was intense even at antiserum dilutions of 1:2,000, suggesting that the staining with GL antiserum was not complement-mediated, nonspecific staining (Buffa et al., 1979). The antisera stained appropriate islet cells on sections of rat pancreas, which were always stained in parallel with sections of guinea pig pancreas. The peroxidase-blocking reaction showed that none of the islet cells showed detectable endogenous peroxidase activity by light microscopy.

## Electron Microscopy

Low-magnification electron micrographs of the islet showed three morphologically distinct cell types (Fig. 5). Immunostaining ultrathin sections indicated that the most numerous of these cell types (B cells and A cells) had morphologically distinctive granules. Identification of cells containing somatostatin and pancreatic polypeptide required immunocytochemical staining because the D cell granules were morphologically similar to PP cell granules.

*B cells* contained large numbers of roughly spherical, membrane-bound secretion granules (350–550 nm) with contents of variable electron opacity (Fig. 6a). Most of these vesicles contained a core of light or medium electron opacity, whereas fewer contained a more electron-dense substance which often was fragmented and condensed toward the center of the vesicle. Profiles of the fragmented granules were often cup-shaped or lighter in the center. Frequently, the smaller granules had the most electron-opaque contents. Immunoperoxidase staining of these cells with highly diluted gpIN antiserum (1:200,000) showed the CN reaction product deposited almost exclusively over B cell granules (Fig. 6b). Although we did not make quantitative measurements, it appeared as if granules that were most electron opaque (darkest) showed more immunoperoxidase reaction centers deposited over the granule cores.

The *A cells* contained large numbers of spherical (250–350 nm in diameter), very electron-opaque granules with a sharply defined limiting membrane (Fig. 7a). Immunoperoxidase staining with GL antisera produced CN reaction product only over the granules of these cells and not of any other islet cell type (Fig. 7b).

The *D* cells contained granules which were similar in morphology to those of A cells. However, the D cells were more angular in shape and their nuclei had a more irregular profile. The D cell granules were also slightly smaller (150–250 nm) and distinctly less opaque, and the limiting membrane was usually not as sharply defined as in the A cell granules (Fig. 8). Electron microscopic immunostaining for SO was required for positive identification of D cells (Fig. 9).

The *F cells* were immunostained by both bPP and pPP antisera. It was not possible to distinguish reliably between D and F cells solely on the basis of granule morphology. Both cell types had granules of same approximate size and electron opacity, although the F cell granules (identified on immunostained cells) were slightly smaller (100-200 nm in diameter; Fig. 10). F cells, however, frequently had fewer granules than D cells, and the granules tended to be situated near the periphery of the cell. Immunostained F cells were relatively rare in ultrathin sections of islets.

# DISCUSSION

This study has demonstrated the presence of four distinct peptide cell types in the guinea pig islet on the basis of light and immunoperoxidase electron microscopic staining. The insulin-containing B cells are the most numerous islet cell type in the guinea pig and can be reliably identified by electron microscopy on the basis of granule morphology. It has been estimated that B cells comprise about 78% of the volume of normal guinea pig islets, based on aldehyde fuchsin staining (Petersson et al., 1970). Like the B cells, guinea pig A cells also have a morphologically distinctive granule which is a reliable identifying ultrastructural feature. In contrast, islet D and F cell granules



Fig. 5. Electron micrograph of portion of a guinea pig islet, showing B cells (B), A cell (A), and probable D cell (D).  $\times$ 7,000.

are very similar in morphology and electron opacity. Thus, identification of D and F cells in the guinea pig pancreas by electron microscopy should be considered equivocal unless immunocytochemistry has been used.

The cores of the secretory granules in guinea pig B cells have a highly variable electron opacity and often are fragmented, kidney-shaped, or cup-shaped in electron micrographs. The kidney-shaped core of the fragmented granule has been reported previously (Caramia et al., 1965; Munger and Lang, 1973). The B cell granules do not display the highly crystalloid or angular structure that is characteristic of the B cell granules of other species (Boquist, 1977; Munger, 1981), although there is a suggestion of crystallinity in some granule cores. The lack of a crystalline core has been attributed to the markedly different primary structure and amino acid composition of guinea pig insulin (Munger, 1981). Guinea pigs are thus an exception to the general case in mammals, in which B cell granules usually have a paracrystalline core (Munger et al., 1965; Munger and Lang, 1973).

The granules of A cells are slightly larger and markedly more electron opaque than granules of D and F cells, like the A cell granules of other species (Munger, 1981). We were unable to distinguish the three classes of A cells in the guinea pig islets described by Caramia et al. (1965) on the basis of variable granule diameters and morphological features. Their A<sub>a</sub> and A<sub>b</sub> types appear to correspond to typical islet A cells which contain granules with glucagonlike immunoreactivity. The cell type that they called  $A_c$ cell resembles morphologically cells containing the smaller granules that stained for pancreatic polypeptide. The variable diameters of granules described by Caramia et al. (1965) in different guinea pig A cells was challenged by Bencosme and Lechago (1968), who were unable to document this finding with quantitative studies on granule size. The identity of secretory granules of small size in supposed A cells has remained unanswered (Munger and Lang, 1973). However, it appears from our immunostaining results that these small granules contain pancreatic polypeptide and are thus diagnostic of F cells. Since Caramia et al. (1965) also reported that  $A_c$  cells were very rare in islets, it seems likely that their A<sub>c</sub> cell type corresponds to the F cell, which is very infrequently found

in electron micrographs of islets immunostained for pancreatic polypeptide.

