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Fine Structure of Mammalian and Avian Pancreatic Islets with Special Reference to D Cells and Nervous Elements

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Summary. The pancreatic islets of the dog, guinea pig and pigeon were studied under the electron microscope and the following results were obtained.

1. Besides A and B cells, D cells are constantly found in the islets of these three species. The D cell granules, when fixed in glutaraldehyde especially by perfusion, are represented by a solid substance of medium electron density and fine granular texture closely encircled by a membrane sac. Generally acknowledged appearance of large D granules with coarse texture and low electron density and of interrupted membrane sacs is ascribed to be an artefact caused by OsO_4 fixation.

2. Figures indicating emiocytotic release of the D granules were obtained in the dog and pigeon.

3. In the dog many nerve fibers containing accumulations of synaptic vesicles and mitochondria are either in close contact with or a certain distance apart from the islet cells. These nerve terminals are divided into two types: those containing mainly small cored vesicles and those with small non-cored vesicles. Large cored vesicles occasionally occur in both types. There was no special relation between a certain type of islet cell and a certain type of nerve terminal. In the guinea pig the nerves, though also in direct contact with islet cells, are much fewer than in the dog.

4. In the dog and guinea pig Schwann cells investing the axons are frequently found in close contact with the islet cells. Some of these cells are incorporated in the islet as an agranular, epithelial cell-like element with attenuated processes inserted among the islet cells.

5. The neuro-insular complex of type II (FUJITA, 1959), i.e. the conglomeration of a nerve fiber bundle and islet cells was found frequently in the dog. Both axons, with or without synaptic vesicles, and Schwann cells are closely juxtaposed with islet cells. Every transitional type was found between this complex and the islet receiving an ordinary amount of nerve elements.

6. In the pigeon, no nervous element was found in the islet tissue.

The D cell of the endocrine pancreas has gained increasing attention since this type of cell, inconspicuous in routine granule stainings, was identified with the argyrophil element believed to correspond to the A cell (FUJITA, 1964) and since the secretory nature of this third cell type of the pancreatic islet was suggested by electron microscopic observations. The hormone secreted by this cell was postulated by some authors to be a gastrin-like substance (SOLCIA and SAMPIETRO, 1965) and by others to be an agent which might regulate fat metabolism (EPPLE, 1966; GABE et MARTOJA, 1969; for review see FUJITA, 1968).

The previous descriptions on the fine structure of the D cells, especially of their secretory granules, vary from author to author (Table). BENCOSME and PEASE (1958), the first who identified D cells under the electron microscope, recognized in this type of cell "vacuoles containing an amorphous material of appreciable electron density" and occasionally "specific granules distinguished

from those of alpha cells by their low electron density". WINBORN (1963) found only vacuoles or empty sacs in the D cells of the monkey whereas MEYER and BENCOSME (1965) found both empty and solid types of granules in the rabbit. The descriptions of most of the later authors coincided with each other in that the D cell granules of various animals were characterized by an electron lucent content with coarse filamentous or granular texture closely encircled by a limiting membrane which was commonly incontinuous (MUNGER, CARAMIA, and LACY, 1965; MEYER, and BENCOSME, 1965; MACHINO, SAKUMA, and ONOE, 1966; SATO, HERMAN, and FITZGERALD, 1966). Only a few authors showed D granules with a considerably high electron density and finely granular texture (GOMEZ-ACEBO, PARRILLA, and R. CANDELA, 1968).

Examination of previous literature strongly suggests that the above mentioned features of the D granules, i.e., an empty or electron lucent appearance and loose texture and the interruptions in the membrane sac are related to artefacts caused mainly by OsO_4 fixation. Applying both single OsO_4 fixation and double glutaraldehyde-OsO_4 fixation to his electron microscopic study of reptilian islet cells, TITLBACH (1968) showed that the D cell granules could be preserved and shown as much more dense and solid by the use of the latter fixation than in the former.

Thus, the first purpose of the present paper is to deal with the fine structure of the D cell granules of the mammalian and avian pancreas with special reference to the differences caused by different fixation techniques.

The second aim of this paper is to examine the processes of the release of the secretory granules of D cells. A diacrine type of secretion was proposed by MEYER and BENCOSME (1965). As the empty type of granules were found to gather in the apical end of the D cells of the rabbit and as the granule sac appeared often incontinuous, it was postulated that the granule content might be released into the cytoplasm adjacent to the apical plasma membrane of the cells. The possibility of this diacrine type of secretion was also proposed by BOQUIST (1967) based on his similar findings in the hamster. MACHINO, SAKUMA, and ONOE (1966), on the other hand, indicated that the D granules in the domestic fowl are released by emiocytosis or by fusion of the granular sac to the plasma membrane of the cell and opening of the fused part. Recently GOMEZ-ACEBO, PARRILLA, and R-CANDELA (1968) descibed that in the islet of the rabbit incubated in vitro with different concentrations of glucose in the medium they "could only rarely observe what appeared to be elimination of a D granule from the D cell by emiocytosis".

The third problem treated in the present paper will be the fine structural relation between insular and nervous elements. A juxtaposition either of ganglion cells or of unmyelinated fiber bundles to islet cells has long been known by light microscopists under the name of neuro-insular complex (for reference see FUJITA, 1959). Recent electron microscopic works, on the other hand, indicate that the islets of some mammals receive an unusually ample supply of autonomic nerve fibers which, at least partly, form synaptic endings directly to the islet cells (LEGG, 1967; WATARI, 1968). Thus, the islet seems to occupy a special position among endocrine tissues as its nervous control is concerned and deserves further investigation under the electron microscope. The difference among animals in the nervous element of the islet tissue will also be treated in this paper.

Materials and Methods

Eleven dogs, five guinea pigs and three pigeons were used in this investigation.

Immersion Fixation of the Pancreas. From adult dogs and guinea pigs anesthetized by an intraperitoneal injection of sodium isomital, a small piece of the tail of the pancreas was dissected out and cut into small blocks not exceeding one millimeter and fixed. Fixatives used were osmium dichromate solution (DALTON, 1955), 1.3% osmium tetroxide buffered at pH 7.3 with a 0.1 M phosphate buffer containing 50 ppm calcium chloride or 2.5% glutaraldehyde in the phosphate buffer with calcium chloride followed by 1.3% osmium tetroxide in the same buffer.

Perfusion Fixation of the Pancreas. Young dogs, guinea pigs and pigeons were decapitated whereas adult dogs were anesthetized with sodium isomital. The thorax and abdomen were opened widely; the thoracic aorta was dissected and a ligature placed around this vessel. A glass canule was inserted into the vessel and tied in place. In the adult dog the canule was inserted into the truncus coeliacus. The canule was then connected with an irrigator filled with the fixative (2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.0—7.1 containing 50 ppm calcium chloride). Perfusion was maintained for 3—10 minutes at the pressure 40—60 cm H_2O. The blocks were cut from the tail of the pancreas (splenic lobe in the pigeon), fixed in a fresh glutaraldehyde solution for 1—4 hrs, and then rinsed in the same phosphate buffer with calcium chloride.

All fixed blocks were dehydrated and embedded in Epon (LUFT, 1962). Thin sections cut on a Porter-Blum MT-1 microtome were studied, after staining with 1.0% uranylacetate and lead (MILLONIG, 1962), with a Hitachi HS 7-s electron microscope.

Results

Dog

Three types of granular cells were identified in both adult and young dogs under the electron microscope (Figs. 1, 2). The majority of the cells were B cells which showed cytoplasmic granules consisting of electron dense, rod or disk-like crystalloid and round spaces surrounded by a membrane sac, the well-known criterion of the B cells of this species since the early work of LACY (1957). The cells with granules consisting of a characteristically electron opaque round core and a distinct clear zone between the former and the membrane sac were identified as A cells as in all the previous investigations. The third cell type was the D cell whose granules showed less electron dense cores surrounded by a membrane sac, and the space between both was either inconspicuous or much narrower than in the A cell granules as pointed out by many previous authors in different animals (e. g. CARAMIA, 1963; MUNGER, CARAMIA, and LACY, 1965; MEYER and BENCOSME, 1965). No transitional types between these three cell types were encountered.

The D cells were scattered among other cell types, and their prolonged cytoplasmic portion to which the specific granules accumulated faced the tissue space often containing blood capillaries. In the materials fixed in osmium tetroxide, the granules (200—400 mμ) showed a coarse, granular or reticular texture and such a low electron density that it was sometimes difficult to discern them from the cytoplasmic matrix (Figs. 2, 3). The membrane sac surrounding the granule core or content showed repeated interruptions and this further caused the difficulty in differentiating the granules. In Dalton's fixation the coarse and electron lucent appearance of the granules and the interruptions in their sacs were more conspicuous than in the fixation with simple osmium tetroxide solution. In the

