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Cell Types of the Endocrine Pancreas in the Shark *Scyliorhinus stellaris* as Revealed by Correlative Light and Electron Microscopy*

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Summary. In the pancreas of *Scyliorhinus stellaris* large islets are usually found around small ducts, the inner surface of which is covered by elongated epithelial cells; thus the endocrine cells are never exposed directly to the lumen of the duct. Sometimes, single islet cells or small groups of endocrine elements are also incorporated into acini. Using correlative light and electron microscopy, eight islet cell types were identified:

Only B-cells (*type I*) display a positive reaction with pseudoisocyanin and aldehyde-fuchsin staining. This cell type contains numerous small secretory granules (⌀ 280 nm). *Type II*- and *III*-cells possess large granules stainable with orange G and azocarmine and show strong luminescence with dark-field microscopy. Type II-cells have spherical (⌀ 700 nm), type III-cells spherical to elongated granules (⌀ 450 × 750 nm). Type II-cells are possibly analogous to A-cells, while type III-cells resemble mammalian enterochromaffin cells. *Type IV*-cells contain granules (⌀ 540 nm) of high electron density showing a positive reaction to the Hellman-Hellerström silver impregnation and a negative reaction to Grimelius' silver impregnation; they are most probably analogous to D-cells of other species. *Type V*-cells exhibit smaller granules (⌀ 250 × 500 nm), oval to elongated in shape. *Type VI*-cells contain small spherical granules (⌀ 310 nm). *Type VII*-cells possess two kinds of large granules interspersed in the cytoplasm; one type is spherical and electron dense (⌀ 650 nm), the other spherical and less electron dense (⌀ 900 nm). *Type VIII*-cells have small granules curved in shape and show moderate electron density (⌀ 100 nm). Grimelius-positive secretory granules were not only found in cell types II and III, but also in types V, VI, and VII. B-cells (type I) and the cell types II to IV were the most frequent cells; types V to VII occurred occasionally, whereas type VIII-cells were very rare.

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In the pancreatic islets of cartilaginous fishes, at least three or four types of cells have been described at the light microscopic level (Fujita 1962; Östberg et al. 1966; Patent and Epple 1967; Kern 1964, 1971). In the present study, correlative light and electron microscopic methods were used to describe the endocrine cell types and their ultrastructure in an elasmobranch, *Scyliorhinus stellaris*.

Materials and Methods

Sharks, *Scyliorhinus stellaris*, were captured at Helgoland in the North Sea, Federal Republic of Germany. For light microscopy, blocks of pancreatic tissue were fixed in Bouin's fluid and embedded in paraffin. Sections were stained with azan, aldehyde-fuchsin-orange G (AFLO) or silver impregnation according to Hellman and Hellerström (H-H) (1960). Materials for electron microscopy were fixed in 10% glutaraldehyde, buffered at pH 7.0 with sodium cacodylate buffer, and postfixed in 1% osmic acid with phosphate buffer. After dehydration in a series of acetone solutions, the tissues were embedded in epoxy resin and cut on an LKB ultramicrotome. Sections were mounted on uncoated grids, stained with uranyl acetate and lead citrate and photographed using a Philips EM 201-G electron microscope. Some materials were sliced with a razor-blade, stained en bloc with Grimelius' silver nitrate method (after glutaraldehyde fixation) and embedded in epoxy resin after dehydration. Thin sections were observed after staining with uranyl acetate only. To identify identical cells by both light and electron microscopy, a few thin sections were stained with uranyl acetate and lead citrate and the adjacent two thick sections were stained with aldehyde-fuchsin and pseudoisocyanin (PIC), respectively, after removal of resin. The same method was applied to the tissues stained en bloc according to Grimelius; the next thick section was stained with aldehyde-fuchsin or PIC. Indirect immunostaining was attempted for glucagon, insulin and somatostatin according to Lange et al. (1975).

Results

Pancreatic islets of the shark, *Scyliorhinus stellaris*, are scattered in the exocrine pancreas and show the typical relationship to ducts as characteristic of other cartilaginous fishes. Large islets are elongated in shape and surrounded by a thin layer of connective tissue (Fig. 1). Only rarely the islet cells display a direct contact with the exocrine cells of the pancreas. Two long slender cytoplasmic processes extending from both sides of a ductular epithelial cell appear to anchor one or a few islet cells to the basal lamina, and in some cases the epithelial cell appears to envelop islet cells completely (Fig. 4, 5). Numerous capillaries surround the outer surface of the islet (Figs. 1, 2), and nerve fibers sometimes are found within the thin surrounding connective tissue layer (Fig. 7). In addition to these large islets, single endocrine elements or small groups of islet cells may occur among the exocrine acinar cells. They are never exposed directly to the acinar lumen, being surrounded by centroacinar and acinar cells.

By means of correlative light and electron microscopic techniques, eight islet cell types were identified on the basis of their light- and ultrastructural characteristics (Table 1). In the order of appearance these cells are classified here as type I-(B-cell) to type VIII-cells. Each cell type occurs singly or in small mixed groups and does not show a regular distributional pattern. Only B-cells are positive with pseudoisocyanin and aldehyde-fuchsin staining. Non-B-cells show different tinctorial

