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EVIDENCE FOR THE PRESENCE OF SEROTONERGIC NERVE FIBERS IN THE FETAL AND ADULT MAMMALIAN PANCREAS

by STEVEN B. KOEVARY

A dissertation submitted to the Graduate Faculty in BioMedical Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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ABSTRACT

EVIDENCE FOR THE PRESENCE OF SEROTONERGIC NERVE FIBERS IN THE FETAL AND ADULT MAMMALIAN PANCREAS

by

Steven B. Koevary

Advisor: Dr. Robert C. McEvoy

No evidence for the existence of serotonergic neurons in the mammalian pancreas has been previously reported. This question was examined by measuring the ability of rat pancreatic fragments to accumulate ^{3}H-5HT at low concentrations. This technique has been used widely for the demonstration of brain and gut serotonergic nerves. After incubating pancreatic tissue ^{3}H-5HT was demonstrated to be taken up avidly. Maximal uptake occured after 15 minutes of incubation. The uptake was demonstrated to be saturable, and was reduced by increasing concentrations of unlabelled serotonin. The apparent uptake constant for this saturable uptake was 8.75 x 10^{-7}M, and the Vmax was 873 pmoles/gram. Adrenergic nerves were an unlikely site for uptake of ^{3}H-5HT, since incubation in the presence of excess norepinephrine did not reduce the uptake. In addition, the serotonin receptor blocker, metergoline, did not affect the uptake, suggesting a lack of a binding component in the uptake. However, in vitro

incubation of pancreatic fragments with parachloroamphetamine and/or 5,7-dihydroxytryptamine, both serotonin neurotoxic drugs, completely obliterated the specific uptake.

The saturable and specific uptake of serotonin was found to be uniform throughout the pancreas, and no sexual differences could be demonstrated. Rabbit, hamster and monkey pancreatic fragments were also demonstrated to specifically accumulate ^{3}H-5HT. Radioautography on sections of the tissue slices after uptake revealed the specific uptake sites as dense aggregates of silver grains in the connective tissue spaces of the exocrine and endocrine pancreas, as well as in the walls of the pancreatic vasculature, all areas known to be traversed by nerve fibers. Such a localization of grains suggests a possible innervation of these structures by serotonergic nerves.

Fenfluramine, a drug which has been demonstrated to release brain serotonin, significantly augmented the pancreatic release of newly uptaken ^{3}H-5HT. Veratridine, a drug which causes nerve depolarization, similarly stimulated the release of ^{3}H-5HT.

Fetal rat pancreata (18, 20 and 22 days) were also demonstrated to specifically take up serotonin. Radioautography revealed similar uptake sites as in the adult, except that in addition,

cells, possibly representing primitive neuronal perikarya, were demonstrated to be heavily labelled. This suggested a possible intrapancreatic location for the cell bodies of these nerves. The preservation of the specific serotonin uptake in 18 day fetal tissue after 4 days in organ culture, substantiated this view.

The presence of serotonergic nerve fibers suggested that the pancreas may contain the biosynthetic enzymes necessary for the synthesis of neurotransmitter. Indeed, rat pancreatic homogenates revealed tryptophan hydroxylase activity. Tryptophan hydroxylase is the rate limiting enzyme in serotonin synthesis from tryptophan.

Demonstration of serotonin containing nerve fibers in the rat pancreas by specific immunocytochemical techniques was attempted. Only pancreatic mast cells were stained. Islet cells could be stained, but only after prior administration of 5-hydroxytryptophan. The immunocytochemical staining of gut enterochromaffin cells for serotonin served as a positive control. Gut serotonergic nerve fibers were also unstained.

THIS DISSERTATION IS DEDICATED TO

MY FATHER

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figures 23-25 represent tissue immunocytochemically stained for serotonin (Figure 25 also stained for glucagon).

I. INTRODUCTION

Serotonin was first identified and isolated from serum in 1948 by Rapport et al. The name implied its ability to act as a potent vasoconstrictor. Serotonin was subsequently identified as a hydroxylated form of carboxylated tryptophan or 5-hydroxytryptamine (5-HT), which is usually found complexed with creatinine sulfate. In 1951 Hamlin and Fisher synthesized it. It was then discovered that 5-HT was identical to a substance, extracted from the gastrointestinal mucosa by Erspamer (1955), which contracted smooth muscle, and which he named 'enteramine.' Serotonin is widely distributed in various mammalian tissues as well as throughout the animal kingdom. In invertebrates, serotonin plays a role in regulating carbohydrate metabolism, by a mechanism analogous to epinephrine's action in vertebrates. Serotonin is structurally similar to auxin, a hormone-like factor found in plants (Garattini and Valzelli, 1965).

In the epithelium of the gut mucosa, the source of serotonin is the enterochromaffin cell (EC), so named because it stains with chromic salts. These cells are found in greatest numbers in the duodenum, and generally decrease in the more distal portions of the gut, although they are more frequent in the caecum and proximal colon (Vialli, 1966). Since the chromaffin granules of these cells are polar toward the basal lamina and not the lumen, these cells are classified as endocrine cells. The EC cells are responsible for the

excess production of serotonin in the carcinoid syndrome. The physiological role for EC serotonin is unknown, but the following hypothesis has been proposed. It is believed that serotonin facilitates the peristaltic reflex by lowering the threshold of mucosal sensory receptors and sensitizing the muscle to acetylcholine (Page, 1968). Scratching the serosal surface, as well as increasing intraluminal pressure, were found to increase serotonin release from the EC cells. Serotonin is not essential for propulsion, however, since peristalsis was unchanged after rats were fed for one month on a tryptophan free diet (Boullin, 1964). The demonstration that vagal stimulation causes the release of 5-HT from the enterochromaffin cell (Tobe et al., 1976), however, lends further credence to the idea that serotonin modulates gut motility.

Serotonin has also been found in mast cells of rats and mice, and in platelets. Benditt et al. (1955) were the first to demonstrate the occurrence of 5-HT in mast cells. Mast cells of the rat and mouse were found to contain measurable amounts of the amine in the normal state. However, small quantities of the amine in humans with the carcinoid syndrome were demonstrated in mast cells (Parrat and West, 1956). Serotonin is probably synthesized in mast cells, since tryptophan hydroxylase, the rate limiting enzyme in serotonin biosynthesis, has been demonstrated in these cells (Kuhn et al., 1980). In addition, mast cells grown in culture for

several weeks, maintained, and even increased, their intracellular 5-HT (Page and Green, 1959). The function of mast cell serotonin is also unknown, but again, it has been hypothesized that it regulates contraction of the vascular smooth muscle (Lewis, 1957).

Serotonin has also been demonstrated in the dense granules of blood platelets. Platelets do not synthesize 5-HT, since they lack tryptophan hydroxylase (Morrissey, et al., 1977). They possess a high affinity uptake system for serotonin, and it is believed that this mechanism is responsible for the 5-HT in the platelet (Lingjearde, 1979). It was thought that the role of the platelet was simply to transport 5-HT from the enterochromaffin cells of the gut to the endothelial cells of the lung, where it was metabolized (White et al., 1975). Such an intracellular transport of serotonin prevents any action on the vasculature. If that were it's sole function, however, it would be superfluous for the platelet to have a specific 5-HT stimulatory receptor on it's membrane (Woolley, 1958). Binding of serotonin to that receptor, initially potentiates 'plug' formation, but prolonged occupation of the receptor appeared to inhibit subsequent responses of the platelets to aggregating agents, restricting the intravascular growth of the plug (Baumgartner and Born, 1968). Serotonin release from platelets appears to be dependent on pH and anion transport (Bennett et al., 1980). Because platelets and serotonergic nerve terminals are similar with respect to the active transport and granular

storage of serotonin, platelets are being studied as a model for the serotonergic synaptosome.

The interest of neurobiologists in serotonin was stimulated by the work of Twarog and Page (1953), who first showed serotonin to be present in brain tissue. Paasonen et al. (1957) demonstrated that serotonin was preferentially distributed in the phylogenetically older regions of the dog's brain. Brodie and Shore (1956) were the first to propose that serotonin was a neurotransmitter in the mammalian CNS. Since then, much work has been done using a variety of techniques on the brain serotonergic system, and more recently, evidence has been presented suggesting the presence of such neurons in the gut.

II. SEROTONERGIC NERVOUS SYSTEM

A. SEROTONERGIC NEURONS IN THE CENTRAL NERVOUS SYSTEM

Evidence that serotonin is localized within neurons was made possible by the development of the formaldehyde-induced fluorescence technique by Falck et al. (1962). Using this technique, serotonergic nerve cell bodies were identified mainly in the raphe nuclei of the lower brain stem. The dorsal and median raphe nuclei were shown to contain most of the 5-HT producing neurons of the midbrain (Dahlstrom and Fuxe, 1965). More recently, Steinbush, using a specific anti-serotonin antibody and indirect fluorescence in the rat, demonstrated serotonin cell bodies in other areas of the mesencephalon and rhombencephalon, but was unable to demonstrate cell bodies in either the telencephalon, diencephalon, or spinal cord. Serotonin nerve terminals, however, seemed to permeate the entire central nervous system, including the spinal cord, where a high proportion of terminals were seen in the region of the lateral horn, suggesting a role for these fibers in modulating sympathetic outflow (Carlsson et al., 1973). It should be mentioned that recent evidence by Frankfurt et al., demonstrated the presence of serotonergic cell bodies along the peri-ependymal region of the hypothalamus, by immunocytochemistry.

Serotonergic axons have been described as unmyelinated, and highly branched (Azmitia, 1978). As the serotonergic axons

approach their regions of termination, they ramify to form preterminal axonal plexuses, characterized by multiple varicosities (Smith and Sweet, 1978). These varicosities have been shown to be filled with vesicular organelles (Descarries et al., 1975), but do not seem to be engaged in classical synaptic relationships. In fact, EM radioautography has demonstrated very few classic synapses of serotonergic nerves (Azmitia, 1978). This suggests that serotonin, released from such varicosities, diffuses as a neurohumoral agent to reach relatively distant targets. Such 'loose' contacts of serotonergic terminals may be a facilitating factor in the vigorous sprouting seen in these nerves, when damaged (Bjorklund et al., 1973).

Several lines of physiological evidence support the anatomical inferences that serotonin is indeed a central neurotransmitter; 1) Serotonin, microiontophoretically applied, influences neuronal polarization (Segal, 1974); 2) Serotonin is synthesized in neurons containing 5-HT (Aghajanian, 1972); 3) Electrical stimulation of the raphe nuclei causes synaptic 5-HT release (Smith and Sweet, 1978); 4) Serotonergic agonists mimic the action of 5-HT on postsynaptic receptors; and 5) a means for serotonin metabolism and inactivation has been demonstrated in presynaptic nerve endings (Snyder et al., 1973). Electrophysiological studies of serotonergic neurons have shown that they are characterized by slow, tonic firing patterns, i.e., one spike per second, suggesting a homeostatic function

for these neurons (Aghajanian, 1972).

Physiological and behavioral studies have ascribed to the raphe system a variety of specific functions. 5-HT has been demonstrated to elevate body temperature when microinjected into the anterior hypothalamus (Green and Graham-Smith, 1975). Such treatment causes release of acetylcholine in the caudal hypothalamus, suggesting that 5-HT raises body temperature by activating a cholinergic pathway. Raphe serotonin has also been implicated in the mechanism for triggering, and maintaining sleep (Bremer, 1977). Parachlorophenylalanine (PCPA), a drug which has destructive effects on serotonergic nerves by inhibiting tryptophan hydroxylase, has been demonstrated to cause insomnia in the cat, characterized by a marked reduction in the total amount of slow wave, and paradoxical sleep. However, stimulation of the raphe nuclei did not induce sleep. Thus, serotonergic involvement in the mechanism of sleep appears complex.

Since serotonin has been demonstrated to be a vasoconstrictor in the periphery, it is not surprising that there have been investigations into a possible vasomotor role for serotonin in the regulation of cerebral blood flow. Several studies in humans and cats have demonstrated contractile effects of serotonin, in vitro, on both extra- and intracranial vessels, the latter being more sensitive to stimulation (Hardebo et al., 1978). The relationship of serotonin to the control of vasomotor tone and blood flow is

complicated by the innervation of cerebral vessels by norepinephrine, substance P, neurotensin, and serotonergic nerves (Reinhard et al., 1979). It is interesting to note that serotonergic fibers, projecting to the vasomotor area of the brain stem, seem to be involved in regulating peripheral vascular tone (Smith and Sweet, 1978).

Serotonergic neurons have also been implicated in the release of prolactin and thyroid stimulating hormone in the rat (Jordan et al., 1979; Garthwaite and Hagen, 1979).

One of the most provocative hypotheses that has come out of serotonin research has been proposed by Woolley and Shaw, in the U.S., and Gaddum, in England. They have suggested that a faulty metabolism of serotonin could be the basis for psychotic behavior. There are several pieces of evidence which support this hypothesis: 1) Since LSD has been demonstrated to block serotonin induced smooth muscle contraction, the hallucinogenic effect of LSD may also be due to it's antagonism of the action of endogenous cerebral serotonin (de la Torre, 1972); 2) Tranquilizing drugs, such as reserpine, decrease brain serotonin content (Blackburn et al., 1967); 3) Antidepressant drugs, such as the monoamine oxidase inhibitors, increase brain monoamine content, and such treatment has been shown to exacerbate the symptoms of patients with schizophrenia (Coppen and Wood, 1980); 4) There is an abnormal excretion of the serotonin metabolite, 5-hydroxyindoleacetic acid, in the urine of schizophrenic patients (de la Torre, 1972). Since over 90%

of the serotonin in the body is in the gut enterochromaffin cells, the relevance of this finding to brain serotonin is difficult to determine. Similarly, the role played by platelets, whose uptake of serotonin has been shown to be compromised in schizophrenics (Modai et al., 1979), remains to be elucidated.

B. SEROTONERGIC NEURONS IN THE PERIPHERAL NERVOUS SYSTEM

Investigations of the gastrointestinal autonomic nervous system have, in the past, concentrated on the role played by the two classic autonomic neurotransmitters, acetylcholine and norepinephrine. However, recent evidence for the presence of other transmitters, by histochemical and physiological techniques (Brinn, 1975; Baumgarten et al., 1970; Cook and Burnstock, 1976; Schultzberg et al., 1978; Fahrenkrug, 1979), has forced a reevaluation of gut innervation. Bulbring and Gershon suggested in 1967, that serotonin may act as a neurotransmitter in the mammalian myenteric plexus, perhaps participating in the vagal inhibitory pathway to the stomach. 5-HT was shown to stimulate ganglion cells, both excitatory and inhibitory, through an action on specific receptors, distinct from the nicotinic receptors for acetylcholine (Drakontides and Gershon, 1968; Gershon, 1967; Bulbring and Gershon, 1967; Schulz and Goldstein, 1973). In addition, serotonin was shown to be released from the gut wall when intestinal neurons were stimulated (Bulbring and Gershon, 1967). Drugs which antagonize responses to 5-HT, were shown to interfere with the vagal relaxation of the stomach (Bulbring and Gershon, 1967). Specific desensitization of ganglionic receptors for 5-HT, reduced the vagal relaxation response. Furthermore, ^{3}H-5-Hydroxy-dl-tryptophan (^{3}H-5HTP) was metabolized to ^{3}H-5HT in the mouse myenteric plexus, and was considered bound in the plexus since it's rate of diffusion from the

tissue was slow (Gershon et al., 1965). Addition of nonradioactive serotonin to such a preparation resulted in a net release of radioactive serotonin from the plexus, which appeared to result from activity in the intramural nervous system, since it was blocked by lidocaine, an anesthetic.

The intestinal plexus also has an adequate mechanism for the inactivation of serotonin. This mechanism involves the reuptake of serotonin by the axon terminals. Gershon and Altman (1971), analyzed this uptake in vitro in a dissected preparation of guinea pig longitudinal muscle and myenteric plexus. Uptake was linear for 30 minutes, and demonstrated a Km of 7.4×10^{-7}M and a Vmax of 220 pmol/g/min. Radioautography revealed that the uptake was limited to the myenteric plexus. In addition, the uptake was found to be highly temperature dependent, could be reduced by inhibitors of glycolysis, but not by inhibitors of aerobic respiration, was sodium dependent and could be inversely correlated to the ambient potassium concentration. Using this technique, Goodrich et al. (1980) were able to demonstrate that enteric serotonergic neurons arise early in vertebrate evolution, possibly in an anscestral chordate resembling amphioxus.

Rothman et al. (1976) demonstrated that two 5-HT uptake mechanisms exist in the ileum of adult rabbits. The first uptake could be blocked by norepinephrine and was believed to represent uptake of 5-HT by adrenergic nerves. This first uptake had a low affinity and was not saturable. The second

uptake mechanism was specific, saturable and exhibited a high affinity for 5-HT (Km = 2 x 10^{-6}M). This uptake was not blocked by norepinephrine until the norepinephrine concentration exceeded that of 5-HT by 1000 fold. It should be noted that two similar uptake mechanisms have been described for the serotonin uptake in the brain (Shaskan and Snyder, 1970). In the brain, the low affinity uptake was believed to result from the nonspecific uptake of 5-HT into an adrenergic compartment, while the high affinity uptake was believed to be specific for serotonergic nerves.

More persuasive evidence in favor of the existence of serotonergic nerves in the myenteric plexus comes from work done by Dreyfus et al. (1977a), which demonstrated the synthesis of ^{3}H-5HT in the plexus following the administration of ^{3}H-tryptophan. The guinea pig myenteric plexus has been demonstrated to take up ^{3}H-tryptophan by a high affinity uptake mechanism (Km = 10^{-5}M). Since enteric neurons grown in culture continued to synthesize serotonin from tryptophan, it was also concluded that these serotonergic fibers must be intrinsic to the gut. The ability to synthesize serotonin from tryptophan is a sine qua non for serotonergic nerves, since the rate limiting enzyme for the synthesis of serotonin from tryptophan, tryptophan hydroxylase, has been found only in such nerves. Similarly, 5-HT fluorescence was abolished by pretreatment of the animals with PCPA, a specific inhibitor of tryptophan hydroxylase. In a related experiment,

tryptophan hydroxylase was identified directly by immunohistochemistry in the myenteric plexus of the rat (Gershon et al., 1977).

In the ileum of the fetal rabbit, the serotonergic reuptake system develops on day 16 of gestation, five to eight days before the norepinephrine reuptake in adrenergic nerves (Rothman et al., 1976). The kinetics of this uptake resemble the high affinity uptake in the adult. In the developing chick, precursors of serotonergic neurons, which are believed to be derived from the neural crest, acquire the ability to concentrate 5-HT even before they take up residence in the myenteric plexus (Epstein et al., 1980; Gershon et al., 1980). Once in the plexus they extend axons which then take over the ability to concentrate transmitter. These precursor cells were found to first colonize the stomach and rectum, but, as development proceeded, the density of cells decreased at the extremes of the gut and increased in the middle.

Thus, the evidence for a peripheral serotonergic system in the mammalian gut is substantial: 1) A specific, high affinity 5-HT uptake mechanism is present in the myenteric plexus, exhibiting similar kinetics as serotonergic fibers in the brain. This uptake is believed to play a role in transmitter inactivation; 2) The enzymes required for de novo synthesis of 5-HT from tryptophan are present in the plexus, and such a synthesis has been demonstrated; 3) Possible synapses have been described in the plexus between serotonergic nerves and

cholinergic, noncholinergic excitatory and nonadrenergic inhibitory neurons; 4) The intrinsic nature of the gut serotonergic nerves has been confirmed by the demonstration that synthesis and uptake of 5-HT persist in cultured plexus; and 5) A specific 5-HT binding protein, which has been found in the brain in areas containing serotonergic pathways, has similarly been localized in the rabbit myenteric plexus (Gershon and Dreyfus, 1977).

III. PANCREATIC INNERVATION

A. MACROSCOPIC

The mammalian pancreas is supplied by both sympathetic and parasympathetic fibers (Woods and Porte, 1974). The parasympathetic fibers emerge from the dorsal motor nucleus of the vagus in the medulla (Lenninger, 1974). These myelinated fibers run along the serosal surface of the esophagus and emerge as two vagal trunks after piercing the diaphragm (Teitelbaun, 1933). From there, the right and left vagal trunks join prevertebral nerve plexuses, consisting of parasympathetic and sympathetic fibers, and sympathetic ganglion cells. Generally, fibers from the right vagus innervate the body and tail of the pancreas, while left vagal fibers supply the head and isthmus (Tiscornia et al., 1974), although extensive overlap may occur.

The sympathetic fibers innervating the pancreas are derived from cells in the intermediolateral cell columns of the 5th through 7th thoracic segments of the spinal cord. These myelinated fibers enter the abdomen as part of the greater and lesser splanchnic nerves. After synapsing in the prevertebral plexus, postganglionic fibers enter the pancreas. These nerves, like vagal preganglionics, carry visceral efferent fibers. In addition, they convey pain impulses (presumably resulting from distention) back to the spinal cord (Gardner et al., 1975). Both the parasympathetic and sympathetic fibers

enter the gland along blood vessels.