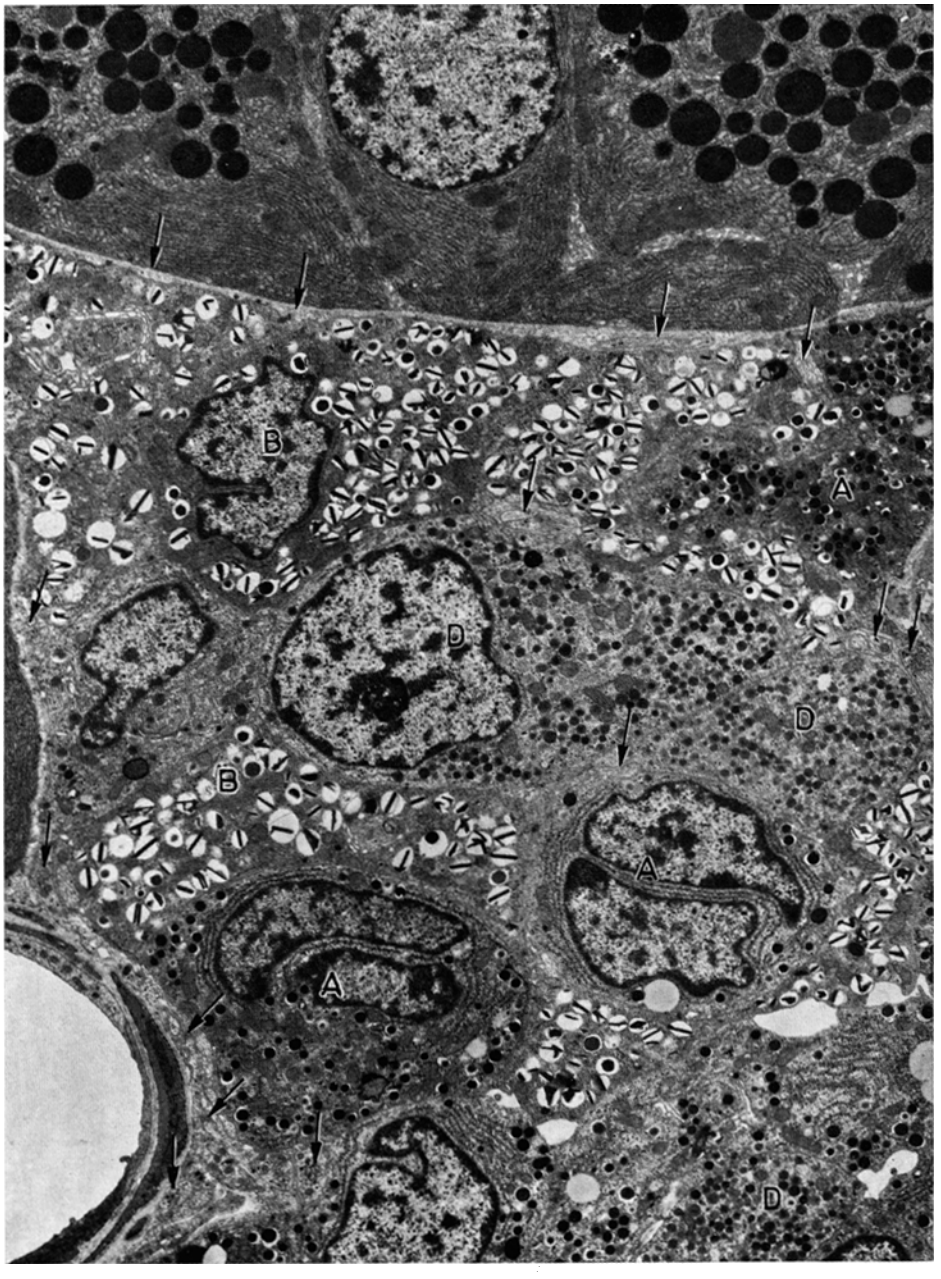


Fig. 1. An electron micrograph of the dog pancreatic islet, showing A cells (*A*) with dense round secretory granules, B cells (*B*) which contain granules with disk-like cores and D cells (*D*) showing round granules of medium electron density. The distribution of nerve fibers ensheathed by Schwann cells is shown by arrows. Perfusion fixation with glutaraldehyde and postfixation with OsO_4. × 7,500

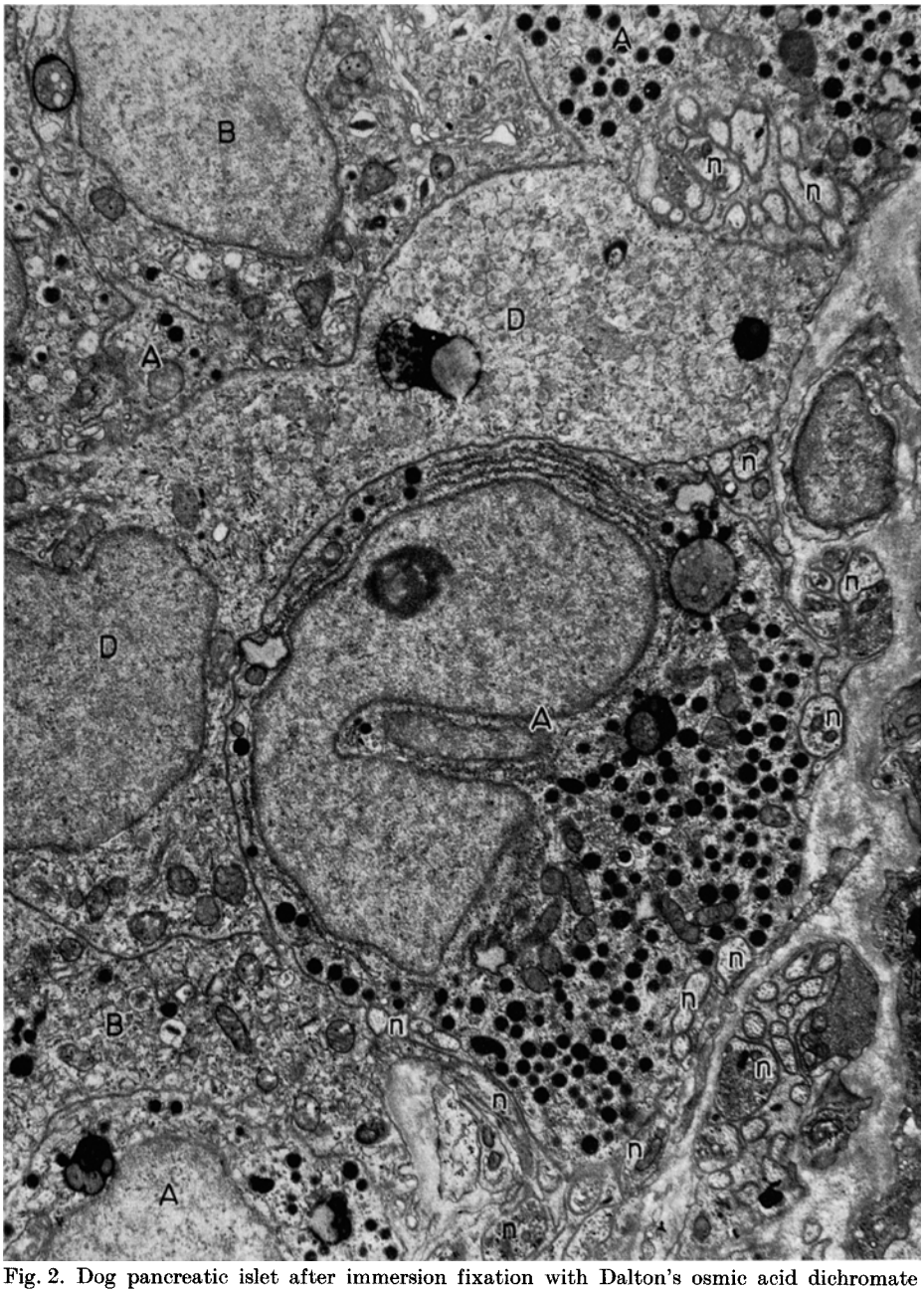


Fig. 2. Dog pancreatic islet after immersion fixation with Dalton's osmic acid dichromate solution. In this fixation the granules of D cell (*D*) appear as poorly definable substance of low electron opacity, encased by a broken membrane sac. Note also many nerve fibers (*n*) in the connective tissue space some of which are in contact with the islet cells. × 9,000

materials perfused with glutaraldehyde and postfixed with osmium tetroxide, the D cell granules appeared *smaller* (150—300 mμ) and more solid, and were composed of finely granular substances with more or less considerable electron