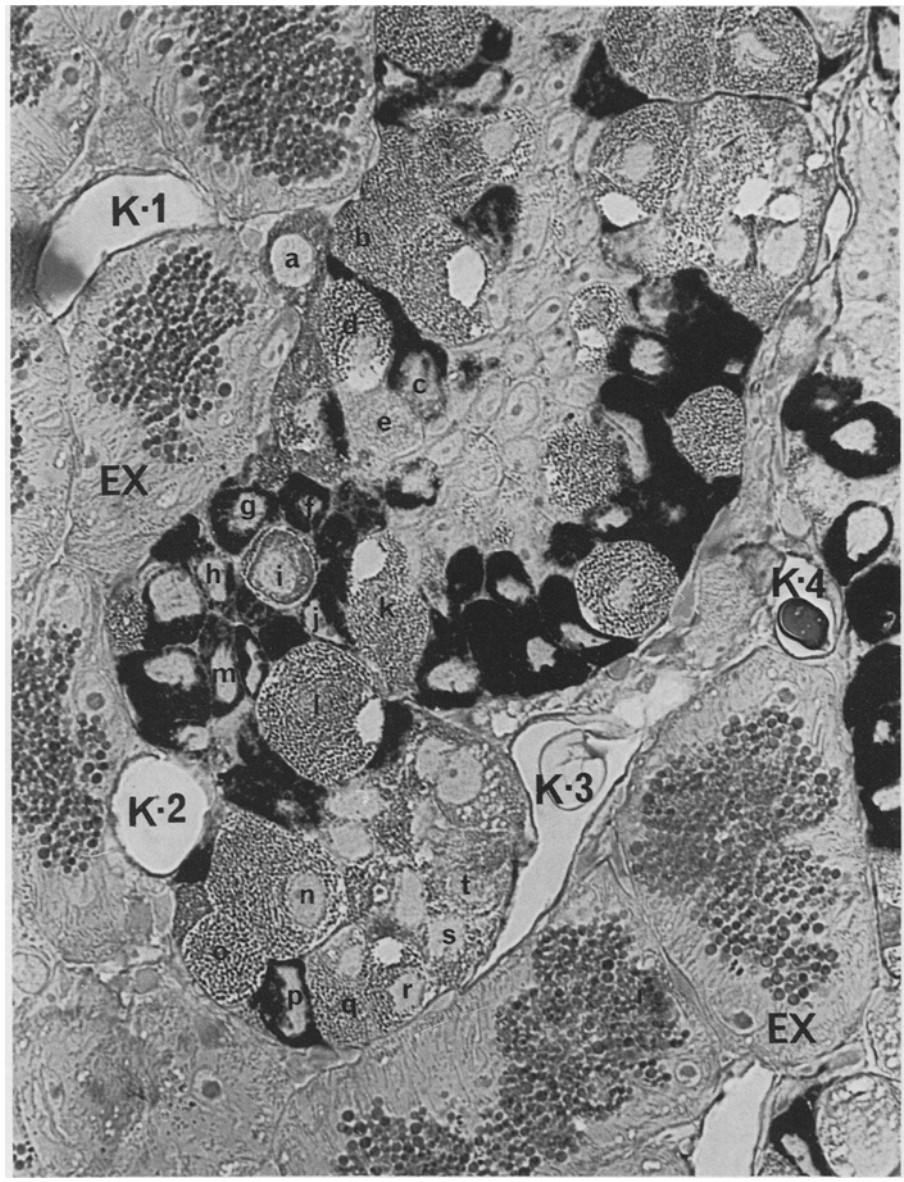


Fig. 1. A portion of an islet from *Scyliorhinus stellaris* surrounded by thin connective tissue with capillaries (*K-1, K-2, K-3, K-4*). Exocrine acinar cells (*EX*) with numerous zymogen granules located outside of the islet tissue. Semithin section stained with pseudoisocyanin. Standard double-fixation. The darkly stained cells are B-cells displaying an oval, light nucleus. Symbols: *a* type V-cell; *c, f, g, h, j, m, p* type I-cells; *e* type VIII-cell; *b, d, h, i, l, n, o, q, r, s, t* type II-cells have been used in Figs. 1–4. ×800

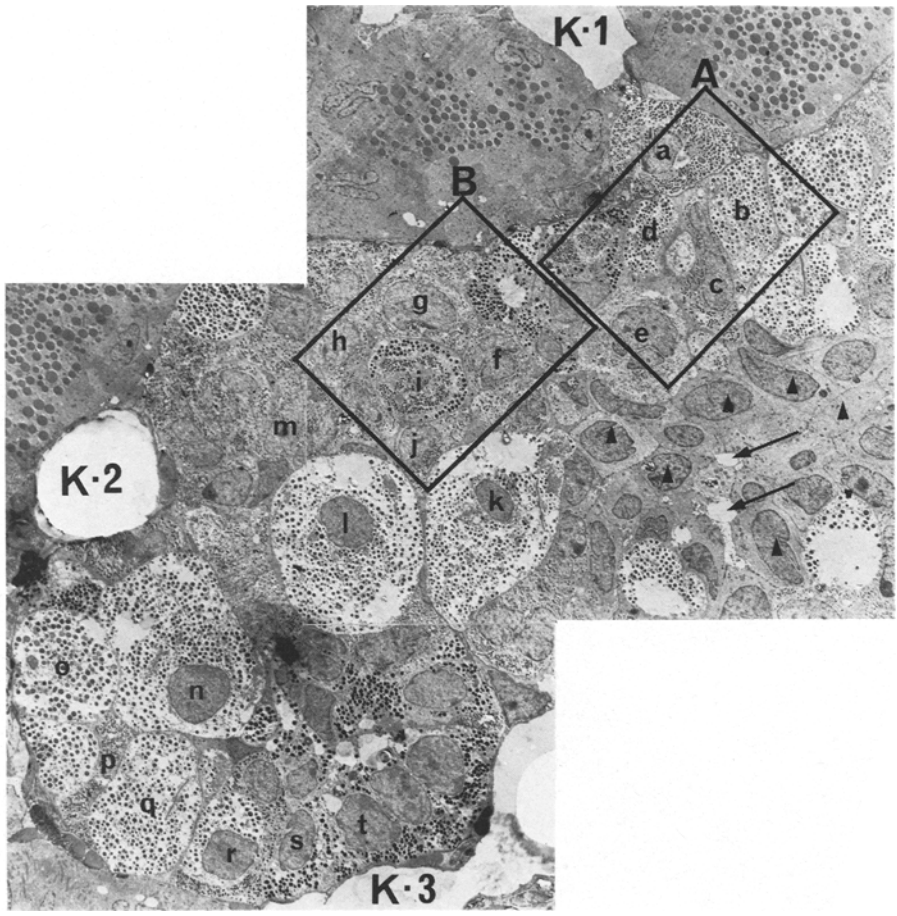
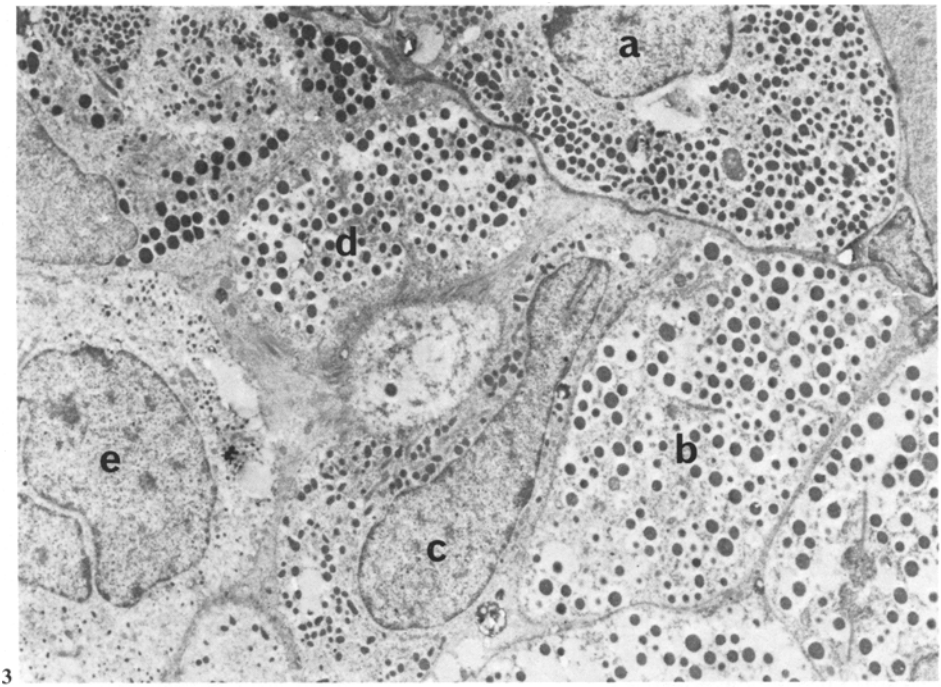


