Quantitative estimation of islet tissue of pancreas in Australian mammals (Comparative histological study)

by

Nalini Edwin, MBBS, M.Sc.
Department of Anatomy and Histology
The University of Adelaide

Thesis submitted for the Degree of Doctor of Philosophy
in the University of Adelaide
November 1986

I hereby certify that the text of this thesis is entirely my own composition, that the findings reported herein (except where due reference is made) are the result of my own personal investigations, and that no part of this work has been previously submitted for a Degree in this or any other University.

N. Edwin

Department of Anatomy and Histology

The University of Adelaide

November 1986

ACKNOWLEDGEMENTS

My sincere thanks to Dr. R.A. Barbour, my supervisor, for his guidance in this work; Mr. Phil. Leppard, for his guidance in the statistical part of the work; Miss E. Batty for technical assistance; Mr. Chris Leigh and Mr. Alan McNeil for assistance in obtaining the material; Mr. Rob Murphy, Mrs. Gail Hermanis and Miss E. Vinall for photographic assistance and Mrs. J. Brazier and Mrs. B. Sheldon who assisted in the typing.

My sincere thanks to Professor J. Priedkalns for his guidance in the final statistical part of this investigation.

CONTENTS

INTRODUCTION AND HISTORICAL SURVEY

(a) General Introduction

In 1869, Paul Langerhans, (as cited by Wellmann and Volk, 1977), presented the first anatomical description of the microscopic islets of tissue within the pancreas (in the rabbit). In 1893, Laguesse advanced the suggestion that these structures are the anatomical counterpart of an internal secretory function of the organ. Schafer (1895) and Diamare (1899), studying the islets, corroborated the existence of a relationship between the islets and carbohydrate metabolism.

The histology of the pancreatic islets continued to become a field of extensive study. Diamare in 1899 and Schulze one year later, were the first to suggest that the islets of Langerhans contain more than one type of cell. Schulze (1900) working with the guinea pig observed small cells with chromatin rich nuclei and larger cells with darker cytoplasm and centralised chromatin clumps. Other authors (Ssobolew, 1902; Mankowski, 1901) soon confirmed these observations. In 1906 Tschassownikow devised a method that permitted the tinctorial differentiation of the two cell types then known. He used Fleming's orange method, using safranin and methyl green. Further progress in fixation and staining technology as applied to the pancreatic islets was achieved in 1907 by Lane who presented the first report on guinea pigs. He found that after fixation of the guinea pig pancreas in 50-70% alcohol the granules of the A cells stained violet with Bensley's neutral gentian while the B cell granules remain unstained. He introduced the designation "αcell" and "βcell". Bensley in 1911 changed Lane's term "β cell" to B cell" and "α cell" to "A cell". Lane (1907) also deserves credit for clearly recognizing the nature of these cells; he stated that the islets of Langerhans are structures which in all probability have the function of producing a twofold substance which, poured into the blood stream, has an important effect on metabolism.

Bensley (1911) published results of several fixation and staining experiments conducted on the pancreas of the guinea pig. He identified a

few agranular or 'clear' cells in the pancreas of this species which he believed represented a 'stage in the physiological activity' of the cells of one of the two known types, or perhaps the source of the A cells.

In 1924, Bowie, studying a teleost pancreas, identified A and B cells as well as a third cell type characterised by a lightly stained, faintly granular cytoplasm and an oval pale nucleus; using neutral ethyl violet and Biebrich's scarlet for staining. These 'γ cells' were believed by him to be precursors of A and B cells. In 1926, Ukai utilized neutral formol and Zenker's fixative with a modified Mallory aniline blue method, applied to pancreatic islets of the cat and of the guinea pig, and found that the A cells stained red and B cells stained blue. Bloom (1931), using Mallory's azan stain in an investigation of human pancreases derived from autoposies, demonstrated A cells which were red, B cells staining orange and was able to identify a third cell type displaying a pale blue cytoplasm with or without darker blue granules. He called it "D cell" and wondered whether it was a transitional element, intermediate between A and B cell, or whether it might be related to Bowie's γ cell, or to Bensley's agranular cell. Thomas (1937) investigated the pancreases of 41 mammalian species and found that A, B and D cells were present in all of them, but C cells were found only in guinea pig.

Some additional cell types have been described in the pancreatic islets of certain species. One of these is the 'Mankowski cell' of the guinea pig (Mankowski, 1901). In the opossum, Thomas (1937) identified the 'E cell' characterized by magenta-coloured granules in tissue fixed with Helly's fluid and stained with Heidenhain's azan. Baumann (1939), Wolter (1950) and Ferner (cited by Miller, 1942) found groups of polygonal cells with brown cytoplasmic pigmentation in the central portions of the pancreatic islets of the horse. The cells are silver-negative and react similarly to A cells in granule stains. They were called 'X cells' by Ferner (cited by Miller, 1942). Bencosme and Liepa (1955) applied the same term to a cell found in the islets, acini and ducts of the dog and cat pancreas. Bencosme and Liepa thought that

the cell might correspond to the Mankowski cell of the guinea pig (Mankowski, 1901) and to certain cells described in the pancreases of snakes (Thomas, 1942). It is silver negative (Lazarus and Shapiro, 1971) and has certain ultrastructural features including secretory granules varying in electron density depending on what fixative has been used (Lazarus and Shapiro, 1971; Munger, Caramia and Lacy, 1965). The 'X cell' of Bencosme and Liepa was called 'F cell' by Munger, Caramia and Lacy (1965).

During the purification of chicken insulin, Kimmel, Polak and Hazelwood (1968) detected a peptide, which they named avian polypeptide (APP). Later, APP was found in pancreatic extracts of birds and reptiles. Larsson, Sundler, Hakanson, Pollock and Kimmel (1974), established by light and electron microscopic immunocytochemical studies in chicken and several mammals, including humans, that pancreatic polypeptide (PP) is stored in insular and extrainsular pancreatic cells (PP) distinct from A, B and D cells.

In ultrastructural studies, two further cell types have been described in the human pancreas by Deconinck, Potvliege and Gepts (1971). These authors distinguished between A cells, B cells, D cells (called 'type IV' by them) and cells of types III and V. Type III cells, termed 'G' cells by Kubes and Jiraseck, (1971) resemble the gastrin-secreting cells of the gastric and duodenal mucosa (Orci, Pictet, Forssmann, Renold and Rouiller, 1968). Type V cells were to be found similar to the serotonin producing cells of the gastrointestinal tract of rats (Orci, Pictet, Forssman, Renold and Rouiller, 1968) and of man (Pearse, Cauling, Weaver and Friesca, 1970).

Solcia, Pearse, Grube, Kobayashi, Bussolati, Creutzfieldt and Gepts (1973) suggest that there are four islet cell types : A, B and D corresponding to type III of Deconinck, Potvliege and Gepts and D_1 corresponding to type IV of same authors. Buffa, Solcia, Capella, Fontana, Trinci and Said (1976) have suggested that the cells secrete vasoactive intestinal peptide (VIP). Larsson, Sundler and Hakanson, (1976) favour the type V cells of Deconinck for

the origin of pancreatic polypeptide (PP). The fact that the PP cells are numerous in the uncinate process of the dog induced Baetens, Rufener and Orci (1976), Buffa, Capella, Solcia, Frigerio and Said (1977) to suggest that these cells correspond to F or X cells described by Bencosme and Liepa (1955) and Munger, Caramia and Lacy (1965). Biogenic monamines have been shown by induced fluorescence in the A_2 cells of owl monkey (**Aotes trivirgatus**) (Cegrell and Falck, 1969).

(b) Silver impregnation methods

It was Piazza in 1911 (cited by Fujita, 1968) who first recognised in the rabbit pancreas, cells with argyrophil granules in the peripheral regions of the islets and occasionally between the acinar cells. He considered these cells as representing a certain secretory stage of islet cells. The identification of argyrophil cells with A cells dates back to the description by Takahashi in 1927 (cited by Fujita, 1968). Hamperl (1932) recognised the argyrophil cells in the peripheral part of human pancreatic islets, acknowledging it as the favourite site of A cells. It was Kon, teacher of Takahashi, in 1933, who identified argyrophil with A cells. Ferner (1938) adopted silver impregnation as the best method for selective demonstration of A cells. Ferner's method of study was relied on and used by many investigators (Hess, 1946; Terbruggen, 1947, 1948; Hultquist, 1946; Hultquist and Tegner, 1949; Bargmann and Creutzfeldt, 1949; Sendrail, Bazex and Bolte, 1949).

The capriciousness of silver impregnation claimed by Hamperl (1932) was denied by Hultquist, Dahlen and Helander (1948) who counted the cell population in a large number of islets in the rat and human pancreas and showed that the percentage occupied by silver blackened cells was constant. In the methodology of islet impregnation, the identity of argyrophil cells with A cells was doubted by Burkl (1951) because the central A cells were not found to be argyrophilic. In the reptilian pancreas in which A cells are predominant in the islet, Miller and Wurster (1958) concluded that argyrophilia is not necessarily a characteristic of A cell granules in the lizard. Clausen (1953) concluded that in elasmobranch pancreas there was a certain number of A cells which were not stained in the silver impregnation. Using the pancreas of the duck (Hellman and Hellerstrom, 1960), rat (Hellerstrom and Hellman, 1960) and dog (Hellman, Wallgren and Hellerstrom, 1962), Hellman and his co-workers bleached silver impregnated paraffin sections (modified Davenport's technique) with $KMnO_4$ and restained them either with chrome-haematoxylin-phloxine or with aldehyde-fuchsin-ponceaux fuchsin

to examine the same cell successively stained by both methods. Confirming that it was only a portion of A cells that gave the argyrophil reaction, they divided the A cells into two groups - argyrophil 'A cells' and nonargyrophil 'A_2 cells'. Thus, not all non-B cells are silver impregnated. Argyrophil cells correspond to only some of the non-B cells (Fujita, 1968).

Epple (1964) and Fujita (1964a) respectively, stated a new interpretation of the argyrophil cells of the islet. Independently from each other and using different animals they reached the same conclusion that the argyrophil cells are identical with D cells. Epple's material was the pancreas of pigeon. Using the modified Davenport technique (Hellerstrom and Hellman, 1960), he found that silver blackened cells showed precisely the same distribution as D cells. Fujita (1964a) noticed the identify of argyrophil and D cells in his study on holocephalian fish (**Chimaera monstrosa**), using sections cut serially and stained alternately with Palmgren's silver impregnation and with aldehyde fuchsin-trichrome staining.

Cavallero and Solcia (1964) and Solcia and Sampietro (1964, 1965) concluded that the argyrophil cell of several mammalian species examined by them corresponded neither to the aldehyde fuchsin positive B cell, nor to tryptophan positive A cell and that this cell was nothing but a D cell. They also noticed that the metachromatic and argyrophil cells in toluidine blue and azure A stained material was nothing but D cells.

Munger, Caramia and Lacy (1965) came to the conclusion that argyrophil cells are D cells. Using rabbits, dogs, possums, they recognised that the argyrophil cells corresponded to the phosphotungstic acid haematoxylin negative and aldehyde fuchsin negative cells.

Silver impregnation methods have added another dimension to the study of pancreatic islet cells since silver impregnable cells were first demonstrated by Piazza in 1911. All such cells are argyrophilic rather than argentaffin and most of the earlier workers employed he Gros-Schultze method of silver impregnation. This technique stains the A cells but is noted for its capriciousness (Wellmann and Volk, 1977). The Bodian's silver proteinate

method also stains the A cells, as do the procedures of Grimelius (1968). Grimelius' silver method stains A and D cells (Epple and Lewis, 1973). On the other hand, modifications of the Davenport's silver impregnation technique devised by Hellerstrom and Hellman (1960) will stain D cells rather than A cells.

(c) Immunocytochemical methods

During the 1970's, immunocytochemical (ICC) methods - immunofluorescence and/or the PAP (peroxidase - antiperoxidase) procedures have been developed, permitting observations that have revolutionised views on the histophysiology and evolution of the GEP neuroendocrine system (Falkmer and Van Noorden, 1983).

In 1971, Faulk and Taylor inaugurated the immunocolloid method for electron microscopy by using a complex of antibodies and colloidal gold particles for cell surface antigen localization. Since then gold particles have been used more and more as markers in immunocytochemistry (Romano and Romano, 1977). Recently, a post embedding staining technique has been developed for antigen localisation on plastic thin sections by the use of protein A-gold complex which allowed the detection of antigen material with high specificity and resolution (Roth, Bendayan and Orci, 1980).

Using these immunocytochemical methods, a major breakthrough in the research front of the endocrine pancreas was made in 1975 when it was found that the hypothalamic peptide, somatostatin, occurred not only in neuronal cells, but in endocrine cells in the pancreatic islets (Polak, Pearse, Grimelius, Bloom and Arimura, 1975; Falkmer and Van Noorden, 1983). Thus the D cells, previously suspected of producing gastrin, were now shown to be not only the storage site, but also the place for the actual production of somatostatin (Falkmer and Van Noorden, 1983). Since then, practically all the GEP hormonal peptides have been demonstrated to have this dual distribution and thus to form the brain-gut axis of the diffuse neuroendocrine system (Van Noorden and Polak, 1979; Falkmer and Van Noorden, 1983). Glucagon is one of the latest editions to this group, having recently been localised in several

areas of the mammalian (including human) brain (Tager, Hohenbok, Markese and Dinerste, 1980; Falkmer and Van Noorden, 1983).

(d) Specific identification and functional correlation of main islet cell types

It is now generally accepted that there are four main kinds of islet parenchymal cells in vertebrates, B, A, D and PP cells (Sato, Herman and Fitzgerald, 1966; Hellman, 1970; Falkmer and Patent, 1972).

The B cell

The B cells are the most numerous constituents of the pancreatic islets in most species. The cells are granulated, nonargyrophilic, and give a characteristic tinctorial reaction with aldehyde fuchsin (Falkmer and Patent, 1972).

Various hormones have an effect on the pancreatic B cell. In 1938, Richardson and Young (cited by Wellmann and Volk, 1977) observed destruction of B cells in dogs made permanently diabetic by the injection of anterior pituitary extracts. These histological changes represented functional exhaustion induced by the diabetic state and suggested that B cells were the source of insulin. It was observed in experimental animals, that the injection of anterior pituitary extracts caused B cell degranulation and often further changes such as hydropic degeneration and even necrosis of B cells. The insulin content in the pancreas of such animals is diminished (Miller, 1942). Degranulation of the cytoplasm of B cells is also observed in both normal and hypophysectomised white rats with daily administration of growth hormone for 6 weeks or longer (Goldner and Volk, 1956).

This evidence suggested that B cells were the source of insulin. Conclusive evidence for this supposition was not available until 1943, when Dunn (cited by Wellmann and Volk, 1977) showed that the selective destruction of the pancreatic B cell by alloxan induces severe diabetes in experimental animals. These investigations have gone far in providing evidence for the origination of insulin, from the pancreatic B cell.

Dietary factors also influence B cell granulation and insulin content of the pancreas even though the observed effects are not the same in all species (Wellmann and Volk, 1977). Lazarus and Bencosme have suggested that while starvation does not diminish the granules in rabbits, degranulation has been recorded in the rat according to Barron (1948) and Nerenburg (1953), and in starved pigeons, B cell 'inactivity' has been recorded (Miller, 1942). Somatostatin, a hypothalamic peptide, that inhibits the secretion of pituitary growth hormone, and is also secreted by D cells inhibits basal insulin secretion in fasted cats and rats (Koerker, Ruck, Chideckel, Palmer, Goodner, Ensinck and Gale, 1974).

The development of widely applicable immunocytochemical techniques for the cellular localization of hormones, has greatly facilitated the identification of islet cell types (at both the light microscopic and ultrastructural level) responsible for the production of insulin, glucagon, somatostatin and pancreatic polypeptide in a wide variety of species, including man (Erlandsen, Hegre, Parsons, McEvoy and Elde, 1976).

El-Salhy and Grimelius (1981) have investigated the endocrine pancreas of the grass lizard, **Mabuya quinquetaeniata** and of the desert lizard, **Uromastyx aegyptia** and observed four cell types, insulin (B), glucagon (A), somatostatin (D) and pancreatic polypeptide (PP). In both species the B, A, D and PP cells could be detected by their reactivity with antisera raised against mammalian insulin, glucagon, somatostatin and pancreatic polypeptide.

Kaung and Elde (1980) have shown in the frog, **Rane pipiens**, using PAP techniques, cells reactive to anti-anglerfish insulin.

Helmstaedter, Feurle and Forssmann (1976) investigating the equine pancreas with immunohistochemical methods to study the distribution of endocrine cells immunoreactive to anti insulin, found that anti insulin reactive B cells formed a large zone around the centre of the islets.

The use of specific immunocytochemical methods, coupled with electron microscopy has allowed the correlation of endocrine cell secretory granules with the production of individual polypeptides (Buchan and Polak, 1980).

Insulin is produced in the alcohol soluble B cells originally named for their basophilic reaction (Buchan and Polak, 1980). The cells are defined ultrastructurally by large, highly pleomorphic granules, many of a crystalline appearance. Morphologically, the cells are rounded with the secretory granules, which sometimes show polarisation, located around the central nucleus, (Buchan and Polak, 1980). The product is liberated into blood capillaries in the islets and so into the circulation (Lacy, 1959).

Data obtained from ultrastructural studies support the concept first enunciated by Lacy, that emiocytosis is the major and probably the only mechanism of insulin secretion (Lacy 1970, cited by Volk and Wellmann, 1985). Emiocytosis, also called 'exocytosis', involves the fusion of the membranous sac of the secretory granule with the plasma membrane of the cell, the rupture of the cell membrane at this point, and the release of the granule into the extracellular space (Volk and Wellmann, 1985).

A cell

The nature and function of the pancreatic A-cells have been a matter of much discussion ever since their discovery (Ssobolew, 1902; Hellerstrom, Hellman, Petersson and Alm, 1964). One reason for the controversy was the lack of satisfactory differential staining methods. The introduction of Gomori's granule staining methods (Gomori, 1939) offered a way for evaluating the frequency of A cells in the islets of Langerhans.

These granulated cells are most accurately characterised by immunocytochemistry and show histochemical reaction for indoles (Munger, 1972). This reaction is specific, due to the relatively large content of tryptophan in glucagon (Munger, 1972). They show tryptophan staining and this reaction is specific (Hellerstrom, Hellman, Petersson and Alm, 1964; Falkmer and Patent, (1972.

A precise cytological delineation of A cells is possible using phosphotungstic acid haematoxylin (PTAH) as described by Gomori (1939). The reliability of the PTAH technique has been demonstrated in mammals (Munger, Caramia and Lacy, 1965), reptiles (Rhoten, 1971), fish (Brinn, personal communication, cited by Munger, 1972) and birds (Machino, personal

communication, cited by Munger, 1972).

The A cells remain nonargyrophilic with the Hellerstrom-Hellman procedure (Hellerstrom and Hellman, 1960), whereas in man and in several other species they are distinctly silver-impregnanted with the Grimelius technique (Grimelius 1968). These cells are synonymous with the A_2 or $\mathcal{A}_2$ cells of the Swedish authors (Epple, 1968; Falkmer and Patent, 1972).

In the rat and mouse, the A cells are typically located in the islet periphery, whereas in the horse they are found in the central core of the islets (Hellerstrom, Hellman, Petersson and Alm, 1964). In man they occur both in the islet periphery and dispersed among other islet cell types (Grimelius, 1968; Hellerstrom, 1977).

The A cells are the production site of glucagon (Lundquist, Brolin, Unger and Eisentraut, 1970). Glucagon was administered to rats and guinea pigs as daily injections of 0.3-0.6mgm/Kg. crystalline glucagon given during 5 days, and with continued treatment for a month there was a marked reduction of the percentage of A cells in the islet tissue (Hellerstrom, Hellman, Petersson and Alm, 1964). In addition these cells showed a pronounced degranulation and a marked diminution in the intensity of the histochemical reaction for tryptophan. This view is further supported by the observation that A cells were found to be absent from the uncinate process of dog pancreas, which had been previously reported to lack extractable glucagon-like activity (Bencosme and Liepa, 1955). This evidence supports the view that glucagon is secreted by A cells.

Immunocytochemically pancreatic glucagon is present in the A cell (Baum, Simms, Unger and Madison, 1972) as shown by immunofluorescent techniques.

Lange (1970) has demonstrated glucagon-containing islet cells by the indirect immunofluorescence technique on semithin and ultrathin sections of epoxy-resin embedded tissue in rat and frog.

Klein and Van Noorden (1980) in teleost fish (**Xiphophorus helleri**) have done immunohistochemical studies using antibodies to pancreatic polypeptide (PP) and glucagon and have shown that separate cell types are responsible

for the production of these peptides.

El-Salhy and Grimelius (1981) have investigated the endocrine pancreas of the grass lizard, **Mabuya quinquetaeniata**, and of the desert lizard, **Uromastyx aegyptia** and observed glucagon (A) cells. In both species, the A cells could be detected by their reactivity with antisera raised against mammalian glucagon.

Kaung and Elde (1980) have shown in the frog, **Rana pipiens**, using PAP techniques cells reactive to antiporcine glucagon.

Helmstaedter, Feurle and Forssmann (1976) investigating the equine pancreas with immunohistochemical methods to study the distribution of endocrine cells immunoreactive to antiglucagon found A cells to be located in the centre of Langerhans islets and frequently in the duct epithelium.

Fuchs, Hahn von Dorsche, Ziegler (1984) have shown the passive transfer of rabbit anti-glucagon antibodies into mice induces peri-insular and intra-insular inflammatin and hyperplasia of alpha cells. Active immunization of rabbits with heterologous glucagon induces alpha cell hyperplasia.

Ultrastructurally the secretory granules show a characteristic halo between the electron dense core and the limiting membrane which is argyrophilic by Grimelius' method (Buchan and Polak, 1980). The granules are insoluble in alcohol.

D cell

This name refers to a granulated parenchymal cell showing argyrophilia by the Hellerstrom-Hellman (1960) modification of Davenport's procedure, but nonargyrophilic by Grimelius' silver nitrate method (Grimelius, 1968). The A_1 cells of the Swedish authors are synonymous with D cells (Epple, 1964; Fujita, 1964b).

The D cells were shown to produce gastrin (Munger, 1968). Furthermore, with the Hellerstrom-Hellman silver staining technique, Hokfelt, Efendic, Hellerstrom, Johansson, Luft and Arimura (1975) have shown that virtually all somatostatin-positive cells are argyrophilic and vice versa and have concluded that it was the D cell that produced somatostatin. D cells have been shown to

contain somatostatin (Polak, Pearse, Grimelius, Bloom and Arimura, 1975; Hokfelt, Efendic, Hellerstrom, Johansson, Luft and Arimura, 1975; Falkmer and Ostberg, 1977) but may contain some kind of gastrin hormone as well (Erlandsen, Hegre, Parsons, McEvoy and Elde, 1976) as shown in humans.

Somatostatin inhibits insulin and glucagon release (Fujimoto, Ensinck and Williams, 1974). Somatostatin also suppresses pancreatic exocrine secretion (Bodan, Sivitz, Owen, Essa-Kouwar and Landor, 1975).

Somatostatin-like activity in extracts of foetal rat pancreas has been found (Vale, Brazeau, Rivier, Brown, Boss, Rivier, Burgae, Ling and Cuillemin, 1975). Somatostatin was identified by radioimmunoassay in the rat pancreas in a concentration similar to that in the hypothalamus (Arimura, Sato, Dupont and Niche, 1975).

The suggested derivation of pancreatic endocrine cells from the neural crest anlage (Goldsmith, Rose, Arimura and Ganong, 1975) could rationalize how pancreatic cells can secrete a peptide observed originally in cellular elements of the central nervous system (Dubois, 1975).

A positive reaction for somatostatin in the rat was demnstrated employing immunocytochemical techniques at light microscopic level (Pelletier, Leclere, Arimura and Schally, 1975). It was mostly located at the periphery of the islet.

Kalliecharan and Steves (1982) have shown a discrete population of cells containing immunoreactive somatostatin using the peroxidase antibody bridge technique at both the L.M. and E.M. level to be present in the endocrine and exocrine portions of pancreas of chick embryo (**Gallus domesticus**).

El-Salhy and Grimelius (1981) have investigated the endocrine pancreas of the gass lizard **Mabuya quinquetaeniata** and of the desert lizard, **Uromastyx aegyptia** and observed somatostatin (D) cells. In both species, the D cells could be detected by their reactivity with antisera raised against mammalian somatostatin.

Kaung and Elde (1980) have shown in the frog, **Rana pipiens**, using PAP techniques, cells reactive to anti-synthetic somatostatin.

Helmstaedter, Feurle and Forssmann (1976) investigating the equine pancreas with immunohistochemical methods to study the distribution of endocrine cells immunoreactive to antisomatostatin and demonstrated D cells as a discontinuous outermost zone around the Langerhans islets.

Endocrine cells producing somatostatin have been identified ultrastructurally as D cells (Buchan and Polak, 1980). The granules are large, round, of medium electron density and stain positively with the Hellerstrom-Hellman silver stain. They are arranged around the periphery of the islets with the granules surrounding the nucleus in humans (Bloom, 1931; Polak, Pearse, Grimelius, Bloom and Arimura, 1975).

Agranular C cells

The C cells are chromophobic, clear, sparsely granulated islet parenchymal cells rather poorly known (Falkmer and Patent, 1972). According to Boquist and Falkmer (1970) they are actually immature precursor cells to the granular islet parenchymal cells, without any specific hormone production, and are often neglected in classifications of GEP (gastro-enteropancreatic) cells. Their most characteristic feature is their inability to give any 'positive' light microscopical staining reactions or characteristic cytochemical feature at all (Boquist and Falkmer, 1970).

Pancreatic polypeptide (PP cells)

A specific polypeptide was isolated from the chicken pancreas (Kimmel, Pollock and Hazelwood, 1968). Later, a similar factor was isolated from the bovine pancreas (BPP) (Lin, Evans, Chance and Spray, 1977).

The pancreatic polypeptide (PP) cell has recently been characterized by light and E.M. immunocytochemistry in a number of species including man, mouse, rat, hamster, guinea pig, rabbit, opossum, cat and dog (Larsson, Sundler, Hakanson, Pollock and Kimmel, 1974; Larsson, Sundler and Hakanson, 1975, 1976). This cell is the source of pancreatic polypeptide (Kimmel, Pollock and Hazelwood, 1968).

A small population of human pancreatic islet cells showed strong immunofluorescence after staining with anti-human pancreatic polypeptide (HPP) serum (Larsson, Sundler and Hakanson, 1975). HPP cells were mainly localized at the periphery of the islets, and sometimes they were scattered in the exocrine pancreas, as well as within the epithelium of small to medium-sized ducts. They exhibit negative staining reactions to both aldehyde fuchsin (Gomori 1950) and the silver staining of Hellerstrom-Hellman (1960) and Grimelius (1968).

Using the immunofluorescence and immunoperoxidase technique on the uncinate process of the dog, Forssmann, Helmstaedter, Metz, Greenberg and Chance (1977) demonstrated that antiserum against bovine pancreatic polypeptide (BPP) reacts with the cell which is localized at the same sites as the F cell, and have concluded that the pancreatic F cell is identical to the pancreatic polypeptide producing (PP) cell.

Paulin and Dubois (1978) have detected PP-containing cells by using anti-bovine pancreatic polypeptide (BPP) serum in the pancreas and gastrointestinal tract of human foetus and adult man.

Rahier, Wallon and Henquin (1981) using immunoperoxidase techniques examined the pancreases from normoglycemic neonates, infants and adults, and have shown that in the posterior part of the head, the proportion of PP cells tended to be higher in adults than in neonates or infants.

Johnson, Noe and Bauer (1982) have demonstrated pancreatic polypeptide (PP) immunoreactivity in the endocrine pancreas of the anglerfish (**Lophius americanus**) and the channel catfish (**Ictalurus punctatus**) using immunocytochemistry. In both species, PP immunoreactive cells were localized at the periphery of endocrine tissue.

Alumets, Hakanson and Sundler (1978) have studied the pancreatic polypeptide (PP) cells in the pancreas of chickens by immuncytochemistry and have demonstrated that the pancreas PP cells were numerous and disseminated in the exocrine parenchyma.

Grieder, Gersell and Gingerich (1978) have identified the F cell of the dog pancreas as the specific cell type containing pancreatic polypeptide. This localization of pancreatic polypeptide was accomplished by immunocytochemical staining of ultrathin sections and direct E.M. identification. They have proposed that the name F cell be used for defining in all species the islet cell that contains pancreatic polypeptide.

Gersell, Gingerich and Grieder (1979) have studied the regional concentrations of pancreatic polypeptide (PP) cell distribution in human and canine pancreases by immunoperoxidase localization and cell quantitation and found that PP concentration was highest in both the uncinate process and the head of the human pancreas and in the right lobe of the canine pancreas. Human F-cells, which contain PP, were located primarily at the periphery of the islets, although a few F-cells were scattered throughout the ducts and acini. Cellular quantitation of canine F-cells in both species correlated significantly with the tissue concentration of PP in all regions studied, validating the use of morphometric techniques to quantitate the reginal distribution of PP.

Ultrastructurally the secretory granules present in these cells are small, round and electron dense with a closely applied membrane. They are Grimelius' silver positive (Buchan and Polak, 1980). The cells are present in the endocrine islets and scattered in the exocrine tissue. The morphology of the cells reflects the glandular nature of the pancreas. The granules are found surrounding the nucleus (Larsson, Sundler and Hakanson, 1976).

(e) Review of literature on quantitation of islet tissue

In the literature, some investigations on the quantitatin of islet tissue are available. According to Rahier, Wallon and Henquin (1981), an investigation of cell population in human neonates and infants, has shown that the volume density of total endocrine tissue is 15% in neonates, 6-7% in infants, and 2-3% in adults. The relative proportion of all cell types varied only slightly between the different regions, except in the posterior part of the head, shich contained 90% of all PP (pancreatic polypeptide) cells.

Sato and Herman (1981) have shown that the rabbit pancreas contains islets equal to 2.2% of its volume.

Kaung and Elde (1980) have shown by morphometric quantitation of immunohistochemically stained sections of the frog (Rana pipiens) pancreas showed that about 2% of the pancreas is endocrine tissue, of this, 43% are insulin positive cells, 43% are occupied by glucagon-pancreatic polypeptide cells and 14% of total islet volume by somatostatin positive cells.

Malaisse-Lagae, Stefan, Cox, Perrelet and Orci (1979) have shown that systematic sampling of human necropsy pancreases has revealed that pancreatic polypeptide (PP) cells are the most abundant cell type in the posterior part of pancreatic head while they are scarce or absent in the remainder of the gland. A quantitative evaluation of insulin, glucagon, somatostatin and pancreatic polypeptide containing cells in PP-rich and in PP-poor regions of the pancreas was carried out in 8 pancreases from subjects ranging in age from 33 foetal weeks to 80 years. This evaluation confirmed the marked non-homogeneity of distribution of PP cells in different parts of the pancreas. In addition, a difference in the respective proportions of endocrine cells was found between young individuals and adults. The differences affected particularly somatostatin-containing D cells which were more numerous in the young than in adults in all regions of the pancreas.

Rahier, Wallon and Henquin (1980) studied the quantitation of somatostatin cells in the human neonatal pancreas and adult pancreas using an immunoperoxidase technique. Their volume density in the head, isthmus, corpus and tail of the gland has been estimated by morphometry. Somatostatin cells were about 20 times more frequent in the neonate (5% of all pancreatic cells) than in the adult.

Light and E.M. studies on pancreatic islets of the lizard, Lygosoma laterale, have been performed by Rhoten (1971). Quantitation has shown A cells to be approximately 45%, B cells about 40% and D cells 10-15%.

Roth (1968) has analysed the cellular composition of the islets in adult pigeon pancreas. In addition to light and dark islets (B and A islets

respectively), mixed islets have been distinguished. All islets mainly composed of D and A cells but also containing B cells, were assigned to the latter group. Studies of the size-frequency distribution of the light islets revealed that the medium-sized islets constituted the greater part of the total volume of the light islets. In the dark and mixed islets, the D cells predominated in the ventral lobe and the A cells in the splenic lobe. The number of D cells seems to be particularly high in the pigeon.

In assessing the relationship between pancreas weight and body weight, Padour (1950) has put forward a thesis, that in mammals, as body size increases, the relative volume of the pancreas decreases. This is referred to later in this thesis.

Jaffe (1951) found that the pancreas constitutes from .106% to .171% of the body weight in rabbit. Takahashi, Yamasaki and Kamiya (1975) have shown in the dolphin, **Pontaporia blainvillei** that the ratio of pancreatic weight to body weight is in the range of 0.19 to 0.35%.

Investigations on some other cetacea have shown that the ratio of pancreas weight to body weight is 0.16% in **Phocoena phocoena** (Slijper, 1958, cited by Takahashi, Yamasaki and Kamiya, 1975), and .04-.09% in **Platinista** (Kamiya and Yamasaki, 1974).

According to Slijper in 1962 (cited by Takahashi, Yamasaki and Kamiya, 1975), the cetacean pancreas seems to be similar to that of most mammals in respect of relative weight (0.1-0.2% in small, and .03-0.15% in large cetaceans).

Regarding the islet distribution in the head, neck, body and tail regions of the pancreas, Clark (1913) found the greatest concentration of islets in the head of the human pancreas, while Overholser (1925) found the greatest islet concentration in the body of the rat's pancreas. Jaffe (1951) found the greatest incidence in the tail of the rabbit's pancreas.

Various authors studied the total volume or weight of the pancreatic islets in humans (Volk and Wellmann, 1977). Laguesse in 1905 noted that the islets occupy 1% of the total pancreatic mass. De Witt in 1906 observed

that they comprised 2% of the organ. Heiberg in 1909 estimated 3% of islet tissue in the pancreas. Weichselbaum in 1910 calculated that 4.3% of the pancreas is occupied by islet tissue. Gundisch in 1934 estimated that 0.6 to 2.11% of the pancreas and according to Susman in 1942, 0.9 to 3.5% of the pancreas is occupied by the islets.

Regarding islet mass, Ogilvie (1937) in agreement with Seyfarth (1924) and Nakamura (1924) estimated that the weight of the average islet fluctuated over a wide range with a mean varying from 0.478 to 2.738 µg.

