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Light and Electron Microscopy of the Islets of Langerhans of the Saimiri Monkey Pancreas.

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LIGHT AND ELECTRON MICROSCOPY OF THE
ISLETS OF LANGERHANS OF THE SAIMIRI
MONKEY PANCREAS.

Louisiana State University, Ph.D., 1963
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LIGHT AND ELECTRON MICROSCOPY OF
THE ISLETS OF LANGERHANS
OF THE SAIMIRI MONKEY PANCREAS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
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in

The Department of Anatomy

by
William Burt Winborn
B. S., University of Texas, 1956
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ABSTRACT

The distribution of alpha, beta, and other islet cells in the tail of the pancreas of the Central American monkey (*Saimiri sciurea*) revealed by the light and electron microscopes, is similar to that occurring in man. Differentiation of acinar, islet and duct cells is based on the presence or absence of specific granules and the special morphology of the cytoplasm and its membrane systems. Conspicuous lipid droplets appear in the islets, largely confined to beta and delta cells. An "intergrade" cell is present, possessing features of both alpha and beta cells, as well as a non-granular variety of delta cells.

Nerve processes which display synaptic vesicles and clusters of mitochondria are present within the islets. Islet and acinar cells are sometimes separated by connective tissue or a reticulum, as observed in light microscopy, but there may be simple apposition of their respective plasma membranes. The fine structure of this reticulum presents several levels of organization. Duct cells possess lipid masses similar to those in islet cells. These duct cells, unlike islet cells, are agranular and possess microvilli and cytoplasmic blebs, both of which show internal structures.

I. INTRODUCTION

The islets of Langerhans were first reported in electron microscopy by Dalton ('51) and later by Robertson ('54). Lacy ('57a, b) differentiated the specific cell types and analyzed fine structure in the guinea pig, rat, rabbit and dog islet cells. Ferreira ('57) described beta cells in embryonic and new born rats and showed specific granules in the Golgi apparatus. Experimental work was reported by Williamson and Lacy ('59) who injected alloxan into rabbits and observed degranulation changes in beta cells. Lacy and Hartroft ('59) described three experimental results; (1) glycogen accumulation in beta cells of cats following injections of glucose, (2) changes in alpha cells induced by cobalt chloride and (3) degranulation of beta cells following Orinase treatment. Normal and neoplastic beta cells in human tissue have also been described (Lacy and Williamson, '60; Bencosme et al., '63). A mode of granule secretion under experimental conditions from beta cells has been postulated by Lacy ('61).

The improved image quality provided by thermostable embedments (Ryter and Kellenberger, '58; Low and Clevenger, '62; Freeman and Spurlock, '62) suggested that further descriptive work on a different animal would be profitable. The Central American monkey (*Saimiri sciurea*) was chosen because the islet tissue of this form had not been described in either light or electron microscopy.

II. MATERIALS AND METHODS

Five Central American squirrel monkeys were anesthetized with nembutal and the tail of each pancreas was removed. The tissue was cut into blocks not exceeding one millimeter in any dimension. These were immediately immersed in buffered osmium tetroxide (Palade, '52) or potassium permanganate (Luft, '56). Fixation was carried out for two to three hours at 0-3° Centigrade. All tissues were dehydrated in acetone and then embedded in Vestopal-W (Ryter and Kellenberger, '58), Selectron-methacrylate (Low and Clevenger, '62) or Maraglas (Freeman and Spurlock, '62). Vestopal-W embedments were initiated with t-butyl perbenzoate¹ and activated with cobalt naphthenate² (Ryter and Kellenberger, '58). This catalyzing procedure, when used for osmium-fixed Selectron-methacrylate embedments, made unnecessary the post-fixation in neutral formalin recommended by Low and Clevenger ('62). Triallyl cyanurate³ in 10% concentration with Vestopal-W improved both the sectioning quality of the blocks and image contrast in the mounted sections. Sections were cut on the Porter-Blum and LKB microtomes.