Fig. 2. Correlative electron micrograph of a thin section cut subsequently to that shown in Fig. 1. *Arrow* small lumen of the duct; *arrowhead* epithelial cell of the duct. ×1,140

properties of their cytoplasm after application of different counterstains. Using aldehyde-fuchsin/light-green/orange G (AFLO), the cytoplasm of various types of non-B-cells stained orange to yellow. The number of these cells varies greatly. Electron microscopically, each cell type was seen to be well granulated and displayed different shapes of secretory granules. Epithelial cells of the ducts were readily distinguished from endocrine cells due to their scarce organelles and to the absence of secretory granules in the cytoplasm. All types of islet cells contact each other. Desmosomes between endocrine cells, and between endocrine cells and epithelial cells of the duct were rare, but they were found frequently between epithelial cells (Figs. 2, 10).

Cell Type I (B-Cell)

As shown in Fig. 1, B-cells staining selectively with pseudoisocyanin are cylindric or polygonal in shape, of medium size and arranged frequently in groups of variable



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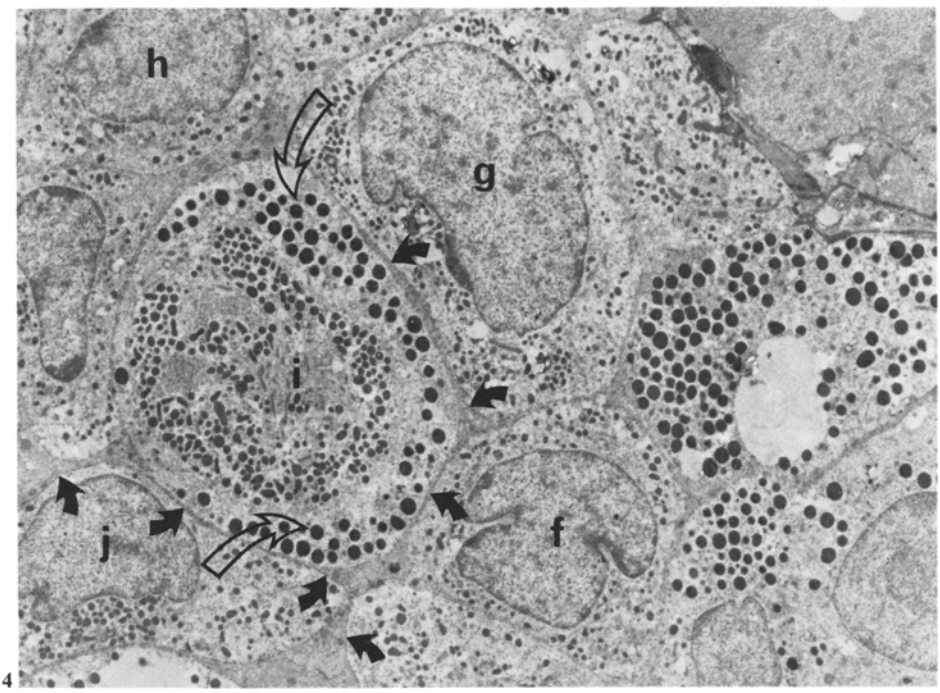

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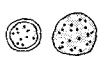
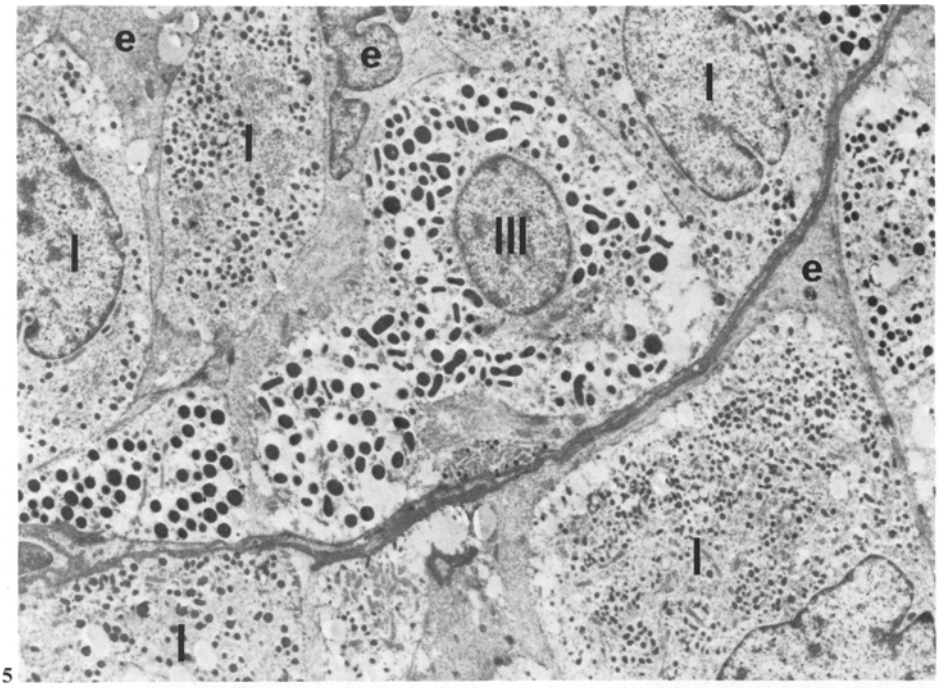
Fig. 3. Electron micrograph of the same group of cells shown in the framed area of Fig. 2A: *a* type V-cell; *b, d* type II-cells; *c* type I-cell (B-cell); *e* type VIII-cell. ×4,100

Fig. 4. Electron micrograph of the same group of cells shown in the framed area of Fig. 2B. The cytoplasm of the type IV-cell (*white arrows*), which contains numerous dense spherical granules, surrounds the cytoplasm of the type V-cell (*i*) completely. *Black arrows* indicate slender cytoplasmic processes of ductular epithelial cells extending between islet cells; *f, g, h, j* type I-cells (B-cells). ×4,100

Table 1. Morphological characterization of islet cells in *Scyliorhinus stellaris*