(f) Comparative Embryological Aspects

The embryological basis of the vertebrate pancreas consists of three pancreatic anlagen or primordia : a dorsal outpocketing of the gut from the budding liver and two ventral buds (sometimes with very early fusion) from the base of the biliary duct and floor of the gut (Frye, 1962: Pictet and Rutter, 1972; Bonner-Weir and Weir, 1979). The dorsal anlage which gives rise to the tail, body and superior part of the head fuses with the ventral anlagen which gives rise to the lower posterior part of the head including the uncinate process of the pancreas (Rahier, Wallon, Gepts and Haot, 1979). Islet tissue is described as more abundant in the dorsal anlage derived pancreas (Hard, 1944; Pictet and Rutter, 1972; Bonner-Weir and Weir, 1979). Pancreatic polypeptide cells are considerably more numerous in a small lobe located at the postero-inferior part of the head region and it is suggested that this lobe corresponds to the part of the pancreas that is derived from the ventral primordium (Rahier, Wallon, Gepts and Haot, 1979). In the neonate pancreas, the D or somatostatin containing cells are more numerous (Rahier, Wallon and Henquin, 1980).

This pattern of regional heterogenity which has an embryological basis is used as a basis for investigation of a generalised endocrine pancreas.

(1) Embryological origin of tissue

Four possible embryological origins of islet cells have been suggested in recent publications (Epple and Lewis, 1973; Andrew and Kramer, 1979).

(a) An origin from mesodermal precursors (Wessels, 1968). Mesenchymal cells accumulate around the primitive gut and form a 'cap' or 'blanket' over the pancreatic diverticulum. Subsequently, proliferation of the epithelial cells requires mesenchymal tissue in close proximity (Pictet and Rutter, 1972).

(b) a neural crest origin, in common with certain anterior pituitary cells, the thyroid C cells, cells of the carotid body, chromaffin tissue and gastrointestinal endocrine cells (Pearse and Polak, 1971). Pancreatic islet cells are members of the APUD cell series (Amine Precursor Uptake and Decarboxylation) (Pearse 1966, Andrew 1976a). All APUD cells are derived from the neural crest, or at least, the neuroectoderm (Pearse 1969; Pearse and Polak, 1971; Pearse, Polak and Bussolati, 1972). Many authors have accepted this view uncritically, whereas Pearse (1975, 1977) has come to believe evidence to the contrary. It has been shown that APUD cells present in the dorsal pancreatic bud of chick embryos are not derived from trunk neuroectoderm (Andrew 1976b), and Fontaine, Le Lievre and Le Douarin (1977) have shown that APUD islet cells do not arise from vagal levels of the neural crest in chicks. Andrew and Kramer (1979) have presented evidence against the neuroectodermal origin by transplanting the neural tube of Japanese quail embryos to chick embryos and showed no pancreatic APUD cells exhibited the quail nuclear marker. Their most likely origin remains the endoderm.

(c) a common entodermal origin with the majority of cells from a 'vertebrate enterosecretory system' whose descendents include exocrine and endocrine cells in very different locations (Adelson, 1971).

(d) a common entodermal origin with the exocrine pancreas, with which the endocrine cells share a 'protodifferentiated phase' before a regulatory factor causes a differentiation into different endocrine and exocrine cells (Pictet and Rutter, 1972). While Pictet and

Rutter's (1972) view is in accordance with most morphological studies, the hypothesis of Pearse and Polak (1971) and of Adelson (1971) are supported by molecular-genetic and clinical data (Epple and Lewis, 1973).

(g) Comparative aspects in Vertebrates

(1) Phylogenetic Origin of Tissue

Based on the classical proposition that the original islet cells are modified mucosal epithelial cells (Epple and Lewis, 1973; Falkmer, 1972; Barrington, 1964) and the interpretation of islet tissue as modified 'taste cells of the gut' (Fujita 1973), the evolution of the endocrine pancreas involves the following steps:

(1) in the original situation, open receptor-secretors of the intestinal mucosa with apical microvilli release hormones into the lamina propria in response to stimuli perceived by receptor sites on the apical microvilli,

(2) open cells give rise to closed cells which become associated with an intestinal gland and respond to stimuli from blood; this transformation involved migration of receptor sites to the basal or capillary pole of the cell,

(3) closed cells proliferate into the submucosa through the lamina propria,

(4) when the exocrine pancreas leaves the intestine to become an extramural gland, the endocrine pancreas follows (Epple and Brinn, 1980).

(2) Phylogenetic Aspects of Pancreatic Development

The four organs : the duodenum, liver, exocrine and endocrine pancreas are always physically, developmentally and functionally related (Pictet and Rutter, 1972).

It is relevant to compare the evolution of the exocrine and endocrine tissues with their differentiation, since many ontogenetic processes apparently recapitulate phylogenetic processes (Pictet and Rutter, 1972).

In vertebrates, there appears to be a phylogenetic tendency for each of the functional cell types of the hepatopancreatic complex to separate physically into separate tissues or organs (Pictet and Rutter, 1972).

In the most primitive living vertebrates, the Myxinoidea, the exocrine pancreas is partially found within the liver, most of the endocrine tissue forms a separate mass connected to the bile duct, from which it originates (Falkmer and Winbladh, 1964; Pictet and Rutter, 1972).

In Chondrichthyes (Elasmobranchs and Holocephali), the exocrine pancreas is completely separated from the liver, and the endocrine cells are disposed as a monolayered peripheral coat around the pancreatic duct (Ferner and Kern, 1964; Thomas, 1940; Fujita, 1962, 1964a; Pictet and Rutter, 1972).

In Osteichthyes, the situation varies. In most teleosts, the endocrine pancreas forms an organ isolated from the exocrine pancreas (Kent, 1969; Rennie, 1903; Pictet and Rutter, 1972). The crossopterygians present an intermediate stage in the formation of the typical islets found in higher vertebrates. Some endocrine cells form an outer layer around the pancreatic duct. In addition, some of the endocrine cells accumulate between the acini to form clusters that are oriented with the capillaries (Grossner, 1968; Pictet and Rutter, 1972).

In all other higher vertebrates, most of the endocrine cells are found in vascularised clusters that are incorporated in the exocrine gland (Miller, 1960; Thomas 1942; Pictet and Rutter, 1972), that is, classical islets of Langerhans.

(3) Phylogenetic Review in Vertebrates

The morphology of the islet organ varies enormously among vertebrates (Epple and Brinn, 1975, 1980).

The pancreatic islets are unique to the vertebrates. They represent an anatomical specialization of the widely scattered endocrine cells of open or closed type in the mucosa of the gut and bile ducts. In the vertebrate ancestors, the protochordates, insulin, somatostatin, glucagon and PP cells all occur as cells of the open type in the mucosa of the digestive tract (Falkmer, 1985).

Cells producing somatostatin, glucagon and PP are present in the mucosa of the digestive tract in all vertebrates. Insulin cells, however, do not occur in the normal gut of any vertebrate. Nevertheless, a close functional connection exists between the insulin cells and the GI mucosa via the enteroinsular axis, mainly consisting of GIP. GIP cells are a phylogenetically late addition to the neuroendocrine system, essentially occurring only in the GEP organs of vertebrates. Thus, the enteroinsular axis is also a feature unique to the vertebrates (Falkmer, 1985).

CYCLOSTOMES (Agnatha)

Both hagfish and lamprey represents the first step in evolution of a separate islet organ, occurring even before the development of an exocrine pancreas (Falkmer and Ostberg, 1977). The islet tissue of cyclostomes represents an evolutionary link between gut-connected, dispersed insulin producing parenchyma of invertebrates and islets of higher vertebrates (Falkmer and Patent, 1972; Falkmer, Emdin, Hava, Lundgren, Marques, Ostberg, Steiner and Thomas, 1973; Falkmer and Ostberg, 1977).

In the hagfish all of the B cells and most of the D cells have left the gut mucosa and are restricted to the bile duct and adjacent islet organ where they appear as endocrine cells, while the glucagon and gastrin producing cells remain in the gut mucosa (Falkmer and Ostberg, 1977; Bonner-Weir and Weir,

1979). They have the first islet organ in evolution. It is a grossly visible, two hormone organ, producing insulin and somatostatin. There is no exocrine pancreatic parenchyma (Falkmer, 1985). The absence of A cells from islet tissue has been considered a basic difference between cyclostomes and gnathostomes (Epple and Brinn, 1980).

In the sea lamprey, **Petromyzon marinus**, two types of A-like cells (one Grimelius-positive; the other one PTAH-positive) have been demonstrated recently and may change this picture (Epple and Brinn, 1980).

GNATHOSTOMATA

The most primitive islet parenchyma in this main subphylum of the vertebrates viz. those equipped with jaws, occurs in cartilaginous fish (Falkmer and Patent, 1972; Epple and Lewis, 1973; Falkmer and Ostberg, 1977).

PISCES

From the ancestral jawed fish, evolved both the cartilaginous fish (Chondrichthyes) and the bony fish (Osteichythyes) (Bonner-Weir and Weir, 1979).

CARTILAGINOUS FISH

The class **Chondrichthyes** is subdivided into two subclasses, the Elasmobranchs consisting of sharks, skates and rays and Holocephali, the ratfish (Falkmer and Ostberg, 1977).

At the level of holocephalon cartilaginous fish (ratfish), the first exocrine pancreatic gland appears. The endocrine pancreas is usually a three-hormone organ, producing not only insulin and somatostatin, but also glucagon (Falkmer, 1985).

Grossly, the chondrichthyan pancreas is a compact gland of mammalian type, where the endocrine parenchyma is disseminated as islets of Langerhans in the large acinar gland (Falkmer and Ostberg, 1977). Light microscopically, in both Elasmobranchs and Holocephali the most conspicuous feature is the close association of islet cells with the epithelium of the pancreatic ducts (Falkmer and Patent, 1972; Falkmer and Ostberg, 1977).

In the cartilaginous fish, in which the first appearance of a pancreas with all four main islet cell types occurs (B, D, A and PP), there seems to be no regional difference in their distribution (Bonner-Weir and Weir, 1979). It is now a four hormone organ (Falkmer 1985).

In two very distantly related groups of fish, the holocephalians and the genus **Lepisosteus**, a fifth type of islet cell is common (Epple and Brinn, 1980).

BONY FISH

The class Osteichthyes is subdivided into three major subclasses, **Crossopterygii** (fringe fins), **Dipneusti** (lung fishes) and **Actinopterygii** (ray fins).

The islet tissue of the sole extant Crossopterygian fish, **Latimeria chalumnae** (the 'living fossil') occurs mainly as an outer layer of cells around small ducts, although there are islet-like formations and scattered endocrine cells within the exocrine parenchyma (Epple and Brinn, 1975; Bonner-Weir and Weir, 1979). In **Protopterus annectens**, a representative lungfish (Dipneusti), islet tissue is found as nodules of variable diameters of 100um to 1mm next to the pancreatic ducts and two major blood vessels (Epple and Brinn, 1975; Bonner-Weir and Weir, 1979). In the three less prevalent actinopterygian orders, Polypteridae, Chondrostei, Holostei, the islets are rather of the mammalian type, widely scattered throughout the exocrine pancreatic parenchyma (Epple and Brinn, 1975; Falkmer and Ostberg, 1977), with the occurrence of partly intrahepatic pancreatic islets, often closely associated with the bile ducts.

In the common bony fishes (Teleosts), much evolutionary progress has been made in the morphology of the pancreatic islets, particularly in regard to the cytological compositions (Falkmer and Ostberg, 1977). The teleosts are the only class outside the mammals where 'spontaneous diabetes mellitus has been discovered and histopathologically analysed' (Nakamura, Yamada and Yokote, 1971; Falkmer and Ostberg, 1977). The endocrine pancreas of teleosts is characterised by the presence of Brockmann bodies - also called principal

islets - in addition to small islets of Langerhans. These are present in higher teleosts (sculpin, anglerfish, salmon) (Falkmer and Ostberg, 1977; Bonner-Weir and Weir, 1979). The Brockmann bodies are usually constant in location in the mesentery near the spleen or the pylorus while smaller accessory islets are scattered randomly throughout the exocrine tissue (Falkmer, 1961; Planas and Garcia, 1964; Epple and Brinn, 1975; Bonner-Weir and Weir, 1979). Islet tissue in some lower teleosts accumulates near the common bile duct (Epple and Brinn, 1975). In the eel, **Anguilla rostrata**, a large islet is found under the dorsal pancreatic surface near the spleen (Brinn and Epple, 1972).

In highly developed teleosts the giant principal islets, called Brockmann bodies, are the most characteristic features, containing about equal numbers of A, B, D and chromophobic cells (Falkmer and Ostberg, 1977).

In the rainbow trout (**Salmo gairdneri**), there are four cell types, A, B, D and PP (Wagner and McKeown, 1981). Likewise, in the pancreatic islets of the flatfish, **Paralichtys olivaceus**, the Brockmann body consisted of a large principal islet and a smaller islet. B, A and D cells were found in both islets. PP cells were restricted to the periphery of the smaller islet (Yoshida, Iwanagu and Fujita, 1983). In the pancreatic islets of a teleost (**Xiphophorus helleri**), A and PP cell distribution have been demonstrated (Klein and Van Noorden, 1980).

In **Sparus auratus** (Teleosti), the islet cells are concentrated in two or three principal islets, or Brockmann bodies, and numerous smaller islets embedded in the exocrine tissue. Insulin, glucagon, somatostatin and pancreatic polypeptide (PP) - immunoreactive cells were identified using an indirect immunocytochemical (PAP) method. Insulin cells were found in the central region of the islet, glucagon cells at the periphery, and in the exocrine tissue surrounding the large principal islet. Somatostatin - immunoreactive cells were distributed throughout the islets. PP - immunoreactive cells were numerous in the smaller islets and found in the whole peripheral area (Abad, Agulleiro, and Rombout, 1986).

In the sea bass, **Dicentrarchus labrax**, a distinct patterned arrangement in both small islets and Brockmann bodies has been observed, using immunocytochemical techniques. There is a central core of insulin and somatostatin cells surrounded by an outer peripheral layer of glucagon and pancreatic polypeptide cells suggesting definite functional interrelationship by this arrangement (Carillo, Zanuy, Duve and Thorpe, 1986).

AMPHIBIANS

In the three extant subclasses, **viz.** the Urodeles, the Apoda and the Anura, the pancreatic islets are as a rule, small, with a marked predominance of B cells (Falkmer and Ostberg, 1977). For some time, it was even claimed that the urodele islets have no A cells (Falkmer and Marques, 1972). There is close anatomical connection with the bile ducts, in particular, but also with the duodenum and spleen (Penhos and Ramey, 1973). In the splenic portion of the pancreas, the islets are larger and more numerous (Falkmer and Ostberg, 1977). Significant variation of islet cell frequency has been described, even within the same order (Miller, 1960; Hellman and Hellerstrom, 1962). Urodeles are described as having islets almost exclusively of B cells as well as those with a central B cell core and numerous peipheral A and D cells (Epple, 1966; Bonner-Weir and Weir, 1979). In addition to the A, B and D cells, amphiphil cells have been found (Epple, 1966) apparently corresponding to intermediary cells of acinoinsular type (Falkmer and Patent, 1972).

This pattern may be real and may reflect a regional variation of islets (Bonner-Weir and Weir, 1979). Most amphibians have at least three distinct pancreatic lobes : hepatic, duodenal and splenic (Penhos and Ramey, 1973). Regional differences in the pancreas of the toad, **Bufo arenarum**, have been shown (Von Lawzewitsch, 1963). There are five pancreatic processes, and between them is variation of islet frequency, size and distribution of cell type, one region having the greatest islet mass and greatest non-B cell population, a second having the smallest islet mass and an almost exclusive B cell population, and a third region intermediate in both islet mass and cell type frequency. A preliminary immuno-cytochemical study of **Rana pipiens**

confirms this pattern of distribution (Bonner-Weir and Weir, 1979).

Regional differences have been noted in the bullfrog (**Rana catesbeiana**) with the hepatic process showing small islets with abundant B, A and PP cells and few D cells and the duodenal process showing large islets with abundant B, few PP, and scattered A and D cells (Tomita and Pollock, 1981).

REPTILES

All the three main extant subclasses of the reptiles - **Chelonia** (turtles and tortoises), **Squamata** (snakes and lizards) and **Crocodilia** (alligators and crocodiles) have been studied (Falkmer and Ostberg, 1977).

The gross aspects of the compact reptilian pancreas, comprising all the main subclasses have been reviewed by Penhos and Ramey (1973). Particularly interesting from a comparative point of view is the tendency of the islets to be larger but fewer in the splenic portion with the oocasional occurrence of giant islets without exocrine parenchyma in the spleen, forming a structure homologous to a Brockmann body of teleosts in some species (Miller, 1962; Falkmer and Patent, 1972; Therat, Alliet, Comlan and Gourdier, 1975; Falkmer and Ostberg, 1977). This region of the pancreas is ideal for **in vitro** studies (Rhoten, 1974; Epple and Brinn, 1980).

It appears likely that the reptiles generally have at least four different cell types (Rhoten and Smith, 1978; El-Salhy and Grimelius, 1981). There is a marked predominance of A cells (Falkmer and Ostberg, 1977; Bonner-Weir and Weir, 1979), and this has been supposed to be responsible for a rather high fasting blood glucose level and for the observations that some reptiles become hypoglycemic rather than hyperglycemic after pancreatectomy (Khanna and Kumar, 1974). This is a situation that becomes more marked in birds (Falkmer and Patent, 1972). The secretion granules are often as large as zymogen granules (Rhoten, 1971, 1973; Therat, Alliet, Comlan and Gourdier, 1975). Thus, in two species of lizards, the relative frequencies of the three main islet cell types have been given as: A cells, 45-50%, B cells 40%, and D cells 10-15% (Rhoten, 1971, 1973; Falkmer and Ostberg, 1977). In **Varanus niloticus**, the A cells have been found to constitute even more than 70% in the largest islet (a

Brockmann body homologue), with B cells around 10%, whereas D cells, agranular C cells and intermediate acinoinsular cells make up the rest (Theret, Alliet, Comlan and Gourdier, 1975; Falkmer and Ostberg, 1977).

Studies in **Pseudomys scripta elegans** (Chelonia), using immunocytochemical methods have shown insulin, glucagon, somatostatin and pancreatic polypeptide-immunoreactive cells. Each endocrine cell type was distributed differently in the duodenal and splenic regions of the turtle pancreas. Insulin and glucagon-containing cells were seen as single scattered cells which were more numerous in the duodenal region, and the cell groups becoming progressively smaller from splenic to duodenal regions. Round or fusiform somatostatin cells with thick processes and spindly pancreatic polypeptide cells were isolated in the duodenal zone also (Agulleira, Garcia Ayala, and Abad, 1985).

BIRDS

Birds have been of great importance in the study of the comparative endocrinology of gastro-entero-pancreatic hormones, due to some anatomical pancreatic and physiological peculiarities (Falkmer and Patent, 1972; Falkmer and Marques, 1972; Falkmer and Ostberg, 1977).

Grossly, the avian pancreas has three morphologically distinct lobes, the dorsal, ventral and splenic, but the relationship of these lobes to each other and the duct system is highly variable among the various groups of birds (Ziswiler and Farner, 1972; Guha and Ghosh, 1978; Bonner-Weir and Weir, 1979). In some species, for example chicken and quail, an additional lobe called the third lobe has been described (Mikami and Ono, 1962), which is found in an area of fusion between the dorsal and ventral lobes (Oakberg, 1949; Bonner-Weir and Weir, 1979). The splenic lobe is contiguous with the spleen and is small (Smith, 1974; Falkmer and Ostberg, 1977). The islet distribution of the Japanese quail (**Coturnix coturnix**) is very similar to that of the chicken, and in both, the splenic lobe consists of islet parenchyma (Smith, 1974; Falkmer and Ostberg, 1977).

Embryologically, the third and splenic lobes are derived from the dorsal anlage whereas the dorsal and ventral lobes derive from ventral anlage

(Dieterlen-Lievre, 1970; Bonner-Weir and Weir, 1979).

A classical concept is that in birds there is to some extent a segregation of A and B cells into 'dark' and 'light' islets respectively (Falkmer and Patent, 1972; Falkmer and Marques, 1972; Smith, 1974; Watanbe, Paik and Yasuda, 1975; Falkmer and Ostberg, 1977). The 'dark' A islets are large, irregularly shaped and located exclusively to the third (if present) and splenic lobes, whereas the 'light' B islets are small, spherical, and evenly distributed in all lobes (Smith, 1974; Falkmer and Ostberg, 1977). In addition, ordinary islets of Langerhans occur, called islets of 'mixed type' (Watanabe, Paik and Yasuda, 1975; Falkmer and Ostberg, 1977). A islets are composed of A and D cells, B islets contain B and D cells (Oakberg, 1949; Roth, 1968; Bonner-Weir and Weir, 1979). In the large 'dark' A islets, not only A cells and irregularly disseminated D cells occur, but there are also a few B cells (Smith, 1974; Watanbe, Paik and Yasuda, 1975; Falkmer and Ostberg, 1977). Mostly, the D cells are located peripherally (Orci, Baetens, Dubois and Rufener, 1975). In the small light 'B islets' the predominating B cells occupy the central part of the islets, whereas the D cells occur in the periphery together with a few A cells (Smith, 1974; Watanbe, Paik and Yasuda, 1975; Falkmer and Ostberg, 1977). Thus it is suggested that 'mixed' islets should be reclassified as either A or B islets (Bonner-Weir and Weir, 1979).

PP cells have been described in birds as extrainsular singlets or small clumps (Larsson, Sundler, Hakanson, Pollock and Kimmel, 1974). In the chicken, PP cells are scattered as singlets or small groups often adjacent to islets in the dorsal and ventral lobes, but few if any, are seen in either the third or splenic lobes (Bonner-Weir, unpublished). By radioimmunoassay concentrations of glucagon, insulin and somatostatin in the four lobes of the chicken pancreas (Weir, Goltos, Steinberg and Patel, 1976; Bonner-Weir and Weir, 1979) show variations which correspond appropriately with the variations of A, B and D cells distribution (Bonner-Weir and Weir, 1979). Recently it has been shown by immunofluorescence that the avian pancreas, particularly that of young birds, indicates a rich supply of D cells (Weir, Goltos,

Steinberg and Patel, 1976; Falkmer and Ostberg, 1977) and thus, abundant amounts of somatostatin (Orci, Baetens, Dubois and Rufener, 1975; Weir, Goltos Steinberg and Patel, 1976).

MAMMALIA

The living members of the class Mammalia fall into two subclasses, Prototheria (egg laying mammals) and Theria, the latter containing the Metatherian or marsupials and the Eutherian or Placentalia (Falkmer and Patent, 1972).

Reports on the endocrine pancreas in prototherian mammals include a cytological report on echidnas (Griffiths, 1968). The islets exhibit the usual alpha and beta cells, which secrete glucagon and insulin respectively, as well as C or indifferent cells. Many of the islets consist of alpha cells alone, or of indifferent cells alone, but the majority consist of a core of beta cells surrounded by a ring of alpha cells (Griffiths, 1968). One report on the quantitative estimation of islet tissue (Edwin, 1979) will be discussed later in this thesis.

Reports on metatherian mammals include a report on macropodid marsupials (White and Harrop, 1975). The islets were studied in the pancreas of red kangaroo, grey kangaroo, and euro, and compared with those of brush-tailed possum (**Trichosurus vulpecula**) and of various eutherian species. The low proportion of B cells in the islets of kangaroos was accompanied by a high proportion of glucagon producing A cells (White and Harrop, 1975). This is referred to later in this thesis. Another report on the immunolocalization of insulin, glucagon, pancreatic polypeptide and somatostatin in the pancreatic islets of possum (**Trichosurus vulpecula**) using the immunofluorescence procedure has demonstrated localization of the four hormones in cells. This immunofluorescence study demonstrates differences in the topographical distribution of the four major hormones between a marsupial species and several of the eutherian mammals (Reddy, Bibby, Fisher and Elliott, 1986). This is referred to later in the thesis. Reports on quantitative estimations in possum (**Trichosurus vulpecula**) (Edwin, 1982) and grey kangaroo (**Macropus fuliginosus**) (Edwin, 1984) will also be discussed later in this thesis.

Various studies in Eutherian mammals have been done Reddy and Elliott (1985) have examined the cellular distribution of the four hormones in the bovine neonate using the immunofluorescence procedure and have demonstrated the four immunoreactive cells, suggesting that they may be important in intra and extra-islet metabolism in the foetus.

Khatim, Gumaa, Petersson, Lundquist, Grimelius and Hellerstrom (1985) have shown in the one-humped camel (**Camelus dromedarius**), staining of pancreatic sections with haematoxylin-eosin or aldehyde fuchsin, showed numerous islets evenly distributed in all regions of the pancreas.

Reddy and Elliott (1985) have demonstrated insulin, glucagon, PP and somatostatin immunoreactive cells in the islets of the goat pancreas by the immunofluorescence procedure.

Kaung (1985) has shown in rat pancreas using an immunocytochemical method, that glucagon and pancreatic polypeptide co-exist in a population of rat islet cells. In another study, Kaung (1985) has confirmed the above finding in an immunocytochemical study at E.M. level, and also that the granules of these cells were morphologically distinct from glucagon granules but similar to pancreatic polypeptide granules and somatostatin granules.

Reddy, Bibby and Elliott (1985) have done a comparative immunocytochemical study on the cellular distribution of the islets in foetal and adult guinea pigs using immunofluorescence techniques. The topographical distribution of the four cell types was found to be significantly different particularly for cells immunostaining for insulin, glucagon and somatostatin. These observations suggest changes in histogenesis during transition from foetal to postnatal and adult life. The presence of the four islet hormones in the foetal pancreas implies that they may be important in foetal metabolism and growth.

Other reports in Eutheria exist and for a number of these there are reports on the endocrine pancreas (Falkmer and Patent, 1972). They deal with the endocrine pancreas of primates (monkeys, apes, man), carnivores (dogs, cats), lagomorphs (rabbits), rodents (mice, rats, hamsters, guinea pigs),

insectivores (hedgehogs, moles), chiroptera (bats), perissodactyla (horses) and artiodactyla (pigs and cattle) (Ferner and Kern, 1969; Hellman and Hellerstrom 1969; Falkmer and Patent, 1972).

These reports are discussed more fully later in this thesis but the general conclusion from these studies is that in the mammalian islets of Langerhans, B cells are by far the most abundant with A and D cells following in that order (White and Harrop, 1975). PP cells are numerous in the uncinate process of the dog pancreas (Baetens, Rufener and Orci, 1976). The fact that the PP cells are numerous in the uncinate process of the dog pancreas induced suggestions (Baetens, Rufener and Orci, 1976) that these cells correspond to F or X cells described by Bencosme and Liepa (1955), Munger, Caramia and Lacy (1965) and Lazarus and Shapiro (1971).

The interrelationship between the exocrine and endocrine pancreas is of great interest in the comparative morphology of the pancreatic islet in animals (Falkmer and Ostberg, 1977). In early embryonic life of most animals and in adult specimens of lower order vertebrates (the Cyclostomata), there is no association between exocrine and endocrine pancreatic parenchyma. As soon as D and A cells first appear in the vertebrate series and in foetal life, there is an intimate topographic relationship between islet cells and acinar tissue (Falkmer and Patent, 1972). It has recently been found that under particular circumstances acino-insular transformation with mixed exocrine and endocrine cells can occur (Orci, Rufener, Pictet, Renold and Rouiller, 1970). Such an acino-insular transformation is said to give rise to D and A cells rather than B cells (Faller, cited by Falkmer and Patent, 1972) because in tetrapod pancreatic islets the afferent blood vessels first reach the A and D cells and then pass to the B cells (Fujita, 1973). The intimate structural relationship between exocrine and endocrine pancreatic parenchyma essentially promotes the mutual regulation between glucagon, gastrin, secretin and pancreozymin (Henderson, 1969).

A few recent reports on mammalian islet morphology was the so called 'X' cell in the central region of the islets of the horse. These 'X' cells are

ordinary glucagon-producing A cells, and that in the periphery of the horse islets not only B and D cells occur, but also occasional G cells and a cell type (called 'S' cells) with small granules (Forssmann, 1976, Falkmer and Ostberg, 1977).

The peculiar topographical distribution of the different parenchymal cells in the horse islets has been investigated in studies in horse pancreas (Fujita, 1973). It was found that the afferent artery to the horse islets enters in the middle of the islet instead of from the periphery as in most mammalian islets (Fujita, 1973). Moreover, it was found that the exocrine acinar pancreatic parenchyma receives its blood supply by the efferent vessels from the islets via an insuloacinar portal system. This anatomical arrangement facilitates a direct hormonal action, (Epple and Lewis, 1973), not only by islet hormones on the exocrine pancreatic parenchyma, but also as a direct influence of the A cells on the D cells and the A and D cells on the B cells (Fujita, 1973). As a matter of fact, it seems to be a rule that in the tetrapod pancreatic islets - and in Brockmann bodies of higher teleosts as well - the afferent blood vessels first reach the A and D cells and then pass to the B cells, thus allowing modifying effects of glucagon and somatostatin on insulin synthesis (Fujita, 1973).

Comparative studies on the topographic distribution and the quantitative relationships between the four main kinds of islet parenchymal cells in mammals have introduced new concepts (Hellman and Hellerstrom, 1969; Hellman, 1970; Falkmer and Patent, 1972). In studies of this kind mammals living under extreme climatic conditions have been included, such as arctic and desert rodents (Quay, 1960). It is likely that metabolic modifications or specializations associated with climatic adaptations are related to modifications in the endocrine glands (Quay, 1960; Falkmer and Patent, 1972). There seems to be a general correlation between central position of cells in the islets and life in an arid environment (Quay, 1960).

During the last 10 years, an important field in which investigations in mammals have contributed to a breakthrough in the comparative endocrinology of

the islet hormones, is the brain-gut axis (Falkmer, 1985). The observation that insulin, somatostatin, glucagon and PP cells seem to belong to those GEP neurohormonal cells that have a dual distribution in the CNS and the GEP organs was made in mammals and has implied several new aspects of the origin and evolution of the whole neuroendocrine system (Falkmer, 1985).

As can be seen from the preceding phylogenetic review, much of the apparent islet variations between species may be the result of sampling different potions of regionally heterogenous pancreases. The embryological pattern of the vertebrate pancreas is compatible with a regional heterogeneity (Bonner-Weir and Weir, 1979). The parts derived from the dorsal anlage, namely the tail, body, superior part of head, are more abundant in islet tissue in fish (Epple, 1969), amphibia (Frye, 1962), birds (Dieterlen Lievre, 1970) and mammals (Hard, 1944; Pictet and Rutter, 1972).

One of the most important advances in the morphology of the pancreatic islets in mammalian animals made during the last decade is that in conventional laboratory rodents there is a PP-rich/glucagon-poor lobe in the posterior part of the head of the pancreas (also seen in man), corresponding to the ventral anlage of the pancreas (Orci, 1982; Falkmer, 1985). The observation that the four types of islet hormone-producing cells are not evenly distributed has a firm phylogenetic basis. The combination of PP-rich/glucagon-poor areas of islet parenchyma near the gut and PP=poor/glucagon-rich pancreatic islets in the parts close to the spleen appears already in some bony fish and is well established in reptiles, birds and mammals (Falkmer, 1985). This fact is of particular importance when quantitative assessments, as in this investigation are performed. Adequate knowledge of the site of origin of the specimen investigated is fundamental for the results of the analysis (Falkmer, 1985). One report on PP cells in Australian mammals is referred to later (Edwin, 1987).

The pattern of regional heterogenity which has a phylogenetic origin is used as a basis for a hypothesis of a generalised endocrine pancreas (Bonner-Weir and Weir, 1979). This hypothesis does not deny species

differences but rather is presented to unify the concept of the vertebrate endocrine pancreas. Such a hypothesis has several elements to it:

1. The splenic portion has a larger concentration of islet tissue than most other parts of the pancreas
2. The islets in the splenic portion are larger and contain A, B and D cells but only few or no PP cells
3. The duodenal portion has smaller islets, that are principally B cells with numerous D cells and occasional A cells. PP cells are found in greater number than elsewhere in this region.

This hypothesis is important, because it unifies and simplifies, the phylogenetic development of the endocrine pancreas as well as rationalizing the regional heterogenity. The question of whether mammals fit this hypothesis has important implications for the understanding of the mammalian pancreas (Bonner-Weir and Weir, 1979). Mammals can be considered in regard to each element of the hypothesis. Firstly, a large portion of islets are in the splenic portion in some mammals (Hard, 1944; Pictet and Rutter, 1972; Volk and Wellmann, 1977), but one report found no marked variation of islet frequency in humans (Hellman, 1959). Secondly, the question of regional variation of islet size is unresolved. Regional differences of size are reported in several species (Bencosme and Liepa, 1955) but denied in adult humans (Hellman and Hellerstrom, 1969). Thirdly, some mammals do have regional differences in the distribution of islet cell types. Two types of islets, one with A, B and D cells and found in the tail or splenic portion and another with B, D and PP cells and found in the head or duodenal portion have been shown in many mammalian species (Larsson, Sundler and Hakanson, 1975; Orci, Baetens, Ravazzola, Stefan, and Malaisse-Lagae, 1976; Forssmann, Helmstaedter and Chance, 1977; Bonner-Weir and Like, 1980). A regionally distinct third type has been identified in the calf (Bonner-Weir and Like, 1980), in which all four cell types are present.

AIMS OF PRESENT STUDY

It is suggestive that mammals do fit the hypothesis of a generalised

endocrine pancreas and this study has been undertaken to investigate the first and third elements of this hypothesis (namely, predominance of islet tissue in the splenic portion and also regional differences in the distribution of islet cell types) in a range of Australian mammals.

Another of the aims of the present investigation is to study the relationship between the weight of the pancreas and body weight, and to quantify the islet tissue mass and also the relationship between average islet tissue mass and body weight in a range of Australian mammals.

This hypothesis (Bonner-Weir and Weir, 1979) based on regional heterogenity of islet composition and distribution encompasses the vertebrate classes of bony fish, amphibians, reptiles and birds. Mammals seem to fit this hypothesis, and this investigation has been undertaken in Australian mammals.

MATERIALS AND METHODS

Six animals, three male and three female, of each of the following species were used and the following procedure employed.

Prototheria

Echidna (**Tachyglossus aculeatus**) - weighed and then anaesthetised with 3ml intraperitoneal nembutal.

Metatheria

Possum (**Trichosurus vulpecula**) - weighed and then anaesthetised with 3ml intraperitoneal nembutal.

Grey kangaroo (**Macropus fuliginosus**) - shot and then weighed.

Eutheria

Hopping mouse (**Notomys alexis**) - anaesthetised with 0.5ml intraperitoneal nembutal and weighed.