¹tertiary butyl perbenzoate; Wallace and Tiernan Inc., Lucidol Division, Buffalo, New York.

²cobalt naphthenate; Heyden Newport Chemical Corp., Nuodex Products Co., Elizabeth, New Jersey.

³Triallyl cyanurate is a compound used in heat-resistant polyester resins. A sample was kindly supplied to this laboratory by Mr. H. J. West, Technical Coordinator, American Cyanamid Co., Plastic and Resin Div., Wallingford, Conn. It is commercially available in small quantities.

Polyester sections were stained with 0.1% PTA in equal parts of acetone and ether (Low, '61b) and Maraglas sections were stained in aqueous lead hydroxide (Millonig, '61).

Formalin-fixed tissue embedded in paraffin was stained with aldehyde fuchsin (Gomori, '50) and phloxine (Gomori '39) for use in light microscopy. Thick Maraglas sections for light microscopy were stained with aqueous toluidine blue (Spurlock et al., '63).

III. OBSERVATIONS

A preliminary examination was made with the light microscope. Aldehyde fuchsin (Gomori, '50) stains beta cells deep purple, positive areas appearing dark in figures 1 and 2. These beta cells usually represent from 60 to 90% of the total islet cell population. Phloxine (Gomori, '39) stains the alpha and other islet cells pink and these appear pale in figures 1 and 2. Electron microscopy shows that the pale groups are composed mostly of alpha cells and a small number of non-granulated delta cells, as reported in the cat (Bencosme and Pease, '58).

Previous electron microscopic observations of islets of several experimental animals (Lacy, '57a, b; Bencosme and Pease, '58) makes possible positive identification of specific cell types whenever entire islets are not visible. The nuclear chromatin of islet cells is usually evenly distributed throughout the karyoplasm (figs. 3, 6, 7) but is occasionally concentrated in the periphery of the nucleus. The nucleoli are much less conspicuous than those of acinar cells. The two components of the nuclear membrane are frequently separated, suggesting a perinuclear cistern (fig. 7) rather than a double membrane. Continuity of the outer layer of the nuclear membrane with the cytoplasmic membranes (Watson '55) sometimes occurs in the islet cells (fig. 7). These nuclei are irregular in outline and often appear lobulated (figs. 6, 7), as contrasted with the round and oval nuclei of duct and acinar cells.

The cytoplasmic matrix of alpha cells is of low density (fig. 3). Mitochondria and specific granules characteristically stand out in sharp contrast against this background (figs. 3, 4, 8). Beta cells possess a matrix of higher density (figs. 3, 7, 9). The structures responsible for this are the granular and agranular endoplasmic reticulum, ribosomes and what appears to be the irregular tubulo-membranous component (ITM) described by Battig and Low ('61). All of these fine structures are in general poorly defined in alpha cells and more highly developed in beta cells. In both cell types ribosomes are scattered singly or, more often, are aggregated in clusters (fig. 8). The beta cells appear to have the higher concentration. Cytoplasmic protuberances of islet cells invaginate adjacent acinar cells as well as other islet cells (fig. 7).

The mitochondria of alpha cells (fig. 8) are fewer and more elongated than those of beta cells. Beta cell mitochondria are round, oval and somewhat irregularly shaped (fig. 9). Islet cell mitochondria possess, at high magnification, small areas of discontinuity in their outer membrane that give the impression of "pores" (fig. 8).

Sections passing through or near the center of beta and delta cells almost invariably contain lipid droplets (figs. 3, 7, 11, 12). They are rarely seen in alpha cells. Often as many as three lipid masses are present in a single section of one cell. These lipid masses are usually circular in outline but oval forms are not uncommon. Each mass is surrounded by a limiting membrane (figs. 7, 11, 12) which is denser than the lipid itself. The size of the droplets appear to be uniform within a single cell but varies from cell to cell. These droplets may exhibit circular osmiophobic areas (fig. 12). The granular

endoplasmic reticulum is more prominent in the beta cells than in the alpha cells. However, neither of the two cell types possess the extensive development typical of acinar cells (fig. 16). This membrane system may appear as multiple pairs of parallel membranes (fig. 6) bounding very narrow cavities, but it may form short channels, isolated and irregularly dilated, whose membranes are randomly studded with ribosomes (fig. 7). The cisternae themselves appear empty. The granular reticulum is best demonstrated where the specific granules are less densely concentrated. The agranular reticulum of alpha cells lacks the extensive definition seen in the beta cells. Here, it consists of narrow tubules or sacs, the caliber of which is more uniform than its granular counterpart.