B. MICROSCOPIC (Special reference to islets)

Morphological evidence for the presence of nerve fibers in the pancreas has existed since the beginning of the century. Cajal (1891) and Muller (1892) demonstrated a rich innervation of the mammalian pancreas with nerve fibers and multipolar cells associated with blood vessels and acinar tissue. Later, Gentes (1902) and Pensa (1905) described a well developed network of nerve fibers in association with islet tissue in the rat, cat and dog. Van Campenhout (1927) described for the first time a peculiar structure which he termed "complex sympathetico insulare" in a variety of mammalian pancreas. This complex was described as containing autonomic nerve cells and islet cells in juxtaposition. This formation was ascribed to a budding of islet cells from the primitive duct system and their migration into adjacent nervous tissue. Van Campenhout believed that these nerve cells influenced, in some undefined way, the differentiation of duct cells into islet cells. Simard (1937) studied the pancreata of men of different ages and found that the islet cells were in contiguity with nerve cells, without the interposition of connective tissue. He coined the term "neuroinsular complex" to describe this phenomenon, and considered the possibility of direct islet cell secretion into the nervous tissue, as well as the possibility that the nerve cells arose directly from the islet cells in the complex. The presence of neuroinsular complexes was observed by Ritter (1946) in the newborn cat, Coujard and Theret (1952)

in the guinea pig, and Hagen (1956) in the dog. The relevance of neuroinsular complexes in pancreatic physiology is unknown. It is possible that such findings represent the exception rather than the rule for islet geography.

Fujita (1959) described another type of complex consisting of islet cells and nerve fibers. Islets were described which lay adjacent to, or in the midst of, nerve bundles which ran through the interlobular connective tissue. He termed these complexes - type II. Simard's neuroinsular complexes, which he was also able to demonstrate, were referred to as type I. The identification of islet cell granules oriented toward the capillary surface rather than toward the nerves refuted Simard's theory of islet secretion into the nervous tissue.

Until 1958, investigations into the innervation of the pancreas, and islet in particular, relied mainly on silver impregnation techniques for the demonstration of nerve fibers. Coupland (1958) used a cholinesterase technique to study neuroinsular complexes and nerve fibers in the pancreas. It should be remembered that this technique stains primarily cholinergic neurons. He observed that the islets were associated with a peri-insular plexus from which fibers could be traced into the interior of the islet. Coupland stated that although he was able to find some neuroinsular complexes, most neurons lay along the connective tissue septae. Morgan and Lobl (1959) used the aldehyde-fuchsin-ponceau (AFP) and cholinesterase techniques to study neuroinsular complexes in

albino rats. Every islet examined was seen to be densely innervated. In newborn rats, the nervous innervation was less apparent, possibly due to inherent limitations in the technique, though some nerve elements were present. The adult pattern was seen 48 hours after birth. They described the rat neuroinsular complex as being composed of a peri-insular plexus (first identified by Coupland), supplying primarily the peripheral region of the islet, and continuous with it, an intra-insular plexus, penetrating into the islet to supply the region of the beta cells. In addition, these authors demonstrated a diminution of acetylcholinesterase activity following insulin and glucose administration, suggesting that the neuroinsular complexes regulated the release of islet hormones. It is interesting to note that islets of alloxan treated animals were seen to possess dystrophic nerve terminals near degranulated beta cells (Shorr and Bloom, 1970). The authors suggested that the appearance of the beta cells may have resulted from neuronal hyperactivity in an attempt to secrete insulin from the post-alloxan insulin depleted cell. It is possible, however, that alloxan may have had a direct effect on nerve terminals.

Pancreatic autonomic nerves have also been demonstrated by using a fluorescent histochemical technique. Falck and Hellman (1963) observed formaldehyde-induced fluorescent nerve fibers primarily associated with pancreatic blood vessels, but also sparsely distributed throughout the exocrine pancreas. The

fluorescence corresponded to that of catecholamines. Legg (1968) used a fluorescence technique to demonstrate nerve fibers throughout the tail of the pancreas of the cat. Adrenergic fibers were seen in relationship to major pancreatic ducts, arterioles, large veins, islet cells, and non-adrenergic nerve cell bodies in the intrapancreatic ganglia. It should be mentioned that Forssman and Greenberg (1977) demonstrated a few yellow fluorescent nerves and varicosities, indicating the presence of serotonin, around blood vessels and close to the islets in the primate pancreas.

Early ultrastructural studies demonstrated that the nerve endings within the islets can be divided into 2 distinct groups. Those nerve terminals which contain 30-50nm agranular vesicles were believed to be cholinergic in nature, whereas other, presumably adrenergic endings contained similar sized but dense-cored vesicles (Richardson, 1964). Watari (1968) classified the nerve endings within different vertebrate pancreata into 4 groups. Type 1a contained agranular vesicles of 50 nm diameter, and type 1b, which were similar to type 1a, but also contained 100 nm diameter dense cored vesicles, were thought to be cholinergic in nature. Type 2a, containing small, dense cored vesicles and agranular vesicles along with some large, dense cored vesicles, and type 2b, containing vesicles of the same size as those of the agranular synaptic vesicles, with a majority of these vesicles containing bar-shaped crystalloid material, were thought to be adrenergic

in nature. These nerve endings represented beaded swellings along the course of the nerve fibers in a fashion similar to that described by Iversen (1967) as "synapse en passage." Legg (1967) identified many nerve fibers in the cat which displayed adrenergic type vesicles in close relationship with the beta cells. These nerve terminals were seen to lie between the basement membrane and the islet cell that they appeared to be innervating. Kobayashi and Fujita (1969) demonstrated small cored and uncored synaptic vesicles in similar locations in the dog pancreas, but could observe no special relationship between the type of islet cell and its nerve terminal.

A third type of islet nerve terminal has been described, containing 60-200 nm dense-cored vesicles that were histochemically distinct from those of other autonomic nerves (Brinn, 1975). Burnstock described a similar type of nerve terminal in connection with mammalian smooth muscle, where he postulated they function as "purinergic" nerves, which release ATP as their neurotransmitter. Purinergic nerves as such, have not been described in mammalian islets, but appear to be present in some fish islets (Lever, 1971). These terminals may also represent serotonergic terminals, since similar terminals were found to accumulate labelled serotonin, as demonstrated by EM radioautography, in the bat pancreas (Nunez et al., 1980).

Recently, a third division of the peripheral autonomic nervous system termed the peptidergic system, has been proposed (Polak and Bloom, 1979a). In this system, peptides are

believed to be released from nerve terminals. Baumgarten et al. (1970) were the first to identify this third type of autonomic fiber in the GI tract. Peptidergic fibers were distinguishable at the ultrastructural level by the presence of neurosecretory granules, similar to those responsible for the storage of vasopressin and oxytocin in the posterior pituitary, containing large granular vesicles of 85-160 nm, and faintly opaque, 40-60 nm vesicles (Baumgarten et al., 1970). They designated these fibers as p-type fibers (polypeptide fibers). Eight morphologically distinct types of axon terminal granular arrangments in Auerbach's plexus in the guinea pig, with more than one type of profile appearing to form synapses with one type of cell body, have been demonstrated (Cook and Burnstock, 1976).

Recently, immunocytochemical techniques have revealed peptidergic nerves in pancreata of different mammalian species (Sundler et al., 1978; Larsson, 1979; Fahrenkrug et al., 1979). Substance P and enkephalin were demonstrated in only a few scattered nerves (Larsson, 1979; Larsson et al., 1976), where they seemed to innervate small ganglia. VIP immunoreactive nerves displayed a similar pattern as substance P and enkephalin nerves, but in addition, were seen to be associated with blood vessels and more infrequently, with exocrine and islet cells (Sundler et al., 1978; Larsson, 1979). The localization of VIP nerves around blood vessels suggests a role for VIP in the control of pancreatic blood flow. Using an

antiserum which recognizes gastrin/CCK, nerves were found in pancreatic ganglia, as well as in moderate amounts in the pancreatic islets (Larsson, 1979). In addition, both gastrin and CCK have recently been shown to be individually present in pancreatic nerves by independent investigators (Rehfeld, 1971; Larsson et al., 1976).

It is noteworthy that intrapancreatic ganglia seem to receive peptidergic nerve fibers. Classically, these ganglia were thought to receive cholinergic, preganglionic fibers, and possibly some adrenergic fibers. In receiving many different types of fibers, these ganglia may play a vital role in the regulation of pancreatic secretion by intergrating all incoming signals in much the same way that the cell bodies in Auerbach's and Miessner's plexa integrate secretion and motility in the gut. Richins (1945) demonstrated that the islet innervation was virtually unaltered in the totally denervated cat pancreas, implicating the intrapancreatic ganglia as the main source of the intraislet nerves. Since of all the peptidergic nerves localized in the pancreas only VIP cell bodies have been demonstrated in these ganglia (Larsson, 1980), it may be assumed that the cell bodies of the other neurons are below the level of immunohistochemical detection, or that they are extrapancreatic.

Richins (1945) observed that most islet nerves were unmyelinated. Both adrenergic and cholinergic fibers were sometimes found within the same Schwann cell sheath (Kobayashi

and Fujita, 1969; Porte et al., 1976). Watari (1968) showed that some nerve fibers entered the islets without a Schwann cell (naked nerve fibers). This latter finding suggests that at such a synapse the neurotransmitter may be released into the intercellular space and simultaneously stimulate or inhibit a large number of islet cells. Orci et al. (1973) have demonstrated the presence of gap junctions between various islet cells, and between the terminals of nerve fibers and islet cells. Since gap junctions are known to be areas of low electrical resistance, it is possible that the secretory response of a functionally coupled islet could be rapidly altered by signals from nerves. Meissner and Schmelz (1974) performed electrophysiological studies on the islets and demonstrated that they have a continous electrical discharge, and that their firing pattern can be altered when challenged with different concentrations of glucose. Similarly, Pace et al. (1978) showed that the electrical activity of the beta cell membrane of rat islets maintained in culture is affected by alterations in the level of glucose in the culture medium. In addition, treatment of batch-incubated rat islets with veratridine (200 uM), a drug which depolarizes membranes, elicited a secretory response in the presence of low glucose (Pace and Blaustein, 1979). Electrical signals may, therefore, play a role in the regulation of islet cell function.

Kobayashi and Fujita (1969) were the first to demonstrate Schwann cell incorporation in the dog and guinea pig islet as

an agranular, epithelial cell-like element with attenuated processes inserted between the islet cells. Later, Smith (1975) also showed that Schwann cells in dog islets have cytoplasmic processes that are contiguous with large expanses of the islet cell surface. These processes were shown to be interposed between the islet basal lamina and the endocrine parenchyma. It has been speculated that these Schwann cells may act as cellular barriers that impede the diffusion of locally released transmitter, as from naked axons mentioned above, and may also help in electrically insulating the islets (Smith and Porte, 1976). This relationship between the Schwann cells and the endocrine parenchyma in the dog suggests that the islets in this species are structurally similar to autonomic ganglia.

A case has recently been presented suggesting that pancreatic islet nerves act as paraneurons i.e. secreting their contents into the blood. Two pieces of evidence substantiate this hypothesis. 1. Fujita and Kobayashi (1979) demonstrated that many islet nerve terminals were exposed to the peri- and paracapillary spaces. While this was interpreted by others as representing a "synapse en passage" type of innervation of islet cells, it was postulated by these authors that the nerves might release their products into those spaces and, through the basal lamina and pored endothelium, into the blood. This discovery lead to the idea that many of the axon-islet cell juxtapositions seen with the electron

microscope may have represented merely a parallel juxtaposition in which both axons and islet cell apices secreted their products into the blood. 2. Early studies, as described above using silver impregnation techniques, have demonstrated the occurrence of ample nerve fibers along the vascular wall in the islet. Since, as capillaries, these vessels may not be functionally innervated (Alm et al., 1980), it is possible that the nerves seen around these vessels may secrete their products into the vasculature.

It has been proposed that islet cells are developmentally derived from neuroectoderm (Pearse and Takor, 1976). The apparent lack of ability of islet Schwann cells to discriminate between nerves and endocrine cells, as mentioned above, supports this view. Thus, islet cells may have functional similarities to nerves e.g. a paraneuronal mode of secretion. Pancreatic islets have been demonstrated, using vascular cast scanning electron microscopy (Fujita and Murakami, 1973), to possess portal vessels radiating from their capillary networks into the pancreatic exocrine tissue. Paraneuronal secretion from islet nerves as well as endocrine cells, therefore, may play a physiological role in regulating certain aspects of exocrine secretion. Two likely candidates for such regulation are insulin and VIP. Insulin, released from islet beta cells, has been demonstrated to act directly on the pancreatic acinar cells to potentiate and uphold pancreozymin-induced protein release (Fujita et al., 1976). VIP, as described in more

detail later, possibly released from islet paraneurons, has been demonstrated to have a secretin-like effect on exocrine secretion (Konturek et al., 1976).

IV. NORMAL PHYSIOLOGY OF INSULIN, GLUCAGON, SOMATOSTATIN AND PANCREATIC POLYPEPTIDE SECRETION

A. INSULIN

Since the primary action of insulin is related to fuel storage, nutrients are considered to play the predominant role in the regulation of it's release. Glucose and most other simple sugars have been shown to elicit insulin secretion in the human (Grodsky et al., 1974). Two theories have been put forth as to the mechanism of this stimulation. The glucose receptor theory envisions a receptor for glucose on the surface of the beta cell. Binding of glucose to this receptor elicits a train of events which ultimately results in the release of insulin (Kobayashi et al., 1980). From studies with alloxan, it has been speculated that the glucoreceptor site on the membrane is adjacent to the hexose transport site (Rossini et al., 1975). A second theory relates the ability of the beta cell to metabolize a given sugar to it's ability to stimulate insulin release. Indeed, there is evidence that reduced pyridine nucleotides produced during glycolysis may be the trigger for insulin release (Zawalich, 1979). In addition to carbohydrates, amino acids and proteins induce insulin secretion (Fajans et al., 1967). Other nutrients, such as glucose and/or gastrointestinal hormones, can augment this stimulation. Fats can also stimulate insulin secretion. Although the mechanism remains unclear, it appears that glycerol, long chain free fatty acids, and ketones do not

directly stimulate the beta cell (White et al., 1978). Gastrointestinal factors apparently also augment fat induced insulin secretion (Creutzfeldt, 1979). The magnitude of the insulin response to fat is less than that seen for carbohydrates and proteins. It is interesting to note that non-nutrient hyperosmolar drinks also stimulate insulin secretion (Goodman, 1974).

The effects of glucose as a prototypical secretagogue will be discussed in some detail. Once stimulated by glucose, the following events are thought to occur within the beta cell. 1. Insulin synthesis is initiated. Glucose causes the initiation of proinsulin synthesis in the endoplasmic reticulum. Proinsulin consists of a single polypeptide chain of 86 amino acids in humans (Rubenstein, 1981). It has recently become clear that a precursor, larger than proinsulin (pre-proinsulin), is the original gene product in the biosynthesis of insulin. The identification of pre-proinsulin suggests that the 51 residue insulin molecule is derived from a single gene which codes for a polypeptide containing 110 amino acids. The amino terminal 49 amino acids are believed to be involved in providing information necessary to direct it's sequestration into the intracellular membrane system and eventual storage within secretory granules (Rubenstein et al., 1975). After synthesis and cleavage of pre-proinsulin to proinsulin, the nascent peptide chain folds, with the aid of oxidation of sulfhydryl groups in the molecule. The newly

synthesized proinsulin molecule is transported to the Golgi region by an energy requiring process. In the Golgi apparatus, membrane bound proteases initiate the conversion to insulin by removal of the connecting or C-peptide. The newly converted insulin and proinsulin are packaged into membrane-bound secretory granules and pinched off into the beta cell cytoplasm. Conversion of proinsulin to insulin continues, and zinc enters the granules, complexing with insulin to form microcrystals exhibiting a 50A periodicity (Lacy, 1977). 2. Insulin secretion is initiated. Although the intermediate steps are not yet clear, glucose stimulation results in a change in membrane permeability to calcium, an enhanced intracellular calcium uptake, and a decreased efflux from the islets (Lacy, 1977). Synchronously, glucose stimulates the intracellular production of cyclic AMP, as well as the activation of beta cell cyclic nucleotide-dependent protein kinases. The purported role of cyclic AMP within the beta cell is to release bound calcium from intracellular organelles, notably the mitochondria, and to polymerize microtubulin into microtubules. The microtubular network is stimulated to contract in the presence of high calcium, and is thought to be involved in transport of the secretory granules to the cell surface, where they are released by the process of exocytosis (Lacy, 1977).

The kinetics of insulin secretion have been extensively examined in the isolated perfused pancreas and isolated islet

perifusion systems (Grodsky et al., 1974; Gerich et al., 1976a). The most striking characteristic of the beta cell response to glucose is it's biphasic pattern of insulin release. An abrupt increase in glucose concentration, from 5.5 to 16.5mM, followed by a continuous infusion (16.7 mM), resulted in peak insulin release within 5 minutes. This was followed by a second phase, which achieved a maximal value approximately 30 minutes after stimulation (Curry et al., 1968). Using in vitro models, different patterns of insulin secretion have been described in response to stimulation by the sulfonylurea drugs and arginine at low to absent glucose concentrations. These findings are not applicable to data derived in humans and other mammals, where ambient glucose levels must be maintained at 4-5 mM.

Several hypotheses have been put forth to explain this biphasic pattern of insulin release. It has been suggested that the insulin secreted during the first phase temporarily inhibits further secretion by a negative feedback mechanism (Lacy, 1977). However, most acute in vitro and perfusion studies have failed to demonstrate such an inhibitory effect of insulin (Malaisse et al., 1967b). A second possibility is that different islet beta cells have differing sensitivities to glucose stimulation. However, the relative consistency in height and duration of the biphasic response suggests that this is unlikely (Lacy, 1977). The third and most popular theory assumes that the first peak of secretion results from release

of insulin secretory granules already associated with the microtubular system, whereas the second represents release from a storage pool of more newly formed secretory granules becoming associated with the tubules (Lacy, 1977).

The dose response curve for insulin release, determined during constant glucose infusions of differing concentrations, was found to be sigmoidal (Grodsky, 1972). This suggests that release involves a series of interacting processes, rather than a single one-step phenomenon. The consequences of such a dose response curve are clear. At the beginning of a meal the blood glucose is low and the insulin secretory response is most sensitive. Since the top of the curve is flat, insulin secretion is seldom maximally stimulated.

In addition to the concentration of stimulant, the rate of stimulation has also been considered important for the magnitude of the insulin response. When five grams of glucose were infused at two different rates, the glucose concentrations achieved was similar at both rates (Chen and Porte, 1976). However, a submaximal insulin response resulted at the lower rate.

In addition to the aforementioned insulin secretogogues, other factors modulate insulin secretion. In 1964 McIntyre et al. demonstrated that the rise in plasma glucose after administration of a given dose by an oral route, was lower than that after intravenous infusion. Dupre et al. (1975)

demonstrated that a crude secretin preparation increased the disappearance rate of intravenous glucose, and elevated serum insulin levels. Thus it was postulated that glucose ingestion stimulates the release of a hormone, named 'incretin' by La Barre (1930), which has stimulatory effects on the pancreatic beta cell. Incretin is currently believed to be gastric inhibitory polypeptide (GIP), a 43 amino acid peptide, which inhibits secretion and motor activity of the stomach (Cataland and O'Dorisio, 1980). It has been localized in the K cells in the middle zone of the duodenal and jejunal glands (Solcia et al., 1975). GIP fits the criteria for an incretin i.e., it stimulates insulin secretion at concentrations found in the plasma after a meal, and it's concentration increases in plasma following oral glucose ingestion. GIP stimulates insulin secretion only in the presence of glucose, and is normally stimulated to be released by a glucose concentration above 8 mM (Schafer and Schatz, 1979). The glucose dependence of the action of this peptide resulted in the renaming of the compound, glucose-dependent insulinotropic peptide. Other substances which stimulate insulin secretion include glucagon, growth hormone, oxytocin, vasopressin, and prostaglandins (White et al., 1978).

Studies with ^{125}I-labelled insulin revealed that it is rapidly cleared from the blood, with a half life of 40 minutes (Duckworth and Kitabchi, 1981). The process appears to involve two enzymes: 1. glutathione-insulin transhydrogenase, which

catalyzes the cleavage of the disulfide bonds, and 2. insulinase, which hydrolyzes the separated alpha and beta chains.

B. GLUCAGON

The concentration of glucose in the blood is the most important signal for glucagon secretion; a decrease in blood sugar signals increased glucagon secretion, while increased blood sugar inhibits secretion (Buchanan et al., 1969). It is likely that the critical value for the stimulation of glucagon release is below 50 mg% glucose concentration, and suppression by glucose is above 150 mg% (Unger et al., 1968). The mechanisms responsible for glucagon suppression by glucose have not been fully clarified. There is no evidence that glucose alone has a strong direct inhibitory effect on the alpha cell, but this is a difficult question to study because isolated pancreatic alpha cells have not been available for investigation. Suppression of alpha cell activity may rely on it's juxtaposition to beta cells (Unger, 1981). Alpha cells without adjacent beta cells are found in glucagonomas, where glucagon suppression is compromised. Support for a beta cell role comes from observations that high concentrations of insulin can inhibit glucagon secretion. Edwards and Taylor demonstrated that metabolism of glucose within the alpha cell inhibits glucagon secretion. Indeed, stimulation of glucagon secretion has been demonstrated, using inhibitors of the glycolytic pathway (Muller et al., 1971).

