CHAPTER I

HISTOLOGICAL AND IMMUNOCYTOCHEMICAL STUDIES ON THE ENDOCRINE PANCREAS OF ANABAS TESTUDINEUS BLOCH
INTRODUCTION

Generally, endocrine glands contain masses of cells without ducts and arranged in columns or groups at some more or less restricted region or regions within the organism. They have a rich vascular supply and their secretions, the hormones, are special chemical co-ordinators possessing specific physiological actions, usually elsewhere in the body. Hormones vary widely in chemical composition and actions of hormones are complex and diverse. Hormones do not produce any new biochemical actions, but, they influence the rate and intensity of existing ones. Many of them indirectly influence the metabolism of carbohydrate, fat, proteins or minerals.

The islets of langerhans are clusters of cells and their cellular organisation has become the subject of extensive research correlated with an increasing interest in the pathology of diabetes. Besides histological investigations, other investigations such as histochemistry, electron microscopy,
immunohistochemistry and immunofluorescence have led to increased knowledge of this organ and thus the field of comparative islet histophysiology has recently progressed very much. Formerly, emphasis used to be placed mostly on the study of the histophysiology of the mammalian islets, but, during the last decade, the endocrine pancreas of poikilothermic vertebrates has become the subject of extensive studies. Moreover, in contrast to the mammalian endocrine pancreas, great diversity is seen in the structure and cellular composition of the endocrine pancreas of lower forms (Reviews, Miller and Lagois, 1970; Falkmer and Patent, 1972; Epplle and Lewis, 1973; Brinn, 1973; Epplle and Brinn, 1975).

The fact that Beta cells produce insulin and Alpha cells produce glucagon in the pancreatic islets of all vertebrates above the level of Cyclostomes is accepted by almost all investigators (Falkmer and Patent, 1972; Brinn, 1973; Epplle and Lewis, 1973; Epplle and Brinn, 1975; Gepts and Pipeleers, 1977). The third islet cell, the D-cell as a separate type secreting the pancreatic somatostatin, is now generally accepted and confirmed histophysiological, ultrastructurally and immunocytochemically (Epplle and Lewis, 1973; Dubois, 1975; Pelletier, et al, 1975; Johnson et al., 1976;
Helmstaedter et al., 1976; Unger and Orci, 1977a; Unger et al., 1977; Pelletier, 1977; Rizzakalla and Emsheri, 1978; Klein and Van Noorden, 1978; Saralamma, 1979; Van Assche et al., 1980; Findlay and Thomas, 1980) even though, its separate entity was questioned by Gomori (1941). Somatostatin has been isolated from both poikilotherms (Noe et al., 1979; Oyama et al., 1980) and homiotherms (Robert et al., 1980). The discovery of another hormone, from the endocrine pancreas of many species including man (Kimmel et al., 1968; Lin et al., 1973; Lin and Chance, 1974; Larsson et al., 1974, 1975, 1976; Kimmel et al., 1975; Chance et al., 1975; Pelletier, 1977; Pelletier et al., 1977; Van Noorden and Patent, 1978; Klein and Van Noorden, 1980; Van Assche et al., 1980; Nakamura et al., 1980; Stefan and Falkmer, 1980) led to the distinct localisation of one more cell type in addition to the well known A, B and D cells. The hormone is now named pancreatic polypeptide. Moreover, recent identification of other cells by immunocytochemical and immunofluorescent methods secreting hormones like VIP (Van Noorden and Patent, 1980), Glicentin and GIP (Larsson and Moody, 1980), Luteinizing hormone releasing factor (Seepala et al., 1979 and 1980), Gastrin (Greider and McGuigan, 1971, Erlandsen et al., 1976) have made the field of islet histophysiology a highly complicated one and this has led to extensive studies on this particular gland. The discovery of identical hormone producing cells in the gut, brain and other regions has become
a contributing factor to the complexity of the islet histophysiology (Polak et al., 1975; Helmstaedter et al., 1977; Loren et al., 1979 a,b; Girod et al., 1979; Pearson et al., 1980).

The first description of the islet tissue as an agglomeration of endocrine cells in the rabbit pancreas was by Langerhans (1869), but, the name Langerhans was given by Laguesse (1893). The gland holds an exceptional position among the endocrine glands in that it is scattered into different sized bodies within the parenchyma. Formerly, it was thought that the islets are present only in the vertebrates and the hormones insulin and glucagon are the products of islet cells only. But many of the recent studies have demonstrated the presence of B-like cells in the gastrointestinal tracts of several invertebrates (Davidson, et al., 1971; Falkmer, 1972; Falkmer et al., 1973). The islet organisation in the Cyclostomes is of significance as it makes a sort of evolutionary link between the gut-dispersed insulin producing cells of the invertebrates and islets of higher vertebrates (Falkmer, 1969; Falkmer and Patent, 1972; Falkmer et al., 1973). Islets were observed in the different classes of vertebrates following the discovery of Laguesse, who attributed an endocrine function to the islets after a detailed study in snakes in 1900 and 1906.

The comparative aspects of endocrine pancreas have been reviewed by Falkmer (1969), Falkmer and Patent (1972), Apple
and Lewis (1973), Eppler and Brinn (1975), Gepts and Pipeleers (1977). Eppler and Brinn (1975), have distinguished 5 types of exocrine and endocrine relationships in the pancreas. They are,

1. Cyclostomian type,
2. Primitive gnathostome type,
3. Protopterus type,
4. Actinopterygian type and
5. Tetrapod type.

Much diversity is seen in morphological relation between the exocrine and endocrine pancreas especially in lower vertebrates. The differences are due to variations of developmental factors rather than to functional factors.

Three types of cells have been identified in the endocrine pancreas of all classes of vertebrates except in Cyclostomes, using the various well established tinctorial and silver impregnation methods. These cell types are functionally independent granular cells, viz. the Alpha, Beta and Delta cells. In addition, a number of other cell types are also described, the characteristics and nature of their secretions are still under controversy. An excellent staining technique for the differentiation of A cells is the Phosphotungstic acid-hematoxylin (PTAH) method of Levene and Feng (1964) by which the cells are stained blue. Eppler (1967a) has developed a sequential method of staining for the differentiation of the various cell
types on the same section by successive application of silver impregnation and other stains. Hellman and Hellerstrom (1960) earlier introduced a silver impregnation method in which the D-cells of the islet can be visualised, studied and photographed first in the silver impregnated state and then after removing the stain, the same sections can be visualised by specific stains for other cell types. Based on the method of silver impregnation, silver positive A1 and silver negative A2 cells were demonstrated by Hellerstrom et al. (1964) Alm and Hellman (1964), Hellerstrom and Asplund, (1966). Another silver-protein method has been reported by Grimelius (1964) by means of which the A2 cells are stained black. Even though, there is a wealth of recent publications regarding the nature and classification of islet cells, there are still controversial opinions. The most noteworthy among them concerns the A and D-cells. Some differentiate A cells into silver positive A1 cells and silver negative A2 cells (Hellman and Hellerstrom, 1960; Ostberg et al, 1966; Hellerstrom and Asplund, 1966). Others consider the A1 cells equivalent to D-cells (Epple, 1965; Rhoten, 1970; Brinn, 1973).

Immunohistochemistry is the study of the chemistry of immune reactions—antigens and antibodies employing the precise analytical methods of chemistry and highly specific reactions.
Now-a-days, immunohistochemical staining is recognized to be the most sensitive and powerful method for the detection of cell types and their distribution in the pancreatic islets at both the light and electron microscopic level. The most successful fixatives for the preservation of antigenicity have been those containing formaldehyde and picric acid. Bouin's fixative contains formaldehyde, picric acid and acetic acid and has been used successfully for the detection of most islet hormones. The use of mammalian hormone sera is helpful in the identification of the islet cell types of lower vertebrates. Munger (1981) has critically reviewed the morphological characterization of islet cell diversity, where he has described different types of islet cells like A, B, C, D, E, F (PP) and certain other types of cells occurring in different vertebrates.

**Alpha cells (A-cells)**

Alpha cells are well demonstrated by PTAH staining technique of Levene and Feng (1964) with which they are stained blue. These cells are argyrophilic with the Grimalius method (1964), but are non-argyrophilic with the method of Hellman and Hellestrom (1960). Munger (1972) has reviewed the details of histology, histochemistry and ultrastructure of A-cells. The cells contain acidophilic granules staining red with phloxin,
eosin and ponceau-de-xylidine. A prominent component of glucagon molecule is tryptophan and this has been proved by immunofluorescence technique also (Baum et al., 1962). Bussolati et al. (1971) have suggested on the basis of histochemical studies on mammalian islets, that the central core of the alpha granule contains glucagon, whereas the surrounding material is of lipoprotein nature.

