The Microanatomy of Human Islets of Langerhans, with Special Reference to Somatostatin (D-) Cells*

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Received December 8, 1982

Summary. The arrangement of the various endocrine cells within mammalian islets of Langerhans indicates that the regulation of insulin and glucagon secretion by pancreatic somatostatin may occur mainly by paracrine mechanisms.

In the present study, the relationship of somatostatin-containing D-cells to blood vessels and to other endocrine cells in the islets of the human pancreas were investigated using immunohistochemically stained serial semithin sections (0.5-1.0 µm). Morphologic features of 335 D-cells were examined and their anatomical relationship to other endocrine cell types and capillaries was determined by morphometric analysis and graphic or three-dimensional reconstructions. The majority of D-cells (84%) was located in close proximity to the capillaries. The intracellular immunoreactive material was accumulated in those cell parts facing the capillaries or their perivascular spaces. The remaining D-cells did not come into contact with the capillaries and showed only moderate or weak immunoreactivity.---A further characteristic feature of islet D-cells, pertinent to about 67% of these cells, is their tendency to be arranged in contiguity to other D-cells.

The present findings indicate that somatostatin after its release from the D-cell reaches other islet cells mainly via the intrainsular circulation or along the perivascular space.

Concerning the general microarchitecture of human islets of Langerhans, the present data are not sufficient to give a conclusive morphological description, because heterogeneities among the islets were observed. These variations appear to be related to the type of vascular supply which differs among islets.

The function of the islets of Langerhans and the release of their hormones is regulated by a variety of stimuli, including signals conveyed by the circulation or via the autonomic nervous system. In addition, the exchange of information between the various islet cell types obviously plays another important role in the regulation of islet cell function. Therefore, studies on the microarchitecture of the islets and the anatomic relationship between islet cells are of great interest for understanding the regulatory mechanisms involved in the function of these endocrine microorgans. Since somatostatin was found to inhibit the secretion of glucagon and insulin (Alberti et al., 1973; Hall et al., 1973; Gerich et al., 1974; Koerker et al., 1974), morphologic investigations have focused on the distribution and cytologic features of somatostatin-containing D-cells. Three mechanisms have been delineated for the influence of somatostatin on the

*This study was supported by a grant from the Deutsche Forschungsgemeinschaft SFB 87/G2.
secretory activity of the other endocrine cell types in the islets (OrcI et al., 1974; OrcI, 1976; Unger and OrcI, 1976; Forssmann et al., 1978; Unger et al., 1978; OrcI and Perrelet, 1979; Efendic et al., 1980; Larsson, 1980; Schaunder, 1980; Arimura and Fishback, 1981; Honey et al., 1981): a) membrane specializations (gap and tight junctions), b) secretion into the intercellular space, the “Parakrinie” of Feyrter (1953), and c) secretion into the capillaries (endocrine route). The apparent interposition of D-cells between the A-cells and B-cells, at first reported in rodent islets and later on also for human islets, favored the hypothesis of a prevailing paracrine mode of action of D-cells (OrcI and Unger, 1975; Unger and OrcI, 1977, 1981a, b). On the other hand, there is an increasing amount of evidence suggesting a hormonal status for pancreatic somatostatin (Miller, 1980; Grube and Aebert, 1981; Unger et al., 1981; Zyznar et al., 1981; Kawai et al., 1982).

In answer to this apparently controversial question, the present investigation was mainly designed to characterize morphologic features of islet D-cells and their microanatomic relationship to other pancreatic endocrine cells and the islet circulation. In addition, more general aspects of the microarchitecture of human islets of Langerhans were studied. These investigations were performed on immunocytochemically stained serial semithin sections (0.5-1.0 μm) and corresponding graphic or three-dimensional reconstructions. These techniques have been found useful for these purposes in preliminary investigations and they fill—concerning optical resolution—the gap between conventional light microscopy (paraffin sections) and electron microscopy (Grube and Aebert, 1981).

MATERIALS AND METHODS

Tissue and tissue preparation

Tissue material: The tissues investigated were selected from 409 histologic specimens of human pancreases obtained by surgery from 9 patients, who had suffered from endocrine adenomas and had undergone partial resection of the gland. The specimens were taken from non-tumorous and macroscopically unaltered regions. After orientation by histologic, immunohistochemical, and electron microscopic examinations the tissue materials from two patients were found suitable for the purposes of the present study and studied further. Specimens from the body of the pancreas (according to region VI of the classification of Malaisse-Lagae et al., 1979) were taken from patient F., a 45-year-old woman suffering from an insulinoma, and specimens from the tail of the pancreas (according to region VII of Malaisse-Lagae et al., 1979) came from patient M., a 67-year-old woman with a glucagonoma syndrome.

Tissue preparation: For light microscopy and immunohistochemistry, small specimens were snap frozen in Freon 22 cooled by liquid nitrogen, freeze-dried over a period of 3 days, fixed by vapor phase p-formaldehyde, and embedded in araldite (Grube, 1980; Grube and Weber, 1980). Serial semithin sections (0.5 μm or 1.0 μm) were cut on an ultramicrotome (Ultracut Reichert-Jung). The constancy and reproducibility of the thicknesses of the sections were examined by electron microscopic measurements of reembedded semithin sections viewed from the “edge” of the sections. Deviation was found to be ± 11% for 0.5 μm and ± 5% for 1.0 μm sections.—The sections were mounted on glass slides by heat (+90°C for 30 min).

For electron microscopy, small specimens were immersed for 3 hr in a solution containing 2% p-formaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer pH
7.3. The specimens were postfixed for 1.5 hr in 2% OsO₄ and embedded in araldite. Ultrathin sections were placed on grids and contrasted with lead citrate and uranyl acetate.

**Serial semithin sections:** According to the purposes of morphometric analyses or reconstructions, serial semithin sections of specimens containing islets were either stained alternatively by all antisera against pancreatic hormones ("sequential staining") or exclusively by somatostatin antisera ("monovalent staining"). The series for sequential staining: from 5 tissue blocks of the pancreatic tail and from 4 tissue blocks of the pancreatic body altogether 470 sections were cut in series of 10–30 sections. Moreover, a series of 1040 semithin sections was cut from a specimen of the pancreatic tail. This series contained several completely sectioned islets; all sections from this series were sequentially immunostained and every 5th section stained by Azur II and methylene blue (RICHARDSON et al., 1960). The series for monovalent staining: a series of 200 semithin sections was cut from a tissue block of the pancreatic tail and 2 series of 130 and 100 sections were cut from specimens of the pancreatic body. In addition, several series of 40 sections were cut from specimens of both pancreatic regions.