Amino acids can strongly stimulate glucagon secretion. This appears to be an important mechanism to prevent insulin mediated hypoglycemia following a protein meal (Goodman, 1974).

It has been speculated that amino acids, most notably alanine, may control glucagon secretion during the starvation state.

It has been postulated that there may be feedback control between free fatty acids and the alpha cell (Luyckx and Lefebvre, 1970). Since there is little evidence that glucagon induced lipolysis is important in man (Goodman, 1974), the physiological importance of free fatty acid suppression of the A cell is probably modest.

Unlike insulin, whose source in the body is believed to be limited to the pancreatic islets, glucagon i.e., glucagon-like immunoreactivity (GLI) has been demonstrated in other organs. While the major source of circulating 'true' glucagon is the pancreatic alpha cell, the gastrointestinal tract is the major source of the GLI, which has been found in extracts of every portion of mammalian gut from esophagus to rectum (Moody, 1977). Pancreatic glucagon has now been found to be present in the stomach as well as the salivary glands (Matsuyama and Foa, 1974). While the concentration of salivary gland glucagon is high in the rat and mouse, in man it is very low.

Glucagon, like insulin, is synthesized as a pre-prohormone in the endoplasmic reticulum. From there, it is transferred to the Golgi, where the secretory granules are formed (Buchanan, 1977).

The secretory mechanism of glucagon has been difficult to determine, because of the difficulty in isolating alpha cells.

It was speculated that the same factors involved in insulin secretion may be involved in glucagon release.

Studies of the role of cAMP in the regulation of glucagon secretion have been ambiguous. Exogenous cyclic AMP stimulated glucagon secretion from the perfused canine and rat pancreas (Iversen and Miles, 1971; Weir et al., 1975). However, dibutyryl cAMP in the rat inhibited glucagon secretion (Toyota et al., 1975). The neonatal rat pancreas, similarly tested, showed release (Jarrousse and Rossselin, 1975). It was suggested by Wolheim et al. (1976a) that the effect of cAMP on glucagon secretion depends on the ambient calcium concentration; with 1 mM Ca cAMP stimulated glucagon release, whereas at 2.5 mM Ca cAMP caused delayed inhibition. These descrepent results may result from the fact that in some experiments acute changes were being measured, while in others long term effects were investigated. It may be that both stimulatory and inhibitory effects could occur at different times.

The role of calcium in alpha cell secretion is also unclear. While several workers have found that an abrupt increase of extracellular calcium evokes and increase in glucagon secretion, an equal number using the same species have demonstrated the reverse (Iversen and Hermansen, 1977; Leclercq-Meyer et al., 1977). Wolheim et al. (1976b), using monolayer cultures of fetal pancreata, observed glucagon secretion when calcium was absent or present at 2.5 mM; at

intermediate calcium concentrations of 0.3 or 1.0 mM, secretion was reduced. Marked stimulation was seen at 30 mM calcium. Use of the ionophore A23187, which caused enhancement of glucagon secretion, suggests that increases of intracellular calcium may trigger exocytosis. It is very difficult to interpret results from experiments where the concentration of extracellular calcium was varied, because this could lead to multiple plasma membrane and intracellular changes which could influence secretion. Paracrine effects from the beta and delta cells can also influence secretion.

Glucagon is rapidly degraded by enzymes in the liver and kidneys, as well as in the blood, by plasma proteolytic enzymes. The half life of infused glucagon in the general circulation is five to ten minutes (Duckworth and Kitabchi, 1981).

C. SOMATOSTATIN

Several substrates have been shown to affect somatostatin secretion in vitro. Glucose, arginine, and leucine stimulate biphasic somatostatin release in a biphasic pattern from perfused pancreata and perifused islets (Schauder et al., 1977; Patton et al., 1977). Glucose did not cause consistent release at concentrations below 7 mM. The maximal somatostatin responses to physiological concentrations of glucose (<30 mM) involve only a two- to threefold increment above basal secretion rates (Gerich et al., 1974a). This suggests that the pancreatic delta cell is less sensitive to glucose than are the alpha and beta cells. While no acute effect of insulin on somatostatin release has been found to date, glucagon, at concentrations less than 1 ng/ml, stimulates biphasic release of somatostatin (Patton et al., 1977). This suggests that islet alpha cells may exert a paracrine effect on delta cells.

Other factors which have been shown to affect somatostatin secretion include sulfonylurea drugs and gastrointestinal hormones, such as GIP, CCK-PZ, secretin, and VIP (Ipp et al., 1977; Ipp et al., 1978b). It has been speculated that these hormones are involved in a negative feedback loop whereby the increased levels of gastrointestinal hormones released following meal ingestion would stimulate pancreatic somatostatin, which in turn would decrease the rate of influx of dietary nutrients by inhibiting these hormones, as well as pancreatic secretion, gut motor activity, and intestinal

absorption. However, in most of these studies, pharmacological concentrations of gut hormone were used, raising concern over the physiological relevance of these findings.

There is evidence that at least three factors may be involved in the mechanism of somatostatin release. Augmentation of somatostatin release by theophylline and 8-bromo-cAMP, both of which would serve to increase intracellular cAMP, suggests that alterations in pancreatic delta cell cAMP levels may influence somatostatin secretion (Schauder et al., 1976). Again, since this was not performed on isolated delta cells, and since glucagon may also be stimulated by such treatment, glucagon may have been the somatostatin inducer. Inhibitors of glucose metabolism decreased somatostatin responses to glucose, suggesting that metabolism of glucose may be an important factor for glucose stimulated somatostatin release (Schauder et al., 1976). While very little is known about the role, if any, calcium plays in somatostatin release, preliminary studies suggest that calcium may be essential for somatostatin secretion (Gerich, 1981).

In man, normal circulating somatostatin-like immunoreactivity has been reported to average between 100 and 400 pg/ml (Conlon et al., 1978). This broad range may be at least partially due to serum-containing proteolytic enzymes, which destroy somatostatin. The half life of exogenously infused somatostatin is about 1-2 minutes in man and dogs. Sites of degradation include serum, liver, muscle, and kidney

(Goodman, 1974). Induction of hypothyroidism has been reported to decrease somatostatin degradation for an, as yet, unknown reason.

D. PANCREATIC POLYPEPTIDE

Human pancreatic polypeptide was discovered independently by Kimmel et al. (1968), and Chance (1972). It is a 36 amino acid peptide which has been localized to a pancreatic endocrine-type cell (PP cell), which is distinct from the other hormone producing islet cells. In contrast to the other islet cells, PP cells are found both in the islets and scattered in the exocrine parenchyma. They appear to be more numerous in the duodenal end of the pancreas, and become more numerous with age (Orci et al., 1976).

All ingested nutrients studied have elicited an increase in plasma PP, even those low in caloric value i.e., celery (Floyd et al., 1978). The response to the ingestion of beef is rapid, typically biphasic, and dependent in magnitude upon the amount ingested. Fats and carbohydrates are less potent stimulators of release than beef, but when all three are present together, as in a mixed meal, a potentiated release is demonstrated (Adrian et al., 1977). The stimulation of PP release seen after the ingestion of nutrients does not appear to result from direct stimulation of the cells by certain digestive products. Levels of PP in peripheral venous plasma did not change during intravenous infusion of 25 grams of amino acids (Adrian et al., 1977), free fatty acids (Floyd et al., 1977), or glucose (Marco et al., 1978). In fact, there is evidence that the intravenous administration of glucose transiently suppresses basal plasma PP levels (Marco et al.,

1977). Thus, stimulation of release may be due to neural influences (discussed later) or gastrointestinal hormones released during ingestion. Of the GI hormones tested, only secretin and pentagastrin stimulated release.

PP appears to be secreted in response to the ingestion of food and seems to modify the rate or amount of pancreatic juice and bile reaching the duodenum. In man, the mechanism of these effects might involve direct inhibition of hepatic bile flow and pancreatic exocrine production or secretion, as well as an increase in the tone of the sphincter of Oddi (Greenberg et al., 1979). There is, as yet, no indication in man of effects of PP on secretion of other islet hormones.

V. NERVOUS CONTROL OF PANCREATIC ISLET SECRETION

A. PARASYMPATHETIC REGULATION

Britton, in 1925, first observed that electrical stimulation of the vagus nerve of the cat resulted in a decrease in blood sugar. He attributed this effect to an increased release of insulin from the pancreas. Later studies showed directly that vagal stimulation results in an increase in insulin release (Findlay et al., 1969). In dogs, electrical stimulation of the right, left, and dorsal vagal trunks caused an increase in immunoreactive insulin (IRI) levels in the pancreatic vein, whereas stimulation of the ventral trunk had no effect (Kaneto et al., 1967). Acetylcholine was found to augment insulin release from islets of rats and dogs in vitro. It was believed to act via activation of muscarinic receptors, since the response was blocked by atropine (Woods and Porte, 1978). In man, acetylcholine also acts on muscarinic receptors, since both oral and infused glucose induced insulin secretion were diminished by atropine. While the effects of atropine in modifying insulin secretion induced by oral glucose are complex, it's effect could not be attributed to a delay in glucose absorption (Henderson et al., 1976). Malaisse (1967c) showed that the stimulatory effects of parasympathomimetic drugs required the presence of glucose. Cholinergic drugs, such as methacholine, have been shown to stimulate insulin secretion in humans (Kajinuma et al., 1968). While it is believed that these drugs act directly on the islets, indirect

effects, such as through release of gastrointestinal hormones, can not be excluded.

Vagotomy yielded mixed results: in dogs, a small decrease in glucose tolerance was reported in one study, while no significant change was recorded in another (Hakanson et al., 1971; Nelson et al., 1967). The latter investigators concluded that vagal innervation of the pancreas was not of major importance for normal insulin secretion. Vagotomy in monkeys resulted in a decrease in IRI levels during a fast, and a decreased glucose tolerance after fasting (Miller and Whittenberger, 1968). In rats, pancreatic vagotomy significantly decreased basal levels of plasma insulin in bilaterally adrenalectomized animals. It was not known what factor from the adrenal gland may have been responsible for this finding. In man, several studies have demonstrated that after selective, partial vagotomy or selective, gastric vagotomy, basal serum insulin levels and the postprandial insulin output were not changed (Becker et al., 1979). After truncal vagotomy, the insulin response to an oral glucose tolerance test or a test meal was either diminished or unchanged.

A neurally mediated reflex secretion of insulin during meals has been demonstrated in man (Parra-Covarrubias et al., 1971). The presentation of psychological conditions that normally presage the ingestion of food resulted in an increase in insulin secretion, believed mediated by the vagus, in the

absence of any food ingestion. There is evidence that this secretion can be controlled through classical conditioning procedures (Woods and Porte, 1978). The condition of reactive hypoglycemia, characterized by a tendency to secrete excess insulin only after eating a carbohydrate meal, suggests that this neural reflex may be hyperactive in these cases. Indeed, individuals with this condition may have learned to secrete insulin inappropriately (Woods and Porte, 1974). Hyperactivity of this reflex, leading to hyperinsulinemia during meals, may also contribute to the development of obesity.

Glucagon secretion, by the canine islets, was increased after stimulation of the vagus nerve (Gerich et al., 1976b). Treatment with atropine abolished the increase, indicating that the alpha cells are also supplied with muscarinic receptors. Atropine had no effect on the increase in glucagon resulting from hypoglycemia in the calf (Bloom et al., 1973). It appears that when the glycolytic pathway in the glucagon producing cell is suppressed, as in hypoglycemia or after the administration of specific blockers such as 2-deoxyglucose, glucagon is released by a mechanism which does not involve the nervous system (the blockade of sympathetic nerve receptors was without effect) (Buchanan, 1977). In a study of the pancreatic glucagon release during insulin induced hypoglycemia in patients after selective gastric vagotomy, no change in basal glucagon levels was found (Russell et al., 1974). Section of both splanchnic nerves in the adrenalectomized calf caused no

change in resting glucagon, but when atropine was added hypoglycemic convulsions occured, apparently due to the elimination of glucagon secretion compounded by the absence of the sympathetic responses. It was concluded that the vagus nerve regulates tonic glucagon secretion under basal conditions (Woods and Porte, 1978). More recently it was found that vagotomy did not affect alpha cell suppression in response to hyperglycemia (Findlay et al., 1979).

Available evidence on the role of the parasympathetic system in the regulation of the release of the other islet hormones is limited. Infusion of acetylcholine in the canine pancreas was shown to inhibit somatostatin release (Samols et al., 1977). This effect was blocked by atropine, indicating that the delta cells also possess muscarinic receptors. In the perfused chicken pancreas, however, acetylcholine (10 uM) potentiated somatostatin secretion (Honey and Weir, 1980). Parasympathetic regulation has also been implicated in the release of pancreatic polypeptide (PP)(Samols et al., 1977). Stimulation of the vagus nerves in anaesthetized pigs increased the portal levels of PP. Acetylcholine itself has been shown to be a potent stimulator of PP release from the isolated perfused porcine pancreas. In man, vagotomy markedly reduced the PP response to a meal. In addition, atropine diminished the PP response to hypoglycemia. These findings indicate that vagal, cholinergic stimulation is a major regulator of PP secretion (Floyd, 1979).

There is evidence that cholinergic effects on the beta cell are modulated by cyclic GMP (Howell and Montague, 1974). Levels of cGMP were found to be increased when isolated guinea pig islets were incubated with acetylcholine, and dibutyryl cGMP had significant stimulatory effects on rates of insulin biosynthesis.

B. SYMPATHETIC REGULATION

One of the earliest studies on the effects of the sympathetic nervous system on insulin release was that of Coore and Randle (1964). These investigators showed that the glucose stimulated insulin release from rabbit islets was abolished by adrenaline (200 ng/ml). The second peak of the biphasic release of insulin in response to glucose in the perfused rat pancreas was inhibited by epinephrine (Weir et al., 1974). In humans, epinephrine infusion (3 ng/Kg/min) inhibited glucose induced insulin secretion by 50% (Efendic et al., 1978). There is also some evidence from the same authors, that epinephrine shifts the dose response curve of glucose induced insulin secretion to the right, an effect compatible with, but not directly proving, competitive inhibition. Epinephrine, in physiological doses, does not block tolbutamide or arginine induced insulin secretion (Weir et al., 1974). Norepinephrine, a sympathetic neurotransmitter, has been shown to decrease basal and glucose induced insulin secretion in humans (Porte, 1967). Electrical stimulation of the splanchnic nerves (Porte et al., 1973), or the ventromedial hypothalamic nucleus (believed to be responsible for brain sympathetic outflow) elicited a decline in basal insulin secretion (Frohman and Bernardis, 1971). Blunted plasma insulin responses were also seen in patients with pheochromocytoma, an adrenal medullary tumor secreting large quantities of catecholamines (Colwell, 1969).

Norepinephrine and epinephrine act primarily on islet cell adrenergic receptors. The inhibitory effect of these catecholamines was prevented by blockade of the alpha receptors with either phentolamine or ergotamine tartrate (Coore and Randle, 1964; Porte, 1967). Administration of these drugs in man also resulted in an increased basal insulin level (Robertson and Porte, 1973). This suggests a physiological role of alpha adrenergic activity upon basal insulin secretion. Mechanisms of epinephrine inhibition have been associated with lowered islet cyclic AMP levels in rats (Turtle and Kipnis, 1967). However, epinephrine has also been reported to inhibit cyclic AMP induced insulin release in rats (Malaisse et al., 1967a). Thus, the effects of epinephrine appear incompletely modulated by cAMP.

Beta adrenergic receptors are also present in islets. Pharmacological stimulation of these receptors in rats not only induces insulin secretion, but also elevates islet cyclic AMP (Burr et al., 1971). Administration of the beta adrenergic stimulant, isoproteronol, to men resulted in a rise in immunoreactive insulin (IRI) levels. Work by Kaneto et al. (1975) showed that adrenergic stimulation of the endocrine pancreas of the dog was primarily via $beta_2$ receptors, since practolol, a specific $beta_1$ adrenoreceptor blocking compound, did not counteract the effect. Glucagon-induced insulin secretion was not blocked by propranolol, a mixed beta antagonist, leading the authors to postulate that glucagon's

effect was not mediated by adrenergic receptors. However, the possibility of glucagon blockade of the inhibitory alpha adrenergic receptor, resulting in an increase insulin release, can not be ruled out.

The effect of alpha adrenergic receptor stimulation appears to be dominant to the beta receptor effect on the beta cell, at least as judged from studies using mixed alpha and beta agonists. However, the opposing effects of alpha and beta receptor stimulation upon insulin secretion may occur simultaneously. Although glucose is ineffective in stimulating insulin release during an epinephrine infusion because of alpha receptor stimulation, insulin levels nonetheless rise over time, regardless of changes in glucose, as a result of simultaneous beta stimulation (Porte and Robertson, 1973). It was hypothesized that epinephrine produces its primary inhibitory effects by selectively blocking the insulin output from a small storage pool via the alpha receptor. In addition, epinephrine simultaneously stimulates both the mRNA synthesis related pool and the storage pool (Porte and Robertson, 1973), via the beta receptor.

The physiological modulation of insulin secretion by adrenergic agents appears established. However, the role played by the catecholamines in the regulation of basal insulin secretion requires further investigation. In experimental animals, alpha adrenergic tone appears to be involved in the maintenance of basal insulin levels. However, in men

exhibiting chronic adrenergic insufficiency in the form of either bilateral adrenalectomy or cervical sympathectomy, there were no abnormalities in basal insulin secretion or glucose induced insulin release (Brodows et al., 1974). These results suggest that neither central adrenergic tone, nor circulating catecholamines, play a significant role in the regulation of insulin secretion.

Beta adrenergic stimulation has been demonstrated to be a factor responsible for the elevation of glucagon levels following sympathetic stimulation (Iversen, 1973; Gerich et al., 1974b). However, exercise induced hyperglucagonemia in rats was shown to be mediated by the alpha receptor (Harvey et al., 1974). Stimulation of the alpha receptor caused a decrease in glucagon secretion in another experiment, whereas alpha blockade resulted in an increase in glucagon, suggesting that alpha adrenergic tone may play a role in governing basal glucagon levels (Gerich et al., 1974b). The rise in glucagon after VM hypothalamic nucleus stimulation was not abolished by either alpha or beta blockers (Walter et al., 1974). To confuse matters even more, it has just recently been demonstrated that the alpha cell is relatively insensitive to isoproterenol and that beta adrenergic regulation of plasma glucagon, therefore, probably occurs by a mechanism other than beta receptor activation (Palmer et al., 1979). By whatever mechanism catecholamines stimulate glucagon secretion, it has been noted that glucagon so stimulated, exhibits a monophasic

secretory response, compared to the biphasic response elicited by hypoglycemia or amino acid injestion (Iversen, 1973). It is interesting to note that the glucagon response to epinephrine was greater in insulin-dependent juvenile diabetics, than in normal volunteers (Gerich et al., 1976b).

Although there is now substantial evidence that manipulation of pancreatic neural input alters insulin and glucagon secretion, no similar data are presently available for the other pancreatic hormones. Samols et al. (1977) recently showed, however, that stimulation of islet cell beta receptors through the use of isoproterenol caused an increase in somatostatin release. Though isoproteronol also stimulates alpha receptors, a beta receptor mechanism seemed likely here, since propranolol prevented its action. But since insulin and glucagon have also been reported to stimulate somatostatin release, it is unclear whether these results indicate a direct action of isoproteronol on the delta cells, or an indirect effect mediated by insulin or glucagon. In contrast, epinephrine was reported to inhibit somatostatin release from isolated rat islets during stimulation by glucose, when insulin levels are expected to be high (Schauder et al., 1976). Human pancreatic polypeptide (PP), the recently discovered polypeptide in pancreatic endocrine cells, has also been implicated as being regulated by the sympathetic system. Adrenergic modulation of PP release, however, is not as easily demonstrated as the parasympathetic effects. The current

literature suggests that beta adrenergic stimulation augments PP release (Floyd, 1979).

C. CNS REGULATION

Glucose is the major fuel that is utilizable by neural tissue; severe hypoglycemia can lead to coma or even death. Changes in the availability of fuels to the brain have been shown to reflexly modify the secretion of the pancreatic hormones involved in systemic glucose uptake and metabolism. La Barre (1930) designed a complex parabiotic experiment to examine the role of the CNS in islet secretion. The blood from one dog perfused only the brain of a second. The pancreatic vein of the second dog drained into the vasculature of a third. Injection of glucose into the first dog caused a marked hypoglycemia in the third dog. This hypoglycemic response was not affected by the removal of the cerebral hemispheres of the second dog, suggesting involvement of the hypothalamus. The hypoglycemia persisted for two hours after cessation of the cross perfusion, and was interpreted as being due to insulin, released from the pancreas of the second dog, after it sensed an increase in glucose.

Although most brain cells are not dependent upon insulin for glucose uptake, there is evidence that some cells, particularly those of the ventral hypothalamus, are sensitive to insulin (Oomura, 1976). It has been reported that injection of insulin or glucose into the carotid arteries or CSF results in a rapid decrease of systemic blood glucose (Szabo and Szabo, 1972). The peripheral hypoglycemia caused by the injection into the carotid arteries appeared to be the result of a direct

neural influence on the liver, whereas that which occurred following CSF injection was due to an increased insulin secretion, mediated by the vagus nerve. Thus, the brain can reflexly increase peripheral glucose uptake when it senses a glucose surplus.

