

Close association of centroacinar/ductular and insular cells in the rat pancreas

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Summary. Close contacts between endocrine insular cells and exocrine acinar, centroacinar and ductular cells occur frequently in the rat pancreas as seen by both light and electron microscopy. Islets of Langerhans are surrounded incompletely by a thin connective tissue capsule or mantle but numerous exocrine-endocrine cell contacts occur at the periphery, which is irregular with considerable "intermingling" of the two cell types. Centroacinar and ductular cells are seen to be in contact with all endocrine cell types but most commonly insulin-secreting B-cells. The basal surface of centroacinar cells in the region of contact may be extensive, sometimes with overlap of basal processes of these cells and their lateral extension between acinar and insular cells. The areas of contact contain no connective tissue or basal lamina and show no surface specializations. The presence of both the "open" and "closed" type of enteroendocrine cells within acini is confirmed, some also being in contact with centroacinar cells. The functional significance of these exo-endocrine cell contacts is discussed in terms of the endocrine-acinar portal system, possible direct paracrine secretion, compartmentalization within the islet, and the known effects of islet hormones on exocrine secretion. Also relevant is the developmental origin of islets from ductal tissue and the cellular origin of some tumours, e.g., insulinomas, from duct cells.

Key words: Pancreas - Centroacinar cells - Islets of Langerhans

Introduction

Langerhans (1869) provided the first detailed description of the histology of the pancreas, suggesting that the gland was racemose in type with scattered collections or islets of cells. Since that time, the gland has been studied extensively and its morphology and functions are well known. Most studies, however, have been concerned either with the exocrine or the endocrine tissue and, as pointed out recently (Leeson and Leeson, 1985), little attention has been given either to exocrine-endocrine relationships or to the nature of the connection between exocrine acini and the duct system. That study (Leeson and Leeson, 1985) confirmed that the rat exocrine pancreas is a compound, tubuloalveolar gland, racemose in type, with acini draining singly or in small groups via short branches to intercalated ducts. Centroacinar cells, the terminal cells of the duct system that are invaginated partially into acini, are in contact with both the lumen and the basal lamina of the secretory unit and show terminal processes or pseudopodia that extend between proximal acinar cells in a complex manner. Additionally, the study indicated that, in some regions, islet cells show close contact not only with acinar cells but also with centroacinar and ductular cells. In that pancreatic hormones are known to influence exocrine secretion of both enzymes (from acinar cells) and an alkaline fluid rich in sodium bicarbonate (from centroacinar and ductular cells), it appeared important to investigate further the extent of this close association and the possibility of a direct paracrine secretory mechanism.

A few previous studies have demonstrated a close relationship between centroacinar/ductular cells and endocrine insular cells. In a light microscopy

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study of islet cells as a component of pancreatic ductal neoplasms in the hamster, Pour (1978) reported that a meshwork of fine ductules surrounded islets and extended within islets as tiny channels. He demonstrated hyperplasia of the intercalated ducts and the formation of new islets. Sacchi et al. (1979), in a study of functioning human insulinomas, published an illustration of a ductular cell in direct contact with islet cells and an illustration showing contact between a centroacinar cell and A-cells in the starling has been published by Williams and Kendall (1982). Bendayan (1982), in human and rat, demonstrated close contacts between acinar and endocrine cells and pointed out that pancreatic hormones have been shown to influence exocrine activity in both normal and diabetic conditions. In a study of three cases of mixed ductuloinsular tumours of the pancreas, Reid et al. (1982) emphasized the origin of islets from ducts. Thus, while the association between centroacinar-ductular cells and insular tissue is known in both normal and pathological situations, the morphological association of the two cell types has been reported only infrequently and the extent of this relationship is unknown. This study was undertaken in an attempt to establish the frequency of such a close morphological association, its extent and nature.

Materials and methods

Eight young adult rats of 50 to 100 days, four males and four unmated females, of the Wistar strain and weighing from 150 to 200 gm were fed food and water ad libitum. All were anesthetized at about 0900 h with ether, the thoraces opened and the animals perfused with fixative via the left ventricle. The fixative was 3% glutaraldehyde plus 3% formaldehyde in 0.1 M cacodylate buffer at pH 7.2. Pancreatic tissue was removed, minced, and placed in fresh fixative for two hours. For light microscopy, some of the tissue was dehydrated in graded ethanols and infiltrated and embedded in glycol methacrylate (Leeson and Rennie, 1973). After trimming, blocks were cut at two micrometers on a JB4A Porter-Blum microtome, the sections flattened in a bath of 5% ethanol, picked up on clean slides and dried briefly on a hot plate. Sections were stained by various methods including hematoxylin and eosin, Masson's trichrome, periodic-acid Schiff, cresol violet, Mallory azan, silver impregnation (Kodousek, 1982), and by methylene blue-basic fuchsin (Leeson and Venketrman, 1984). For electron microscopy, tissue was washed in buffer, placed for an hour in 1% buffered osmium tetroxide (Millonig, 1961), rinsed in buffer, dehydrated through graded ethanols over two hours, and infiltrated and embedded in a standard Epon-araldite mixture. Ultrathin sections were cut on a Porter-Blum II ultramicrotome and stained with uranyl acetate and lead citrate (Reynolds, 1963). Electron microscopy was performed with a Philips EM 410 at 80 KV.

Results

Light Microscopy

Lobules of the pancreas are delineated by thin, delicate interlobular connective tissue. Within lobules, acini are packed closely in an irregular fashion with sparse connective tissue between and around them, this connective tissue containing numerous blood capillaries. Ducts are not prominent and larger blood vessels, of the order of arterioles (Fig. 3) and venules, lie adjacent to ducts and to islets. Islets, varying in size dependent upon the plane of section, are scattered throughout the gland. Connective tissue is sparse within islets and associated mainly with blood vessels of the islets (Fig. 1) and, in most, appears as a thin "capsule" or mantle delineating islets from surrounding exocrine tissue. However, this capsule in many islets appears incomplete with regions of limited extent where acinar and insular tissues are in close contact and apparently not separated by connective tissue (Figs. 2-5). Additionally there are quite frequently regions of close opposition between insular cells and centroacinar cells (Figs. 2-5) and intercalated ducts (Figs. 2, 5) at the circumference of islets. Rarely, a larger intralobular duct lies closely adjacent to an islet.