**Antisera**

Commercially obtained antisera: Anti-pork insulin guinea pig serum LAA (Novo, Denmark), anti-pork glucagon rabbit serum K 964 and K4023 (Novo).—Somatostatin antisera were kindly supplied by Dr. A. ARIMURA (New Orleans, USA) and Dr. H. ETZRODT (Ulm, FRG). Antisera against bovine pancreatic polypeptide (BPP) and human pancreatic polypeptide (HPP) were generously donated by Dr. R. E. CHANCE (Indianapolis, USA), and antiserum against α-endorphin (Melinda) by Drs. B. A. EIPPER and R. E. MAINS (Denver, USA), an antiserum against ACTH₁⁻³₉ by Dr. K. H. VOIGT (Ulm, FRG), and an antiserum against gastrin¹⁻¹⁷ by Dr. W. SCHLEGEL (Münster, FRG). Unlabelled porcine anti-rabbit IgG and soluble peroxidase anti-peroxidase (PAP) complexes were purchased from Dakopatts, Denmark.

**Immunohistochemistry**

Throughout this study the "PAP" method of STERNBERGER (1979) as modified for semithin sections (GRUBE, 1980) was used. Briefly, after removal of the epoxy resin with sodium methoxide (MAYOR et al., 1961), the sections were incubated in 2% non-immune swine serum for 30 min (to minimize a possible background staining caused by the second antibody). They were then exposed to the peptide antisera (first antibodies) diluted 1,000–8,000 for 40 hr at +4°C. The second antibody, unlabelled porcine anti-rabbit IgG, was applied at dilutions of 1:20–1:40 for 30 min at room temperature. The site of antigen-antibody reaction was revealed by 10 min exposure to a solution containing 0.0125% 3,3′-diaminobenzidine-HCl and 0.002% H₂O₂ in 0.05 M Tris-HCl buffer pH 7.6. (The buffer used for dilution of the antisera and as rinsing solution between the various steps of the immunohistochemical procedure was 0.01 M phosphate buffered 0.15 M saline pH 7.3=PBS) Finally the sections were dehydrated in graded ethanol solutions, cleared in xylene and mounted in Eukitt.

For identification of immunoreactive cells at the ultrastructural level, the semithin ultrathin section technique was used (LANGE, 1967, 1970). Semithin sections from specimens fixed for routine electron microscopy (see above) were placed on microscope slides and treated with sodium methoxide. Subsequently osmium deposits were removed with 5% H₂O₂ for 5 min. Using somatostatin antisera, this "etching" of semithin sections revealed only a faint staining of presumed D-cells. However, a short
proteolytic treatment (BROZMAN and BROZMANOVÁ, 1966) of the sections with 0.05% trypsin for 3 min at +37° C, subsequent to the etching with H₂O₂, was found suitable in overcoming this problem, and greatly enhanced the intensity of immunostaining (GRUBE et al., 1982b). All sections pretreated in this way were then processed for immunohistochemistry as described above. Ultrathin sections adjacent to immunostained semithin sections were processed for electron microscopy as described.

**Immunohistochemical specificity controls:** All immunohistochemical findings were submitted to extensive controls to exclude method and antibody non-specificities (BUFFA et al., 1979; STERNBERGER, 1979; GRUBE, 1980; GRUBE and WEBER, 1980; LARSSON, 1981). Control experiments were performed in human pancreases and in pancreases of rats and dogs. Moreover, pituitaries and the pyloric mucous membrane of rats served as reference organs for immunostaining with the antisera against ACTH, α-endorphin, and gastrin. All specificity controls were carried out in serial semithin sections alternatively stained according to the normal immunohistochemical protocol. Thus—because about 20 serial sections pass through the same endocrine cells—immunostaining and the corresponding specificity controls regularly were performed in the same cells.

The specificity controls included: a) Replacement of the primary antisera by an hyperimmune serum of unrelated specificity (α-fetoprotein antiserum, Dakopatts) or by nonimmune rabbit sera (Miles, USA, and Dakopatts) and by PBS (cf. OBSERVATIONS). b) Use of ascending dilutions of the antisera and control sera (cf. OBSERVATIONS). c) Preadsorption of the antisera with 10–100 μg/ml diluted antisera of their homologous and with heterologous peptides for 24 hr at +4°C. The peptides used for adsorption tests included pork insulin (Hoechst, FRG), synthetic glucagon (Serva, FRG), synthetic somatostatin (Peninsula, USA) and BPP (Dr. R. E. CHANCE, Indianapolis, USA). Immunostaining was abolished when the antisera had been preadsorbed with 20 μg/ml or more of their homologous peptides. Except for glucagon immunoreactivities which decreased when high amounts of BPP (100 μg/ml or more) were used for preadsorption, heterologous peptides added to the antisera were without effect on immunostaining. d) Omission of the second and third antibodies and of the DAB-H₂O₂ step or their replacement by PBS. No staining was observed under these conditions. e) Finally, the identification of somatostatin-immunoreactive cells at the ultrastructural level (consecutive semithin ultrathin section technique) confirmed that distinct endocrine cells (D-cells) were recognized by our somatostatin antisera.

**Microscopical techniques**

**Light microscopy:** The immunostained semithin sections were viewed with phase contrast optics or interference contrast optics according to Nomarski in a Leitz Orthoplan microscope. Phase contrast optics greatly enhance the contrast of DAB polymers in semithin sections and eliminate the necessity of counterstaining. Moreover, at higher magnifications even those parts of endocrine cells lacking immunoreactive material can be distinguished. Photomicrographs at magnifications of 400–1,000× were taken with a Leitz Orthomat camera.

**Electron microscopy:** Ultrathin sections were viewed and photographed in an electron microscope Zeiss EM 9S-2 at magnifications of 1,800–28,000×.

**Morphometric analyses**

**Amount of endocrine tissue:** During preliminary investigations of the pancreases included in the present study, the impression was gained that the number of islets was increased, at least in the pancreas of patient M (glucagonoma syndrome). Therefore,
5 μm paraffin sections through tissue blocks (size: 0.5×1.0×2.0 cm) of this pancreas, fixed and embedded concomitantly, were cut at various levels and stained either by hematoxylin eosin or by an argyrophilic reaction (Grimelius, 1968). In these sections the number of islets/mm² tissue was determined and compared with the corresponding values of 8 control pancreases (necropsy specimens from patients without endocrine or pancreatic disorders) and of 2 pancreases from hyperinsulinenic patients (surgical samples from 2 children who had undergone partial resection of the pancreas).

Proportion of endocrine cell types: Islets in sequentially immunostained serial semithin sections were photographed, and copies at a total magnification of 1,000× were prepared from the negatives. The fractional area occupied by B-, A-, D- and PP-cells was determined using the image analyser system IBAS 1 (Videoplan, Zeiss-Kontron, FRG) and expressed as a percentage of the total area occupied by these cells. It should be noted that the measurements included the actual area of the endocrine cells and were not restricted to only the immunoreactive cell parts. As detailed in Table 1, the percentages of the endocrine cell types were determined in 5 islets which had also been examined for other histologic parameters of D-cells. In 3 of these islets, 100–200 consecutive semithin sections had been immunostained exclusively for somatostatin cells. Within these islets, the proportions of the endocrine cell types were determined in sequentially immunostained serial sections preceeding and following the segments of the islets stained for D-cells. In the remaining 2 islets, 150 and 250 serial sections were stained alternatively with all 4 antisera against pancreatic hormones. Here, the various endocrine cell types were measured in a series of 4 consecutive sections at every 10 μm distance. In addition, the actual cell numbers of the major endocrine cell types were determined in the completely sectioned islet. The results of the quantitative determinations in these 5 islets were compared to the findings obtained in 9 other islets from other specimens of the same pancreases and found to be representative for the material investigated.