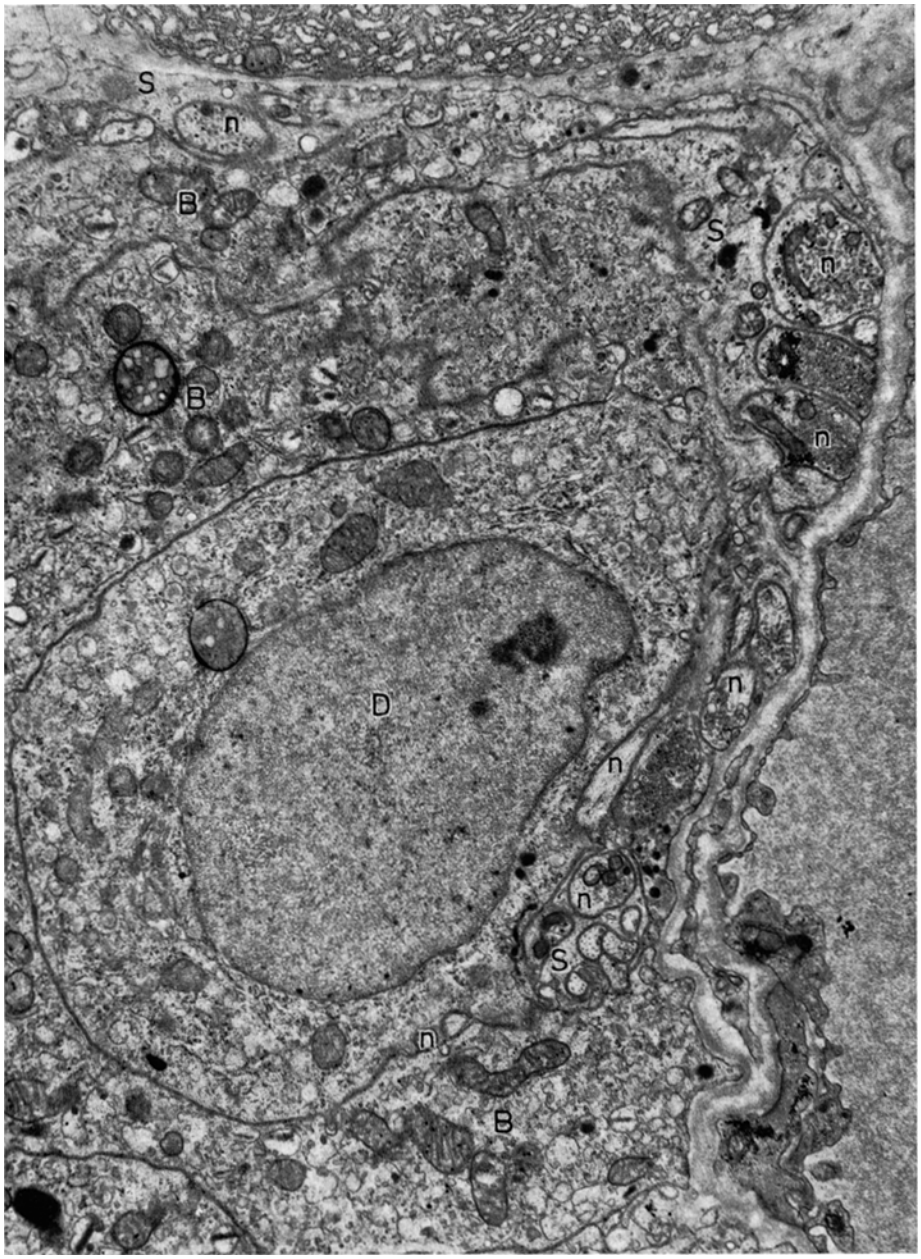
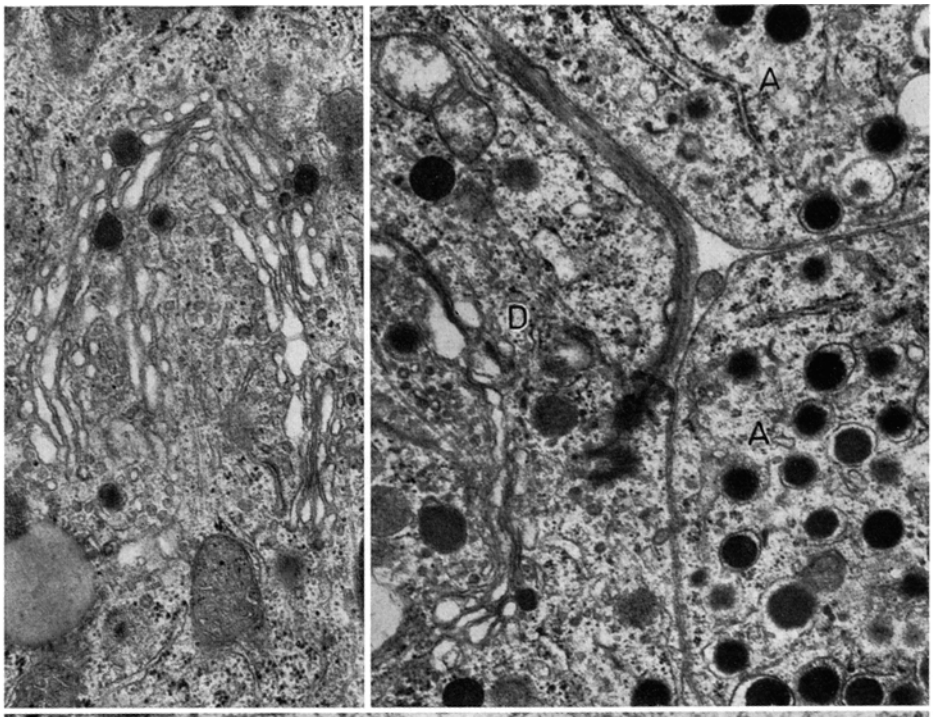


Fig. 3. Dog pancreatic islet. Numerous nerve fibers (*n*) surrounded by the cytoplasm of Schwann cell (*S*) occur in the space between the blood capillary and the islet cells. It seems that one islet cell receives several nerve fibers at the same time. Dalton's osmic acid dichromate fixation. × 10,000

density closely encased by a limiting membrane which showed few interruptions (Fig. 6). Granules of this type were, though less constantly, seen also after immersion fixation in glutaraldehyde. It may be worthwhile to add that, in the dog,



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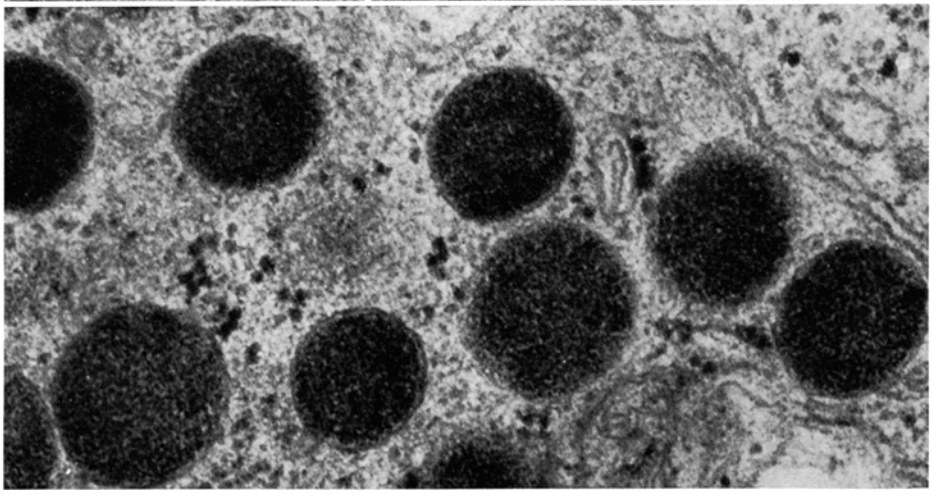

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Figs. 4—6. Perfusion fixation with glutaraldehyde and postfixation with OsO_4

Fig. 4. Golgi area of a D cell of the dog. Small osmiophilic granules can be seen in the Golgi vesicles. × 20,000

Fig. 5. Parts of two A cells (*A*) and a D cell (*D*) of a dog pancreatic islet. The D cell shows a single cilium arising from a basal body, one of a pair of centrioles. × 20,000

Fig. 6. D cell granules in a high magnification. Note the fine granular texture and the uninterrupted limiting membrane. × 70,000

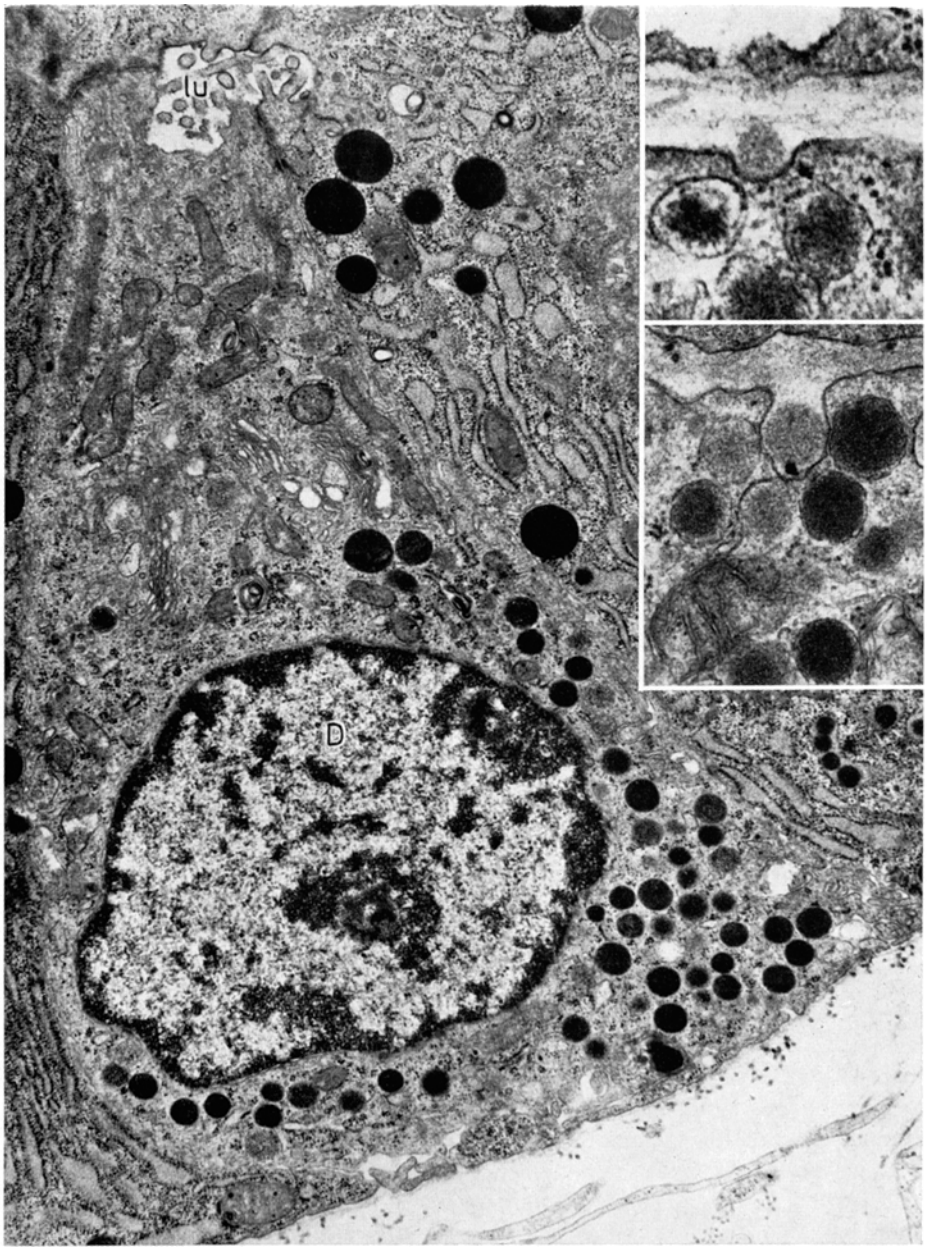
perfusion fixation tended to cause the opening of the space between the capillary wall and islet cells.

