# Morphological Evidence for Pancreatic Polarity of β-Cell Within Islets of Langerhans

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**Reconstructions from serial paraffin sections were used to define the relationships of the pancreatic endocrine cells and the microvasculature of the islets of Langerhans of the rat. We have reported that the afferent vessel to an islet, an arteriole, enters the islet directly in the β-cell core. Immediately after entering the islet, this arteriole branches into capillaries. Herein, we report that β-cells have two capillary faces. When viewed in cross section, 8–10 β-cells form a tubelike structure around a central capillary. The outer side of each β-cell also abuts a capillary. A clear polarity of secretory granules was seen on the ultrastructural level when the β-cells were partially degranulated by prior in vivo treatment with glyburide. Of the remaining granules, 75.7 ± 1.7% were accumulated in the apical portion of the cell, even though this portion was only 50.4 ± 3.2% of the cytoplasm. β-Cells around a particular cross-sectioned capillary shared the same orientation of granular accumulation. These findings provide morphological evidence of in situ β-cell polarity that could be an anatomical basis for functional compartmentalization. *Diabetes* 37:616–21, 1988**

The islet of Langerhans is a microorgan with an organization that is both complex and functionally significant (1,2). The morphological evidence suggests that the four islet cell types [α,β,δ, and pancreatic polypeptide (PP)] have a nonrandom organization within the islet (1). Islet hormones have been shown to be capable of influencing the secretion of other islet cells, even though many of these potential interactions may not occur physiologically (for review see ref. 2).

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It has been found in the isolated perfused canine pancreas that glucagon (3) and somatostatin (4), at concentrations that are only a small fraction of their estimated intraislet concentration, have profound effects on islet cell functions. In addition, in humans it has been suggested that relatively small amounts of insulin can suppress insulin secretion (5). These findings led to the hypothesis that the interstitial space within the islet is compartmentalized such that an exocytotic-venous capillary pathway is separate from an arterial-receptor pathway (4). Tight junctions were suggested as possibly sealing off areas of interstitial space. However, tight junctions, although common and extensive in isolated islets (6), are uncommon and small in islets fixed in situ (7,8). Therefore, they are less likely to be the anatomical basis for compartmentalization.

The islets have a direct arterial blood supply and are so well vascularized that the mass of islet capillaries often is described as glomerular-like (9). The afferent vessel, an arteriole, enters the islet at a gap in the noncontinuous mantle of non-β- (α- δ-, and PP-) cells and plunges directly into the β-cell core (10). Immediately after entering the islet, the arteriole branches into several capillaries that follow tortuous paths and branch occasionally with the β-cell core. Because the arrangement of the islet cells and the microvessels had not been well defined, we analyzed this arrangement at the light- and electron-microscopic levels in an effort to determine whether islet architecture provides an anatomical basis for intraislet compartmentalization.

## MATERIALS AND METHODS

Male Sprague-Dawley rats (Flow, Dominion, VA) weighing 250–350 g were heparinized by tail vein injection and then anesthetized with an overdose of amobarbital (1.2 ml/100 mg i.p.). Three pancreases were infused with black india ink (Pelican ink) via a cannulated aorta. There was little resistance of this aqueous solution to the slow pushing of a hand-held syringe. These perfused pancreases were excised and fixed in Bouin's fixative overnight, embedded in paraffin, and serially sectioned at 5 μm. Sections were stained by the immunoperoxidase method of Sternberger (11) with a cock-

tail of antisera (rabbit antisynthetic somatostatin, our D-10, 1:1500 final dilution; rabbit anti-bovine PP, 1:3000 final dilution, gift of R. Chance, Lilly, Indianapolis, IN; rabbit anti-porcine glucagon, 1:3000 final dilution, gift of M. Appel, Univ. of Massachusetts, Worcester, MA) to stain the non-β-cells, as previously reported (12). For light-microscopic reconstruction, sections of 14 islets were photographed. Tracings of these islets were made at ×225 (final) on transparent acetate sheets and were superimposed for analysis.