Distribution and anatomical relationship of D-cells: Within the 5 islets just mentioned (Table 1) D-cells were more closely examined. Photomicrographs of the corresponding serial sections were prepared at a total magnification of 1,000–1,600×. From all D-cells included in these series (altogether 335 cells), the following parameters were determined (cf. Table 2): 1) position within the insular tissue: location near the “cortex” of the islet versus location within the inner cell mass (“medulla”) of the islet; 2) anatomical relationship to capillaries or the perivascular space; 3) anatomical relationship to other D-cells (“homologous contacts”). Within the completely sectioned islet, in addition, the exact numerical relationship of D-cells to other endocrine cell types (“heterologous contacts”) was determined. Finally, within this islet, the kind and number of cell types bordering the intrainsular capillaries and their connective tissue investments was analysed.

Reconstruction methods
In one islet from the pancreatic body and in two islets from the pancreatic tail (cf. Table 1, islets No. III–V), the serial semithin sections were immunostained with antisomatostatin. These serial sections were photographed and copies were prepared at a total magnification of 1,600–2,600×. The contours of the islets, of the intrainsular capillaries, and of the D-cells were drawn from these copies on transparent foils of 0.1 mm thickness. When these foils were stacked against a bright background (light box), transparent pictures of the corresponding islet parts were obtained, though of course
in a “compressed” state, because the thickness of the foils did not correspond to the final magnification of the sections. However, by examining these foils, the distribution of D-cells within the islets and their anatomical relationship to capillaries as well as their homologous contacts could be clearly judged. Based upon these foils three-dimensional reconstructions of single D-cells or of D-cell groups were made as wax plate models or balsa wood plates.

From a series of 20 foils, the contours of all D-cells were filed into a computer (NEU system of neurographic programs (Speck, 1981), European Molecular Biology Laboratory, Heidelberg, FRG). The pictures displayed by the computer system were isometric projections of 8 different views of these D-cells.

**OBSERVATIONS**

1. **Tissue preparation and immunohistochemical techniques**

   Since light microscopic studies of the microarchitecture of the islets of Langerhans have previously been performed only in paraffin embedded tissues and by immunofluorescence methods, we will briefly report on the major advantages of the techniques applied during the present investigation.

   To minimize the shrinkage of cells and tissues during fixation, the specimens were snap frozen, freeze-dried, fixed by vapor phase p-formaldehyde and embedded—omitting any intermedia—in epoxy resin. After this procedure, the tissue structure was excellently preserved, and the endocrine elements of the islets especially did not show any fixation artefacts. Similarly, the preservation of antigenicities of the pancreatic hormones proved to be optimal under these conditions. A minor disadvantage of the present method is the relatively poor preservation of connective tissue fibers. Embedding in araldite—as compared to other epoxy resins—facilitated the preparation of serial semithin sections. Thus, only 1–2% of the sections were lost during sectioning, even in series of up to 1,000 sections. In addition, embedding in resin or other plastics is a prerequisite for exact reconstructions of histologic specimens.

   When serial semithin sections are sequentially immunostained for the various pancreatic hormones, all corresponding endocrine cells of the islets are shown almost within the same plane (Fig. 1). Hence, anatomical relationships between the various endocrine cell types can be clearly determined. Moreover, from monovalent immunostained serial sections, single cells or the corresponding cell population can be reconstructed graphically or three-dimensionally rendering exact informations about their shapes, their relations to homologous cells or to blood vessels.

   Finally, semithin sections stained by immunoperoxidase methods and viewed with phase contrast optics reveal cellular details of pancreatic endocrine cells barely discernable by conventional light microscopic or immunofluorescent techniques.

2. **Histology and immunohistochemistry**

   The tissues investigated came from patients suffering from endocrine adenomas of the pancreas. Therefore, even though the specimens were taken from non-tumorous regions, we gave careful attention to pathologic changes which might possibly occur in the endocrine tissue of these specimens.

   In determining the number of islets per area of pancreatic tissue in sections through the pancreas of patient M., we found $2.50 \pm 0.24$ islets/mm$^2$. Corresponding data in
Fig. 1. Arrangement of the major endocrine cell types within a human islet of Langerhans (cauda pancreatis). Phase contrast microscopy of 3 consecutive semithin (0.5 μm) sections immunostained by the PAP method for insulin (a), glucagon (b), and somatostatin (c). All sections pass through the same cells. × 370. d. Most of the somatostatin cells have a close relationship to capillaries (labeled by asterisks) and cell parts remote from capillaries (labeled by arrowheads) often show less dense immunostaining. Phase contrast microscopy. × 920
the pancreases of controls were 1.12 (±0.32) islets/mm², whereas the pancreases of the 2 children suffering from hyperinsulinemic hypoglycemia contained 4.48 (±1.33) and 5.87 (±0.62) islets/mm². The amount of endocrine tissue in the pancreas of patient F. has not been determined. However, within this pancreas also the number of islets seemed to be increased when compared to the controls.

Concerning the size of the islets in the pancreases of both patients, most of the islets were medium-sized and showed their largest diameters to be between 140 and 240 μm. The largest islet observed in our material measured 280 × 250 × 180 μm and small islets present in both pancreases 50-70 μm. These small islets mostly lacked intrainsular capillaries; they were composed of all major endocrine cell types in the pancreas of patient M., whereas small islets in the pancreas of patient F. contained in most cases only B- and D-cells.

Immunohistochemistry revealed the staining of individual endocrine cell types when anti-insulin and anti-somatostatin in appropriate dilutions were used. Glucagon (A-) cells were immunoreactive to both the C-terminal specific antiserum K 964 and the N-terminal specific antiserum K 4023. A-cells within the islets of specimens from patient F. (insulinoma) showed a considerable decrease of immunoreactive material which was mostly confined to one cell pole. All A-cells of both pancreases showed weak staining when anti-somatostatin was applied in dilutions 1:2,000 or less. This dilution-dependent staining of A-cells is thought to be caused by non-specific mechanisms, because an antiserum of surely unrelated specificity (α-fetoprotein antiserum) also caused moderate or weak staining of these cells when it was utilized in dilutions.
of up to 1:600. Likewise, A-cells showed a faint "immunostaining" when non-immune rabbit sera diluted 1:200 or less were used as first layers in the immunohistochemical procedure. Thus, pancreatic A-cells of man concerning non-specific staining may be compared to pancreatic A-cells of other mammals and to pyloric gastrin cells, intestinal GLI-cells or pituitary corticotrophs which all bind immunoglobulins by presumable non-immunologic mechanisms (GRUBE and WEBER, 1979, 1980; GRUBE, 1980; GRUBE and AEBERT, 1981). Accordingly, all A-cells showed a dilution-dependent staining by PP antisera. However, a minority of glucagon-immunoreactive cells localized preferentially in extrainsular sites showed strong immunoreactivities towards PP antisera, and that independent from the concentrations of the primary antisera within a wide range. These findings confirm observations in other mammals (GRUBE et al., 1982a), indicating the existence of a subpopulation of pancreatic A-cells that concomitantly contain glucagon and PP-like substances (refer also to the findings on intestinal GLI-cells, FIOCCA et al., 1980; RAVAZZOLA and ORCI, 1980; GRUBE and AEBERT, 1981; LEHY et al., 1981; GRUBE, 1982). "True" PP-cells, immunoreactive exclusively to BPP and HPP antisera were sparse in our material which included specimens from the pancreatic body and tail.