A series of electron mircrographs were obtained which seem to indicate different stages of granule release in the D cells (Figs. 8, 9). The granules were gathered beneath the plasma membrane facing the capillary and some of them were often seen attached to the plasma membrane. The limiting membrane of the granule came into fusion with the plasma membrane and a pore opened at the fused part through which the granule content seemed to be discharged. Thus, we believe that we could reveal the mechanism of D cell granule release to be called emiocytosis or reverse pinocytosis.

A well developed Golgi complex was found in the paranuclear region on the side opposite to the capillary and sometimes contained in its sacs small, dense granules which were believed to be the precursors of the D granules, because every intermediate form was found between the small granules and mature D granules (Figs. 4, 5). A pair of centrioles occurred in close association with the Golgi complex (Fig. 5). Some centrioles were continuous with a single cilium, over the surface of which the plasma membrane was reflected down to the attachment to the centriole. Granular endoplasmic reticulum was represented by relatively small, flattened cisternae scattered among the granules, especially on the side opposite to the capillary. Free ribosomes, cytoplasmic microtubules and filaments were dispersed in the cytoplasm. Mitochondria having a few cristae mostly occurred on the side of the nucleus opposite to the accumulation of the specific granules. Lysosomes were frequently recognized and were represented by round or elongate bodies measuring up to 2—3 μ in diameter limited by an agranular membrane. They contained a finely particulate matrix, vesicular components, myelin figures and remants of degenerating cell elements such as mitochondria. Irregularly shaped lipid droplets measuring up to 2 μ were encountered though rarely.

While the majority of D cells were included in the islets a small number of them occurred singly among the acinar cells. In such cases the secretory granules were accumulated in the basal part of the cell facing the periacinar connective tissue. A slender extension often reached the glandular lumen, and its free end bore several microvilli. This cell portion was attached to the neighboring acinar cells by a typical terminal bar. Thus, the fine structures of the extra-insular D cells were quite reminiscent of those of some endocrine cells in the gastro-intestinal mucosa of the rat described by SOLCIA, VASSALLO, and SAMPIETRO (1967) and by FORSSMANN and his co-workers (1969).

Small non-myelinated nerve fibers invested by Schwann cells were common in the space between the blood capillary and the islet cells. Favoring the side of the islet cell facing the capillary, many of the nerve fibers accompanied by thin Schwann cell cytoplasm came into intimate contact with the surface of the islet cell often indented to receive them. There were distinguished three different modes of neuro-insular connection. Most frequently the attenuated cytoplasm of a Schwann cell, ensheathing an axon either completely or incompletely, intervened between the latter and the islet cell. In the second type a naked axon came into direct contact with the islet cell. In the third case a Schwann cell was situated with its perikaryon within the boundary of an islet and was more or less widely



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Figs. 7—9. Perfusion fixation with glutaraldehyde and postfixation with OsO_4

Fig. 7. An extrainsular D cell of the dog. The apex of the D cell reaches the acinar lumen (*lu*). × 10,000

Figs. 8 and 9. Electron micrographs of the dog D cell, showing a series of figures indicating the release of D granules into tissue space by emiocytosis. × 20,000

adhered to the islet cell. Long cytoplasmic processes of the Schwann cell were often recognized inserted deeply between the islet cells (Figs. 12, 13, 15). The distance between the nervous element, i.e. either Schwann cell or axon, and the islet cell surface measured about 300 Å. A special differentiation in the membranes such as occurrence of desmosome-like thickening was not recognized.

Some of the nerve fibers thus related to islet cells contained accumulations of synaptic vesicles, small-sized mitochondria and glycogen particles whereas some others only well-defined neurotubules. The former type of fibers which deserve the designation of nerve terminals could also be found in the pericapillary space keeping some distance (3—5 μ) from an islet cell (Fig. 2). The synaptic vesicles both in the direct and "en distance" type terminals were divided into three different types: small non-cored (30—50 mμ in diameter), small cored (30—50 mμ) and large cored vesicles (100—500 mμ). There seemed to be at least two types of nerve endings on the basis of the combinations of these three vesicle types: the one contained numerous small non-cored and occasional large cored vesicles whereas the other, numerous small cored and occasional large cored vesicles. Small vesicles with a rod-like core described by WATARI (1968) as a special type of synaptic vesicles and also shown in Fig. 6 of the paper by LEGG (1967) could be encountered occasionally in the specimen fixed in osmium containing solutions, and the authors are inclined to ascribe this to a change in the core shape related to the effect of fixation. It may be worthwhile to mention that non-cored vesicles with an elongate profile first mentioned in the central nervous system by UCHIZONO (1965), HIRATA (1966), and BODIAN (1966) and later described also in peripheral nerve endings (UCHIZONO, 1968; KOBAYASHI, 1968) often occurred mixed with the ordinary, spherical non-cored vesicles (Fig. 11). Every islet cell type including the D cell turned out to have an equal possibility of receiving either one of these types of nerve terminals. Two, three or more nerve terminals, sometimes of different types could occur in a single islet cell (Fig. 10).

The Schwann cell, if located within the islet as above described, could be identified as such by following features: It invested mostly one or a few profiles of axons; the nucleus was characterized by a conspicuously dark zone beneath its membrane which corresponds to the "fibrous lamina" designated by FAWCETT (1966) in some cell types (Fig. 15); the cytoplasm contained numerous filaments and microtubules and relatively sparce cell organelles. Occasionally undulating tubules represented by a mass of tubular structures arranged, in cross section, in a crystalloid pattern occurred in the paranuclear cytoplasm of the Schwann cell (Fig. 14).

Neuro-insular complexes of Type II (FUJITA, 1959), i.e., the conglomeration of a bundle of nerve fibers and islet cells were encountered not infrequently (Figs. 12, 13). Typical ones were located in the interlobular space of the pancreas and ensheathed by a connective tiusse capsule common to the nervous and insular elements. In the complexes the islet cells were in close contact either with Schwann cells or with axons which partly were free from the Schwann cell investment. A part of the axons contained synaptic vesicles of different types mentioned above, small sized mitochondria and glycogen particles, thus

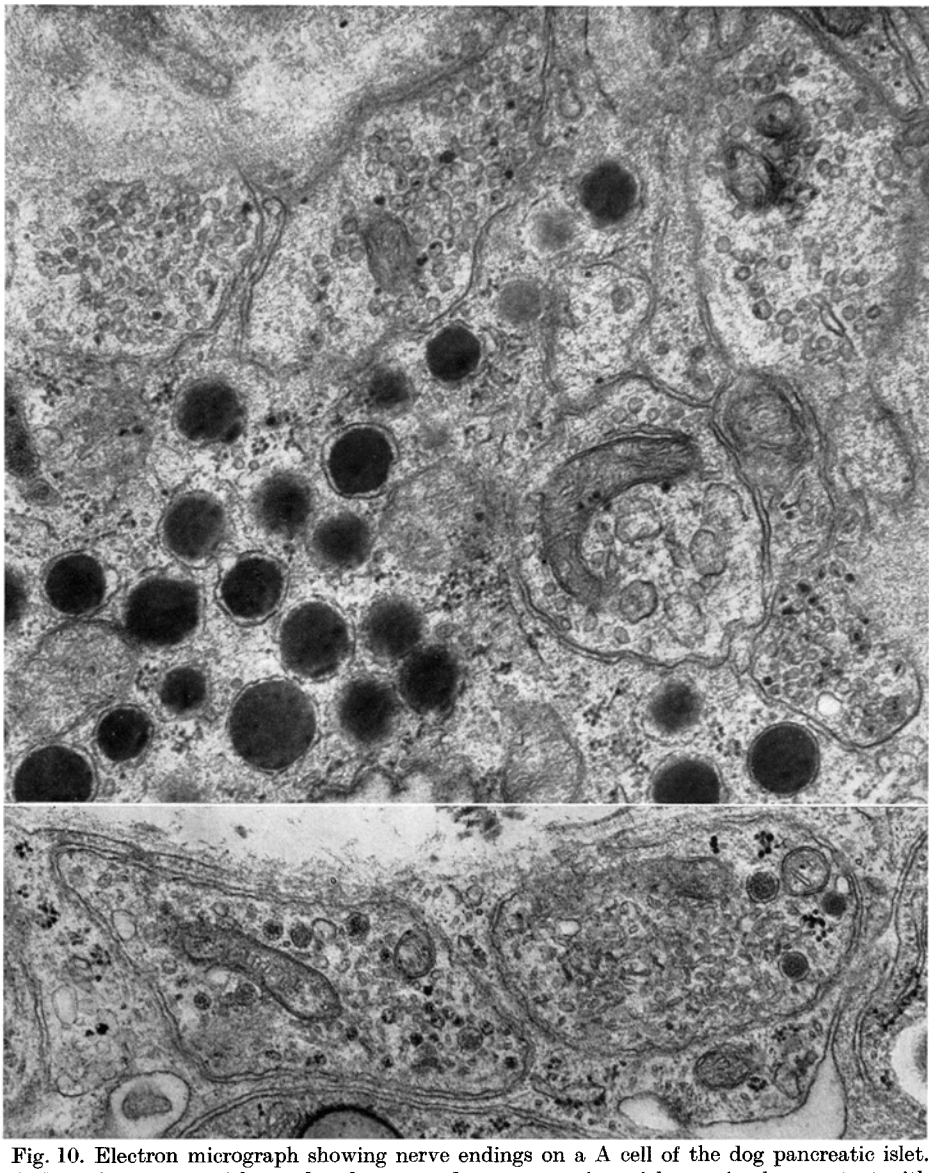

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Fig. 10. Electron micrograph showing nerve endings on a A cell of the dog pancreatic islet. At least four axons with cored and non-cored type synaptic vesicles are in close contact with the cell. Dalton's osmic acid dichromate fixation. × 50,000

Fig. 11. Two types of nerve endings ensheathed by a Schwann cell found in a dog islet. The one contains small cored vesicles and a few large cored ones, whereas the other agranular cylindrical vesicles and a few large cored ones. Perfusion fixation with glutaraldehyde and postfixation with OsO_4. × 35,000

indicating the characteristics of nerve terminals. There were transitional forms between the typical complexes and the ordinary islets containing smaller amounts of nervous elements.