Water rat (**Hydromys chrysogaster**) - anaesthetised with 1.5ml intraperitoneal nembutal and weighed.

In each animal, the pancreas was then isolated, removed and weighed. The relationship between the weight of the pancreas and the weight of the animal was calculated.

Fig. 1. Showing scanning lines, 12 in number, 16.6cm long and 1cm apart within the projected field (two thirds original size)

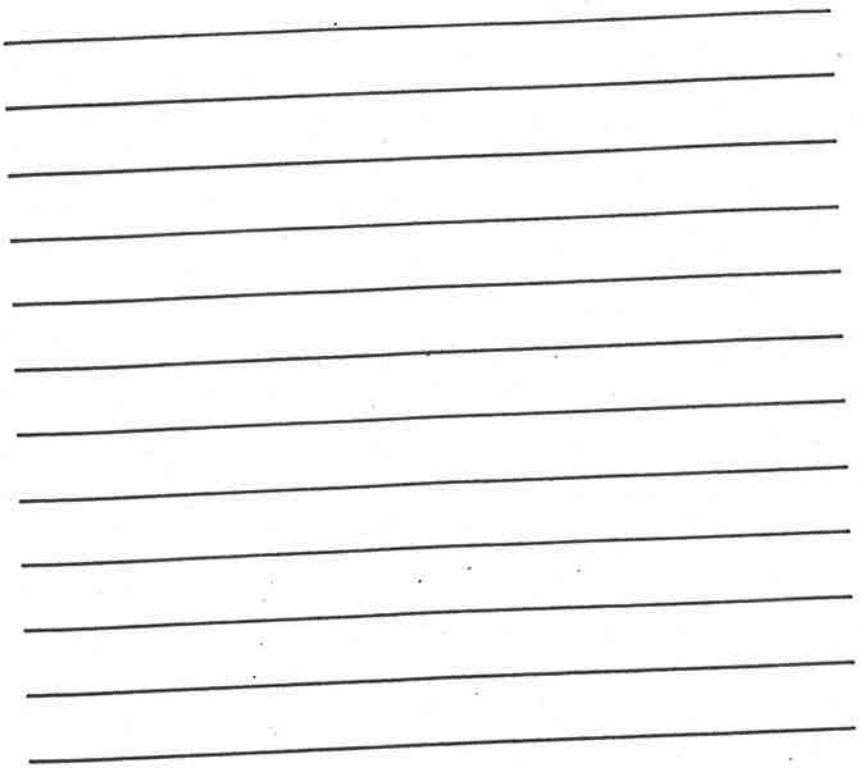
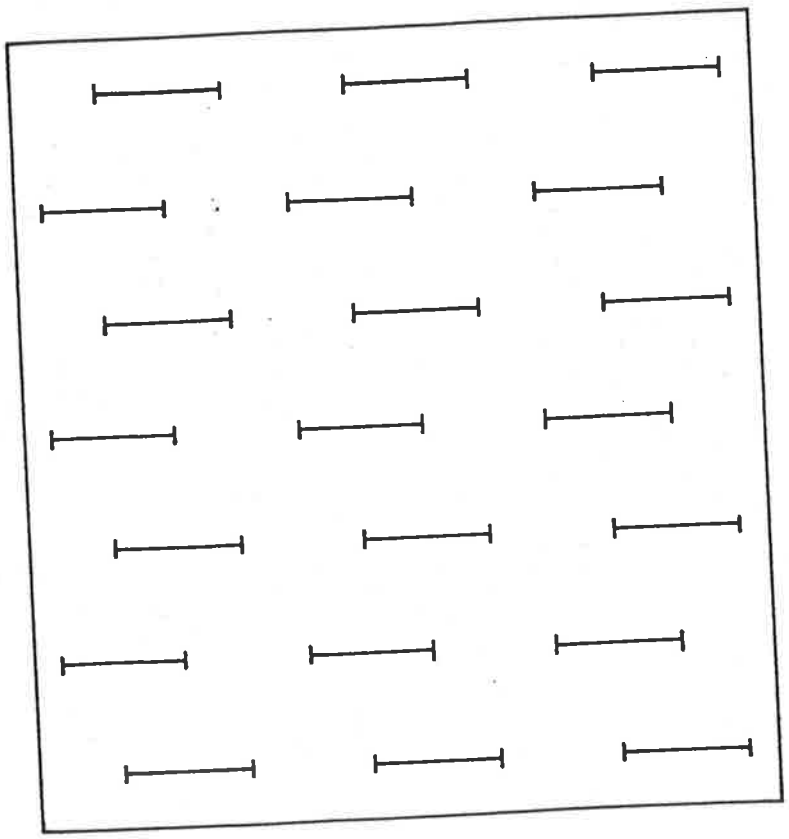


Fig. 2. Weibel graticule showing intercepts (two thirds original size).



In the echidna, the pancreas was well defined. In the possum, it was diffuse, situated on the posterior abdominal wall. In all the other species, the pancreas was diffuse, situated in the mesentery extending onto the dorsal abdominal wall. In these species, difficulty was experienced in identifying the head, neck, body and tail regions. The head region was adjacent to the intestine and the tail region towards the splenic region. The neck and body were succeeding regions to the head. The neck was the constricted region adjoining the head and the body, the part that extended transversely across the posterior abdominal wall. Samples of selected regions, head, neck, body and tail, were fixed in Bouin's solution. The tissues were paraffin embedded and sectioned at 5um.

Some sections were stained with haematoxylin and eosin. Each section was projected using a X40 objective, onto paper with 12 lines 16.6cm long and 1cm apart within the projected field, and using a scanning method (Carpenter and Lazarow, 1962) a linear measurement of islet tissue along these lines was obtained (Fig. 1). Five hundred different fields were covered for each of the four regions in each animal (so that a minimum distance of scanning necessary for 5% accuracy was achieved in each case) and expressed in a ratio with the distance actually scanned, by a formula of Carpenter and Lazarow (1962).

$$\frac{\text{Total distance scanned}}{\text{Required distance of scanning for desired accuracy}} = \frac{(\%\ \text{Desired accuracy})^2}{(\%\ \text{Observed accuracy})^2}$$

where the observed accuracy $= \frac{\text{standard error}}{\text{volume component}} \times 100$

and the desired accuracy $= 5\%$

Linear component fractions were taken to represent area fractions and these, in turn, volume fractions (Loud, 1962; Weibel, 1979).

The relationship between percentage islet tissue and the weight of the pancreas was examined. In calculating the percentage islet tissue, it is assumed that the regions, head, neck body and tail are of equal size, as seen by naked eye appearance.

The relationship between islet tissue mass and sex was examined.

The relationship between average islet tissue mass and the body weight was examined.

Paraffin sections of 5um thickness were stained with aldehyde fuchsin (Gomori, 1950) for the assessment of B cells in all the species.

Using a Wild-M 501 semiautomatic sampling microscope, and a Weibel graticule (Fig. 2) (Weibel, Kistler and Scherle, 1966) counts were made of the intercepts over islet tissue and also intercepts over B cells. The number of intercepts over B cells in relation to 500 intercepts of islet tissue was thus determined, and assuming that the point fraction is equal to the volume fraction (Weibel, Kistler and Scherle, 1966), the percentage volume of B cells in islet tissue was calculated. The procedure was systematically performed through the different regions, head, neck, body and tail, and through the series of six animals of each species.

For the assessment of alpha cells, the same procedure was employed using sections stained by the following:

Grimelius' silver nitrate stain (Grimelius, 1968) in echidna and possum.

Phosphotungstic acid haematoxylin in grey kangaroo and water rat.

PAP immunoperoxidase technique for glucagon, using Immulok histoset reagents in hopping mouse. The Immulok method was modified with final incubation at 37°C for 40 minutes.

For the assessment of D cells, the same procedure was employed using sections stained by the following:

Epple's modification (1967) of the modified Davenport technique (Hellerstrom and Hellman, 1960) in grey kangaroo.

PAP immunoperoxidase technique for somatostatin using Immulok histoset reagents in echidna, possum, hopping mouse and water rat. The Immulok method was modified with final incubation at 37°C for 40 minutes.

For the assessment of PP cells, the same procedure was applied using sections stained by the PAP immunoperoxidase technique for pancreatic polypeptide using Immulok histoset reagents in all species investigated. The Immulok method was modified with final incubation at 37°C for 40 minutes.

For the staining of the A and D cells, different methods of staining have been used in different species because the established specific cell stains were found to be unsuccessful in some species. This may be due to species specificity. Silver stains are well known for their capriciousness in staining. The PAP immunoperoxidase technique (immuno-cytochemical method) was a reliable method but not used entirely as it is an expensive method.

Staining methods were compared. The PAP immunoperoxidase technique for insulin has been compared with aldehyde fuchsin. The immunocytochemical method is reliable and specific, but expensive method, and is here used in a different situation to other immunocytochemical stains in this study and is used to check the specificity of aldehyde fuchsin which has been used for estimating B cells in all the species studied. Using the head, neck, body and tail regions of one water rat, sections were stained with PAP immunoperoxidase technique for insulin and positioned on the stage of a microscope using a vernier scale, and photographs taken of several islets. The B cells were then estimated using a Weibel graticule. The sections were decolourised using 70% alcohol and restained with aldehyde fuchsin and scanned areas of the same section, the islets repositioned on the microscope stage and the same islets photographed. The B cells in the same islets were estimated using a Weibel graticule. The above two staining methods were compared, and this is a double approach. The photographs show whether the same cells are staining in a small sample. The figures obtained show whether the two methods are giving comparable figures with a large sample.

A similar procedure was used on sections of one grey kangaroo to compare the PAP immunoperoxidase technique for glucagon with phosphotungstic acid haematoxylin. The comparison between the two stains is more informative as different stains have been used in the series.

A similar procedure was used on sections of one possum to compare the PAP immunoperoxidase technique for glucagon with Grimelius' silver nitrate technique (Grimelius, 1968). The two staining methods were done to see if the results were comparable.

A similar procedure was used on sections of one grey kangaroo to compare the PAP immunoperoxidase technique for somatostatin with Epple's modification (1967) of the modified Davenport technique (Hellerstrom and Hellman, 1960). The two staining methods were done to see if the results were comparable.

RESULTS

TABLE I Relationship between the weight of the pancreas (P) and body weight (W)

PROTOTHERIA

Echidna (Tachyglossus aculeatus)

The pancreas is a well defined organ presenting a head, neck, body and tail. The head region is adjacent to the duodenum and the tail projects to the spleen.

The relationship between the weight of the pancreas and body weight is tabled, and the statistical calculation was done to seek correlation in all the species investigated.

	Body Weight (in gm) W	Pancreas wt. (in gm) P	P/W	Mean	S.E.
E1	2590	7.32	0.0028	0.002283	0.000199
E2	1810	4.32	0.0023		
E3	3265	6.2	0.0018		
E4	3695	9.4	0.0025		
E5	2450	4.11	0.0016		
E6	2595	7.19	0.0027		

Simple linear regression.

$a = 0.62 \qquad b = 2.342$

$SE(b) = 425.6616 \qquad t = \frac{b}{SE} = .0055 \qquad d.f = 4$

Conclusion t = .0055, accepts the hypothesis that b is o and no linear association exists between the weight of the pancreas (P) and body weight (W) in echidna (Tachyglossus aculeatus).

METATHERIA

Possum (Trichosurus vulpecula)
The pancreas is a diffuse gland and extends onto the posterior abdominal wall.

TABLE I(b)

	Body Weight (in gm) W	Pancreas wt. (in gm) P	P/W	Mean	S.E.
P1	1575	1.08	0.00068	0.0011	0.0018
P2	1350	2.00	0.0014		
P3	1535	1.70	0.0011		
P4	1630	2.29	0.0014		
P5	1695	2.00	0.0011		
P6	1415	1.10	0.0077		

Simple linear regression.

a = 1405.84 b = 75.22

SE (b) = 904.6214 $t = \frac{b}{SE} = .0831$, d.f = 4

Conclusion t = .0831, accepts the hypothesis that b is o and no linear association exists between the weight of the pancreas (P) and body weight (W) in possum (Trichosurus vulpecula).

Grey Kangaroo (Macropus fuliginosus)
The pancrease is very diffuse. It is situated in the mesentery and extends onto the dorsal abdominal wall. The head region is adjacent to the intestineand the tail extends to the spleen.

TABLE I(c)

	Body Weight (in gm) W	Pancreas wt. (in gm) P	P/W	Mean	S.E.
K1	80000	47.8	0.0005975	0.00072	0.000092
K2	20800	24	0.001153		
K3	21000	12	0.000571		
K4	16000	12	0.00075		
K5	29500	16	0.000542		
K6	26000	18	0.000692		

Simple linear regression.

a = -3621.68 b = 1656.63

SE (b) = 107.92608 $t = \frac{b}{SE} = 2.4$ d.f = 4

Conclusion t = 2.4 accepts the hypothesis that b is o and no association exists between the weight of the pancreas (P) and body weight (W) in grey kangaroo (Macropus fuliginosus).

EUTHERIA

Hopping Mouse (Notomys alexis)

The pancreas is very diffuse. It is situated in the mesentery and extends onto the dorsal abdominal wall. The head region is adjacent to the duodenum, and the succeeding regions, the neck and body. The tail region extends to the spleen.

TABLE I(d)

	Body wt. (in gm) W	Pancreas wt. (in gm) P	P/W	Mean	S.E.
M1	29.3	0.137	0.004675	0.004374	0.000501
M2	24.1	0.080	0.003319		
M3	30.45	0.123	0.004039		
M4	31.10	0.125	0.004019		
M5	23.94	0.084	0.003508		
M6	29.9	0.20	0.006688		

Simple linear regression.

a = 21.61 b = 52.25

SE (b) = 225.353805 $t = \frac{b}{SE} = .2318$ d.f = 4

Conclusion t = .2318 accepts the hypothesis that b is o and no linear association exists between the weight of the pancreas (P) and body weight (W) in hopping mouse (Notomys alexis).

Water rat (Hydromys chrysogaster)
The pancreas is diffuse and situated in the mesentery and extends onto the dorsal abdominal wall. The head region is adjacent to the duodenum and the tail extends to the splee .

TABLE I(e)

	Body Weight (in gm) W	Pancreas wt. P	P/W	Mean	S.E.
R1	702	3.502	0.004988	0.004363	0.000180
R2	499.5	2.271	0.004546		
R3	638	2.648	0.004150		
R4	833	3.164	0.003798		
R5	659	3.082	0.004676		
R6	744	3.468	0.004661		

Simple linear regression.

a = 617.022 b = 24.669

SE (b) = 224.731162 $t = \frac{b}{SE} = .109771$, d.f = 4

Conclusion t = .109771 accepts the hypothesis that b is o and no linear association exists between the weight of the pancreas (P) and body weight (W) in in the water rat (Hydromys chrysogaster).

TABLE II Showing mean and standard error of islet tissue mass based on 500 observations (each observation 199.2cms)

PROTOTHERIA

TABLE IIa Echidna (Tachyglossus aculeatus)

Animal	Head cm	Head SE	Neck cm	Neck SE	Body cm	Body SE	Tail cm	Tail SE	Average	Average %
E1	.4036	.0120	.5583	.0170	.3618	.0160	.2226	.0090	.3865	.1940
E2	.4672	.0209	.1514	.0060	.5264	.0235	.1490	.0066	.3235	.1623
E3	.3386	.0140	.1688	.0075	.3464	.0155	.2228	.0099	.2691	.1350
E4	.2294	.0099	.7152	.0315	.1936	.0086	.1758	.0078	.3285	.1649
E5	.1120	.0050	.1966	.0088	.1548	.0069	.5976	.0260	.2652	.1331
E6	.1918	.0084	.2018	.0090	.2552	.0114	.2446	.0109	.2233	.1120
	.2904		.3320		.3063		.2687		.2993	.1502

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.0133	3	.0044	.1375 F3, 15 (5%) = 5.41(NS)
Animals	.0625	5	.0125	.3767 F5, 15 (5%) = 2.90
Error	.4978	15	.0331	
Total	.5738	23		

Conclusion. There is no significant difference in the amount of islet tisue in different regions.

METATHERIA

TABLE II(b) Possum (Trichosurus vulpecula)

Animal	Head cm	Head SE	Neck cm	Neck SE	Body cm	Body SE	Tail cm	Tail SE	Average	Average %
P1	.1116	.0049	.1080	.0048	.1088	.0048	.1148	.0051	.1108	.0556
P2	.1264	.0056	.1240	.0055	.1164	.0052	.1134	.0050	.1200	.0602
P3	.1134	.0051	.1128	.0050	.1122	.0050	.1138	.0050	.1130	.0567
P4	.1224	.0054	.1116	.0049	.1244	.0055	.1134	.0050	.1179	.0591
P5	.1048	.0046	.1216	.0054	.1182	.0052	.1154	.0051	.1150	.0577
P6	.1110	.0049	.1126	.0050	.1214	.0054	.1092	.0048	.1135	.0569
	.1149		.1151		.1169		.1133		.1150	.0578

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.000038	3	.000012	.365
Animals	.000183	5	.000036	1.038
Error	.000529	15	.000035	
Total	.000751	23		

F3, 15 (5%) = 5.41(NS)

F5, 15 (5%) = 2.90

Conclusion. There is no significant difference in the amount of islet tissue in different regions.

TABLE II(c) Grey Kangaroo (Macropus fuliginosus)

Animal	Head cm	Head SE	Neck cm	Neck SE	Body cm	Body SE	Tail cm	Tail SE	Average	Average %
K1	.3096	.0138	.2858	.0127	.3120	.0139	.2932	.0131	.3001	.1506
K2	.3056	.0136	.2838	.0127	.2974	.0133	.2794	.0125	.2915	.1463
K3	.2704	.0121	.2744	.0121	.2694	.0120	.2742	.0122	.2721	.1365
K4	.2878	.0128	.2840	.0127	.2582	.0115	.2840	.0127	.2785	.1398
K5	.2798	.0125	.2804	.0125	.2822	.0126	.3048	.0136	.2868	.1439
K6	.2598	.0116	.2768	.0123	.2908	.0130	.3046	.0136	.2830	.1420
	.2855		.2808		.2850		.2900		.2853	.1431

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.160917	3	.053639	5.0777 F3, 15 (5%) = 5.41(NS)
Animals	.001950	5	.000390	.036922 F5, 15 (5%) = 2.90
Error	.158467	15	.010564	
Total	.004399	23		

Conclusion. There is no significant difference in the amount of islet tissue in different regions.

EUTHERIA

TABLE II(d) Hopping Mouse (Notomys alexis)

Animal	Head cm	Head SE	Neck cm	Neck SE	Body cm	Body SE	Tail cm	Tail SE	Average	Average %
M1	.4084	.0182	.4604	.0206	.3482	.0155	.5770	.0258	.4485	.2251
M2	.4200	.0188	.4326	.0193	.4016	.0179	.5272	.0236	.4453	.2234
M3	.4212	.0188	.4112	.0184	.3990	.0178	.5408	.0242	.4430	.2195
M4	.3800	.0170	.4164	.0186	.4098	.0183	.5586	.0250	.4412	.2214
M5	.4126	.0184	.4064	.0181	.4344	.0194	.5216	.0233	.4437	.2227
M6	.4164	.0186	.4294	.0192	.4420	.0197	.5560	.0248	.4609	.2313
	.4097		.4260		.4058		.5468		.4471	.2239

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.080959	3	.026986	7.1897
				F3, 15 (5%) = 5.41(Sig)
Animals	.001030	5	.000206	.5490
				F5, 15 (5%) = 2.90
Error	.056301	15	.003753	
Total	.025688	23		

Conclusion. The tail region shows a greater proportion of islet tissue.

EUTHERIA

TABLE II(e) Water rat (Hydromys chrysogaster)

Animal	Head cm	Head SE	Neck cm	Neck SE	Body cm	Body SE	Tail cm	Tail SE	Average	Average %
R1	.5072	.0220	.5578	.0240	.5792	.0259	.7524	.0336	.5991	.3007
R2	.5616	.0251	.5464	.0244	.5788	.0259	.7362	.0329	.6057	.3040
R3	.5592	.0250	.5594	.0250	.5738	.0256	.7228	.0323	.6038	.3030
R4	.5952	.0266	.5292	.0236	.5804	.0259	.7434	.0332	.6120	.3071
R5	.5802	.0259	.5814	.0260	.5824	.0260	.7212	.0322	.6163	.3093
R6	.5622	.0251	.5402	.0241	.5042	.0225	.7012	.0313	.5769	.2896
	.5609		.5524		.5664		.7295		.6023	.3022

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.130041	3	.043347	75.4 F3, 15 (5%) = 5.41(Sig)
Animals	.003831	5	.000766	1.3 F5, 15 (5%) = 2.90
Error	.008619	15	.000574	
Total	.142491	23		

Conclusion. The tail region shows a greater proportion of islet tissue.

TABLE III Relationship between percentage islet tissue (I) and pancreas weight (P)

PROTOTHERIA

TABLE III(a). Echidna (Tachyglossus aculeatus)

Animal	Pancreas (in gm) P	% Islet tissue I	Wt. of islet tissue IXP (mgm)	Mean	S.E.
E1	7.32	0.1940	14.20	0.976	0.166929
E2	4.32	0.1623	7.01		
E3	6.2	0.1345	8.33		
E4	9.4	0.1649	15.50		
E5	4.11	0.1331	5.47		
E6	7.19	0.1120	8.05		

Simple linear regression.

$a = 3.92 \qquad b = 16.68$

$SE\ (b) = 42.7841 \quad t = \frac{b}{SE} = .3898 \quad d.f = 4$

Conclusion. There is no significant regression of P (weight of pancreas) on I (% islet tissue). There is no clear relationship between percentage islet tissue and size of pancreas.

METATHERIA

TABLE III(b). Possum (Trichosurus vulpecula)

Animal	Pancreas (in gm) P	% Islet tissue I	Wt. of islet tissue IXP (mgm)	Mean	S.E.
P1	1.08	0.0556	0.60048	0.983	.012787
P2	2.00	0.0602	1.204		
P3	1.70	0.0567	0.963		
P4	2.29	0.0591	1.353		
P5	2.00	0.0577	1.154		
P6	1.10	0.0568	0.624		

Simple linear regression.

a = -11.97 b = 236.86

SE (b) = 29.38 $t = \frac{b}{SE} = 8.06$ d.f = 4

Conclusion. There is a significant regression of P (weight of pancreas) on I (% islet tissue). There is a direct relationship between percentage islet tissue and size of pancreas.

TABLE III(c). Grey Kangaroo (Macropus fuliginosus)

Animal	Pancreas (in gm) P	% Islet tissue I	Wt. of islet tissue IXP (mgm)	Mean	S.E.
K1	47.8	.1506	71.98	32.55	0.8490
K2	24	.1463	35.11		
K3	12	.1365	16.38		
K4	12	.1397	16.76		
K5	16	.1441	23.05		
K6	18	.1780	32.04		

Simple linear regression.

a = -.76 b = 150.10

SE (b) = 144.9953 $t = \frac{b}{SE} = 1.0352$ d.f = 4

Conclusion. There is no significant regression of P (weight of pancreas) on I (% islet tissue). There is no clear relationship between percentage islet tissue and size of the pancreas.

EUTHERIA

TABLE III(d). Hopping mouse (Notomys alexis)

Animal	Pancreas (in gm) P	% Islet tissue	Wt. of islet tissue IXP (mgm)	Mean	S.E.
M1	.137	.2251	0.308	0.280	0.04207
M2	.080	.2234	0.178		
M3	.123	.2195	0.269		
M4	.125	.2214	0.276		
M5	.084	.2227	0.187		
M6	.200	.2313	0.462		

Simple linear regression.

a = -1.62 b = 7.78

SE (b) = .55754 $t = \frac{b}{SE} = 13.95$ d.f = 4

Conclusion. There is a significant regression of P (weight of pancreas) on I (% islet tissue). There is a direct relationship between percentage islet tissue and size of pancreas.

TABLE III(e). Water rat (Hydromys chrysogaster)

Animal	Pancreas (in gm) P	% Islet tissue I	Wt. of islet tissue IXP (mgm)	Mean	S.E.
R1	3.502	.3007	10.530	9.124	0.562
R2	2.271	.3040	6.903		
R3	2.648	.3030	8.023		
R4	3.164	.3071	9.716		
R5	3.082	.3093	9.532		
R6	3.468	.2896	10.043		

Simple linear regression.

$a = 11.70 \qquad b = -28.71$

$SE\ (b) = 9.998 \qquad t = \frac{b}{SE} = 2.871 \qquad d.f = 4$

Conclusion. There is a significant regression at 5% level of P (weight of pancreas) on I (% islet tissue). There is an inverse relationship between percentage islet tissue and size of pancreas.

TABLE IV Relationship between islet tissue mass (T) and body weight (W)

PROTOTHERIA

TABLE IV(a) Echidna (Tachyglossus aculeatus)

	Body Weight (in gm) W	Wt of islet tissue T (mgm)	T/W mgm/kg	Mean	S.E.
E1	2590	14.20	5.48	3.57	0.489
E2	1810	7.01	3.87		
E3	3265	8.33	2.55		
E4	3695	15.50	4.19		
E5	2450	5.47	2.23		
E6	2595	8.05	3.10		

Simple linear regression.

a = 17867.92 b = 970.67

SE (b) = 2801.4002 $t = \frac{b}{SE} = .3464$ d.f = 4

Conclusion t = .3464, accepts the hypothesis that b is o and no linear association exists between islet tissue mass (T) and body weight (W) in echidna (Tachyglossus aculeatus).

METATHERIA

Table IV(b) Possum (Trichosurus vulpecula)

	Body Weight (in gm) W	Wt of islet Tissue T (mgm)	T/W mgm/kg	Mean	S.E.
P1	1575	0.60048	0.3812	0.642016	0.083241
P2	1350	1.204	0.8918		
P3	1535	0.963	0.6273		
P4	1630	1.353	0.8300		
P5	1695	1.154	0.6808		
P6	1415	0.624	0.4409		

Simple linear regression.

a = 14343.31 b = 1007.06

SE (b) = 15597.23 $t = \frac{b}{SE} = .0645$, d.f = 4

Conclusion t = .0645, accepts the hypothesis that b is o and no linear association exists between islet tissue mass (T) and body weight (W) in possum (Trichosurus vulpecula).

TABLE IV(c) Grey Kangaroo (Macropus fuliginosus)

	Body Weight (in gm) W	Wt of islet Tissue T (mgm)	T/W mgm/kg	Mean	S.E.
K1	80000	71.98	0.899	1.06	0.177
K2	20800	35.11	1.687		
K3	21000	16.38	0.78		
K4	16000	16.76	1.04		
K5	29500	23.05	0.7813		
K6	26000	32.04	1.23		

Simple linear regression.

a = -27256.09 b = 10733.86

SE (b) = 9896.5799 $t = \frac{b}{SE} = 1.08$ d.f = 4

Conclusion t = 1.08, accepts the hypothesis that b is o and no linear association exists between islet tissue mass (T) and body weight (W) in grey kangaroo (Macropus fuliginosus).

TABLE IV(d) Hopping mouse (Notomys alexis)

	Body Weight (in gm) W	Wt of islet Tissue T (mgm)	T/W mgm/kg	Mean	S.E.
M1	29.3	0.308	10.5	9.7	1.22
M2	24.1	0.178	7.3		
M3	30.45	0.269	8.8		
M4	31.1	0.276	8.8		
M5	23.94	0.187	7.8		
M6	29.9	0.462	15.4		

Simple linear regression.

a = 222.35 b = 210.6

SE (b) = 830.47 $t = \frac{b}{SE} = .2535$, d.f = 4

Conclusion t = .2535, accepts the hypothesis that b is o and no linear association exists between islet tissue mass (T) and body weight (W) in hopping mouse (Notomys alexis).

TABLE IV(e) Water rat (Hydromys chrysogaster)

	Body Weight (in gm) W	Wt of islet Tissue T (mgm)	T/W mgm/kg	Mean	S.E.
R1	702	10.530	15.0	13.45	.5364
R2	499.5	6.903	13.8		
R3	638	8.023	12.5		
R4	833	9.716	11.6		
R5	659	9.532	14.4		
R6	744	10.043	13.4		

Simple linear regression.

$a = 837.79 \quad b = 652.61$

$SE(b) = 744.4243 \qquad t = \frac{b}{SE} = .8766 \quad d.f = 4$

Conclusion t = .8766, accepts the hypothesis that b is o and no linear association exists between islet tissue mass (T) and body weight (W) in water rat (Hydromys chrysogaster).

TABLE V Relationship between islet tissue and sex.

PROTOTHERIA

TABLE V(a) Echidna (Tachyglossus aculeatus)

Animal	Sex	Mean islet tissue mass
Echidna 1	Male	0.1940
Echidna 2	Female	0.1623
Echidna 3	Female	0.1345
Echidna 4	Male	0.1649
Echidna 5	Male	0.1331
Echidna 6	Female	0.1120

One way analysis of Variance

$F_{1,4} = 0.92$

$F_{1,4}$ (5%) = 7.71

Conclusion. There is no significant difference between sexes.

METATHERIA

TABLE V(b) Possum (Trichosurus vulpecula)

Animal	Sex	Mean islet tissue mass
Possum 1	Female	0.0556
Possum 2	Female	0.0602
Possum 3	Male	0.0567
Possum 4	Male	0.0591
Possum 5	Male	0.0577
Possum 6	Female	0.0568

One way analysis of Variance

$F_{1,4} = .04$

$F_{1,4}$ (5%) = 7.71

Conclusion. There is no significant difference between sexes.

TABLE V(c) Grey Kangaroo (Macropus fuliginosus)

Animal	Sex	Mean islet tissue mass
Kangaroo 1	Male	0.1506
Kangaroo 2	Female	0.1463
Kangaroo 3	Male	0.1365
Kangaroo 4	Female	0.1397
Kangaroo 5	Male	0.1441
Kangaroo 6	Female	0.1780

One way analysis of Variance

$F_{1,4} = .05$

$F_{1,4}$ (5%) = 7.71

Conclusion. There is no significant difference between sexes.

EUTHERIA

TABLE V(d) Hopping mouse (Notomys alexis)

Animal	Sex	Mean islet tissue mass
Hopping mouse 1	Female	0.2251
Hopping mouse 2	Male	0.2234
Hopping mouse 3	Female	0.2195
Hopping mouse 4	Male	0.2214
Hopping mouse 5	Male	0.2227
Hopping mouse 6	Female	0.2313

One way analysis of Variance

$F_{1,4} = 1.86$

$F_{1,4}$ (5%) = 7.71

Conclusion. There is no significant difference between sexes.

TABLE V(e) Water rat (Hydromys chrysogaster)

Animal	Sex	Mean islet tissue mass
Water rat 1	Male	0.3007
Water rat 2	Female	0.3040
Water rat 3	Female	0.3030
Water rat 4	Female	0.3071
Water rat 5	Male	0.3093
Water rat 6	Male	0.2896

One way analysis of Variance

$F_{1,4} = .70$

$F_{1,4}$ (5%) = 7.71

Conclusion. There is no significant difference between sexes.

TABLE VI Quantitative estimation of beta (β) cells showing mean (based on 500 observations) using a Weibel graticule.

PROTOTHERIA

TABLE VI(a) Echidna (Tachyglossus aculeatus)

Animals	Head	Neck	Body	Tail	Average	Average %
E1	.3170	.2559	.2879	.2925	.2883	28.83
E2	.3380	.3104	.3306	.3612	.3350	33.50
E3	.3030	.3010	.3134	.3494	.3167	31.67
E4	.3258	.2996	.3448	.3446	.3262	32.62
E5	.3103	.3181	.3044	.3146	.3118	31.18
E6	.3093	.3073	.3142	.3160	.3117	31.17
	.3172	.2987	.3158	.3280		31.49

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.0129	3	.0043	.0713 F3, 15 (5%) = 5.41(N.S.)
Animals	.0256	5	.0051	.0845 F5, 15 (5%) = 2.90
Error	.9056	15	.0603	
Total	.9441	23		

Conclusion. The β cells are uniformly distributed in the head, neck, body and tail regions.

HERIA

VI(b) Possum (Trichosurus vulpecula)

imals	Head	Neck	Body	Tail	Average	Average %
1	.5980	.5920	.5780	.5980	.5915	59.15
2	.5840	.6000	.5940	.6040	.5955	59.55
13	.6040	.5960	.6080	.5980	.6015	60.15
14	.5920	.5980	.5920	.5840	.5915	59.15
15	.5960	.6060	.5860	.5940	.5955	59.55
16	.5920	.5900	.5900	.5960	.5920	59.20
	.5943	.5970	.5913	.5956		59.45

lysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.0001	3	.00003	.69 F3, 15 (5%) = 5.41(N.S.)
Animals	.0003	5	.00006	1.14 F5, 15 (5%) = 2.90
Error	.0008	15	.00005	
Total	.0012	23		

nclusion. The β cells are uniformly distributed in the head, neck, body and tail regions.

TABLE VI(c) Grey Kangaroo (Macropus fuliginosus)

Animals	Head	Neck	Body	Tail	Average	Average %
K1	.2046	.2358	.2348	.2470	.2305	23.05
K2	.2108	.2064	.2288	.2462	.2230	22.30
K3	.2292	.2596	.2185	.2272	.2336	23.36
K4	.2724	.2426	.2291	.2439	.2428	24.28
K5	.2184	.2635	.2696	.2069	.2396	23.96
K6	.2067	.2378	.2129	.2078	.2762	27.62
	.2236	.2409	.2322	.2298		22.84

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.0009	3	.0003	2.79
Animals	.0024	5	.0004	F3, 15 (5%) = 5.41(N.S.) 4.47
Error	.0016	15	.0001	F5, 15 (5%) = 2.90
Total	.0100	23		

Conclusion. The β cells are uniformly distributed in the head, neck, body and tail regions.

EUTHERIA

TABLE VI(d) Hopping mouse (Notomys alexis)

Animals	Head	Neck	Body	Tail	Average	Average %
M1	.2128	.2572	.2380	.2783	.2465	24.65
M2	.2504	.2765	.2212	.2267	.2437	24.37
M3	.2148	.2305	.2474	.2387	.2328	23.28
M4	.2317	.2529	.2879	.2617	.2585	25.85
M5	.2857	.2448	.2886	.2041	.2558	25.58
M6	.2155	.2262	.2136	.2027	.2145	21.45
	.2351	.2480	.2494	.2353		24.19

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.00109	3	.000365	.2813 F3, 15 (5%) = 5.41(N.S.)
Animals	.034182	5	.006836	5.266 F5, 15 (5%) = 2.90
Error	.019472	15	.001298	
Total	.015805	23		

Conclusion. The β cells are uniformly distributed in the head, neck, body and tail regions.