Electron Microscopy

In most areas, small amounts of connective tissue intervene between exocrine and endocrine tissue. This connective tissue consists of small bundles of collagen microfibrils with occasional elongated fibroblasts and, usually, contains capillary blood vessels. However, in many sections there are areas of close contact between exocrine, acinar cells and endocrine cells of islets, without any intervening connective tissue or basal lamina (Figs. 6, 7, 9, 11). Often, the endocrine-exocrine interface is highly irregular with one or more insular cells interposed between acinar cells (Figs. 6, 9, 11, 14). Where centroacinar cells are present in peri-insular acini, they are often separated from islet cells by small amounts of connective tissue, usually with associated sinusoidal capillary blood vessels within the connective tissue. Similarly, small intercalated ducts infrequently lie adjacent to islets but separated by small amounts of connective tissue. However, in many cases, centroacinar cells that lie between acinar cells and which are usually seen extending to the acinar lumen, show bases that are in direct contact with islet tissue (Figs. 6-13, 18). In such a location, centroacinar cells occur singly or as two, rarely more, adjacent cells; the cells usually being pyramidal with a narrow apex abutting on the acinar lumen and showing a few microvilli. Lateral borders make interfaces with acinar cells and show junctional complexes near the luminal border, often with scattered spot desmosomes. Basal surfaces lie adjacent to insular cells (Figs. 6, 7). The cells show all features of centroacinar cells as previously described (Ekholm et al., 1962; Ichikawa, 1965; Leeson and Leeson, 1985) including inconspicuous organelles, both primary and secondary lysosomes (Figs. 10, 17), and occasional apical cilia (Fig. 16).

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However, variations in shape and relationships do vary. The basal surface in contact with endocrine cells may be very slender (Fig. 6) or very extensive (Figs. 7, 9, 10, 14-17), or may in part be related to connective tissue (Fig. 18). Frequently, the basal region of one or more centroacinar cells shows a large, foot-like process that passes deeply to lie between endocrine cells (Figs. 9, 11, 14) or between acinar and endocrine cells (Figs. 10, 12), making close contact with them. Quite commonly, adjacent centroacinar cells show complex interdigitation (Figs. 10, 14) and overlapping of basal processes (Figs. 16, 17), often with numerous spot desmosomes on the centroacinar-centroacinar interface (Fig. 16). Obviously, due to the plane of section,

Abbreviations

A = A cell of islet	d = spot desmosome
a = arteriole	E = acinar cell
B = B cell of islet	I = intercalated duct
b = basal lamina	L = lumen
C = centroacinar cell	n, N = nucleus
D = D cell of islet	T = connective tissue
arrow = close contact. centroacinar-islet cell	
arrowhead = close contact. acinar-islet cell	

Fig. 1. Photomicrograph of an islet (center) surrounded by acinar tissue. Thin connective tissue delineates acini and separates insular from endocrine tissue. Within the islet, it is associated mainly with blood vessels. Periodic acid Schiff stain. x 350

Fig. 2. Photomicrograph showing acinar and insular cells in close contact (arrowheads), also with close contact between centroacinar-insular cells (arrows) and with an intercalated duct (I). H.E. x 450

Fig. 3. Photomicrograph showing close contact between islet and acinar cells (arrowhead) and between centroacinar and islet cells (arrow). Note the arteriole (a). Methylene blue-basic fuchsin. x 1,200

Fig. 4. Centroacinar cells (nuclei labelled 'n') pass to an intercalated duct (I) with close contacts between acinar-insular (arrowheads) and centroacinar-insular (arrow) cells. Methylene blue-basic fuchsin. x 1,200

Fig. 5. Close contacts are seen between insular-acinar (arrowhead), insular-centroacinar (arrows) and insular-intercalated duct (I) (asterisk) cells. H.E. x 1,200

Fig. 6. Electron micrograph showing (lower left) the lumen (L) of an acinus bordered by two acinar (E) and two centroacinar (C) cells, one showing a nucleus (n), the other as a thin slip of cytoplasm extending between the two acinar cells. The base of this cell shows a small area of direct contact (arrow) with an islet A-cell that also makes contact with the two acinar cells (arrowheads). Other A cells are also in contact (open arrowheads) with an acinar cell. x 7,500

Fig. 7. The lumen (L) of an acinus appears above, bordered by two acinar (E) and two centroacinar cells (C), the lower one pyramidal and with its base showing close contact (arrows) with B-cells below. Close contact also occurs between one B-cell and an acinar cell (arrowhead). x 7,500

Fig. 8. A higher magnification of Figure 7 showing the interface between the base of the centroacinar cell and a B-cell, with no surface specialization. x 15,000

Fig. 9. An acinar lumen (L) is bordered (below) by two centroacinar cells, the base of each making contact with B-cells. One centroacinar cell shows a basal tongue of cytoplasm extending between B-cells (arrow). x 7,500

Fig. 10. Similar to Figure 9, but showing parts of 3 centroacinar cells with a cytoplasmic extension from a fourth (below, C₄) with complex interdigitation with its adjacent cell. x 17,500

Fig. 11. A centroacinar cell here makes contact with A-cells. x 7,500

Fig. 12. A higher magnification of part of Figure 11 showing a basal cytoplasmic extension of the centroacinar cell between acinar (E) and A-cells. x 24,500

Fig. 13. The base of a centroacinar cell (C) lies between acinar cells (E) and makes contact with a D-cell, with no surface specialization of the interface. x 17,500

Fig. 14. Both 'light' and 'dark' centroacinar cells border an acinar lumen (L), show complex interdigitation, and extend (downward) between B-cells. x 9,000

Fig. 15. A montage of a higher magnification of Figure 14 showing interdigitation between light and dark centroacinar cells that are in close contact with a B-cell (arrows). x 15,500

Fig. 16. The lumen (L) of an acinus contains dense material and is bordered partially by a centroacinar cell, that (below) makes contact with a second centroacinar cell (nucleus N) with spot desmosomes (d) between the two. The nucleated cell shows an extensive close contact (arrows) with an islet B-cell. x 22,000