Immunohistochemical localization of glucagon was done in vertebrates using the unlabelled antibody peroxidase antiperoxidase technique and indirect immunofluorescence (Klein and Van Noorden, 1978, 1980; Rhoten and Hall, 1981). The immunohistochemical and electron microscopical studies on the pancreatic islets of the teleost fish, *Xiphophorus helleri* using antibodies to pancreatic polypeptide and glucagon show that separate cell types are responsible for the production of these peptides. But it is reported that glucagon and pancreatic polypeptide are present in the same cell during development and in the adults of *Rana pipiens* (Kaung and Elde, 1980; Kaung, 1981).

**Beta Cells (B-cells)**

Beta cells constitute about 75% of the total islet cell population in many vertebrates so far described including man. There are excellent staining techniques by which the B-cells
can be demonstrated by the light microscope, viz., the Chromealum-hematoxylin-phloxin and aldehyde fuchsin procedures of Gomori (1939, 1941 and 1950), the Victoria blue method of Ivic (1959) and the pseudoisocyanin technique of Schiebler and Schießler (1969). The ultrastructural identification of islet cell is based mainly on the characteristics of the secretory granules. Beta granules are electron dense, polymorphous enclosing a wide membranous sac in human endocrine pancreas (Gepts and Pipeleers, 1977). The selectivity of aldehyde-fuchsin for B-cell granule is considered to be due to its action with sulphonic acid groups formed by the oxidation of the disulphide bridge occurring in insulin (Scott and Clayton, 1953). Pretreatment of deparaffinized sections of islets with 5% glutaraldehyde or Bouin's fluid and then oxidation with acid permanganate and bleaching in oxalic acid gives very good results of aldehyde fuchsin (Hanslyon and Prento, 1980).

It has been accepted since a long time that B-cells are responsible for insulin production and release and that the beta granules are the primary storage sites of insulin. Dixit and Patel (1964) have reported the presence of insulin in the royal jelly of honey bees, though radio immunoassay failed to demonstrate the same (Ishay et al, 1976). Cells producing insulin like substance also occur in the mucosa
of the alimentary canal of the Prochordates (Van Noorden and Pearse, 1976; Bevis and Throndyke, 1978) and of molluscs (Fritsch et al., 1976; Plisetskaya et al., 1978). Using the fluorescent antibody technique insulin was localized in the B-cells (Lacy and Davis, 1957) and using immunohistochemical methods the B-cell was identified in fishes, *Lophius americanus* and *Ictalurus punctatus* (Johnson et al., 1976), in the salmon (Maksimovich et al., 1978), in the ray, *Dasyatis akajei* (Yuriko and Yul, 1981), in *Gottius scorpius* (Stefan and Falkmer, 1980), in Amphibia adults, (Tomita and Pollock, 1981; Kaung and Elde, 1980) and larvae (Kaung, 1981), in Reptiles (Rhoten and Hall, 1981; ElSalhy and Grimalius, 1981), in birds (Guha and Ghosh, 1978; Sitbon and Mialhe, 1980) and in mammals (Van Assche et al., 1980; Baskin, et al., 1981) including man (Pelletier, 1977). The ultrastructural characteristics of the light and dark B-cells were studied by Ulekhin (1979) in the endocrine pancreas of rats.

**D-cells**

D-cell as a separate islet cell type secreting somatostatin is now generally accepted and confirmed histophysiologically, ultrastructurally and immunocytochemically (Happle and Lewis, 1973; Erlandsen et al., 1976, Alumets et al., 1977; Unger and Orci, 1977a). In aldehyde-fuchsin preparations the
D-cells are stainable light green. They are metachromatic with toluidene blue (Manochio, 1960; Solcia et al., 1968) and give ultraviolet fluorescence with pseudoisocyanin (Epple 1967a). It is negative to tryptophan reaction. D-cell is beautifully stained by the silver impregnation method of Hellman and Hellerstrom (1960) where it appears dark brown and is identical to the D-cell originally described by Bloom (1931). D-cells are also demonstrated and described by Epple (1965), Fujita (1968) and Rhoten (1970, 1971).

There is a wealth of data supporting the view that somatostatin is the product of D-cells. This tetradecapeptide was isolated first from the sheep hypothalamus (Brazeau et al., 1973) and afterwards in many other vertebrates. It is localised in the D-cells by immunocytochemical and immunofluorescent techniques in fishes (Johnson et al., 1976; Klein and Lange, 1977; Klein and Van Noorden, 1978; Riska1la and Emsheri, 1978; Stefan and Falkmer, 1980; Yuriko and Yul, 1981), in amphibians (Kaung and Elde, 1980; Kaung, 1981), in reptiles (Girod et al., 1979; Rhoten and Hall, 1981; Tomita and Pollock, 1981), in birds, (Orci et al., 1975; Weir et al., 1976) and in mammals (Goldsmith et al., 1975; Pelletier et al., 1975; Polak et al., 1975; Hokfelt et al., 1975; Orci et al., 1975; Dubois, 1975; Pelletier 1977; Dorn et al., 1980; Van Assche et al., 1980). By using highly specific antisera,
B-endorphin and somatostatin were identified simultaneously in the D-cell of the rat, guinea pigs and man by immunocytochemical technique (Watkins et al., 1980). Erlandsen et al. (1976) have demonstrated both gastrin and somatostatin in the same population of pancreatic D-cell by using multiple histochemistry, i.e., by using different chromogens. Some authors have attributed D-cell to be the source of gastrin. The immunofluorescent detection of gastrin cells as identical to the silver positive D-cells in the islet tissue of mammals in vivo (Lomsky et al., 1969; Greider and McGuigan, 1971) and in vitro (Braaten et al., 1976) together with the identification of gastrin in non-Beta cell tumours of Zollinger-Ellisson type (Cavallero et al., 1967; Creutzfeldt et al., 1970) led to the view that gastrin is secreted by the D-cells. But this view is not accepted generally as many studies presented contradicting results. No gastrin cells were identified in pancreatic islets of Zollinger-Ellisson tumours (Lotstra et al., 1974; Pelletier, 1977; Rhoten and Smith, 1978). Moreover, gastrin containing cells were identified in the G-cells of the horse and not in the D-cells (Forssman, 1976). In the rat pancreatic islets somatostatin containing cells were peripherally disposed (Luft et al., 1974; Dubois, 1975; Hokfelt et al., 1975; Orci et al., 1975; Erlandson et al., 1976; Ito et al., 1978).
Pancreatic Polypeptide secreting cells (PP cells)

Pancreatic polypeptide, a 36 amino acid peptide that was originally recognized as a contaminant during the isolation of chicken insulin, is now isolated from the pancreas of many animals (Kimmel et al., 1968; Lin and Chance, 1974; Kimmel et al., 1975; Chance et al., 1976; Larsson et al., 1974, 1975, 1976; Pelletier, 1977; Pelletier and Leclerc, 1977). This has been localised immunocytochemically in the granules of a distinct cell type other than the well known A, B and D cells of the pancreas and its amino acid sequence has been established (Kimmel et al., 1975; Chance et al., 1976). Nakamura et al. (1980) have shown that PP cells are abundant in the pancreatic portion of the rat hooking around posterior to the superior mesenteric vessels and the elements of the nucleus, granular endoplasmic reticulum and the golgi apparatus are well developed in these cells.