LE VI(e) Water rat (Hydromys chrysogaster)

nimals	Head	Neck	Body	Tail	Average	Average %
R1	.2602	.2145	.2333	.2114	.2298	22,98
R2	.2141	.2354	.2491	.2594	.2395	23.95
R3	.2223	.2418	.2373	.2562	.2394	23.94
R4	.2135	.2643	.2557	.2086	.2355	23.55
R5	.2585	.2508	.2509	.2419	.2505	25.05
R6	.2553	.2500	.2574	.2323	.2487	24.87
	.2373	.2428	.2472	.2349		24.05

lysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.000552	3	.000184	.6401 F3, 15 (5%) = 5.41(N.S.)
Animals	.001235	5	.000247	.859 F5, 15 (5%) = 2.90
Error	.004312	15	.000287	#
Total	.006099	23		

lusion. The β cells are uniformly distributed in the head, neck, body and tail regions.

Quantitative estimation of alpha (A) cells showing mean (based on 500 observations) using a Weibel graticule.

PROTOTHERIA

TABLE VII(a) Echidna (Tachyglossus aculeatus)

Animals	Head	Neck	Body	Tail	Average	Average %
E1	.2077	.1992	.1791	.1920	.1945	19.45
E2	.1780	.1821	.1880	.1902	.1845	18.45
E3	.1843	.1820	.1981	.1860	.1876	18.76
E4	.1861	.1843	.1840	.1821	.1828	18.28
E5	.1901	.1981	.1860	.1843	.1896	18.96
E6	.1880	.1822	.1903	.1880	.1871	18.71
	.1890	.1879	.1875	.1871		18.76

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.000290	3	.000097	.0710 F3, 15 (5%) = 5.41(N.S.)
Animals	.007165	5	.001433	1.049 F5, 15 (5%) = 2.90
Error	.020488	15	.001366	
Total	.027943	23		

Conclusion. The A cells are uniformly distributed in the head, neck, body and tail regions.

ETATHERIA

ABLE VII(b) Possum (Trichosurus vulpecula)

Animals	Head	Neck	Body	Tail	Average	Average %
P1	.3120	.3160	.3100	.3140	.3130	31.30
P2	.3220	.3240	.3220	.3080	.3190	31.90
P3	.3160	.3100	.3100	.3120	.3120	31.20
P4	.3240	.2920	.3220	.3220	.3150	31.50
P5	.3260	.3160	.3180	.3160	.3190	31.90
P6	.3120	.3140	.3140	.3240	.3160	31.60
	.3186	.3120	.3160	.3160		31.56

alysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.000163	3	.000054	.205 F3, 15 (5%) = 5.41(N.S.)
Animals	.000205	5	.000041	.154 F5, 15 (5%) = 2.90
Error	.003973	15	.000264	
Total	.004341	23		

nclusion. The A cells are uniformly distributed in the head, neck, body and tail regions.

ABLE VII(c) Grey Kangaroo (Macropus fuliginosus)

Animals	Head	Neck	Body	Tail	Average	Average %
K1	.3532	.3980	.3490	.3514	.3629	36.29
K2	.3557	.2942	.3297	.3622	.3354	33.54
K3	.3257	.3956	.3064	.3089	.3341	33.41
K4	.3183	.3001	.3153	.3660	.3249	32.49
K5	.3306	.3478	.3167	.3029	.3245	32.45
K6	.3300	.3187	.3383	.3427	.3324	33.24
	.3355	.3424	.3259	.3390		33.87

nalysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.009457	3	.003152	.369 F3, 15 (5%) = 5.41(N.S.)
Animals	.157377	5	.031475	3.68 F5, 15 (5%) = 2.90
Error	.128101	15	.008540	
Total	.038733	23		

nclusion. The A cells are uniformly distributed in the head, neck, body and tail regions.

TABLE VII(d) Hopping mouse (Notomys alexis)

Animals	Head	Neck	Body	Tail	Average	Average %
M1	.1645	.2144	.2360	.2241	.2097	20.97
M2	.1618	.2378	.2141	.2442	.2144	21.44
M3	.1682	.2529	.2647	.2644	.2375	23.75
M4	.1656	.2130	.2567	.2475	.2207	22.07
M5	.1684	.2520	.2514	.2241	.2239	22.39
M6	.1682	.2058	.2642	.2224	.2151	21.51
	.1661	.2293	.2478	.2377		22.02

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.612550	3	.204183	6.24 F3, 15 (5%) = 5.41(Sig.)
Animals	.001931	5	.000386	.099 F5, 15 (5%) = 2.90
Error	.584394	15	.038959	
Total	.030087	23		

Conclusion. The head region shows a lower proportion of A cells.

TABLE VII(e) Water rat (Hydromys chrysogaster)

Animals	Head	Neck	Body	Tail	Average	Average %
R1	.1541	.1954	.1789	.1791	.1768	17.68
R2	.1428	.1891	.1957	.1853	.1782	17.82
R3	.1480	.2041	.2043	.1806	.1842	18.42
R4	.1532	.1753	.1931	.1971	.1796	17.96
R5	.1582	.1949	.1934	.1841	.1826	18.26
R6	.1581	.1934	.1923	.1945	.1845	18.45
	.1524	.1920	.1929	.1867		18.09

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.006695	3	.002231	23.02 F3, 15 (5%) = 5.41(Sig.)
Animals	.000210	5	.000042	.4335 F5, 15 (5%) = 2.90
Error	.001033	15	.000096	
Total	.007939	23		

Conclusion. The head region shows a lower proportion of A cells.

TABLE VIII Quantitative estimation of delta (δ) cells showing mean (based on 500 observations) using a Weibel graticule.

PROTOTHERIA

Echidna (Tachyglossus aculeatus)

Conclusion: No evidence of δ cells in the six specimens I have worked on. Two other samples of Bouin fixed platypus and echidna stained concurrently gave positive results and thus served as positive controls.

METATHERIA

TABLE VIII(a) Possum (Trichosurus vulpecula)

Animals	Head	Neck	Body	Tail	Average
P1	.1040	.1147	.1035	.1100	.1080
P2	.1240	.1071	Not worked	.1140	
P3	NOT		WORKED		
P4	.1177	Not worked	.1042	Not worked	
P5	Not worked	Not worked	.1086	Not worked	
P6	.1000	.1010	.1087	Not worked	
	.2236	.2409	.2322	.2298	

Conclusion. Because of missing values, it was decided not to proceed with statistical calculations.

TABLE VIII(b) Grey Kangaroo (Macropus fuliginosis)

Animals	Head	Neck	Body	Tail	Average	Average %
K1	.1716	.1702	.1679	.1942	.1759	17.59
K2	.1730	.1742	.1985	.1853	.1820	18.20
K3	.1920	.1887	.1753	.1918	.1869	18.69
K4	.1860	.1872	.1809	.1745	.1821	18.21
K5	.1975	.1933	.1910	.1786	.1901	19.01
K6	.1988	.1798	.1788	.1929	.1875	18.75
	.1864	.1822	.1820	.1862		18.42

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.000106	3	.000035	.343 F3, 15 (5%) = 5.41(N.S.)
Animals	.000510	5	.000102	.991 F5, 15 (5%) = 2.90
Error	.001544	15	.000102	
Total	.002161	23		

Conclusion. The D cells are uniformly distributed in the head, neck, body and tail regions.

EUTHERIA

TABLE VIII(c) Hopping Mouse (Notomys alexis)

Animals	Head	Neck	Body	Tail	Average	Average %
M1	.1853	.1878	.1875	.1829	.1858	18.58
M2	.1976	.1902	.1819	.1867	.1891	18.91
M3	.1945	.1856	.1941	.1860	.1900	19.00
M4	.1824	.1835	.1774	.1947	.1845	18.45
M5	.1810	.1818	.1950	.1860	.1859	18.59
M6	.1708	.1873	.1770	.1754	.1776	17.76
	.1852	.1860	.1854	.1852		18.54

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.000002	3	.00000077	.02 F3, 15 (5%) = 5.41(N.S.)
Animals	.000438	5	.0000876	2.5 F5, 15 (5%) = 2.90
Error	.000518	15	.0000345	
Total	.000959	23		

Conclusion. The D cells are uniformly distributed in the head, neck, body and tail regions.

TABLE VIII(d) Water rat (Hydromys chrysogaster)

Animals	Head	Neck	Body	Tail	Average	Average %
R1	.1374	.1327	.1345	.1484	.1382	13.82
R2	.1340	.1311	.1261	.1346	.1314	13.14
R3	.1295	.1415	.1344	.1439	.1373	13.73
R4	.1274	.1270	.1463	.1333	.1335	13.35
R5	.1438	.1276	.1428	.1355	.1374	13.74
R6	.1328	.1282	.1371	.1378	.1339	13.39
	.1341	.1313	.1368	.1389		13.52

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.034925	3	.011641	5.116 F3, 15 (5%) = 5.41(N.S.)
Animals	.000148	5	.000029	.013 F5, 15 (5%) = 2.90
Error	.034129	15	.002275	
Total	.000944	23		

Conclusion. The D cells are uniformly distributed in the head, neck, body and tail regions.

TABLE IX Quantitative estimation of pancreatic polypeptide (PP) cells showing mean (based on 500 observations) using a Weibel graticule

PROTOTHERIA

TABLE IX(a) Echidna (Tachyglossus aculeatus)

Animals	Head	Neck	Body	Tail	Average	Average %
E1	.3613	.2386	.2403	.2234	.2659	26.59
E2	.3718	.2340	.2402	.2551	.2752	27.52
E3	.3302	.2335	.2617	.2415	.2667	26.67
E4	.3423	.2424	.2406	.2549	.2700	27.00
E5	.3213	.2364	.2470	.2417	.2616	26.16
E6	.3301	.2306	.2463	.2430	.2625	26.25
	.3428	.2359	.2460	.2432		26.69

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.046322	3	.015440	90.1 F3, 15 (5%) = 5.41(Sig.)
Animals	.000513	5	.000102	.59 F5, 15 (5%) = 2.90
Error	.002569	15	.000171	
Total	.049406	23		

Conclusion. The head region shows a greater proportion of PP cells.

METATHERIA

TABLE IX(b) Possum (Trichosurus vulpecula)

Animals	Head	Neck	Body	Tail	Average	Average %
P1	.3292	.2064	.2003	.2147	.2376	23.76
P2	.3200	.2007	.2050	.2000	.2314	23.14
P3	.3273	.1920	.1954	.2000	.2286	22.86
P4	.3160	.1820	.2123	.2086	.2297	22.97
P5	.3282	.2105	.2042	.2097	.2376	23.76
P6	.3194	.2077	.1970	.2015	.2314	23.14
	.3233	.1998	.2023	.2057		23.27

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.065597	3	.021865	38.03 F3, 15 (5%) = 5.41 (Sig.)
Animals	.000283	5	.000056	.98 F5, 15 (5%) = 2.90
Error	.008624	15	.000574	
Total	.066774	23		

Conclusion. The head region shows a greater proportion of PP cells.

IX(c) Grey kangaroo (Macropus fuliginosus)

No evidence of PP cells in the six specimens I have worked on. Samples of Bouin fixed euros (Macropus robustus) stained concurrently with sections of the investigating series of Macropus fuliginosus gave positive results and thus served as positive controls.

IX(d) Hopping mouse (Notomys alexis)

No evidence of PP cells in the six specimens I have worked on. Samples of Bouin fixed Possum (Trichosurus vulpecula) stained concurrently with sections of the investigating series of Notomys alexis gave positive results and thus served as positive controls.

TABLE IX(e) Water rat (Hydromys chrysogaster)

Animals	Head	Neck	Body	Tail	Average	Average %
R1	.3402	.2454	.2481	.2471	.2702	27.02
R2	.3357	.2539	.2547	.2451	.2723	27.23
R3	.3401	.2589	.2519	.2454	.2740	27.40
R4	.3300	.2453	.2466	.2470	.2672	26.72
R5	.3247	.2453	.2425	.2518	.2660	26.60
R6	.3304	.2544	.2508	.2432	.2697	26.97
	.3335	.2505	.2491	.2466		26.99

Analysis of Variance

Comparison	Sum of Squares	Degrees of Freedom	Mean Square	F
Regions	.032386	3	.010795	49.9 F3, 15 (5%) = 5.41 (Sig.)
Animals	.000181	5	.000036	1.67 F5, 15 (5%) = 2.90
Error	.000324	15	.000021	
Total	.032891	23		

Conclusion. The head region shows a greater proportion of PP cells.

TABLE X Summary of statistically significant findings.

Animals (No.)	Islet tissue volume as % of total pancreatic vol.		% B cell vol. in islet tiss.	% A cell vol. in islet tiss.		% D cell vol. in islet tiss	% PP cell vol. in islet tiss.	
	Total pancreas	Tail of pancreas	Total pancreas	Total pancreas	Head of pancreas	Total pancreas	Total pancreas	Head of pancreas
Echidna (6)	.1502		31.49	18.76		No staining obtained	26.69	34.28^{5}*
Possum (6)	.0578		59.45	31.56		Irregular staining	23.27	32.33^{6}*
Grey Kangaroo (6)	.1431		22.84	33.87		18.42	No staining obtained	
Hopping Mouse (6)	.2239	$.5468^{1}$*	24.19	22.02	16.61^{3}*	18.54	No staining obtained	
Water Rat (6)	.3022	$.6023^{2}$*	24.05	18.09	15.24^{4}*	13.52	26.99	33.35^{7}*
Column 1	2	3	4	5	6	7	8	9

Column 1 shows the species and number of animals investigated.
Column 2 shows islet tissue volume as % of total pancreatic volume
Columns 2-3 show that in the tail region of the hopping mouse and water rat there is a greater % of islet tissue than in the pancreas as a whole.
Column 4 shows the volume of β cells as a % of total islet volume.
Column 5 shows the volume of A cells as a % of total islet volume
Columns 5-6 show that in the head region of the hopping mouse and water rat there is a lesser % of A cell volume in islet tissue than in the pancreas as a whole.
Column 7 shows the volume of D cells as a % of total islet volume in the grey kangaroo, hopping mouse and water rat. No staining was obtained in the echidna, and irregular staining results were obtained in the possum.
Column 8 shows the volume of PP cells as a % of total islet volume in the echidna, possum and water rat. No staining was obtained in the grey kangaroo and hopping mouse.
Columns 8-9 show that in the head region of the echidna, possum and water rat there is a greater % of PP cell volume in islet tissue than in the pancreas as a whole. No staining was obtained in the grey kangaroo and hopping mouse.

Analysis of Variance

At the 5% level of significance, comparing the respective mean values of the four regions of the pancreas - the head, neck, body, and tail - the F3, 15 value is 5.41 (Cambridge Elementary Statistical Table, Cambridge University Press (1966), p.8). In 7 of the comparisons made, the observed F values were greater than 5.41 (1* F = 7.19, 2* F = 75.40, 3* F = 6.24, 4* F = 23.02, 5* F = 90.10, 6* F = 38.03, 7* F = 49.90). Thus the respective means of the four regions were not statistically equal at the 5% level in these 7 comparisons. On inspection of these means, it was apparent that the regional means indicated by the 7 asterisks in the Table showed the greatest difference from the corresponding means of the pancreas as a whole and were, therefore, assumed to explain the 7 significant differences observed.

NOTE: These comments on the Analysis of Variance apply also to the Tables presenting the original data from which the data for the Summary Table were taken.

PLATE 3a. Islet tissue of pancreas of water rat (Hydromys chrysogaster) showing β cells using immunoperoxidase technique for insulin (x10). Scale 50um.

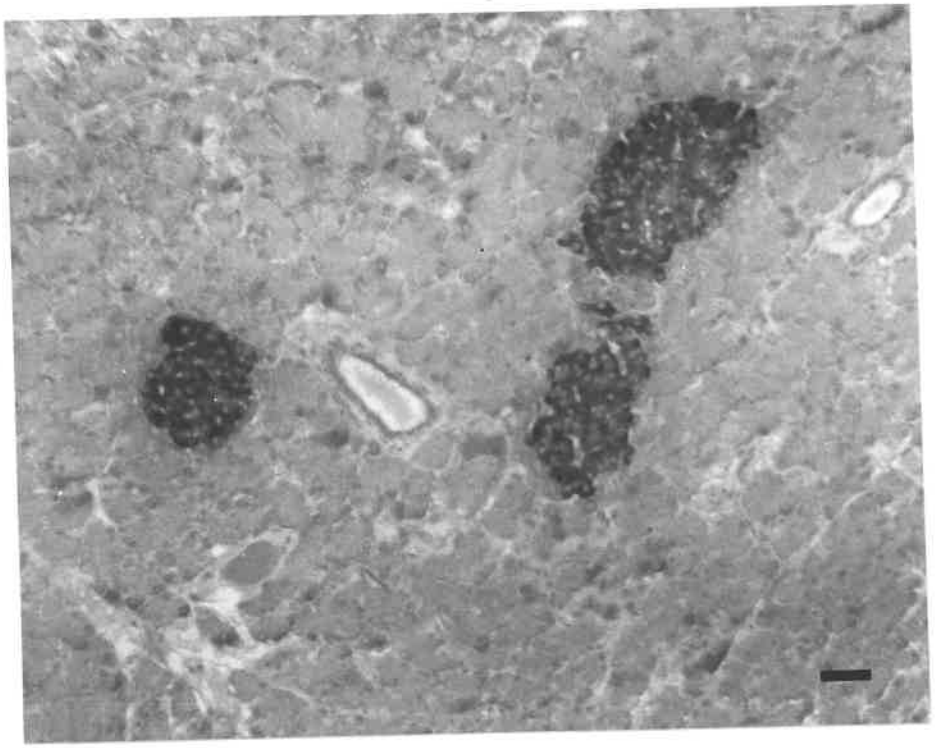


PLATE 3b. Same section (as above), decolourised and restained with aldehyde fuchsin showing β cells in the same islet in water rat (Hydromys chrysogaster) (x10). Scale 50um.

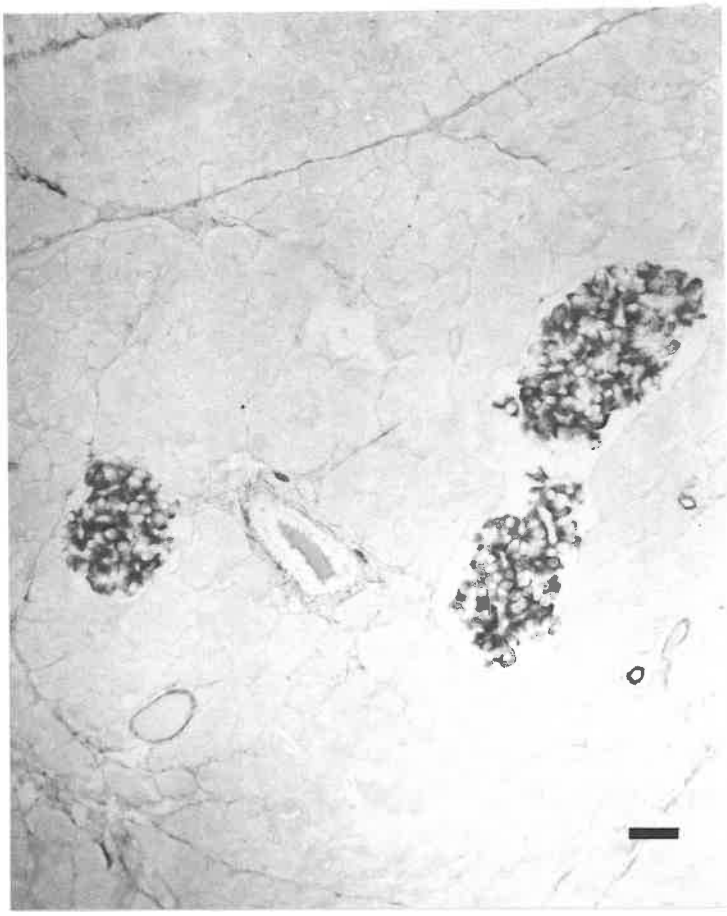


PLATE 4a. Islet tissue of pancreas of water rat (Hydromys chrysogaster) showing β cells using immunoperoxidase technique for insulin (x10). Scale 50um.

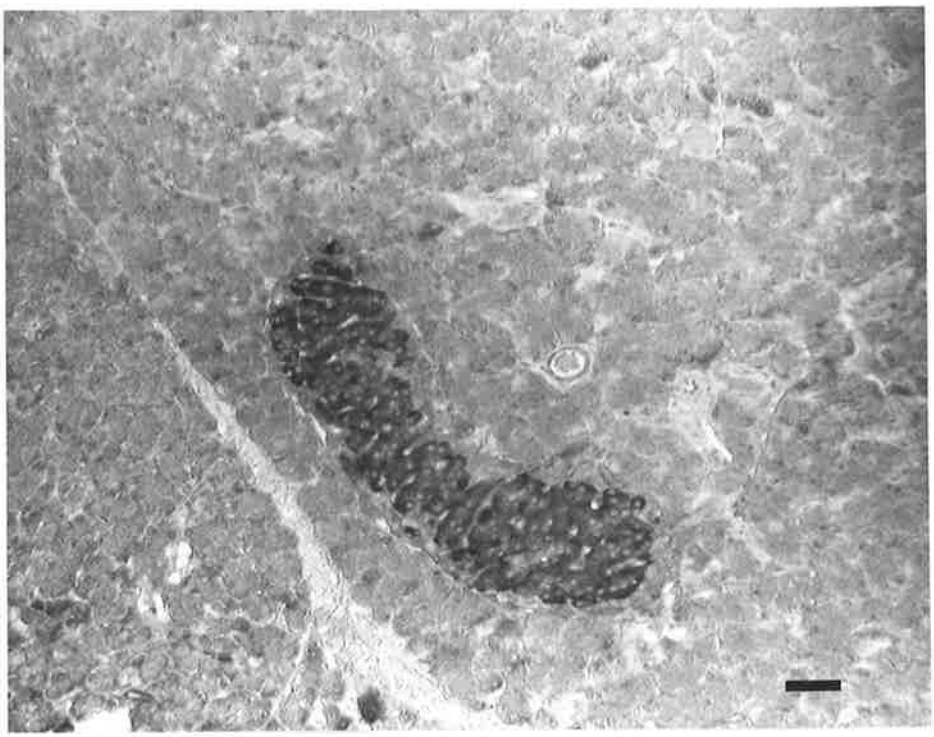
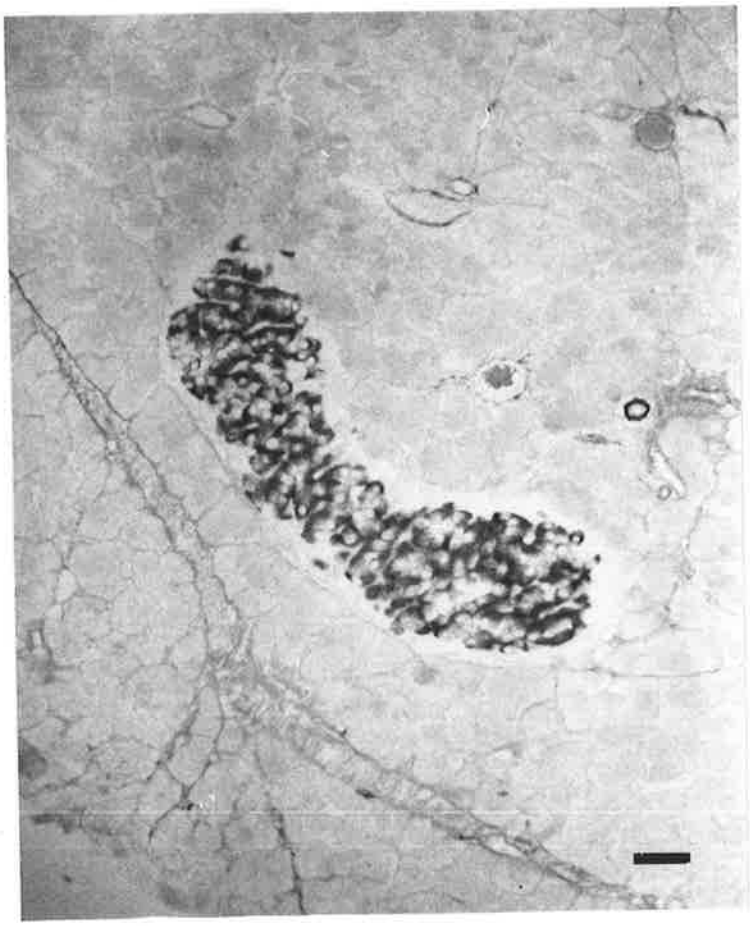


PLATE 4b. Same section (as above), decolourised and restained with aldehyde fuchsin showing β cells in the same islet in water rat (Hydromys chrysogaster) (x10). Scale 50um.



ABLE XI COMPARATIVE STAINING

(a) Quantitative estimation of beta (β) cells showing mean stained with immunoperoxidase for nsulin, photographed, decolourised and stained with aldehyde fuchsin and photographed in water at (Hydromys chrysogaster)

Sections of water rat stained with immunoperoxidase for insulin are shown in Plate 3a, 4a ections are decolourised and stained with aldehyde fuchsin, and the same field is shown in late 3b, 4b.

mmunoperoxidase for insulin

Animals	Head	Neck	Body	Tail	Average
R1	.2195	.2427	.2338	.2220	.2295

ldehyde fuchsin

Animals	Head	Neck	Body	Tail	Average
R1	.2255	.2628	.2458	.2417	.2439

nclusion. There is no statistical comparison. The figures obtained look similar.

PLATE 5a. Islet tissue of pancreas of grey kangaroo (Macropus fuliginosus) showing A cells using immunoperoxidase technique for glucagon (x10). Scale 50um.

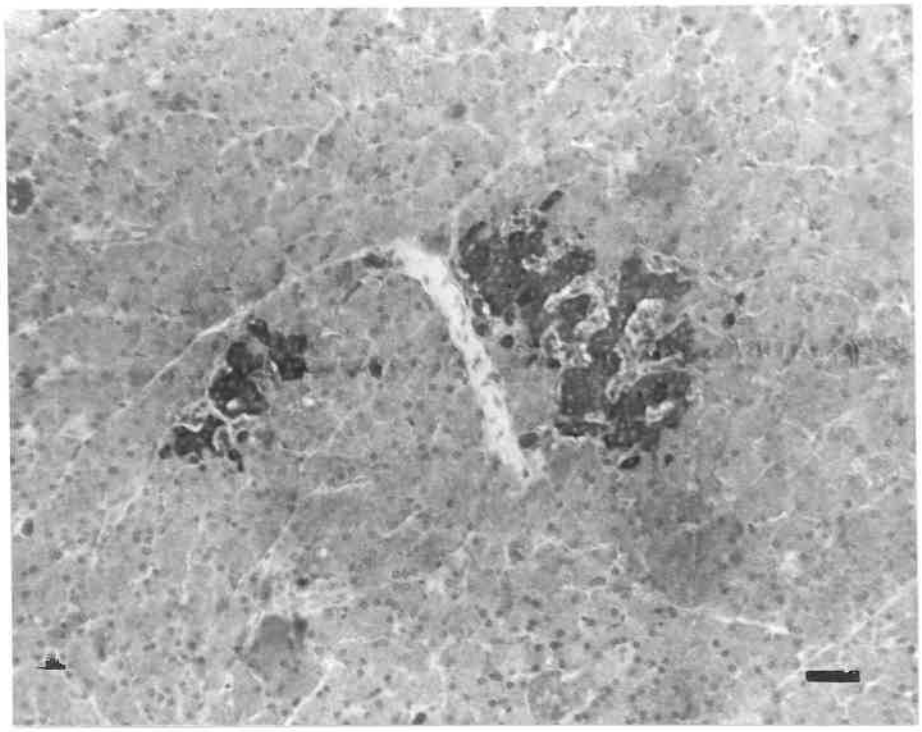


PLATE 5b. Same section (as above), decolourised and restained with phospho-tungstic acid haemotoxylin showing A cells in the same islet in grey kangaroo (Macropus fuliginosus) (x10). Scale 50um.

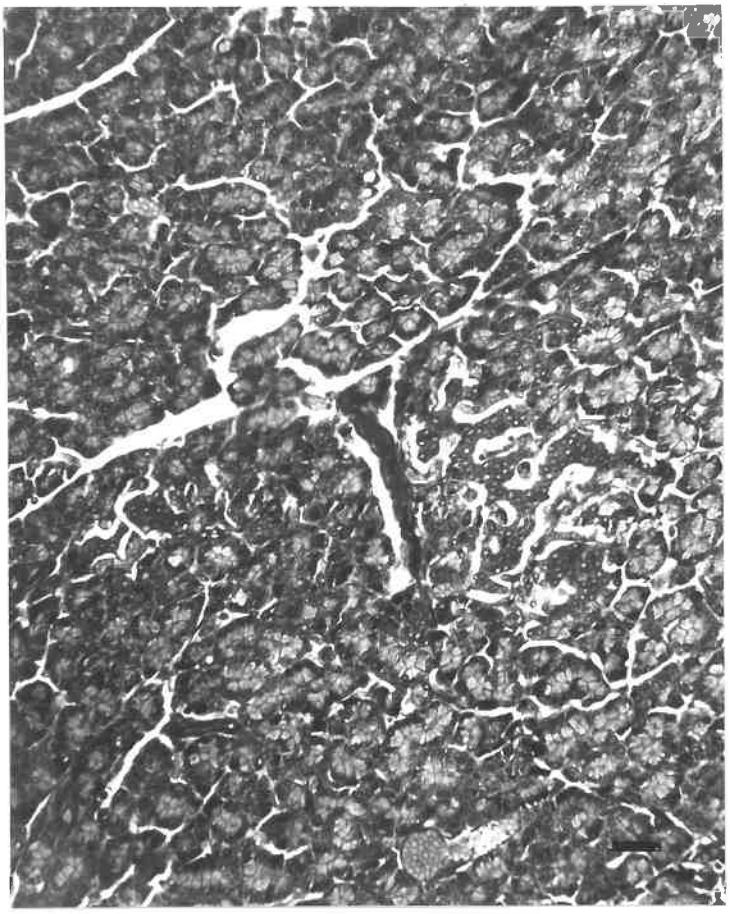


PLATE 6a. Islet tissue of pancreas of grey kangaroo (Macropus fuliginosus) showing A cells using immunoperoxidase technique for glucagon (x10) Scale 50um.

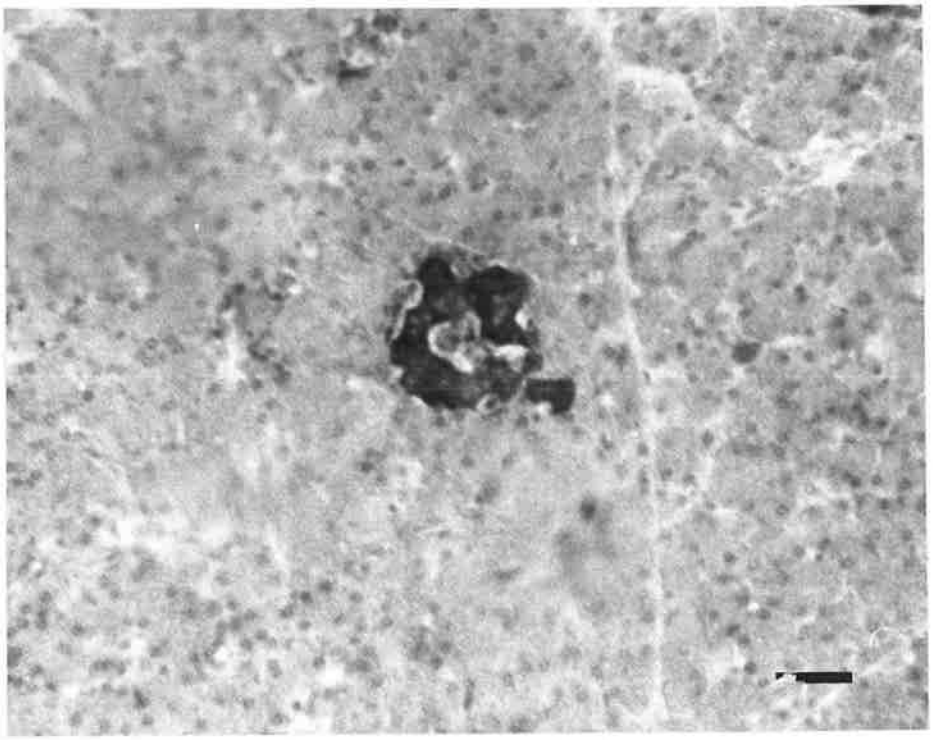
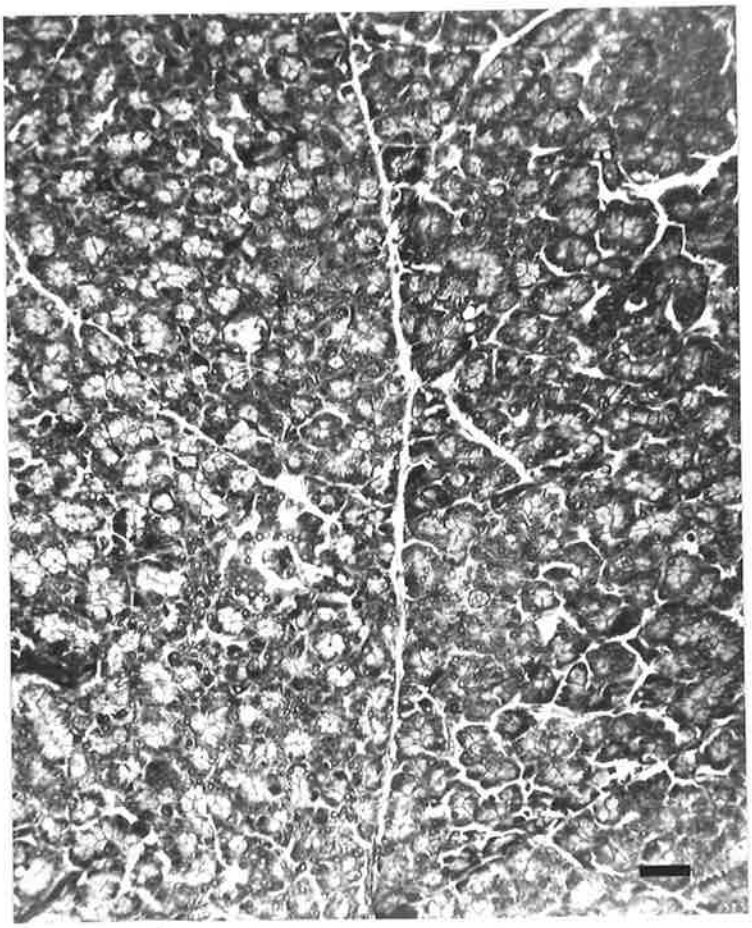


PLATE 6b. Same section (as above), decolourised and restained with phosphotungstic acid haemotoxylin showing A cells in the same islet in grey kangaroo (Macropus fuliginosus) (x10). Scale 50um.