Observation	Cell type (suggested function)							
	I (Insulin)	II (Glucagon)	III (Entero-chromaffin)	IV (Somato-statin)	V	VI (Pancreatic polypeptide)	VII	VIII
LM of cells								
Size and shape	Medium and polygonal	Large and oval	Large and oval	Medium and polygonal	Medium and oval-polygonal	Large and oval	Large and oval	Small and oval-angular
Stainability								
AFLO	Purple	Dark brown	Light brown	Orange	Dark yellow	Light green	Dark blue	Light blue
Pseudoisocyanin	+++	−	−	−	−	−	−	−
Azocarmine	−	++	++	−	(+)	+	±	−
Brightness in dark field	−	+++	++	+	−	−	−	−
Silver impregnation (H-H)	−	−	−	+++	−	−	−	−
EM of secretory granules								
Scheme of granule								
Size, shape and density	Small, angular, less dense	Large, spherical, moderately dense	Large, spherical-elongated, dense	Medium, spherical angular, dense	Medium, elongated, dense	Small, spherical, moderately dense	Large, spherical, dense or less dense	Small, angular, dense
Mean diameter (nm)	280	700	450 × 750	540	250 × 500	310	650, 900	100
Silver impregnation (Grimelius)	−	++	++	−	++	++	++ ++	−

(+) very weak; + weak; ++ moderate; +++ strong; − negative. *AFLO* aldehyde-fuchsin / light green / orange G; *H-H* Hellmann and Hellerström; *LM* light microscopy; *EM* electron microscopy



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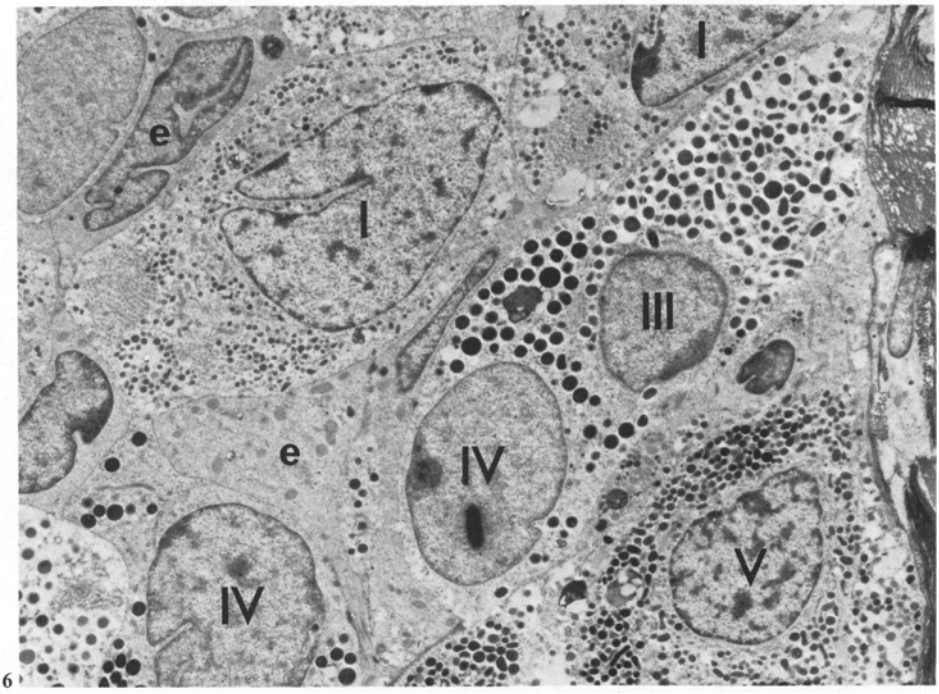

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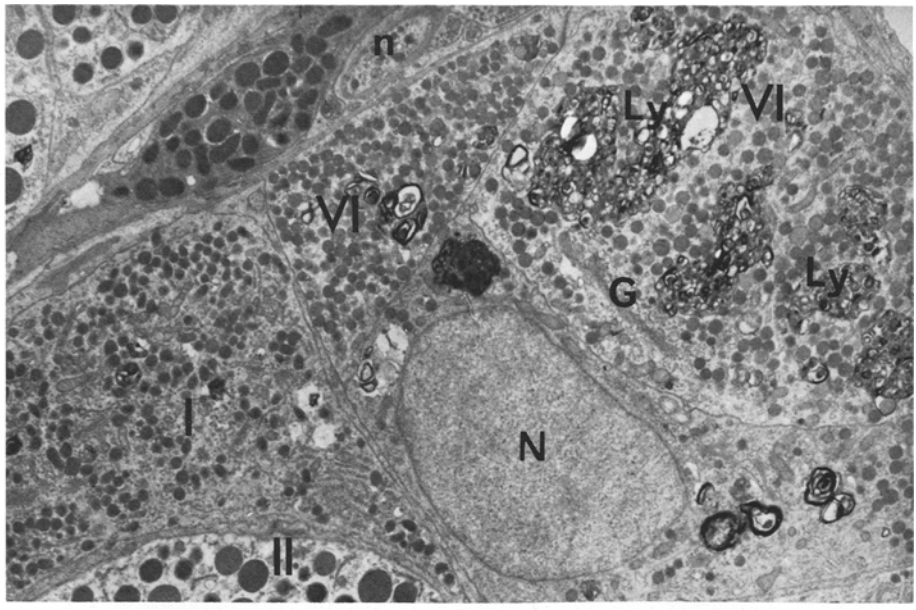
Fig. 5. Electron micrograph of portions of islets in *Scyliorhinus stellaris*. Standard double-fixation. Certain type I-cells = B-cells (*I*) possess small secretory granules (I-a), whereas other elements contain somewhat larger granules (I-b); *e* epithelial cell of the duct; *III* type III-cell. × 4,100

Fig. 6. Electron micrograph of portions of islets in *Scyliorhinus stellaris*. Standard double-fixation; *e* epithelial cell of the duct; *I* type I-cell (B-cell); *III* type III-cell; *IV* type IV-cell; *V* type V-cell. × 4,100



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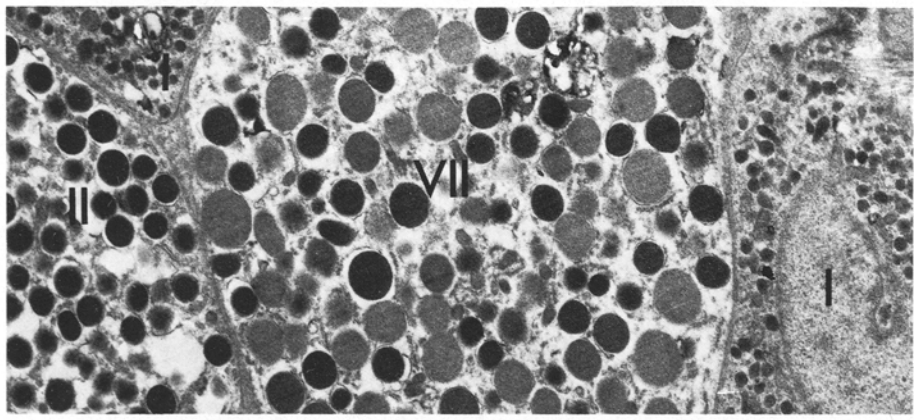