In birds and mammals, these cells occur as isolated cells or in small clusters rarely at the periphery of the islet tissue, but more frequently in the exocrine pancreas (Larsson et al., 1975; Polak et al., 1976a; Erlandsen et al., 1976; Pelletier and Leclerc, 1977; Pelletier, 1977; Sundler et al., 1977a). Immunocytochemically and electron microscopically, PP secreting cells were identified in fishes

In the fish, Xiphophorus helleri, it is shown that separate cell types are responsible for the production of PP and glucagon (Klein and Van Noorden, 1980). In Cottus scorpius, PP cells are present only in the juxta pyloric Brockmann body and absent in the juxta splenic Brockmann body (Stefan and Falkmer, 1980). In the frog, Rana pipiens, glucagon and PP are present in the same cell both in the larvae and in the adults (Kaung, 1981). In Anolis carolinensis, Rhoten and Hall (1981) have shown that PP immunoreactive cells occurred predominantly as single cells or groups of two or three, scattered around the non-endocrine portion of the pancreas, although, PP cells were occasionally found in association with other endocrine cells. The human pancreatic polypeptide is clearly associated with a 4th cell type which is characterised by the presence of small secretory granules (Pelletier, 1977). Ultrastructurally, Pancreatic polypeptide has been localised in the F-cell of
of the dog pancreas (Greider et al., 1978). Since the pancreatic polypeptide is contained in the secretory granules of an endocrine type cell and is present in circulating blood (Polak et al., 1976a) it is suggested that this polypeptide is a hormone and can be considered as the 4th pancreatic hormone. The presence of immunologically similar PP like substances in two classes of vertebrates as far removed from each other as fishes and mammals indicates that a molecule of this type occurred very early in the evolutionary history of the vertebrates before the separation of the lines leading to fish and mammals (Van Noorden and Patent, 1978).

In addition to the above described granular cell types several other parenchymal cells have been described in the islets of many species of lower vertebrates.

**Clear Cells** have been described in many (Nakamura and Yokote, 1971; Falkmer and Patent, 1972; Klein and Lange, 1977; Agulleiro et al., 1982) which appear as agranular with a faint non-specific background tinge with low cytoplasmic electron density and hence are also called agranular cells. Secretory granules are either sparse or absent (Falkmer and Olsson, 1962; Boquist and Falkmer, 1970; Kobayashi and Takahashi, 1974). These types of cells are predominant in fish islets but occur rarely in mammals (Falkmer et al., 1964a; Boquist and Falkmer, 1970). Some consider these cells as functional stages of
A or B-cells (Mosca, 1957). However, they are considered to be the precursors of granular cells, especially B-cells, but without any specific hormonal production (Falkmer et al., 1964a; Blair et al., 1969; Boquist and Falkmer, 1970; Falkmer and Patent, 1972; Brinn, 1973). But, according to Klein (1977) the clear cells do not represent a single cell type in teleosts. The function of this cell type has not yet been precisely defined. Nakamura and Yokote (1971) consider the clear cell to be a variety of B-cell in Carps.

In the islets of many lower vertebrates another cell type has been identified which shows both the staining characters of B and D-cells and hence they are named 'amphiphil' cells. They are pseudoisocyanin metachromatic like the B cells (Epple, 1967b) and argyrophilic with Hellman and Hellerstrom silver impregnation technique (1960). This cell type is described by Epple (1965, 1967b) in the islets of fishes and amphibians and according to him these cell types represent intergrade stages between two types of granular cells.

In Holocephali, the endocrine pancreas possesses a unique type of cell, almost occupying 50% of the islet parenchyma, the details of which have been worked out by Fujita (1964), Patent and Epple (1967) and Patent (1972).
These cells are argyrophilic with the Hellman and Hellerstrom silver impregnation technique, they do not stain with aldehydefuchsin or pseudoisocyanin (Apple and Lewis, 1973). It differs from the D-cells in shape and topography. There is no information regarding the nature and role of their hormone. The argyrophilic cells of some teleost fishes correspond to the X cells of Holocephali (Apple and Lewis, 1973).

The islets of both groups of Cyclostomes have several unique topographical and histological features. The light cells which may or may not be granular and the acidophil cells of Petromyzon marinus (Apple and Brinn, 1975, Brinn and Apple, 1976) are other cell types, whose physiological significance is not clear.

At present, it is an established fact that the hormones, glucagon, insulin, somatostatin and pancreatic polypeptide are produced by the Alpha, Beta, Delta and PP cells respectively of the endocrine pancreas of vertebrates, even though, the cells secreting these hormones are found in the extra pancreatic regions also, viz., the alimentary canal, brain etc.

The islet organ of the Gnathostomes always develops in association with the exocrine pancreas. The main advantage of this association is the guarantee that the islet hormone
will be transported quickly to the liver without further dilution in the systemic circulation. The differences in size and intra-pancreatic islet distribution are largely a reflection of the growth dynamics of both the developing islets and of exocrine pancreas (Epplle and Brinn, 1975).

Pancreatic cells represent a primitive distribution among Elasmobranchs (Saralamma, 1979; Yuriko and Yul, 1981). There is a well-developed pancreas in them, but it is dispersed mainly along the walls of the small and medium-sized pancreatic ducts (Saralamma, 1979) or occupies the outer layer of the double-layered duct epithelium of the pancreas (Yuriko and Yul, 1981) or on the walls of the pancreatic ducts as single or multiple layers (Kern, 1964, Ostberg, et al., 1966, Brinn and Epplle, 1975). Detailed studies by the light and electron microscopy on the pancreatic islets of Holocephali are available (Fujita, 1962, 1964; Patent and Epplle, 1967; Patent, 1972).

The holocephalian pancreas is a compact organ consisting of one pear-shaped lobe very closely associated with the liver at one end and with the spleen at the other. The endocrine tissue is scattered throughout the exocrine tissue and occurs as groups of cells associated with the pancreatic duct. There are at least four endocrine cell types, A,B,D and a unique type of cell with characteristic staining properties—X-cells (Fujita, 1964, Patent and Epplle, 1967). The X-cells are
the most abundant occupying the central portion of an islet in rat fish (Patent, 1976).

The endocrine pancreas of many teleosts consists of macroscopically visible Brockmann bodies or Principal islets as well as smaller accessory islets. They are often scattered through out the body cavity. Because of their size and accessibility for experimental manipulations, the Brockmann bodies have afforded unique properties for the elucidations of islet structure and function. The endocrine pancreas has been studied fairly extensively in the teleosts (Bowie, 1925; Falkmer, 1961, 1969; Epple, 1969; Kobayashi and Takahashi, 1970; Nakamura and Yokote, 1971; Patent, 1972; Brinn, 1971, 1973, 1975; Epple and Brinn, 1975; Klein, 1977; Saralamma, 1979; Stefan and Falkmer, 1980; Agulleiro et al., 1982). Three types of pancreas have been differentiated among the teleosts:

1. A compact pancreas with scattered islets similar to that in most gnathostomes.

2. A diffuse pancreas, split into lobes that might extend to various sites of the body cavity and

3. A disseminated pancreas which is scattered in small portions over the body. Various transitions between these types occur.
Greatest species variation in the structure, cytology and
distribution is seen in the teleostean endocrine pancreas.
Only a limited number of teleosts have a compact pancreas
with scattered islets similar to that of higher vertebrates
(Khanna, 1966; Khanna and Mehrotra, 1968; Brinn and Epple,
1972; Epple and Brinn, 1975; Saralamma, 1979). However,
in the majority of teleosts, the endocrine pancreas is con-
centrated into discrete Brockmann bodies or Principal islets
and a number of accessory islets in close contact with the
principal islet (Falkmer, 1961; Sivadas, 1964; Epple, 1969;
Thomas, 1970, 1975; Kobayashi et al., 1976; Khanna and
Singh, 1971; Brinn, 1973, 1975; Epple and Brinn, 1975;
Saralamma, 1979; Rombout et al., 1979; Agulleiro et al., 1982).
Intrahepatic and intra-splenic, sometimes intra-ovarial
islets have been found in various species (Epple, 1969).

Epple and Brinn (1975) after an extensive study of the
Brockmann body of a number of teleost fishes, opined that the
Brockmann bodies are not made of pure endocrine cells alone,
but consists of both exocrine and endocrine tissue of varying
proportions, thus contradicting the observations of Falkmer
and Patent (1972) who reported that the Brockmann bodies of
some fishes—Cottus scorpius, Lophius piscatorius and Scorpaena
scorpha are completely devoid of acinar parenchyma.