ABLE XI(b) Quantitative estimation of alpha (A) cells showing mean stained with mmunoperoxidase for glucagon, photographed, decolourised and stained with phosphotungstic acid aemotoxylin (PTAH) and photographed in grey kangaroo (Macropus fuliginosus)

ections of grey kangaroo stained with immunoperoxidase for glucagon are shown in Plate 5a, 6a ections are decolourised and stained with phosphotungstic acid haemotoxylin (PTAH), and the me field is shown in Plate 5b, 6b.

Immunoperoxidase for glucagon

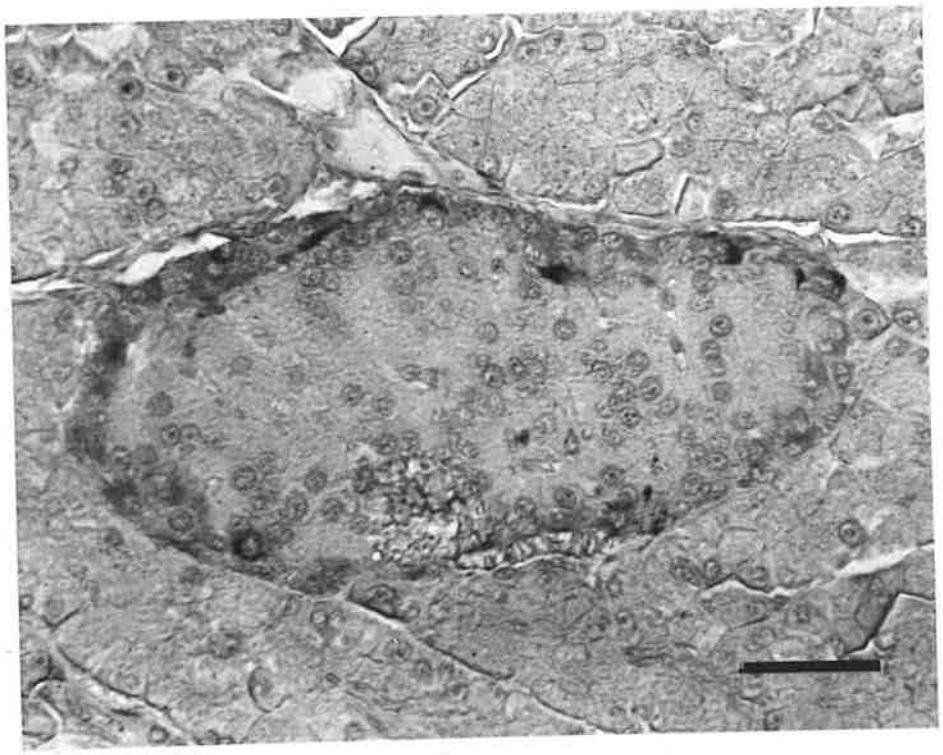
Animal	Head	Neck	Body	Tail	Average
K1	.3134	.3193	.3177	.3155	.3164

Phosphotungstic acid haemotoxylin (PTAH)

Animal	Head	Neck	Body	Tail	Average
K1	.3144	.3147	.3098	.3156	.3136

nclusion. There is no statistical comparison. The figures obtained look similar.

PLATE 7. Islet tissue of pancreas of possum (Trichosurus vulpecula) showing A cells using immunoperoxidase technique for glucagon (x20). Scale 50um.



ABLE XI(c) Quantitative estimation of alpha (α) cells showing mean stained with mmunoperoxidase for glucagon, photographed, decolourised and stained with Grimelius' silver itrate stain in possum (Trichosurus vulpecula) and photographed.

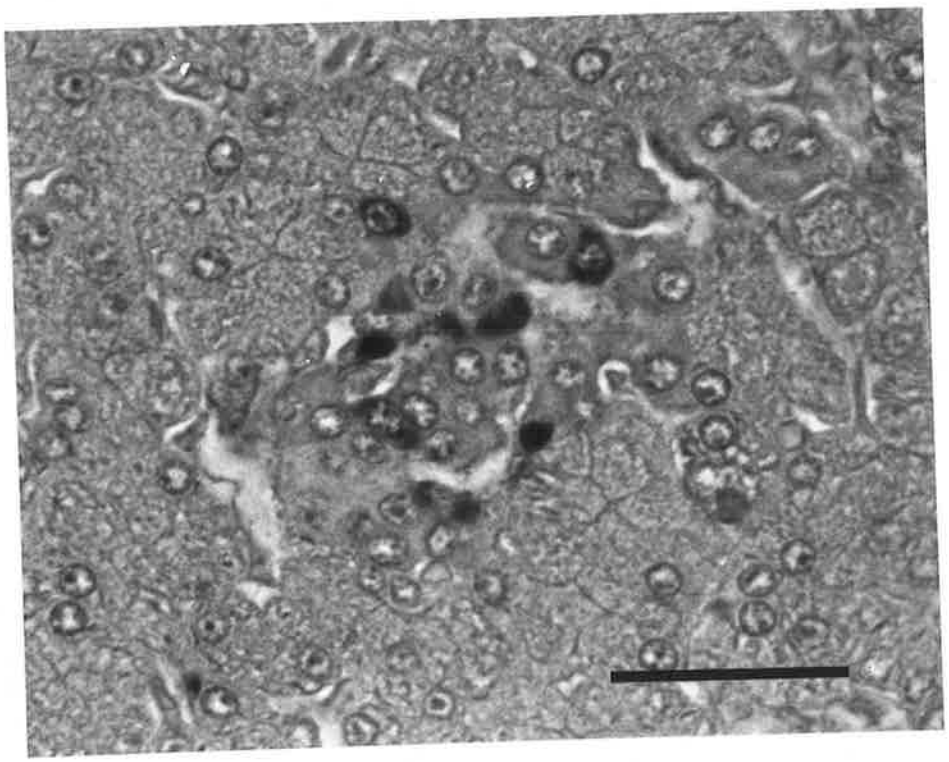
ections of possum stained with immunoperoxidase for glucagon are shown in Plate 7.

Immunoperoxidase for glucagon

Animal	Head	Neck	Body	Tail	Average
P1	.3273	.3118	.3200	.3034	.3156

Conclusion. There is no statistical comparison.
Grimelius' silver nitrate stain was tried twice and did not work.

PLATE 8. Islet tissue of pancreas of grey kangaroo (Macropus fuliginosus) showing D cells using immunoperoxidase technique for somatostatin (x20). Scale 50um.



ABLE XI(d) Quantitative estimation of delta (δ) cells showing mean stained with immunoperox-dase for somatostatin, photographed, decolourised and stained with modified Davenport's silver echnique (Hellerstrom and Hellman, 1960) and using Epple's modification (Epple 1967) in grey angaroo (Macropus fuliginosus).

ections of grey kangaroos stained with immunoperoxidase for somatostatin are shown in Plate 8. mmunoperoxidase for somatostatin

Animal	Head	Neck	Body	Tail	Average
K4	.1836	.1818	.1920	.1943	.1879

egative result with modified Davenport's silver technique.
fter staining with immunoperoxidase techniques, silver stains have been negative although positive results were obtained in this species when stained only with silver. The staining was tried twice.

PLATE 9. Islet tissue of pancreas of echidna (Tachyglossus aculeatus) stained with H and E (x20). Scale 50um.

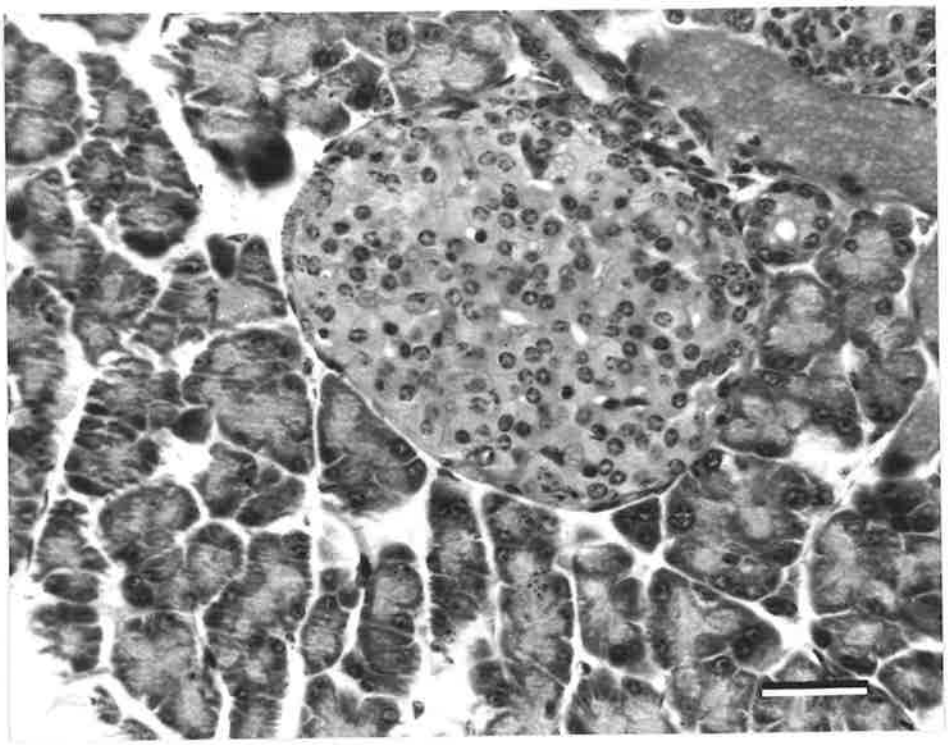


PLATE 10. Islet tissue of pancreas of possum (Trichosurus vulpecula) stained with H and E (x20). Scale 50um.

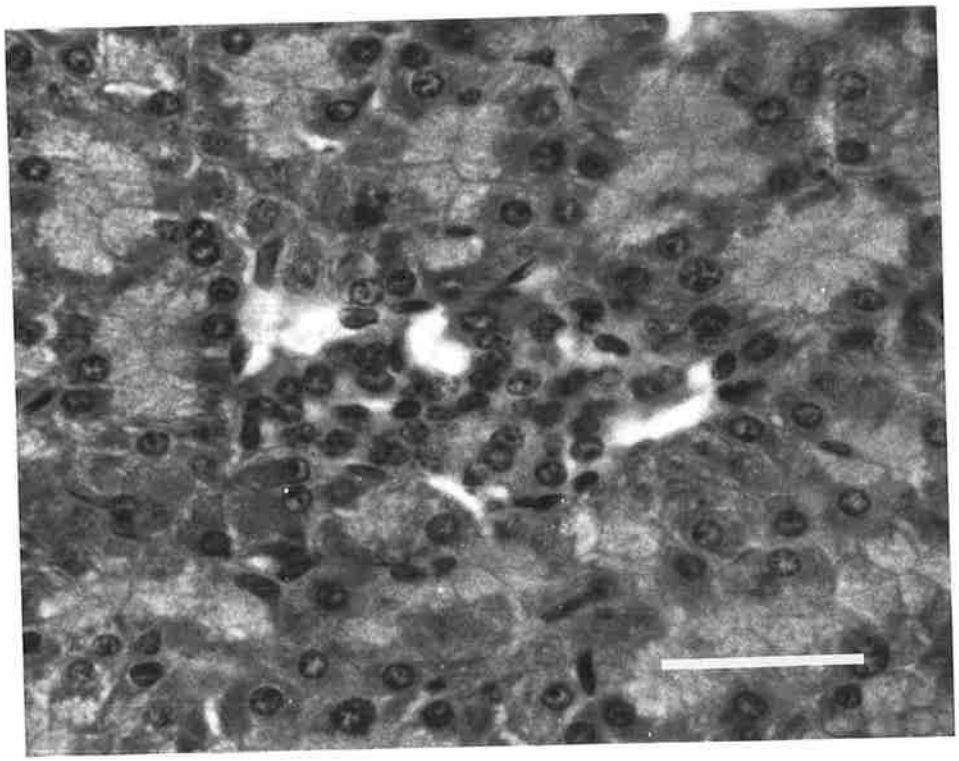


PLATE 11. Islet tissue of pancreas of grey kangaroo (Macropus fuliginosus) stained with H and E (x20). Scale 50um.

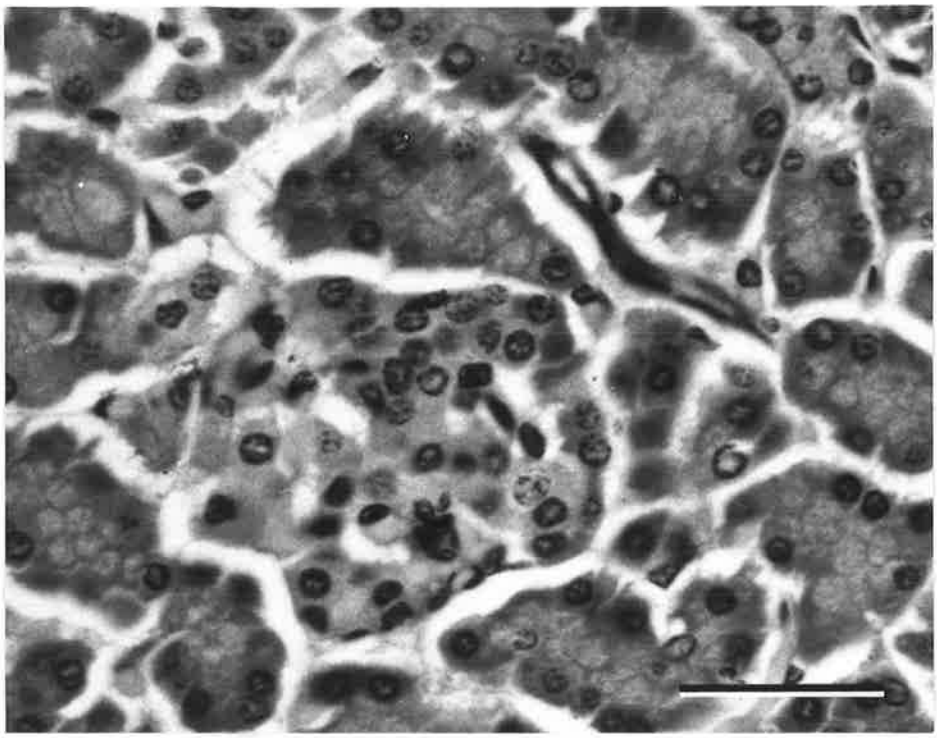
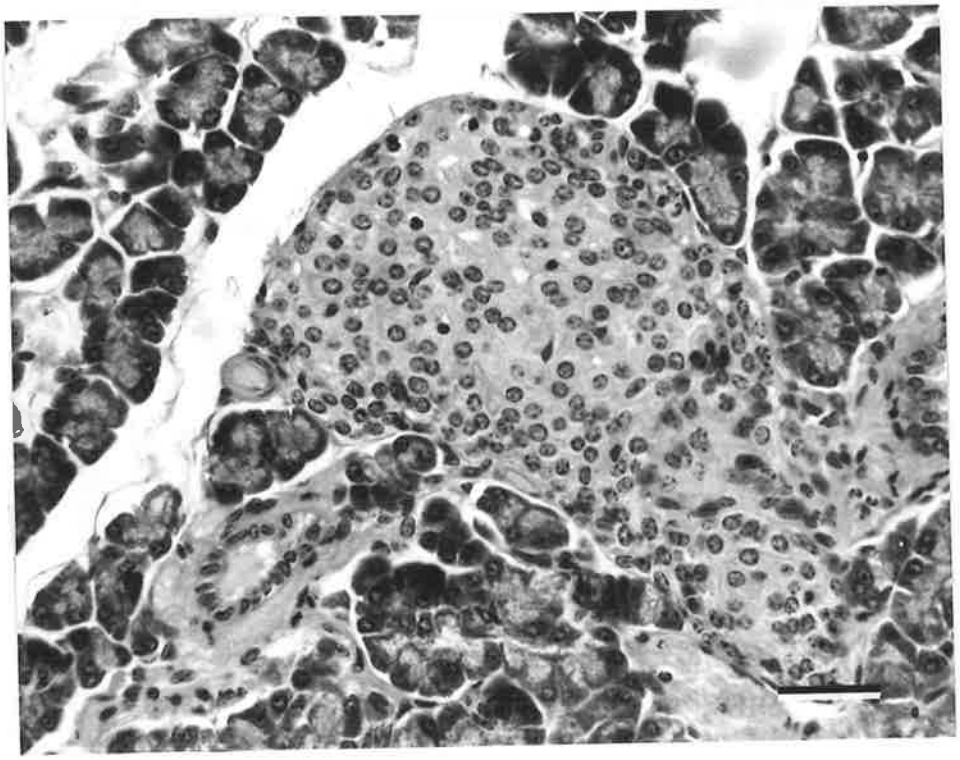


PLATE 12. Islet tissue of pancreas of hopping mouse (Novomys alexis) stained with H and E (x20). Scale 50um.



DISCUSSION

(a) Histology of pancreas

(1) Echidna (Tachyglossus aculeatus)

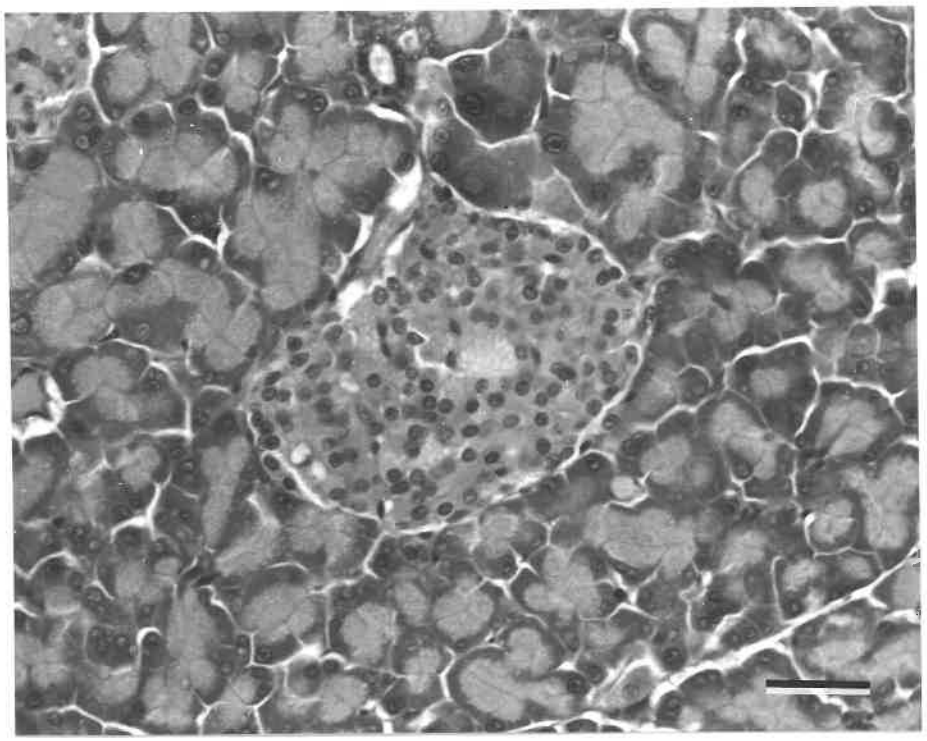
The islets are compact spheroidal groups of cells (Plate 9) distributed throughout the gland. Griffiths (1965) in his study, using Gomori stain, showed the presence of A and B cells. Studies in this department have demonstrated the presence of D cells by an immunoperoxidase technique (Barbour, unpublished observation) though only negative results were achieved with the material used for this study. In this study, PP cells have been shown both in an interacinar position and within the islets. Studies in the department have shown similar observations (Barbour, unpublished observation).

(2) Possum (Trichosurus vulpecula)

The islets are well defined (Plate 10). Thomas (1937), in Didelphis marsupialis has shown that the cellular components consist of B cells which form the central mass with other types clumped in groups around the periphery, consisting of A and E cells. In the possum (Trichosurus vulpecula), the islets contain A, B, D and PP cells as shown in this study. In this study, PP cells have been shown both in an interacinar position and within the islets. Studies in the department have shown similar observations (Barbour, unpublished observation).

Using the immunofluorescence procedure, Reddy, Bibby, Fisher and Elliott (1986) have demonstrated differences in the topographical distribution of each cell type in possum (Trichosurus vulpecula). Insulin immunoreactive cells occurred as groups of cells peripherally and within the islet. In several islets glucagon cells were the predominant cell population and were distributed peripherally as well as centrally. PP cells were fewer in number and usually occurred as single cells within the islet. Cells immunoreactive to anti-somatostatin serum were observed in varying numbers in the peripheral and central regions of the islet.

PLATE 13. Islet tissue of pancreas of water rat (Hydromys chrysogaster) stained with H and E (x20). Scale 50um.



(3) Grey Kangaroo (Macropus fuliginosus)

The islets are well defined (Plate 11) and are of varying shapes and sizes (White and Harrop, 1975). The islets show the presence of A, B, and D cells in this study. Studies in the department, on euros (Macropus robustus) have shown the presence of PP cells mostly within the islets but also interacinar in position (Barbour, unpublished observation). Negative results were achieved with the material used for this study.

(4) Hopping Mouse (Notomys alexis)

The islets are well defined (Plate 12) and are of varying shapes and sizes. The cytology shows the presence of A, B and D cells in this study. Negative results were obtained for PP cells.

(3) Water rat (Hydromys chrysogaster)

The islets (Plate 13) are similar to those in hopping mouse (Notomys alexis), although PP cells were demonstrated to be interacinar in position and also peripherally situated in the islets.

(b) Relationship between pancreas weight and body weight

Padour (1950) has put forward a thesis that in different species of mammals as body size increases the relative volume of the pancreas decreases. In this study, the ratio of pancreas weight to body weight found was echidna 0.228%, possum 0.110%, grey kangaroo 0.072%, hopping mouse 0.437% and water rat 0.436%. These results partly conform to Padour's hypothesis, except for the possum, where the possum which is smaller in size than an echidna but nevertheless has a relatively smaller pancreas. In the hopping mouse and water rat, they have the same percentage but very different body weights, the hopping mouse being much smaller than the water rat. In none of the species investigated in this study is there any correlation between pancreas weight and body weight.

Investigations on some cetaceans have shown that the ratio of pancreas weight to body weight is 0.16% in Phocoena phocoena (Slijper, 1958, cited by

Takahashi, Yamasaki and Kamiya, 1975), and 0.04%-0.09% in Platinista (Kamiya and Yamasaki, 1974).

According to Slijper in 1962 (cited by Takahashi, Yamasaki and Kamiya, 1975), the cetacean pancreas seems to be similar to that of most mammals in respect of relative weight (0.1-0.2% in small, and 0.03-0.15% in large cetaceans). This is in keeping with Padour's hypothesis (1950).

(c) Islet distribution in regions of the pancreas

A uniform distribution of islet tissue exists in the head, neck, body and tail regions in echidna (**Tachyglossus aculeatus**), possum (**Trichosurus vulpecula**), and grey kangaroo (**Macropus fuliginosus**). A greater relative amount of islet tissue was found in the tail region in hopping mouse (**Notomys alexis**) and water rat (**Hydromys chrysogaster**).

Studies on the distribution of islets have been carried out in some other species. Clark (1913) found the greatest concentration of islets in the head of the human pancreas, while Overholser (1925) found the greatest islet concentration in the body of the rat's pancreas. Jaffe (1951) found the greatest distribution in the tail of the rabbit's pancreas.

Khatim, Gumaa, Petersson, Lundquist, Grimelius and Hellerstrom (1985) have shown in the one-humped camel (**Camelus dromedarius**) numerous islets evenly distributed in all regions of the pancreas. There were no obvious differences between the frequency of the various islet cells in different regions, and concluded that the endocrine pancreas is dispersed into islets of the same size and cellular composition as has been described in many other mammalian species. In this study, echidna, possum and grey kangaroo have shown a uniform distribution in all regions of the pancreas.

The embryological pattern of the vertebrae pancreas is compatible with a regional heterogeneity (Bonner-Weir and Weir, 1979). Islet tissue is described as more abundant in dorsal anlage derived pancreas in mammals (rat) (Hard, 1944) and humans (Volk and Wellmann, 1977), but one report found no marked variation in humans (Hellman, 1959).

The dorsal anlage gives rise to the tail, body and superior part of the head of the pancreas. The findings of Overholser (1925), and Jaffe (1951) and this study on hopping mouse and water rat, fit the concept that islet tissue is more abundant in the dorsal anlage derived pancreas. The findings of Clark (1913) and the studies on echidna, possum and grey kangaroo, do not fit this embryological concept.

(d) Percentage islet tissue in the pancreas

The percentage of islet tissue in the pancreas has been assessed in all the species investigated.

In echidna and grey kangaroo there is no relationship between percentage islet tissue and the weight of the pancreas.

In possum, hopping mouse and water rat, there is a clear relationship between percentage islet tissue and the weight of the pancreas. In possum and hopping mouse it is a direct relationship between percentage islet tissue and size of pancreas. In water rat, it is an inverse relationship between percentage islet tissue and size of pancreas.

Various authors studied the total volume or weight of the pancreatic islets in humans (Volk and Wellmann, 1977). Laguesse in 1905 noted that the islets occupy 1% of the total pancreatic mass in humans. DeWitt in 1906 observed that they comprised 2% of the organ, Heiberg in 1909 estimated 3% of islet tissue in the pancreas, Weichselbaum in 1910 calculated that 4.3% of the pancreas is occupied by islet tissue; Gundisch in 1934 estimated that 0.6 to 2.11% of the pancreas, and according to Susman in 1942, 0.9 to 3.5% of the pancreas is occupied by the islets.

Sato and Herman (1981) have shown that the rabbit pancreas contains islets equal to 2.2% of its volume. Kaung and Elde (1980) have shown by morphometric quantitation of immunohistochemically stained sections of the frog, Rana pipiens, pancreas, showed that about 2% of the pancreas is endocrine tissue.

In this study, 0.15% of islet tissue is found in echidna, 0.05% in possum,

PLATE 14. Islet tissue of pancreas of echidna (Tachyglossus aculeatus) stained with aldehyde fuchsin showing β cells (x20). Scale 50um.

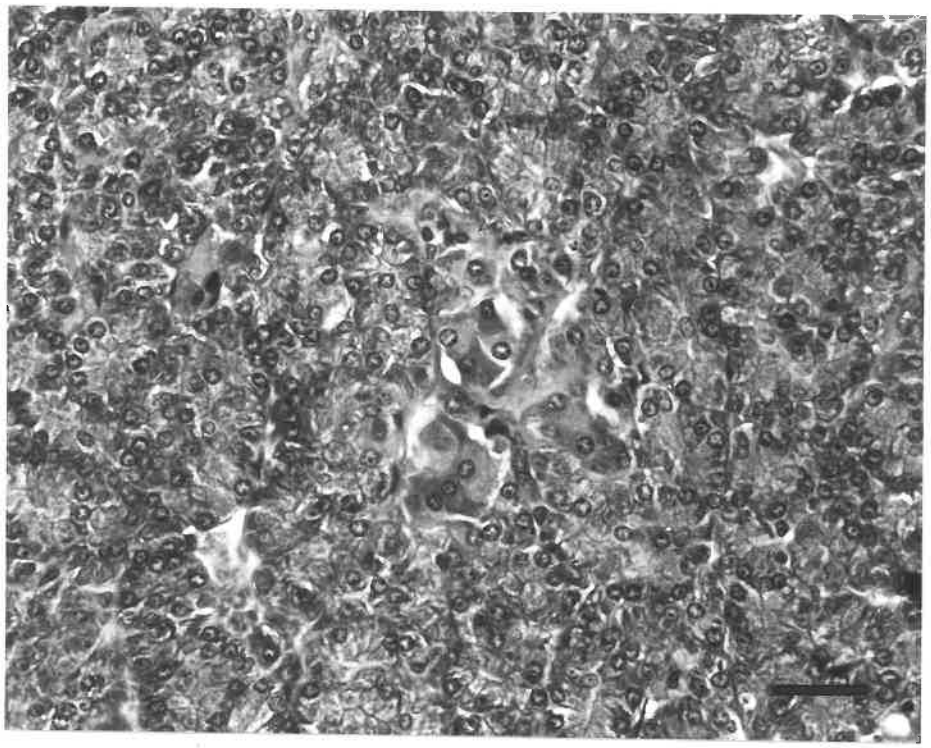
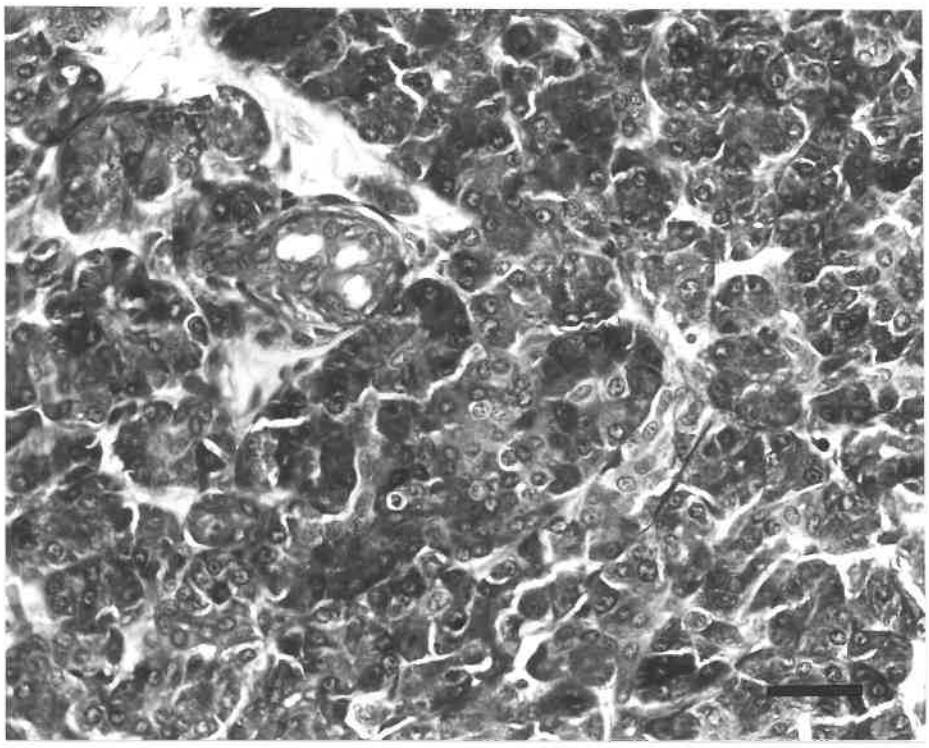


PLATE 15. Islet tissue of pancreas of possum (Trichosurus vulpecula) stained with aldehyde fuchsin showing β cells (x20). Scale 50um.



0.14% in grey kangaroo, 0.22% in hopping mouse and 0.30% in water rat. The results are lower than other studies.

(e) Islet mass in relation to body weight

The mass of islet tissue in relation to body weight has been calculated. There is no correlation in any of the species investigated. The values extend over a wide range of .6420-13.5. The values for the investigated species are 3.57 in echidna, 0.6420 in possum, 1.06 in grey kangaroo, 9.7 in hopping mouse and 13.45 in water rat.

Ogilvie (1937) in agreement with Seyfarth (1924), Nakamura (1924), estimated that the weight of the islets in humans fluctuated over a wide range with a mean varying from 0.478 to 2.738ug.

(f) Relationship between islet tissue and sex

There is no preponderance in either sex in any of the species investigated.

(g) Quantitative estimation of B cells in pancreas

In the echidna the majority of the islets consist of a core of B cells surrounded by a ring of A cells (Griffiths, 1968). This study has shown that both B and A cells are dispersed randomly in the islet (Plates 14 & 19) and B cells found to be 31.5%.

Very little information exists about the cellular composition of islets of Langerhans in marsupials (White and Harrop, 1975). It has been found in the opossum, **Didelphis marsupialis**, that B cells are the most abundant type (Thomas, 1937). White and Harrop (1975) have reported 52.7% B cells in the possum (**Trichosurus vulpecula**).

In this study, B cells are 59.5% in possum (Plate 15) and has confirmed the predominance of B cells.

Non-ruminant herbivorous marsupials like the brush-tailed possum (**Trichosurus vulpecula**) rely on glucose as an energy source, rather than volatile fatty acids, while the reverse is true of ruminant herbivorous

PLATE 16. Islet tissue of pancreas of grey kangaroo (Macropus fuliginosus) stained with aldehyde fuchsin showing β cells (x20). Scale 50um.

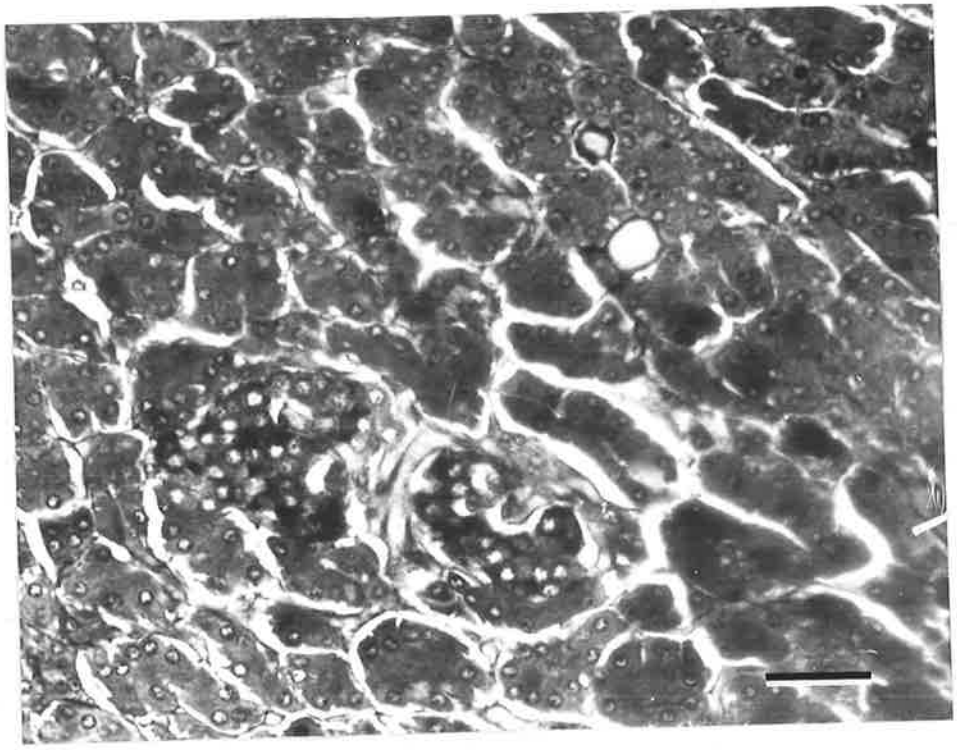


PLATE 17. Islet tissue of pancreas of hopping mouse (Notomys alexis) stained with aldehyde fuchsin showing β cells (x20). Scale 50um.