When serial semithin sections which were sequentially immunostained by antisera against insulin, glucagon, somatostatin, and PP were thoroughly examined, we regularly found endocrine cells unreactive to all of these antisera. These cells (and likewise

Fig. 3. Arrangement of somatostatin cells at different planes of a human pancreatic islet. Two semithin (0.5 mm) sections through the same islet were cut at 40 μm distance, immunostained (PAP method) for somatostatin, and viewed by phase contrast microscopy. Within the section shown in (a) somatostatin cells are grouped together facing the vas afferens (?) of the islet, whereas they are rather evenly distributed within the endocrine tissue on the other plane (b). ×400
all other endocrine cell types) also lacked immunoreactivities to ACTH, α-endorphin, and gastrin antisera.

Ultrastructural analyses revealed that a variety of endocrine cell types could be recognized according to the morphologic criteria of their secretion granules (Fig. 2). Confirming the results of other authors (Orči et al., 1974; Alumets et al., 1977; Capella et al., 1977; Pelletier, 1977), we regularly observed B-, A-, D- and D₁-cells. Cell types tentatively classified as F-, P- and C-cells were less frequently found; only once did we observe a cell identified as an EC-cell.

Endocrine cells immunoreactive to anti-somatostatin were identified at the electron microscopic level by a consecutive semithin ultrathin section technique: all cells exhibiting somatostatin immunoreactivities in semithin sections were identified in adjacent thin sections as typical D-cells.

Concerning possible pathologic changes in endocrine tissues at the ultrastructural level, the only ones to be observed were those already recognized by light microscopy in A-cells of the pancreas of patient F., i.e., the rarity of secretory granules in these cells. The presence of lipofuscin inclusions seen mainly in B-cells (Fig. 2) and occasionally also in other endocrine cell types are regarded as age-dependent changes.

3. Morphometric analyses and microarchitecture of the islets

A detailed examination of the D-cells was performed in 5 islets. Before entering into a description of D-cell features, we will refer to the proportion of endocrine cell types contained in these islets and the general arrangement of the islet endocrine cells.

The islets selected for closer investigation were medium-sized and corresponded to the "compact" and "ribbon" types as described by Ferner (1952) (see DISCUSSION). The relative proportion of B-, A- and D-cells within these islets are listed in Table 1. Because PP-cells made up only about 0.5%, they were omitted here. The data refer to mean values of 7–12 determinations carried out in sequentially immunostained serial semithin sections at 10 μm distance in each islet. In islet No. I the values are based upon determinations throughout the completely sectioned islet; in islet No. II the quantitative analysis included 75 μm of the islet; in the islets Nos. III, IV and V the determinations were performed adjacent to those parts of the islets in which sections had been immunostained exclusively for somatostatin cells. Even though the relative proportion of each endocrine cell type varied from one islet to the other, the cellular composition of the islets showed no striking differences from data given in literature for islets of the corresponding pancreatic regions (Orči et al., 1976, 1979; Orči, 1977; Malaisse-Lagae et al., 1979).

Concerning the arrangement of the major endocrine cell types within the islets, we found it necessary to distinguish between descriptions based upon observations of single sections (two-dimensional image) and descriptions which also take into account findings obtained by examinations of serial semithin sections (three-dimensional image). Viewed two-dimensionally (Fig. 1), the insular tissue seems to be divided into smaller "lobules" by capillaries and their connective tissue investments (Orči, 1977; Ungar et al., 1978). The B-cells are located either in the center of these lobules or adjacent to the capillaries (Fig. 1a). The A-cells are distributed corresponding to a modified "mantle" islet, inasmuch as they form an incomplete ring at the periphery of the islets and "enter" the islets along the capillaries (Fig. 1b). Similar to this distribution pattern, the D-cells are preferably located next to intrainsular capillaries but are only infrequently found at the periphery of the islets (Fig. 1c). When the arrangement of islet tissues was studied in larger series of sections, a subdivision of the islets into
"lobules" could not be observed. All endocrine cells of the islets were found to be in contiguity. In other words, the endocrine cells make up a compact mass of cells which is permeated but not subdivided by capillaries and the connective tissue investments (closely applied) to them. The intrainsular capillaries are surrounded by layers of endocrine cells which apparently constitute the cortices of islet "lobules" in two-dimensional images. However, the composition of these layers varies from one plane to the other. They may consist either of all types of endocrine cells or only of one or two endocrine cell types. Moreover, endocrine cells which appear to be rather remote from capillaries (in the second "row") on one plane often extend to capillaries at other planes (note cytoplasmic extensions of B-cells in Fig. 1a). The exact number of endocrine cell types bordering the intrainsular capillaries was determined throughout the completely sectioned islet No. I. This islet contained 703 B-cells, 122 A-cells, and 65 D-cells. An anatomical relationship to intrainsular capillaries was found for 308 B-cells, 40 A-cells and 51 D-cells. Thus, the layer of endocrine cells adjacent to intrainsular capillaries in this islet was preferentially made up of B-cells. With regard to the possible functional significance (Larsson, 1980) the percentage of cells from each endocrine cell population bordering the intraislet capillaries should be emphasized. These were 44% of all B-cells, 33% of all A-cells, and 79% of all D-cells present in islet No. I. When we take into account that a considerable proportion of the remaining cells—especially the A-cells—will have contact with the perinsular capillary network, only a minority of endocrine islet cells may lack any vascular contact. Hence, it seems also difficult to distinguish islet lobules in a functional sense.

Remarkably, the vascular supply of the islets and consequently the arrangement of the endocrine cell populations varied between the islets. We will refer to this aspect of islet microarchitecture within the DISCUSSION of the present study.