The opposite reflex presumably also occurs. Injection of 2-deoxyglucose, a nonmetabolizable competitor of glucose, into the carotid artery of the rabbit, resulted in CNS glucopenia and caused a marked increase in peripheral blood glucose, mediated, in part, by adrenal secretions (Sakata et al., 1963). The administration of 2-deoxyglucose to the CSF resulted in an increase in plasma glucagon levels, and to a decrease in insulin secretion (Muller et al., 1973). The reflex increase of glucose is considered part of an overall sympathetic arousal, mediated by the hypothalamus and splanchnic nerves. These experiments indicate that the brain responds to an apparent decrease in glucose availability by causing a reflex increase of plasma glucose.

In summary, hypoglycemia has a direct stimulatory effect on the hypothalamus in exciting the sympathetic system, which in turn, causes the release of glucagon, and inhibits the release of insulin from the pancreatic islets. Epinephrine release from the adrenal medulla is also stimulated. Epinephrine and glucagon act on the liver to stimulate glycogenolysis, and thereby restore blood sugar to normal. Hyperglycemia stimulates hypothalamic parasympathetic centers

to cause a vagally mediated increase in insulin secretion, which then acts to decrease blood sugar by opening glucose transport channels in receptive tissues. In addition, direct innervation to the liver stimulates glycogen synthesis.

D. PEPTIDERGIC REGULATION

The role played by peptidergic nerves in the control of pancreatic exocrine and endocrine secretion remains obscure. Since gastrin (Rehfeld, 1971) and CCK (Pederson and Brown, 1979; Konturek, 1978) are potent stimulators of insulin release, it can be speculated that nerves containing these peptides in the islet may be of physiological importance. Both peptides are also known to stimulate the release of pancreatic enzymes and bicarbonate (Konturek, 1978). More information is available in connection with VIP. VIP has been shown to have a secretin-like effect on the water and bicarbonate secretion from the porcine pancreas (Fahrenkrug, 1979). In addition, it has been shown to stimulate insulin and glucagon release from the isolated, perfused porcine pancreas (Lindkaer et al., 1978), and canine pancreas (Kaneto et al., 1977). In the perfused rat pancreas, VIP was found to stimulate somatostatin release under basal and glucose stimulated conditions (Szecowka et al., 1980). The occurence of VIPergic terminals in relation to the pancreatic exocrine and endocrine parenchyma, together with the fact that the vagally induced release of VIP is blocked by atropine, suggest a physiological role for VIP in the nervous modulation of pancreatic exocrine and endocrine secretion.

Substance P did not influence insulin release in the rat, in vivo or in vitro in one study (Lockhart-Ewart et al., 1976), while in another, inhibited glucose and arginine induced

insulin release, and lowered fasting insulin levels (Brown et al., 1976). Systemic injections of large doses of Substance P (2-6 ug) caused inhibition of insulin secretion. Infusion of Substance P (20 pmoles/Kg/min) into the dog pancreas in vivo, caused a stimulation of insulin secretion and elevated peripheral glucose levels (Kaneto et al., 1978). It is apparent that more work needs to be done to reconcile these opposing effects.

Experiments testing the effects of beta endorphin on islet hormone release have yielded mixed results. Using the perfused dog pancreas, Ipp et al. (1978a) demonstrated that at basal glucose concentrations beta endorphin suppressed somatostatin (SRIF) release, while the secretion of insulin and glucagon was stimulated. Omission of glucose from the perfusate was associated with higher baseline levels of glucagon, and an exaggerated glucagon response to beta endorphin; SRIF levels were suppressed as in the presence of glucose. The effects of B-endorphin on canine islet hormone release were completely abolished by the opiate antagonist, naloxone. These authors concluded therefore, that endogenous opiate peptides were capable of altering islet cell function, probably via a specific receptor mechanism. However, experiments in the rat failed to show an alteration in insulin secretion with naloxone (Tannenbaum et al., 1979). B-endorphin may play a role in the response to a hypoglycemic stress in the human (Nakao et al., 1979): it's concentration is elevated substantially after

insulin injection. The release of beta endorphin under these circumstances may play a role in augmenting glucagon secretion as noted above in the dog.

In addition to being present in nerve fibers, many of the above mentioned peptides have been demonstrated by immunocytochemistry in cells in the intestinal mucosa and the pancreas. VIP immunoreactive cells have been detected in the dog, guinea pig, and man, where they were shown to be similar to the delta cells (Buffa et al., 1977). The presence of these cells may help explain the occurence of pancreatic tumors secreting VIP in the watery diarrhea syndrome (Polak and Bloom, 1979b). Endorphins and enkephalins have also been localized immunocytochemically in endocrine cells in the stomach, duodenum, gallbladder and pancreas (Powell and Skrabanek, 1979). Some evidence suggests a localization of B-endorphin in the delta cells in the human pancreas (Bruni et al., 1979), while in rat islets, endorphin-like immunoreactivity seemed to be contained within the alpha cells (Grube et al., 1978b). It is interesting to note that B-endorphin was absent from the pancreas of a human with Type II diabetes (Lockhart-Ewart et al., 1976). In addition, CCK-PZ was found to be localized in the rat and human alpha cell (Grube et al., 1978a). While these authors could not specifically identify gastrin immunoreactivity within the islet cells, Erlandsen et al. (1976) localized this peptide within the somatostatin containing delta-cells in the human. In addition, growth

hormone has been identified in peripherally placed islet cells in the rat (Hakanson et al., 1978), ACTH has been identified in the pancreatic polypeptide containing cells of the endocrine pancreas (Larsson, 1978), and luteinizing hormone-releasing factor (LRF) immunoreactivity has been identified, but not localized to any cell type (Seppala et al., 1979). It should be noted that a high concentration of thyrotropin-releasing hormone has been isolated from the rat pancreas (Martino et al., 1978; Koivusalo and Leppaluoto, 1977; Leppaluoto et al., 1978). Most of this TRH immunoreactivity was localized within the islets (Martino et al., 1978), but it's particular origin within the islet has yet to be determined. Fetal and newborn rats were found to have pancreatic levels of TRH higher than in the hypothalamus (Koivusalo and Leppaluoto, 1977), whereas the reverse was true for the adult. No effect of TRH on the islets was demonstrated in the rat, in vitro or in vivo (Lockhart-Ewart et al., 1976).

There are additional gut peptides that have been shown to have effects on pancreatic exocrine and endocrine secretion. Neurotensin, which has been identified in gut endocrine cells from the esophagus to the colon (Powell and Skrabanek, 1979), has recently been shown to have a dual effect on the rat endocrine pancreas (Dolais-Kitabgi et al., 1979). At low glucose concentrations it stimulated insulin, glucagon and somatostatin release, but under stimulatory conditions with either glucose or arginine, it inhibited the release of all

three hormones. Motilin, a 22 amino acid peptide whose presence in the serotonin containing enterochromaffin cells is still disputed, is a potent inhibitor of secretin-induced bicarbonate secretion (Singh and Webster, 1978). Since this peptide was released by duodenal alkalinization, it was postulated by these authors that the peptide may be involved in the feedback inhibition of pancreatic exocrine secretion. Bombesin, a 14 amino acid peptide first isolated from the skin of frogs, has been shown to have an effect on pancreatic secretion. In the endocrine pancreas, it stimulated insulin, glucagon and pancreatic polypeptide release, the latter being cholinergic dependent (Polak and Bloom, 1979a). Somatostatin was unaffected by the perfusion of the canine pancreas with bombesin (Ipp and Unger, 1979). In the exocrine pancreas, bombesin stimulated enzyme secretion and calcium efflux (Polak and Bloom, 1979b). It was hypothesized that the effects of bombesin were as a releasing factor for other GI hormones (Singh and Webster, 1978).

VI. SEROTONIN AND THE PANCREAS

A. LOCALIZATION OF SEROTONIN IN THE PANCREAS

In 1963, Falck and Hellman, using formaldehyde to induce fluorescence, demonstrated the presence of biogenic amines in the islets of the guinea pig, cat, dog, and horse. This fluorescence was markedly decreased by pretreatment of the animals with reserpine (2 mg/Kg), a catecholamine and monoamine depletor. No specific fluorescence was observed in the islets of the rat or mouse. Injection of tritiated 5-hydroxytryptophan (^{3}H-5HTP, 60 mg/Kg) into mice resulted in a marked uptake into the pancreatic acinar cells (Gershon and Ross, 1966). Initially, the labelling was diffuse and did not appear concentrated in any region of the cells. By 30 minutes, however, the labelled material was concentrated in the apical portion of the cells and was discharged into the duct system within one hour. The islet cells were also labelled, but specific cell types were not identified. Much of the radioactivity in the pancreas was shown to have been metabolized to labelled 5-HT, owing to the high concentration of aromatic amino acid decarboxylase in this tissue. In 1968, Cegrell examined the biogenic amines in different species using the same technique of formaldehyde induced fluorescence. A storage of monoamines in endocrine cells was demonstrated in the human fetus, pig, dog, cat and guinea pig, but not in the cynomolgus monkey, golden hamster or rat. In the islets of the rabbit and mouse, a monoamine store could be demonstrated but

only in young, pigmented animals. In albino mice, the histochemical fluorescence characteristic of dopamine or serotonin could be demonstrated only after L-DOPA or 5-HTP injection, respectively. Using a glutaraldehyde-dichromate technique for electron microscopy, Jaim-Etcheverry and Zieher (1968) demonstrated that 5-HT, present in beta cells of the guinea pig pancreas, was localized within the same secretory granule as insulin. Following the intravenous injection of ^{3}H-5HTP (8×10^{-7} moles), Ekholm et al. (1971) examined uptake in the islet cells as demonstrated by electron microscopic radioautography. They found most of the silver grains over alpha and beta cell granules, with very few grains being demonstrated over the delta cells. Morphometrically, the greater portion of silver grains resided over the peripheral, as opposed to the dense cored region of the secretory granules. Pretreatment of the islets with a monoamine oxidase (MAO) inhibitor caused an increased retention of label over the cells, most notably over the alpha cells, while pretreatment with reserpine abolished the radioautographic reaction. Similarly, Gylfe et al. (1973) incubated ob/ob mouse islets with ^{14}C-5HTP. After homogenization and ultracentrifugation, the majority of label sedimented with the secretory granule fraction. Using the dansylation method, they determined that the labelled material was largely 5-HT. Dansyl chloride reacts with tryptophan and its derivatives which are then separated by two-dimensional thin-layer chromatography and detected by fluorescence. It could be noted, in retrospect,

however, that neuronal synaptosomes would also sediment with the secretory granule fraction during centrifugation. Further studies by Gylfe (1980), in which islets from obese-hyperglycemic mice were preloaded with ^{3}H-5HTP and pargyline (an MAO inhibitor) demonstrated a complete parallelism between serotonin efflux and insulin release. In addition, the efflux of ^{3}H-5HT was found to be blocked by metabolic inhibitors, suggesting that the maintenance of 5-HT within the beta cell depends on the expenditure of metabolic energy (Gylfe, 1980).

B. PHYSIOLOGICAL ROLE OF SEROTONIN ON ENDOCRINE PANCREATIC SECRETION

After the observation that precursors of 5-HT are taken up by the islet cells, interest has centered on the role of these intracellular amines as regulators of islet cell function. Effects of 5-HT have been assessed by examining the changes in insulin secretion produced by the administration of amine precursors, or of inhibitors of the breakdown of intracellular monoamines. Infusion of amine precursors such as 5-HTP, in vivo, resulted in a state of reduced insulin responsiveness to glucose when isolated islets or pieces of rat, mouse, or hamster pancreata were subsequently incubated with glucose in vitro (Lundquist et al., 1971; Rossini and Buse, 1973; Lebovitz and Feldman, 1973; Lernmark, 1971). In rabbits, infusion of 5-HTP (50 mg/Kg) resulted in a marked reduction in the glucose stimulated insulin release (Wilson et al., 1974). It was verified that the reduction in secretion was due to the conversion of the precursor to 5-HT; administration of a decarboxylase inhibitor (300 mg/Kg) blocked the inhibitory effect. On the contrary, the accumulation of 5-HT following 5-HTP administration to rats resulted in no alteration in the pattern or total amount of insulin released following either glucose or tolbutamide stimulation (Pulido et al., 1978). In the rat, both calcium ions and the intracellular serotonin accumulated after precursor administration were found to occupy the halo of the insulin secretory granule after glucose

stimulation (20 mM), while no such arrangement was seen during low glucose (3 mM) (Kloppel and Bommer, 1979). One can therefore speculate that serotonin may block insulin release in some species by interfering with the intracellular calcium, which is known to be an absolute requirement for release. Indeed, in at least one case (Lindstrom and Sehlin, 1979), 5-HT has been shown to reduce calcium ion uptake by isolated islets, possibly by stimulating efflux.

While it is clear that islet cells can incorporate the immediate biosynthetic precursor, and decarboxylate it to serotonin, the circulating levels of 5HTP are probably negligible (Ekholm et al., 1971). The physiological significance, therefore, of the demonstration of alterations in insulin release following administration of a high concentration of 5-HTP is open to question. 5-HTP has never been detected in the circulation of normal rats or mice at a concentration close to the Km of the decarboxylase enzyme for 5-HTP. In these species, concentrations of 5-HTP up to 10,000X the physiological range, were required before 5-HT could even be detected in the pancreas (Ekholm et al., 1971). It should be noted here that most of the evidence presented above for the presence of serotonin in the pancreas relied on the prior administration of 5-HTP. Though serotonin was identified using biochemical and fluorescent techniques in the pancreas without administration of precursor, the amount detected biochemically did not seem to correlate with the level of fluorescence seen.

This data does not rule out the possibility of a presence of a histochemically undetectable but biochemical store of the monoamine in another tissue compartment, such as a nerve terminal, which could be detected biochemically but not histochemically.

The effects of monoamines on the islet cells have also been studied using inhibitors of MAO. Monoamine oxidase, one of the enzymes involved in the degradation of serotonin, has been shown to be present in the islets of many species (Feldman and Chapman, 1975a; Quansah et al., 1981). The intracellular localization of MAO has not been determined. Islet MAO was shown to be very similar to liver MAO in its Km for tryptamine, and its sensitivity to inhibitors. MAO inhibitors can be classified into two groups: hydrazine type and non-hydrazine type. The data on modification of insulin release using MAO inhibitors is confusing. Aleyassine and Lee (1971) found that many of the MAO effects on insulin secretion were concentration dependent. All the MAO inhibitors were found to be effective in inhibiting monoamine oxidase at relatively low concentrations, but neither the stimulation nor inhibition of insulin secretion could be directly correlated with the degree of MAO inhibition (Feldman and Chapman, 1975a). It was also noted that some MAO inhibitors elicited hypoglycemia unrelated to insulin secretion. It is very difficult, therefore, to interpret the results from such experiments. In addition, it was recently discovered that hydrazine stimulates insulin

release at concentrations which do not inhibit MAO (Feldman and Chapman, 1975b). In that same experiment, it was determined that rabbit islet MAO had 10x the specific activity against dopamine as serotonin, and so MAO may not be involved in the major pathway for serotonin inactivation, at least in rabbits.

The effects of exogenously administered serotonin on islet hormone secretion were studied by the direct incubation of pancreatic tissue with high concentrations of 5-HT (10^{-4} to 10^{-3}M), and by administration of serotonin receptor antagonists. By these techniques, serotonin was shown to inhibit the glucose stimulated insulin secretion from the golden hamster, rabbit and mouse pancreas (Feldman et al., 1972; Quickel et al., 1971; Feldman, 1979). Serotonin inhibited insulin release induced by glucose, tolbutamide and dibutyryl cAMP. When the rat pancreas was perfused with a solution containing serotonin (10^{-5}M), insulin secretion in response to a glucose challenge was inhibited, while basal levels were unaffected (deBold and Bencosme, 1975). It is interesting to note that in this experiment, exogenous serotonin was shown to inhibit only the first phase of the biphasic insulin response. To the contrary, injection of serotonin (6 mg) into the portal vein of dogs enhanced glucose stimulated insulin release (Faud et al., 1975). A stimulatory effect of 5-HT on insulin release in albino rats has also been reported (Gagliardino et al., 1971). Quickel et al. (1971) have discovered that after treatment of hamsters with reserpine

(0.5 mg/Kg) there was a marked decrease in the sensitivity to serotonin inhibition, but no alteration in sensitivity to exogenous norepinephrine. This experiment mimicked the normal condition found in albinos, as these animals have no detectable norepinephrine in the nerve endings near islet cells, or in the islet cells themselves. These results suggest that serotonin inhibition of insulin secretion in hamsters may be partially dependent upon the release of endogenous norepinephrine from adrenergic nerves in the islet (Ekholm et al., 1971). Alternatively, serotonin could stimulate insulin secretion via beta receptors either directly or in conjunction with catecholamines. This mechanism could provide one explanation for the inhibitory and stimulatory action of serotonin on insulin release. At least in one study (Feldman and Lebovitz, 1970), serotonin was reported to interact directly with the alpha adrenergic receptor.

Serotonin antagonists have been employed to study the effects of serotonin on insulin release. Feldman et al. (1972), using an in vitro rabbit pancreas sytem, were able to potentiate both glucose and tolbutamide stimulated insulin secretion by preincubating slices with methysergide maleate, a potent serotonin antagonist. However, other investigators concluded that pretreatment with methysergide had no effect on insulin release (deBold and Bencosme, 1975). Cyproheptadine inhibited insulin secretion from intact rats, perifused rat islets and from hamster pancreatic segments (Richardson et al.,

1975; Feldman et al., 1974; Wold et al., 1971). These effects may have resulted from cyproheptadine's action as an inhibitor of MAO, rather than it's effect as a serotonin receptor blocker. In contrast, cyproheptadine potentiated the glucose stimulated insulin secretion from segments of rabbit pancreas (Feldman et al., 1972), and induced large increases in insulin in rats fasted overnight (Bryce and Jacoby, 1978). No consistent alteration was observed with methysergide or cinanserin, in the rat.

Exogenous serotonin (4×10^{-3}M) decreased the basal and arginine stimulated glucagon release from mouse alpha cells (Marco et al., 1977). Other investigators however, found serotonin to be without effect on glucagon release (Pontiroli et al., 1978).

Serotonin caused no observable changes in insulin secretion after infusion of glucose or arginine in normal human subjects (de Leiva et al, 1978). Serotonin antagonists, such as methysergide, had only minimal effects on stimulating secretion in normal subjects after oral or infused glucose, whereas basal insulin levels were increased in Type II diabetics. PCPA (parachlorophenylalanine), an inhibitor of serotonin synthesis, resulted in a slight reduction in insulin release. In patients with the carcinoid syndrome, which is associated with elevated serotonin levels, improved insulin secretion was observed after methysergide (Feldman et al., 1972). Glucagon secretion in man was potentiated by 5-HT

receptor antagonists (Weir, 1981). It is difficult to interpret results after the intraperitoneal injection of such drugs, however, since the effects may have been the result of their interaction with other organs, such as the brain or liver.

VII. RATIONALE AND OUTLINE OF INVESTIGATION

The pancreas has classically been regarded as being innervated only by sympathetic and parasympathetic nerves. Widespread application of immunocytochemical techniques to the localization of certain peptides in nerve fibers has stimulated a reexamination of this question. Using these techniques, neurosecretory nerves, believed to synthesize and secrete chemical mediators, have been localized in the pancreas of certain mammalian species. The peptides which have been described in these nerve fibers include VIP, Substance P, and CCK. Recently, nerve fibers which use serotonin as their neurotransmitter have been described in the mammalian myenteric plexus. The cell bodies of these serotonergic nerves have been shown to be intrinsic to the gut. Since the gut and pancreas have a similar ontogeny, and exhibit a striking parallelism in their possession of neurosecretory nerves and endocrine cells, it is expected that the pancreas may also be innervated by serotonergic nerves.

The pancreatic islets are capable of hormone secretion in response to a glucose infusion in the absence of extrinsic nerve input. The autonomic nervous system is believed to act as a 'fine tuning' control for modulating hormone secretion in the normal subject. There is evidence that innervation may play a role in the pathogenesis of abnormal islet function in the diabetic patient. For example, administration of serotonin was shown to significantly enhance or depress insulin secretion

in Type II diabetic humans, and administration of serotonin receptor antagonists to these individuals resulted in an increase in insulin secretion. This suggests the possibility that serotonin, released from nerve fibers in the pancreas, may be physiologically relevant in the control of pancreatic function. Thus, an investigation into the presence of serotonergic fibers in the pancreas may add to our understanding of islet cell function.

In order to demonstrate the presence of serotonergic nerve fibers in the pancreas, advantage was taken of the high affinity uptake system for this neurotransmitter. Tissue was incubated in a low concentration of ^{3}H-5HT, with and without an excess of unlabelled 5-HT, and specific uptake was determined by dissolving and counting the radioactivity in the tissue. The time course, kinetic and saturation curves for this uptake were determined. Sex, glandular regional, and species differences in uptake were also investigated. In addition, the effects of norepinephrine, serotonin receptor and uptake blockers, and specific serotonin neurotoxins on the pancreatic uptake were investigated. Uptake in rat fetuses was also investigated. Radioautography was performed on tissue after uptake in order to visualize the sites of the serotonin uptake. The uptake of radiolabeled serotonin, followed by radioautography, has been used extensively to study the distribution of serotonergic nerves in the brain and gut, and it is now similarly being used in the pancreas to demonstrate

the presence of serotonergic fibers.