The most conspicuous structures in the alpha cells are their specific granules. These intensely osmiophilic, homogeneous spheres appear to be morphologically identical with those already described in other animals (Lacy, '57a, b; Bencosme and Pease, '58). Each granule is surrounded by a membranous sac with a distinct intervening space between granule and membrane (figs. 4, 10, 13, 14). The granule may lie in an eccentric or concentric position within the sac. Alpha granules are about $2000\overset{\circ}{\text{\AA}}$ in diameter. These granules are uniform in size except when associated with the Golgi apparatus, where many small ones are found (figs. 3, 14). This interpretation in a single section is based upon the observation that a granule appears most dense when the section passes through or near the center of the granule. A mature granule of comparable size (figs. 4, 8), cut tangentially, is much less dense.

The membranes forming the sacs about the specific granules are devoid of ribosomes and appear much like those forming the vesicular portion of the Golgi apparatus (figs. 4, 14). Most often this membrane limits a single granule but two and rarely three have been observed (fig. 10). Alpha cell granules frequently occur in high concentration in the vascular pole of the cell (fig. 3).

The specific granules of the beta cell differ considerably from those of the alpha cells. They display a lower density and the substance of the mature granule is finely particulate (fig. 5). An "internal porous structure" (Lacy, '61) is demonstrable in these granules at high magnification (fig. 9). Like the alpha cell, the beta cell granule is surrounded by a membranous sac which is usually obscure (fig. 5). This is because the mature granule occupies the entire space enclosed by the membrane. Immature beta cell granules display a dense amorphous structure that does not completely fill the enclosing sac (fig. 9). They may be observed in the vesicular position of the Golgi apparatus, but are also present in other parts of the beta cell. A non-granular variety of delta cells (Bencosme and Pease, '58) is occasionally observed in the islets (fig. 11). It is similar in appearance to the beta cells, save for the specific granules. This cell type does not possess electron dense structures such as those visualized as granules in alpha and beta cells. They do, however, possess comparable membranous sacs similar to those seen surrounding specific granules in alpha and beta cells. No masses are visible within the sacs of these cells.

Material embedded in Vestopal-W often shows cells with both dense homogeneous and finely particulate granules (fig. 6). This type has been arbitrarily categorized as an "intergrade" cell (Winborn, '62).

Single or isolated islet cells are present, being completely surrounded by acinar cells (figs. 12, 13). These cells are apparently not in proximity to larger islet masses. Both alpha and beta cells exhibit this variation in cell arrangement. Beta cells have occasionally been observed forming portions of small duct systems. This identification is based on the presence of specific granules in the islet cell cytoplasm.

The Golgi apparatus appears to be similar in all islet cell types. It is not noticeably more prominent in one cell type than in another. This structure consists of parallel membranes, piled one upon another, numerous vesicles and irregular sacs or vacuoles (figs. 3, 9, 14). Immature forms of granules in both alpha and beta cells are present in the saccular portion of the Golgi apparatus.