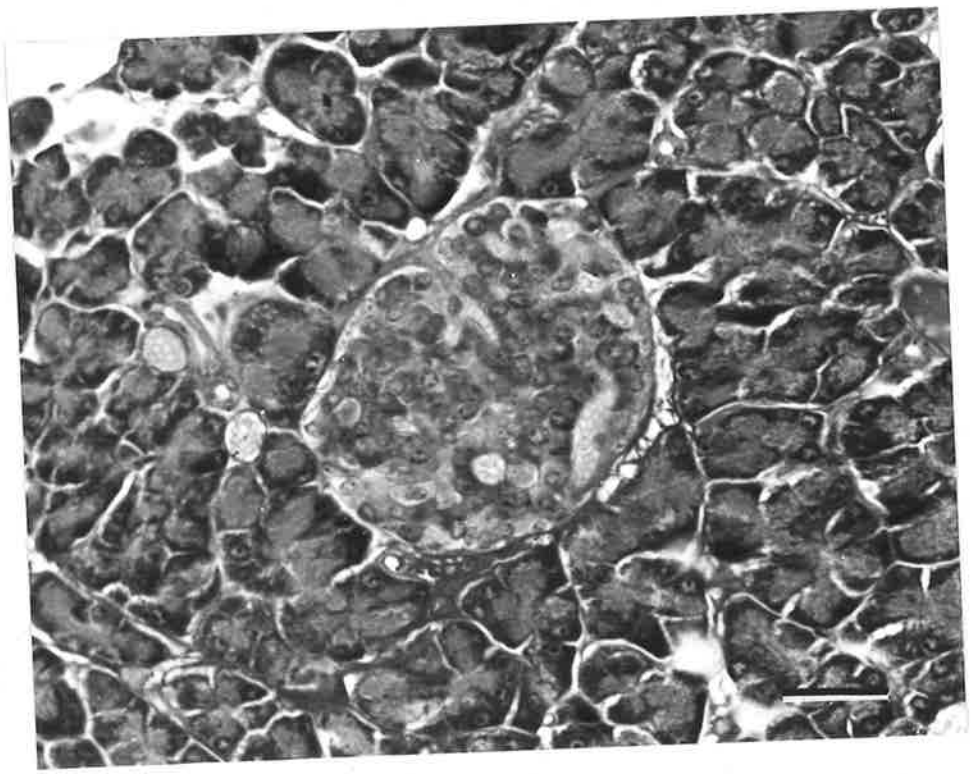


PLATE 18. Islet tissue of pancreas of water rat (Hydromys chrysogaster) stained with aldehyde fuchsin showing β cells (x50). Scale 50um.

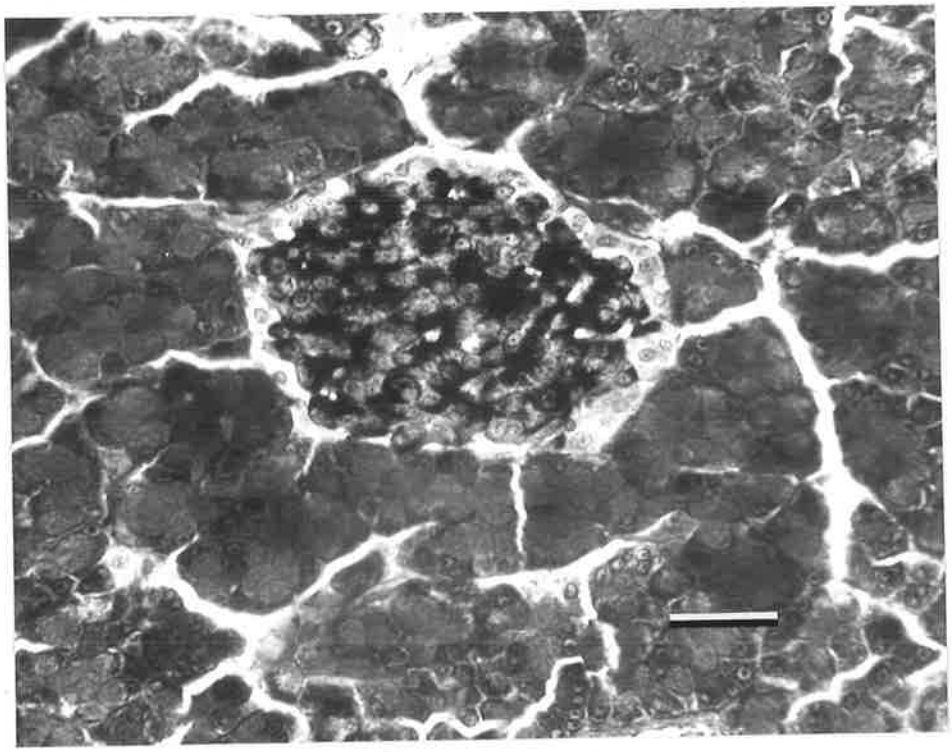
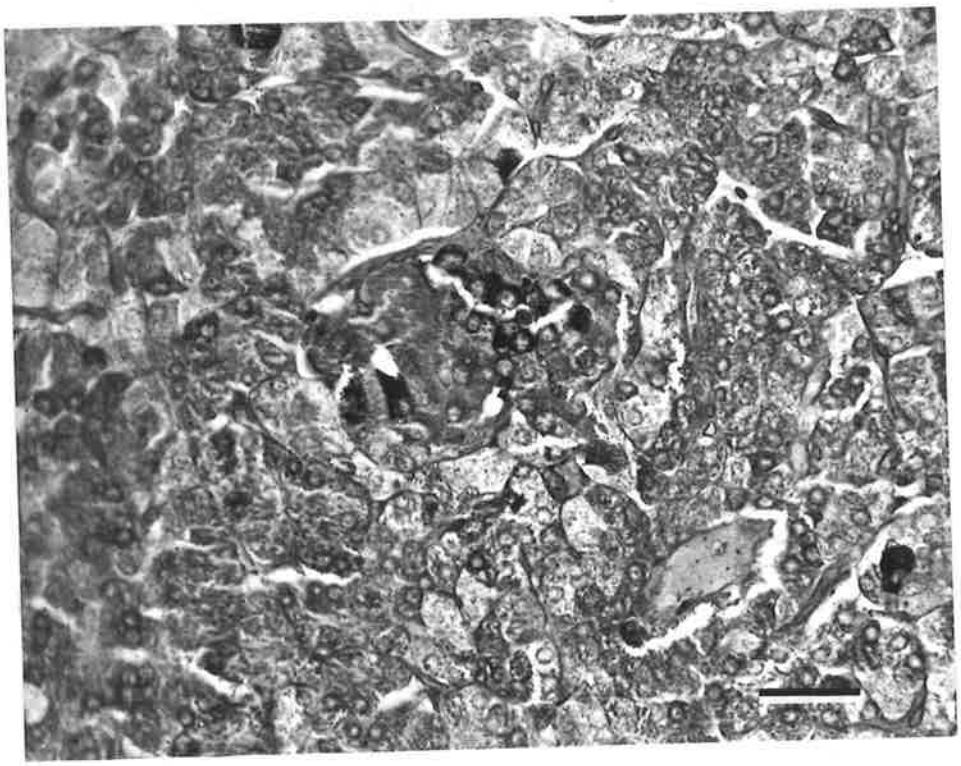


PLATE 19. Islet tissue of pancreas of echidna (Tachyglossus aculeatus) stained with Grimelius' silver stain showing A cells (x20). Scale 50um.



marsupials. The species studied was **Setonix brachyurus** (Moir, Somers and Waring, 1956; Barker, 1961; White and Harop, 1975). The possum shows a preponderance of B cells (White and Harrop, 1975). No other nonruminant herbivorous marsupial has been studied.

A previous study of the grey kangaroo, **Macropus fuliginosus** showed B cells to be 15.9% (White and Harrop, 1975). This study has shown a low proportion of B cells (22.8%) (Plate 16) relative to A cells, and has confirmed the findings of the previous study.

A study of the cellular composition in rodentia show the B cells to be the most predominant cell (White and Harrop, 1975) and the present study confirms this in hopping mouse (24.2%) (Plate 17).

In this study, the B cells are the most predominant cell in water rat (24.1%) (Plate 18). This is compared with 70% in the rat (Alm and Hellman, 1964; White and Harrop, 1975), 72.5% in the guinea pig (Alm and Hellman 1964; White and Harrop, 1975), 80% in the rabbit (Ferner and Kern, 1969; White and Harrop, 1975), 78.9% in the bat (**Myotis myotis**) (Mosca, 1956; White and Harrop, 1975), 61% in the beluga whale (Quay, 1957; White and Harrop, 1975), 75% in the dog (Hunt, 1936; White and Harrop, 1975), 83.2% in the cat (Petkov, 1967; White and Harrop, 1975), 56% in the horse (Ferner and Kern, 1969; Hellman, Rothman and Hellerstrom, 1962; White and Harrop, 1975), and 80% in cattle (Petkov, Gospodinov and Galabova, 1970; White and Harrop, 1975).

The B cell has long been known to manufacture insulin (Hellerstrom, 1977). In the study of the mammalian islets of Langerhans, B cells are generally the most abundant (White and Harrop, 1975), and this study confirms this in echidna, possum, hopping mouse and water rat. In the metatherian marsupials, B cell is the most predominant cell in possum, whereas in grey kangaroo, A cell is most predominant. This reflects their energy source, which is glucose in possum (non-ruminant herbivore) and volatile fatty acid in grey kangaroo (ruminant herbivore) (Moir, Somers and Waring, 1956; Barker, 1961; White and Harrop, 1975)

PLATE 20. Islet tissue of pancreas of possum (Trichosurus vulpecula) stained with Grimelius' silver stain showing A cells (x20). Scale 50um.

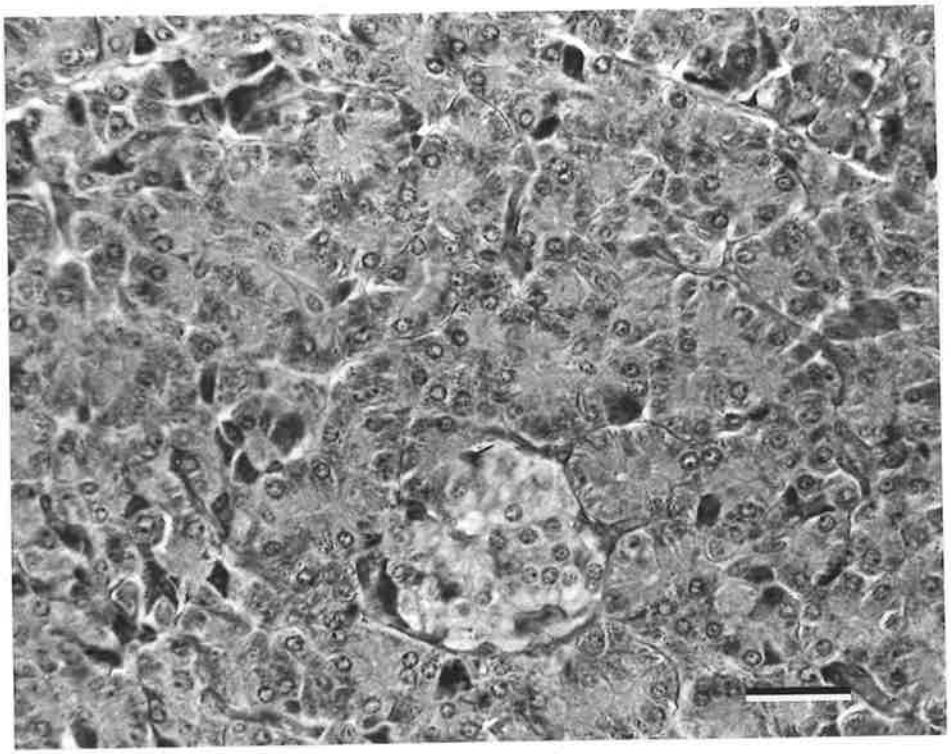


PLATE 21. Islet tissue of pancreas of grey kangaroo (Macropus fuliginosus) stained with phosphotungstic acid haematoxylin showing A cells (x20). Scale 50um.

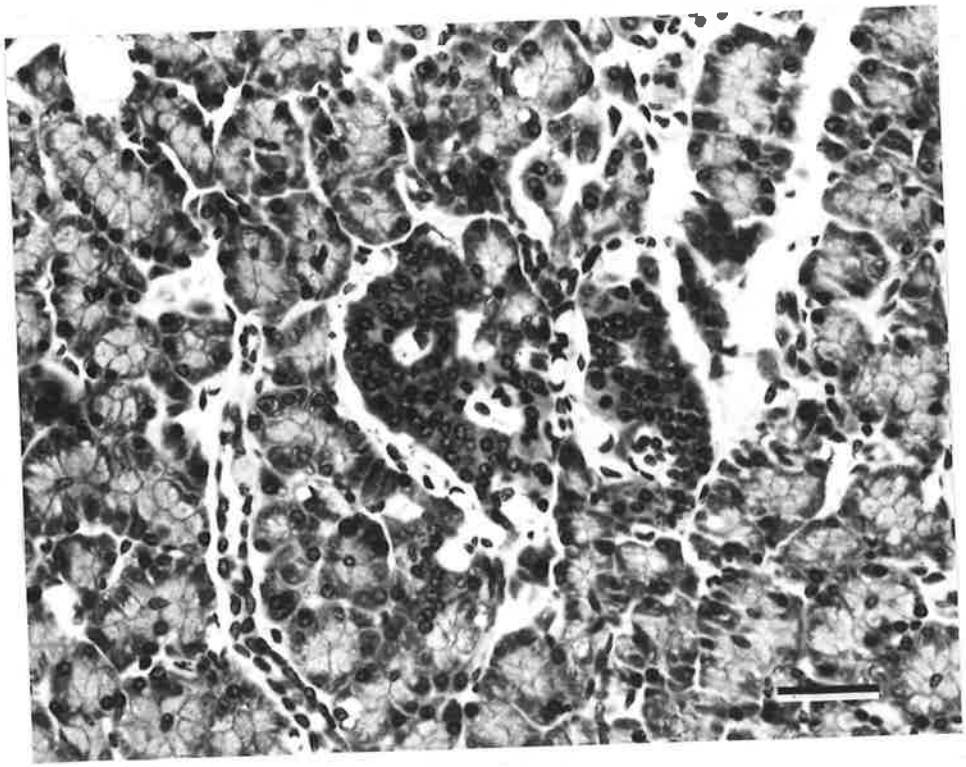


PLATE 22. Islet tissue of pancreas of hopping mouse (Notomys alexis) using immunoperoxidase technique for glucagon showing peripheral A cells (x20). Scale 50um.

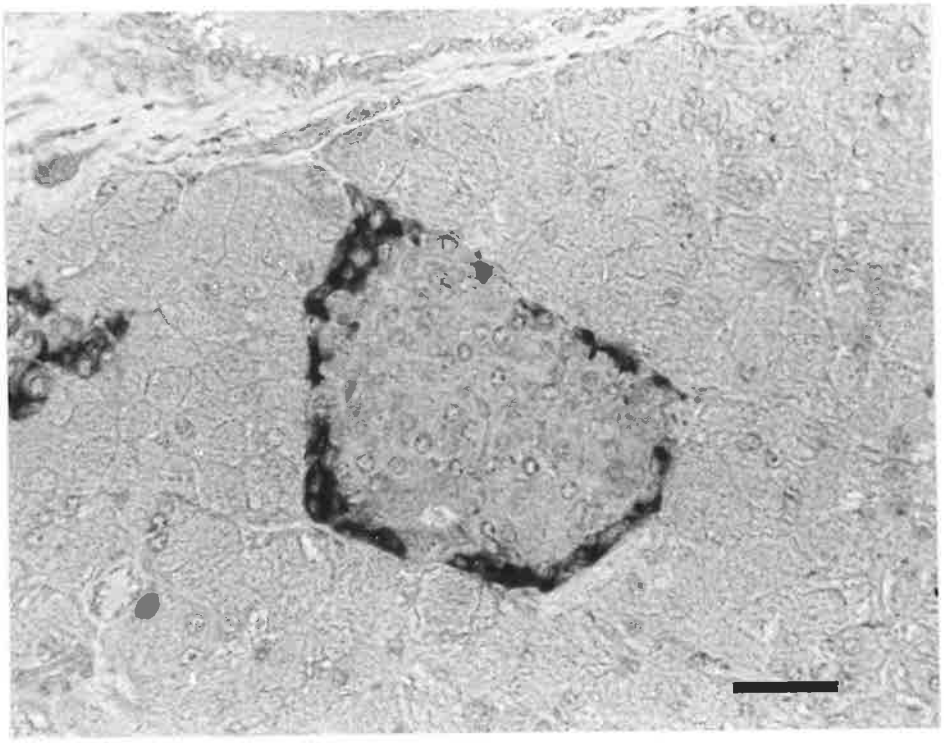
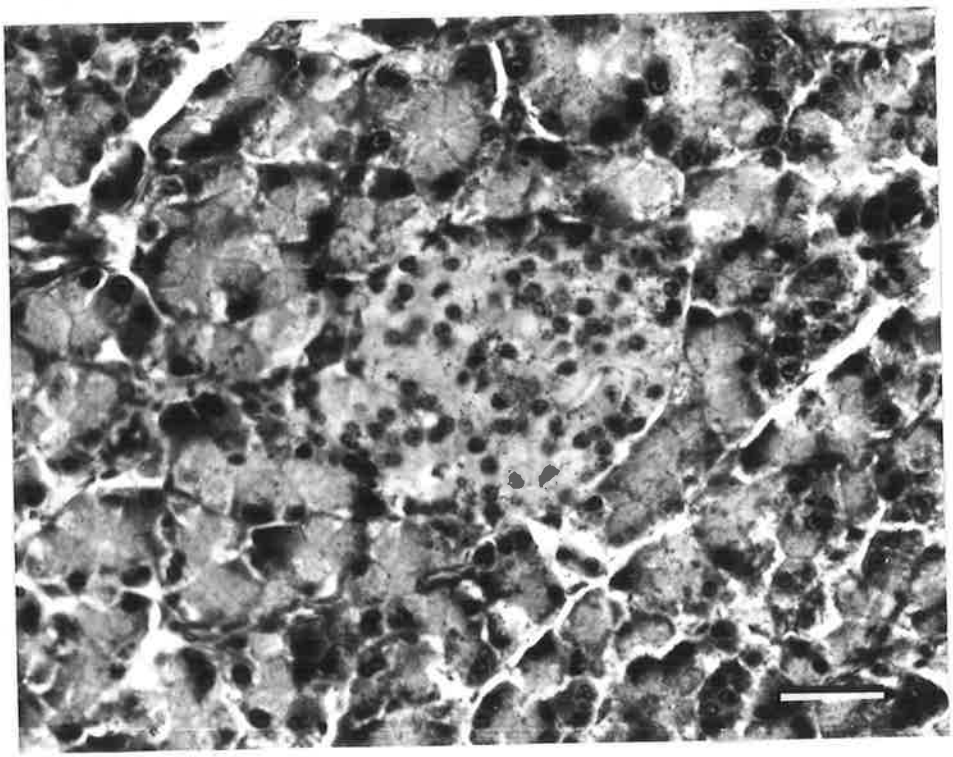


PLATE 23. Islet tissue of pancreas of water rat (Hydromys chrysogaster) stained with phosphotungstic acid haematoxylin showing peripheral A ce (x20). Scale 50um.



(h) Quantitative estimation of A cells in pancreas

In this study the A cells constitute 18.7% in echidna and show a uniform distribution in all regions (Plate 19).

The A cells secrete glucagon, the hyperglycemic principle (Hellerstrom, Hellman, Petersson and Alm, 1964). Crude insulin preparations made from echidna pancreas, exhibited a glucagon effect, suggesting that more glucagon than insulin is stored in the pancreas (Griffiths, 1965, 1978). There is no other literature to report any work done on the endocrine pancreas in prototherian mammals.

In this study, A cells constitute 31.5% in possum (Plate 20) and they are uniformly distributed. One report has shown A cells to be 42.5% in possum (White and Harrop, 1975), otherwise very little information exists about the cellular composition in marsupials. One report showing preponderance of A cells (67.2%) has been reported in grey kangaroo (White and Harrop, 1975). This study confirms the above finding in showing a preponderance of A cells (33.6%) (Plate 21) with uniform distribution. Grey kangaroos use volatile fatty acids as their energy source, rather than glucose (Moir, Somers and Waring, 1956; Barker, 1961). Ruminant and ruminant-like herbivores are better equipped to deal with hypoglycemic rather than hyperglycemic conditions, by virtue of their excess of glucagon producing A cells in the islets (Barker, 1961; Harrop and Barker, 1972; White and Harrop, 1975).

In this study on the Australian desert rodent, hopping mouse, the A cells constitute 22% and are situated in the periphery of the islet (Plate 22) and diminished in the head or duodenal region. In other arid environment species, as in the kangaroo mouse (**Microdipodops pallidus**), the A cells have been shown to occupy a central position in the islets (Quay, 1960; Falkmer and Patent, 1972).

Study on the water rat (**Hydromys chrysogaster**) in this investigation, shows the A cells to be situated in the periphery of the islet and constitute 18.1% (Plate 23) and diminished in the head or duodenal region. The A cells constitute 30% in the rat, (Alm and Hellman, 1964; White and Harrop, 1975),

PLATE 24. Islet tissue of pancreas of possum (Trichosurus vulpecula) using immunoperoxidase technique for somatostatin showing D cells (x20). Scale 50um.

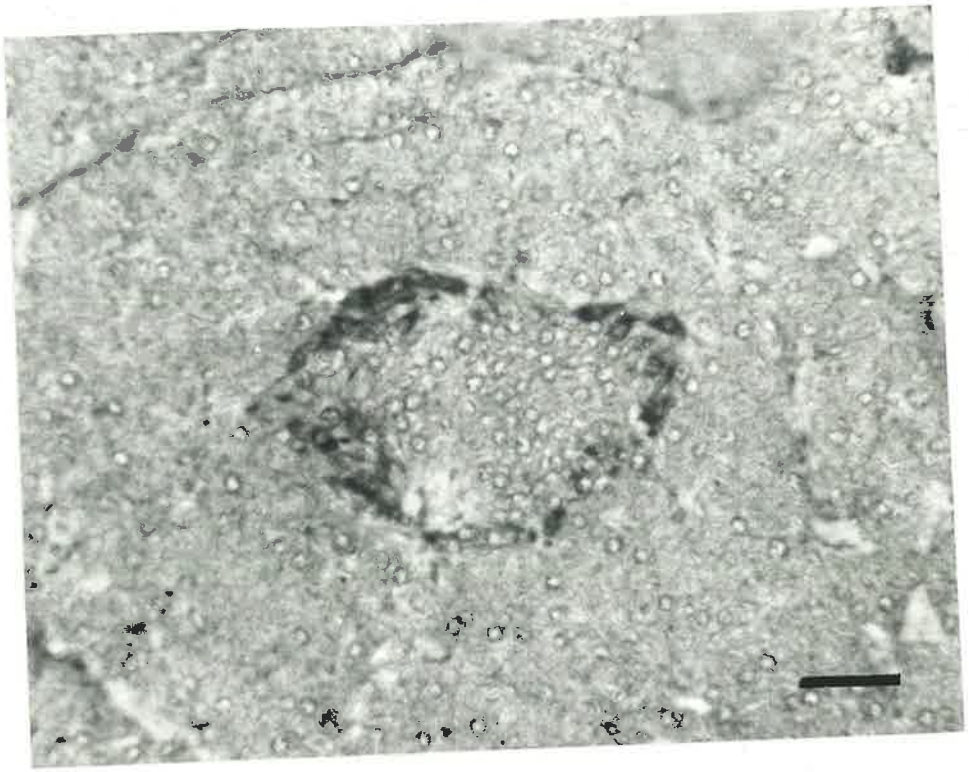


PLATE 25. Islet tissue of pancreas of grey kangaroo (Macropus fuliginosus) stained with the modified Davenport silver technique showing D cells (x20). Scale 50um.

PLATE 26. Islet tissue of pancreas of hopping mouse (Notomys alexis) using immunoperoxidase technique for somatostatin showing D cells (x20). Scale 50um.

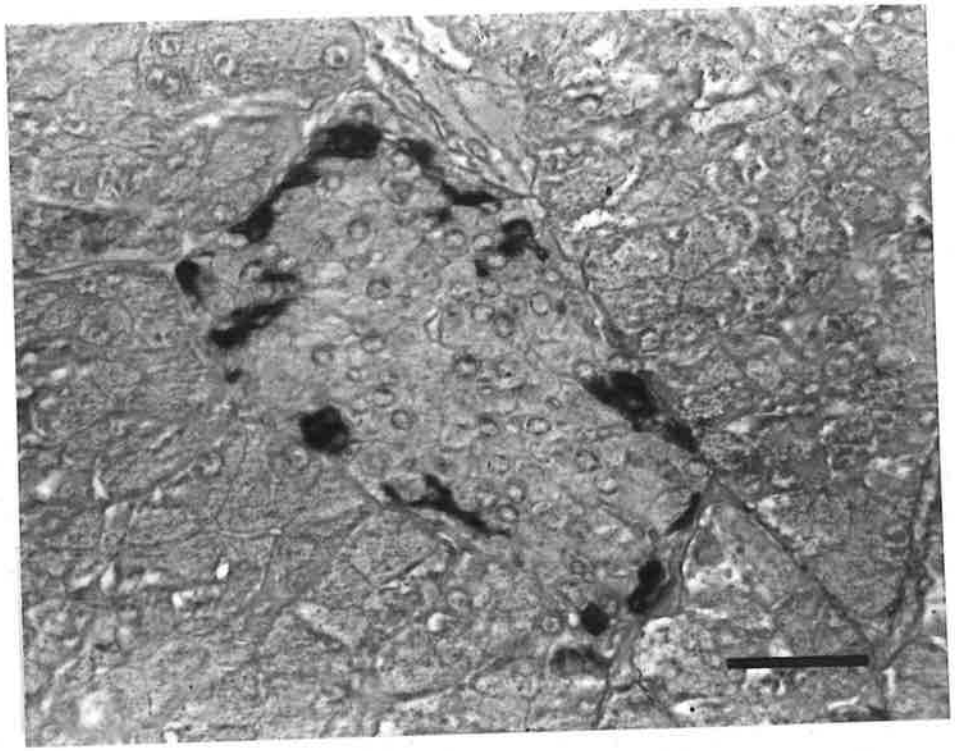
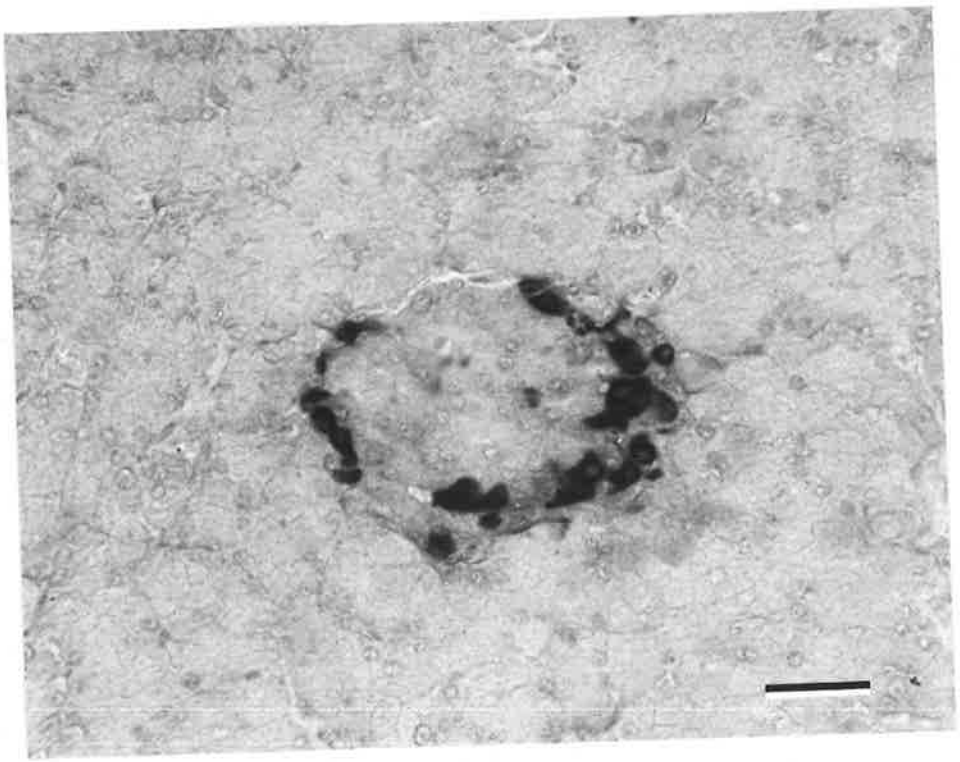


PLATE 27. Islet tissue of pancreas of water rat (Hydromys chrysogaster) using immunoperoxidase technique for somatostatin showing D cells (x20) Scale 50um.



27.5% in the guinea pig (Alm and Hellman, 1964; White and Harrop, 1975), 20% in the rabbit (Ferner and Kern, 1969; White and Harrop, 1975), 39% in the beluga whale (Quay, 1957; White and Harrop, 1975), 25% in the dog (Hunt, 1936; White and Harrop, 1975), 16.8% in the cat (Petkov, 1967; White and Harrop, 1975), 44% in the horse (Ferner and Kern, 1967; White and Harrop, 1975), 20% in cattle (Petkov, Gospodinov and Galabova, 1970; White and Harrop, 1975), 60% in sheep (Alm and Hellman, 1964; White and Harrop, 1975), 19.5% in the pig (Alm and Hellman, 1964; White and Harrop, 1975), and 33% in the monkey **Macaca cynomolgus** (Alm and Hellman, 1964; White and Harrop, 1975).

The diminished number of A cells in the head or duodenal region of pancreas in hopping mouse and water rat fits the pattern of regional heterogeneity of cell types within the mammalian endocrine pancreas. This hypothesis has as one of its concepts, the element that the head or duodenal portion has islets that are principally B cells and PP cells with occasional A cells (Bonner-Weir and Weir, 1979).

(i) Quantitative estimation of D cells in pancreas

In echidna, (**Tachyglossus aculeatus**), there is no evidence of D cells in the specimens investigated, although positive results were obtained with control specimens. Studies in the department have shown the presence of D cells in different tissue samples using an immunoperoxidase technique (Barbour, unpublished observation).

The D cells secrete somatostatin (Dubois, 1975). In this study, the cells constitute 11.1% in possum (Plate 24) (a negative result was obtained on some sections), 18.4% in grey kangaroo (Plate 25), 18.5% in hopping mouse (Plate 26), 13.5% in water rat (Plate 27).

(j) Quantitative estimation of PP cells

In echidna (**Tachyglossus aculeatus**), PP cells are found scattered among the exocrine cells and within the islets (Plate 28) and constitute 26.6%. About 80% of these PP cells are in the islets and represent 21.1% of islet

PLATE 28. Islet tissue of pancreas of echidna (Tachyglossus aculeatus) using immunoperoxidase technique for pancreatic polypeptide showing PP cells (x20). Scale 50um.

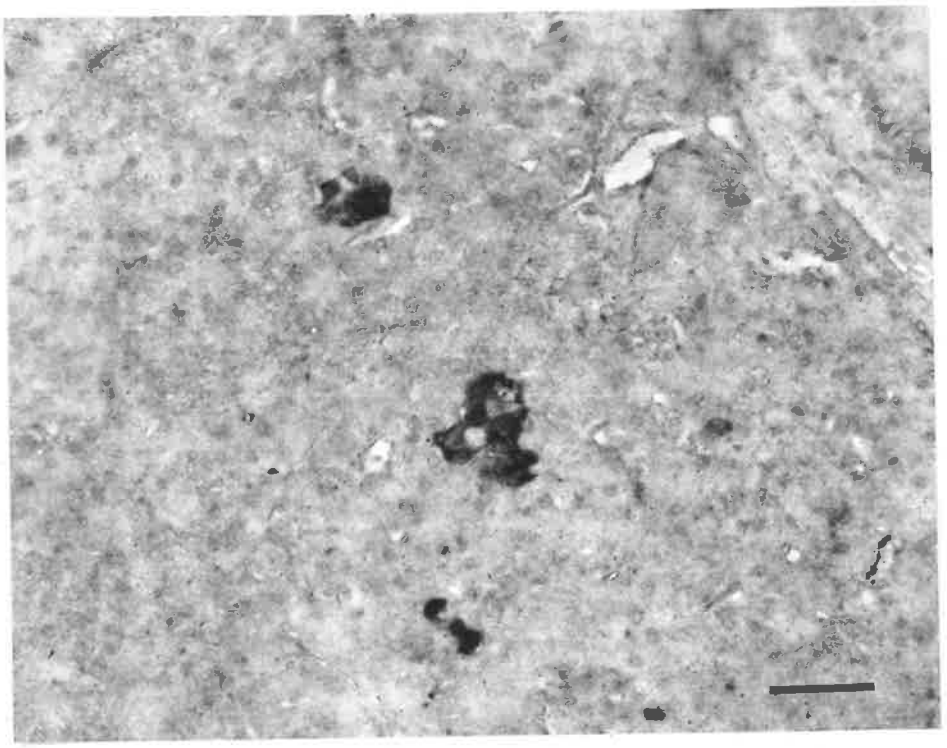


PLATE 29. Islet tissue of pancreas of possum (Trichosurus vulpecula) using immunoperoxidase technique for pancreatic polypeptide showing PP cells (x20). Scale 50um.