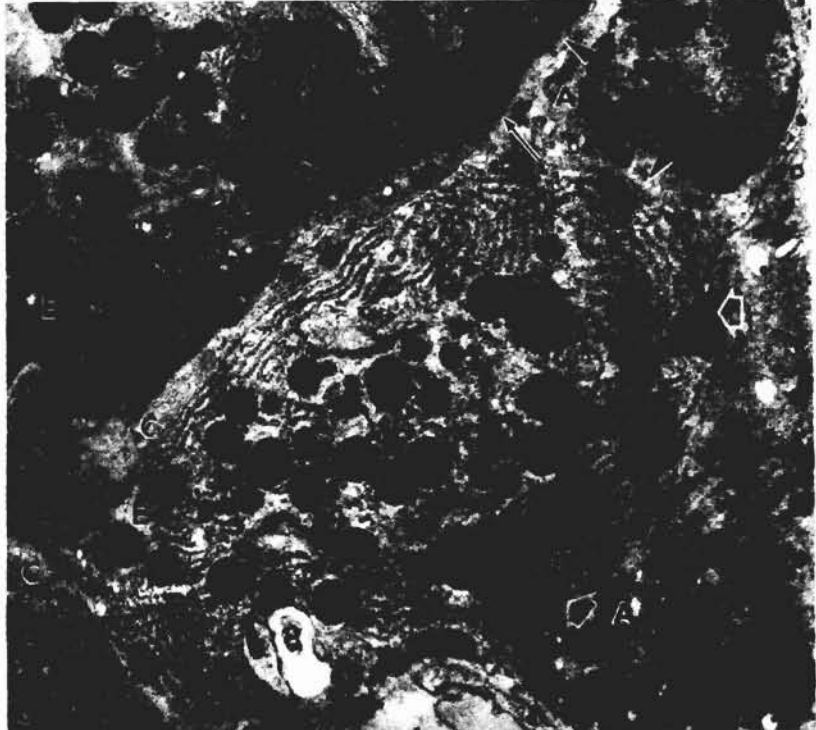
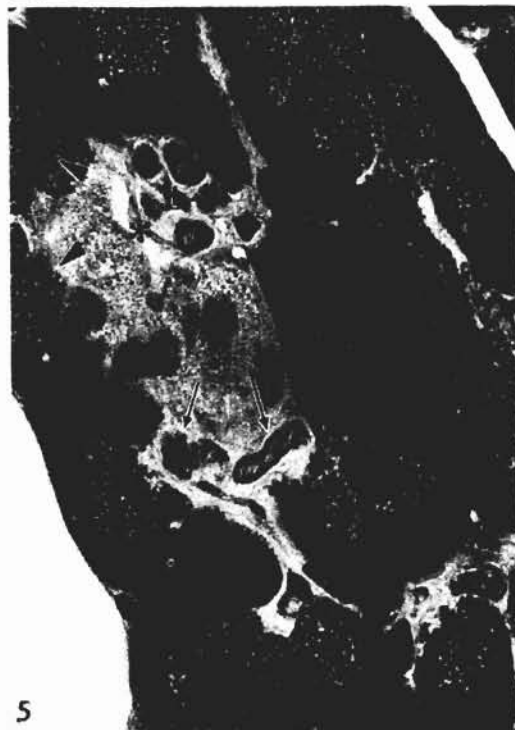
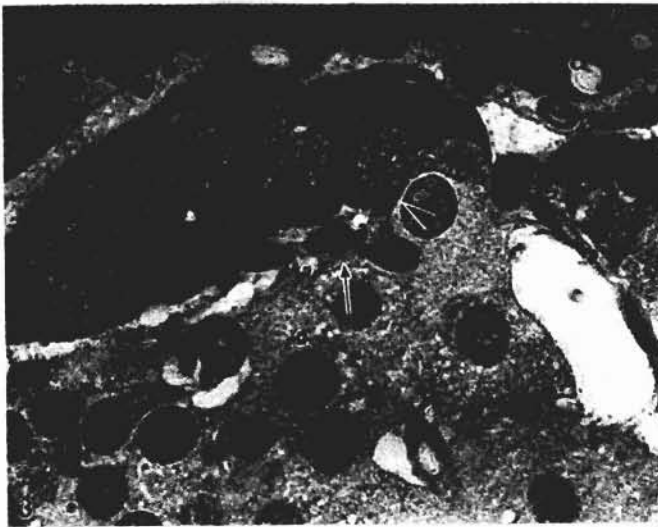
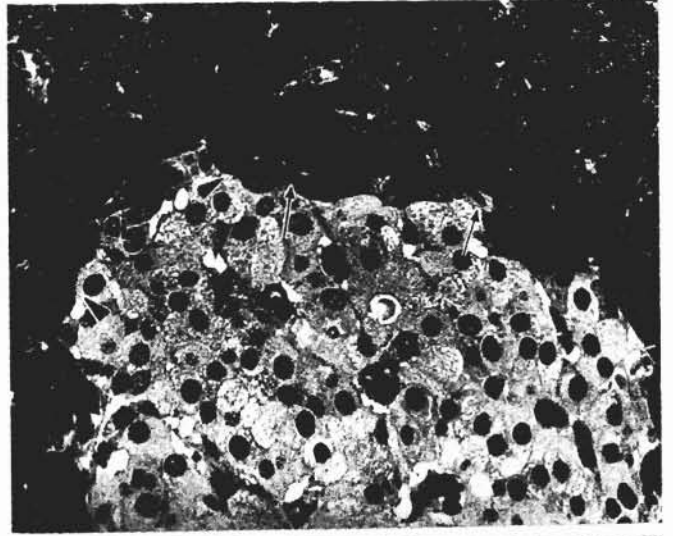
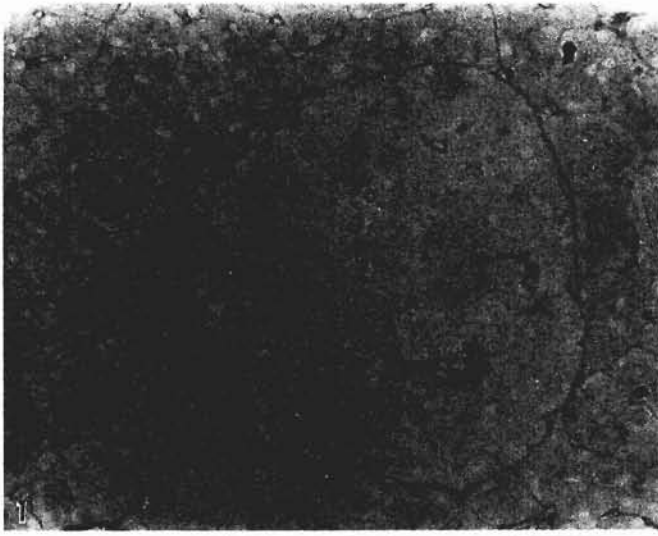
Fig. 17. Similar to figure 16 with overlap of dark and light centroacinar cells, the lower of which makes contact with a B-cell. x 17,500