Cells of small intralobular and intercalated ducts, like islet cells, show lipid masses (fig. 17). These structures, however, do not appear in every cell section as is usually true with beta cells. Thick Maraglas sections stained with Toluidine blue for light microscopy demonstrate these masses well. The duct cells, unlike the islets, lack specific granules but often possess cytoplasmic blebs and microvilli (fig. 17). Cross sections (fig. 19) of the microvilli sometimes demonstrate seven marginally placed annuli and a single central one. Longitudinal and slightly diagonal sections suggest that these annuli represent small internal tubules or cylinders (fig. 18). Microvilli are occasionally observed with internal structures below the free

surface of the cell (fig. 18). The basal portion of this internal structure may extend into the region of the Golgi apparatus. The marginal limits of the internal organization appear to be associated with intracellular fine filaments (fig. 18). These fine filaments in turn converge on terminal bars, thus forming a terminal web (fig. 18). The duct cell in addition possesses cytoplasmic blebs which project from the free surface of the cell. These blebs also display internal structures (figs. 17, 18). Interdigitating processes of adjacent duct cells are present (fig. 17).

Boundaries between islet and acinar cells vary considerably. The simplest boundary situation is direct apposition of the cell membranes of islet and acinar cells (figs. 12, 13). This arrangement may be modified in the form of reciprocal interlocking processes from adjacent cells. However, direct apposition is more common and often the acinar and islet cell membranes appear as one. Separation of the two membranes by an appreciable space is made apparent by slender and extremely elongated cytoplasmic processes of fibroblasts or finger-like processes from adjacent acinar cells. Boundary (basement) membranes (Low, '61a) are closely applied to both islet and acinar cell membranes when a tissue space between these two cells is evident (figs. 3, 7). Unit fibers of collagen and fibroblasts are occasionally seen in the space between the two boundary membranes.

The islet cells are richly supplied with capillaries (fig. 10). Specific granules display a vascular polarity in both alpha and beta cells (figs. 3, 10). The pericapillary space or tissue space is also bounded by two boundary membranes (figs. 3, 10). One is immediately adjacent to the endothelial cell and the other directly applied to the

parenchymal islet cells. Fibroblasts and unit fibers of collagen may occasionally lie in the compartment part of the tissue space formed by the two boundary membranes. The endothelium of the capillary wall is attenuated to form fenestrations (Farquhar, '62) which are bridged by diaphragms (figs. 3) similar to those described by Rhodin ('62). Golgi membranes are limited to areas near the nucleus of the endothelial cell. The granular reticulum, ribosomes, and vesicles are usually present where the fenestrations are not prominent. Here also the granular reticulum is continuous with the perinuclear cisternae of the endothelial cell.

Nerve processes are observed frequently within islets. Most often they are typical ones without cytoplasmic specializations. Several cases, however, revealed nerve fibers with clusters of mitochondria and synaptic vesicles. One particular area was examined in serial sections. This revealed a nerve process with numerous synaptic vesicles and clusters of mitochondria (figs. 20, 21, 22). An encircling cell (figs. 20, 21, 22) was observed about the synaptic process. It possessed elongated cytoplasmic extensions which came into intimate contact with a beta cell. This encircling cell possessed an elongated and large mitochondrion (fig. 22) immediately adjacent to the beta cell membrane.

ILLUSTRATIONS

Explanation of plates

All electron micrographs are from tissue fixed in OsO_4 .

A brief explanation of figures precedes each illustration.

Legends for all plates

A, alpha cell	I, intergrade cell
AC, acinar cells	IM, intermembranous space
AR, agranular endoplasmic reticulum	IP, interdigitating process
B, beta cell	L, lipid droplet
BM, boundary membrane	LS, longitudinal striations
CB, cytoplasmic bleb	M, mitochondria
D, delta cell	N, nerve process
DE, desmosome	N_1 , nerve process ₁
E, encircling cell	N_2 , nerve process ₂
EN, endothelial cell	NU, nucleus
F, fibroblast	P, protuberance
FE, fenestrations	PO, pore
FI, cytoplasmic filaments	R, ribosome
G, granules	S, membranous sac
GA, Golgi apparatus	T, tissue space
GR, granular endoplasmic reticulum	U, unmyelinated nerve
Z, zymogen granule	

PLATE I.

EXPLANATION OF FIGURES

The center of each field is occupied by an islet. Beta cells appear dark and other islet cells light. A prominent tissue space separates islets from acinar cells. Formalin fixation. Stained with aldehyde fuchsin and phloxine. X750.