4. Features of islet somatostatin (D-) cells

Frequency and arrangement of D-cells. In Table 1 mean values of the relative proportions of the three major endocrine cell types are given. It was shown that the percentage of D-cells varied to a certain degree from one islet to the other. It must now be emphasized that also within the same islet a considerable deviation of the number of D-cells occurred at different "parallels of latitude" (segments) of the islets. Therefore, the percentage of D-cells varied between 3% and 13.2% within islet No. I or

Table 1. Characterization of human islets of Langerhans morphometrically analyzed during the present study

| Pancreatic region | Islet (Code No.) | Greatest diameters (μm) | Relative proportions of the major endocrine cell types (in %; deviations in parentheses) |
|-------------------|-----------------|------------------------|---------------------------------------------------------------------------------------|
|                   |                 |                        | A                                      | B                                      | D                                      |
| Tail              | I               | 125 × 70               | 9.4 (7.6–12.6)                        | 83.5 (76.8–89.5)                       | 6.8 (3.0–13.2)                         |
| Tail              | II              | 230 × 185              | 22.3 (21.8–22.7)                      | 61.9 (58.2–66.4)                      | 7.5 (5.6–11.8)                        |
| Tail              | III             | 240 × 125              | 19.5 (13.9–24.6)                      | 73.9 (67.9–80.9)                      | 6.6 (5.2–8.8)                         |
| Body              | IV              | 160 × 70               | 15.1 (12.9–20.2)                      | 80.1 (78.1–83.7)                      | 4.8 (1.0–8.4)                         |
| Body              | V               | 110 × 80               | 14.4 (10.7–18.1)                      | 78.9 (78.6–81.5)                      | 6.7 (2.5–10.1)                        |
between 1% and 8.4% in islet No. IV (Table 1). Accordingly, the number of D-cells that was counted in the islets ranged from 21 D-cells per 50 μm (=100 semithin sections) to 102 D-cells per 75 μm insular segment (Table 2, 2nd and 3rd column). When the D-cells were identified with regard to their position within the islets, it was found that the great majority (78.5%) is located within the medulla of the islets (Table 2, 4th and 5th column).

The examination of D-cells with respect to their anatomical relationship to capillaries revealed that about 84% of these cells were localized adjacent to capillaries or their connective tissue investments (Table 2, 6th column). This percentage might have been even higher except that the relationship of D-cells, localized at the cortex of the islets, to the perinsular capillary network could not always be determined unequivocally in our material. In any case, the anatomical relationship of pancreatic D-cells to capillaries is one of the distinctive features of this cell population.

Regarding the distribution of D-cells within the various segments of the islets, no common pattern could be recognized. D-cells may be distributed in a random conformation among other endocrine cells on one plane or grouped together or occupy an excentric position on another plane of the same islet (Fig. 3). Once noticed, we found such accumulations of D-cells at certain segments almost regularly in all human islets examined in this direction. Within islet No. I which was completely sectioned and immunostained, we observed “peaks” of D-cells at 2 levels at a distance of about 30 μm and 40 μm from the upper and lower poles of this oval-shaped islet. Here and in all other corresponding cases, the D-cells were grouped in close proximity to capillaries that passed the islet cortex or were adjacent to centrally located capillaries.

A further and probably important feature of islet D-cells is related to their mutual anatomical “connections” as revealed by examinations of serial sections. About 67% of all D-cells were found in close proximity to each other: this juxtaposition is referred to as “homologous” contacts (Table 2, 7th column). In islets or islet segments that contain relatively few D-cells (e.g. islets Nos. I and V) these D-cell groups are made up of only a few cells. On the other hand, and that preferably in larger sized islets (islets Nos. II and III), the anatomical inter-D-cell relationship reminds one of a “syncytium-like” arrangement of this cell population within the islet. This viewpoint is illustrated in Figure 4, where 12 consecutive semithin sections from islet No. III are presented, and the basic principles of the arrangement of D-cells present in this region are outlined in a schematic drawing. A more complete and three-dimensional image of the D-cells of this region as was obtained by computer analysis of 20 serial sections is shown in Figure 5.

| Islet (Code No.) | Number of serial sections (0.5 μm) | Number of D-cells | Location in the Islet | Anatomical relation to capillaries | “Homologous contacts” |
|------------------|-----------------------------------|-------------------|-----------------------|----------------------------------|----------------------|
| I                | 250                               | 65                | 14                    | 51                               | 37                   |
| II               | 150                               | 102               | 13                    | 89                               | 83                   |
| III              | 200                               | 100               | 21                    | 79                               | 77                   |
| IV               | 100                               | 21                | 10                    | 11                               | 21                   |
| V                | 130                               | 47                | 14                    | 33                               | 43                   |
| **Total**        | **335**                           | **72**            | **263**               | **280**                          | **223**              |

(=100%) (21.5%) (78.5%) (83.6%) (66.6%)
Fig. 4. Phase contrast microscopy of 12 serial semithin (1.0 \(\mu m\) distance) sections through a human pancreatic islet immunohistochemically (PAP method) stained for somatostatin (a-m). Most of the somatostatin cells show close relationships to capillaries and are localized in contiguity to other somatostatin cells. \(\times 320\). These principles of the arrangement of islet somatostatin cells are illustrated in a schematic drawing based upon the 12 serial sections (n). The shape of the cells as seen in the first and upper section of this series is represented by dark-grey, while that of the deeper layers by light-grey. C capillaries.
In addition to the homologous contacts between D-cells which have been determined in all 5 islets, the anatomical relationship of D-cells to the other major endocrine cell types (B- and A-cells) was examined quantitatively in islet No. I. Out of 65 D-cells, 25 (38%) showed juxtaposition to both B- and A-cells. The remaining 40 D-cells (62%) showed "heterologous" contacts only to B-cells.

Shape of D-cells. Already in single sections through D-cells, the manifold appearance

Fig. 5. Graphic reconstructions (NEU system of neurographic programs, European Molecular Biology Laboratory, Heidelberg, FRG) of somatostatin cells in a 20 μm "disc" of a human pancreatic islet (same islet as in Fig. 4). In a the cells are viewed from "above" (perpendicular to the sections). In b the cell complex is viewed from "lateral," as indicated by the arrow in the upper left corner of a. Note contiguities between most of the somatostatin cells.
of this cell type is obvious (Fig. 1c, d, 3). Also, in three-dimensionally reconstructed cells, it is rather difficult to give a generalized description of the shape of D-cells. However, apart from rounded or pyramidal-shaped cells with diameters between 10 μm and 16 μm, the majority of D-cells shows a polygonal form and exhibits cytoplasmic processes of varying length. D-cells that are located at some distance from capillaries generally possess long processes extending to intrainsular capillaries (Fig. 6, 7). The maximal length of such processes was 22 μm. When these processes are cut along their longitudinal axis and viewed in single sections they appear slender and rounded. Examination of serial sections reveals, however, that these processes are actually better described as sheet-like extensions of D-cells. Repeatedly we observed D-cells showing relationships to 2 different capillaries via these cytoplasmic extensions (Fig. 9, 10).

Fig. 6. Somatostatin-immunoreactive cells in human islets of Langerhans (semithin sections: PAP method; phase contrast microscopy). Long cytoplasmic processes, cut here along their longitudinal axes, extend to intraislet capillaries (labeled by asterisks). x 1,000

Fig. 7. Four semiadjacent sections through 2 islet somatostatin cells cut at 6.0 μm distances (techniques and magnification as in Fig. 6). The perikarya of these cells show moderate immunostaining (a); densities of immunoreactivities increase within the (obliquely sectioned) cytoplasmic extensions (b-d) the nearer they come to the capillary bed (labeled by asterisks).