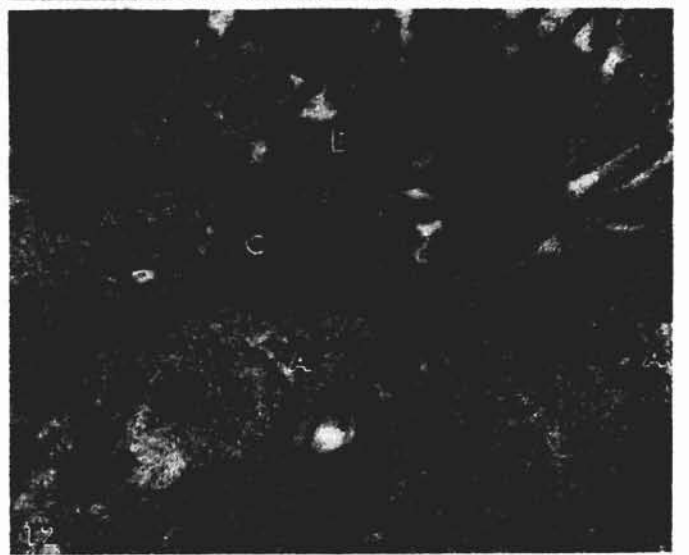
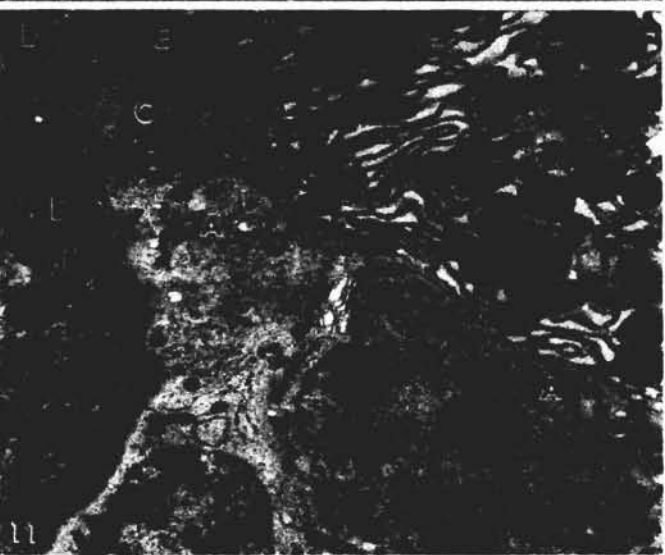
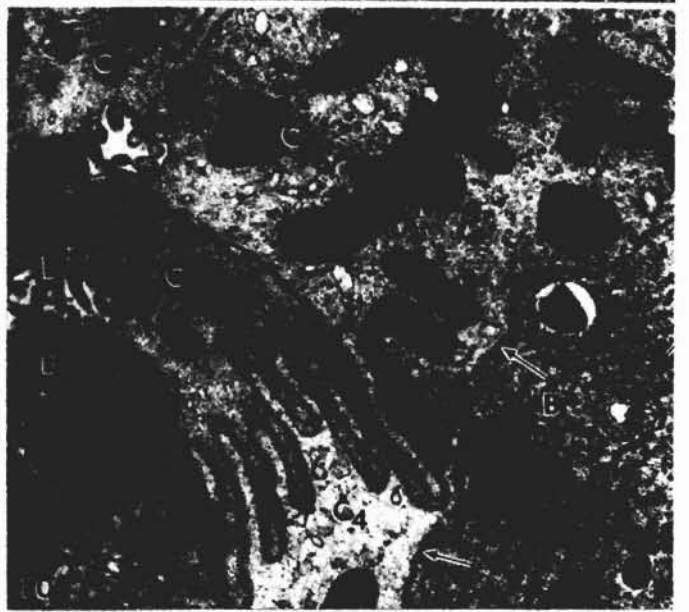
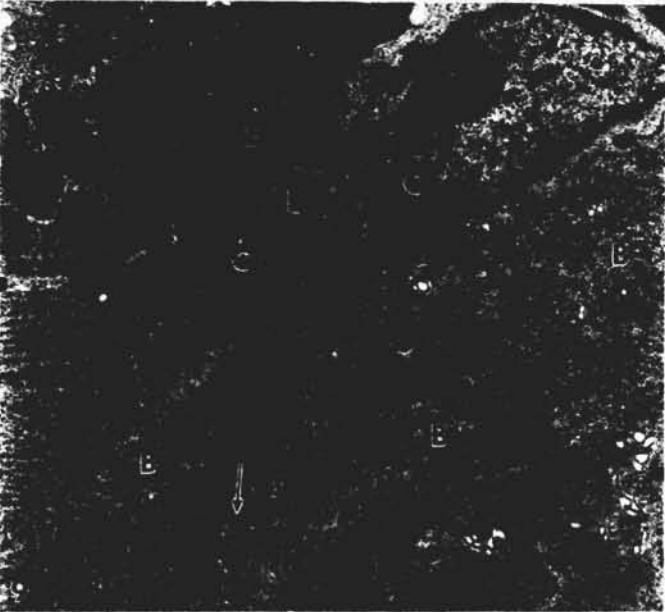
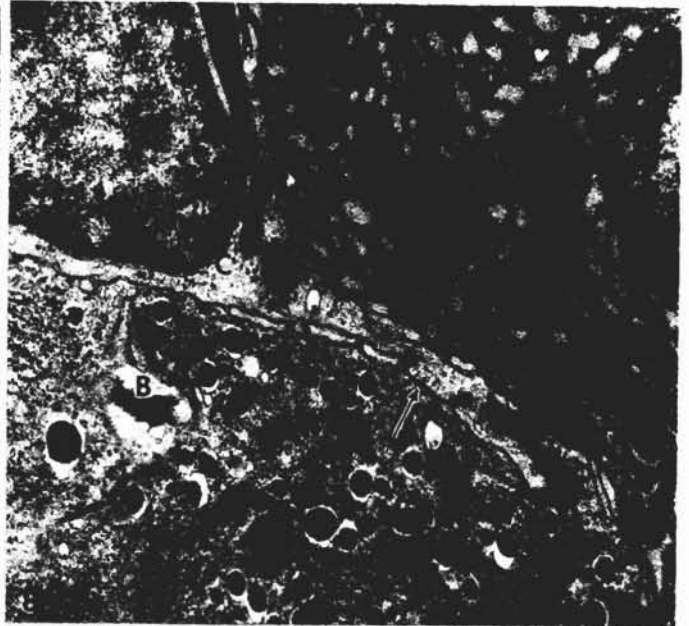
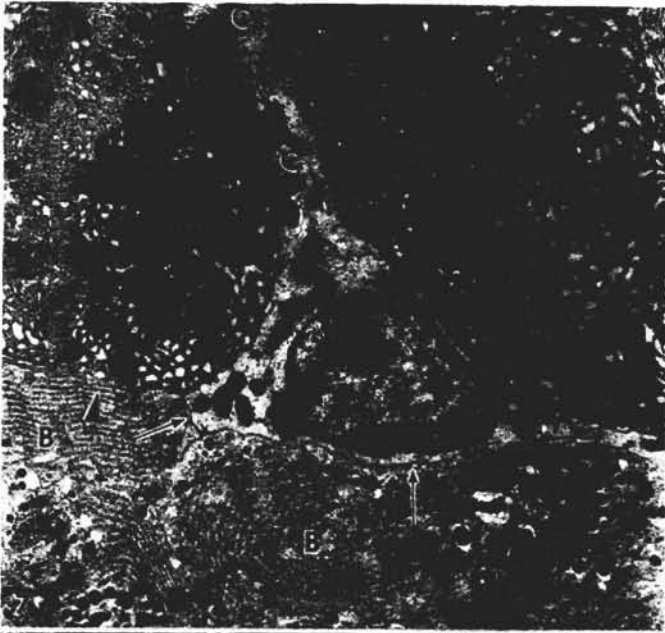
Fig. 18. The nucleated (N) centroacinar cell makes contact with an acinar lumen (above) and its base shows close contact with the process of a D-cell. On each side is connective tissue (T), partially separating the D-cell from exocrine tissue. x 13,500