The endocrine pancreas of all teleosts studied so far,
contain at least 3 cell types, which are clearly distinguishable
using the conventional staining techniques, viz., A, B and D cells. In some, 4 types (Nakamura and Yokote, 1971; Yoakim, 1977; Saralamma, 1979) and in others 5 types (Klein, 1977; Rombout et al., 1979) are also identified. In addition, other granular and agranular cell type have been frequently identified (Falkmer and Patent, 1972; Apple and Lewis, 1973; Brinn, 1973; Apple and Brinn, 1975; Klein, 1977; Rombout et al., 1979; Saralamma, 1979). The agranular cells or clear cells are not specifically stained by any one of the methods used so far (Falkmer, 1961; Falkmer et al., 1964a; Qureshi and Matty, 1969; Saralamma, 1979). They do not produce any hormone of their own (Blair et al., 1969) and they are supposed to be the functional stages (Mosca, 1957) or the precursors of other granular cells (Bencosme et al., 1965; Falkmer et al., 1964a; Boquist and Falkmer, 1970). Agranular cells may be undifferentiated cells, agranular phases of cell cycle or sometimes may be badly preserved as a result of poor fixation (Klein, 1977).

A 4th granular cell was detected in the endocrine pancreas of many teleosts when light and electron microscopic studies were done (Thomas, 1970, 1975; Kobayashi et al., 1976; Klein, 1977; Saralamma, 1979; Rombout et al., 1979). Apple and Brinn, 1975, opined that all the cell types identified in the endocrine pancreas of many teleosts are granular and there may be 4 or 5 types easily distinguishable. The 4th cell type is
argyrophilic (Brinn and Epple, 1972; Hirane and Honma, 1971) in *Anguilla rostrata* and in *Istiophorus platypterus*. Immunocytochemically, Klein and Van Noorden, (1980) have identified that A-cells with round granules are actually PP cells, whereas, the earlier studies revealed the occurrence of two types of A-cells in *Xiphophorus helleri* (Klein, 1977).

A 5th cell type has been located in the endocrine pancreas of *Barbus conchonius*, characterised by small granules with a moderately electron dense core by Rombout et al., (1979), of *Xiphophorus helleri*, by Klein and Lange, (1977) of Carps by Faller and Lange, (1969) and by Kudo and Takahashi, (1973).

Amphiphill cell type, described by Epple, (1965 and 1967b) in a few teleosts, shows both E-and D-cell staining characteristics (pseudoisocyanin fluorescence and argyrophilia according to Hellman and Hellerstrom 1960) whose function has not been established clearly. They are identified in *Tilapia mossambica* (Epple, 1965) and in *Salmo trutta* (Epple, 1967). In Ictalurus, there is a high incidence of D-cells in the islet, (Brinn, 1973) and somatostatin has been detected in them immunohistochemically (Johnson et al., 1976). In *Lepisosteus* and *Xiphophorus* also somatostatin has been detected immunohistochemically by Johnson (1976) and Klein (1977) respectively.
Acinar islet cell has been reported in many vertebrates and has features of both exocrine and endocrine pancreatic cells. It has been described in *Ictalurus nebulosus* (Bencosme et al., 1965). The cellular cytoplasm contains zymogen granules, A and B granules, mitochondria and endoplasmic reticulum. The sole observation of an acinar islet cell in *Kipphorbus helleri* (Klein, 1977) appears questionable, since islet granules and zymogen granules are present in two distinct parts of the cell. Seven distinct cell types were observed in the Brockmann bodies of *Mugil* by Agulleiro et al., (1982).

There is no general agreement regarding the different cell types found in the endocrine pancreas of teleosts. Some authors have reported three islet cell types (Bencosme et al., 1965; Kobayashi and Takahashi, 1970), some have reported four (Qureshi and Matty, 1969; Thomas, 1970, 1975; Brinn, 1973; Apple and Lewis, 1973; Kobayashi and Takahashi, 1974; Falkmer and Ostberg, 1977; Saralamma, 1979; Stefan and Falkmer, 1980) or five different types (Kudo and Takahashi, 1973; Wagner and McKeown, 1981). Immunocytochemical studies are also on record regarding the nature of the endocrine pancreas in teleosts (Johnson et al., 1976; Falkmer et al., 1977, 1978; Stefan et al., 1978; Klein and Van Noorden, 1980; Stefan and Falkmer, 1980; Wagner and McKeown 1981).
In addition to the insulin producing B-cells, glucagon producing A-cells, somatostatin producing D-cells and pancreatic polypeptide producing PP cells, other cells like Vaso active Intestinal peptide secreting D1 cells, Secretin producing S cells, Gastrin producing G cells were also identified tentatively, (Erlandsen et al. 1976), but the convincing studies are to be conducted for a better understanding of islet cytology. There is increasing evidence that islet cell types exhibit variations among vertebrates, especially among the lower vertebrates. Variations exist among different species within the same class. The islet histology of the teleosts appears to be very complex and there are many contradicting views regarding the structural and functional aspects of the different cell types. Immunocytochemical and immunofluorescent studies made using the different antisera have added more and more types of cells to the already existing islet population of cells. Thus the field of islet cytology has become more and more complex. Numerous studies are made on the islet histology of vertebrates and hence a wealth of data is available. However, immunocytochemical and immunofluorescent studies among the teleosts are very scarce. Moreover, the function of islet hormones among fishes differ to some extent from those of mammals. Therefore, for a better and thorough understanding of the islet
histology of teleosts, using the conventional staining and also using the immunocytochemical techniques, this work was undertaken. The fish *Anabas testudineus* was not utilised previously in the studies of the endocrine pancreas of teleosts and this study is expected to provide a new data on the histology and physiology of the endocrine pancreas of teleosts.
MATERIAL AND METHODS

The fish Melanotaenia hirundo (family, Characididae, subfamily, Melanotaeniinae, subgenus, Tullioperca), the assumed alliling perch, has been selected for the experiment by its accessibility in local waters of Yellowknife Lake, Yellowknife Territory. The fish were reared for two weeks in large earthen tanks (1.27 m x 2.29 m) in the laboratory. The tanks were provided with aeration and water changes to prevent carbonic acid accumulation. After they were acclimatized to the laboratory conditions, during that period, they were fed three times daily on a diet consisting of fish meal, lanoline, and vitamin E. Each fish was transferred to a tank with experimental conditions. Adult male and female were used for the histological study of the pancreas. Preparation of Material

The fish was killed by decapitation. Immediately after decapitation, the pancreas was dissected free of fat and surrounding connective tissue. The pancreas was removed and immersed in appropriate fixatives. The fixatives used for the present study were 10% neutral formalin, Bouin's fluid, alcohol, Bouin's fluid and Barbour-formalin or Bolly's fluid. Barbour-formalin was prepared by mixing 5 mL of formalin and 95 mL of alcohol. After fixation, the tissue was dehydrated in graded alcohol-water in methyl benzoate. Before wax impregnation, the methyl benzoate was replaced by benzene. The
MATERIAL AND METHODS

The fish, Anabas testudineus (Family, Labyrinthicii, Actinopterygii, Teleostei), the common climbing perch, has been selected due to its accessibility in local waters of Vellayani lake, near Trivandrum. They were stocked for two weeks in large cement tanks (4 x 3 x 2 ft) in the laboratory. The tanks were previously washed with potassium permanganate to prevent fungal infection. Thus they were acclimatized to the laboratory conditions. During that period, they were fed twice daily commercial fish feed containing egg, liver and vitamins etc. bought from a local pet's centre, Trivandrum. Water in the tank was changed twice a week. After two weeks, apparently healthy fishes ranging in weight from 30-40 grams were transferred from the stock to small aquarium glass tanks kept under laboratory conditions. Adult males and females were used for the cytological study of the endocrine pancreas.

Preparation of sections

The fishes were killed by decapitation, immediately dissected and as there was no distinct pancreas, the Brockmann bodies - the Principal islets, the accessory islets and the surrounding mesenteries were removed and immersed in appropriate fixatives. The fixatives used for the present study were 10% neutral Formalin, Aqueous Bouin's fluid, Alcoholic Bouin's fluid and Zenker-formol or Helly's fluid. Zenker-formol was prepared just before use by mixing 5 ml. of Formalin and 95 ml of stock Zenker's fluid. After fixation, the tissues were dehydrated in graded alcohol and cleared in methyl benzoate. Before wax impregnation, the methyl benzoate was replaced by benzene. Two
changes of paraffin wax at 58-60°C were used for embedding. After 50-55 minutes, blocks were prepared using wax for embedding purposes. Thin serial sections were cut at 4-5μ thickness.

**Histological Staining methods**

The following staining procedures were applied to the sections:

3. Phosphotungstic acid hematoxylin. PTAH. Levene and Feng (1964)
4. Silver impregnation (Hellman and Hellerstrom - 1960 and Hellerstrom and Hellman 1960) or its modification by Epple (1967a).