D cells of the guinea pig islets have numerous granules that are slightly smaller and of lower opacity than A cells, but these cells cannot be identified with certainty in the absence of immunostaining. Their granules closely resemble those of F cells (which contain pancreatic polypeptidelike immunoreactivity), being only slightly larger than F cell granules. However, the elongated processes and angular shape assist in tentative recognition of D cells in the absence of immunostaining. Thus, the cell identified by Caramia et al. (1965) as a D cell could be either a D cell or a cell containing pancreatic polypeptide. This is unlike the situation in other species, where cells with pancreatic polypeptide often can be distinguished from other islet cells on the basis of granule characteristics (Larsson et al., 1976; Forssman et al., 1977). However, since D cells are very numerous in guinea pig islets, whereas islet F cells are very scarce, most islet cells encountered with this granule morphology are probably D cells rather than F cells.

With their elongated processes, guinea pig islet D cells resemble somatostatin cells of the rat gastric mucosa, whose elongated processes "contact" exocrine cells (Larsson et al., 1979; Alumets et al., 1979). In contrast to these gastric D cells, however, the processes of D cells in guinea pig islets end adjacent to capillaries in the islet rather than contacting other endocrine cells. The elongated shape of these islet D cells may only be a consequence of their being intercalated between B cells or A cells, and have no functional significance related to regulation of A cells or B cells. Although we have not made quantitative measurements on D cell numbers, our visually subjective evaluation suggests that guinea pig islets have a much larger D cell population than is characteristic of rat or human islets (Orci, 1981). The physiological significance of the large numbers of D cells in guinea pig islets is not understood.

The topographical distribution of the endocrine cells in the guinea pig islet is markedly different than that found in rats, humans, and many other mammals, where A cells and D cells are located principally at the islet periphery (Epple and Lewis, 1973; Lacy and Greider, 1979). It has been suggested that the arrangement of islet cells in rats and humans is related to microcirculation of the



islets, presumably reflecting some functional control of A cells and D cells over B cell secretion (Unger and Orci, 1976, 1977). However, microvascular cast and serial reconstruction studies of the rat islet have questioned the validity of this hypothesis (Bonner-Weir and Orci, 1982). The anatomical arrangement of guinea pig islet cells may reflect a different intraislet regulatory pattern and islet microcirculation, as has been suggested for horse islets, which resemble guinea pig islets in the arrangement of islet endocrine cells (Fujita, 1973). However, distinctly different patterns of islet cell topography are associated with rich vascularization in the islets of various mammals. An alternate hypothesis suggests that the topographical distribution of islet cells is based on patterns of intracellular communication among islet cells, and, hence, on the integrated functioning of islets (Appel and Like, 1982). The possibility remains that the islet organization in guinea pig may be related to intercellular communication and paracrine interactions of the islet cells.

Recent morphological studies on guinea pig islets are conspicuous for having used classical tinctorial and morphological criteria as a basis for identifying islet cell types, whereas islet cell types in other common laboratory animals are now almost universally demonstrated by immunocytochemical staining. Part of the reason for this situation with respect to B cells is probably based on a report that guinea pig B cells did not stain with a fluorescent antibody technique (Lacy, 1957), and also because the differences in antigenicity (Moloney and Coval, 1955) and peptide

structure (Smith, 1966; Humbel et al., 1972) of guinea pig insulin compared with other mammalian insulins suggested that guinea pig B cells would probably not react with antisera to the insulins of other mammals. However, we recently demonstrated that guinea pig B cells can be identified by immunoperoxidase staining with light and electron microscopy, using not only antisera to guinea pig insulin but, in certain cases, also antisera to porcine insulin (Baskin et al., 1981a, 1982). Since the present work shows that all four guinea pig islet cell types can be easily demonstrated by immunoperoxidase staining, morphological studies on guinea pig islet cells should rely on immunocytochemistry for identification of islet cell types.

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Fig. 7. Electron micrographs of guinea pig A cells. a) A cell (A) granules with uranyl acetate, lead citrate staining. Adjacent B cell (B) granules are shown for comparison. Note characteristic fragmented, cup-shaped B cell granule (arrowpoint).  $\times 18,000$ . b) Electron micrograph of A cell granules immunostained for glucagonlike immunoreactivity. The paler granules (several indicated by arrowpoints) show fewer reaction foci. Darkest A cell granules show most intense immunostaining reaction.  $\times 24,000$ .

Fig. 6. Electron micrographs of guinea pig B cells. a) Variable size and opacity of B cell granules. Arrowpoint indicates characteristic fragmented B cell granule with cup-shaped core. Uranyl acetate, lead citrate.  $\times 18,000$ . b) Electron micrograph of B cell immunostained with antiserum to guinea pig insulin. Reaction product foci (several examples indicated by arrowpoints) are deposited exclusively over B cell granule cores. The paler granules show fewer reaction foci.  $\times 24,000$ .



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Fig. 8. Electron micrograph of D cell (D) immunostained with antiserum to somatostatin. Heavy immunoperoxidase deposits almost obscure D cell granules. Granules in adjacent A cell (A) do not stain.  $\times 18,000$ .

Fig. 9. Electron micrograph of immunostained D cell granules. Reaction product is deposited principally over granules having lower electron opacity and smaller size than the granules of A cells.  $\times 27,500$ .

Fig. 10. Electron micrograph of an islet F cell immunostained with antiserum to bovine pancreatic polypeptide. Reaction product is deposited over granules that resemble D cell granules, but are slightly smaller in diameter. ×27,500.

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