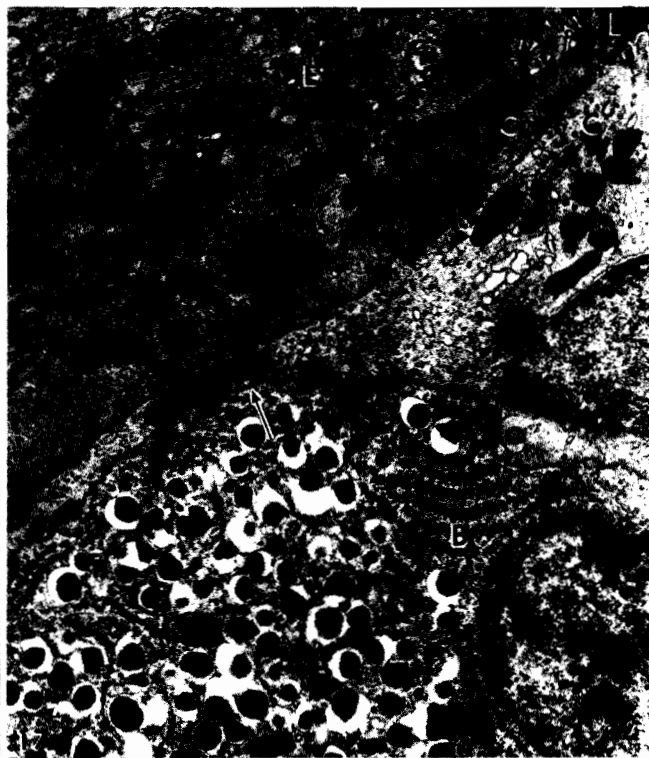
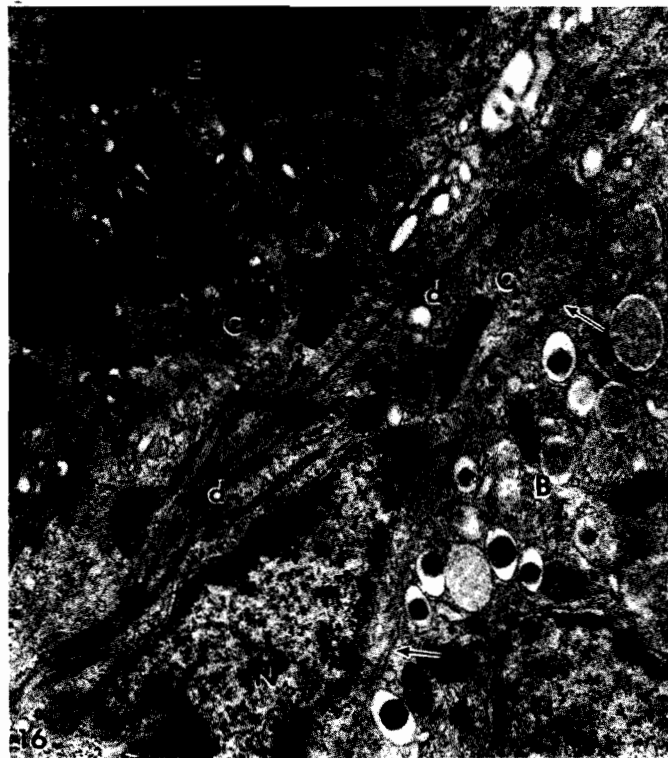
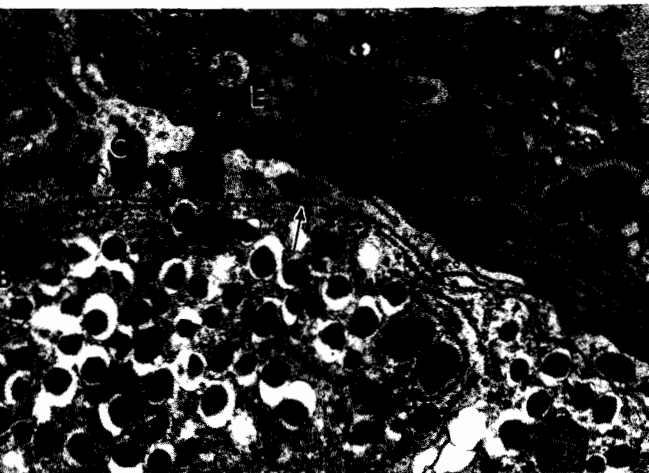
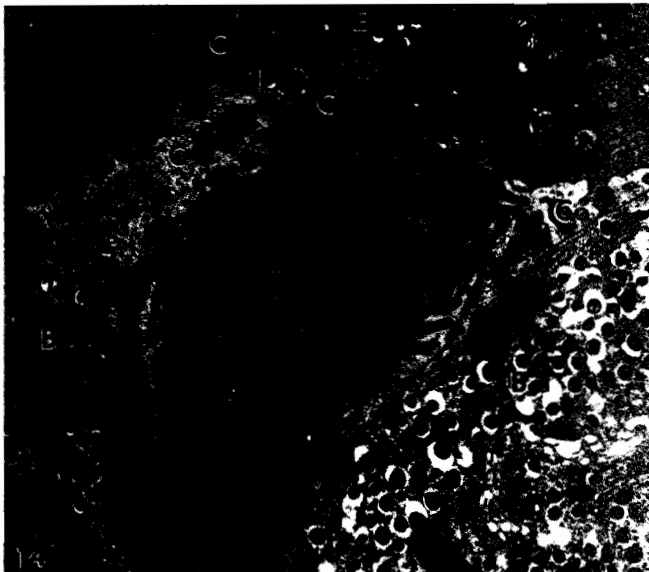
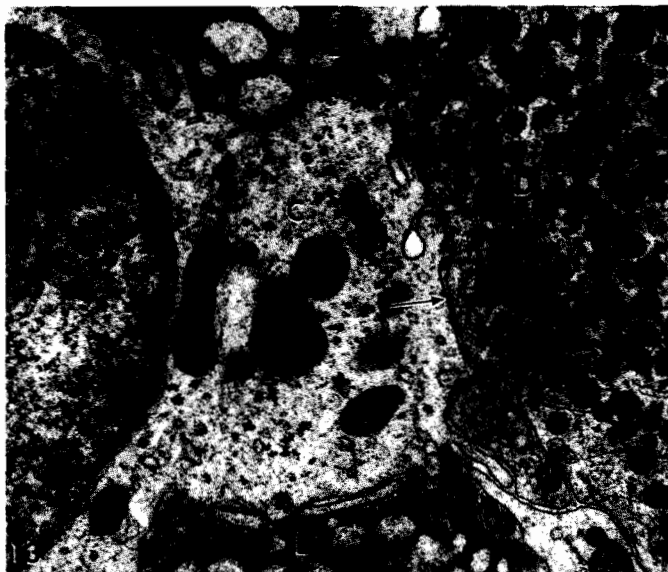
Fig. 19. A B-cell makes close contacts with (arrows) cells of an intercalated duct (I). x 11,000