Bouin fixation followed by AF-trichrome has been used by some investigators (Gabe, 1969, 1970). Fresh paraldehyde was used for the preparation of the stain to give good results. Pre-treatment of the deparaffinized sections with 5% glutaraldehyde or Bouin's fluid and then oxidation with acid permanganate and bleaching in oxalic acid gave very good staining results with aldehyde-fuchsin (Hanslyon and Prento, 1980).

The D-cells were mainly identified by the method of silver impregnation according to the method of Hellman and Hellerstrom, 1960, but with slight modifications introduced by Epple (1967a). The sections, after refixation in Bouin's fluid for 2 hours at 37°C were dehydrated in graded ethanol and then treated with Silver nitrate solution kept at 20°C for 48 hours. Therefore no pH adjustment was done. The sections were then treated with Pyrogallic acid for 60 seconds,
transferred to absolute alcohol, cleared in xylene and mounted in DPX.

For all staining procedures fresh grades of alcohol and xylene were used.

**Immunohistochemical Staining Methods**

The tissue sections were processed for the indirect enzyme-labelled antibody method (Nakane and Kawaoi, 1974) Deparaffinized sections were hydrated, washed with phosphate-buffered saline, (PBS, pH 7.2) and incubated with the various conjugants in petri-dishes, the bases of which contained wet cotton, for nearly 30 minutes. Then the sections were washed with buffer thrice and peroxidase activity was demonstrated with the chromogen, Diaminobenzidine-Hydrogen peroxide technique. The DAB solution was prepared just before use, by dissolving 20 mg. of DAB in 50 ml. of PBS and then adding 20/ul. of 30% hydrogen peroxide. The sections were incubated in the DAB solution for nearly 30 minutes, at room temperature. Then washed in double distilled water twice, dehydrated in graded alcohols, cleared in xylene and mounted in DPX.

Controls were used for identifying specific immunohistochemical staining. Each specific antiserum was either
completely blocked or greatly diminished by absorption with purified antigens. Guinea pig anti-insulin serum was absorbed with bovine insulin, rabbit anti-glucagon serum was absorbed with glucagon, anti-somatostatin serum was absorbed with somatostatin.

Guinea pig anti-insulin serum was purchased from Cambridge Medical Diagnostics, Inc., Turnpike, MA. U.S.A. Rabbit anti-glucagon serum (30 K) was generously provided by Dr. R.H. Unger, South Western Medical School, Dallas, Texas. Rabbit anti-somatostatin serum, R-108, was kindly supplied by Dr. Akira Arimura, Tulane University, New Orleans, U.S.A. Crystalline glucagon and somatostatin were purchased from Sigma Chemicals, U.S.A.
OBSERVATIONS
OBSERVATIONS

The endocrine pancreas of *Anabas testudineus* is a highly diffuse structure which is concentrated into distinct bodies called the Principal islets or Brockmann bodies visible with the naked eye just beneath the anterior part of the dark red spleen. In comparatively larger fish, two rounded macroscopic white principal islets could be observed whereas in the smaller ones, mostly, a single islet could be seen. Thus, it appears that proportionately larger fish have somewhat larger amount of islet tissue than the smaller ones. The principal islets are surrounded by a connective tissue capsule (Plate I, Fig. 1). The islets are easily noticeable as small white rounded structure just beneath the spleen. In addition to the principal islets, there are about six to seven very small accessory islets scattered along the mesenteries of the anterior part of the intestine (Plate I Fig. 4). They could not be easily distinguished with the naked eye. These islets are of various size, shape etc., but most of them are found to be spherical, under the microscope. In a majority of cases, the Principal islets are found to be oval, however, spherical ones are also seen. In sections, the oval principal islets are about 720 μ in length and 480 μ in breadth. Both the Principal islets and the accessory islets are highly vascularised and are found to be encapsulated by a delicate thin layer of connective
tissue. The parenchyma within the capsule did not show any admixture of exocrine and endocrine pancreatic tissue.

Various conventional staining techniques had been applied on successive paraffin embedded sections which were cut at 4-5 μm thickness. The main B-cell staining methods are Aldehyde-fuchsin trichrome and Chromealum-hematoxylin phloxin methods, the main A-cell staining technique is the phosphotungstic acid hematoxylin method and the main D-cell staining is based on the silver impregnation technique.

In aldehyde-fuchsin preparations, most of the cells in the islets were found to be AF-positive showing dark purple granules in their cytoplasm (Plate I, Fig.2). These cells correspond to the B-cells of other vertebrates. They are packed or clumped into small groups or clusters scattered throughout the islet and not concentrated in the central region of the islet as in higher vertebrates, however, slight concentrations of these cells at some particular regions were also noted. Still, no strict shell like arrangement, as the one noted in mammals with the B-cells in the centre and the A- and D-cells in the outer zone was observed here. Moreover, distinct patterns of distributions of the different cell types were also not always recognizable. Any type of cell could be seen in any part of the islet. As for example, the B-cells were also found in the periphery (Plate I, Fig.5). These B-cells are non-argyrophilic according to the silver-impregnation method of Grimelius (1964) and Hellman and Hellerstrom (1960). Pre-treatment of deparaffinized sections of islets with aqueous Bouin's fluid and then oxidation with acid permanganate
Figs. 1 - 9. Sections from the Endocrine Pancreas of *Anabas testudineus* Bloch.

Fig. 1. Principal islet and an accessory islet. Chromealum hematoxylin-phloxin x 100.

Fig. 2. Principal islet. Arrows indicate B-cells. Aldehyde-fuchsin staining x 120

Fig. 3. Accessory islet. Arrows indicate B-cells. Aldehyde-fuchsin staining x 140.

Fig. 4. Six accessory islets. Aldehyde fuchsin staining x 300

Fig. 5. High power view of a part of the principal islet showing A and B cells. Chromealum-hematoxylin phloxin staining x 400

Fig. 6. Principal islet showing A cells. PTAH staining x 100

Fig. 7. High power view of a part of the principal islet showing A cells. PTAH staining x 400

Fig. 8. Principal islet after silver impregnation showing D cells x 140

Fig. 9. Accessory islet after silver impregnation showing D cells x 400

A - alpha cells
B - Beta cells
D - Delta cells
Is - Accessory islets
and bleaching in oxalic acid gave very good staining results, in the AF-preparations. In chrome-hematoxylin-phloxin method of staining these B-cells are stained dark blue in colour and they show the features of the purple coloured AF positive cells (Plate I, Fig.1). These tinctorial reactions suggest that the B-cells of *Anabas testudineus* are identical with the B-cells of the mammalian islets. The cytoplasm is full of a large number of evenly distributed granules. The ultrastructural identification of islet cell is based on the characteristics of the secretory granules, but the details of the granular structure could not be studied under the light microscope. On the basis of the tinctorial characteristics of the B-cells of *Anabas testudineus*, it may be inferred that they are associated with insulin secretion. These B-cells constitute about 75% of the total islet cell population.

In PTAH—phosphotungstic-acid-hematoxylin preparations, certain cells are stained dark blue in colour (Plate I, Fig. 6 and 7) and they correspond to the A-cells of other vertebrates. In this staining, the AF positive cells remained colourless. These A-cells also showed a highly scattered arrangement throughout the islet. The cells are spherical and at one region of the islet, in the periphery, a slight concentration of these cells is observed. These cells are non-argyrophilic with the silver-impregnation method of Hellman and Hellerstrom (1960). These A-cells contain acidophilic granules staining red with phloxin, eosin and ponceau-de-xylidine. They are not seen as groups as in the case of the B-cells, but are seen as single cells with a
scattered arrangement throughout the islet. They are round with small nuclei and three to four granules are seen in the cytoplasm. These granules are variable in size. It appears that the PTAH staining is specific for A-cells in the fish under present investigation.

In the silver-impregnation staining method, certain cells are found to be silver positive, which are stained as dark brown cells with a scattered arrangement throughout the islet (Plate I, Fig.8). These islet cells correspond to the D-cells of the other higher vertebrates. The AF positive cells of the islet are seen to be intimately intermingling with the AF negative cells especially in the central region. These D-cells are stained light green in colour in the AF-trichrome preparations. It is found that there are a large number of D-cells both in the principal islets and also in the accessory islets (Plate I, Fig.9). They are, like the A-cells, spherical with granules in their cytoplasm. However, the secretory granules are strikingly smaller than the other islet cell granules. These D-cells are not restricted to the periphery, but are found among the B- and A-cells. Perhaps, this arrangement of the D-cells may have a functional significance also. Moreover, a large number of D-cells are present in the islets. The presence of a large number of silver positive D-cells indicates that the hormone
produced by them, viz., somatostatin may play an important role in the entire homeostasis of the organism.