Fig. 8. Somatostatin cells in human islets of Langerhans. Semithin sections were overstained by use of less diluted somatostatin antiserum (1:1000). Immunoreactivities are accumulated at the vascular pole of these cells (capillaries labeled by asterisks). The remaining cytoplasmic areas are weakly stained or clear (labeled by arrowheads). x 1,250
Fig. 9. Phase contrast microscopy of 12 serial semithin (1.0 μm) sections through a group of somatostatin cells in a human pancreatic islet. The somatostatin cell labeled by arrows in a–m shows 2 strongly immunoreactive processes extending towards different capillaries. This cell is in contiguity with 2 other somatostatin cells; one of them, labeled by arrowheads in a–i has no vascular contact. ×780

Fig. 10. Schematic drawings of 2 somatostatin cells, graphically reconstructed from immunostained serial semithin (1.0 μm) sections through human islets of Langerhans. The cell shown in a corresponds to that labeled by arrows in Fig. 9. The somatostatin cell shown in b was similarly reconstructed from another series of sections. Intensities of immunostaining are represented by dark spots. C capillaries. According to their morphological features and the distribution of immunoreactivities, this kind of islet somatostatin cells may remind one of neurons with receptor and effector poles.
The processes reaching capillaries can easily be recognized due to their dense immunostaining. D-cells have, however, also shorter cytoplasmic processes that are less densely stained or lack immunoreactivities. These "silent" (with respect to immunostaining) processes extend between other endocrine cells (Fig. 8-10). The latter processes and the corresponding cytoplasmic areas near the nuclei (perikarya) were difficult to distinguish, especially when they were cut tangentially, in sections that had been stained with highly diluted somatostatin antisera (according to the usual staining protocol). However, these cell parts could clearly be demonstrated when sections were overstained by the use of less dilute antisera (1:1,000) as shown in Figure 8. It should be emphasized that a considerable proportion of inter-D-cell contiguities consist of juxtapositions of those cell parts lacking immunoreactive material. Therefore, they often could be distinguished only by careful examination of serial sections at high microscopic magnifications.

Intracellular distribution of somatostatin immunoreactivity. As already indicated in the preceding paragraph, the intensity of immunostaining and the intracellular distribution of immunoreactive material depend on the shape of the D-cells or on their position within the islet tissue. In D-cells that show a rounded shape and border adjacent capillaries with broad areas, immunoreactivities are strong and nearly evenly distributed within the cytoplasm (e.g., some of the D-cells shown in Fig. 4 a–m). In D-cells that lack vascular contacts, the immunoreactive material is relatively sparse and localized in the perinuclear cytoplasm (D-cell indicated by an arrowhead in Fig. 9). The prototypes of D-cells, characterized by their polygonal shape, show an uneven distribution of immunoreactive material. Within these types of cells, cell parts extending towards capillaries or cytoplasmic processes facing capillaries are densely immunostained (Fig. 6, 8–10). When such cytoplasmic extensions are cut transversely and the densities of immunostaining are compared at various planes, it becomes evident that staining intensities increase the nearer these processes come to the capillary wall (Fig. 7). The remaining cytoplasmic areas of polygonal D-cells including cell parts remote from capillaries and "silent" processes show only moderate staining intensities or a complete lack of immunoreactive material (Fig. 6–10).

DISCUSSION

The present investigation predominantly aimed to characterize the D-cells of human islets of Langerhans according to cytologic and immunocytochemical criteria and to correlate the findings with the prevailing mode of function of these cells. The studies were performed with techniques newly introduced in this field, namely graphic or three-dimensional reconstructions based upon immunostained serial semithin sections. Because appropriate specimens can not be obtained from healthy subjects, tissues from patients who had undergone partial resection of the pancreas because of endocrine adenomas were investigated. Therefore at first data related to the tissue material investigated and to the techniques applied have to be discussed. Thereafter, we will consider cytologic and histotopographic features of islet D-cells and the functional implications of our findings. Finally, more general aspects of the microarchitecture of pancreatic islets will be dealt with, so far as this can be judged from our results.

1. Material and methods
To determine possible pathologic changes we examined our tissue material according
to various histologic and cytologic parameters and compared them to data as given in the literature.

Proportion of endocrine tissue. Estimations of the number of islets per area of pancreatic tissue sections revealed about 1.1 islets/mm² in the pancreases of the control group and about 2.5 islets/mm² in the pancreas of patient M. Even though this method may give only semi-quantitative data (LAGUESSE, 1905; BARGMANN, 1939; FERNER, 1952), the latter pancreas obviously showed an augmentation of the endocrine tissue. Such an increase in the proportion of the endocrine tissue in non-tumorous regions has also been described in other cases of endocrine adenomas where it has tentatively been referred to as trophic actions of the peptides secreted from the adenomas (CREUTZFELDT, 1980).

Size of the islets. Within the human pancreas the sizes of the islets vary within a wide range. However, about 70% of all islets have a greatest diameter between 75 μm and 225 μm (NEUBERT, 1927; BARGMANN, 1939; FERNER, 1952). Concerning this parameter, we found no basic differences from these values in our tissue material. For detailed morphometric analyses we selected islets with diameters between 110 and 240 μm, which thus corresponded to the most frequent islet types.

Angioarchitecture. This aspect will be discussed below. It should, however, be already mentioned here that the islets coded as Nos. I-V were not uniform with respect to their vascular supply. This finding confirms the observations of other authors (FERNER, 1952; SAPIN and VDOVIN, 1981).

Frequency and distribution of the major endocrine cell types. Up to now exact determinations of the relative proportions of B-, A-, D- and PP-cells in human islets of Langerhans have rarely been performed. The proportion of each endocrine cell type turned out to be dependent on the pancreatic region and the individual age (FERNER, 1952; GEPTS, 1965; GERSELL et al., 1979; MALAISSE-LAGAE et al., 1979; ORCI et al., 1979; MILNER et al., 1981). The mean values of our quantitative estimations (Table 1) did not show striking differences from data obtained in the islets of corresponding pancreatic regions of healthy human subjects. Especially was the frequency of D-cells in our tissue material (4.8-7.5%) within a range that corresponded to normal values. Likewise, the distribution of the main endocrine cell types in the islets (Fig. 1) corresponded to findings as given in the literature (LANGE, 1973; ERLANDSEN et al., 1976; ALUMETS et al., 1977; ORCI, 1977; ORCI and PERRELET, 1979; ERLANDSEN, 1980; WATKINS et al., 1980; UNGER and ORCI, 1981a, b), but some peculiarities of human nesohistology have to be considered in more detail (see below).

Cytology and immunohistochemistry. At the electron microscopic level we observed all types of endocrine cells as described in human islets of Langerhans (ORCI et al., 1974; ALUMETS et al., 1977; CAPELLA et al., 1977; PELLETIER, 1977). Confirming the results of other authors, somatostatin-immunoreactive cells were identified at the ultrastructural level by a semithin ultrathin section technique as typical D-cells. Changes classified as pathologic ones were those observed in glucagon cells of islets from the pancreas of patient F (insulinoma). These cells only showed less dense immunostaining at the light microscopic level, and they contained only few secretory granules seen ultrastructurally. Since insulin has an inhibitory effect upon glucagon metabolism (SAMOLS et al., 1972) these changes may be caused by the increased insulin secretion from the corresponding adenoma.
Analogous to findings in endocrine cells of intestinal epithelia (Fiocca et al., 1980; Ravazzola and Orci, 1980; Grube and Aebert, 1981; Lehy et al., 1981; Grube, 1982) and in the endocrine pancreas of various species (Grube et al., 1982a) we sporadically found an “overlapping” of glucagon and PP immunoreactivities also in endocrine cells of the human pancreas. At present these findings are difficult to interpret; they point, however, to a close relationship between glucagon- and PP-cells. A definite explanation for these immunohistochemical findings will perhaps only be possible after the isolation and sequence analysis of glucagon and PP precursor proteins.