For the ultrastructural studies, six animals were given glyburide (3 mg/kg i.p., Upjohn, Kalamazoo, MI) 24 and 3 h before being killed to partially degranulate the β-cells. Pancreatic tissue was fixed in 2% glutaraldehyde and 0.1 M phosphate buffer at pH 7.4, postosmicated, and then stained en bloc with uranyl acetate. Islets were sectioned at several steps to do partial reconstructions at the ultrastructural level. The tissue sections on copper grids were stained with uranyl acetate and lead citrate and viewed on a Zeiss EMIOCA microscope.

For morphometric studies on distribution of granules, micrographs (×4800 or ×9500 final magnification) of β-cells spanning two capillaries were chosen for quantification. An arbitrary line was drawn at the approximate middle of the cell. This line was tangent to the nucleus and parallel to the cell surface facing the capillary; the portion without the nucleus was called the *apical portion*. Granules were counted on the micrographs. Photocopies of the micrographs were cut appropriately and weighed to quantify the proportions of cross-sectional area [percent of portion = weight of portion/(weight of whole section of individual cell − weight of nucleus)].

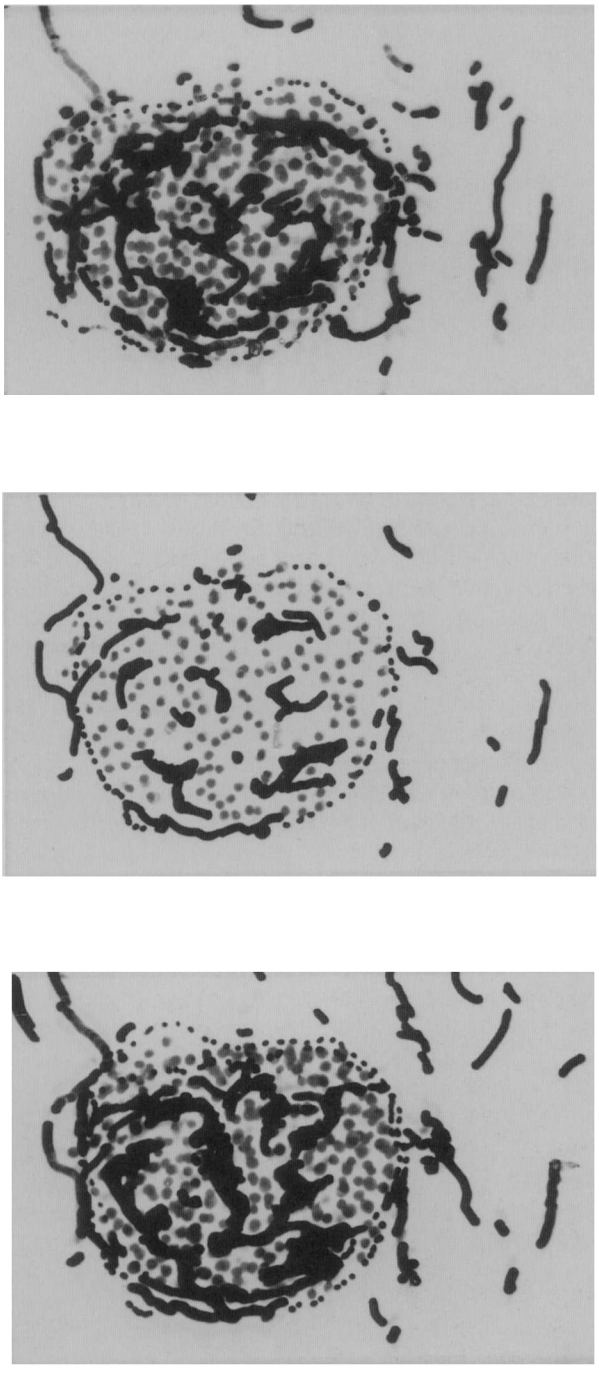


**FIG. 1. Micrographs of composite tracings of 1 islet such as were used in light-microscopic reconstructions. *Black lines* are vessels injected with India ink; *gray dots* are nuclear profiles; *small black dots* delineate border of islet. *B*: composite tracing from 3 serial sections of islet. *A*: composite of the 3 preceding serial sections with *tracing B* overlaid. *C*: composite from the 3 serial sections after composite with *tracing B* overlaid. *A* and *C* begin to give 3-dimensionality, whereas *B* shows cross-sectional arrays. Note rosettelike array in *B*, pattern seen also in Fig. 3.**