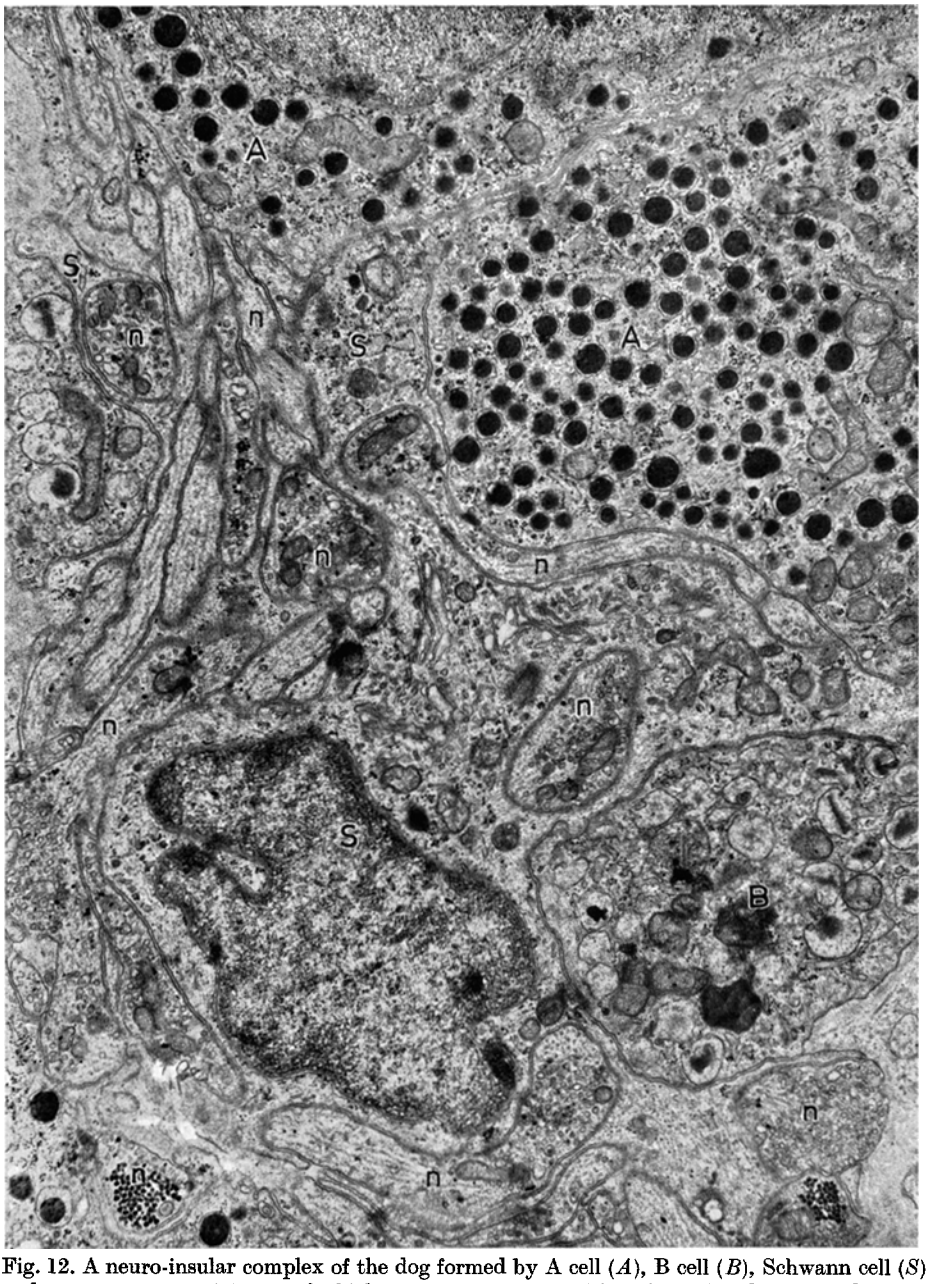


Fig. 12. A neuro-insular complex of the dog formed by A cell (*A*), B cell (*B*), Schwann cell (*S*) and numerous axons (*n*) part of which contain synaptic vesicles of cored and non-cored types. Note also the Golgi complex and centriole of the Schwann cell. Dalton's osmic acid dichromate fixation. × 15,000

Ganglion cells were found in the interlobular spaces not infrequently, especially in poppies. Neuro-insular complexes of the first type, i.e., the juxtaposition of the nerve cells and islet cells were not encountered.

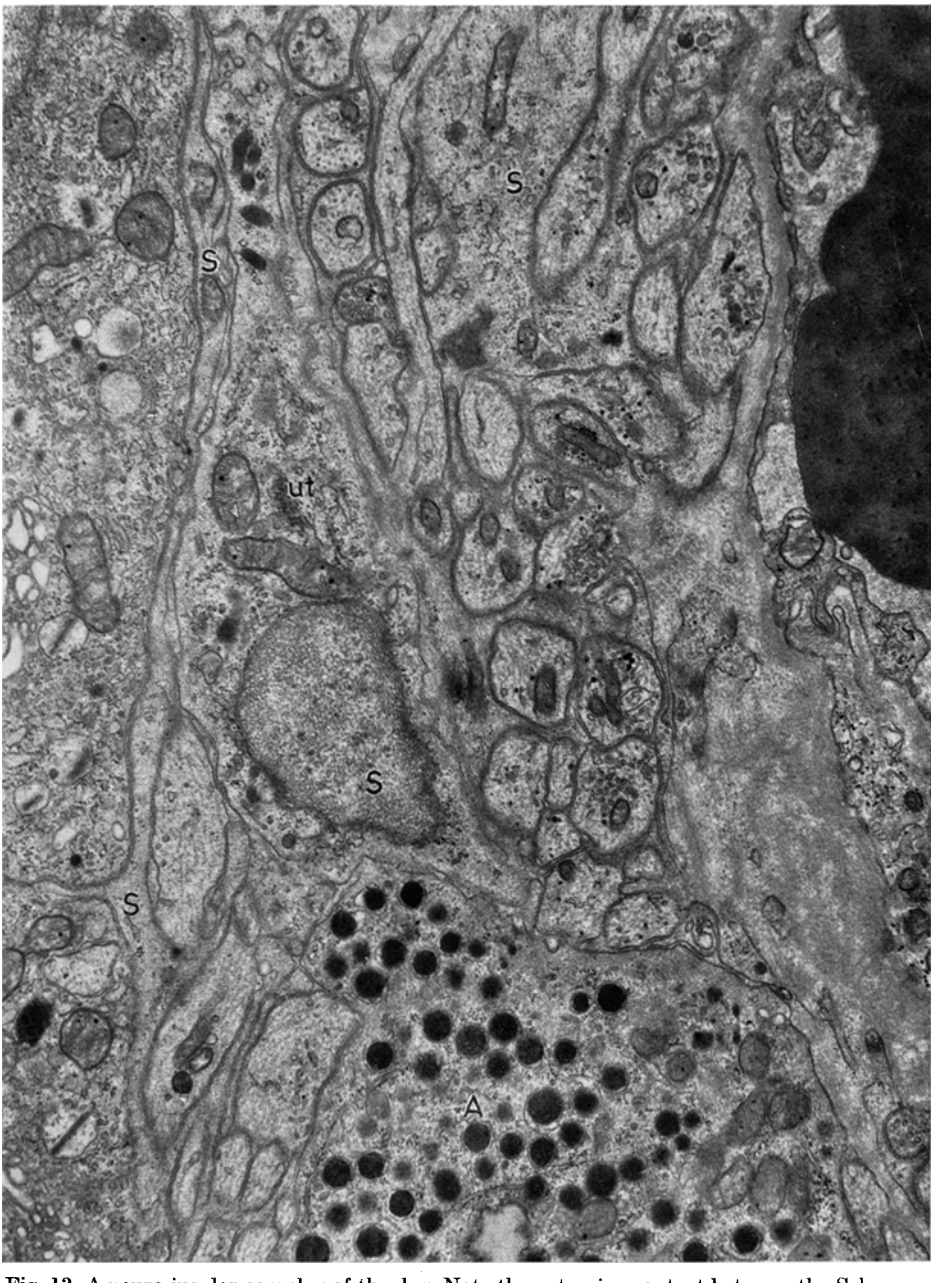
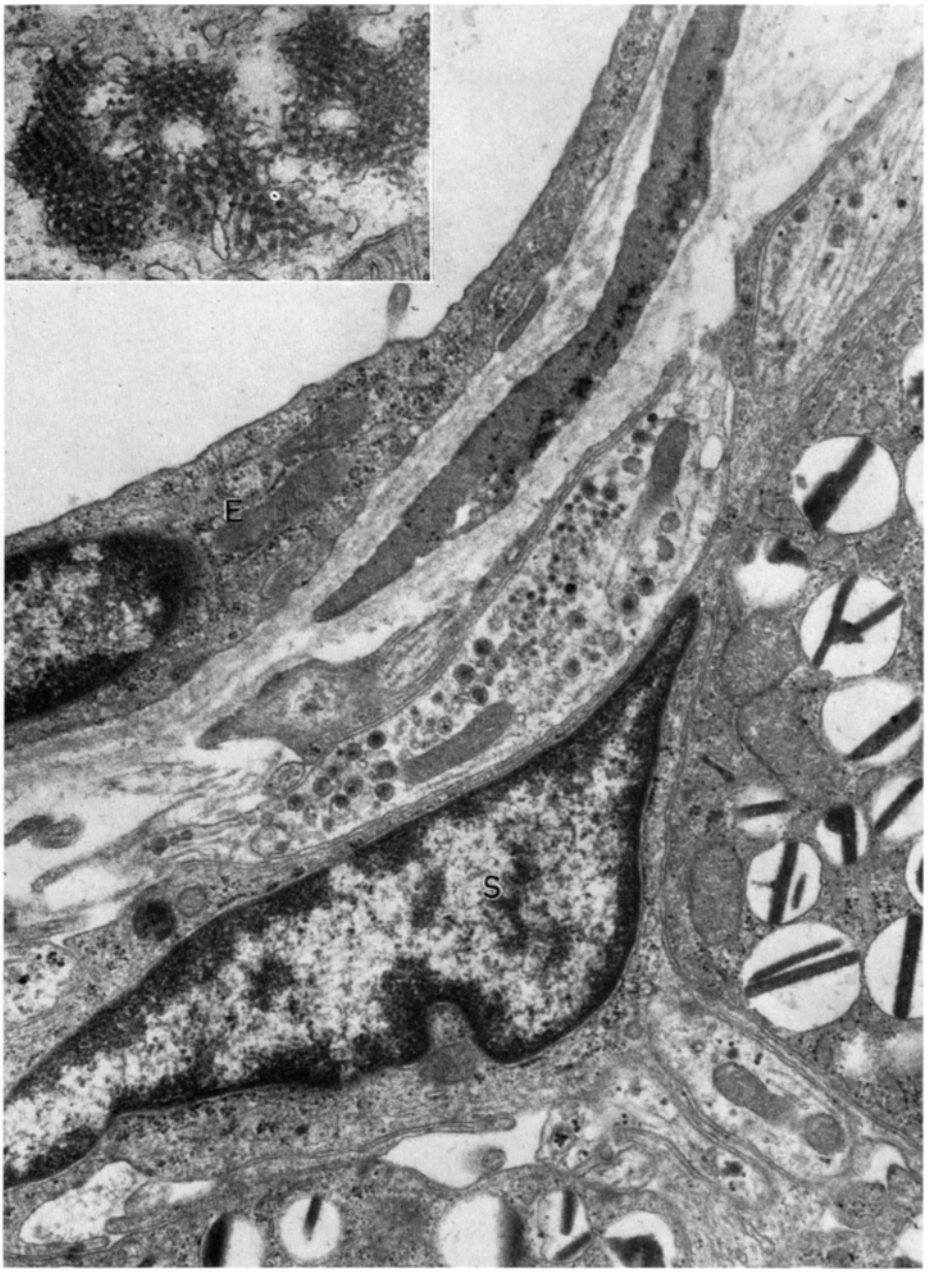