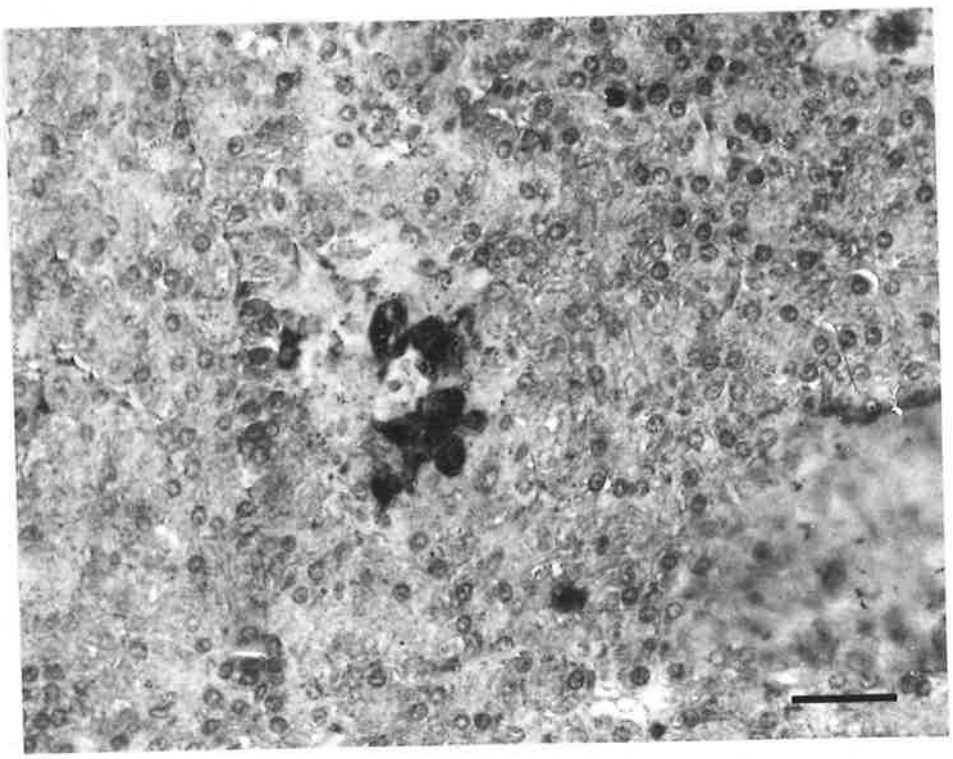
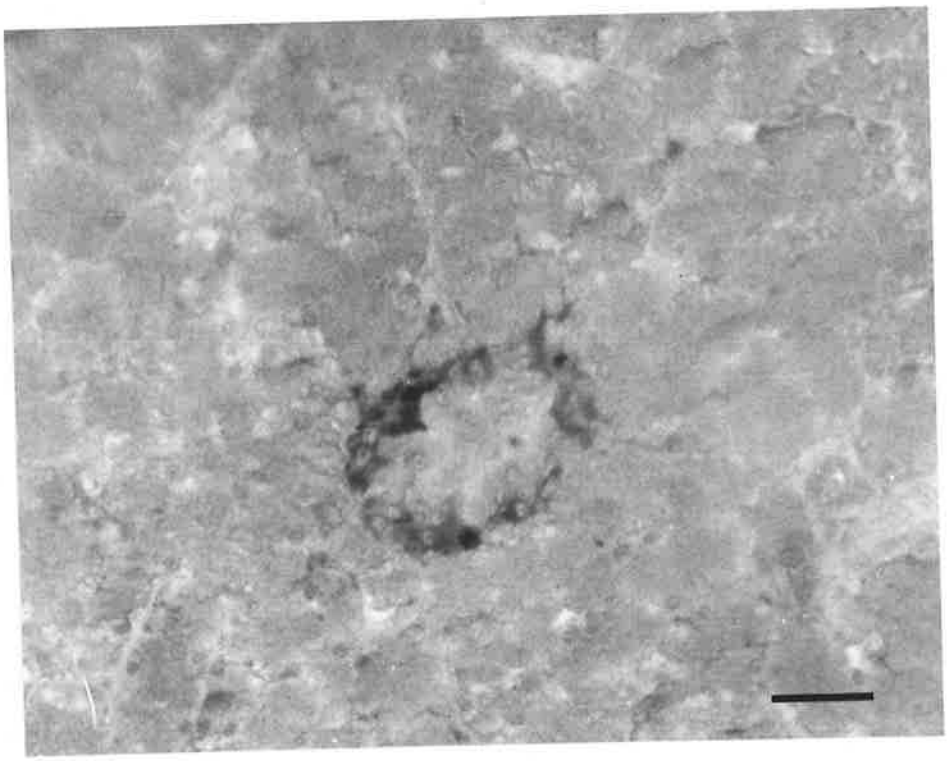


PLATE 30. Islet tissue of pancreas of water rat (Hydromys chrysogaster) using immunoperoxidase technique for pancreatic polypeptide showing peripheral PP cells (x20). Scale 50um.



cell volume. The rest equivalent to an additional 5.5% of islet cell volume, are found interacinar in position. The same proportion was present in an interacinar position in all regions. Of the 26.6% of PP cells a greater proportion was found in the head region. Other studies in the department have shown PP cells present and scattered among the acini and in the islets in other specimens of this species (Barbour, unpublished observation).

In possum (**Trichosurus vulpecula**), PP cells are found scattered among the acini and within the islets (Plate 29) and constitute 23.2%. About 66% of these PP cells are in the islets and represent 15.1% of islet cell volume. The rest equivalent to an additional 8.1% of islet cell volume are found interacinar in position. Of the 23.2% of PP cells a greater proportion was found in the head region. The same proportion was present in an interacinar position in all regions. Other studies in the department have shown many PP cells present and interacinar in position and also present in the islets (Barbour, unpublished observations).

In grey kangaroo (**Macropus fuliginosus**), there was no evidence of PP cells in the entire series investigated, although positive results were obtained with control specimens.

In hopping mouse (**Notomys alexis**), there was no evidence of PP cells in the entire series investigated, although positive results were obtained with control specimens.

In water rat (**Hydromys chrysogaster**), PP cells were found scattered among the exocrine cells and peripherally situated in the islets (Plate 30) and constitute 26.9%. About 75% of these PP cells are in the islets and represent 19.8% of islet cell volume. The rest equivalent to an additional 7.1% of islet cell volume are found interacinar in position. Of the 26.9% of PP cells a greater proportion was found in the head region. The same proportion was present in an interacinar position in all regions.

PP cells are rare in the gastro-intestinal mucosa and occur predominantly in the pancreas (Larsson, Sundler and Hakanson, 1975, 1976). In most species they occur both within the islets and scattered among the exocrine cells

(Gepts, Baetens, DeMey, 1978) and this observation is confirmed in the species investigated in this study. They are preferentially located in the periphery of the islet (Larsson, Sundler and Hakanson, 1975, 1976; Orci, Baetens, Ravazzola, Stefan and Malaisse-Lagae, 1976), and this finding is confirmed in the water rat (**Hydromys chrysogaster**) of this study.

This distribution of PP cells in neonatal humans was 79% of PP cells in the islet (Rahier, Wallon, Gepts and Haot, 1979; Floyd and Vinik, 1981), and more abundant in the head of pancreas. Malaisse-Lagae, Stefan, Cox, Perrelet and Orci (1979) have shown that systematic sampling of human necropsy pancreases has revealed that PP cells are the most abundant cell type in the posterior part of the pancreatic head. This regional predominance is confirmed in the present study in echidna, possum and water rat. It is known that this part of the pancreas represents the ventral primordium of the pancreas and this is the embryological basis for the greater number of PP cells in the head of the pancreas (Rahier, Wallon, Gepts and Haot, 1979; Floyd and Vinik, 1981). It has been suggested in the mammalian pancreas that the PP cells are concentrated to a small region in the lower dorsal part of the head of the pancreas, close to the gut mucosa which is equipped with a separate vascular supply (Falkmer, 1979).

Regional concentration of PP cells is highest in the right lobe of the canine pancreas (Gersell, Gingerich and Grieder, 1979). The F cell of the dog pancreas has been identified as the specific cell type containing PP (Greider, Gersell and Gingerich, 1978).

Paulin and Dubois (1978) consider that PP is the most recently evolved of the four main islet hormones. PP is the last cell type to appear during foetal life. The PP cells have no physiological function until an exocrine gland has developed and the function of the PP cell is associated with the function of the exocrine pancreas and gastrointestinal tract (Falkmer and Stefan, 1978). PP is a hormone involved in regulatory processes for food intake and digestion (Falkmer, 1979).

(k) Discussion on total islet volume

Echidna (Tachyglossus aculeatus)

The B cells constitute 31.5% , A cells 18.7% and PP cells 21.1%. The total of these is 71.3%. Stained sections showed negative results for D cells.

Possum (Trichosurus vulpecula)

The B cells constitute 59.5%, A cells 31.5%, D cells about 11.1% and PP cells 15.1%. The total of these is 117.2% (this result could be due to missing values and patchy results for D cells).

Grey kangaroo (Macropus fuliginosus)

The B cells constitute 22.8%, A cells 33.8% and D cells 18.4%. The total of these is 75.0%. The other components of the islet are reticular cells and blood capillaries. Stained sections showed negative results for PP cells.

Hopping mouse Notomys alexis)

The B cells constitute 24.2%, A cells 22% and D cells 18.5%. The total of these is 64.7%. The other components of the islet are reticular cells and blood capillaries. Stained sections showed negative results for PP cells.

Water rat (Hydromys chrysogaster)

The B cells constitute 24.1%, A cells 18.1%, D cells 13.5% and PP cells 19.8%. The total of these is 75.5%. The other components of the islet are reticular cells and blood capillaries.

(1) Comparative staining

Immunoperoxidase for insulin and aldehyde fuchsin

Same sections of water rat stained with immunoperoxidase for insulin (Plate 3a,4a), decolourised and stained with aldehyde fuchsin (Plate 3b,4b), have shown agreement on quantitation. The photographs taken show staining of the same cells.

Immunoperoxidase for glucagon and phosphotungstic acid haematoxylin (PTAH)

Same sections of grey kangaroo stained with immunoperoxidase for glucagon (Plate 5a,6a) decolourised and stained with PTAH (Plate 5b,6b) have shown agreement on quantitation. The photographs taken show staining of the same cells.

Immunoperoxidase for glucagon and Grimelius' silver nitrate stain

Some sections of possum stained with immunoperoxidase for glucagon (Plate 7) were quantitated, decolourised and stained with Grimelius' silver nitrate stain that showed negative results. The prior treatment of the tissue with immunoperoxidase for glucagon could have resulted in the negative result.

Immunoperoxidase for somatostatin and Epple's modification (Epple, 1967) of modified Davenport technique (Hellerstrom and Hellman, 1960)

Some sections of grey kangaroo, stained with immunoperoxidase for somatostatin (Plate 8), decolourised and stained by the Epple's modification (Epple, 1967) of the modified Davenport technique (Hellerstrom and Hellman, 1960) showed negative results. The prior treatment of the tissue with immunoperoxidase techniques could have resulted in the silver stains being negative.

Schweisthal, Schweisthal and Frost (1978) have shown an argyrophilic fourth cell in the rat pancreatic islet can be differentiated from other silver-staining cells by using a modification of the Grimelius' aqueous silver nitrate technique. Restaining of the tissues using fluorescent techniques with anti-HPP (human pancreatic polypeptide) serum results in bright fluorescence in the fourth cell type. The results of this study present further evidence that there are four cell types in the pancreatic islet of the rat and that the Grimelius' method is not specific for A cells and stains both glucagon and pancreatic polypeptide immunoreactive cells.

Schweisthal, Clark and Shevell (1981) used three staining techniques which were compared to determine whether they were staining the same cell in the pancreatic islet of the rat. The staining techniques used were Hellerstrom-Hellman alcoholic silver nitrate, pseudoisocyanin, and the immunofluorescent technique for somatostatin. These comparisons were made by using staining and restaining procedures on the same islet. The results demonstrated that all three procedures stained the delta cell of the rat pancreatic islet.

Wagner and McKeown (1981) have done a histological study of the pancreatic islets in rainbow trout, **Salmo gairdneri.** Four different cell types were identified. They were B, D and A and PP cells. They have shown that the silver impregnation technique of Hellerstrom and Hellman (1960) stained D cells but their number were fewer than the population of cells revealed subsequently by the PAP technique using antisomatostatin serum.

Wilander and Westermark (1976) observed argyrophil cells in the human foetal pancreas with the silver stain of Grimelius (1968), restained with the modified Davenport alcoholic silver nitrate method (Hellerstrom and Hellman, 1960). In the human foetal pancreatic tissue, there was a more frequent overlapping between two silver stains than in adult pancreatic islets (Grimelius, 1968). This was attributed to a difference in the staining properties of A and D cells or to an increased occurrence of other types of argyrophil cells in the human foetal pancreas. Differences in pH values, concentrations and solvents as well as in the composition of the reduction solutions in the silver impregnations of Hellerstrom and Hellman and of Grimelius are responsible for the discrepant staining reactions (Hellerstrom and Hellman, 1960; Grimelius, 1968).

For the staining of the B cells in the water rat, it has been shown in this study that the same cells stain with immunoperoxidase for insulin and aldehyde fuchsin. Therefore, it is acceptable to use aldehyde fuchsin.

In staining A cells in the grey kangaroo, it has been shown in this study that the same cells stain with immunoperoxidase for glucagon and PTAH. Quantitation of the same tissue stained with both stains are in agreement. Therefore it is acceptable to use PTAH.

In the possum, A cells have been stained with Grimelius' silver (Grimelius, 1968) and assessed. Sections stained with immunoperoxidase for glucagon and quantitated, have shown negative results when decolourised and stained with Grimelius' silver. For the assessment of D cells in grey kangaroo, Epple's modification (Epple, 1967) of the modified Davenport technique (Hellerstrom and Hellman, 1960) has been used for quantitation.

Sections stained with immunoperoxidase for glucagon and somatostatin and quantitated have shown negative results when decolourised and stained with Grimelius' silver (1968), and Epple's modification (1967) of the Hellerstrom and Hellman stain (1960). Results on quantitation with the immunocytochemical stains show agreement with the results on quantitation with the tinctorial stains, and this comparison is on different sections. The difficulty encountered in the use of tinctorial stains may be due in part to the lack of specificity of these stains for the peptide hormones in the cell (Wagner and McKeown, 1981). Wilander, Lundquist, Westermark and Grimelius, (1980) have demonstrated that Grimelius' (1968) silver impregnation technique stains both glucagon and pancreatic polypeptide cells. The Hellerstrom and Hellman stain is not even understood (Klein and Lange, 1977; Wagner and McKeown, 1981). Furthermore, these staining techniques have been optimised in other laboratories, using other species (generally mammals) and may be followed verbatim. It is for these reasons that the immunocytochemical technique appears to be a preferable alternative (Wagner and McKeown, 1981), but is an expensive method. In this study a quantitative comparison (on different sections), have shown agreement with the tinctorial stains and the immunocytochemical stains, and this provides the evidence for the reliability of the Grimelius silver stain and the Hellerstrom and Hellman stain, in this study.

(m) General discussion

Non ruminant herbivorous marsupials, like the brush-tailed possum (**Trichosurus vulpecula**), rely on glucose as an energy source (Moir, Somers and Waring, 1956; Barker, 1961; White and Harrop, 1975). Diabetes with an upset to their carbohydrate metabolism, induced by the injection of alloxan, is tolerated better by nonruminant than ruminant or ruminant-like species (Adams and Bolliger, 1954; Griffiths, McIntosh and Leckie, 1969; White and Harrop, 1975).

The digestion and metabolism of carbohydrates in macropodid marsupials is of a similar pattern to that found in ruminants (Moir, Somers, Sharman and Waring, 1954). In ruminants the fermentation of carbohydrates in the rumen restricts the glucose available for absorption. In kangaroos, though they are not truly ruminant, it is probable they 'chew the cud' or ruminate (Moir, Somers and Waring, 1956). They have a pregastric fermentation chamber akin to that of true ruminants, and occupy an intermediate position between ruminant and nonruminant herbivores (Moir, Somers and Waring, 1956; Barker, 1961; Harrop and Barker, 1972; White and Harrop, 1975). Grey kangaroos use volatile fatty acids as their energy source and this shows the significance of the present finding of a high proportion of glucagon producing A cells. A ruminant type digestion with its exploitation of synthetic and fermentative abilities of bacteria in foregut, where the products of these activities are accessible to absorption, could explain the well known ability of kangaroos to survive on poor grazing (Moir, Somers and Waring, 1956).

Studies on the physiology of the spinifex hopping mouse have shown its ability to survive without free water (Watts and Aslin, 1981). It can utilise water produced during metabolism. Modifications or specialisations in carbohydrate metabolism or utilisation associated with climatic adaptations may be related to modifications of blood cholesteral level and blood non-protein nitrogen level (Quay, 1960). The production and conservation of metabolic water may be greater from carbohydrate and fatty food rather than protein food materials (Quay, 1960). Comparative studies of the significance of the islets and their hormones to the metabolic activities of adult desert rodents are valuable (Quay, 1960).

CONCLUSION

The pancreatic islets are unique to vertebrates. They represent an anatomic specialization of the widely scattered endocrine cells of the open or closed type in the mucosa of the gut and bile ducts. The first islet organ is in jawless fish (hagfish and lamprey) and is a two hormone organ, producing

insulin and somatostatin. At the level of holocephalan cartilaginous fish (rat fish), the first exocrine pancreatic gland appears. The endocrine pancreas is a three-hormone organ, producing insulin, somatostatin and glucagon. As the next step in phylogeny, in plagiostomian cartilaginous fish (sharks, rays), the endocrine pancreas has become a four hormone organ, now also producing PP (Falkmer, 1985).

The phylogenetic order of appearance of the four islet hormone cells in the endocrine pancreas is: insulin, somatostatin, glucagon and PP (Falkmer, 1985).

The observation that the four types of islet hormone producing cells are not evenly distributed has a firm phylogenetic basis (Falkmer, 1985). It may be considered in relation to the hypothesis of Bonner-Weir and Weir (1979) of the mammalian endocrine pancreas.

The first element of the hypothesis suggests a pattern of regional heterogeneity and has shown the presence of a large proportion of islets of Langerhans in the tail or splenic portion of the pancreas (Bonner-Weir and Weir, 1979). This study has shown a uniform distribution of islet tissue in prototherian and metatherian mammals and a greater proportion of islet tissue in the tail or splenic portion of the pancreas of the two species of Eutherian mammals studied which fits the hypothesis.

The third element of this hypothesis postulates regional differences in the distribution of islet cell types showing a lower proportion of PP cells in the same region of the pancreas. This study shows, in regard to the A cells , that no regional differences in the distribution of islet cell types exist in prototherian and metatherian mammals only Eutherian mammals, (hopping mouse and water rat), showing a low proportion of A cells in the head or duodenal region. With regard to the PP cells, regional differences in distribution exist in prototherian (echidna), a metatherian (possum), and a eutherian (water rat) which exhibit a greater proportion of PP cells in the head or duodenal region. Thus, the Eutherian mammals fit Bonner-Weir and Weir's hypothesis (1979) of the mammalian endocrine pancreas with regard to the first

element of the hypothesis and the A cell component of the third element, so far as this work has shown. With regard to the PP cell component of the third element of the hypothesis, one member of each of the prototherian (echidna), metatherian (possum) and eutherian (water rat) group fit the hypothesis in this investi- gation. This pattern of organisation has an embryological basis, which shows a greater proportion of islet tissue exists in the parts of the pancreas derived from the dorsal anlage (Bonner-Weir and Weir, 1979) and a greater proportion of PP cells in the part of the pancreas that represents the derivative of the ventral primordium of the pancreas (Floyd and Vinik, 1981) and is equipped with a separate vascular supply (Falkmer, 1979). The pan- pancreatic regions that provided the two different types of islets have been shown to be irrigated by different arterial system in the rat (coeliac artery for the dorsal region and superior mesenteric artery for the ventral region) (Baetens, Malaisse-Lagae, Perrelet and Orci, 1979). This inverse relationship between glucagon and PP cells means that caution must be exercised when inter- interpreting functional or morphological observations using different pancreatic functions (Orci, Baetens, Ravazzola, Stefan and Malaisse-Lagae, 1976). This may also have a functional significance which needs further investigation (Bonner-Weir and Weir, 1979).

This comparative study of the endocrine pancreas in Australian mammals has been summarised (Table X) and fills a gap in the literature of the gastro- entero-pancreatic system and hence a contribution.

REFERENCES

Abad, M.E., Agulleiro, B and Rombout, J.H.W.M. (1986). An immunocytochemical and ultrastructural study of the endocrine pancreas of **Sparus auratus** L. (Teleosti). Gen. Comp. Endocr. 64:1-12.

Adams, D.M. & Bolliger, A. (1954). Observations on carbohydrate metabolism and alloxan diabetes in a marsupial (**Trichosurus vulpecula**). Aust. J. Exp. Biol. Med. Science 22:101-111.

Adelson, J.W. (1971). Enterosecretory proteins. Nature 229:321-324.

Agulleiro, B., Garcia Ayala, A. & Abad M.E. (1985). An immunocytochemical and ultrastructural study of the endocrine pancreas of **Pseudomys scripta elegans** (Chelonia). Gen. Comp. Endocr. 60:95-103.

Alm, G. & Hellman (1964). Distribution of the two types of A cells in the pancreatic islets of some mammalian species. Acta Endocr. 46:307-316.

Alumets, J., Hakanson, R. & Sundler, F. (1978). Distribution ontogeny and ultrastructure of pancreatic polypeptide (PP) cells in the pancreas and gut of the chicken. Cell Tiss. Res. 194:377-386.

Andrew, A. (1976a). Pancreatic APUD cells in older chick embryos with special reference to their identity. Histochemistry 49:213-219.

Andrew, A. (1976b). An experimental investigation into the possible neural crest origin of pancreatic APUD (islet) cells. J. Embryol. Exp. Morph. 35:577-593.

Andrew, A. & Kramer, B. (1979). An experimental investigation into the possible

origin of pancreatic islet cells from rhombencephalic neuroectoderm. J. Embryol. Exp. Morph. 52:23-38.

Arimura, A., Sato, H., Dupont, A. & Niche, N. (1975). Somatostatin: Abundance of immunoreactive hormone in rat stomach and pancreas. Science 189: 1007-1009.

Baetens, D., Malaisse-Lagae, F., Perrelet, A. & Orci, L. (1979). Endocrine pancreas: three dimensinal reconstruction shows two types of islets of Langerhans. Science 206:1323-1325.

Baetens, D., Rufener, C. & Orci, L. (1976). Bovine pancreatic polypeptide (BPP) in the pancreas and in the gastrointestinal tract of the dog. Experientia 32:785.

Bargmann, W. & Creutzfeldt, W. (1949). Zur Morphologie des Alloxan diabetes. Klin Wochenschr. 1949:268-271.

Barker, J. (1961). The metabolism of carbohydrate and volatile fatty acids in the marsupial **Setonix brachyurus**. Q. Jl. exp. Physiol. 46:54-68.

Barrington, E.J.W. (1964). Hormones and evolution. London: Academic Press, pp. 909-911.

Barron, S.S. (1948). Significance of the beta granules in the islets of Langerhans of the pancreas. Arch. Path. 46:159-163.

Baum, J., Simms, B.E., Unger, R.H. & Madison, L.L. (1972). Localization of glucagon in the alpha cells in the pancreatic islet by immunofluorescent techniques. Diabetes 11:371-374.

Baumann, A. (1939). Uber das sogenannte insulare Gangorgan in der Bauchspeicheldruse der Saugetiere insbesondere des Pferdes. Z. Microsk. Anat. Forsch 46:223-248.

Bencosme, S.A. & Liepa, E. (1955). Regional differences of the pancreatic islet. Endocrinology 57:588-593.

Bensley, R.R. (1911). Studies on the pancreas of the guinea pig. Am. J. Anat. 12:297-388.

Bloom, W. (1931). A new type of granular cell in the islets of Langerhans of man. Anat. Rec. 49:363-371.

Boden, G., Sivitz, M., Owen, O.E., Essa-Kouwar, N. & Landor, J.H. (1975). Somatostatin suppresses secretin and pancreatic exocrine secretion. Science 196:163-165.

Bonner-Weir, S, & Like, A.A. (1980). A dual population of islets of Langerhans in bovine pancreas. Cell Tiss. Res. 206:157-170.

Bonner-Weir, S. & Weir, G.C. (1979). The organization of the endocrine pancreas : a hypothetical unifying view of the phylogenetic differences. Gen. Comp. Endocr. 38:28-37.

Boquist, L. & Falkmer, S. (1970). The significance of agranular and ciliated islet cells. In: 'The structure and metabolism of the pancreatic islets' (eds. S. Falkmer, B. Hellmann & I.B. Taljedal). Oxford: Pergamon Press. pp. 25-35.

Bowie, D.J. (1924). Cytological studies of the islets of Langerhans in a teleost. Anat. Rec. 29:57-73.

Brinn, J. & Epple, A. (1972). Structure and ultrastructure of the specialized islet organ of the American eel, **Anguilla rostrata**. Anat. Rec. 172:277.

Buchan, A.M.J. & Polak, J.M. (1980). The classification of the human gastro-enteropancreatic endocrine cells. Invest. Cell. Pathol. 3:51-71.

Buffa, R., Capella, C., Solcia, E., Frigerio, B. and Said, S.J. (1977). Vaso-active intestinal peptide (VIP) cells in the pancreas and gastrointestinal mucosa. Histochemistry 50:217-227.

Buffa, R., Solcia, E., Capella, C., Fontana, P., Trinci, E. & Said, S.J. (1976) Immunohistochemical detection of vasoactive intestinal peptide (VIP) in a specific endocrine cell of the pancreatic islets. Rendic. Gastroenterol. 8:73-75.

Burkl, W. (1951). Sind die Silberzellen der Langerhansschen Inseln mit den $\mathcal{A}$-Zellen identisch? Acta Anat. 12:358-382.

Carpenter, A.M. & Lazarow, A. (1962). Component quantitation of tissue sections. II. A study of the factors which influence the accuracy of this method. J. Histochem. cytochem. 10:324-328.

Carrillo, M., Zanuy, S., Duve, H. & Thorpe, A. (1986). Identification of hormone-producing cells of the endocrine pancreas of the sea bass (**Dicentrachus labrax**), by ultrastructural immunocytochemistry. Gen. Comp. Endocr. 61:287-301.

Cavallero, C. & Solcia, E. (1964). Cytologic and cytochemical studies on the pancreatic islets. In: 'The Structure and metabolism of the pancreatic islets' (eds. S.E. Brolin, B. Hellman & H. Knutson). London: Pergamon Press. pp.83-97.

Cegrell, L. & Falck, B. (1969). A catecholamine storage in the pancreatic A_2 cells of owl monkey (**Aotes trivirgatus**). Z. Zellforsch 94:530-533.

Clark, E. (1913). The number of islets of Langerhans in the human pancreas. Anat. Anz. 43:81-94.

Clausen, D.M. (1953). Beitrag zur Phylogenie der Langerhansschen Inseln der Wirbeltiere. Biol. Zentralbl. 72:161-182.

Deconinck, J.F., Potvliege, P.R. & Gepts, W. (1971). The ultrastructure of the human pancreatic islets. (I) The islets of adults. Diabetologia 7:266-282.

Diamare, V. (1899). Studii comparativi sulle insole di Langerhans del pancreas Int. Mschr. Anat. Physiol. 16:155-209.

Dieterlen-Lievre, F. (1970). Tissue exocrine et endocrine du pancreas chez l'embryon de poulet: origine et interactions tissulaires dans la differenciatin. Develop. Biol. 22:138-156.

Dubois, M.P. (1975). Immunoreactive somatostatin is present in discrete cells of the endocrine pancreas. Proc. Nat. Acad. Sci. 72 No. 4:1340-1343.

Dunn, J.S., Sheehan, H.L. & McLetchie, N.G.B. (1943). Necrosis of islets of Langerhans produced experimentally. Lancet 1:484-487. Cited by Wellmann & Volk (1977).

Edwin, N. (1979). Quantitative estimation of islet tissue mass and beta and alpha cells of echidna pancreas. I.R.C.S. Med. Sci. 7:119.

Edwin, N. (1982). Quantitative estimation of islet tissue of pancreas in possums (**Trichosurus vulpecula**). J. Zool. Lond. 197:455-461.

Edwin, N. (1984). Quantitative estimation of islet tissue of pancreas in adult grey kangaroos (**Macropus fuliginosus**). J. Zool. Lond. 203:125-133.

Edwin, N. (1985). Quantitative estimation of islet tissue of pancreas in hopping mouse (**Notomys alexis**). J. Zool. Lond. (A) 206:191-201.

Edwin, N. (1985). Quantitative estimation of islet tissue of pancreas in water rat (**Hydromys chrysogaster**). J. Zool. Lond. (A) 207:277-288.

Edwin, N. (1987). Quantitative estimation of pancreatic polypeptide (PP) cells of islet tissue of pancreas in Australian mammals. J. Zool. Lond. (213: 665-671.

El Salhy, M. & Grimelius, L. (1981). Histological and immunohistochemical studies of the endocrine pancreas of lizards. Histochemistry 72:237-247.

Epple, A. (1964). Zur vergleichenden zytologie des Inselorgans. Verh. Dit. Zool. Gesell. 27:461-470.

Epple, A. (1966). Islet cytology in urodele emphibians. Gen. Comp. Endocr. 7:207-214.

Epple, A. (1967). A staining sequence for A, B and D cells of pancreatic islets. Stain. Technol. 42(2):53-61.

Epple, A. (1968). Comparative studies on the pancreatic islets. Endocr. Japon, 15:107-122.

Epple, A. (1969). The endocrine pancreas. In: 'Fish Physiology' (eds. W.S. Hoar & D.J. Randall). New York/London: Academic Press. Vol. 2, pp.275-319.

Epple, A. & Brinn, J.E. (1975). Islet histophysiology: evolutionary correlations. Gen. Comp. Endocr. 27:320-349.

Epple, & Brinn, J.E. (1980). Morphology of the islet organ. In: 'Hormones, Adaptation and Evolution' (eds. S. Ishii, T. Hirano & M. Wada). Japan Sci. Soc. Press, Tokyo:Springer-Verlag, pp. 213-220.

Epple, A. & Lewis, T.L. (1973). Comparative histophysiology of the pancreatic islets. Am. J. Zool. 13:567-590.

Erlandsen, S.L., Hegre, O.D., Parsons, J.A., McEvoy, R.C. & Elde, R.P. (1976). Pancreatic islet cell hormones. Distribution of cell types in the islet and evidence for the presence of somatostatin and gastrin within the D cell. J. Histochem. Cytochem. 24:883-897.

Faller, A. (1969). Electronenmikroskopische Differenzierung verschiedener Inselzelltypen im Pankreas normaler Albinoratten. Z. Zellforsch 97:226-248. Cited by Falkmer & Patent (1972).

Falkmer, S. (1961). Experimental diabetes research in fish. Acta Endocr. Supp. 59:1-122.

Falkmer, S. (1972). Insulin production in vertebrates and invertebrates. Gen. Comp. Endocr. Supp. 3:183-184.

Falkmer, S. (1979). Immunocytochemical studies of the evolution of islet hormones. J. Histochem. Cytochem. 27:1281-1282.

Falkmer, S. (1985). Comparative morphology of pancreatic islets in animals. In: 'The diabetic pancreas'. (Eds. A.W. Volk and E.R. Arquilla). Plenum Medical Book Company. New York. London. pp.17-52.

Falkmer, S., Emdin, S., Hava, N., Lundgren, G., Marques, M., Ostberg, Y., Steiner, D.F. & Thomas, N.W. (1973). Insulin in invertebrates and cyclostomes. Amer. Zool. 13:625-638.

Falkmer, S. & Marques, M. (1972). In: 'Glucagon: molecular physiology, clinical and therapeutic implications' (eds. P.J. LeFebvre & R.H. Unger). London/New York : Pergamonn Press. p.343.

Falkmer, S. & Ostberg, Y. (1977). Comparative morphology of pancreatic islets in animals. In: 'The diabetic pancreas' (eds. B.W. Volk & K.L. Wellmann). New York/London : Plenum Press. pp.15-57.

Falkmer, S. & Patent, G. (1972). Comparative and embryological aspects of pancreatic islets. In: 'Handbook of Physiology' (eds. D.F. Steiner & N. Freinkel). Vol.1 'The endocrine pancreas' Baltimore: Williams & Wilkins pp.1-23.

Falkmer, S. & Stefan, Y. (1978). Pancreatic polypeptide (PP) : phylogenetic aspects in gastrointestinal mucosa and endocrine pancreas. Scand. J. Gastroenterol, 13: Suppl. 49:59.

Falkmer, S. and Van Noorden, S. (1983). Ontogeny and phylogeny of the glucagon cell. In: Handbook of experimental pharmacology. (ed. P.J. LeFebvre). Springer, Berlin, Heidelberg, New York pp.81-119.

Falkmer, S. & Winbladh, L. (1964). An investigation on the pancreatic islet tissue of the hagfish (**Myxine glutinosa**) by light and electron microscopy. In: 'The structure and metabolism of the pancreatic islets' (eds. S.E. Brolin, B. Hellman and H. Knutson). Oxford : Pergamon Press pp. 17-32.

Faulk, W.P. & Taylor, G.M. (1971). An immunocolloid method for the electron microscope. Immunochemistry 8:1081-1083.

Ferner, H. (1938). Uber die Entwicklung der Langerhansschen Inseln nach der Geburt und die Bedentung der versilberbaren Zellen in Pankreas des Menschen. Z. mikr. anat. Forsch. 44:451-488. Cited by Miller (1942).

Ferner, H. & Kern, H. (1964). The islet organ of **Selachians**. In: 'Structure and metabolism of the pancreatic islets' (eds. S.E. Brolin, B. Hellman & H. Knutson). Oxford : Pergamon Press pp.3-10.

Ferner, H. & Kern, H. (1969). Die vergleichende Morphologie die Langerhansschen Inseln. Das Inselorgan der Saugetiere insbesondere der Laboratoriumstiere. In: 'Handbuch des Diabetes Mellitus, Pathophysiologie und Klinik' (ed. E.F. Pfeiffer). Munich: Lehmanns. Vol. 1, pp.39-62.