## RESULTS

**Light-microscope reconstructions.** Reconstructions of islets perfused with india ink were initially used to see the relationship of islet cells to the islet capillaries on the light-microscopic level. Composite tracings of adjacent serial sections revealed four patterns of islet endocrine cells and capillaries (Fig. 1), i.e., *1*) a single row of cells between two

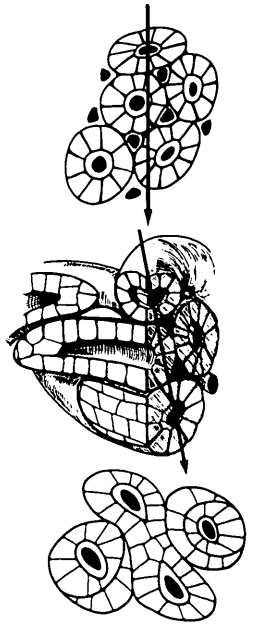

**FIG. 2. Diagram of model that accommodates patterns of islet cells and capillaries seen in randomly sectioned tissue. Profiles seen with different planes of section of basic unit of tubule of β-cells surrounding central capillary are shown. *Top*: section perpendicular to central capillary. *Middle*: section parallel to same central capillary. *Bottom*: section obliquely cut to central capillary. Each β-cell faces 2 capillaries.**

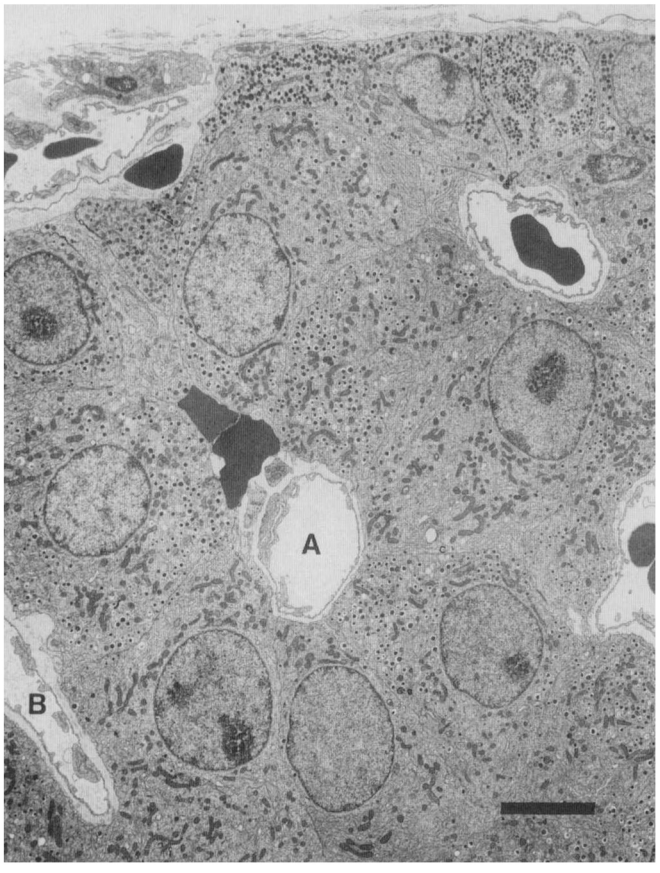


**FIG. 3. Micrograph from glyburide-treated rat showing rings or rosettes of β-cells surrounding capillary *A*. β-Cells face 2 capillaries. By reconstruction of this islet on ultrastructural level, connection between capillary *B* and arteriole entering islet has been traced. Therefore, capillary *B* is considered an arterial capillary. Although capillary *A* is only incompletely traced in this islet, it is presumably a venous capillary based on other islet reconstructions. *Bar* = 5 μm.**

capillaries, *2*) a double row of cells between two capillaries, *3*) a ring of cells around a cross-sectioned capillary, and *4*) a clump of cells between two capillaries. Because the capillaries have previously been shown to follow a tortuous path traversing the β-cell core, it is unlikely that any section of an islet would show only one of these patterns. In fact, at least two of the above patterns were seen in any section. The distances between capillaries and the patterns of islet cell nuclei between the capillaries are consistent with each β-cell having two capillary faces. A three-dimensional model that best accommodates all the data is that of a tubular array of 8–10 β-cells surrounding a central capillary (Fig. 2). The outer side of each cell also faces another capillary, which is usually not in cross section.