Since the presence of serotonergic nerves necessitates the presence of the biosynthetic machinery for the synthesis of neurotransmitter, pancreatic homogenates were assayed for tryptophan hydroxylase, the rate limiting enzyme in serotonin synthesis from tryptophan.

If serotonin acts as a neurotransmitter in the pancreas, it should be possible to demonstrate its release upon nerve stimulation. This question was approached in two ways. After specific uptake of ^{3}H-5HT into nerve fibers in pancreatic slices, the release of the labelled 5-HT, in response to fenfluramine, a drug which is known to cause release of serotonin from brain serotonergic nerves, was determined. Second, a similar experiment was performed using veratridine, a drug that is known to non-specifically stimulate nerve depolarization by opening sodium channels in the axon. If the site of ^{3}H-5HT uptake was indeed serotonergic nerves, it would be expected that such treatments would result in an increased rate of release of radioactivity. A critical assumption in these experiments is that newly uptaken ^{3}H-5HT will be preferentially released. Our results seem to indicate that this is the case.

Since it has been demonstrated by others that the abdominal portions of the vagus and splanchnic nerves are devoid of serotonin fibers (Dreyfus et al., 1977b), the

pancreatic serotonergic cell bodies may reside within the gland, or possibly in the myenteric plexus of the duodenum, an area known to contain serotonergic perikarya (Gershon and Dreyfus, 1980). To investigate this, fetal pancreata were cultured in vitro alone, or with their attached duodenal segment. After 4 days an uptake was performed. If it is assumed that serotonergic axons, severed from their cell bodies for 4 days, lose their ability to accumulate serotonin (Azmitia and Conrad, 1976), then uptake should be preserved in pancreas cultured alone or with duodenum if the cell bodies are intrapancreatic. If the cell bodies were located in the duodenum, then only pancreata cultured with duodenum should demonstrate uptake.

Serotonin immunocytochemistry was also performed on pancreatic slices in order to visualize the presence of serotonin in pancreatic nerves without pretreatment with the monoamine.

MATERIALS & METHODS

I. Animals:

Male Sprague Dawley rats (Zivic Miller Labs, Allison Park, PA), weighing approximately 200 g, were used, unless otherwise stated. The rats were maintained in the central animal facility on a 24 hour control light cycle (12 hours light/12 hours dark). They were fed Purina rat chow ad libitum, and had free access to tap water.

II. Drugs:

Pargyline, p-chlorophenylalanine, p-chloroamphetamine, 5-hydroxytryptamine, veratridine, norepinephrine, tryptophan, and phenylalanine, were purchased from the Sigma Chemical Co. (St. Louis, MO). Other drugs were supplied by the following companies: fluoxetine, Eli Lilly and Co. (Indianapolis, IN); fenfluramine HCL, and metergoline, Robins Research Labs (Richmond, VA).

III. Tritiated serotonin uptake in pancreas:

The procedure was similar to that used by Azmitia and Marovitz (1980). Krebs-Ringer solution (KRBS) was supplemented with pargyline (10^{-4}M), a non-hydrazine monoamine oxidase inhibitor, ascorbic acid (10^{-3}M), and dextrose (10^{-2}M). Rats were decapitated and the pancreas was rapidly removed, placed in KRBS, and bubbled with 95% O_2 and 5% CO_2, on ice. The pancreas was minced with fine scissors into small

pieces, and washed with excess buffer three or four times during the process. Mincing was considered complete when the pieces were able to be passed through a standard Pasteur pipette. Twenty to thirty pieces were then placed into beakers containing KRBS (2 mls). Ten ul of tritium labelled serotonin (New England Nuclear or Amersham, specific activity 16.6 - 30.3 Ci/mmole; alpha and beta hydrogens labelled) was added to all the beakers to a final concentration of 5×10^{-8}M. The beakers were then incubated in a shaking water bath at 37°C. The incubation was stopped at time intervals from 5 to 20 minutes by placing the beakers on ice, and the tissue was transferred to scintillation vials containing a large volume of cold buffer. In subsequent experiments, tissue was incubated for 15 minutes at which time the uptake was maximal. Prior to weighing, the tissue was washed twice with buffer, and then placed into a Petri dish containing fresh buffer. Eight to ten pieces were selected randomly for each weighing, blotted gently on filter paper, placed in a scintillation vial, and weighed. Generally, five to ten samples were weighed from each experimental group. Since weighings took from 30 to 60 minutes, it was necessary to check for radioactivity loss during this process; no loss was demonstrated. Protosol (NEN) (0.4 ml), was pipetted into each vial, which were then placed in a 45°C oven overnight, after which time they were counted in a Beckman scintillation counter, using Econofluor (NEN). The uptake was expressed as pmoles/gram wet weight, by the following calculations:

Counts per minute (CPM) X efficiency = Disintegrations per minute (DPM); DPM ÷ 2.22 x 10^{12} disintegrations per curie ÷ specific activity ÷ tissue weight = pmoles/gram

Since the uptake was performed on tissue fragments, there was concern that the size of the pieces might influence diffusion of the ^{3}H-5HT. If so, larger pieces would yield deceptively low uptake values. To test this, uptake (in pmoles/gram wet weight) was correlated with weight, (range = 2 to 4 mg), with a resulting correlation coefficient of 0.13. Therefore, changes in uptake per unit weight, based on tissue size alone, do not appear to be linearly related.

A. Saturability of uptake:

To determine whether the uptake mechanism for serotonin in the pancreas is saturable, pancreatic slices were incubated as described with ^{3}H-5HT at a final concentration of 9 x 10^{-8}M, which, unless otherwise stated, was the concentration used in all subsequent experiments. To some beakers, unlabelled serotonin (10 ul) was added to final concentrations of 10^{-8}M, 10^{-7}M, 10^{-6}M, or 10^{-5}M. Those beakers not receiving unlabelled serotonin were given ten ul of buffer so as to compensate for the difference in volumes. This compensation procedure was performed in all uptake experiments. Since, as will be seen later, incubation of tissue in the presence of unlabelled 5-HT at 10^{-5}M completely abolished the silver grain aggregates

seen in radioautographs, representing the uptake sites, nonspecific uptake was defined as the number of counts present when tissue was incubated in the presence of 10^{-5}M 5-HT; specific uptake was defined as the difference between uptake in the presence and absence of 5-HT (10^{-5}M).

B. Specificity of uptake:

Specificity was determined by the addition of unlabelled norepinephrine (10^{-5}M) to the incubation medium of some of the tissue, in addition to ^{3}H-5HT.

C. Determination of the kinetics of the specific serotonin uptake:

The kinetics of the serotonin uptake was examined by incubating pancreatic slices with increasing concentrations of ^{3}H-5HT (4.8×10^{-8}M to 3.9×10^{-7}M). This range was used since at higher concentrations uptake by other than neuronal elements might interfere with the determination of the kinetics of the high affinity system. Specificity was determined at each concentration by the addition of unlabelled 5-HT (10^{-5}M). The data were analyzed by a Lineweaver-Burk plot.

D. Sex difference in uptake:

In order to determine if there is a variation in pancreatic ^{3}H-5HT uptake based on sex, the pancreata from two male and two female weight matched rats were removed,

pooled based on sex, and an uptake performed as described.

E. Variation in different areas of the pancreas:

The distribution of the serotonin uptake sites was investigated in the different regions of the pancreas. The splenic, pyloric, and intestinal sections of the gland were separately pooled from three rats, and an uptake was performed.

F. Species variation:

The specific uptake of serotonin in the rat pancreas was compared to that of the rabbit, golden hamster, and monkey. The pancreata from two rats, six rabbits (New Zealand White, 5 to 6 lb females, Perfection Breeders, Douglassville, PA), three hamsters (Golden Syrian, 120 to 130 gram females, Charles River, Mass), and one monkey (Macaca Fascicularis, 2.9 Kg female, Charles River, Mass), were separately pooled and an uptake was performed as previously described. Monkey pancreas fragments were incubated with 1×10^{-7}M ^{3}H-5HT.

G. Effect of pharmacologic agents on serotonin uptake:

The effect of fluoxetine, a drug known to inhibit the high affinity uptake of serotonin in the brain and gut, on pancreatic serotonin uptake was determined. Fluoxetine was injected (10 mg/Kg i.p.), and four hours later the pancreas was removed. It was minced as usual, and fragments were placed into beakers containing two ml of fresh KRBS, containing 10^{-5}M fluoxetine. Uptake was performed as previously

described. The effect of p-chloroamphetamine (PCA), a drug known to have destructive effects on brain 5-HT nerves, was similarly determined. PCA (20 mg/Kg i.p.) was injected on five successive days, and the pancreas was removed and an uptake performed one week later. All animals were kept in the cold from the time of the first injection, to prevent death from hyperthermia. In addition, the effect of PCA in vitro was also investigated. Tissue fragments were incubated with 5 x 10^{-4}M PCA, together with ^{3}H-5HT. Tissue was also preincubated with PCA (5 x 10^{-4}M) for 30 minutes, washed well with buffer, and subsequently incubated with ^{3}H-5HT. The latter was performed in order to rule out a competition of PCA with serotonin, which could account for a reduction in serotonin uptake.

The effect of the neurotoxin 5,7-dihydroxytryptamine (5,7DHT) on the pancreatic uptake of serotonin was also investigated. Tissue fragments were incubated with 5,7DHT (5 x 10^{-4}M) in the presence of ^{3}H-5HT. In addition, fragments of tissue were also incubated with ^{3}H-5HT in the presence of both PCA and 5,7DHT, at concentrations of 10^{-6} to 10^{-4}M.

Metergoline is a serotonin post synaptic receptor blocking agent. To determine whether binding of ^{3}H-5HT to it's receptor contributes to the uptake measurements, metergoline was added to some beakers at a final concentration of 10^{-6}M.

H. Serotonin uptake in fetal tissue:

Developmental change in uptake of ^{3}H-5HT was also examined in 18, 20, and 22 day fetal rat pancreata. Fetuses were removed by hysterectomy on day 18, 20, and 22 of gestation, the day after mating taken as day 1. The entire pancreas from the 18 day fetuses was removed, washed with KRBS, and placed into beakers containing two ml of buffer, and specific uptake of ^{3}H-5HT was determined. The 20 and 22 day pancreata were minced, and fragments were placed in beakers for uptake determination. The tissue after uptake was analyzed radioautographically (see below). Some 18 day fetal tissue was treated with 8.3×10^{-8}M ^{3}H-5HT, and subsequently embedded in plastic and dipped for radioautography in order to allow for greater morphological clarity of radioautographically labelled structures.

IV. Culture procedure:

Fetal rats were removed by sterile hysterectomy on day 18 of gestation. The stomach, duodenum, spleen and pancreas complex was completely removed. Under the dissecting microscope, the stomach and spleen were carefully cut away with iridectomy knives. Some pancreata were also dissected away from the duodenum, while others were kept intact. Specific uptake of ^{3}H-5HT was quantified in some pancreata. Others were subsequently cultured alone, or together with their attached duodenal segment. The pancreatic explants were

cultured using the watch glass method as modified by Hegre et al., (1972). The explants were supported at the air-liquid interface by a polycarbonate membrane filter (Bio-Rad, 5 um pores), placed upon a platform of stainless steel. The medium was Tissue Culture Medium 199 and 50% heat-inactivated chicken serum (McEvoy, 1980). The culture dishes were incubated at 37°C in a humidified atmosphere of 5% carbon dioxide in air. The explants were transfered to fresh medium after 2 days. After 4 days in culture, the explants were removed from the grids, if present, the duodenum was removed, and the pancreas was washed well with buffer, and an uptake procedure performed. Uptake was compared between 18 day fetal pancreas and pancreatic explants after 4 days in culture with or without attached duodenum.

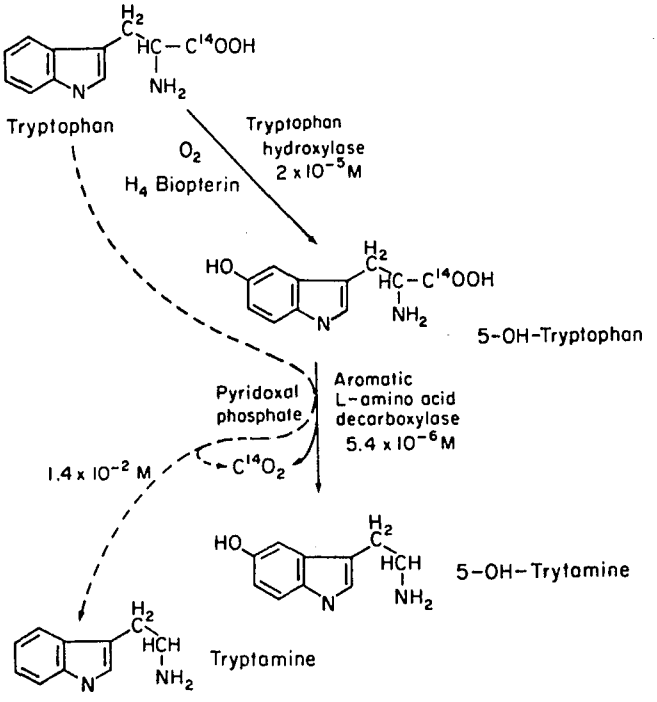
V. Tryptophan hydroxylase activity in the adult rat pancreas:

Tryptophan hydroxylase activity was determined by a modification of the procedure of Azmitia and McEwen (1976). Rats were killed by decapitation, and the pancreas was removed and placed into 0.1M Tris-acetate buffer (pH 8.1), containing sucrose (0.32M), on ice. The pancreas was sliced into four pieces of approximately 150 grams, and weighed on waxed paper. Each piece was then placed into homogenizing tubes containing 0.5 ml of cold buffer. Some pieces were transferred to tubes with buffer containing parachlorophenylalanine (PCPA, 10^{-2}M), or phenylalanine (10^{-6}M). The samples were

homogenized using a Cole-Parmer polytron (Model 4420). The uncentrifuged homogenate was transferred to the outer well of a modified Warburg flask (Kontes), and [1-^{14}C]trytophan (10^{-6}M, specific activity 15.1 mCi/mmole) was added. The center well contained a piece of filter paper saturated with Protosol (0.4 ml). The reaction was maintained in a 37°C water bath for 20 minutes, and was stopped by the addition of 0.6 ml of 10^{-2}M EDTA. The flasks were shaken for an additional 2 hours in an ice bath, and the $^{14}CO_2$ released was collected in the center well on the filter paper soaked with 0.4 ml Protosol. The center wells with their contents, were then transferred to scintillation vials, to which 10 ml of Econofluor was added, and were counted in a Beckman scintillation counter. The assay is based on the preferrential decarboxylation of 5-hydroxytryptophan, compared with tryptophan (Fig. 1). The amount of $^{14}CO_2$ liberated is stoichiometrically related to the amount of [1-^{14}C]tryptophan hydroxylated. Enzyme activity was calculated by a procedure analogous to that described earlier, and was expressed as pmoles of $^{14}CO_2$ released/gram wet wt.

Figure 1. The scheme depicted below illustrates the principle of the tryptophan hydroxylase assay. The terminal carboxy group of tryptophan is split by the decarboxylation of 5-hydroxytryptophan (straight line). However, at high tryptophan levels, this terminal carboxy group can be directly decarboxylated without being hydroxylated (dash line). Numbers represent Km values for the conversions demonstrated.

(adapted from Azmitia, 1973)



VI. Perifusion of pancreatic slices:

Pancreatic fragments were perifused with buffer after uptake, and treated with two drugs, which have been demonstrated to release neuronal stores of serotonin. Tissue was incubated with ^{3}H-5HT as described earlier. After uptake, the tissue fragments were washed twice with buffer, and placed in a Millipore chamber (Millipore Co., Bedford, Mass) in a 37^{o}C water bath, where they were perifused using a Gilson Minipulse II peristaltic pump with oxygenated KRBS at a rate of 0.5 ml/min. Aliquots were collected at one minute intervals using a Buchler Fraction collector. After 20 minutes, the perifusion medium was switched to one containing either fenfluramine or veratridine (10^{-5}M). The tissue was perifused for an additional 25 (fenfluramine) and 30 (veratridine) minutes. Two additional perifusions served as controls. Tissue incubated with ^{3}H-5HT in the presence of unlabelled 5-HT (10^{-5}M) was perifused as above, and tissue treated with ^{3}H-5HT alone was perifused without fenfluramine or veratridine. At the end of each experiment, a sample of tissue from each group was washed, weighed, dissolved in Protosol, and counted in Econofluor, all as previously described, to determine the amount of radioactivity remaining in the tissue. Aliquots (50 ul) from each fraction collected were similarly counted using Aquasol (NEN). Some pancreatic fragments from the experiment using veratridine were fixed for radioautography.

VII. Histological preparation:

A. Plastic and paraffin embedding:

Some tissue, after uptake, was embedded in paraffin, according to the procedure outlined in the manual of the Armed Forces Institute of Pathology (Luna, 1968), using an Autotechnicon (Technicon Co., Chauncey, N. Y.). In addition, 18 day fetal tissue was embedded in plastic to allow for better morphological identification of labelled structures in subsequent radioautographs, by a modification of the procedure of Wilson and Groves (1979). Briefly, 18 day fetal pancreata were fixed by immersion in 2.5% glutaraldehyde in phosphate buffer (0.1M), containing $MgSO_4$ (0.05%) and sucrose (3%). The tissue was postfixed in 2% OsO_4 in phosphate buffer (0.12M) with dextrose (7%), on a mechanical rotator for slow, continuous agitation for 3 hours at room temperature. The tissue was then rinsed in cold sodium acetate (0.1M), and immersed in aqueous uranyl acetate (0.5%) for 30 minutes on ice. Cooling was necessary to prevent precipitates of uranyl acetate throughout the tissue. The tissue was then rinsed in cold 0.1M sodium acetate for two changes at two minutes each. The remaining steps in the dehydration with increasing concentrations of methanol were also done with cold solutions. The blocks were then placed in fresh propylene oxide for two changes of 15 minutes each, and then placed into a 1:1 propylene oxide:Epon mixture for three hours. The fluid was decanted and a fresh mixture was poured in. The tissue blocks

were left in this solution overnight in a rotator. The following day, they were transferred to the Epon embedding mixture and left for six hours on the rotator. Each tissue block was embedded individually in gelatin capsules, and was left overnight in a 37°C oven. They were then transferred to a 45°C oven for eight hours, and then finally to a 60°C oven for two days. One micron sections were cut with glass knives, using a Huxley Ultra-Microtome (LKB Instruments, Inc., Rockville, Md.). The sections were allowed to dry prior to coating with NTB nuclear tracking emulsion, as described below. After development, the slides were counterstained with 1% Toluidine blue, containing 0.1% sodium borate, which was diluted 1:9 with 70% alcohol just prior to use.

B. Perfusion:

In order to determine whether platelets might be the source of radioautographic silver grain aggregates, a rat was perfused with ice cold saline through the ascending aorta for five minutes prior to removal of the pancreas and treatment with ^{3}H-5HT. Blanching of the pancreas was verified before it's removal. The pancreas was then minced and placed into beakers, and an uptake performed. The pieces were subsequently rinsed, embedded in paraffin, and sections were prepared for radioautography.

C. Radioautography:

After incubation with ^{3}H-5HT, pieces of pancreas were

fixed overnight in glutaraldehyde (2.5%) in 0.1M phosphate buffer, containing $MgSO_4$ (0.05%) and sucrose (3%). Following washing and dehydration, the tissue was embedded in paraffin, sectioned (4-7u) and mounted on glass slides. The slides were coated with NTB nuclear tracking emulsion (Eastman Kodak, diluted 1:1 with a 15% glycerin solution), cooled and allowed to dry for four hours. They were then stored in a light tight container at 5°C. After 2-5 weeks, they were developed for two minutes in freshly prepared D-170 developer (Kodak), fixed in Kodak Rapid Fixative, and washed for three hours. The sections were counterstained with 0.8% cresyl violet, a metachromatic stain. After photographing, some slides were left in xylene overnight to remove their coverslips, hydrated, and incubated for five minutes in a 7% potassium ferrocyanide solution, which removed their radioautographic silver grains. This procedure was performed on slides where heavy accumulations of silver grains appeared to be over cells, so that the cells could be identified.