Floyd, J.C. & Vinik, A.I. (1981). Pancreatic polypeptide. In: Gut hormones (eds. S.R. Bloom & J.M. Polak) London/New York : Churchill Livingstone pp. 195-201.

Fontaine, J., LeLievre, C. and LeDouarin, N.M. (1977). What is the developmental fate of the neural crest cells which migrate into the pancreas in the avian embryo? Gen. Comp. Endocr. 33:394-404.

Forssmann, A. (1976). The ultrastructure of the cell types in the endocrine pancreas of the horse. Cell Tiss. Res. 167:179-195.

Forssmann, W.G., Helmstaedter, V. & Chance, R.E. (1977). Ultrastructural and immunohistochemical demonstration of pancreatic polypeptide-containing F cells in the stomach and pancreas of **Tupaia belangeri**. Cell Tiss. Res. 177:481-492.

Forssmann,W.G., Helmstaedter,V., Metz,J., Greenberg,J. & Chance,R.E. (1977). The identification of the F cell in the dog pancreas as the pancreatic polypeptide producing cell. Histochemistry 50:281-290.

Frye, B.E. (1962). Extirpation and transplantation of pancreatic rudiments of the salamanders, **Ambystoma punctatum** and **Eurycea bislineata**. Anat. Rec. 144:97-108.

Fuchs, H., Hahn von Dorsche, H. & Ziegler, M. (1984). Glucagon antibodies alter Langerhans islets. Biomed. Biochim. Acta 43:691-693.

Fujimoto, W.Y., Ensinck, J.W. & Williams, R.H. (1974). Somatostatin inhibits insulin and glucagon release by monolayer cell cultures of rat endocrine pancreas. Life Science 15:1999-2004.

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

Fujita, T. (1964a). Cytologic studies on the pancreatic islets of **Chimaera monstrosa**. In: 'The structure and metabolism of the pancreatic islets' (eds. S.E. Brolin, B. Hellman & H. Knutson). Oxford : Pergamon Press. pp.11-15.

Fujita, T. (1964b). The identification of the argyrophil cells of pancreatic islets with D cells. Arch. Histol. Jap. 25:189-197.

Fujita, T. (1968). D cell, the third endocrine element of the pancreatic islet. Arch. Histol. Jap. 29:1-40.

Fujita, T. (1973). Insuloacinar portal system in horse pancreas. Arch. Histol. Jap. 35:161-170.

Fujita, T. (1973). Gastro-entero-pancreatic system. Tokoyo : Igaku Shoin.

Gepts, W., Baetens, D. & DeMey, J. (1978). In: Gut hormones (Ed. S.R. Bloom). London/New York : Churchill Livingstone pp. 229-233.

Gersell, D.J., Gingerich, R.L. & Grieder, M.H. (1979). Regional distribution and concentration of pancreatic polypeptide in the human and canine pancreas. Diabetes 28:11-15.

Goldner, M.G. & Volk, B.W. (1956). The effect of hypophysectomy and of prolonged growth hormone adminstration on the pancreatic cells of the rat. Ciba Found. Colloq. Endocr. 9:75-88.

Goldsmith, P.C., Rose, J.C., Arimura, A. & Ganong, W.F. (1975). Ultrastructural localization of somatostatin in pancreatic islets of the rat. Endocrinology 97:1061-1064.

Gomori, G. (1939). Studies on cells of pancreatic islets. Anat. Rec. 74: 439-459.

Gomori, G. (1950). Aldehyde fuchsin: a new stain for elastic tissue. Am. J. Clin. Path. 20:665-666.

Grieder, M.H., Gersell, D.J. & Gingerich, R.L. (1978). Ultrastructural localization of pancreatic polypeptide in the F cell of the dog pancreas. J. Histochem. Cytochem. 26:1103-1108.

Griffiths, M. (1965). Digestion, growth and nitrogen balance in an egg-laying mammal, **Tachyglossus aculeatus**. Comp. Biochem. Physiol. 14:357-375.

Griffiths, M. (1968). Echidnas. London/New York : Pergamon Press. pp.123-124.

Griffiths, M. (1978). Biology of monotremes. New York/San Francisco/London: Academic Press. pp. 156-157.

Griffiths, M., McIntosh, D.L. & Leckie, R.M.C. (1969). The effects of cortisone on nitrogen balance and glucose metabolism in diabetic and normal kangaroos, sheep and rabbits. J. Endocr. 44:1-12.

Grimelius, L. (1968). The argyrophil reaction in islet cells of adult human pancreas studied with a new silver nitrate procedure. Acta Soc. Med. Upsal. 73:271-294.

Grimelius, L. (1968). A silver nitrate stain for α_2 cells in human pancreatic islets. Acta Soc. Med. Upsal. 73:243-270.

Grossner, D. (1968). Das inselorgan de Crossoptergiens **Latimeria chalumnae**. Z. Zellforsch 84:417-428.

Guha, B. & Ghosh, A. (1978). A cytomorphological study of the endocrine pancreas of some Indian birds. Gen. Comp. Endocr. 34:38-44.

Hamperl, H. (1932). Was sind orgentaffine Zellen. Virchows Arch. Path. Anat. 286:811-833.

Hard, W.L. (1944). The origin and differentiation of the alpha and beta cells in the pancreatic islets of the rat. Am. J. Anat. 75:369-403.

Harrop, C.J.F. & Barker, S. (1972). Blood chemistry and gastrointestinal changes in the developing red kangaroo (**Megaleia rufa, Desmarest**). Aust. J. Exp. Biol. Med. Sci. 50:245-249.

Hellerstrom, C. (1977). Growth pattern of pancreatic islets. In: 'The diabetic pancreas'. (Eds. B.W. Volk and K.F. Wellmann) New York/London : Plennum Press pp. 61-97.

Hellerstrom, C. & Hellman, B. (1960). Some aspects of silver inpregnation of the islets of Langerhans in the rat. Acta. Endocr. 35:518-532.

Hellerstrom, C., Hellman, B., Petersson, B. & Alm, G. (1964). The two types of α pancreatic cells and their relation to the glucagon secretion. In: 'The structure and metabolism of the pancreatic islets' (eds. S.E. Brolin, B. Hellman & H. Knutson). London/New York : Pergamon Press. pp. 117-130.

Hellerstrom, C., Petersson, B. & Hellman, B. (1960). Some properties of the B cells in the islets of Langerhans studied with regard to the position of the cells. Acta Endocr. 34:449-456.

Hellman, B. (1959). The frequency distribution of the number and volume of the islets of Langerhans in man. Acta Soc. Med. Upsal. 64:432-460.

Hellman, B. (1970). Methodological approaches to studies on the pancreatic islets. Diabetologia 6:110-120.

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

Hellman, B. & Hellerstrom, C. (1962). Cellular composition of the islets of Langerhans in the bullfrog, Rana catesbiana. Acta Anat. 48:149-155.

Hellman, B. & Hellerstrom, C. (1969). In: 'Handbuch des diabetes mellitus' (ed. E.F. Pfeiffer). Munich. Lehmanns p.89.

Hellman, B., Rothman, U. & Hellerstrom, C. (1962). Identification of a specific type of α cell located in the central part of the pancreatic islets of horse. Gen. Comp. Endocr. 2:558-564.

Hellman, B., Wallgren, A. & Hellerstrom, C. (1962). Two types of islet A cells in different parts of the pancreas of the dog. Nature 194:1201-1202.

Helmstaedter, V., Feurle, G.E. & Forssmann, W.G. (1976). Insulin, glucagon and somatostatin immunoreactive endocrine cells in the equine pancreas. Cell Tiss. Res. 172:447-454.

Henderson, J.R. (1969). Why are the islets of Langerhans? (Hypothesis) Lancet 11:469-471.

Hess, W. (1946). Zur histologischen Diabetes diagnose an den Langerhans' schen Inseln. Untersuchungen mit Versilberung nach Gros-Schultze. Schweiz. Z. Path. Bact. 9:46-60.

Hokfelt, T., Efendic, S., Hellerstrom, C., Johansson, O., Luft, R. & Akimura, A. (1975). Cellular localization of somatostatin in endocrine-like cells and neurons of the rat with special reference to the α_1 cells of pancreatic islets and to the hypothalamus. Acta Endocr. Supp. 200-5-41.

Hultquist, G.T. (1946). On the occurrence of s.c. silvercells in islets tumors and in the pancreas in cases of islet tumors. Gastroenterol. 71:193-209.

Hultquist, G.T., Dahlen, M. & Helander, C.G. (1948). Uber die Technik bei Darstellung und Zahlung der sog. Silberzellen in der Langerhans' schen Inseln. Schweiz. Z. Path. Bact. 11:570.

Hultquist, G.T. & Tegner, B. (1949). Beitrag zue Methodik bei Farbung von Granula in den Zellen der Langerhan' schen Inseln. Schweiz. Z. Path. Bakt. 12:717-722.

Hunt, T.E. (1936). Differential counts of the islet cells of the dog pancreas. Anat. Rec. 67 Suppl. No. 3:27.

Jaffe, F.A. (1951). A quantitative study of the islets of Langerhans in the rabbit. Anat. Rec. 111:109-121.

Johnson, D.E., Noe, B.D. & Bauer, G.E. (1982). Pancreatic polypeptide (PP) like immunoreactivity in the pancreatic islets of the Anglerfish (**Lophius americanus**) and the Channel catfish (**Ictalurus punctatus**). Anat. Rec. 204:61-67.

Kalliecharan, R. & Steves, J. (1982). Light and electron microscopic immunohistochemical localization of somatostatin in the endocrine and exocrine portions of the pancreas of the chick embryo (**Gallus domesticus**). Anat. Rec. 202:349-357.

Kamiya, T. & Yamasaki, F. (1974). Organ weights of Pontaporia blainvillei and Platinista gangetica (**Platinistidae**). Sci. Rep. Whales Res. Inst. 26:263-268.

Kaung, H.C. (1985). Glucagon and pancreatic polypeptide coexist in a population of rat islet cells. Experientia 41:86-88.

Kaung, H.C. (1985). Electron microscopic immunocytochemical localization of glucagon and pancreatic polypeptide in rat pancreas : characterization of a population of islet cells containing both peptides. Anat. Rec. 212:292-300.

Kaung, H.C. & Elde, R.P. (1980). Distribution and morphometric quantitation of pancreatic endocrine cell types in the frog, **Rana pipiens**. Anat. Rec. 196:173-181.

Kent, G.C. (1969). Comparative anatomy of the vertebrates. St. Louis : Mosby p. 250.

Khanna, S.S. & Kumar, S. (1974). Cellular composition of the islets of Langerhans in the Indian sand lizard **Uromastix hardwickii**. Acta Anat. 88:67-75.

Khatim, M.S., Gumaa, K.A., Petersson, B., Lundquist, G., Grimelius, L. and Hellerstrom, C. (1985). The structure and hormone content of the endocrine pancreas of the one-humped camel (**Camelus dromedarius**). Anat. Anz. 159:181-186.

Kimmel, J.R., Pollock, G.H. & Hazelwood, R.L. (1968). Isolation and characterization of chicken insulin. Endocrinology 83:1323-1330.

Klein, C. & Lange, R.H. (1977). Principal cell types in the pancreatic islets of a teleost fish **Xiphophorus helleri**. Cell Tissue Res. 176:529-551.

Klein, C. & Van Noorden, S. (1980). Pancreatic polypeptide (PP) and glucagon cells in the pancreatic islets of **Xiphophorus helleri** (Teleostii). Cell Tiss. Res. 205:187-198.

Koerker, D.J., Ruch, W., Chideckel, E., Palmer, J., Goodner, C.J., Ensinck, J. & Gale, C.C. (1974). Somatostatin: hypothalamic inhibitor of the endocrine pancreas. Science 184:482-483.

Kon, Y. (1933). Uber die Silberreaktion der Zellen. Jena: Gustav Fischer 8: 46-49.

Kubes, L. & Jirasek, K. (1971). Finding of the G cells of the human pancreatic islet. Shornik Vedeckych Praci Lek. Fak. K.U. Hradci Kralove 14:481-483.

Lacy, P.E. (1959). Electron microscopic and fluorescent antibody studies on islets of Langerhans. Exp. Cell Res. Supp. 7:296-308.

Lacy, P.E. (1970). Beta cell secretion - from the standpoint of a patho-biologist. Diabetes 19:895-900 (Cited by Volk and Wellmann, (1985).

Lane, M.A. (1907). The cytological characters of the areas of Langerhans. Am. J. Anat. 7:409-422.

Lange, R. (1970). Immunofluoreszenznikruskopische Darstellung gluka-gonbildender Zellen an plastikdunnschnitten von Inselgewebe (Ratte Frosch). Histochemi 22:226-233.

Larsson, L.I., Sundler, F., Hakanson, R., Pollock, H.G. & Kimmel, R.J. (1974). Localisation of APP, a postulated new hormone to a pancreatic endocrine cell type. Histochemistry 42:377-382.

Larsson, L.I., Sundler, F. & Hakanson, R. (1975). Immunohistochemical local-isation of human pancreatic polypeptide (HPP) to a population of islet cells. Cell. Tiss. Res. 156:167-171.

Larsson, L.I., Sundler, F. & Hakanson, R. (1976). Pancreatic polypeptide - a postulated new hormone: identification of its cellular storage by light and E.M. immunocytochemistry. Diabetologia 12:211-226.

Lazarus, S.S. & Bencosme, S.A. (1956). Development and regression of cortisone-induced lesions in rabbit pancreas. Amer. J. Clin. Path. 26:1146-1156.

Lazarus, S.S. & Shapiro, S.S. (1971). The dog pancreatic X cell: a light and E.M. Study. Anat. Rec. 169:487-491.

Lin, T.M., Evans, D.C., Chance, R.E. & Spray, G.F. (1977). Bovine pancreatic peptide: action on gastric and pancreatic secretion in dogs. Am. J. Physiol. 232:311-315.

Loud, A.V. (1962). A method for the quantitative estimation of cytoplasmic structures. J. cell Biol. 15:481-487.

Lundquist, G., Brolin, S.E., Unger, R.H. & Eisentraut, A.M. (1970). The cellular origin of glucagon. In: 'The structure and metabolism of the pancreatic islets' (eds. S. Falkmer, B. Hellman & I.B. Taljedal). Oxford: Pergamon Press. pp.115-121.

Malaisse-Lagae, F., Stefan, Y., Cox, J., Perrelet, A. & Orci, L. (1979). Identification of a lobe in the adult human pancreas rich in pancreatic polypeptide. Diabetologia 17:361-365.

Mankowski, A. (1901). Ueber die mikroskopischen Veranderungen des Pankreas nach Unterbindung einzelner Theile und uber einige mikrochemische Besonderheiten der Langerhans'schen Inseln. Arch. Mikr. Anat. Bonn 59:286-294.

Mikami, S.I. & Ono, K. (1962). Glucagon deficiency induced by extirpation of alpha islets of the fowl pancreas. Endocrinology 71:364-473.

Miller, M.R. (1960). Pancreatic islet histology and carbohydrate metabolism in amphibians and reptiles. Diabetes 9:318-323.

Miller, M.R. (1962). Observations on the comparative histology of the reptilian pancreatic islet. Gen. Comp. Endocr. 2:407-414.

Miller, M.R. & Wurster, D.H. (1958). Further studies on blood glucose and pancreatic islets of lizards. Endocrinology 63:191-200.

Miller, R.A. (1942). Effects of anterior pituitary preparations and insulin on islet cells of the pigeon pancreas. Endocrinology 31:535-544.

Moir, R.J., Somers, M., Sharman, G. & Waring, H. (1954). Ruminant-like digestion in a marsupial. Nature 173:269-270.

Moir, R.J., Somers, M. & Waring, H. (1956). Studies on marsupial nutrition. I. Ruminant-like digestion in a herbivorous marsupial (**Setonix brachyurus quoy S. gaimard**). Aust. J. Biol. Sci. 9:293-304.

Mosca, L. (1956). Changes in the islets of Langerhans associated with age and hibernation. Q. J. Exp. Physiol. Cogn. Med. Sci. 41:433-441.

Munger, B.L. (1968). The pancreatic islet D cell. Pennysyl. Med. 71:62-67.

Munger, B.L. (1972). The histology, cytochemistry and ultrastructure of pancreatic islet A cell. In: 'Glucagon Molecular Physiology - Clinical and Therapeutic Implications'. (eds. P.J., Lefebvre & R.H. Unger). London/New York : Pergamon Press pp.7-25.

Munger, B.L., Caramia, F. & Lacy, P.E. (1965). The ultrastructural basis for the identification of cell types in the pancreatic islets: rabbit, dog, possum. Z. Zellforsch. 67:776-798.

Nakamura, M., Yamada, K. & Yokote, M. (1971). Ultrastructural aspects of the pancreatic islets in carps of spontaneous diabetes mellitus. Experientia 27:75-76.

Nakamura, N. (1924). Untersuchungen uber das Pankreas bei Foten Neugenborenen Kindern und im Pubertatsalter. Virchows Arch 253:286-349.

Nerenburg, S.T. (1953). Regranulation of beta cells of islets of Langerhans following insulin and starvation. Amer. J. Clin. Path. 23:340-342.

Oakberg, E.F. (1949). Quantitative studies of pancreas and islets of Langerhans in relation to age, sex, and body weight in white leghorn chickens. Am. J. Anat. 84:279-310.

Ogilvie, R.F. (1937). A quantitative estimation of the pancreatic islet tissue Quart. J. Med. 6:287-300.

Orci, L. (1982). Macro and micro domains in the endocrine pancreas. Diabetes 31:538-565.

Orci, L., Baetens, I., Dubois, M.R. & Rufener, C. (1975). Evidence for the D cell of the pancreas secreting somatostatin. Horm. Metab. Res. 7:400-402.

Orci, L., Baetens, D., Ravazzola, M., Stefan, Y. & Malaisse-Lagae, F. (1976). Pancreatic polypeptide and glucagon: non-random distribution in pancreatic islets. Life Science 19:1811-1816.

Orci, L., Pictet, R., Forssmann, W.G., Renold, A.K. & Rouiller, C. (1968). Structural evidence for glucagon producing cells in the intestinal mucosa of the rat. Diabetologia 4:56-57.

Orci, L., Rufener, C., Pictet, R., Renold, A.E. & Rouiller, C. (1970). Present state of the evidence for mixed endocrine and exocrine pancreatic cells in spiny mice. In: The Structure and Metabolism of the Pancreatic Islets. (Eds. S. Falkmer, B. Hellman, & I.B. Taljedal). Oxford : Pergamon Press pp.37-52.

Overholser, M. (1925). The number of islets of Langerhans in the pancreas of albino rat. Endocrinology 9:493-504.

Padour, H. (1950). Differenzen endokriner Drusen bei Saugetieren verschiedener Kor pergrosse mit besonderer Berucksichtigung der Langerhansschen Inseln. Zool. Jahrb. Abt. Allg. Zool. Physiol. Tiere 62:102-137.

Paulin, C. & Dubois, P.M. (1978). Immunohistochemical identification and localization of pancreatic polypeptide cells in the pancreas and gastro-intestinal tract of the human foetus and adult man. Cell Tiss. Res. 188:251-257.

Pearse, A.G.E. (1966). Common cytochemical properties of cells producing polypeptide hormones with particular reference to calcintonin and thyroid C cells. Vet. Rec. 79:587-590.

Pearse, A.G.E. (1969). The cytochemistry and ultrastructure of polypeptide hormone producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. J. Histochem. Cytochem. 17:303-313.

Pearse, A.G.E. (1975). The endocrine division of the nervous system : a logical extension of the APUD Concept. Folia Anat. Iugoslavica 4:5-20.

Pearse, A.G.E. (1977). The APUD concept and its implications: related endocrine peptides in brain, intestine, pituitary, placenta and anuran cutaneous glands. Med. Biol. 55:115-125.

Pearse, A.G.E., Couling, I., Weavers, B. & Friesca, S. (1970). The endocrine polypeptide cells of the human stomach, duodenum and jejunum. Gut 11:649-658.

Pearse, A.G.E. & Polak, J.M. (1971). Neural crest origin of the endocrine polypeptide (APUD) cells of the gastrointestinal tract and pancreas. Gut 12:783-788.

Pearse, A.G.E. & Polak, J.M. (1971). Neural crest origin of the endocrine polypeptide (APUD) cells of the gastrointestinal tract and pancreas. Gut 12:783-788.

Pearse, A.G.E., Polak, J.M. & Bussolati, G. (1972). The neural crest origin of gastrointestinal and pancreatic endocrine polypeptide cells and their distinction by sequential immunofluorescence. Folia Histochem. Cytochem. Krakow 10:115-120.

Penhos, J.C., & Ramey, E. (1973). Studies on the endocrine pancreas of amphibians and reptiles. Amer. Zool. 13:667-698.

Pelletier, G., Leclere, R., Arimura, A. & Schally, A. (1975). Immunohistochemical localization of somatostatin in the rat pancreas. J. Histochem. Cytochem. 23:699-701.

Petkov, P.E. (1967). Histochimie du pancreas du chat avec discussion du tissu Langerhansien endocrine. Histochemie 11:305-318.

Petkov, P.E., Gospodinov, C. & Galabova, R. (1970). Histochemistry of Langerhans islets in the pancreas of cattle (Bos taurus L). Histochemie 23:127-137.

Piazza, C. (1911). Sulle variazoni morfo-fisiologiche dell'isolotto di Langerhans. Anat. Anz. 38:127-136. (Cited by Fujita, 1968).

Pictet, R. & Rutter, W.J. (1972). Development of the embryonic pancreas In: 'Handbook of Physiology'. Vol.1. 'The endocrine pancreas'. (eds. D.F. Steiner & N. Freinkel). Baltimore : Williams & Wilkins. pp.25-66.

Planas, J. & Garcia, F. (1964). New data regarding the pancreatic islets in the Tunny fish. Acta anat. 57:185-191.

Polak, J.M., Pearse, A.G.E., Grimelius, L., Bloom, S.R. & Arimura, A. (1975). Growth-hormone release-inhibiting hormone in gastrointestinal and pancreatic D cells. Lancet 1:1220-1222.

Quay, W.G. (1957). Pancreatic weight and histology in the white whale. J. Mammal. 38:185-192.

Quay, W.G. (1960). The pancreatic islets of desert rodents. Am. Midl. Naturalist 64:342-348.

Rahier, J., Wallon, J., Gepts, W. & Haot, J. (1979). Localisation of pancreatic polypeptide cells in a limited lobe of the human neonate pancreas : remnant of the ventral primordium. Cell Tiss. Res. 200:359-366.

Rahier, J., Wallon, J. & Henquin, J.C. (1980). Abundance of somatostatin cells in the human neonatal pancreas. Diabetologia 18:251-254.

Rahier, J., Wallon, J. & Henquin, J.C. (1981). Cell populations in the endocrine pancreas of human neonates and infants. Diabetologia 20:540-546.

Reddy, S.N., Bibby, N.J. & Elliott, R.B. (1983). Cellular distribution of insulin, glucagon, pancreatic polypeptide hormone and somatostatin in the foetal and adult pancreas of the guinea pig : a comparative immunohistochemical study. Eur. J. Cell Biol. 38:301-305.

Reddy, S.N., Bibby, N.J., Fisher S.L. & Elliott, R.B. (1986). Immuno-localization of insulin, glucagon, pancreatic polypeptide, and somatostatin in the pancreatic islets of the possum, **Trichosurus vulpecula**. Gen. Comp. Endocr. 64:157-162.

Reddy, S.N. & Elliott, R.B. (1985). Ontogeny of cells containing insulin, glucagon, pancreatic polypeptide hormone and somatostatin in the bovine pancreas. Aust. J. Biol. Sci. 38:237-243.

Rennie, J. (1903). On the occurrence of a principal islet in the pancreas of **Teleostii**. J. Anat. 37:575-578.

Rhoten, W.B. (1971). Light and electron microscopic studies on pancreatic islets of the lizard **Lygosoma laterale**. 1. Normal and glucose loaded animals. Gen. Comp. Endocr. 17:203-219.

Rhoten, W.B. (1973). The cellular response of saurian pancreatic islets to chronic administration. Gen. Comp. Endocr. 20:474-489.

Rhoten, W.B. (1974). Sensitivity of saurian pancreatic islets to glucose. Amer. J. Physiol. 227:993-997.

Rhoten, W.A. & Smith, P.H. (1978). Localisation of four polypeptide hormones in the Saurian pancreas. Am. J. Anat. 151:595-601.

Romano, E.L. & Romano, M. (1977). Staphylococcal protein A bound to colloidal gold : a useful reagent to label antigen antibody sites in electron microscopy. Immunochemistry 14:711-715.

Roth, A. (1968). Quantitative studies on the islets of Langerhans in the pigeon. Acta Anat. 69:609-622.

Roth, J., Bendayan, M. & Orci, L. (1980). F I T C - protein A - gold - complex for light and electron microscopic immunocytochemistr4y. J. Histochem. Cytochem. 28:55-57.

Sato, T. & Herman, L. & Fitzgerald, P.J. (1966). The comparative ultra-structure of the pancreatic islets of Langerhans. Gen. Comp. Endocr. 7:132-157.

Schafer, E.A. (1895). Internal Secretions. Lancet 2:321-324.

Schulze, W. (1900). Die Beteutung der Langerhansschen Inseln im Pancreas. Archiv. Mikr. Anat. 56:491-509.

Schweisthal, M.R., Clark, L.D. & Shevell, J.L. (1981). The staining of pancreatic delta cells for light microscopy. Stain Technol. 56:25-28.

Schweisthal, M.R., Schweisthal, J.V. & Frost, C.C. (1978). Localization of human pancreatic polypeptide in an argyrophilic fourth cell type in islets of the rat pancreas. Am. J. Anat. 152:257-261.

Sendrail, M., Bazex, A. & Bolte, N. (1949). Sur une nouvelle technique de differenciation cellulaire applicable au pancreas insulaire. Ann. Endocr. 10:374-377.

Seyfarth, C. (1924). Pankreas und diabetes mellitus. Klin Wochenschr 3: 1085-1087.

Slijper, E.J. (1958). On the vascular system of cetacea. Proc. XVth Intern. Congr. Zool. Section 11, No. 38. Cited by Takahashi, Yamasaki and Kamiya (1975).

Slijper, E.J. (1962). Whales. London : Hutchinson pp. 308-309. Cited by Takahashi, Yamasaki & Kamiya (1975).

Smith, P.H. (1974). Pancreatic islets of the coturnix quail. A Light and E.M. study with special reference to the islet organ of the splenic lobe. Anat. Rec. 178:567-586.

Solcia, E., Pearse, A.G.E., Grube, D., Kobayashi, S., Bussolati, G., Creutzfeldt, W. & Gepts. W. (1973). Revised Wiesbaden classification of gut endocrine cells. Rend. Gastroenterol. 5:13-16.

Solcia, E. & Sampietro, R. (1964). Istochimica dei secreti ormonali nelle isole pancreatic normali e nei tumor insulari. Rivista Patol. Clin. Speriment 5:427-437.

Solcia, E. & Sampietro, R. (1965). On the nature of the metachromatic cells of pancreatic islets. Z. Zellforsch 65:131-138.

Ssobolew, L.W. (1902). Zur normalen und pathologischen Morphologie der inner en Secretion der Bauchspeicheldruse (Die Bedeutung der Langerhansschen Inseln). Virchows Arch. Path. Anat. 168:91-128.

Tager, H.S., Hohenbok, M., Markese, J. & Dinerste, R.J. (1980). Identification and localization of glucagon-related peptides in rat brain. Proc. Natl. Acad. Sci. U.S.A. 77:6229-6233.

Takahashi, K. (1927). Uber die Silberreaktion in den Langerhansschen Inseln. Trans. Jap. Path. Soc. 17:65-66. Cited by Fujita (1968).

Takahashi, K., Yamasaki, F. & Kamiya, T. (1975). Some notes on the pancreas of Franciscana (LA Plata dolphin) **Pontoporia blainvillei**. Okajimas Fol. Anat. Jap. 52:27-38.

Terbruggen, A. (1947). Die Bedeutung der Zelltypen des menschlichen Insel-apparates fur Inselfunktion and Diabetes mellitus. Klin. Wochenschr. 1947:434-438.

Terbruggen, A. (1948). Untersuchungen uber Inselapparat und Inseladenome, insbesond ere uber die Zelltypes bei Diabetes mellitus und Spontanhypoglykamie. Virchows Arch. Path. Anat. 315:407-460.

Theret, C., Alliet, J., Comlan, G. & Gourdier, D. (1975). Endocrine pancreas of **Varanus niloticus** - ultrastructural and cytochemical study with electron microscopy and X-ray microdiffraction. C.R. Hepd. Seanc. Acad. Sci. Paris, ser. D 280:2125-2128.

Thomas, T. (1937). Cellular components of the islets of Langerhans. Am. J. Anat. 62:31-57.

Thomas, T.B. (1940). Islet tissue in the pancreas of the **Elasmobranchs**. Anat. Rec. 76:1-17.

Thomas, T.B. (1942). The pancreas of snakes. Anat. Rec. 82:327-345.

Tomita, T. & Pollock, G. (1981). Four pancreatic endocrine cells in the bull-frog (**Rana catesbeiana**). Gen. Comp. Endocr. 45:355-363.

Tschassownikow, S. (1906). Uber die histologischen Veranderungen der Bauchspeichel druse nach Unferbindung des Ausfuhrungsganges. Archiv. Mikr. Anat. 67:758-772.

Ukai, S. (1926). Transplantations versuche bei Kaninchen. Mitt. U. Allg. Path. U. Path. Anat. 3:89-172.

Vale, W., Brazeau, P., Rivier, C., Brown, M., Boss, B., Rivier, J., Burgue, R., Ling, N. & Guillemin, R. (1975). Somatostatin. Rec. Prog. Horm. Res. 31:365-397.

Van Noorden, S. & Polak, J.M. (1979). Hormones of the alimentary tract. In: 'Hormones and evolution' (ed. E.J.W. Barrington). Academic Press. London/New York pp.791-828.

Volk, B.W. & Wellmann, K.F. (1977). Quantitative studies of the islets of non-diabetic patients. In: 'The diabetic pancreas' (eds. B.W. Volk & K.F. Wellmann). New York/London : Plenum Press pp.121-128.

Volk, B.W. & Wellmann, K.F. (1985). Histology, cell types and functional correlation of islets. In 'The diabetic pancreas' (eds. B.W. Volk & E.R. Arquilla). Plenum Medical Book Company. New York/London pp./81-106.

Von Lawzewitsch, I. (1963). Topographical distribution of the islets of Langerhans in the toad, **Bufo arenarum Herschl.** Acta Physiol. Lat. Amer. 13:382-384.

Wagner, G.F., & McKeown, B.A. (1981). Immunocytochemical localization of hormone producing cells within the pancreatic islets of the rainbow trout (**Salmo gairdneri**). Cell Tiss. Res. 221:181-192.

Watanbe, T., Paik, Y.K. & Yasuda, M. (1975). Fine structure of the pancreatic islets in domestic fowl with special reference to the cell type and secretion. Achiv. Hist. Jap. 38:259-274.

Watts, C.H.S. & Aslin, H.J. (1981). The rodents of Australia. Angus & Robertson pp. 108-112.

Weibel, E.R. (1979). In: 'Practical methods for biological morphometry'. Vol. 1. London : Academic Press. p.4.

Weibel, E.R., Kistler, A.S. & Scherle, W.F. (1966). Practical stereological methods for morphometric cytology. J. Cell Biol. 30:23-38.

Weir, G.C., Goltos, P.C., Steinberg, E.P. & Patel, Y.C. (1976). High concentration of somatostatin immunoreactivity in chicken pancreas. Diabetologia 12:129-132.

Wellmann, K.F. & Volk, B.W. (1977). Histology, cell types and functional correlation of islets of Langerhans. In: 'The diabetic pancreas' (eds. B.W. Volk & K.F. Wellmann). New York/London : Plenum Press. pp.99-119.

Wessels, N.K. (1968). Problems in the analysis of determination, mitosis, and differentiation. In: 'Epithelio-mesenchymal interactions. 18th Hahnemann Symposium' (eds. R. Fleischmajer & R.E. Billingham). Baltimore: Williams & Wilkins. pp. 132-151.

White, A.W. & Harrop, C.J.F. (1975). The islets of Langerhans of the pancreas of macropodid marsupials: a comparison with **Eutherian** species. Aust. J. Zool. 23:309-319.

Wilander, E., Lundquist, M., Westermark, P., & Grimelius, L. (1980). Immunocytochemical study of argyrophil (Sevier Munger) endocrine cells of adult human pancreas. Histochemistry 68: 95-98.

Wilander, E. & Westermark, P. (1976). On argyrophil rections of endocrine cells in the human foetal pancreas. Cell Tiss. Res. 168:33-43.

Wolter, J.R. (1950). Die Langerhans'schen Inseln des Pferdes mit besonderer Berucksichtigung der Zelltypen. Z. Zellforsch 35:229-239.

Yoshida, K., Iwanaga, T., Fujita, T. (1983). Gastro-entero-pancreatic (GEP) endocrine system of the flatfish **Paralichtys olivaceus** : an immunocytochemical study. Arch. Histol. Jap. 46:259-266.

Ziswiler, & Farner, D.S. (1972). Digestion and digestive systems. In: 'Avian Biology'. Vol. 11 (eds. D.S. Farner & J.B. King). New York. Academic Press. p.402.

APPENDIX

Edwin, N. (1979). Quantitative estimation of islet tissue mass and beta and alpha cells of echidna pancreas. *IRCS Medical Science, 7,* 119.

Edwin, N. (1982), Quantitative estimation of islet tissue of pancreas in possums (Trichosurus vulpecula). *Journal of Zoology, 197*(4), 455–461.

Edwin, N. (1984). Quantitative estimation of islet tissue of pancreas in adult Grey kangaroos (Macropus fuliginosus). *Journal of Zoology, 203*(1), 125–133.

Edwin, N. (1985). Quantitative estimation of islet tissue of pancreas in Spinifex hopping mouse (Notomys alexis). *Journal of Zoology, 206*(2), 191–201.

Edwin, N. (1985). Quantitative estimation of islet tissue of pancreas in water rat (Hydromys chrysogaster). *Journal of Zoology*, *207*(2), 277–288.

Edwin, N. (1987). Quantitative estimation of pancreatic polypeptide (PP) cells of islet tissue of pancreas in Australian mammals. *Journal of Zoology, 213*(4), 665–671.