**Ultrastructural studies.** At the ultrastructural level, the β-cells were seen to fit the configurations described in Fig. 2. In some sections the tubular pattern was seen (Fig. 3). In other sections the lengthwise aspect of a capillary was seen with "cords" of β-cells along each side. Fortuitous sections showed β-cells spanning two capillaries (Fig. 4). The capillaries could be followed by step sectioning through an islet. They were continuous, with bends at every four to five β-cells. We have not been able to distinguish morphological differences between the capillaries. All are fenestrated and have occasional pericytes. However, at times the connections between capillaries of a tubular array of β-cells could be traced in reconstructions to the arteriole entering the islet (as in the islet shown in Fig. 3). In such cases, the capillaries traced were the peripheral rather than the central ones.

TABLE 1
Distribution of granules in partially degranulated β-cells

| | Apical | Basal |
|---|---|---|
| Total granules (%; $n=36$ cells) | 75.7 ± 1.7* | 24.3 ± 1.7* |
| Total cytoplasm (%; $n=32$ cells) | 50.4 ± 3.2 | 49.6 ± 3.3 |

Values are means ± SE. See MATERIALS AND METHODS for procedures used for morphometric quantification.
*$2P < .001$ compared with portion of cytoplasm, via 2-tailed Student's $t$ test.

In a normal β-cell, insulin granules fill the cytoplasm, and no obvious polarity is evident. However, after treatment with glyburide, the β-cells were considerably degranulated and a clear polarity of insulin granules was seen (Fig. 5). In cellular profiles spanning capillaries, the nucleus is closer to one capillary face, arbitrarily designated the *basal face*. At the opposite face, designated *apical*, the remaining insulin granules were clumped. With quantification the basal and apical portions of the cytoplasm were shown to be equal in proportion (Table 1). However, the insulin granules were unequally distributed and disproportionate in the apical portion. All the β-cells around a particular cross-sectioned capillary showed the same polarity. The apical side faced the central capillary.

The arrangement of the non-β- (α-, δ-, and PP-) cells and the capillaries is less clear cut. The mantle of non-β-cells often appears columnar or cordlike in section, with their interior aspects along a fenestrated capillary, but it is unclear whether the non-β-cells have two faces on blood vessels. Often the exterior aspect of the non-β-cell is in contact with an area of interstitial space bounded by the islet capsule, a thin layer of fibroblasts, and collagen. Collecting or postcapillary venules, without fenestrations and with almost continuous pericyte layers, pass through this bounded space; some of these venules are also found in indentations of the islet.

## DISCUSSION

Although most previous studies of islet organization have dealt only in two-dimensional terms, Goldstein and Davis (13) made three-dimensional reconstructions and concluded that islets are "best visualized as being made up of a compact mass of cells pervaded by a network of capillaries." However, this lack of organization of the islet does not fit well with our current concepts of structural and functional relationships. On the other hand, with our basic observation that each β-cell has two capillary faces, a three-dimensional model of the islet endocrine cells and microvasculature can be devised (Fig. 2). Of course, not every image of a sectioned β-cell will show both capillary faces because of the geometry and size of the components. (The β-cell is an ~13 × 13 × 8-μm truncated pyramid, and the capillary is a 5-μm-diam cylinder, so there would be many sec-

tions of a β-cell that did not show its capillary.) The richness of the vascularization of the islet that has previously been observed may be partially attributable to the dual capillary faces of the β-cells. The arrangement of the non-β-cells is still unresolved.