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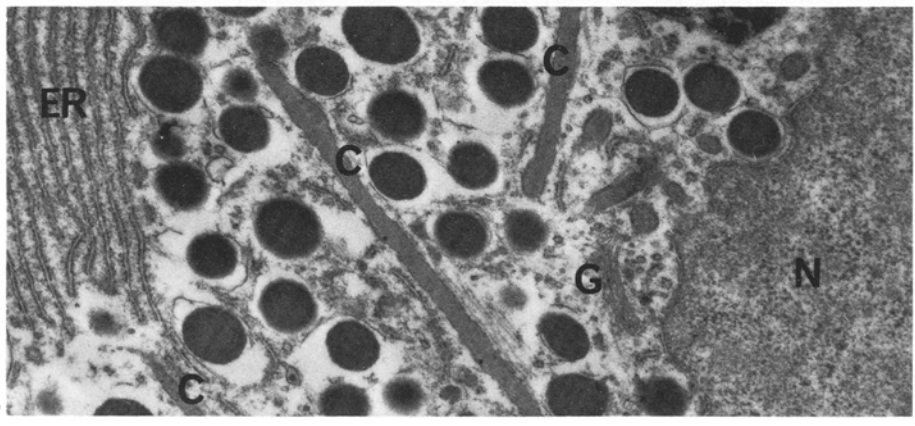


9

numbers. Some of them appear to emit long, slender cytoplasmic processes between neighboring cells. When stained with aldehyde-fuchsin, the cytoplasm shows a variable intensity of purple coloring. Electron microscopically, B-cell granules are very small, angularly shaped and exhibit homogeneous, moderately dense cores (Figs. 3–8). Sometimes two kinds of granules are found in B-cells: (i) a smaller type (I-a: 250 nm in diameter) and (ii) a somewhat larger type (I-b: 300 nm in diameter) (Fig. 5). The average diameter of B-granules was 280 nm. These granules are negative to Grimelius' silver impregnation (Figs. 10, 12).

Cell Type II

Light microscopically, the largest round cells have a scattered distribution and possess oval nuclei. These cells contain large, coarse secretory granules which stain red with azocarmine, dark brown with AFLO-staining and shine brightly in the dark field. Electron microscopically, secretory granules of this cell type show circular cores of moderate density with a surrounding limiting membrane; a narrow space is recognizable between these two structural components (Figs. 3, 8, 9). The granules have an overall diameter of 600 to 850 nm (average 700 nm). Number and size of these structures are variable in each type II-cell. Using Grimelius' silver impregnation method, fine grains appear over both the dense core and at the narrow surrounding halo (Figs. 10, 11). Some cells that contained several crystalline structures filled with numerous parallel, long fibers and/or fine cross-periodicity were encountered; the largest one measured 0.2 μm in width and 7 μm in length (Fig. 9).

Cell Type III

Light microscopically, this cell type resembles very closely type II-cells. They are large and oval in shape with an oval nucleus. However, they contain somewhat slender granules stained light brown with orange G and red with azocarmine. With dark-field microscopy, secretory granules of this cell type shine brightly but less so than granules of type II-cells. Electron microscopically, the secretory granules of this cell type are very variable, having spherical, oval or elongated cores of high electron density with a surrounding smooth limiting membrane; a narrow space is

Fig. 7. Electron micrograph of a portion of an islet of *Scyliorhinus stellaris*. Standard double-fixation. Three type VI-cells (*VI*) are present containing numerous small spherical secretory granules of moderate density, a Golgi apparatus (*G*), and lysosomes (*Ly*); nucleus (*N*). Note somewhat irregular secretory granules of a type I-cell = B-cell (*I*); *n* nerve fiber in connective tissue; *II* type II-cell. × 6,800

Fig. 8. Electron micrograph of a portion of a type VII-cell (*VII*) containing both dense and less dense granules. Two type I-cells = B-cells (*I*) and a type II-cell (*II*) are situated adjacent to the type VII-cell. Standard double-fixation. × 6,800

Fig. 9. Electron micrograph of a portion of a type II-cell. Standard double-fixation. Small Golgi apparatus (*G*) located adjacent to the nucleus (*N*). Portions of three long crystalline structures (*C*) are found among the secretory granules. At the left, lamellar granular endoplasmic reticulum (*ER*). × 16,500

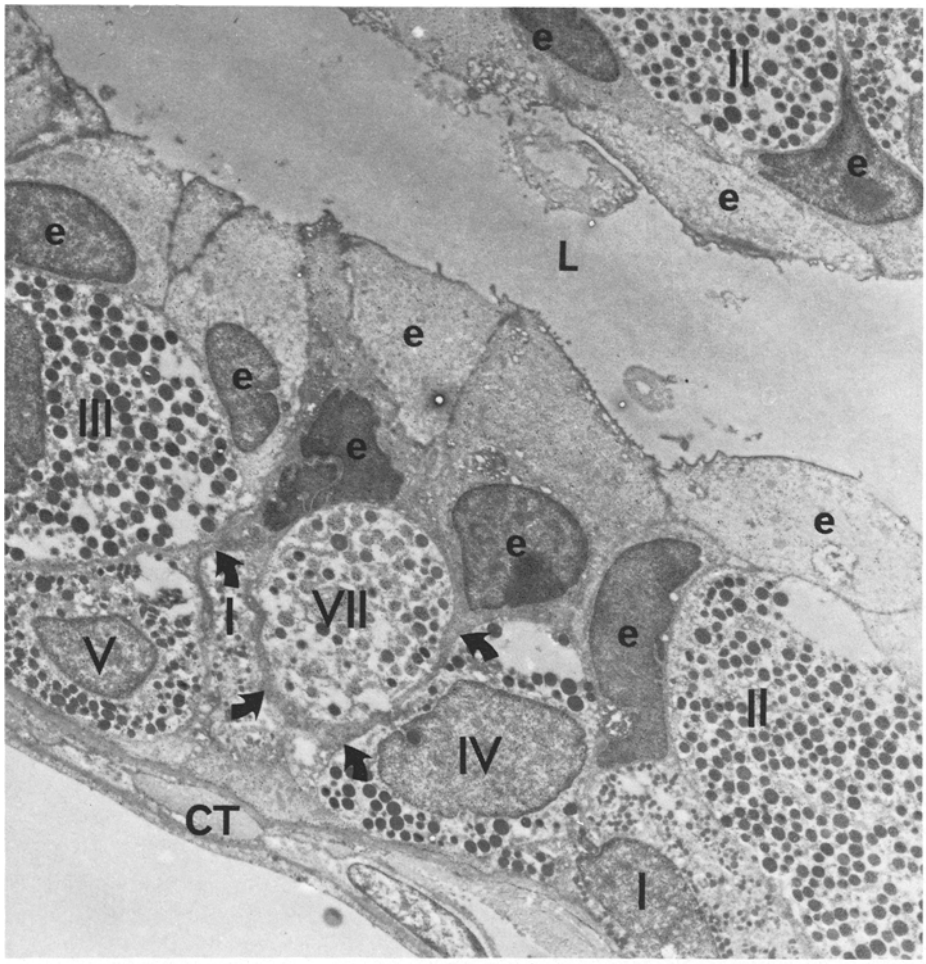
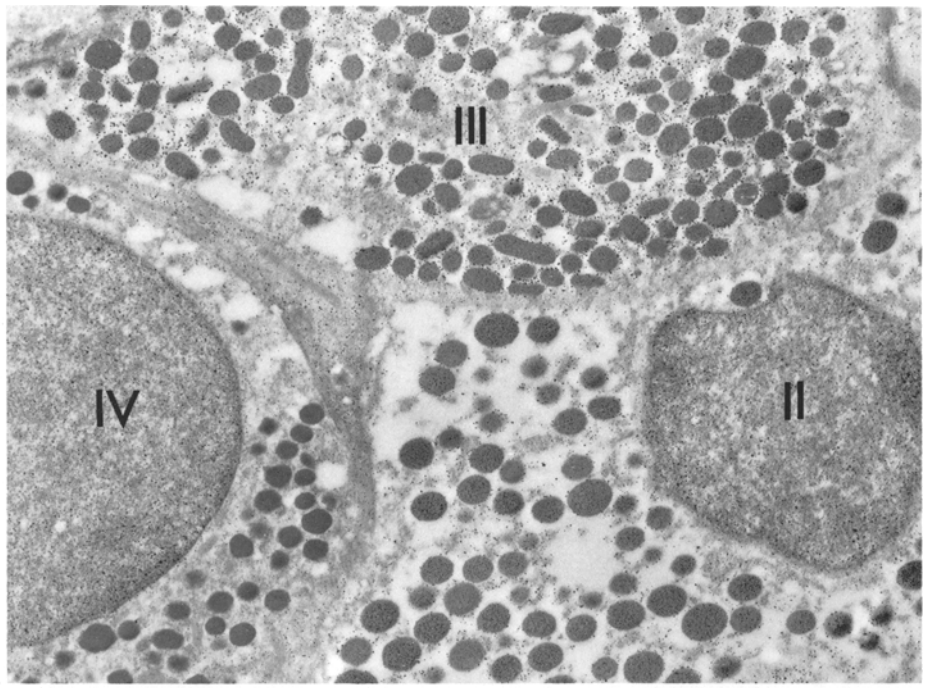