Fig. 13. A neuro-insular complex of the dog. Note the extensive contact between the Schwann cells (*S*) and islet cells. Undulated tubules (*ut*) are seen in the Schwann cell cytoplasm. Dalton's osmic acid dichromate fixation. × 15,000

Guinea Pig

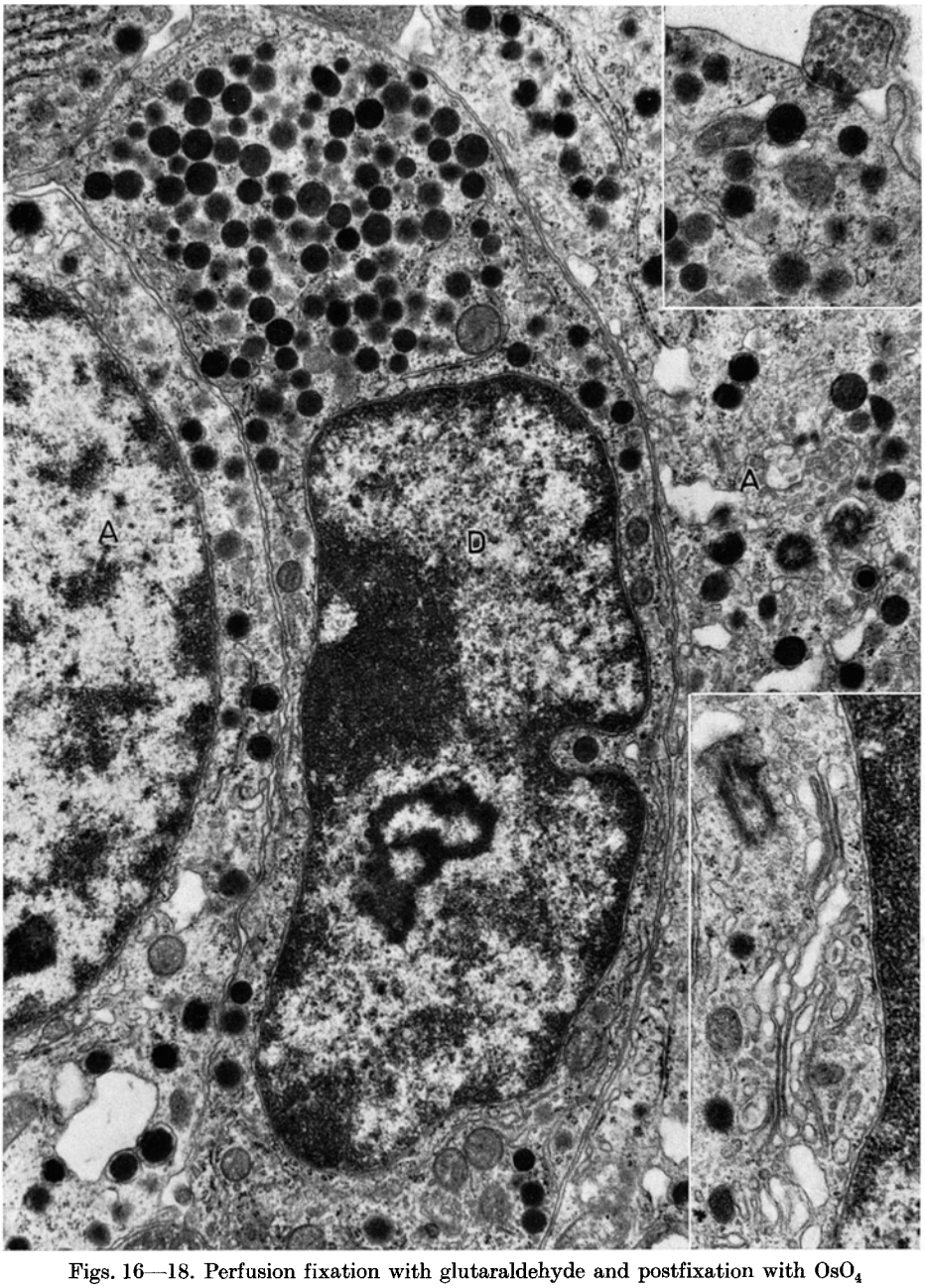
The B cells of the guinea pig were identified by their major population and by their characteristic granules whose core was of irregular contour and very



Figs. 14 and 15. Perfusion fixation with glutaraldehyde and postfixation with OsO_4

Fig. 14. Undulating tubules seen in a Schwann cell of a dog pancreatic islet. × 25,000

Fig. 15. A Schwann cell (*S*) found in a dog islet. Note the well developed "fibrous lamina" in its nucleus. A nerve ending contains many small cored vesicles and a few large cored vesicles. *E* Capillary endothelium. × 20,000



Figs. 16—18. Perfusion fixation with glutaraldehyde and postfixation with OsO_4

Fig. 16. A D cell (*D*) of the guinea pig with A cells (*A*) on both sides. × 25,000

Fig. 17. Golgi area of guinea pig D cell. Note the basal body of a cillium and the probably immature D granules in the Golgi area. × 25,000

Fig. 18. A possible nerve ending on a D cell of a guinea pig. Note the desmosome-like membrane thickenings. × 25,000

loosely encircled by a limiting membrane. The granules of the A cells, though variable in size from cell to cell (200—300 mμ) as recently pointed out by CARAMIA, MUNGER, and LACY (1965) and by BENCOSME and LECHAGO (1968), were characterized by their round and electron dense core surrounded by a distinct clear zone beneath the membrane sac. The granules of the D cells, after glutaraldehyde fixation, measured around 200 mμ and generally showed a lower electron density than the A cell granules (Figs. 16—18). The most reliable criterion of the D granules was the absence of a distinct space between the granule substance and its membrane sac. The long known characteristic of the guinea pig A cells, that their nuclei are conspicuously large and round with distinct nucleoli as compared with other cell types, served as an auxiliary criterion for the cells under the electron microscope.

The D cells mostly showed a columnar, spindle-like or crescent profile and were scattered throughout the islet. The nucleus was generally elongated and the cytoplasm extended to the connective tissue containing blood capillaries. The specific granules were accumulated mainly in this cytoplasmic portion.

In the osmium and Dalton-fixed materials the D cell granules showed large size (250—350 mμ), electron lucent appearance and interruption in their membrane sacs. They corresponded to the figures of the granules after the same fixation shown by BENCOSME and PEACE (1958), SATO, HERMAN, and FITZGERALD (1966). In the specimens fixed with glutaraldehyde the granules were represented by smaller (200—300 mμ) and denser bodies surrounded by a continuous limiting membrane (Figs. 16, 18). They varied conspicuously in electron opacity and the densest ones corresponded to the A cell granules. Perfusion fixation turned out to surpass immersion in the preservation of both the granules and cytoplasm of the D cells.

The findings on the other cell organelles such as the Golgi complex, centrioles and flagellum, cytoplasmic filaments, lysosomes, endoplasmic reticulum and free ribosomes essentially corresponded to those in the D cells of the dog. The specific granules seemed to be formed in the sacs of the Golgi complex; no figures indicating the release of the granules from the cell could be obtained in this animal.

The islet cells were recognized occasionally in contact either with nerve fibers or with the Schwann cell as described in the case of the dog. A part of the nerve fibers showed fine structures characteristic of synaptic terminals. The nature and types of the synaptic vesicles seemed to correspond to those in the dog, though our findings in the guinea pig are insufficient to conclude that. A possible nerve terminal with desmosome-like membrane thickenings was once encountered which is shown in Fig. 18. The quantity of the nervous element per insular element was much less in the guinea pig than in the dog. Schwann cell perikarya occurred, though less frequently than in the dog, in the peripheral region of the islet, being adhered to the islet cells.