Polarity of β-cells is not a new or unexpected concept, but this study is the first direct evidence of polarity of β-cells in situ within islets of Langerhans. One of the essential features of secretory epithelial cells is their polarization. The secretory and sensory poles are usually luminal and adluminal, respectively. In the literature there have been numerous observations of a polarization of β-granules in islets toward "the capillary pole" (14–17). More recently, Lombardi et al. (18) used the asymmetrical budding of two RNA viruses to show that neonatal islet cells in monolayer cultures have polarization of plasma membrane domains. Within the native islet, the β-cells are usually highly granulated, with an estimated 13,000 granules per β-cell throughout the cell (19). It is easy to appreciate how difficult it would be to see a polarization of granules in a well-granulated β-cell. Although the polarity of granules in this study may result from glyburide's dual role as stimulator of insulin release and as inhibitor of insulin biosynthesis, the phenomenon is probably more general, because none of the previous examples used sulfonylureas (14–17). In this report we have visually (Fig. 5) and morphometrically (Table 1) shown that such a polarization occurs at one of two capillary poles arbitrarily designated the apical pole.

We are still confronted with the question of whether there is an anatomical basis for compartmentalization. A strict definition of structural compartmentalization implies physical barriers either by a dual circulation system or by extensive tight junctional sealing of interstitial spaces. We found no evidence of the former; in fact, we were convinced of the continuity of the capillaries. There are bends within the islets and occasional branches within the β-cell core, but there seems to be one continuous system throughout an islet. Two reports with freeze fracture have found few tight junctions in rat islet tissue fixed in situ rather than their frequent occurrences after collagenase-isolation procedures (7,8). Therefore, the evidence is against a strictly defined structural compartmentalization.

However, an anatomical basis for a more loosely defined functional compartmentalization can be envisioned. This basis is twofold, the polarity of the hormone-secreting cells and the microvasculature that imposes a directional flow of blood through the islet. The polarity of the hormone-secreting cells would allow a topographic separation of an apical secretory surface and a basal sensing surface, thus allowing a more subtle discrimination of low levels of hormones without the swamping of locally secreted hormones. Although we have shown such a polarity only for the β-cell, it cannot be ruled out for α- and δ-cells. The second integral part of the anatomical basis for a functional compartmentalization is the microvascular arrangement such that the blood flow is from the point of arteriole entry across the islet in a directional

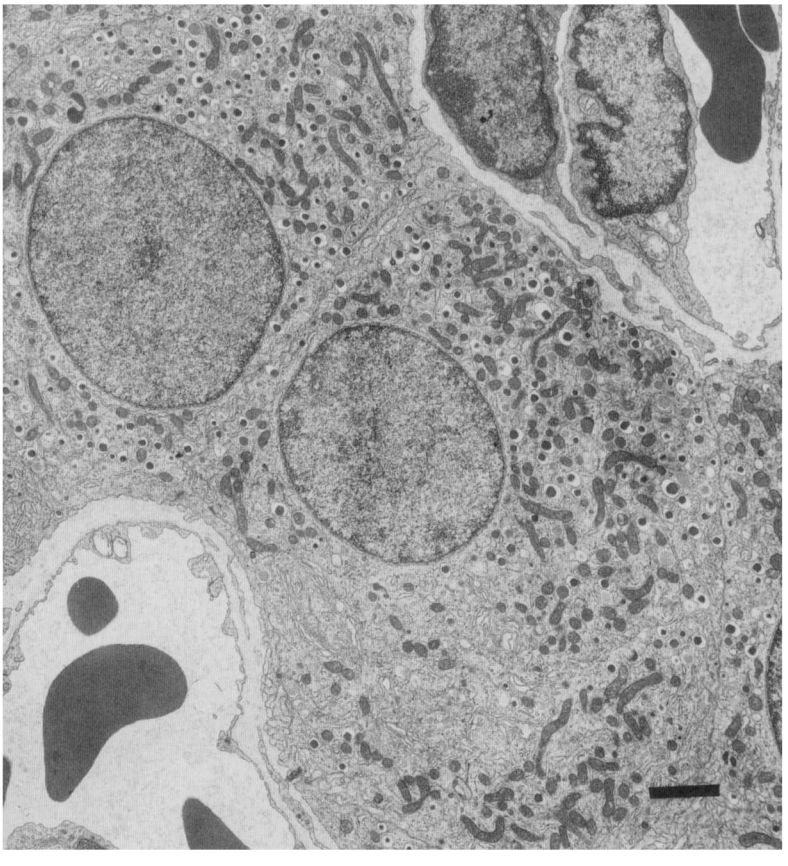

**FIG. 4. Micrograph from glyburide-treated rat showing β-cells have 2 capillary faces and polarity in accumulation of residual granules. *Bar* = 2 μm.**