D. Immunocytochemistry:

1. Insulin and glucagon:

To identify the islets in radioautographs, some sections after ^{3}H-5HT uptake were stained for insulin by a modification (McEvoy and Hegre, 1977) of the indirect antibody enzyme technique of Sternberger et al. (1970). All antisera were diluted in phosphate buffered saline (PBS) containing 0.5%

bovine serum albumin, pH 7.6. After deparaffinizing, and hydrating the slides to PBS, they were removed from the buffer, and the excess water carefully wiped away, getting as close to the tissue as possible. The primary antiserum, guinea pig anti-insulin serum, at a dilution of 1:1000, was applied to each slide, and left on for 24 hours at 5°C in a humidified chamber. After 24 hours, the slides were rinsed in buffer, and the second, linking antibody was applied. This antibody was a sheep anti-rabbit immunoglobulin (Antibodies, Inc., Davis, CA) at a dilution of 1:250, and was left on the slides for one hour at room temperature, after which time the slides were rinsed with buffer, and the marker complex, rabbit peroxidase anti-peroxidase (Cappel Labs, Cochranville, PA) was applied at 1:400 for one hour. Slides were then rinsed with buffer, placed in a staining rack, and immersed in a freshly prepared and filtered medium containing 30 mg/dl of 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma) and 0.0075% hydrogen peroxide (Fisher) in PBS. The slides were incubated in DAB for six minutes and rinsed thoroughly in distilled water. The slides were allowed to air dry prior to dipping for radioautography.

With these test slides was also processed a slide containing normal adult rat pancreas, which served as a positive staining control. Specificity controls for the primary antibody were not performed in these experiments, however, specificity has been verified by others (Madson,

1979).

Adjacent sections to those which were stained for serotonin following administration of 5-hydroxytryptophan, as described below, were stained for glucagon by a procedure identical to that just outlined, except that the insulin antibody was replaced by a glucagon antibody, which was used at a final dilution of 1:2000. Both insulin and glucagon antibodies were prepared in our laboratory (McEvoy et al., 1977).

2. Serotonin:

Rats were injected with pargyline (200 mg/Kg i.p.) 20 minutes prior to an injection of L-tryptophan (200 mg/Kg i.p.), and were kept in a cold room for 90 minutes. In addition, some animals were injected with 5-hydroxytryptophan (200 mg/Kg), the immediate biosynthetic precursor of serotonin, followed by 200 mg/Kg of pargyline. The animals were then perfused with 200 ml of ice cold 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) with 0.1% $MgSO_4$. Animals were also perfused with Bouins solution, or 2.5% glutaraldehyde, in 0.1M phosphate buffer, containing 0.05% $MgSO_4$ and 3% sucrose. The pancreas and duodenum were then removed, and post fixed for 12 hours in the same fixatives at $4^{o}C$. In some untreated animals, the pancreas was removed, minced, and incubated with 1 x 10^{-7}M of unlabelled serotonin, by the procedure outlined earlier, and then fixed by immersion with Bouins. Following

dehydration, the tissues were embedded in paraffin, sectioned (7 u), and mounted on glass slides. After deparaffinization, the slides were hydrated to PBS, they were removed from the buffer, and the excess water carefully wiped away, getting as close to the tissue as possible. A 0.6% pronase solution (Sigma protease fraction VI) was then applied for 10 minutes at room temperature, after which time the slides were rinsed with PBS. The primary antibody (generously donated by Jean Lauder), which was raised in rabbits, was then applied to the tissue at a 1:1000 dilution for 48 hours in a moist chamber at room temperature. The slides were then rinsed well in PBS and a sheep anti-rabbit immunoglobulin was applied at a dilution of 1:100 for six hours. The tissue was rinsed again, and rabbit peroxidase anti-peroxidase was applied at 1:100 overnight. Peroxidase activity was detected by incubation in DAB and 0.0075% hydrogen peroxide for ten minutes at room temperature. The slides were then rinsed well in distilled water, dehydrated, and coverslipped. No counterstain was used.

Since duodenal enterochromaffin cells possess a large store of serotonin, slides containing gut tissue served as positive staining controls. Specificity was demonstrated by incubating tissue with primary antibody that had been pretreated with 5-HT (10^{-3}M).

VIII. Photography:

A Leitz light microscope with 10x, 25x, 40x, and 63x

planapochromatic objective lenses for bright field microscopy and a 35 mm photographic attachment were used with Eastman Kodak Ektachrome 160 (tungsten), and Panatomic-X film. All black and white photographs were printed on Kodak Polycontrast and #4 paper, using Dektol developer (Kodak), and were fixed using Kodak Rapid Fixer.

IX. Statistics:

All statistical analyses utilized the Student's unpaired t-test for determination of significance between group means. P values less than 0.05 were taken as significant. Linear regression analysis was performed to determine the Km value for uptake, and for analysis of time course data.

RESULTS

I. Time course of tritiated serotonin uptake in pancreas:

Rat pancreatic fragments incubated in vitro with ^{3}H-5HT for 0,5,7,10,12,14,15 and 20 minutes, and the total uptake, expressed as pmoles/gram, was plotted as a function of time (Figure 2). Maximal uptake was found to occur after 15 minutes of incubation. Regression analysis demonstrated that the uptake follows an exponential course. In all subsequent experiments, uptake was performed with a 15 minute incubation.

II. Saturability of uptake:

In order to determine the saturability of the uptake system, pancreatic slices were incubated with ^{3}H-5HT in the presence of increasing concentrations of unlabelled serotonin. Figure 3 shows the percentage of the total uptake of ^{3}H-5HT as a function of increasing concentrations of unlabelled serotonin. Total uptake of label was reduced with concentrations of 5-HT up to 10^{-5}M, to about 50%. Nonspecific (low affinity) uptake was subsequently defined as pmoles of ^{3}H-5HT in tissue incubated in the presence of 5-HT (10^{-5}M). Conversely, specific (high affinity) uptake is the difference in uptake (pmoles/gram) between tissue treated with ^{3}H-5HT in the presence or absence of 5-HT (10^{-5}M).

The mean, standard deviation and range of the specific

uptake was determined from the total and nonspecific uptake values from 16 separate experiments (approx. 60 values in each group). For tissue incubated with ^{3}H-5HT (9×10^{-8}M), the mean specific uptake was 78 pmoles/gram, with a standard deviation of 10.6. The range of values for total uptake was 151.5 to 290.8, and the range for nonspecific uptake was 93.1 to 208.2. Though the range of values was great, linear regression analysis revealed a highly significant linear correlation between nonspecific and total uptake (0.95), such that the values calculated for specific uptake fell within a relatively narrow range.

III. Specificity of uptake:

To determine whether the uptake in the pancreas is specific for serotonin, norepinephrine, a catecholamine neurotransmitter, was added to the incubation medium of some tissue during treatment with ^{3}H-5HT. If the uptake is by serotonergic nerves, and if these nerves have similar properties as those in the brain and gut, then it would be expected that norepinephrine would not diminish the ^{3}H-5HT uptake. The data expressed in Table 1 support this hypothesis. Even in the presence of a relatively high concentration of norepinephrine, no significant difference in uptake from control tissue was observed.

IV. Kinetics of the specific serotonin uptake:

Pancreatic slices were incubated at 37°C for 15 minutes with different concentrations of ^{3}H-5HT from 4.8 x 10^{-8}M to 3.9 x 10^{-7}M. To determine specific uptake at each concentration of ^{3}H-5HT, unlabelled 5-HT, at a final concentration of 10^{-5}M, was added to the incubation medium. No evidence for saturability was found within the concentration range examined. Converting the values for the specific uptake into their reciprocal, a Lineweaver-Burk analysis was performed (Figure 4). The straight line was determined by linear regression, and possessed a correlation coefficient of 0.99. The calculated Km value was 8.75 x 10^{-7}M, and the Vmax of uptake was 873 pmoles/gram of ^{3}H-5HT.

V. Sex differences and regional variation in uptake:

The specific uptake of ^{3}H-5HT in the pancreata of male and female rats was compared. As shown in Table 2, no significant difference in specific uptake was obtained. Uptake variation in different regions of the pancreas was also investigated by separately incubating splenic, pyloric and intestinal portions of the gland, with ^{3}H-5HT. Again there were no significant differences among the anatomical regions (Table 3).

VI. Species variation:

Uptake experiments were performed on rabbit, hamster, and rat pancreatic fragments using a concentration of ^{3}H-5HT of 8.3×10^{-8}M. The data in Table 4 indicate a significantly greater uptake of label by the rabbit pancreas, than those of either the rat or hamster, which demonstrated comparable uptake. In contrast, when uptake by the monkey pancreas was investigated using a higher concentration of label (1×10^{-7}M), values were comparable to that in the rat at the lower concentration.

VII. Effects of pharmacologic agents on serotonin uptake:

The effect of fluoxetine, a drug known to inhibit the high affinity uptake of serotonin in the brain and gut, on pancreatic serotonin uptake was determined. The data presented in Table 5 indicate that after such treatment the specific uptake of serotonin was markedly reduced to nonspecific levels.

The effect of p-chloroamphetamine (PCA), a drug known to have destructive effects on brain 5-HT nerves, on the uptake in the pancreas was similarly determined. Multiple injections of PCA in vivo did not result in a reduction in the specific high affinity uptake of serotonin (Table 6). However, when pancreatic fragments were incubated with PCA in vitro together with ^{3}H-5HT, specific uptake was abolished (Table 7). Preincubation of tissue fragments with PCA (10^{-4}M) for 30 minutes prior to incubation with ^{3}H-5HT similarly abolished

the uptake (Table 8).

Incubation of pancreatic fragments with 5,7DHT (5 x 10^{-4}M) in the presence of ^{3}H-5HT, also abolished the specific uptake (Table 7). When incubated in the presence of PCA and 5,7DHT at concentrations of 5 x 10^{-6}, 10^{-5}, and 10^{-4}M, specific uptake was abolished at the two higher concentrations, while some uptake was preserved when concentrations of 10^{-6}M were used.

The effect of metergoline (10^{-6}M) on the ^{3}H-5HT uptake by the pancreas was also investigated. As shown in Table 9, the specific uptake of ^{3}H-5HT in the presence of metergoline was not significantly different from the control tissue.

VIII. Serotonin uptake in fetal tissue:

In order to determine whether the uptake system for serotonin is present in fetal tissue, 18, 20, and 22 day fetal rat pancreata were removed, and an uptake performed on the intact pancreas (18 day), and pancreatic fragments (20 and 22 day). After incubation with ^{3}H-5HT, all tissue demonstrated a specific uptake of label (Table 10).

IX. Uptake by the 18 day fetal pancreas in culture:

Fetal pancreata were cultured alone or with their attached duodenal segment for 4 days, after which time an uptake was performed. While the specific uptake of serotonin was reduced

after 4 days in culture (significantly, in tissue cultured with duodenum), a significant specific uptake of serotonin remained (Table 11). Uptake by pancreata cultured with duodenum showed a similar degree of uptake as those culture without.

X. Trytophan hydroxylase activity in the adult rat pancreas:

Trytophan hydroxylase activity was detectable in pancreatic homogenates (Table 12). The specificity of this activity was examined using PCPA and phenylalanine. Parachlorophenylalanine (PCPA) is a specific inhibitor of tryptophan and phenylalanine hydroxylase. Addition of PCPA significantly reduced the amount of enzyme activity. The addition of phenylalanine did not result in a reduction in apparent enzyme acitivity.

XI. Perifusion of pancreatic slices:

When fenfluramine was added to the medium during perifusion of pancreatic slices after uptake of ^{3}H-5HT, the amount of radioactivity in the subsequent fractions remained significantly higher for a longer period of time than that of either of the two controls (Figure 5). The controls included perifusion of pancreatic slices with fenfluramine after nonspecific uptake of ^{3}H-5HT, and perifusion of slices without the addition of fenfluramine. After perifusion, tissue treated with fenfluramine was dissolved, counted and compared with the tissue perifused for the same amount of time without

fenfluramine. The fenfluramine treated slices showed a lower number of counts, indicating a greater rate of release of radioactivity from the neuronal (presumably), compartment in the tissue (Table 13). Tissue preincubated with excess unlabelled serotonin and perifused with fenfluramine also showed a slightly lower number of counts than similar tissue perifused without fenfluramine, indicating that a small proportion of the nonspecific label may also be released by the drug.

Addition of veratridine during perifusion of pancreatic slices after uptake of ^{3}H-5HT significantly stimulated the efflux of radioactivity from rat pancreatic tissue (Figure 6). No stimulation of radioactive release by veratridine was observed from tissue after uptake of ^{3}H-5HT in the presence of unlabelled serotonin (10^{-5}). Analysis of the experimental tissue after perifusion indicated a significant loss of radioactivity (Table 14).

XII. Radioautography:

A. Visualization of saturable and specific serotonin uptake sites.

Radioautography of pancreatic slices after incubation with ^{3}H-5HT revealed the specific uptake as dense aggregates of silver grains in the connective tissue areas of the inter- and intralobular septae adjacent to blood vessels (Figure 7). The silver grain aggregates seemed to extend along blood vessels

for varying distances. There were no dense accumulations of grains over the exocrine or islet parenchymal cells, although dense aggregates were also seen around acini and in the pericapillary and intercellular areas within the islets (Figure 8). The adventitia of larger pancreatic arteries and veins were shown to contain dense silver grain aggregates (Figure 9). Perfusion of a rat with saline prior to treatment with ^{3}H-5HT did not alter the pattern of uptake (Figure 10).

After uptake of ^{3}H-5HT, scattered silver grains were seen over the exocrine and islet parenchymal cells. These cellularly localized grains were greater than background, but, unlike the dense aggregations, were still present after treatment with excess 5-HT (Figure 11). This cellular compartment is, therefore, not saturable in the concentration range tested. Norepinephrine (10^{-5}M) failed to decrease the aggregates or randomly scattered grains in the pancreas, supporting the hypothesis that noradrenergic nerves are not the sites of uptake (Figure 12).

It is known that mast cells exhibit an uptake of ^{3}H-5HT. As shown in Figure 13A, however, mast cells retained label after treatment with excess, unlabelled 5-HT. Their uptake system is, therefore, not saturable in the concentration range tested, and contributes to what has been defined as 'nonspecific uptake.' The labelled cells in Figure 13A were clearly identified as mast cells after removal of the radioautographic emulsion (Figures 13B,C).

B. Species variation.

As shown earlier, pancreata of the rabbit, hamster and monkey pancreas demonstrate the ability to accumulate serotonin specifically. Radioautographically, the sites of uptake in the rabbit and hamster appear to closely resemble those seen in the rat (Figure 14A,B), with dense aggregates seen coursing along blood vessels and in the connective tissue areas of the exocrine and endocrine pancreas. A similar pattern was seen in the monkey, but was much less pronounced (Figure 14C).

C. Effect of pharmacologic agents on serotonin uptake.

1. Fluoxetine: Radioautographs of tissue treated with fluoxetine were almost completely devoid of dense silver grain aggregates, while the scattered grains remained unchanged (Figure 15).

2. PCA and 5,7DHT: When rats were injected with PCA, no effect on the uptake of serotonin was demonstrated biochemically. Radioautographs from this tissue after uptake showed a normal pattern of labelling with abundant silver grain aggregates in the connective tissue (Figure 16A). However, when PCA was added to the pancreatic fragments in vitro, either during, or prior to the ^{3}H-5HT uptake, the dense silver grain aggregates were absent from the connective tissue areas (Figure 16B,C).

3. Metergoline: Incubation of pancreatic fragments in the presence of metergoline, a serotonin post-synaptic receptor blocking agent, similarly did not affect the uptake of labelled serotonin. Radioautography showed a pattern of labelling in the islets of treated tissue which resembled that of the control (Figure 17).

D. Serotonin uptake in fetal tissue.

After uptake of ^{3}H-5HT, 18, 20, and 22 day fetal pancreata were processed for radioautography, in order to visualize the sites of the serotonin uptake, and to see how they compare to the adult. All fetal tissue examined radioautographically displayed the ability to accumulate serotonin. The labelling pattern closely resembled that of the adult (Figure 18). Eighteen day fetal pancreas (Figure 18A) seemed to be the most densely labelled of the three gestational ages examined. In 20 (Figure 18B) and 22 day fetal tissue (Figure 18C), the uptake sites were represented by dense silver grain aggregates throughout the connective tissue.

In plastic sections of 18 day fetal pancreata, some cells were found to be heavily labelled (Figure 19), whereas none were found in paraffin sections. These labelled cells were primitive in appearance and displayed no characterizing features that permitted their morphological identification.

Further examination of thin section radioautographs of 18 day fetal tissue demonstrated ocassional labelling within the

vasculature (Figure 20). Such labelling represents uptake by platelets. The uptake system for serotonin by platelets is well established, and resembles the pancreatic uptake in it's kinetics.

After 4 days in culture, 18 day fetal rat pancreata were processed for radioautography. As demonstrated earlier, culturing did not result in a loss of their ability to accumulate serotonin. The pattern of dense silver grain aggregates was similar to that seen in all uncultured fetal and adult tissue examined (Figure 21).

F. Effect of veratridine.

After perifusion with veratridine (10^{-5}M), the sites of the ^{3}H-5HT uptake were visualized radioautographically. The dense silver grain aggregates were almost completely absent (Figure 22A), while control tissue demonstrated the usual pattern of silver grain aggregates (Figure 22B).

XIII. Serotonin immunocytochemistry:

Immunocytochemical stainings, performed on pancreata from rats treated with pargyline and tryptophan, revealed the presence of serotonin in gut enterochromaffin cells (Figure 23) and pancreatic mast cells (Figure 24). No staining of nerve fibers could be demonstrated, regardless of the fixative used.

Rat islets contain the enzyme aromatic amino acid decarboxylase. Administration of 5-hydroxytryptophan to these

cells results in the rapid production of serotonin. This could be verified immunocytochemically as illustrated in Figure 25A. Islet cells were found to be positive for serotonin. Serotonin was not detectable without pretreatment with 5-HTP. The pattern of 5-HT positive cells in the center of the islet suggested that serotonin accumulated primarily in the B cells. Comparison of the section in Figure 25A with an adjacent section stained for glucagon (Figure 25B) supported this hypothesis.

Figure 2. Time course for ^{3}H-serotonin uptake. Total uptake at each time point was expressed in pmoles/gram. Each point represents the value obtained from at least five samples. Maximal uptake was found to occur after 15 minutes. Statistical analysis indicated that the uptake follows an exponential function.

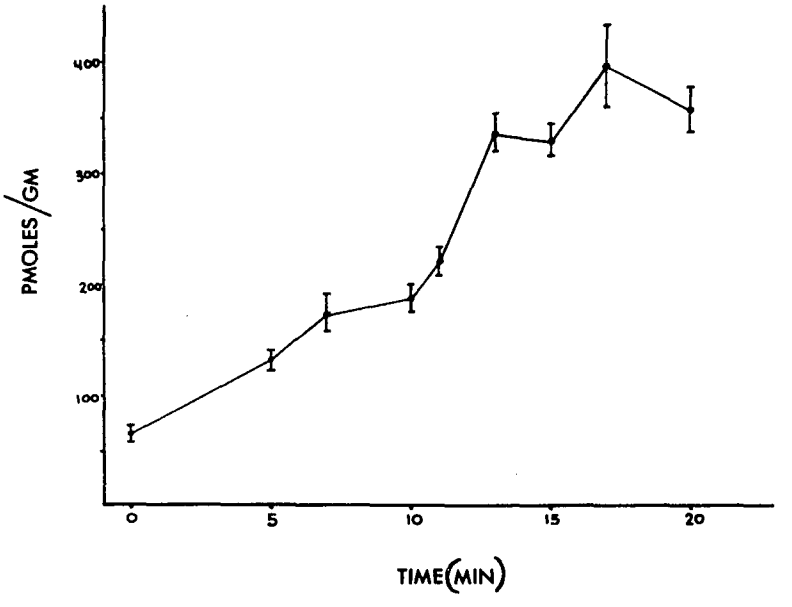


Figure 3. Pancreatic slices incubated with ^{3}H-5HT and varying concentrations of unlabelled serotonin. Uptake was reduced by unlabelled serotonin up to 10^{-5}M. The uptake obtained during incubation with labelled serotonin in the presence of 5-HT (10^{-5}M), was subsequently defined as nonspecific. 100% of the total uptake was that obtained from tissue treated with ^{3}H-5HT in the absence of unlabelled 5-HT.

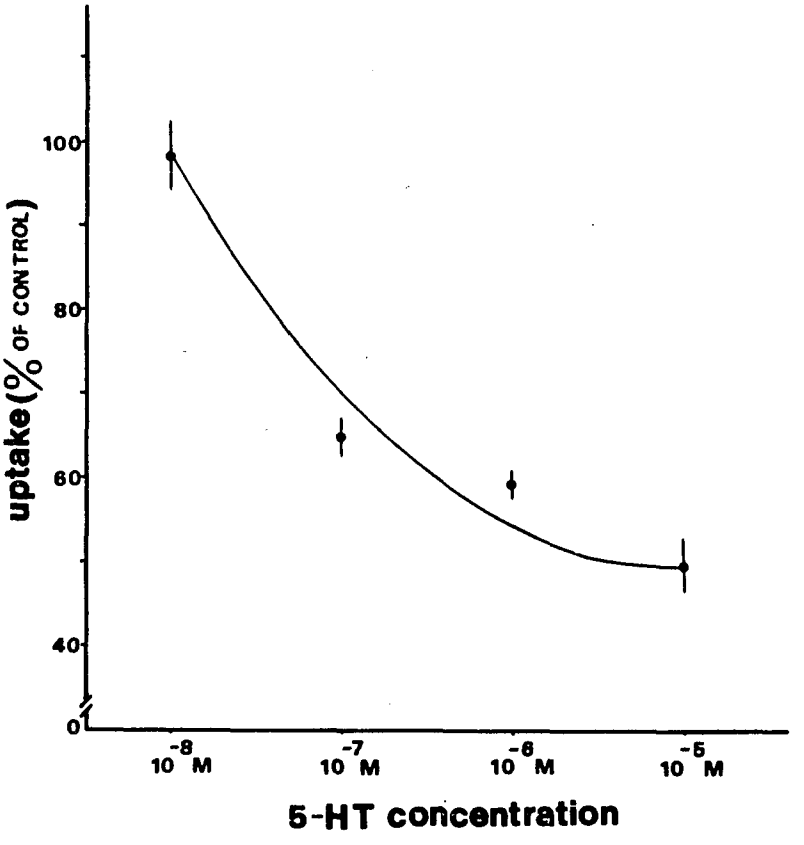


Figure 4. A Lineweaver-Burk plot of specific uptake data obtained from the incubation of adult pancreatic slices in varying concentrations of ^{3}H-5HT, in the presence or absence of 5-HT (10^{-5}M). Reciprocal values for the specific uptake were plotted as a function of the reciprocal substrate concentration (^{3}H-5HT). The Km and Vmax values were obtained from the X and Y intercepts, respectively. The points represent mean values from eight observations. r = 0.99.