Pigeon

The splenic lobe of the pigeon pancreas contains large "dark" and small "light islets". The former have been described as composed of a mixture of A and D cells whereas the latter, of a central group of B cells and D cells arranged

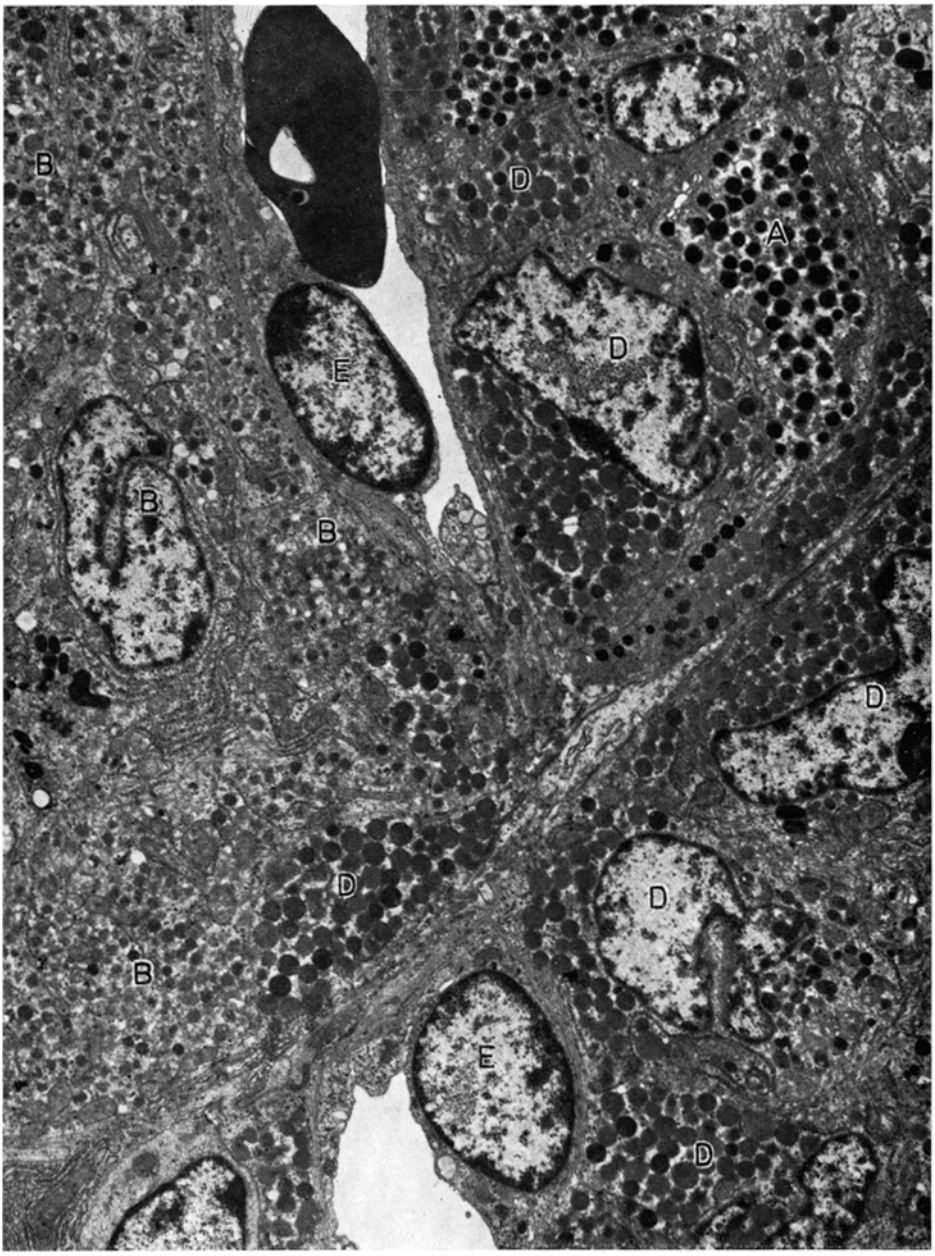
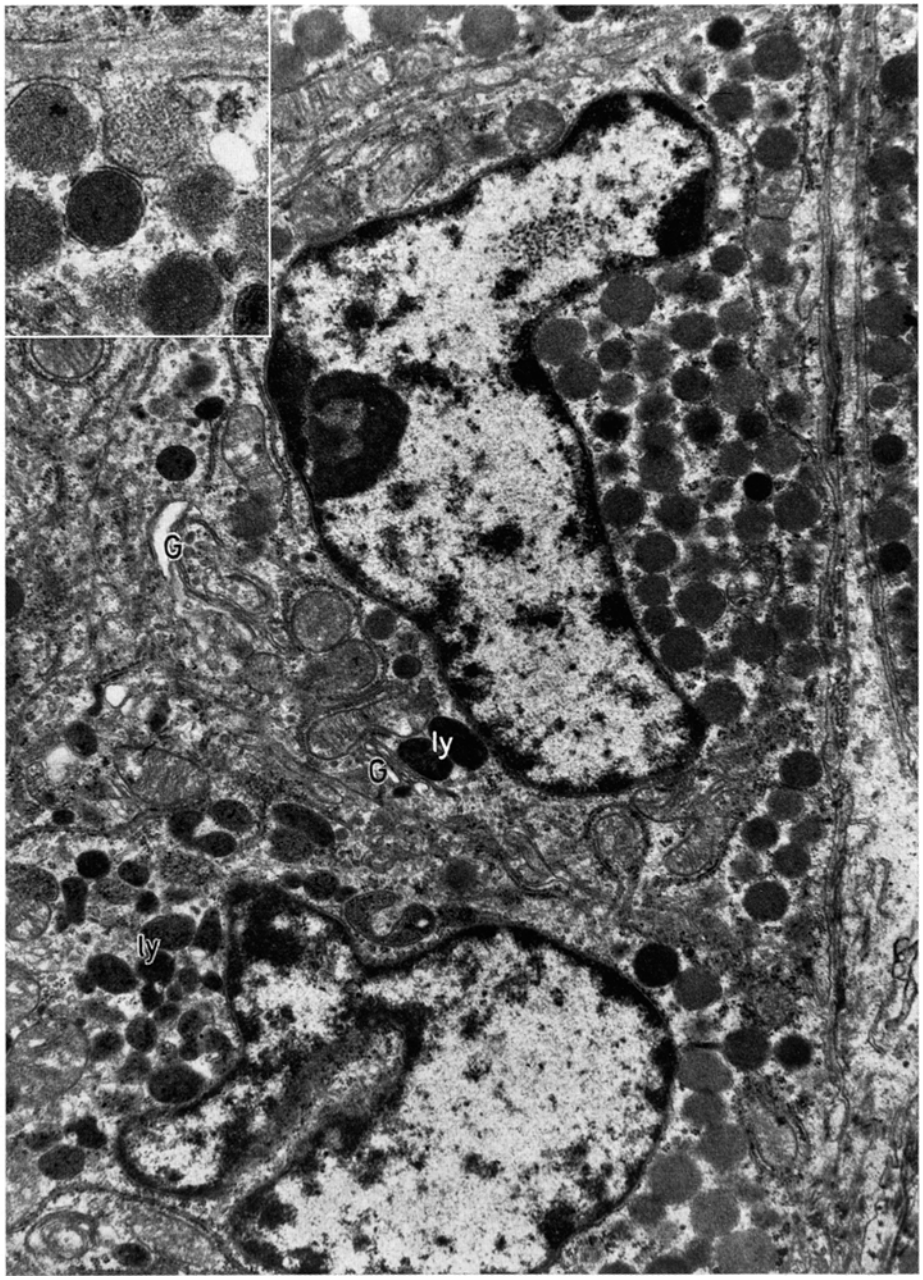


Fig. 19. Islet cells of the pigeon. Note the different appearances of the granules in A (*A*), B (*B*) and D cells (*D*). *E* Endothelial cells. Perfusion fixation with glutaraldehyde and post-fixation with OsO_4. × 5,500

concentrically around them (EPPLE, 1964). This distribution pattern of the cells can be relied on to only a limited extent in the identification of islet cell types under the electron microscope because, as recently pointed out by ROTH (1968)



Figs. 20 and 21. Perfusion fixation with glutaraldehyde and postfixation with OsO_4

Fig. 20. D cells of pigeon pancreatic islet. The specific granules are gathered on one side of the cell facing the tissue space whereas Golgi complex (*G*), mitochondria, lysosomes (*ly*) and granular endoplasmic reticulum are seen on the opposite side. × 15,000

Fig. 21. An electron micrograph suggesting the emiocytotic release of granules in the pigeon D cell. × 20,000

in his light microscopic study, there occurred frequently islets of a "mixed type" which contained all three cell types mingled together.

The A and B cells were identified by their granules each similar in fine structure to those of the corresponding cells in the guinea pig, whereas the D cells were judged as such by their round and large (400—600 mμ) granules of lower electron density and by the membrane sac closely attached to the granule substance (Figs. 19, 20).

The D cells were rounded cells arranged along the blood capillaries. The polarity of the cell, however, was no less conspicuous than in the elongate D cells of the dog and guinea pig: the specific granules were compactly gathered on the side of the cell facing the blood stream whereas the Golgi complex, mitochondria and lysosomes on the other side. As in the other animals described above, the D cell granules could be demonstrated after fixation in glutaraldehyde in the form of a solid and finely granular substance of medium electron density surrounded by an uninterrupted limiting membrane. The cytoplasmic organelles such as centrioles and free flagellum, endoplasmic reticulum, lysosomes and cytoplasmic filaments corresponded in their structure and occurrence to those in the dog and guinea pig. Figures suggesting the formation of granules in the sacs of the Golgi complex were also obtained.