- 1 Beta cells may show random placement and constitute slightly over one-half of the islets.
- 2 This islet is principally composed of beta cells and is typical. A heavy positive stain for beta cells surrounds the capillaries (arrows).

PLATE I.

PLATE II.

EXPLANATION OF FIGURES

Maraglas; grids floated on aqueous lead hydroxide for 20 min.

- 3 The acinar pancreas (AC) is separated from the islet cells by two boundary membranes (BM) which limit the tissue space (T). Two beta cells (B) with finely particulate granules possess lipid droplets (L). Each droplet is surrounded by a dense membrane. An alpha cell (A) with dense homogeneous granules (G) has a prominent Golgi apparatus (GA) containing immature granules (arrows). An endothelial cell (EN) of a capillary borders the islet. X11,000.
- 4 Alpha cells possess dense osmophilic granules. Each is surrounded by a membranous sac (S). X29,000.
- 5 Delta cells possess finely particulate granules surrounded by poorly defined sacs (S). A granule with internal porous structure (arrow) is present. X29,000.

PLATE II.

PLATE III.

EXPLANATION OF FIGURE

Vestopal-W; grid immersed in 0.1% PTA by weight in equal parts of acetone and ether for 30 min.

- 6 An alpha cell with a lobulated nucleus (NU) is surrounded by three cells; an intergrade (I) with both dense and finely particulate granules, a beta cell (B) with multiple pairs of parallel membranes of granular endoplasmic reticulum (GR) and another beta cell with irregularly shaped mitochondria (M). X12,000.

PLATE III.

PLATE IV.

EXPLANATION OF FIGURE

Maraglas; grid floated on aqueous lead hydroxide for 20 min.

- 7 A beta cell (B) with a lipid droplet (L), dilated granular endoplasmic reticulum (GR), agranular endoplasmic reticulum (AG) and a perinuclear cistern in continuity with cytoplasmic membranes (arrows) is adjacent to the acinar cells (AC). Above, another beta cell invaginates a cytoplasmic protuberance (P) into an alpha cell (A). X18,000.

PLATE IV.

PLATE V.

EXPLANATION OF FIGURES

Maraglas; grids floated on aqueous lead hydroxide for 20 min.

- 8 Elongated mitochondria (M) of alpha cells have dilated intermembranous spaces (IM). A tangential section of mitochondrial membranes may show "pores" (PO). Clusters of ribosomes (R), dense specific granules and dilated granular endoplasmic reticulum (GR) occur in the cytoplasm. X41,000.
- 9 Oval or rod-shaped mitochondria (M) with irregular outlines are found in beta cells. The Golgi apparatus (GA) has an immature specific granule (G) associated with it. Ribosomes (R) and dilated cisternae of the granular endoplasmic reticulum (GR) may also be observed. X41,000.

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PLATE V.

PLATE VI.

EXPLANATION OF FIGURES

Maraglas; grids floated on aqueous lead hydroxide for 20 min.

- 10 A capillary showing endothelial fenestrations (FE) borders an alpha cell (A) containing specific granules, three of which are within a single membranous sac (S). A nerve process (N) with synaptic vesicles lies between two alpha cells. X23,000.
- 11 A delta cell (D) containing numerous empty membranous sacs (S) and a large lipid droplet (L) lies adjacent to two alpha cells (A). X30,000.

PLATE VI.

PLATE VII.

EXPLANATION OF FIGURES

- 12 An isolated beta cell (B), with specific granules (G), is completely surrounded by acinar cells (AC). A lipid droplet (L) with a circular osmiophobic area appears in the beta cell. Zymogen granules (Z) are present in the acinar cells. Vestopal-W; grid immersed in 0.1% PTA by weight in equal parts acetone and ether for 30 min. X10,000.
- 13 An isolated alpha cell (A), characterized by specific granules (G) is surrounded by acinar cells (AC). No connective tissue stroma intervenes. Maraglas; grid floated on aqueous lead hydroxide for 20 min. X8000.

PLATE VII.