Fig. 10. Electron micrograph of a portion of an islet from *Scyliorhinus stellaris* surrounded by connective tissue (*CT*). Islet cells containing numerous secretory granules located outside of the two layers of clear epithelial cells (*e*); the latter are arranged on opposite sides of the ductular lumen (*L*). Fixation in glutaraldehyde without osmic acid. Using the Grimelius' method silver grains appear over the secretory granules of type II-(*II*), III-(*III*), and VII-(*VII*) cells, while they are lacking over granules of type I-(*I*) and IV-(*IV*) cells. For cell type V, see text and Table 1. *Arrows* slender cytoplasmic processes arising from the epithelial cells (*e*) of the duct. ×4,100

found between the two structures (Fig. 5). These secretory granules are positive to Grimelius' silver impregnation (Fig. 11). The granules of the third cell type are 450 nm in width and 750 nm in length (mean values).

Cell Type IV

Cells of this type are oval to polygonal in shape with an oval nucleus and appear solitarily or in small groups of several cells within the islets. They are medium in size



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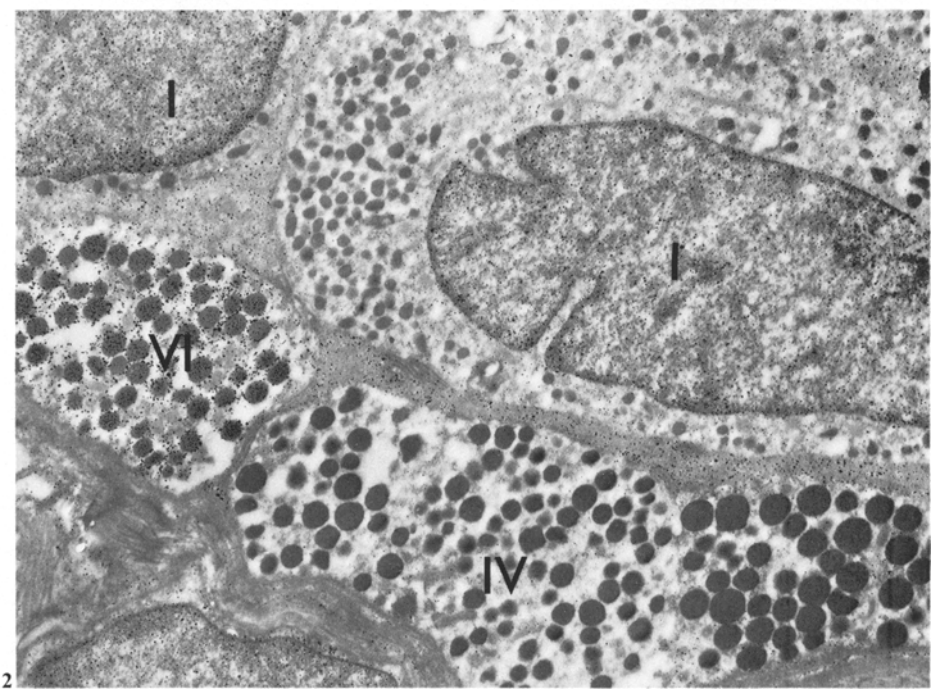

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Fig. 11. Portion of three islet cells from *Scyliorhinus stellaris* after silver impregnation en bloc according to the Grimelius method. Secretory granules of type II-(*II*) and III-(*III*) cells are positive to the Grimelius' stain, type IV-(*IV*) cell is negative. Due to the osmic acid-free fixative the membrane of the granules is not visible. ×9,800

Fig. 12. Secretory granules of type I-(*I*) and type IV-(*IV*) cells are negative to the Grimelius stain; type VI-(*VI*) cell is positive. Due to the osmic acid-free fixative the membrane is not visible. ×9,800

and their secretory granules are stained orange with AFLO staining. Electron microscopically, they have numerous secretory granules of spherical, but also somewhat "angular" shape (Figs. 4, 6, 11, 12). Each granular core shows a homogeneous high electron density and is surrounded by a closely attached limiting membrane. The average diameter of these granules is 540 nm. The secretory granules of the type IV cell are negative to Grimelius' silver impregnation (Fig. 12).

Cell Type V

Cells of this type appear almost always solitarily in the islets. They are medium in size and oval or polygonal in shape. Their cytoplasm colors dark yellow with AFLO staining and contains numerous secretory granules. Electron microscopically, they display medium-sized secretory granules with cores of moderate electron density, which are variable in shape: oval, slender, or irregular (Figs. 3, 4, 6). As shown in Fig. 6, irregular secretory granules of the cell type V are very similar to those of type III-cells, but are somewhat smaller than the latter. They measure 250 nm in width and 500 nm in length. Fine silver grains appear on the granular core after Grimelius' silver impregnation (Fig. 10).