A colocalization of somatostatin and endorphin-like immunoreactivities has been described in D-cells of the human endocrine pancreas (Watkins et al., 1980). We could not confirm these findings during the present investigation. This discrepancy may be caused either by another regional specificity of our endorphin antisera or by differences in interpreting immunohistochemical results. According to our experiences (Grube, 1980; Grube and Weber, 1980; Grube and Aebert, 1981) a specific immunohistochemical reaction—among other criteria—should also be stable when ascending dilutions of the primary antisera are used. This stability could not be achieved during the mentioned investigation (Watkins et al., 1980) for endorphin immunoreactivities in D-cells.

In summary, the islets more closely investigated in our specimens according to most of the parameters taken into consideration were comparable to corresponding findings in pancreatic islets of healthy human subjects. However, it should be emphasized that recent publications on human pancreatic islets only refer to some of these parameters that are necessary for the histologic and cytologic characterization of the endocrine pancreas in health and disease.

Histologic and reconstruction techniques. Previous light microscopic investigations on human islets of Langerhans were mainly performed using 3-10 μm paraffin sections and empirical or immunofluorescent staining techniques. The main advantages of the present methods (apart from those already mentioned) result from the thinness of the tissue sections and from the possibility of using phase contrast optics for examination of immunoperoxidase stained sections. Thus, intracellular distribution of immunoreactive material within single cells as well as their complete shape and their anatomical relationship within the islets can be clearly determined.

Certain problems arose, however, concerning the methods of three-dimensional reconstructions from serial semithin sections. The use of plastic plates (Goldstein and Davies, 1968; Baetens et al., 1979) seemed inappropriate for the purposes of the present study. Therefore, we utilized 1 mm wax plates or 1 mm balsa plates for the reconstructions of single cells. However, due to their brittleness or instability, these materials are less suitable for the reconstruction of cell groups “linked” together by thin cytoplasmic extensions. For the latter objective we found most suitable the NEU system of neurographic programs that originally was designed for computerized graphic reconstructions of neurons from serial sections (Speck, 1981).

2. Cytology and arrangement of D-cells

Our findings show that the D-cells of human islets of Langerhans may be subdivided into two basic types. The one type, represented by only a minority of D-cells, has no apparent relationship to blood vessels. Accordingly, this type of D-cell—extending and modifying the basic subdivision of entero-endocrine cells (Fujita and Kobayashi, 1973)—may be called a “closed type” D-cell. The other type of D-cell, represented by the
great majority of these cells, borders the vascular system and therefore may be called D-cell of the “open type.” These latter D-cells are further characterized by their polygonal shape, and they often display long cytoplasmic extensions towards the intraislet capillaries, whereas shorter processes are localized between adjacent endocrine cells. With regard to their shape these D-cells remind one of neurons and—taking into account the concept of “paraneurons” (Fujita, 1977; Fujita et al., 1981)—of a corresponding bipolar structure (see also: Matsuba et al., 1982). Hence, the long cytoplasmic extensions towards capillaries would represent the effector poles and the perikarya or short processes be analogous to receptor poles. This assumption is supported by the intracellular distribution of immunoreactive material: the vascular pole of these D-cells regularly is strongly immunoreactive, whereas the other cell parts are only weakly stained or show no staining at all. However, this view is probably too abstract, and that mainly because of two reflexions. Firstly, the presumed “effector poles” of D-cells simultaneously have to function also as receptors of blood-born or nerval signals. Secondly, histologic investigations permit, of course, no conclusions with respect to the plasticity of D-cell processes and of their level of hormone content. It seems, for instance, imaginable that islet D-cells in vivo show phasic changes. Thus, there might be an interchange between the “open” and “closed” types according to different patterns of stimulatory and inhibitory signals.

Our determination of “homologous” and “heterologous” anatomical relationships revealed that the contiguity between D-cells is another characteristic feature of this cell population. In view of the relative sparsity of D-cells, the proportion of 67% homologous contacts must be regarded as being beyond a random conformation and demands a functional explanation for this arrangement. We presume that this contiguity is the morphologic counterpart of a functional unit of islet D-cells where all information is delivered to other members of the cell population, thereby synchronizing their function. Of course, this presumption is entirely hypothetical. As yet, morphologic investigations have shown membrane specializations (gap junctions) between all three major types of islet cells (Orci et al., 1974; Orci and Ungar, 1975; Orci, 1976; Forssmann et al., 1978; Orci and Perrelet, 1979) and there is also evidence of the electrotonic and metabolic coupling of cells via gap junctions (Kohen et al., 1979; Meda et al., 1980; Michaels and Sheridan, 1981; Schwarzmann et al., 1981). However, additional specialized intercellular contacts between homologous islet cells have not been detected. Because we found during preliminary studies in the human pancreatic islets an even more pronounced contiguity between A-cells, we, albeit, are convinced that there are peculiar interrelationships between the members of each of the major endocrine cell populations of the islets. Whether these functional connections occur via electrotonic coupling as it has been shown for insulin cells (Meissner, 1976) can only be examined when more sophisticated experimental methods will be available.

3. D-cells, endocrine or paracrine elements?

Since the D-cell has been identified as an independent cell type of the islets of Langerhans (Bloom, 1931; Caramia, 1963; Fujita, 1966, 1968; Kobayashi and Fujita, 1969) its close relationship to capillaries was repeatedly described (Caramia, 1963; Munger et al., 1965; Fujita, 1966, 1968; Kobayashi and Fujita, 1969; Rhoten, 1971; Goldsmith et al., 1975). Moreover, several authors observed exocytotic figures at the vascular pole of D-cells (Machino et al., 1966; Fujita, 1968; Kobayashi and Fujita, 1969; Watanabe et al., 1975; Schusdziarra et al., 1978). Therefore, there was no real doubt that D-cells correspond to typical endocrine cells. However, the discovery of somatostatin in D-
cells (Luft et al., 1974; Dubois, 1975; Goldsmith et al., 1975; Hökfelt et al., 1975; Orci et al., 1975; Pelletier, 1977) and the knowledge of the inhibitory effects of this peptide upon islet A- and B-cells (Alberti et al., 1973; Hall et al., 1973; Gerich et al., 1974; Koerker et al., 1974) favored the assumption of a local action of pancreatic somatostatin. When the apparent interposition of D-cells between A- and B-cells had been emphasized and interpreted in the sense of mutual functional relationships between adjacent cells (Orci and Ungér, 1975), the pancreatic D-cell later on served as a paradigm of a “paracrine” cell.