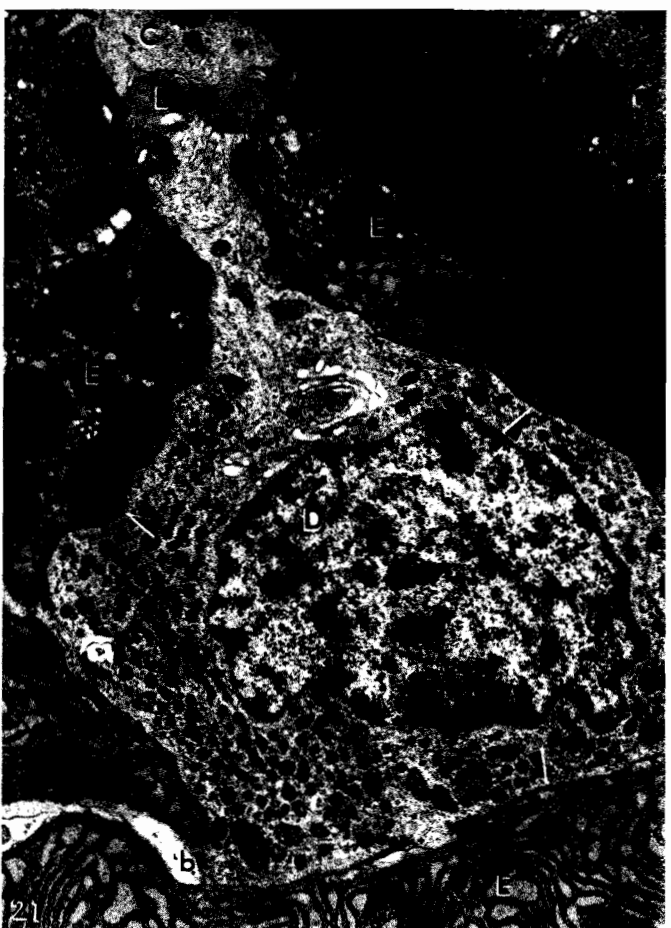
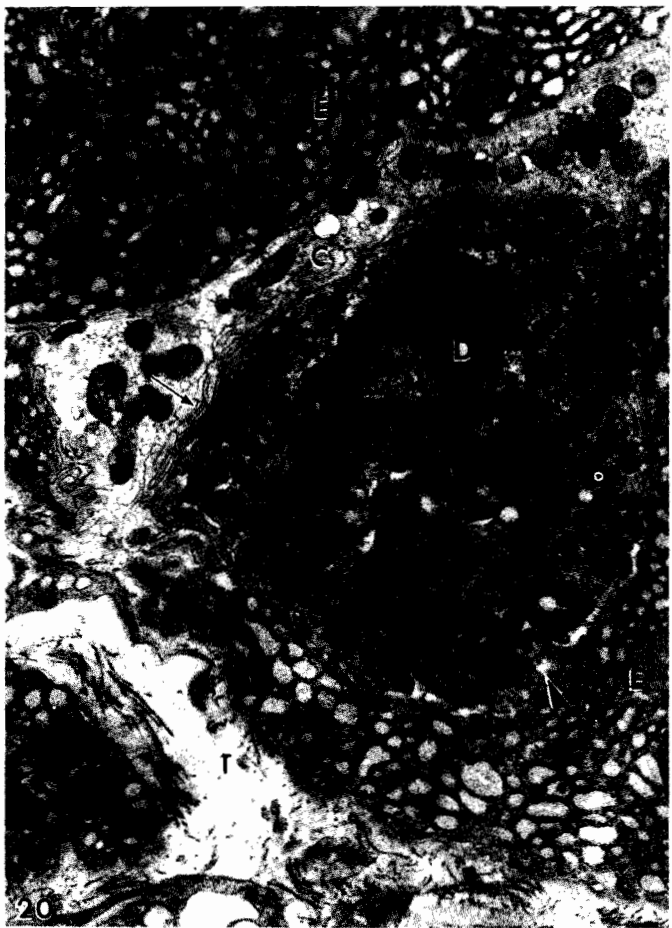
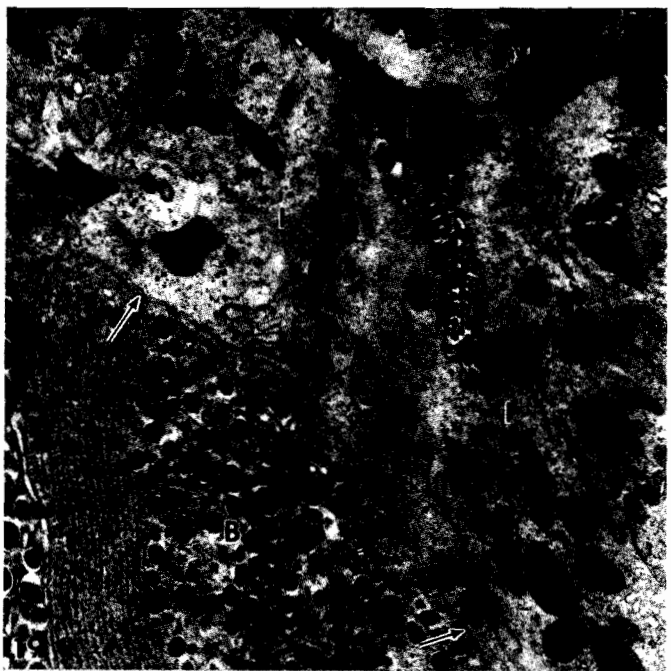
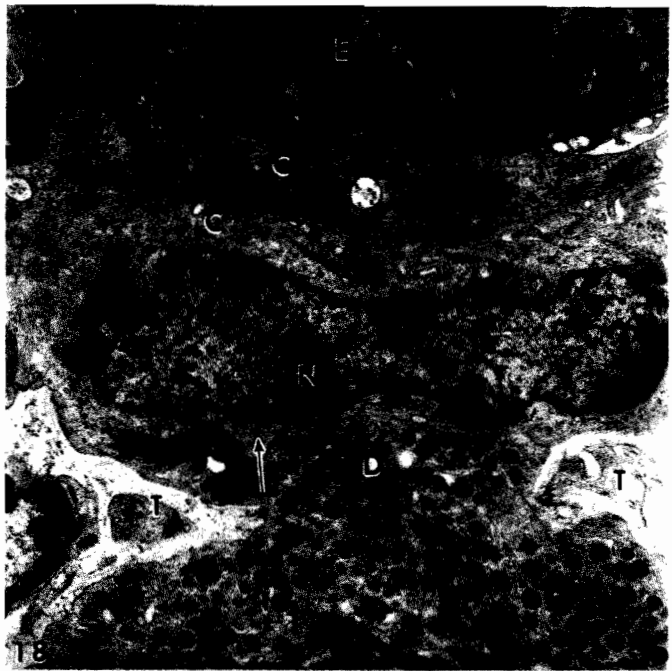
Fig. 20. A single D-cell lies in an acinus, making close contact with both an acinar (E) cell (arrowhead) and a centroacinar (C) cell (arrow). x 9,000