The three types of cells observed in the principal islets after each of the routine staining procedures were also found in the accessory islets. In them, when AF staining was done, about half of the islet cells were found to be dark purple in colour and were arranged more or less in the centre (Plate I, Fig. 3 and 4). These purple cells correspond to the B-cells of other vertebrates. The other half was AF negative. A major portion of the AF negative cells were found to be argyrophilic by the silver impregnation method of Hellman and Hellerstrom (1960). They appeared as dark brown coloured cells, which showed a scattered distribution throughout the entire islet parenchyma (Plate I, Fig. 9). It appears that they correspond to the D-cells of other vertebrates. In addition, another set of granular cells were found located in the periphery and also in the centre and among the B and D cells, which stained as deep blue cells after the PTAH staining specific for the A-cells of the islets. The same cells were stained as pink cells after the AF-trichrome method and after the chrome-alum hematoxylin-phloxin method. It is presumed that they correspond to the A-cells of other vertebrates. Therefore, it can be concluded that there are
mainly three types of cells found in the accessory islets also. These accessory islets resemble the principal islets very much as far as the cytology is concerned.

Immunocytochemical staining using the different antisera showed a very close similarity in the nature and arrangement of the three main types of islet cells identified by the conventional staining techniques. The aldehyde-fuchsin and chrome-hematoxylin staining pattern of the B-cells, the PTAH staining pattern of the A-cells and the silver impregnated pattern of the D-cells, all these correlated very closely with the immunostaining pattern obtained using the antibodies specific to insulin, glucagon and somatostatin respectively (Plate II, Figs. 10, 11, 13 and 15). Because the antiserum to pancreatic polypeptide was not available no immunocytochemical study could be done to detect the PP cells of Anabas testudineus.

In sections treated with anti-insulin serum, positive reacting cells were observed in the islet parenchyma, arranged throughout, without any definite concentrations in the central part of the islet. The specificity of the reactions has been verified by negative controls (Plate II, Figs. 12, 14 and 16). In anti-insulin immunostained sections, groups of
dark brown cells scattered throughout the islet could be observed. The cytoplasm was full of a large number of secretory granules which very closely corresponded to the AF positive granules. In sections stained with antiglucagon serum, single dark brown scattered spherical cells were observed, the nature and arrangement of which resembled the dark blue cells of PTAH staining. Three to four granules were detected in them. These cells are comparatively smaller than the B cells. In sections treated with the anti-somatostatin serum, single spherical cells were found to be stained as dark brown cells, having a scattered arrangement throughout the islet, intermingling with the other two types of cells. The nature and arrangement of the cells, the character of the secretory granule, all these closely resembled the silver positive D-cells of the silver-impregnation technique. The secretory granules were strikingly smaller than the granules of the other islet cells. A large number of somatostatin positive cells were detected. As noted before the presence of such a large number of D-cells may have some functional significance.
Figs. 10 - 16. Sections from the Endocrine Pancreas of *Anabas testudineus* after immunocytochemical staining.

Fig. 10. Principal islet treated with anti-insulin serum x 120

Fig. 11. High power view of the Principal islet treated with anti-insulin serum x 520

Fig. 12. Control treated with anti-insulin serum x 520

Fig. 13. Principal islet treated with anti-glucagon serum x 100

Fig. 14. Control treated with anti-glucagon serum x 520

Fig. 15. Principal islet treated with anti-somatostatin serum x 145

Fig. 16. Control treated with anti-somatostatin serum x 520

Figs. 17 and 18. Endocrine Pancreas of *Anabas testudineus* after Starvation.

Fig. 17. Endocrine Pancreas of normally fed fish. Aldehyde-Fuchsin trichrome staining x 120

Fig. 18. Endocrine pancreas on 120th day of starvation. Intense degranulation of A and B cells.

A - Alpha cells
B - Beta cells
D - Delta cells
DISCUSSION

In Aedes annulatus, the anocluical process is of primitive diffuse type with prominent basal and a few accessory basal insertion points in the mesenteric and the cuticular part of the Haltere. A similar condition has been reported in many related species and there have been reports regarding the role of the Haltere of insects in flight (Hokrner, 1961; Bellman and Bellman, 1961; Falkner et al., 1964; Hickman, 1970, 1975; Hickman and Singh, 1971, 1977).

Some, in some Coleoptera, well developed postgenital process in the male and present in the female in the mesolunation, a condition similar to that found in the vertebrates (Buchanan and Rose, 1969; Hinton and Sykes, 1972; Hickman and Bellman, 1972; Kobayashi and Yokoyama, 1974). (Sugi and Horie, 1973). The presence of the aedeagus with postgenital process in the classification of Coleoptera (1975).

The Aedes annulatus has a female form, called a form, with a female-like ovipositor and a male-like ovipositor. However, the ovipositor in male Aedes annulatus is modified to be inserted into the female ovipositor in the female-like ovipositor.
DISCUSSION

In *Anabas testudineus*, the endocrine pancreas is of primitive diffuse type with Brockmann bodies and a few accessory islets suspended in the mesenteries near the anterior part of the spleen. A similar condition has been reported in many teleost fishes and therefore reports are available regarding the cytology of the islets of Langerhans in them (Falkmer, 1961; Falkmer and Hellman, 1961; Falkmer et al., 1964a; Sivadas, 1964; Thomas, 1970, 1975; Khanna and Singh, 1971; Brinn, Jr, 1971; Brinn, 1973; 1975; Epple and Brinn, 1975; Kobayashi et al., 1976; Klein and Lange, 1977; Saralamma, 1979; Stefan and Falkmer, 1980). However, in some teleosts well developed compact pancreas or islets are present being scattered throughout the exocrine pancreas, a condition similar to that found in higher vertebrates (Khanna and Mehrotra, 1968; Brinn and Epple, 1972; Khanna and Gill, 1973; Kobayashi and Takahashi, 1974; Epple and Brinn, 1975; Saralamma, 1979). The pancreas of *A. testudineus* can be considered as Actinopterygian type according to the classification of Epple and Brinn (1975).

The Brockmann bodies of *Anabas testudineus* are free from acinar parenchyma as reported in *Lophius piscatorius*, *Scorpaena scropha* and *Cottus scorpius* (Falkmer and Patent, 1972). But in the opinion of Epple and Brinn 1975, the Brockmann body is penetrated and surrounded by varying degrees of exocrine acini and connective tissue, which condition was confirmed in *Ophicephalus punctatus* by Saralamma (1979). However, the secondary islets in those fishes are devoid of exocrine penetration, but are surrounded by connective tissue
and exocrine acini. In *Xiphophorus helleri*, originally, Klein and Lange (1972), reported that, the Brockmann body or the giant islet is totally devoid of acinar tissue. Contrary to this, Eppele and Brinn (1975), have shown that the giant islet of *X. helleri*, is not completely separated from the exocrine tissue. In *Sparus auratus*, the Brockmann body is surrounded by a thick layer of exocrine tissue which can form strands between the endocrine cells (Agulleiro et al., 1982). It is believed that the early teleosts had an islet tissue organisation of scattered islets in the acini, that resembled the condition in other vertebrates. Such a condition was observed in the Elopomorph fishes, which were placed at the beginning of the teleost pedigree (Winbladh Biuw, 1972). Therefore, it can be presumed that the occurrence of the Brockmann bodies as small concentration of endocrine tissues may be a secondary acquisition in teleosts. Scattered small islets in the acini were also observed and reported in *Saccobranchus fossilis* by Saralamma (1979).