A series of electron micrographs suggesting the emiocytotic release of the D granules were obtained in the pigeon as in the case of the dog, though the figures of granule discharge occurred only rarely (Fig. 21).

In all the sections observed under the electron microscope no nerve fibers could be found in relation to the endocrine pancreas of the pigeon. Nerves were encountered only in the vicinity of the arterioles in the interlobular connective tissues.

Discussion

Cell Identification

In spite of the descriptions by some light microscopists (reference in FUJITA, 1968) and electron microscopists (LEGG, 1967) who found either A or B granules mingled within the same cell with D granules, we could find no intermediate type of cell which might indicate a transition from one cell type to another. The theory on the independent nature of the D cell proposed by FUJITA (1964) and EPPLE (1964) in the light microscopic studies seems valid at the electron microscopic level.

Since the early works of THOMAS (1937) the C cells have been believed characteristic of the guinea pig, and it has been equivocal whether this animal possesses C *besides* D cells or *in place of* D cells. FUJITA (1968) studied this problem mainly on the basis of the attitudes of the cells in different stainings and concluded that the name, C cell, was only traditional, and this type of cell was nothing but D cells in other species. This view has been supported by the present electron microscopic study as only three types of islet cells could be differentiated in the guinea pig and as the granules in the third cell type varied unessentially in fine structure from that of the D cells in other species. SATO, HERMAN, and FITZGERALD (1966) distinguished the C cells of the guinea pig from the D cells on the basis of an inconspicuous difference in the density of their granules under the electron microscope, but this view of theirs seems to be caused by their predisposition to the traditional designation of guinea pig islet cells.

D Cell Granules

As described in the introduction the fine structural characteristics of D cell granules generally known by previous electron microscopists, i.e., their low electron density and coarse reticular or granular texture and the interruptions in its membrane sac, are believed to be artefacts caused by the initial fixation in OsO_4. The electron microphotographs by MACHINO, SAKUMA, and ONOE (1966), SATO, HERMANN, and FITZGERALD (1966) and LEGG (1967) seem to be typical examples of the effect of this fixation. Fixatives containing dichromate such as Dalton's fluid give corresponding or even worse results as the paper by LACY (1957) and MUNGER (1962) and our own examination indicate. The damage to the D granules by OsO_4 was recently pointed out by TITLBACH (1968) using reptilian material.

A survey of previous literature (see Table) and the present results indicate the clear tendency of the D granules: the larger the size of the granules, the coarser and the more electron lucent appear the granules. OsO_4 or Dalton's fixation is

Table. *A survey of previous literature concerning the fine structure of D cell granules*

Authors	Animals	Fixatives (GA: Glutaraldehyde)	D cell granules (Release mode)	Limiting membrane
LACY (1957)	Guinea pig	Dalton's fix.	Not distinct	Almost completely lost
BENCOSME and PEASE (1958)	Cat	OsO_4	Vacuole-like, but partly solid	Interrupted or lost
MUNGER (1962)	Rabbit	Dalton's fix.	Not clearly discernible (diacrine)	Interrupted or lost
WINBORN (1963)	Monkey	OsO_4, $KMnO_4$	Empty vesicles	Interrupted, but distinct
MEYER and BENCOSME (1965)	Rabbit	OsO_4	Partly lost, partly solid, (diacrine)	Partly damaged
MUNGER *et al.* (1965)	Rabbit, dog	GA-OsO_4	Solid, moderately dense	Continuous, partly interrupted
CARAMIA *et al.* (1965)	Guinea pig	GA-OsO_4	Solid, semi-opaque	Partly continuous
SATO *et al.* (1966)	Guinea pig, dog	OsO_4	Diffuse and obscure	Interrupted or fragmentary
MACHINO *et al.* (1966)	Chicken	OsO_4	Coarse and reticular (emiocytosis)	Interrupted
LEGG (1967)	Cat	OsO_4	Empty and obscure	Interrupted, but distinct
GOMEZ-ACEBO *et al.* (1968)	Rabbit	GA-OsO_4	Solid and dense (emiocytosis)	Continuous, closely applied to the granule
TITLBACH (1966, 1967, 1968)	Reptiles	OsO_4	Diffuse and obscure	Interrupted, but distinct
		GA-OsO_4	Solid and dense	Continuous, closely applied to the granule

believed to cause not only the loosening of the granule architecture but also, as an inevitable parallel phenomenon, enlargement of their size. The interruptions of the granule sacs seem to be the result of this swelling.

It is noticed that the damage by OsO_4 or Dalton's fixation occurs not only in the granules but also in the entire cytoplasm of the D cells. Either swollen-and-watery or dark-and-compressed appearance of the cytoplasm is a general occurrence. This change in the D cells is conspicuous because the adjacent A and B cells look satisfactorily fixed. It is unknown whether the change in the granules causes the damage in the cytoplasm or vice versa, or both are the common results caused by an unusual state of the cell triggered by the arrival of OsO_4. The reason of the selective sensitiveness of the D cell to osmium is unknown also.

The present observations of the possible formation and growth of the D granules in the sacs of the Golgi complex coinside with the descriptions by MEYER and BENCOSME (1965) and other authors.

The postulation of diacrine secretion by the D cell (MEYER and BENCOSME, 1965; BOQUIST, 1967) which was based on the occurrence of empty granules near the plasma membrane and of interrupted granule sacs in the OsO_4 fixed D cells seems not tenable any more. On the contrary, the present study provides electron micrographs suggesting the processes of emiocytotic release of D granules in the dog and pigeon. This corresponds to the finding by MACHINO and his associates (1966, 1968) in the domestic fowl, and to the fragmental observation by GOMEZ-ACEBO and his coworkers (1968) in the rabbit D cells incubated in vitro. It seems worthy of mention that the release of secretion from the A cells was first proposed to be of diacrine type (MUNGER, 1962) but recently seems to have been established as emiocytosis (PRZYBYLSKI, 1967; FUJITA and MATSUNO, 1967; GOMEZ-ACEBO, PARRILLA, and R-CANDELA, 1968). The secretion mechanism in the B cells has not been elucidated as yet.

The present and previous studies indicate that figures of the granule release from the D cell can be obtained only rarely. It seems important for the elucidation of the function of this type of cell to find either physiological or pharmacological conditions which would stimulate the secretion of this cell.

Nerve supply in Islet Cells

The present study confirms the electron microscopic observations by BENCOSME (1959), WINBORN (1963), STAHL (1963), LEGG (1967), and WATARI (1968) that mammalian islet cells receive nerve terminals with accumulation of synaptic vesicles and mitochondria. STAHL (1963) in the rat and WINBORN (1963) in the monkey recognized a cell process which accompanies the nerve fiber and is intercalated between the latter and the islet cell when they come into contact. STAHL identified this intercalated element with Schwann cell cytoplasm. This finding has been confirmed by the present study though it was revealed that the axons could also become naked to be attached to the islet cell without an intercalated element. The present authors cannot agree the view of WATARI (1968) that "*naked* axons enter the islet and terminate on the islet cell surface without any special attachments" and that "there are no interpositions between the nerve ending and the islet cell." As far as the authors know, the present study first proposed the occurrence of Schwann cell perikaryon *within* the islet. The

"agranular cells" of the islet whose occurrence and nature have been much disputed are believed at least partly to correspond to these Schwann cells.

The nerve fibers with synaptic vesicles which approach but remain apart from the islet cell were described in the present study and regarded as "en distance" type nerve terminals. The possibility cannot be excluded, however, that the terminal, though isolated from the cell in the section observed, may be in contact with another cell which is not included in the section under study. Complete serial sections covering a certain thickness are needed to solve this problem.

Attention has been focused on the possible differentiation of islet cell types with regard to their nervous control, whether sympathetic or parasympathetic. However, the present study in accordance with LEGG (1967) shows that none of the three islet cell types can be connected to any one of morphological categories of nerve terminals. A, B and D cells may receive the terminals containing small cored vesicles, i.e., of presumptive sympathetic nature. The large cored vesicles whose nature is unknown occur in the nerve terminals to islet cells as in the synapses of many other tissues. This type of vesicle was described in the islet nerve terminals by LEGG (1967) and WATARI (1968).

WATARI (1968) emphasized the large number of nerve fibers distributed to the islet cells of the bat. The dog islet cells observed in the present paper receive such an ample number of nerve fibers that there seems to be no corresponding instance in any endocrine tissue of any animal. Even if one takes into account that not all the nerve fibers which are in a close contact with the islet cell surface correspond to nerve terminals but some may represent passing fibers and that a winding nerve may possibly be cut several times in a section, the apparently superfluous nerve supply to the islet cells may be explained only by special affinity of the nerves to insular elements. This seems the very same affinity which brings about the conspicuous structure called the neuro-insular complex. FUJITA (1959) observed under the light microscope that in the developing pancreas islet cells emigrated into nerve tissue or nervous elements were incorporated into islets to form neuro-insular complexes. He described, besides the known type of the complex formed by the nerve cells and islet cells, the occurrence of a second type which is represented by a conglomeration of nerve *fibers* and islet cells. Typical forms of this latter type complex were encountered in the dog pancreas examined in the present study. It should be emphasized, however, that the morphological relation between the axons, Schwann cells and islet cells in these complexes are identical with the ordinary islets receiving nerve supply and only the quantitative ratio of the nervous and insular components vary gradually from the complexes to the ordinary islets. It may be worthy of mention that the neuro-insular complexes are especially abundant in the dog, the animal in which the present study revealed the most ample nerve supply to the general islets under the electron microscope.

Neither the present nor the previous (MACHINO, SAKUMA, and ONOE, 1966; MACHINO and SAKUMA, 1968; PRZYBYLSKI, 1967) studies include a single electron micrograph showing a nerve fiber in the islet tissue of the bird. It seems worthwhile to conduct a more systematic examination as to whether the islet system of avian and lower vertebrates is innervated at all. The conspicuous contrast in the nervous distribution between mammalian and avian islets suggests a possible difference in the control mechanism of secretion between both groups of animals.

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