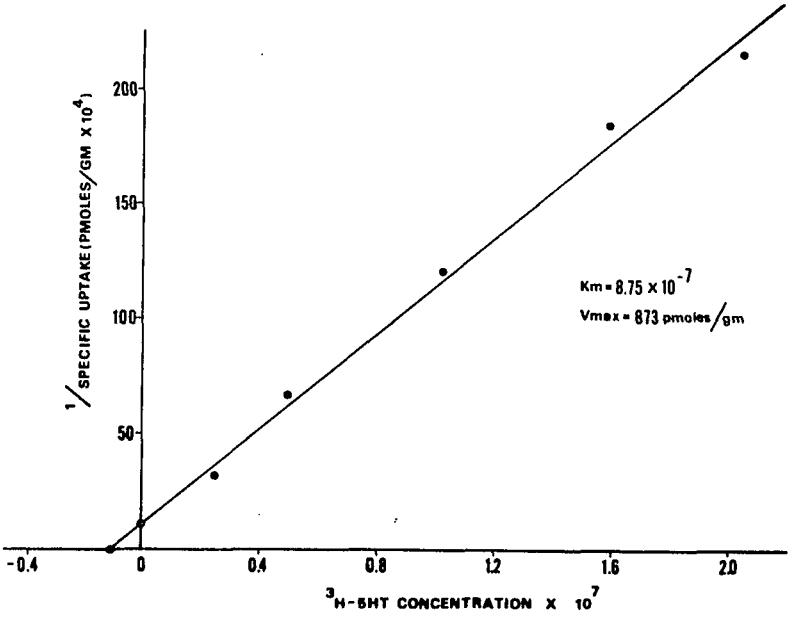


TABLE 1

SPECIFICITY OF ^{3}H-SEROTONIN UPTAKE

BY ADULT RAT PANCREATIC FRAGMENTS

IN VITRO

Treatment	N	^{3}H-5HT Uptake (% of control)
Control	5	100.0±3.5
5-HT	5	53.5±1.3*
NE	12	95.0±5.7

Data are Mean ± SEM

5-HT and norepinephrine concentrations = 10^{-5}M.

*Different from control, P<0.001

N = number of samples counted

TABLE 2

^{3}H-5HT UPTAKE IN THE MALE AND FEMALE RAT PANCREAS

Sex	N	Specific uptake (pmoles/gram)
Male	10	90.4±16.3
Female	10	83.0±5.8

Data are Mean ± SEM

No significance between sexes was obtained

N = number of samples counted

TABLE 3

COMPARISON OF ^{3}H-5HT UPTAKE BETWEEN VARIOUS REGIONS OF THE ADULT RAT PANCREAS

Region	Specific Uptake (pmoles/gm)
Splenic	87.4±16.9
Pyloric	86.1±8.4
Duodenal	84.1±4.6

Data are Mean ± SEM

No significance between groups was obtained

TABLE 4

SPECIES VARIATION IN PANCREATIC SEROTONIN UPTAKE

Species	N	Specific uptake (pmoles/gram)
Rat	10	59.7±5.4
Rabbit	13	122.4±14.6*
Hamster	20	52.4±6.0
Monkey	10	42.0±10.6

Data are Mean ± SEM

^{3}H-5HT in rabbit, hamster, and rat = 8.3×10^{-8}M

^{3}H-5HT in monkey = 1×10^{-7}M

*Different from rat, $P<0.001$

N = number of samples counted

TABLE 5

EFFECT OF FLUOXETINE ON THE SPECIFIC UPTAKE OF ^{3}H-5HT IN THE PANCREAS

Treatment	N	^{3}H-5HT uptake (% of control)
Control	5	100.0±6.2
5-HT	5	60.5±1.2*
Fluoxetine	5	65.3±3.2*

Data are Mean ± SEM

*Different from control, P<0.005

N = number of samples counted

TABLE 6

EFFECT OF INJECTIONS OF PCA IN VIVO

ON THE ^{3}H-5HT UPTAKE BY ADULT RAT PANCREATA

IN VITRO

Rat	Treatment	N	% specific uptake
1	Control	10	100.0±17.3
2	PCA	10	94.1±30.8
3	PCA	10	99.1±24.4
4	PCA	10	98.6±19.5

Data are Mean ± SEM

PCA dosage = 20 mg/Kg i.p.; 3 animals tested

No significance between groups was obtained

N = number of samples counted

TABLE 7

EFFECT OF PCA, 5,7 DHT OR BOTH ON THE UPTAKE OF ^{3}H-5HT BY PANCREATIC SLICES IN VITRO

Treatment	% specific uptake
Control	100.0±9.9
PCA (5×10^{-4}M)	18.7±6.6**
5,7DHT (5×10^{-4}M)	8.0±11.1**
Both (5×10^{-4}M)	14.2±11.6**
Both (5×10^{-5}M)	-1.9±10.3**
Both (5×10^{-6}M)	45.0±14.7*

Data are Mean ± SEM; N = 10

*Different from control, $P<0.01$, ** $P<0.001$

**Not significantly different than both at 5×10^{-6}M

TABLE 8

EFFECT OF 30 MINUTE PREINCUBATION OF PANCREATIC FRAGMENTS WITH PCA ON THE ^{3}H-5HT UPTAKE

Treatment	N	^{3}H-5HT uptake (% of control)
Control	5	100.0±11.9
5-HT	5	51.8±2.9*
PCA	5	43.3±1.1** ***

Data are Mean ± SEM

PCA concentration = 10^{-4}M

*Different from control, P<0.005

**Different from control, P<0.001

***Different from 5-HT treated, P<0.05

TABLE 9

EFFECT OF METERGOLINE ON THE SPECIFIC UPTAKE OF ^{3}H-5HT

Treatment	N	% of specific uptake
Control	5	100.0±13.7
Metergoline(10^{-6}M)	5	106.0±8.8

Data are Mean ± SEM

N = number of samples counted

TABLE 10

^{3}H-5HT UPTAKE BY 18, 20, AND 22 DAY FETAL RAT PANCREAT

Age	N	Specific uptake (pmoles/gram)
18 day	7	239.9±22.9
20 day	7	255.9±74.8
22 day	7	144.6±28.7

Data are Mean ± SEM

^{3}H-5HT concentration = 1 x 10^{-7}M

No significance between ages was obtained

N = number of samples counted

TABLE 11

SPECIFIC UPTAKE OF ^{3}H-5HT BY 18 DAY FETAL RAT PANCREATA BEFORE AND AFTER 4 DAYS IN CULTURE

Treatment	N	Specific uptake (pmoles/gram)
Prior to culture	7	239.9±22.9
4 day culture	7	169.4±38.2
4 day culture with duodenum	12	115.8±12.8*

Data are Mean ± SEM

*Different from uncultured, P<0.001

N = number of samples counted

TABLE 12

TRYPTOPHAN HYDROXYLASE ACTIVITY

IN THE ADULT RAT PANCREAS

Treatment	Enzyme Activity (Pmoles/gram)
^{14}C-tryptophan	19.0±1.9
^{14}C-tryptophan + PCPA	8.8±2.0*
^{14}C-tryptophan + Phenylalanine	20.4±3.8

Data are Mean ± SEM; N = 5

^{14}C - tryptophan, phenylalanine conc. = 10^{-6}M

PCPA (parachlorophenylalanine) conc. = 10^{-2}M

*Different from ^{14}C-tryptophan, $P<0.005$

Figure 5. The effect of fenfluramine on the release of radioactivity from adult rat pancreatic slices after uptake of ^{3}H-5HT. A = Perifusion of slices with fenfluramine after total uptake. B = Perifusion of slices treated with fenfluramine after nonspecific uptake i.e., in the presence of unlabelled serotonin (10^{-5}M). C = Perifusion of slices after total uptake in the absence of fenfluramine. The amount of radioactivity released from the experimental tissue remained significantly higher for a longer period of time than those of the two controls. Baseline = average of three values before addition of fenfluramine (at arrow). Each point represents mean of three perifusions. Vertical lines = standard error.

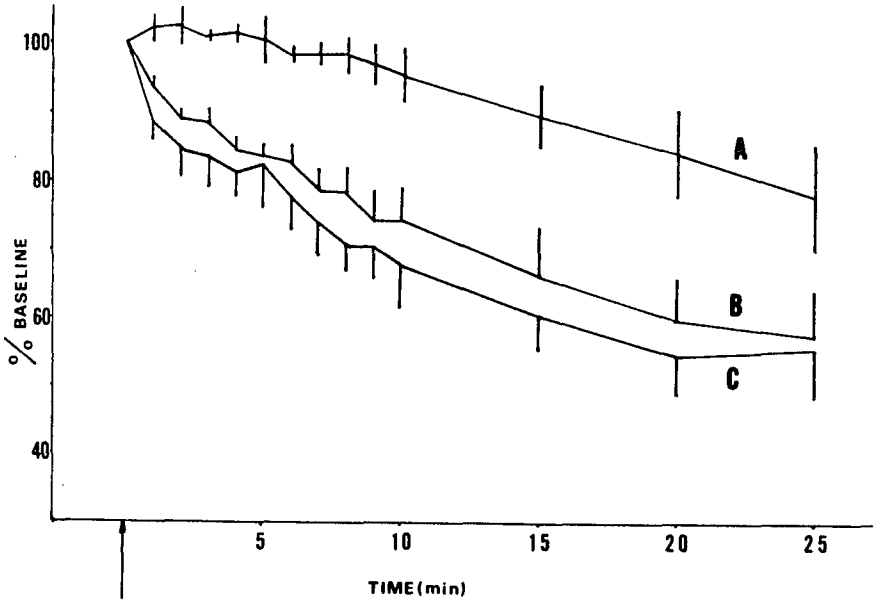


TABLE 13

^{3}H-5HT REMAINING IN ADULT RAT PANCREATIC FRAGMENTS

AFTER PERIFUSION WITH OR WITHOUT DL-FENFLURAMINE

Uptake	Perifusion	^{3}H-5HT in tissue (% of control)
^{3}H-5HT	KRBS alone	100.0±6.9
^{3}H-5HT and 5-HT (10^{-5}M)	KRBS alone	36.7±6.9*
^{3}H-5HT	Fenfluramine	41.4±2.3*
^{3}H-5HT and 5-HT (10^{-5}M)	Fenfluramine	25.1±1.5

Data are Mean ± SEM; N = 15 in all groups

*Different from ^{3}H-5HT, KRBS alone, $P<0.001$

KRBS = Krebs Ringer Bicarbonate Solution

Figure 6. The effects of veratridine on the release of radioactivity from adult rat pancreatic fragments after uptake of ^{3}H-5HT. A = Perifusion of slices of tissue with veratridine after total uptake of ^{3}H-5HT. B = Perifusion of slices of tissue with veratridine after nonspecific uptake i.e., in the presence of excess, unlabelled 5-HT. Baseline is the average of three samples from tube 20 of each group. Arrow represents point of addition of veratridine. Veratridine administration resulted in a significant increase in the rate of radioactivity released. Vertical lines = standard error.

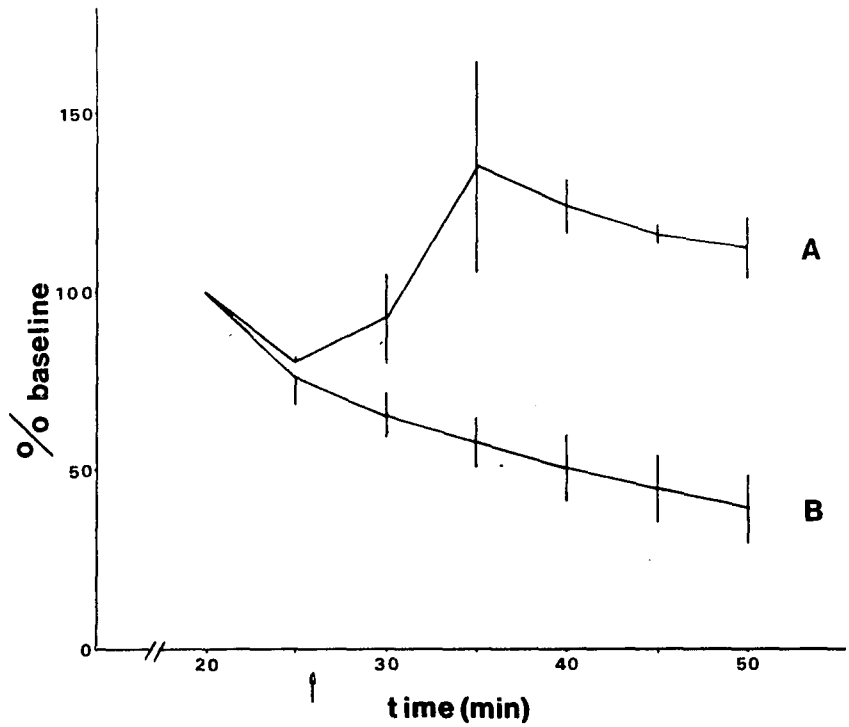


TABLE 14

^{3}H-5HT REMAINING IN RAT PANCREATIC SLICES

FOLLOWING PERIFUSION WITH VERATRIDINE

Uptake	Perifusion	^{3}H-5HT in tissue (% of control)
^{3}H-5HT	KRBS	100.0±9.0
^{3}H-5HT + 5-HT (10^{-5}M)	KRBS	27.5±2.0*
^{3}H-5HT	Veratridine	49.3±3.0* **

Data are Mean ± SEM; N = 5 in all groups

Veratridine and 5-HT concentrations = 10^{-5}M

^{3}H-5HT concentration = 10^{-8}M

*Different from control, P<0.001

**Different from 5-HT treated, P<0.001

Figure 7. A radioautograph of a rat pancreatic slice incubated in vitro with ^{3}H-5HT. Silver grain aggregates (arrows) were visible along blood vessels. There were no dense accumulations of silver grains over acinar or islet tissue. * = blood vessel. Final magnification 1800x. Cresyl violet counterstain. (All photomicrographs taken with Leitz Orthoplan microscope, bright field illumination, using a Zeiss Plan-apo objective).

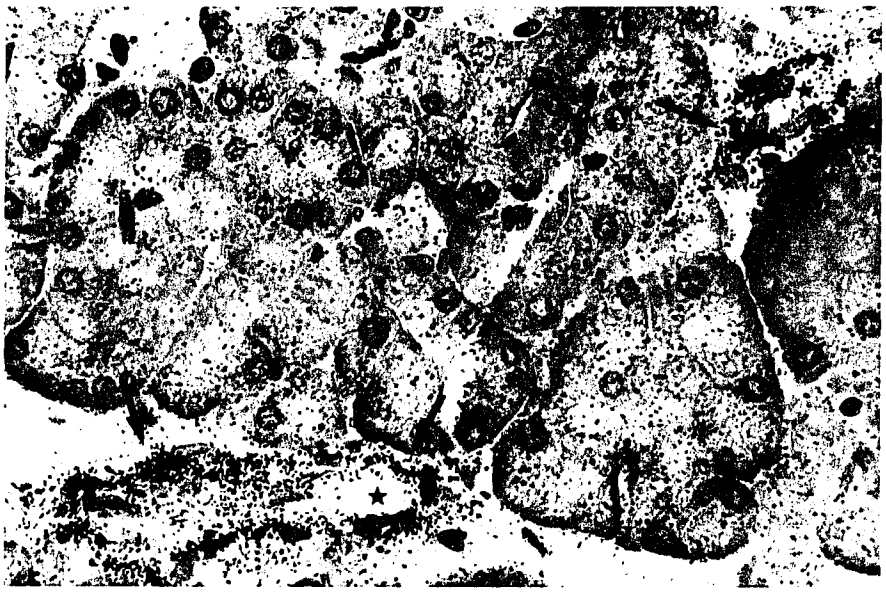


Figure 8. A radioautograph of a rat pancreatic slice incubated in vitro with ^{3}H-5HT. Prior to dipping for radioautography, slides were stained immunocytochemically for insulin, to more easily identify islets in the tissue. Silver grain aggregates (arrows) were seen in the interstitial spaces of the islet in juxtaposition to some positively stained cells. Final magnification 1800x. Cresyl violet counterstain.

Figure 9. Radioautograph of pancreatic slice after uptake with ^{3}H-5HT. Dense aggregates of silver grains were seen in the smooth muscle wall of large pancreatic vessels, between the tunica media and externa (arrows). These aggregates may represent terminations of the fibers on smooth muscle cells. Final magnification 1000x. Cresyl violet counterstain.

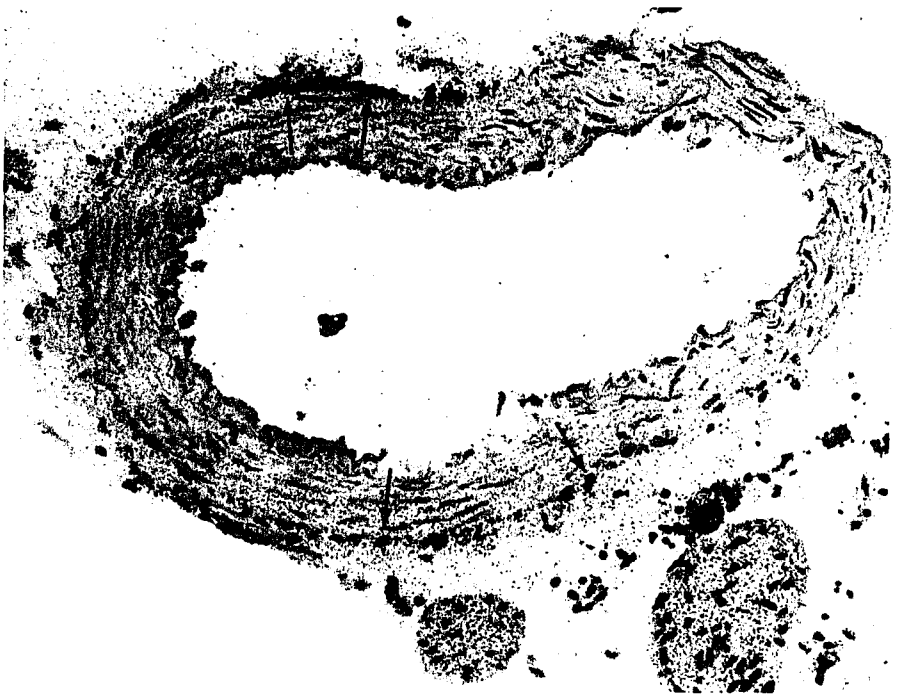


Figure 10. Radioautograph of a pancreatic slice from a rat perfused with saline for 5 minutes prior to removal of the pancreas for uptake. Such treatment removed all red blood cells, suggesting that the perfusion was successful. The pattern of labelling was very similar to that seen in unperfused animals (arrows), suggesting that platelets were not responsible for the uptake. Final magnification 1000x. Cresyl violet counterstain.

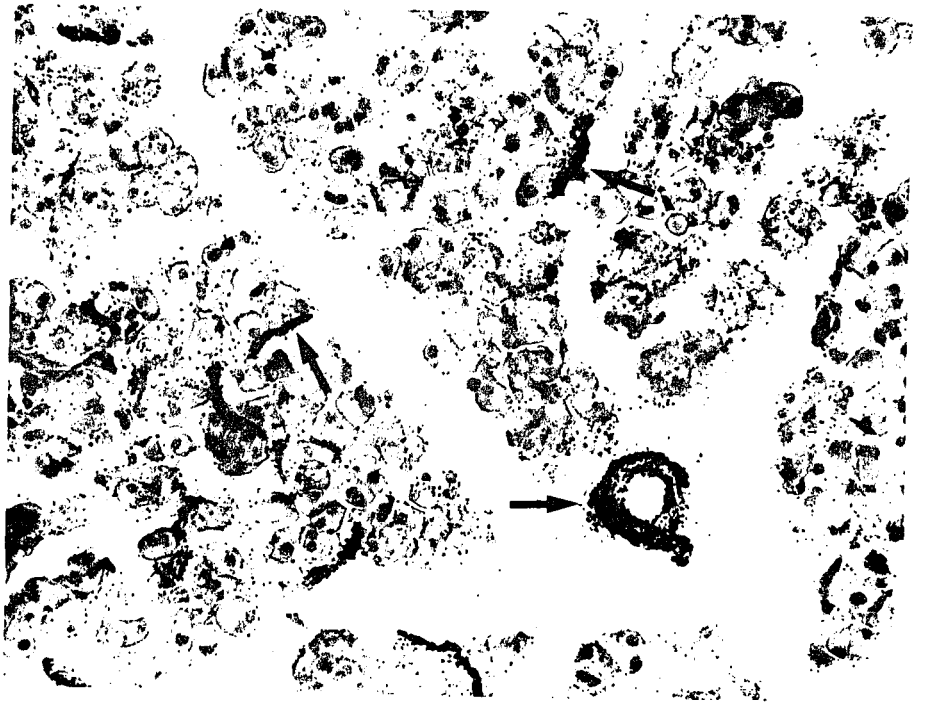


Figure 11. Radioautograph of tissue incubated with ^{3}H-5HT in the presence of unlabelled 5-HT (10^{-5}M). Only scattered silver grains were seen over the exocrine and islet cells. These grains represent nonspecific uptake since they were not displaced by the unlabelled 5-HT. BV = blood vessel. Final magnification 1800X. Cresyl violet counterstain.