In *Anabas testudineus*, the cell types are seen to be intermingling with each other, an arrangement that is seen in Ictalurid fishes, (Brinn, 1971, 1975), in *Clarias batrachus*, (Khanna and Mehrotra, 1968), in *Saccobranchus fossilis* (Saralamma, 1979). Generally in them, more A-cells are arranged in the periphery, even though, a few cells are found
among the B-cells also. B and D-cells are found filling the entire islet. B-cells are more and are seen scattered throughout the islet. In *Fugu rubripus* (Kobayashi et al., 1976) and in *Ophicephalus punctatus* (Saralamma, 1979) the B-cells are scattered in the central region intermingling with the D-cells, while the periphery and the remaining central region are occupied by the A and 4th cell types. In *Xiphophorus helleri*, (Klein and Lange, 1977), the A-cells are distributed at the periphery, whereas the B- and D-cells are situated at the centre. In the marine teleost fish, *Lithognathus mormyrus*, the A- and C-cells occupy the outer zone and the B- and D-cells occupy the inner zone, in number, the C cells dominate in the outer zone, while, the B-cells dominate in the inner zone, (Rizkalla and Emsher, 1978). In *Carassius auratus*, the B-cells form cords or anastomose with each other. A-cells intermingle with the B-cells often in small groups of a few cells, whereas the D-cells are scattered and isolated. The arrangement of the B- and D-cells in *Anabas testudineus* resembles that of *Carassius*, but however, the distribution of A-cells differ very much. In the fish under present investigation, the D-cells are also scattered among the B-cells and no 'shell' arrangement as the one noted among the mammals was seen. In the Principal islet, the A-cells form a group at one region in the periphery and some A-cells are found among the B- and D-cells in the central central region also. From the observations done so far,
regarding the distribution of the various cell types of the endocrine pancreas of the different teleost species, it can be concluded without any doubt that the teleost fishes exhibit greatest species variation in the pattern of islet cell distribution among the vertebrates. In Anabas testudineus, the alpha cells are stained deep blue when treated with PTAH and are stained pink when treated with phloxin or ponceau-de-xylidine. However, these alpha cells do not show a positive reaction to PTAH in Saccobranchus fossilis (Saralamma, 1979) and in Limanda limanda (Thomas, 1975). At the same time, in Saccobranchus fossilis, these islet cells are stained by phloxin and azocarmine, which indicate that they correspond to the alpha cells of other vertebrates. Brinn (1975), reported that in Ictalurid fishes, PTAH stained many more cells than did ponceau-de-xylidine, the number being 50% or more and thus indicated that PTAH is non-specific at least in them. Contrary to this, in Ophicephalus punctatus, the PTAH stainable cells correspond in number and distribution to the acidophil cells in AF trichrome preparation (Saralamma, 1979). In Anabas testudineus also, PTAH appears to be specific for the A-cells as revealed by staining. The number and distribution of the PTAH positive cells is in correspondence with the number and pattern of arrangement to the cells of the AF trichrome stain.
Two types of $A_2$ cells, ($A_{2r}$ with round granules and $A_{2f1}$ with floculent granules) were reported in the endocrine pancreas of Barbus conchonius by Rombout et al., (1979). The $A_{2f1}$ cells producing glucagon and the $A_{2r}$ cells containing granules resembling enteroglucagon producing ones of mammals. Two types of $A_2$ cells were also found by Klein and Lange (1977) in Xiphophorus helleri, which suggestion was withdrawn after a detailed immunohistochemical and electron microscopical study in 1980, in the same fish. But, in Barbus conchonius, the $A_{2r}$ cells are Gremlielius positive whereas they are negative in Xiphophorus helleri. The earlier suggestion of Klein and Lange (1977) was that, these two categories of cells may represent two stages in the evolution of the same cell type. Later, it was confirmed that, the two types of A-cells were PP cells and A-cells. PP cells are A cells with round granules and glucagon cells are $A_2$ cells with crystalline granules (Klein and Van Noorden, 1980).

In Anabas testudineus, the argyrophil cells are numerous arranged as small round cells and intermingling with the B-cells and scattered in the islet parenchyma. It is proposed that they produce somatostatin, the release-inhibiting factor for the growth hormone in vertebrates. This hormone may be of particular interest for regulating
pancreatic hormones of teleosts because the number of D-cells is much larger in teleosts than in mammals (Rombout et al., 1979). The argyrophil cells are found to be numerous in the islets as reported by Havu, (1969), Khanna and Singh, (1971), Johnson et al., (1976), Rombout et al., (1979), Stefan and Falkmer, (1980). However, in Saccobranchus fossilis, the argyrophil cells are found to be fewer in number (Saralamma, 1979), where they occur as single isolated cells interspersed among the A- and B-cells and confined to the central zone only. A similar distribution of argyrophil cells has been reported in Cottus scorpius, (Falkmer and Hellman, 1961), Scorpaena scropha, (Scoli and Sampietro, 1965), Gadus callarias (Thomas, 1970), Xiphophorus helleri (Klein and Lange, 1977). Somatostatin positive cells are generally low in number in mammalian islets, whereas those cells are much more in number in Lophius and Ictalurus (Johnson et al., 1976) which may be indication of an important role for this hormone in teleost physiology. In Anabas testudineus also, there are a number of silver positive D-cells, as many as the B-cells and they are shown to be stained immunocytochemically when treated with the antisomatostatin serum. In the Nile Catfish, Schilbe mystus, Yoakim, (1977) identified A$_1$ cell types, which were the only cell type that revealed an argyrophilic reaction. Since teleost principal islets appear
to be regulated by factors differing from those which regulate islet function in higher forms, the presence of large amounts of somatostatin, known to influence insulin and glucagon is intriguing. Somatostatin may be an intermediate between the primary regulating factor in fish and the final insulin or glucagon response. Somatostatin cells may not only be involved in local regulation. Their presence in such large numbers may indicate an involvement in the homeostasis of the entire organism (Johnson et al., 1976). Two types of argyrophil cells have been demonstrated in the pancreatic islets of the ratfish, *Hydrolagus colliei*, one type accounting for approximately 50% of all islet cells, is identical to the X-cells of *Chimaera monstrosa*, by Fujita and other category corresponds to the D-cells of *Chimaera* (Patent and Apple, 1967). The large number of somatostatin positive cells in many teleosts, as mentioned earlier, is certainly of interest considering the generally low number of similar cells in mammalian islets. Therefore it appears that, in *Anabas testudineus* also, the presence of somatostatin positive cells in large numbers, about two thirds as many as B-cells and one and one half times the number of A-cells, is indicative of the fact that the hormone is not only involved in local regulation but also in the homeostasis of the entire organism.

Detailed electron microscopic, immunohistochemical and immunofluorescent studies were done on the endocrine pancreas

Immunocytochemical studies on the teleost pancreas revealed the existence of cells containing insulin, glucagon and somatostatin in the goose fish Lophius americanus, and in the channel cat fish, Ictalurus punctata, by Johnson et al., (1976), in the daddy sculpin, Cottus scorpius by Falkmer et al., (1977, 1978), Stefan and Falkmer, (1980). Immunohistochemical staining of the pancreatic islets revealed the presence of somatostatin in the D-cells of Xiphophorus helleri by Klein and Van Noorden (1978). In addition to the above mentioned cell types, a 4th endocrine cell type, containing pancreatic polypeptide (PP) has been found in some teleost species (Klein, 1977; Van Noorden and Patent, 1978; Klein and Van Noorden, 1980; Stefan and Falkmer 1980). Moreover, by the use of immunohistochemical techniques, the four endocrine cell types were detected in the islets of an elasmobranch, viz., the ray, Dasyatis akajei, by Yuriko and Yul (1981). There, the PP cells
could not be differentiated from somatostatin immunoreactive cells, although the former were smaller in number. Using immunofluorescence and electron microscopic studies, four endocrine cell types were identified in the daddy sculpin, 
1.**Cottus scorpius** by Stefan and Falkmer (1980). In the daddy sculpin, there are two Brockmann bodies, one located close to the pyloric region and the other close to the spleen. A noteworthy point in their observation is that, in the Brockmann body of the pyloric region and in the small islet aggregates around this giant accumulation of islet parenchyma, there are a number of PP cells in addition to the other three cell types, typically located at the utmost periphery of both the small and large islets. However, in contrast, the Brockmann body close to the spleen and the small islets around it are completely devoid of PP cells, but the other three cell types are present. It has been shown that somewhat similar conditions exist in the mammalian pancreas where the PP cells are mainly located in the lowermost and posterior parts of the head of the pancreas (Gersell et al., 1979).