PLATE VIII

EXPLANATION OF FIGURES

Maraglas; grids floated on aqueous lead hydroxide for 20 min.

- 14 The Golgi apparatus (GA) of this alpha cell contains immature (smaller) specific granules (arrows). Mature (larger) granules (G) are also in the field. X45,000.
- 15 Duct cell mitochondria (M) possess dense intramitochondrial granules (arrows) which are absent in islet cell mitochondria (figs. 8 and 9). The lucid cytoplasm contains only a few membranes of the agranular endoplasmic reticulum (AR) and scattered ribosomes (R). X27,000.
- 16 Acinar cell mitochondria (M) also possess dense intramitochondrial granules (arrows). The dense cytoplasm has a high concentration of randomly distributed ribosomes (R) and membranes of granular endoplasmic reticulum (GR). X24,000.

PLATE VIII.

PLATE IX.

EXPLANATION OF FIGURES

Maraglas; grids floated on aqueous lead hydroxide for 20 min.

- 17 The cells of small intralobular ducts possess microvilli (MV) and cytoplasmic blebs (CB) projecting into the lumen of the duct. One of the duct cells shows a lipid droplet (L) and others show interdigitating processes (IP). A typical tissue space (T) is limited by two boundary membranes (BM) and contains an unmyelinated nerve (U) and part of a fibroblast (F). X17,000.
- 18 Duct cells also show desmosomes (DE) and fine cytoplasmic filaments (FI) that appear to converge on longitudinal striations (LS) of a microvillus near the region of the Golgi apparatus (GA). Two other microvilli, cut in different planes of section, display internal structures (arrow). A large cytoplasmic bleb (CB) showing internal structure is present. X32,000.
- 19 A cross-section of one of three microvilli (arrow) shows seven peripherially arranged annuli and a single central one. X54,000.

PLATE IX.

PLATE X.

EXPLANATION OF FIGURES

Maraglas; grids floated on aqueous lead hydroxide for 20 min. These figures show serial sections of nerve processes (N_1), (N_2), an encircling cell (E) and adjacent alpha (A) and beta (B) cells. X17,000.

- 20 A single nerve process (N_1) shows synaptic vesicles and clusters of mitochondria. An encircling cell (E) surrounds the synaptic process and appears to continue around and make contact with a beta cell (B).
- 21 Another nerve process (N_2) appears in this field. The encircling cell (E) continues to make contact with the beta cell.
- 22 The same encircling cell (E), as shown in the above two figures, displays a large and elongated mitochondrion (M).

PLATE X.

IV DISCUSSION

The fine structure of the islets of Langerhans of the Saimiri monkey, as revealed in this study through the use of several new embedding media, supports the observations of other investigators (Lacy '57a, b; Bencosme and Pease '58). Islet cells can be distinguished from acinar and duct cells by the character of the granular endoplasmic reticulum, with nuclear shape and lipid masses also constituting useful criteria. Islet cells themselves are identified by the special morphology of specific granules, agranular as well as granular reticulum and by their respective cytoplasmic densities.

The morphology of the alpha cell granules is constant in the dog, rabbit, guinea pig, rat, (Lacy, '57a, b) bat, (page 497 in Bloom and Fawcett, '62) and man (Bencosme et al., '63). They consist of intensely osmiophilic spheres loosely enclosed in membranous sacs. Mature granules, usually found in the vascular pole of the cell (figs. 3, 10), are about 2000 $\text{\AA}$ in diameter and of uniform density (fig. 4). Alpha granules in the Golgi apparatus, probably immature (Fawcett, '61), are of the same density but are smaller (figs. 3, 4). The argentaffine cell granules of the bat stomach, so well illustrated by Ito and Winchester ('63) are remarkably similar to these alpha granules but are more than twice the diameter.