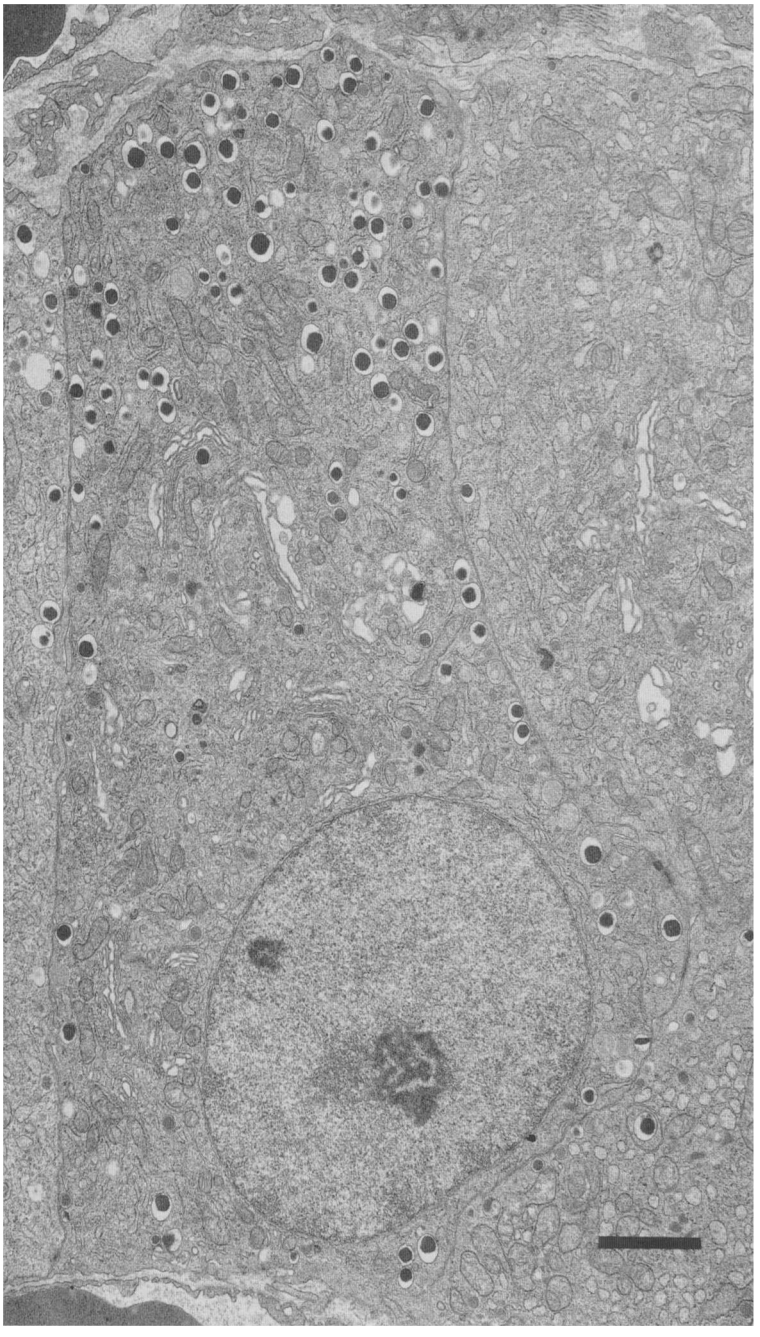

**FIG. 5. Micrograph showing polarity of granules accumulated at apical end of β-cell. *Bar* = 2 μm.**

sense. This directionality of blood flow was shown dynamically with the passing of a bolus of dye in the islet of a living rabbit (20). A consequence of this directional blood flow is that the islet hormone-secreting cells are perfused in a sequence of β-cells and then the non-β-cells. Maruyama et al. (21) showed, by perfusing anti-insulin antibody, which remained in the islet microvasculature, that blood-borne insulin normally restrains glucagon secretion. The concept of the functional compartmentalization based on the sequence of perfusion of the islet cells has been further supported by the retrograde perfusion studies of Stagner and Samols (22), although similar experiments recently published by Kawai et al. (13) inexplicably give differing results.