Correlative immunohistochemical and electron microscopical studies on the pancreatic islets of the teleost fish **Xiphophorus helleri**, using antibodies to glucagon and PP show that separate cell types are responsible for the production of
these peptides (Klein and Van Noorden, 1980). The A₂ cells with round granules are the PP cells while the A₂ cells with crystalline granules are the true glucagon cells. The presence of PP cells was revealed by immunohistochemical staining in many teleosts (Van Noorden and Patent, 1978) and the cells were frequently found at the periphery of the islets as in mammals and were of irregular shape. In many teleosts, the glucagon cells were frequently found in association with the PP cells at the periphery of the islets, but occupying a much larger area of the islet, extending towards the centre (Van noorden and Patent, 1978). Immunohistochemical observations in *Anabas testudineus* revealed the presence of the glucagon cells when the antiserum for glucagon was used. The arrangement of the A-cells was similar in both the immunostained and conventionally stained sections. Few cells occupy the periphery and others were found intermingling with the B and D-cells. As the antiserum for pancreatic polypeptide was not available, no immunohistochemical study could be done to detect the PP cells in the fish under present study. In many teleosts, the PP cells were detected and found to be in frequent association with the glucagon secreting A-cells. However, in the elasmobranch, *Dasyatis akajei* (Yuriko and Yul, 1981), it was found that the PP positive cells, after an immunohistochemical study, could not be differentiated from the somatostatin positive
immunoreactive cells, although, the former were smaller in number. It may be that, an unknown substance in the cells reacted to both anti-PP and anti-somatostatin sera or that the PP positive cells might contain an unknown peptide cross reacting to the anti-somatostatin serum. As no report regarding the presence of PP and somatostatin in the same cells is available, at present it is not possible to make any conclusive remarks on that observation.

The immunohistochemical studies using the anti-guinea pig insulin serum in *Anabas testudineus* gave positive results, whereas the controls were negative. As noted in the case of the A-cells, there was a typical agreement in the general arrangement of the B-cells in both the immunostained and other histologically stained preparations. The cells are located in groups of scattered cells through out the islet parenchyma. Immunohistochemically the B-cells were detected in many vertebrates (Helmstaedter et al., 1976; Johnson et al., 1976; Klein, 1977; Rombout et al., 1979; Stefan and Falkmer, 1980, Rhoten and Hall, 1981; Baskin et al., 1981). There are many reports available regarding the detailed nature of the B-cells based on immunohistochemical and electron microscopic studies not only in mammals but also in the lower vertebrates. The arrangement of the B-cells in *Anabas testudineus* differs from that in the *Barbus conchonius* (Rombout et al., 1979) where the cells were located in groups or strands in the central part of the islet, a condition similar to that of the mammals. A
similar type of arrangement of the B-cells in the central part of the islet was observed in the Dab, *Limanda limanda*, by Thomas, (1975) after light and electron microscopic investigations. In the Daddy sculpin, *Cottus scorpius* also, the B-cells are found to be in the centre of the islet, along with the somatostain positive cells as shown by immunohistochemistry (Stefan and Falkmer, 1980). However, in *A. testudineus*, the immunoreactive B-cells are not confined to the centre only, by the strands or groups of cells are scattered throughout the islet parenchyma.

Somatostatin containing cells have been demonstrated by immunohistochemistry in many teleosts (Johnson et al., 1976; Klein, 1977; Klein and Van Noorden, 1978; Van Noorden and Patent 1978; Stefan and Falkmer 1980) and in elasmobranchs (Yuriko and Yul, 1981). Johnson et al. (1976) opined that in the anglerfish, *Lophius americanus* there were large areas of somatostatin positivity surrounded by a rim of glucagon positive cells and then by insulin positive cells. In the catfish, *Ictalurus punctatus*, also there were a large number of somatostatin positive cells but were intermingled with the endocrine tissue. As mentioned earlier the presence of such a large number of somatostatin positive cells in the islets of these fishes is certainly of interest considering the generally low number of similar cells in the mammalian islets.
The condition of the D-cells in *Anabas testudineus* is in accordance with the above investigations. As reported earlier, their presence in large numbers may indicate that the somatostatin is also involved in the entire homeostasis of the organism in addition to its original function of inhibiting the release of insulin and glucagon. The somatostatin positive cells in *Anabas testudineus* are arranged throughout the islet. A similar type of arrangement of the D-cells was observed in *Xiphophorus helleri*, where the D-cells are distributed throughout the islet except for the peripheral area, the cells were usually narrow and often triangular in shape (Klein and Van Noorden, 1978).

In *Anabas testudineus*, the staining pattern of islet cells obtained after silver impregnation method of Hellman and Hellerstrom (1960) correlated very closely with the staining obtained by using antibody specific for somatostatin. Specificity controls in which specific antisera for these islet hormones were absorbed with excess purified antigen completely eliminated positive staining of the respective cell type.

Immunohistochemical staining has revealed that the pancreatic islets of various teleost fishes contain a pancreatic polypeptide like substance which cross reacts with antibodies to mammalian and avian pancreatic polypeptide in *Xiphophorus helleri*, *Anguilla anguilla*, *Ictalurus nebulosus*, *
Leoplagosteus osseus and Gillichthys mirabilis (Van Noorden and Patent, 1978). Glucagon cells were frequently found in association with the PP cells at the periphery of the islet, but occupying a much larger area of the islet extending towards the centre. The PP cells were frequently found at the periphery of the islets as in mammals and were of irregular shapes. The presence of immunologically similar PP like substance in two classes of vertebrates far removed from each other as fishes and mammals indicates that a molecule of this type occurred very early in the evolutionary history of vertebrates before the separation of the lines leading to fish and mammals. The survival of these presumably related molecules suggest that PP like substances are probably of physiological importance (Van Noorden and Patent, 1978). An interesting observation with regard to the topography of the PP cells in the endocrine pancreas was made by Stefan and Falkmer (1980) in the teleost fish, Cottus scorpius, the daddy sculpin. As mentioned earlier, a noteworthy point in their observation is that the juxtapyloric Brockmann body contained insulin, glucagon, somatostatin and pancreatic polypeptide immunofluorescent cells, whereas, the juxtasplenic Brockmann body showed insulin, glucagon and somatostatin containing cells, but no pancreatic polypeptide cells. However, in Anabas testudineus, no immunohistochemical study could be made as the antiserum to PP was not available.
Immunohistochemical localization of pancreatic endocrine cells of the leopard frog embryos and young larvae was made by Kaung (1981). In the leopard frog, *Rana pipiens*, it was found immunohistochemically that both in the adult and young larvae, glucagon and PP are present in the same cells. The dual presence of these two hormones were reported in some other vertebrates also and they further support a close relationship between these two hormones (Kaung and Elde, 1980; Ravazzola and Orci, 1980).

In the rat fish, the islets showed two populations of cells with differing glucagon immunoreactivity (Stefan et al., 1981). Their findings indicate that among glucagon immunoreactive cells, some cells situated exclusively in the pancreas with both C and N terminal glucagon immunoreactant, while the other pancreatic as well as intestinal cells, the sequence that binds C terminal antiglucagon serum appears masked and becomes accessible to the antibody only after proteolytic digestion. Grube et al., (1978) have shown that in the endocrine pancreas of man and rat, the A-cells showed immunoreactivity to CCK-PZ. This finding throws some light on the functional unity of the endocrine pancreas and the gastrointestinal endocrine system.
SUMMARY

1. The structure and cellular composition of the endocrine pancreas of the "Climbing perch" Anabas testudineus is studied by means of conventional staining techniques like chrome-alum-hematoxylin phloxin, phosphotungstic acid hematoxylin, silver impregnation and also by means of immunocytochemical techniques using the different antisera under the light microscope.

2. Anabas testudineus possesses a diffuse pancreas. The endocrine cells are aggregated into one or two large Brockmann bodies or Principal islets and six to seven small accessory islets scattered in the mesenteries and are encapsulated by thin delicate connective tissue capsule. The islet parenchyma is formed of A, B and D cells. No strict shell like arrangement as the one noted in mammals, with the B-cells in the centre and the A and D cells in the outer zone, was observed here. Any type of cell could be seen in any part of the islet. All the cells showed highly scattered arrangement throughout the islet. A large number of D cells is intermingling with the A and B cells. B-cells are grouped into small clusters and are scattered in the parenchyma. A and D cells are spherical and are seen as single cells having a scattered arrangement. A large number of D cells are present in the islet.
3. Immunocytochemical staining using the different antisera, viz., anti-insulin, anti-glucagon and anti-somatostatin showed a very close similarity in the nature and arrangement of the three main types of islet cells identified by the conventional staining techniques.