Fig. 21. A single D-cell lies between acinar (E) cells, making contact with both and a third acinar cell (below) (arrowheads), and the basal lamina (b) of the acinus, and reaches the lumen (L). x 13,500









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the apical (luminal) surface of a centroacinar cell is not always seen and, thus, a centroacinar cell, or a portion of it, appears wedged between acinar and insular cells, but showing no luminal surface. In a few instances, the basal surface of a centroacinar cell appears flat and regular but is in contact with a broad foot-like extension of an insular cell (Fig. 18), this process apparently passing through the mantle of thin connective tissue that surrounds the islet.

In regions of close contact between centroacinar and insular cells, adjacent plasmalemmae of the cells generally lie parallel and separated by an interval of 20 to 25 nm (Figs. 7, 8, 10, 13, 15-17), sometimes with minor irregularities or small cytoplasmic processes between the cells (Figs. 8, 13). However, no surface specializations such as gap or tight junctions or spot desmosomes were present on these interfaces (Figs. 10, 12, 13, 15-18). Most of the cell contacts are between centroacinar and B-cells (Figs. 7, 9, 10, 15-17) but contacts with A (Figs. 6, 11, 12) and D-cells (Figs. 13, 18) are also seen and, occasionally, with presumptive C-cells containing very few secretory granules. Obviously, in all contacts between centroacinar and insular cells as described above, no capillary sinusoidal blood vessels intervene between the two cell types, although they often lie adjacent to regions of close contact (Figs. 6, 18).

Similar relationships are seen, but less frequently, between insular cells and cells of intercalated ducts (Fig. 19) and, again, the most common relationship is with B-cells.

Additionally, occasional, isolated endocrine cells are found located in exocrine acini. Also seen are direct contacts within acini of single endocrine cells and centroacinar cells (Fig. 20) and, in one section only, an apparent D-cell lay between acinar cells and extended from the basal lamina to the lumen, its luminal border showing apical microvilli (Fig. 21).

Discussion

The results of this study demonstrate clearly that close contacts between insular and exocrine tissue are common in the rat pancreas and, therefore, likely to be of functional significance. These contacts can be demonstrated even by light microscopy. Kodousek (1982) used silver impregnation methods and described strong argyrophilia in centroacinar cells and ductular epithelium and one of his illustrations shows the peripheral portion of an islet, but no close contact is visible in this figure. It had been anticipated that the frequency and extent of close contacts between centroacinar and insular cells would be demonstrated more clearly by light microscopy using these silver impregnation methods, but they were inconsistent in our hands, perhaps not surprisingly, as Kodousek notes that the methods had varying success in his study.

By electron microscopy, the contacts between exocrine and endocrine cells are seen to be such as to exclude all connective tissue elements and the basal lamina.

The results also illustrate the irregularity at the border of islets and the apparent "intermingling" of insular and acinar cells in some regions. Nevertheless, over the majority of the islet surface, the peripheral insular cells are separated from exocrine tissues by a mantle of fine connective tissue and a basal lamina. This study confirms the findings of Bendayan (1982) who, in a short paper on human and rat pancreas, reported close contacts between endocrine and exocrine cells. He pointed out, as have other investigators, that there are immunocytochemical and biochemical differences between acinar cells surrounding islets (peri-insular cells) and those at a distance from an islet (tele-insular cells), which suggests a possible direct influence of islet hormones on neighbouring acinar tissue. On these exocrine-endocrine cell interfaces, Bendayan reported the presence of desmosomes, gap and tight junctions but pointed out that they are difficult to demonstrate in thin sections, being postulated by his observation of focal obliteration of intercellular spaces. They were not seen in the current study.