Beta granules are as variable in form in the various species as the alpha granules are constant. The beta granules of other forms are crystalloid (Lacy '57a, b). In the Saimiri monkey the substance of

the granule is finely particulate, being barely resolved in our preparations. These particles occupy the entire space enclosed by the sac when the granules are mature. The granules themselves are about 2500 Å in diameter (figs. 3, 9). The crystalloid organization typical of many other species is not present in the beta granules of this form. Immature granules can be recognized by small size and sparse concentration of the particulate material. They do not always seem to be in the neighborhood of the Golgi apparatus.

Benscome and Pease ('58) described delta cells in the cat that possessed "vacuolar compartmentalization" of the cytoplasm. These authors also described a "granular variant", the granules of which cells possessed internal structure and were less dense than alpha granules. The vacuolar form was regarded as typical. A more recent study (Bencosme et al., '63) also favored this interpretation and it was postulated that delta cells might represent immature beta cells. In our material delta cells of the vacuolar variety are present (fig. 11), but whether or not they represent immature forms is not clear. The possibility that the vacuoles might represent regressive changes should not be ignored.

Three islet cell types: alpha, beta and delta can be identified as indicated above, by their specific granulation (or lack of it). These three types account for most of the islet cells but not for all of them. The term "intergrade" (Winborn, '62) is useful to designate the few cells that possess both high density (alpha) and low density (beta) granules (fig. 6). These intergrades might correspond to the cells seen in light microscopy that are on the borderline between one type and another. The C cells described by Lacy in the guinea pig pancreas are not present in our material. The cells of small ducts often resemble them.

The nuclei of islet cells in this study are consistently irregular in outline (figs. 3, 6, 7), being similar to those observed in dog islets (Lacy, '57). The fibrillar structures in the nuclei of human islet cells described by Bencosme et al. ('63) are also present, although rare, in both islet and acinar cells. The nuclear envelope (fig. 7) resembles the perinuclear cistern of Watson ('55) more than the classic double membrane system of Hartmann ('53). Immature specific granules are sometimes associated with the Golgi apparatus (figs. 3, 9, 14) possibly indicating the site of synthesis (Fawcett, '61; Ferreira, '57). This is conspicuous in alpha cells but immature beta granules are less frequently observed in the same place. According to experiments with tolbutamide, the Golgi apparatus in beta cells is not involved in insulin formation (Lacy '61). In summary, the Golgi apparatus seems to play a dominant part in granule formation in alpha cells but beta cells more closely simulate the mode of synthesis described by Palade in pancreatic acinar cells (pp. 64-83 in Hayashi, '59).

Mitochondria were described by Munger ('58) in the mouse as rod-shaped in both alpha and beta cells, the higher concentration being in the latter. A similar generalization can be made from this study except that beta cell mitochondria tend to be oval with irregular outlines (fig. 9). They closely resemble those with dilated intermembranous spaces, as described by Andrea ('62) in the developing spermatocyte. The intramitochondrial granules which are so conspicuous in acinar and duct cells (figs. 15, 16) are absent in all islet cells, both in the monkey and in other animals.

Conspicuous lipid droplets are present in the islets of the human pancreas (Bencosme et al. '63). Similar structures have been found in

this study, but are limited largely to the beta and delta cells (figs. 3, 7, 11, 12). Many of these droplets closely resemble the homogeneous variety described in interstitial cells by Fawcett and Burgos ('50). These authors make note of the prominence of similar lipid droplets in steroid-producing glands. Lipid masses have been described in dogs (Lacy, '57b) but do not appear to be as prominent as those of human and monkey islets.

Cytoplasmic density differs markedly between alpha and beta cells. It is expressed chiefly by the granular and agranular endoplasmic reticulum, isolated ribosomes and the remainder by the cytoplasmic matrix as defined by Porter (Brachet and Mirsky, '61). However, a finer structure in the matrix has been described (Battig and Low, '61; Battig and Clevenger, '61), the irregular tubule-membranous component (ITM). This latter system appears to be contributory to the different densities between alpha and beta cells.