Cell Type VI

Type VI-cells are large and oval in shape and appear solitarily or in small groups in the islets. Cytoplasm of this cell type stains light green with the AFLO method. Sometimes, several lysosomes appear in the cytoplasm (Fig. 7). Electron microscopically, type VI-cells display small spherical secretory granules of moderate density (Fig. 7). The average diameter of this granule is 310 nm. The small granular cores show a positive reaction to Grimelius' method (Fig. 12).

Cell Type VII

Cells of this type are large and oval in shape and appear solitarily in the islets. Their cytoplasm is dark blue with AFLO staining. Electron microscopically, they have two types of large secretory granules with intermediate forms; one extreme is spherical and dense (650 nm in diameter), the other is spherical and less dense (900 nm in diameter) (Fig. 8). Both types of granules have fine silver grains over their cores after Grimelius' silver impregnation (Fig. 10).

Cell Type VIII

Usually this cell type appears small and angular in shape, but sometimes it may have an oval appearance (Figs. 1e, 2e). These cells are found solitarily or in small groups in the islets. The cytoplasm of this cell type is light blue with AFLO staining. Electron microscopically, they display small secretory granules, angular or irregular in shape and of moderate electron density (100 nm in diameter) (Fig. 3e). These granules are negative to Grimelius' silver impregnation.

General Cytology of Different Types of Cells as Observed Electron Microscopically

In all types of cells the mitochondria are oval to slender in shape, except in type I where they are rod-like. They are scattered among the secretory granules. The

rough endoplasmatic reticulum is sometimes whorled (type I) or lamellar and is abundantly present except in the case of type V cells. The Golgi apparatus is small, consisting of several lamellae and vesicles and is conspicuous except in cell types III, VI, VII and VIII.

Discussion

The fact that the endocrine cells have a close relationship to the excretory duct is one of the characteristic features of the pancreas in cartilaginous fishes (Diamare 1899; Jackson 1922; Thomas 1940; Fujita 1962; Ferner and Kern 1964; Kern 1964; Östberg et al. 1966). Islet cells of *Scyliorhinus stellaris* also appear around small ducts, the inner surface of which, however, is always covered with epithelial cells of the exocrine duct. Frequently, slender cytoplasmic processes of these ductular epithelial cells appear to envelop islet cells completely and sometimes anchor them to the basal lamina. This architecture might suggest the possibility of a functionally intimate relationship between endocrine cells and epithelial cells of these small ducts.

B-cells (*type I*) are the only distinct cell type that reacts positively to pseudoisocyanin- and aldehyde-fuchsin staining. Electron microscopically, some B-cells contain larger secretory granules than others. We could, however, find no convincing evidence for the existence of two different subtypes of B-cells. Irrespective of the conspicuous variation in the fine structure of B-granules among different animal species, the B-cell granules of *Scyliorhinus* should be categorized separately because of their peculiar small and somewhat curved form, not found in any other animal species examined to date.

Concerning A-cells in mammals, birds, reptiles, amphibians and in some teleosts, most of the secretory granules possess a spherical core surrounded by a closely fitting limiting membrane. On the other hand, crystalline A-granules were demonstrated in some teleosts; these crystalline structures of the granular core were classified as rhombic dodecahedral (see Lange 1980). However, we did not observe cells displaying a crystalline granular core in the present study on *Scyliorhinus stellaris*. Light microscopically (Table 1), the coarse granules of *type II*-cells show the strongest brilliance among all islet cells when observed in dark field. According to general experience (Lange 1973), in *Scyliorhinus stellaris* the type II-cell is thus thought to be the most probable candidate for an equivalent of A-cells. Electron microscopically, in contrast to many other animal species (in which A-cell granules show a positive reaction to Grimelius' silver method only within the halo area between the core and the surrounding limiting membrane), silver grains in the type II-cell of *Scyliorhinus stellaris* appear not only within the halo but also on the core of the secretory granules. In the present material after immunostaining using an antiserum against bovine glucagon, a slightly positive reaction of large oval cells was obtained, however, not in a reproducible manner. In conclusion, there is additional evidence from various observations that the type II-cell of *Scyliorhinus* is probably the glucagon-producing element.

The cytoplasm of *type III*-cells is stained red with azocarmine and light brown with AFLO staining. Secretory granules of this cell type shine brightly but less intensely than type II-cells when viewed by dark field microscopy. Secretory granules that appear irregularly round, ovoid or kidney-shaped and are of high

electron density, resemble strikingly those of the enterochromaffin cells (EC cells) found in the adrenal gland, or in the epithelium of stomach or intestines exhibiting a strong reaction to Grimelius' silver method. Lazarus and Shapiro (1971) have pointed out that the X-cell secretory granules in islets of the dog show similarities in profile and electron density to the corresponding features reported for enterochromaffin cells. Enterochromaffin cells have also been observed as rare components of pancreatic islets of other animals species (Like and Orci 1972; Grube 1976; Böck and Gorgas 1976).

We were able to demonstrate the presence of D-(A_1-) cells using the silver impregnation method of Hellman and Hellerström (1960). These cells were examined, however, in preparations obtained from paraffin-embedded blocks after fixation in Bouin's fluid, so that strictly alternating serial sections for light and electron microscopy were not available in the present study. Nevertheless, *type IV*-cells are the most probable candidate for the D-cell based on shape, number, and distribution in the islets, and due to negative staining of their secretory granules with Grimelius' silver impregnation. Recently, somatostatin has been demonstrated in the pancreatic D-cells of many mammals, and has further been revealed by radioimmunoassay and by immunohistochemical staining in some species of fish (Johnson et al. 1976; Falkmer et al. 1977; van Noorden and Patent 1978; Klein and van Noorden 1978). The demonstration of somatostatin in the type IV-cells of the shark would constitute a definite argument for their identification as D-cells.

Pancreatic polypeptide (PP)-cells were identified as an independent cell type (distinct from A-, B-, and D-cells) by immunocytochemical, ultrastructural, histological, and histochemical techniques in Langerhans islets of different animals, including fish (Langer et al. 1979; Klein and van Noorden 1980; Tagliafierro et al. 1980). Previous electron microscopic studies have revealed the presence of cells possessing spherical granules of smaller size than those in the A-, B-, and D-cells of fish (Kudo and Takahashi 1973; Kobayashi et al. 1975; Brinn and Epple 1976; Rombout et al. 1979). Such small granules have been observed in mammals to be covered by silver grains when using the Grimelius' procedure (Cavallero et al. 1976; Larsson et al. 1976). In *Scyliorhinus*, the *type VI*-cells may be equivalent to the PP-cells, based on the positive reaction of their small spherical granules to Grimelius' silver impregnation. However, there is no definite identification possible unless positive immunostaining results have been obtained. A new concept dealing with the gastroenteric and pancreatic (GEP) endocrine elements as a uniform system has been proposed and discussed (see Fujita 1976). In the pancreas of *Scyliorhinus* most of the islet cells present electron microscopic features closely resembling not only the endocrine elements of the pancreas of other vertebrate species but also the cells scattered in the gastro-enteric mucosa. Although the morphology of the granules displays in some cases a certain specificity, the chemistry of a secretion product cannot, in general, be postulated from merely morphological features. Therefore, the present report of eight morphological distinct cell types in *Scyliorhinus* must await confirmation by means of immunocytochemical methods.