Functionally, centroacinar and ductular cells are believed to be responsible for the production of an alkaline fluid rich in sodium bicarbonate, a secretion necessary for the solubilization of zymogen released from acinar cells (Jamieson, 1983) and for its flushing through the duct system (Sarles, 1977). Several investigators have questioned this (Ekholm et al., 1962; Ichikawa, 1965; Williams and Kendall, 1982) mainly on morphological grounds, believing that the structure of the cells is inconsistent with the function. Previous reports on the morphology of centroacinar cells (Ekholm et al., 1962; Ichikawa, 1965; Sarles, 1977; Ferraz de Carvalho, 1979, 1981; Williams and Kendall, 1982; Jamieson, 1983; Leeson and Leeson, 1985) and the observation of their relation to acinar cells and the acinar lumen, as reported in this study, leave no doubt that, in many locations, centroacinar cells are related directly to insular cells, as are acinar cells. As has been summarized by Bauer (1983), many islet hormones may influence the activity of acinar cells, either directly or indirectly. Insulin, cholecystokinin, and vasoactive intestinal peptide stimulate and glucagon, pancreatic polypeptide and somatostatin inhibit pancreatic exocrine secretion. Additionally, there is an interesting and important endocrine-acinar portal system in the pancreas. Basically, one or more afferent arterioles supply each islet, the vessel(s) passing through the mantle around the islet to supply first the peripheral A and D-cells and then the more centrally located B-cells via an extensive capillary network, with efferent capillaries (vasa efferentia) passing back from the islet to form a capillary network in adjacent peri-insular acinar tissue. By such a system, islet hormones may interact with other islet cells and then pass to acinar tissue in high concentrations.

The lack of surface specialization on interfaces between centroacinar/ductular cells and islet cells as observed in this study is, perhaps, surprising. Acinar-acinar interfaces show junctional complexes of

zonulae occludentes and adherentes near the luminal surfaces with scattered spot desmosomes and frequent gap junctions more basally (Ekholm et al., 1962; Ichikawa, 1965; Sarles, 1977; Jamieson, 1983; Leeson and Leeson, 1985). Acinar-centroacinar interfaces are similar but most investigators report that gap junctions are not present, although a study by Metz et al. (1978) reported their presence in this location. Surface specializations between insular cells are well known. Orci et al. (1973), by freeze fracture and lanthanum tracer techniques, demonstrated both gap and tight junctions between B-B and A-B cells and, in further studies (Orci et al., 1975a), considered them important for the secretory behaviour of cells within the islet, the coupling via gap junctions perhaps maintaining glucose homeostasis within tightly controlled limits (Orci et al., 1975b). Further, Orci and Unger (1975) described the arrangement of A and D-cells in the peripheral region of islets with most B-cells located centrally and making contact only with other B-cells, an arrangement considered important to the normal and pathological functioning of islets. Interestingly, an increase in both the size and number of gap junctions between B-cells during stimulation has been described (Meda et al., 1979) and, also, a similar increase occurs when the insulin content of B-cells is depleted (Meda et al., 1980a). Other studies (Meda et al., 1980b) report that gap junctions are twice as numerous between B-cells at the periphery of an islet compared to those at the center, consistent with the hypothesis that B-cells may not form a functionally homogeneous endocrine cell mass. Orci (1982) has summarized the macro- and microdomain arrangement in the endocrine pancreas achieved by tight and gap junctions, describing gap junctions not only between B-cells but also between A and B-cells, although not all islet cells present in a cluster appear to be metabolically coupled with each other at a given time. The level of activity and effectiveness of the endocrine-acinar portal system (and paracrine secretion) may be controlled and/or modified within the islet by morphological compartmentalization of islet tissue (Orci, 1982; Kawai et al., 1982). As Kawai et al. (1982) have pointed out from their study of the effect of circulating somatostatin, the relative sensitivity of the islets to small changes in perfused somatostatin is best explained by compartmentalization of the interstitium of the islets, which is achieved by the presence of tight and gap junctions between all islet cell types (Orci, 1982). While Kawai et al. (1982) had suggested that tight junctions create compartments in the islet and that the microdomains so formed determine the degree of paracrine communication, in't Veld et al. (1984), in a study of normal endocrine pancreas, found that tight junctions declined progressively during culture and they suggested that, rather than being involved in normal islet cell function, the junctions provide an adaptive mechanism intended to seal and protect the islet microdomains against sudden perturbations in local interstitial fluid.

As already mentioned, no surface specializations between centroacinar/ductular cells and endocrine cells

of islets were seen in the regions of close opposition of the two cell types in this study, contrary to the findings of Bendayan (1982). However, the possible functional significance of these contacts and the possibility of a paracrine secretion associated with the endocrine-exocrine portal system is implied by the studies of Romagnoli et al. (1984). They noted that insulin is believed to have a trophic and secretagogue effect on the exocrine pancreas, an effect that may be both direct and indirect in that hypoglycemia causes vagal stimulation with hypersecretion of gastrin, the gastrin itself causing increased pancreatic enzyme secretion. Romagnoli et al. (1984) studied three patients with insulinomas and reported that in hyperinsulinemia both centroacinar and ductular cells became more numerous with more prominent apical microvilli and contained a larger Golgi apparatus and more mitochondria, while acinar cells lost most of their granules and showed other changes. It is of interest that a large proportion of the close contacts seen in this study were between centroacinar/ductular cells and B, insulin-secreting, islet cells.

There are two additional, important relationships between exocrine and endocrine tissue in the pancreas that require brief comment, these being concerned with the developmental origin of the islets and relationship in tumour formation. Fontaine et al. (1977) studied pancreatic differentiation and considered that endocrine cells arise from the duct system of the exocrine pancreas. A marked expansion of the total islet cell mass plus the development of new, additional islets occurs during much of the postnatal period (Hellerstrom, 1977). King et al. (1978), in the opossum, have reported two generations of islets, as in the sheep also, and believe that the terminal ends of duct epithelium give rise to endocrine tissue. Like and Chick (1969) studied genetically diabetic mice and found proliferating ducts after dietary restriction but considered that transformation of duct to islet cells was unlikely. In contrast, in the duck, Braun-Blanquet (1969) considered that centroacinar-insular cell transformation does occur. As mentioned previously, Pour (1978), in a study of experimental tumour formation, demonstrated the formation of new islets associated with hyperplasia of the intercalated ducts, and Sacchi et al. (1979) provided support for previous studies that suggest the genesis of insulinomas from ductular cells. Further, Reid et al. (1982), in a study of mixed ductulo-insular tumours, observed that their occurrence is understood by the concept that islets develop from ducts. With this knowledge of the association between centroacinar-ductular cells and insular tissue in both normal and pathological states, the finding of a morphological close contact between the two is not surprising.

Enteroendocrine cells are distributed widely in the gastrointestinal tract, and even in the ducts of the pancreas (Neutra and Padykula, 1983). The presence of both the "open" type (with a narrow-apical pole) and the "closed" type (not being in contact with the lumen) in pancreatic acini and, in some cases, making contact with centroacinar cells is therefore not surprising and is, perhaps, just another example of the close association of the two cell types that has been demonstrated by this study.

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