In conclusion, this study provides morphological evidence of in situ β-cell polarity that could be the anatomical basis for a functional compartmentalization of the islet in conjunction with the previously reported islet microvasular pattern. Thus, regulation of intraislet interactions is determined to a certain degree by constraints imposed by the three-dimensional architecture of the islets of Langerhans.

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## REFERENCES

1. Orci L, Unger RH: Functional subdivision of islets of Langerhans and possible role of D-cells. *Lancet* 2:1243–44, 1975
2. Samols E, Bonner-Weir S, Weir GC: Intra-islet insulin-glucagon-somatostatin relationships. *Clin Endocrinol Metab* 15:33–58, 1986
3. Samols E, Harrison J: Intraislet negative insulin-glucagon feedback. *Metabolism* 25:1443–47, 1976
4. Kawai K, Ipp E, Orci L, Perrelet A, Unger RH: Circulating somatostatin acts on the islets of Langerhans by way of a somatostatin-poor compartment. *Science* 218:477–78, 1982
5. Asplin CM, Paquette TL, Palmer JP: In vivo inhibition of glucagon secretion by paracrine beta cell activity in man. *J Clin Invest* 68:314–18, 1981
6. Orci L: The microanatomy of the islets of Langerhans. *Metabolism* 25:1303–13, 1976
7. In't Veld PA, Pipeleers DG, Gepts W: Evidence against the presence of tight junctions in normal endocrine pancreas. *Diabetes* 33:101–104, 1984
8. Yamamoto M, Kataoka K: A comparative study on the intercellular canalicular system and intercellular junctions in the pancreatic islets of some rodents. *Arch Histol Jpn* 47:485–93, 1984
9. Wharton GR: The blood supply of the pancreas, with special reference to that of the islands of Langerhans. *Anat Rec* 53:55–81, 1932
10. Bonner-Weir S, Orci L: New perspectives on the microvasculature of the islets of Langerhans in the rat. *Diabetes* 31:883–89, 1982
11. Sternberger LA: *Immunocytochemistry*. Englewood Cliffs, NJ, Prentice-Hall, 1974
12. Bonner-Weir S, Trent DF, Honey RN, Weir GS: Responses of neonatal rat islets to streptozotocin: limited β-cell regeneration and hyperglycemia. *Diabetes* 30:64–69, 1981
13. Goldstein MB, Davis EA Jr: The three dimensional architecture of the islets of Langerhans. *Acta Anat* 71:161–71, 1968
14. Ferreira D: L'ultrastructure des cellules du pancreas endocrine chez l'embryou et le rat nouveau-ne. *J Ultrastruct Res* 1:14–25, 1957
15. Bencosme SA, Pease DC: Electron microscopy of the pancreatic islets. *Endocrinology* 63:1–13, 1958
16. Hellerstrom C: Growth pattern of pancreatic islets in animals. In *The Diabetic Pancreas*. Volk BW, Wellman KF, Eds. New York, Plenum, 1977, p. 73
17. Like AA: Spontaneous diabetes in animals. In *The Diabetic Pancreas*. Volk BW, Wellman KF, Eds. New York, Plenum, 1977, p. 390
18. Lombardi T, Montesano R, Wohlwend A, Amherdt M, Vassalli J-D, Orci L: Evidence for polarisation of plasma membrane domains in pancreatic endocrine cells. *Nature* (Lond) 313:694–96, 1985
19. Dean PM: Ultrastructural morphometry of the pancreatic β-cell. *Diabetologia* 9:115–19, 1973
20. Fraser PA, Henderson JR: The arrangement of endocrine and exocrine pancreatic microcirculation observed in the living rabbit. *Q J Exp Physiol* 65:151–58, 1980
21. Maruyama H, Hisatomi A, Orci L, Grodsky GM, Unger RH: Insulin within islet is a physiologic glucagon release inhibitor. *J Clin Invest* 74:2296–99, 1984
22. Stagner JI, Samols E: Retrograde perfusion as a model for testing the relative effects of glucose versus insulin on the cell. *J Clin Invest* 77:1034–37, 1986
23. Kawai K, Chiba Y, Okuda Y, Yamashita K: Hormone release from pancreatic islets perfused from venous side. *Diabetes* 36:256–60, 1987