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References

Böck P, Gorgas K (1976) Enterochromaffin cells and enterochromaffin-like cells in the cat pancreas. In: Fujita T (ed) Endocrine gut and pancreas. Elsevier, Amsterdam, pp 13–24

Brinn JE, Epple A (1976) New types of islet cells in a cyclostome *Petromyzon marinus* L. Cell Tissue Res 171:317–329

Cavallero C, Spagnoli LG, Villashi S (1976) An electron microscopic study of human pancreatic islets. In: Fujita T (ed) Endocrine gut and pancreas. Elsevier, Amsterdam, pp 61–71

Diamare V (1899) Studii comparativi sulle isole di Langerhans del pancreas. Int Mschr Anat Physiol 16:155–205

Falkmer S, Elde RP, Hellerström C, Petersson B, Efendić S, Fohlman J, Siljevall JB (1977) Some phylogenetical aspects on the occurrence of somatostatin in the gastro-entero-pancreatic endocrine system. A histological and immunocytochemical study, combined with quantitative radioimmunological assays of tissue extracts. Arch Histol Jpn 31:99–117

Ferner H, Kern H (1964) The islet organ of selachians. In: Brolin SE, Hellman B, Knutson H (eds) The structure and metabolism of the pancreatic islets. Pergamon Press, Oxford, pp 3–10

Fujita T (1962) Über das Inselsystem des Pankreas von *Chimaera monstrosa*. Z Zellforsch 57:487–494

Fujita T (ed) (1976) Endocrine gut and pancreas. Elsevier, Amsterdam

Grube D (1976) Biogenic monoamines in the GEP endocrine system of various mammals. In: Fujita T (ed) Endocrine gut and pancreas. Elsevier, Amsterdam, pp 119–132

Hellman B, Hellerström C (1960) The islets of Langerhans in ducks and chickens with special reference to the argyrophil reaction. Z Zellforsch 52:278–290

Jackson S (1922) The islands of Langerhans in elasmobranch and teleostean fishes. J Metab Res 2:141–147

Johnson DE, Torrence JL, Elde RP, Bauer GE, Noe BD, Fletscher DJ (1976) Immunohistochemical localization of somatostatin, insulin and glucagon in the principal islets of the anglerfish (*Lophius americanus*) and the channel catfish (*Ictalurus punctata*). Am J Anat 147:119–124

Kern H (1964) Untersuchungen über das Pankreas einiger Selachier mit besonderer Berücksichtigung des Inselorgans. Z Zellforsch 63:134–154

Kern HF (1971) Vergleichende Morphologie der Langerhans'schen Inseln der Wirbeltiere. In: Dörzbach E (ed) Handbook of experimental pharmacology. New Series. Vol XXXII/1 Insulin. Springer, Berlin Heidelberg New York, pp 1–70

Klein C, van Noorden S (1978) Use of immunocytochemical staining of somatostatin for correlative light and electron microscopic investigation of D cells in the pancreatic islet of *Xiphophorus helleri* H. (Teleostei). Cell Tissue Res 194:399–404

Klein C, van Noorden S (1980) Pancreatic polypeptide (PP)- and glucagon cells in the pancreatic islet of *Xiphophorus helleri* H. (Teleostei). Cell Tissue Res 205:187–198

Kobayashi K, Takahashi Y, Shibasaki S (1975) A light and electron microscopic study on endocrine cells of the pancreas in a marine teleost *Limanda herzensteini* (Jordan et Snyder) 10th Int Cong Anat, Tokyo, pp 285

Kudo S, Takahashi Y (1973) New cell types of the pancreatic islets in the Crucian carp, *Carassius carassius*. Z Zellforsch 146:425–438

Lange RH (1973) Histochemistry of the islets of Langerhans. In: Graumann W, Neumann K (eds) Handbuch der Histochemie, Vol 8/1. Gustav Fischer, Stuttgart, pp 1–141

Lange RH (1980) Crystallography of intracellular insulin and glucagon as revealed by investigations of tissues and models. In: Brandenburg D, Wollmer A (eds) Chemistry, Structure and Function of Insulin and Related Hormones. Walter de Gruyter & Co, Berlin, pp 665–672

Lange RH, Syed Ali S, Klein C, Trandaburu T (1975) Immunohistological demonstration of insulin and glucagon in islet tissue of reptiles, amphibians and teleosts using epoxy-embedded material and antiporcine hormone sera. Acta Histochem 52:71–78

Langer M, van Noorden S, Polak JM, Pearse AGE (1979) Peptide hormone-like immunoreactivity in the gastrointestinal tract and endocrine pancreas of eleven teleost species. Cell Tissue Res 199:493–508

Larsson LI, Sundler F, Håkanson R (1976) Pancreatic polypeptide – a postulated new hormone: Identification of its cellular storage site by light and electron microscopic immunocytochemistry. Diabetologia 12:211–226

Lazarus SS, Shapiro SH (1971) The dog pancreatic X cell: A light and electron microscopic study. Anat Rec 169:487–499

Like AA, Orci L (1972) Embryogenesis of the human pancreatic islets: A light and electron microscope study. Diabetes 21 (Suppl 2):511–534

Van Noorden S, Patent GJ (1978) Localization of pancreatic polypeptide (PP)-like immunoreactivity in the pancreatic islets of some teleost fishes. Cell Tissue Res 188:521–525

Östberg H, Hellerström C, Kern H (1966) Studies on the A_1-cells in the endocrine pancreas of some cartilaginous fishes. Gen Comp Endocrinol 7:475–481

Patent GJ, Epple A (1967) On the occurrence of two types of argyrophil cells in the pancreatic islets of the holocephalan fish, *Hydrolagus colliei*. Gen Comp Endocrinol 9:325–333

Rombout JHWM, Rademakers LHPM, van Hees JP (1979) Pancreatic endocrine cells of *Barbus conchonius* (Teleostei, Cyprinidae) and their relation to the entero-endocrine cells. Cell Tissue Res 203:9–24

Tagliafierro G, Faraldi G, Pozzi MG (1980) Immunohistochemical and ultrastructural identification of glucagon cells on the endocrine pancreas of some selachians. Gen Comp Endocrinol 40:351

Thomas TB (1940) Islet tissue in the pancreas of the elasmobranchii. Anat Rec 76:1–16

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