Single or isolated islet cells (figs. 12, 13) are more conspicuous in electron microscopy than those seen with the light microscope. They have little tendency toward lobulation of the nucleus and concentration of specific granules. The boundary between these isolated cells and the acinar pancreas is not marked by the presence of intervening connective tissue, there being direct apposition of the plasma membranes of the islet and acinar cells. Contiguous membranes occasionally interdigitate. Small groups of islets may be similarly related to acinar cells. If the islets are separated from the acini by an appreciable distance, then connective tissue is present.

Certain portions of the duct system (figs. 17, 18) are included in this report because the individual cells closely resemble islet

cells. However, the absence of specific granules identifies these cells as part of a duct system. Tubules, apparently the anastomosing ones of light microscopy (page 501 in Bloom and Fawcett, '62), are present throughout the monkey pancreas. Some of these tubules connect to islet cell masses. Rarely, single islet cells form part of this system, which is said to consist of undifferentiated epithelium. Lipid masses are present in the duct cells (fig. 17), and there is apparently no difference between these droplets and those of islet cells. Cytoplasmic blebs and microvilli (figs. 17, 18) were shown in the duct system of the rat pancreas by Ekholm et al. ('62). In the centroacinar and intercalary ducts these authors demonstrated annuli in cross-sections of the microvilli and interpreted them as longitudinally arranged cylinders cut in section. Microvilli with the same arrangement of annuli, seven peripheral and one central (fig. 19), are also present in the Saimiri monkey. Longitudinal striations appear in the microvilli (fig. 18), not only beyond the free surface of the cell, but sometimes extending within it near to the Golgi zone. The cytoplasmic filaments associated with microvilli (fig. 18) are comparable to structures of the cell web described by Leblond et al. ('60).

The nerve fibers present in the monkey islets contain synaptic vesicles (figs. 20, 21, 22), which, according to De Robertis ('61), are almost exclusively confined to the presynaptic site. Many of them resemble the nerve endings in the central nervous system (Palay, '56) and at the myoneural junction (Palade, '54). The processes observed in this study do not have the localized thickenings of the cell membranes characteristic of other synaptic sites. What is referred to

as encircling cell (figs. 20, 21, 22) always intervened between islet and nerve process. The identity of this cell is not known, and it does not correspond to any islet cell type observed in this study.

A connective tissue stroma is recognized to be closely associated with the islet cells in light microscopy. Here, a reticulum more or less delimits islets from acini (page 496 in Bloom and Fawcett, '62) and accompanies blood vessels. In electron microscopy this reticulum shows various units of organization and this warrants reinterpretation. The simplest expression of the stroma is seen where there is a small but apparent space between islet and acinar cells. Here, boundary (basement) membranes (Low, '61a) follow both islet and acinar cell plasma membranes (fig. 3). The tissue space intervenes. Other formed elements of the connective tissue such as fibroblasts and/or unit fibers of collagen, may appear between the two boundary membranes in the tissue space as seen between duct and acinar cells (fig. 17). These elements, being part of the connective tissue, lack boundary membranes of their own. It is clear that the connective tissue stroma of the pancreas, where it forms the reticulum of light microscopy, exemplifies an ultrastructural pattern found generally throughout the body. This pattern, recognized in electron microscopy although largely unformulated (Pease, '58; Policard and Collet, '59) was described in the alveolar wall by Low ('61a) in terms applicable to the entire body. The descriptive term "boundary membrane" designates the membranous formed element determining the extent of the tissue space. Battig and Low ('61) later presented an analysis of connective tissue ultrastructure in the heart, where the boundary membrane concept was used to help identify certain cell types. Dougherty's descriptions

of the cervical uterine mucosa (162a, b) interpreted the connective tissue according to the systematics of this boundary membrane concept, and confirmed the presence of the same pattern in the uterus. In the monkey pancreas this ultrastructural situation is present. It is typical and without variation.

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VI. BIOGRAPHY

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EXAMINATION AND THESIS REPORT

Candidate: William Burt Winborn

Major Field: Anatomy

Title of Thesis: Light and Electron Microscopy of the Islets
of Langerhans of the Saimiri Monkey Pancreas.

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

April 29, 1963