Our findings in human islets of Langerhans have now shown that the anatomical position of D-cells must be regarded as more differentiated and that morphologic data permit various conclusions with respect to the mode of function of these cells. Only a minority of D-cells (about 15%) apparently lacks vascular contacts. These “closed” types of D-cells might influence adjacent cells by somatostatin secreted into the intercellular space, that is by paracrine pathways according to the original concept (Freyter, 1953). The great majority of D-cells, however, is closely related to the intrainsular circulation and shows intracellular accumulations of immunoreactive material towards the vascular poles (“open” type D-cells). This observation indeed is not restricted to human islet D-cells; we obtained similar findings in the islets of rats, rabbits, and dogs when serial semithin sections were examined with respect to this parameter. The close relationship between D-cells and the vascular system is also evident from a finding whose functional implications as yet can be interpreted only speculatively. Within certain segments of the islets D-cells gather together forming a cuff-like border around capillaries penetrating the islet cortex. These D-cell cuffs can be found only once or twice in an islet, and we presume that the corresponding capillaries are the afferent vessels of the islets (see also: Ferner, 1952; Thiel, 1954; Sapin and Vdovin, 1981).

Considering the morphologic criteria, most of the islet D-cells thus may secrete into the capillary system or at least into the perivascular space. Accordingly, two pathways can be assumed for the regulation of A-cells and B-cells by pancreatic somatostatin cells of the open type. A secretion into the perivascular space will reach only a limited number of adjacent cells, whereas a somatostatin secretion into the intrainsular capillaries, especially into the vasa afferentia would influence a greater number of islet cells (first target) as well as—via insulo-acinar “portal” vessels—exocrine pancreatic cells, the second target (see also: Thiel, 1954; Fujita and Murakami, 1973; Fujita et al., 1976, 1981; Henderson and Daniel, 1979; Miller, 1980; Kawai et al., 1982).

Our observations that long cytoplasmic extensions of islet D-cells were regularly found to run towards capillaries might also be of interest concerning the functional morphology of D-cells in gastric epithelia. Here, similar processes have been interpreted as the morphologic counterpart of possible cell-to-cell (paracrine) interactions between D-cells and adjacent endocrine and non-endocrine epithelial cells (Alumets et al., 1979; Kusumoto et al., 1979; Larsson et al., 1979). Taking into account our findings in islet D-cells, the relationship of gastric D-cell processes to the subepithelial vascular system should also be examined.

4. Microanatomy of the islets of Langerhans

Most recent publications on the histology of human islets of Langerhans have dealt with the relative proportion of the major endocrine cell types in health and disease. In addition to this nesocytology (“Zellbild”; Ferner, 1952), other morphologic parameters should be taken into consideration to find out the principles of islet function as well as to recognize possible disorganizations within these cellular communities. Apart from
morphologic features to be examined at the ultrastructural level, these other parameters include the arrangement of the endocrine tissue as a whole (nesohistology), the vascular supply of the islets (nesoangiology), and the innervation of the endocrine tissue (nesoneurology). We here will discuss the nesohistology, nesocytology, and nesoangiology of human islets as far as the findings of the present study allow conclusions in this context.

In human islets of Langerhans an arrangement of the endocrine tissue in form of islet “lobules” has been described (ORCI, 1977; UNGER et al., 1978; UNGER and ORCI, 1981 a, b). According to this image the B-cells constitute the core of these subunits and they are surrounded by a heterocellular layer of endocrine cells localized either in the islet cortex or along the intraislet capillaries. Based upon this anatomical arrangement several hypotheses on the intrainsular organization have been put forward (UNGER and ORCI, 1977, 1981a, b; UNGER et al., 1978; LARSSON, 1980). In view of the present findings, however, the images on nesohistology and nesocytology or the corresponding hypotheses on the functional relationships between the various endocrine cell types in our opinion seem to be too schematic.

During our investigations we obtained no morphologic evidence for a subdivision of human islets into lobules. Indeed, only examinations of single histologic sections suggest this kind of organization (Fig. 1). Seen three-dimensionally, however, islet lobules in the anatomical sense apparently do not exist and that for two reasons: firstly, the islets contain only a small amount of connective tissue which is confined to the perivascular spaces; secondly, the arrangement of the capillaries (the border-lines of the presumed lobules) may vary from one histologic section through an islet to an adjacent section. Thus, even adjacent planes of the same islet show different patterns of the capillary network (Fig. 4). We therefore share the opinion that the islets are “made up of a compact mass of cells pervaded by a network of capillaries” (GOLDSTEIN and DAVIES, 1968; see also: NEUBERT, 1927). Moreover, the existence of islet lobules in a functional sense is not supported by our findings. The composition of the layers of endocrine cells bordering the intrainsular capillaries varies from one plane to the other. Thus, a layer to be classified as “heterocellular” in the one plane may turn to a “homocellular” layer (made up of B-cells) in an adjacent segment, and vice versa. Because of these reasons we deny the existence of lobules as morphologic or functional subunits of human islets. Rather, our findings indicate that—apart from the postulated principle of contiguities between the cells of each major endocrine cell population—the arrangement of the various endocrine cell types in the first place depends on the vascular supply of the islets or, more exactly, on the kinds of islet capillaries and their segments actually considered. Most interestingly, and confirming the findings of some other authors (FERNER, 1952; THIEL, 1954; SAPIN and VDOVIN, 1981), the mode of intrainsular ramifications of the vasa afferentia was not uniform. Obviously in consequence of this fact, the arrangement of the major endocrine cell types varied between the islets. In islets of relatively small size (e.g. islets Nos. I and V of the present study) we found a kind of “central vessel” from which the tentative vasa efferentia extended radially to the islet cortex. A heterocellular layer of endocrine cells was observed mainly adjacent to this centrally located capillary, whereas the vasa efferentia were surrounded predominantly by B-cells. Larger sized islets (e.g. islet Nos. II and III) showed another kind of vascular supply. Here, the tentative vas afferens (surrounded by a cuff of D-cells) ramified near the islet cortex into an anastomotic capillary network which gave rise to relatively short vasa efferentia. Only histologic sections passing near the
"equator" through this type of islet (Fig. 1) showed an arrangement of endocrine cell types generally considered as being characteristic for human islets.

Previous studies on the nesoangiology of the mammalian pancreas revealed species-specific differences in the type of vascular supply of the islets, and the interspecies variations of nesocytology have been connected with these differences (FERNER, 1952; FUJITA and MURAKAMI, 1973; FUJITA et al., 1976). To our knowledge, corresponding detailed studies in human islets are still lacking. Due to the techniques applied in the course of the present study, we could only tentatively differentiate between the vasa afferentia and efferentia of the islets. In any case, the apparent heterogeneity of the vascular supply and of the arrangement of endocrine cells—in addition to the reasons already mentioned—makes it difficult to give at present a generalized description of the microarchitecture of human islets of Langerhans.

Acknowledgments. We are indebted to Dr. P. T. SPECK and Dr. H.-J. WAGNER for help with the computer graphics and to Dr. V. SCHUSDZIA for help with the manuscript. The expert technical assistance of Ms. M. HANSER and Ms. G. KRÄUTLE is gratefully acknowledged.

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