Figure 12. Radioautograph of pancreatic slice incubated with ^{3}H-5HT in the presence of excess norepinephrine (10^{-5}M). Addition of norepinephrine did not result in a reduction in specific uptake. A radioautograph from this tissue demonstrated a pattern of labelling around the vasculature and in the interstitial spaces (arrows), identical to that seen in untreated tissue. Final magnification 1000x. Cresyl violet counterstain.

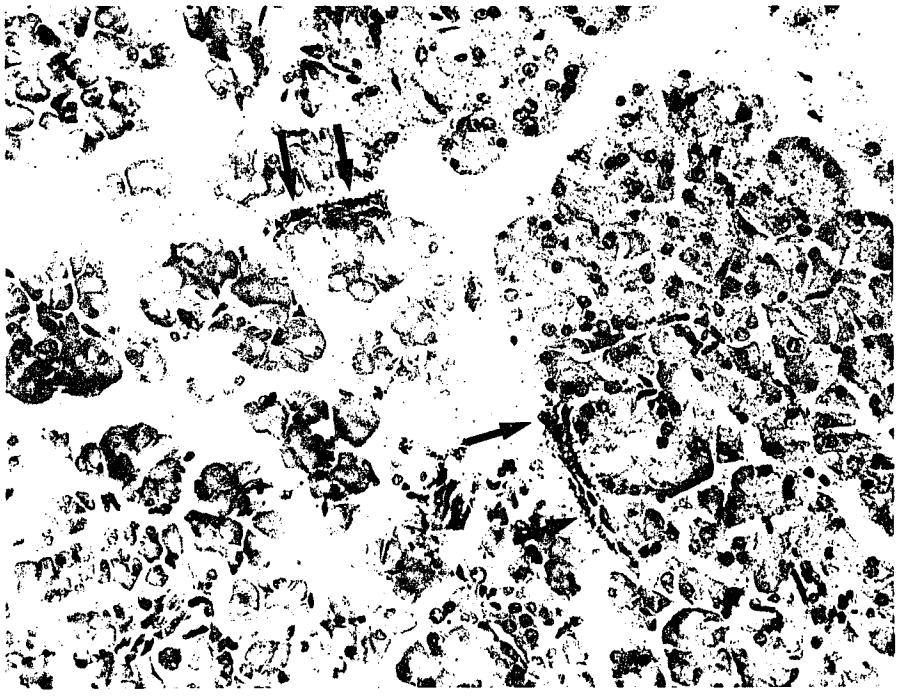
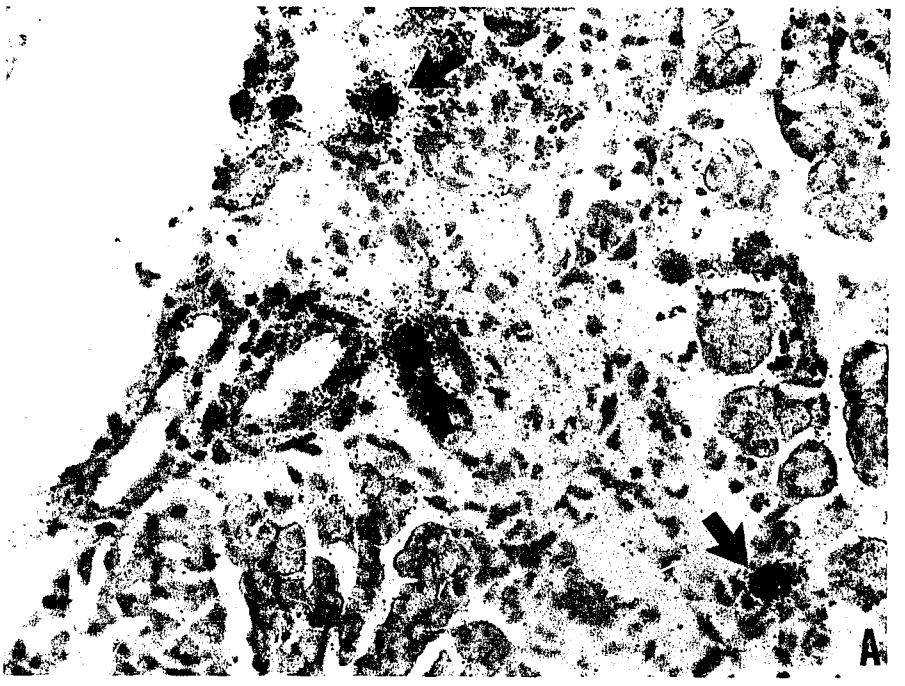
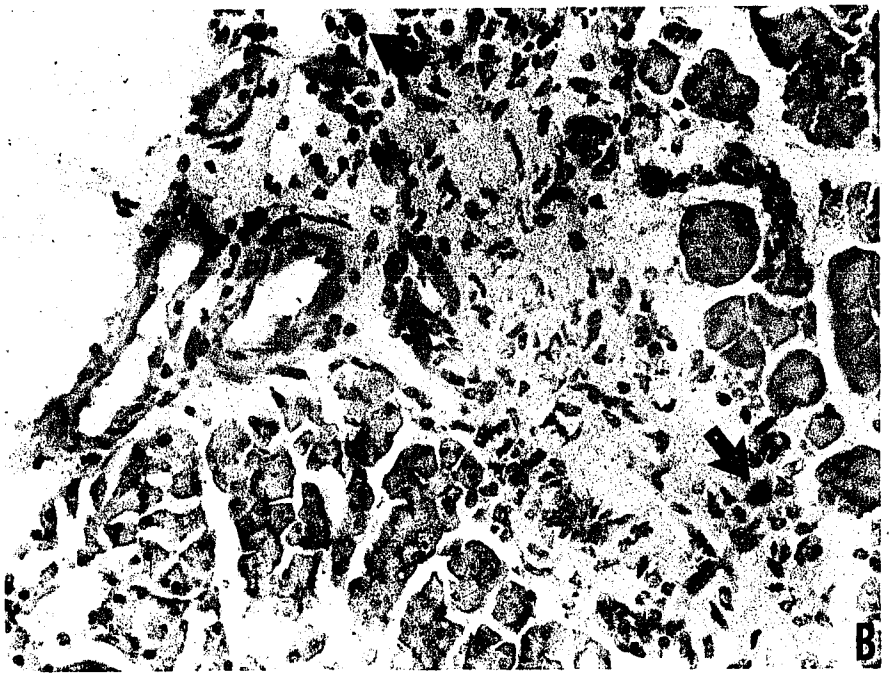


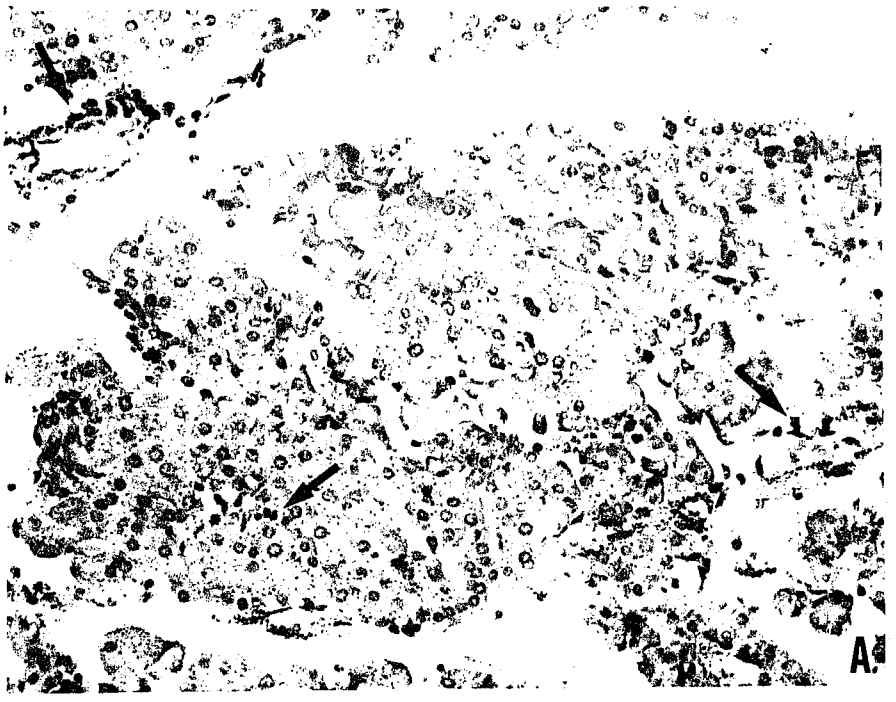
Figure 13. A = Radioautograph of tissue incubated with ^{3}H-5HT. In addition to the usual pattern of labelling, some pancreatic cells were heavily labelled (arrows). B = Photomicrograph of the section in figure A after removal of the radioautographic silver grains. These cells were revealed to be mast cells, characterized by their centrally located nucleus and metachromatically staining granules (arrows). C = Photomicrograph of another section after removal of radioautographic silver grains, revealing a cluster of mast cells in a segment of pancreatic connective tissue which had been heavily labelled in the radioautograph. Final magnification 1000x. Cresyl violet counterstain.

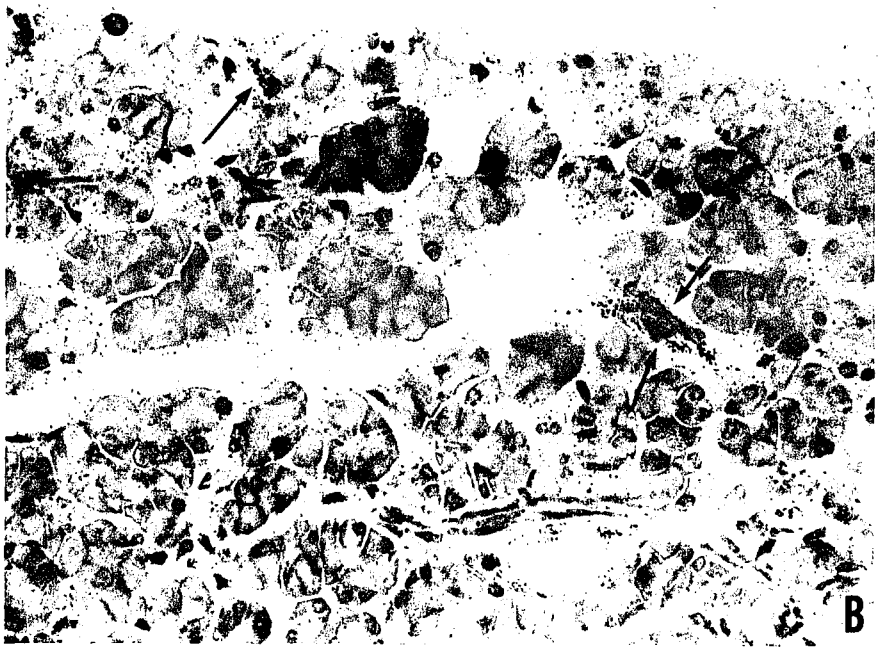



B


C

Figure 14. Species variation in the specific uptake of ^{3}H-5HT. A = Radioautograph of rabbit pancreatic fragments incubated with ^{3}H-5HT. The pattern of dense silver grain aggregates was similar to that seen in the rat, being located along blood vessels and in connective tissue areas (arrows). B = A similar pattern was demonstrated radioautographically in slices of hamster pancreata after ^{3}H-5HT uptake (arrows). C = Radioautograph of monkey pancreatic fragments incubated with ^{3}H-5HT. Dense silver grain aggregates were present (arrows), but were not as abundant as in either the rat or rabbit. Final magnification 1000x. Cresyl violet counterstain.




B

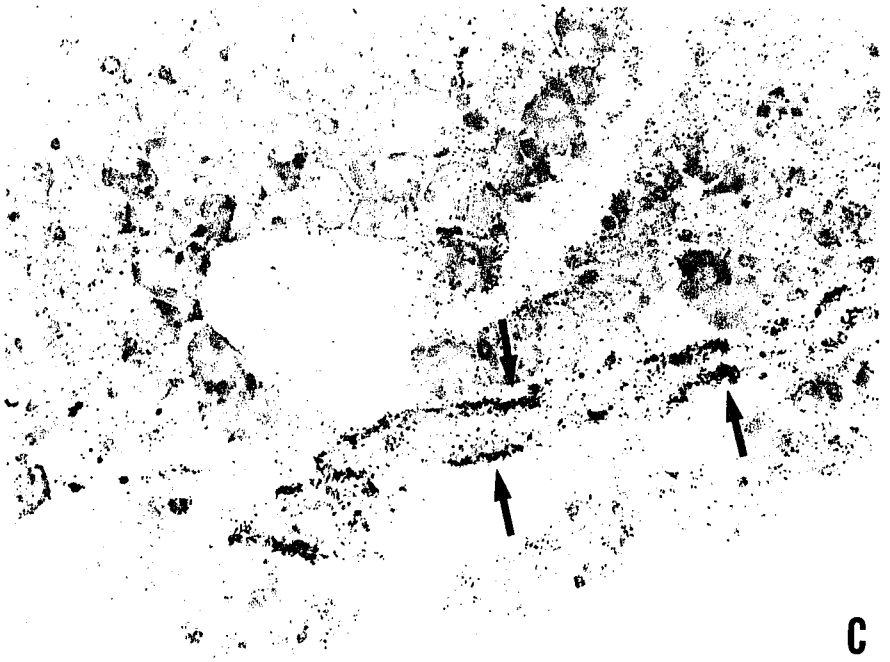

C

Figure 15. Radioautograph of pancreatic slice treated with fluoxetine prior to incubation with fluoxetine in the presence of ^{3}H-5HT. The dense silver grain aggregates seen in untreated tissue were absent. Final magnification 1800x. Cresyl violet counterstain.

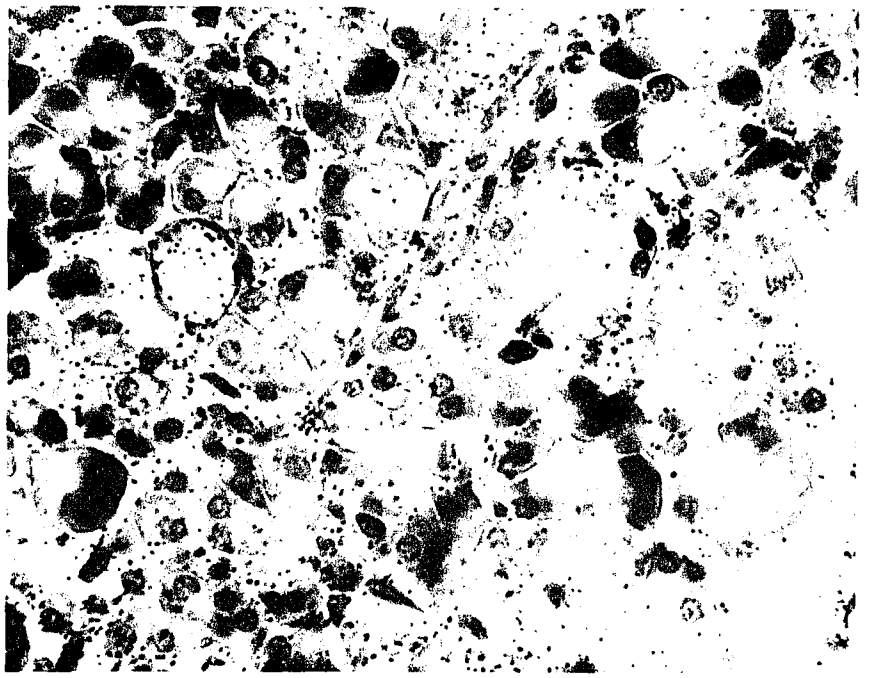
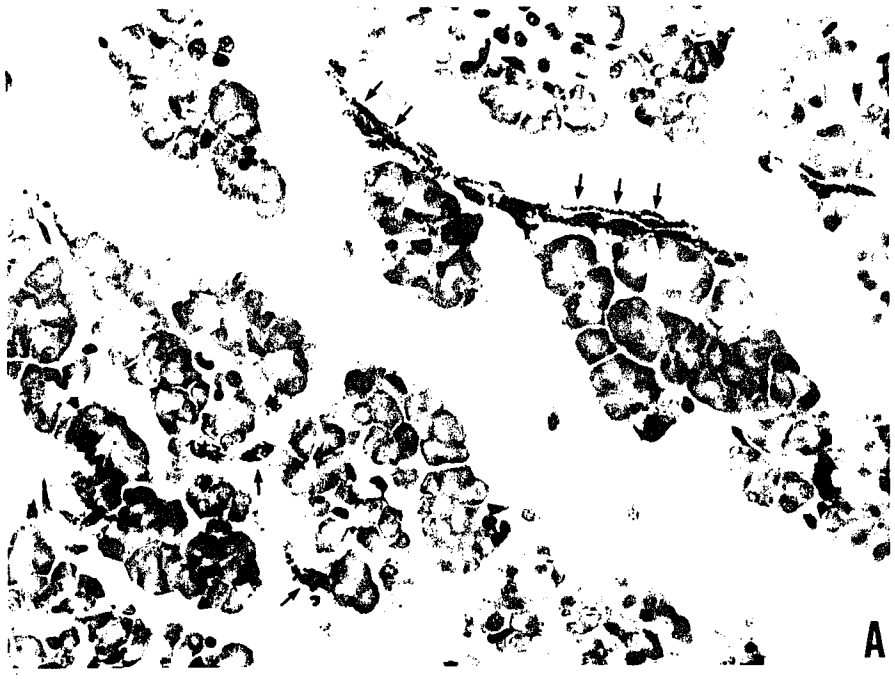
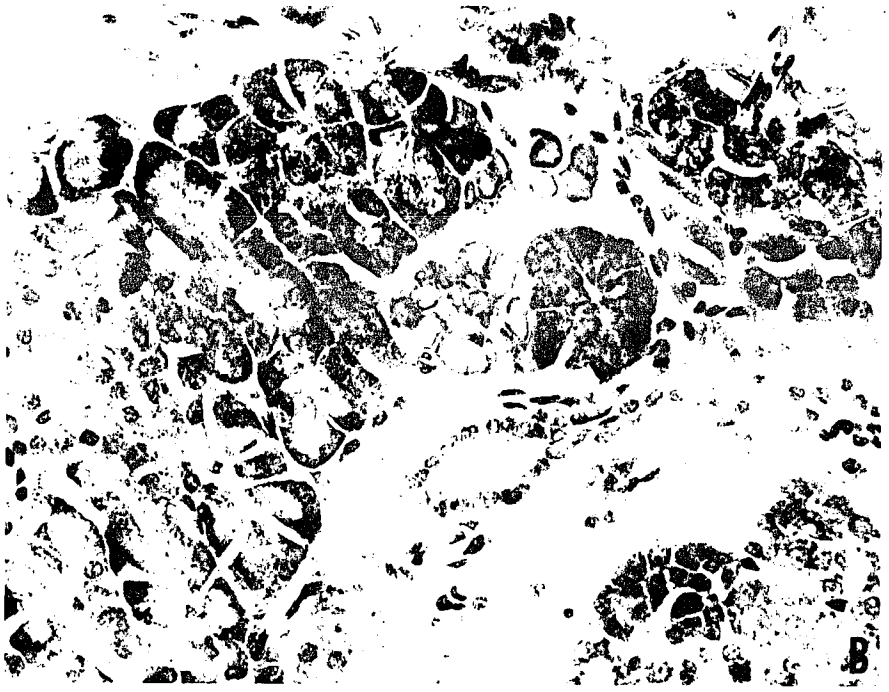


Figure 16. A radioautograph of a pancreatic slice from a rat injected with PCA (20 mg/Kg i.p.), on which an uptake of ^{3}H-5HT was subsequently performed. Radioautography corroborated the data from scintillation counting, which demonstrated no effect of the drug on uptake. The arrangement of dense silver grain aggregates was unchanged (arrows)(A). However, when slices were incubated in vitro with PCA (10^{-4}M) during (B), or prior to incubation with ^{3}H-5HT (C), no dense silver grain aggregates could be demonstrated. Final magnification 1000x. Cresyl violet counterstain.




B

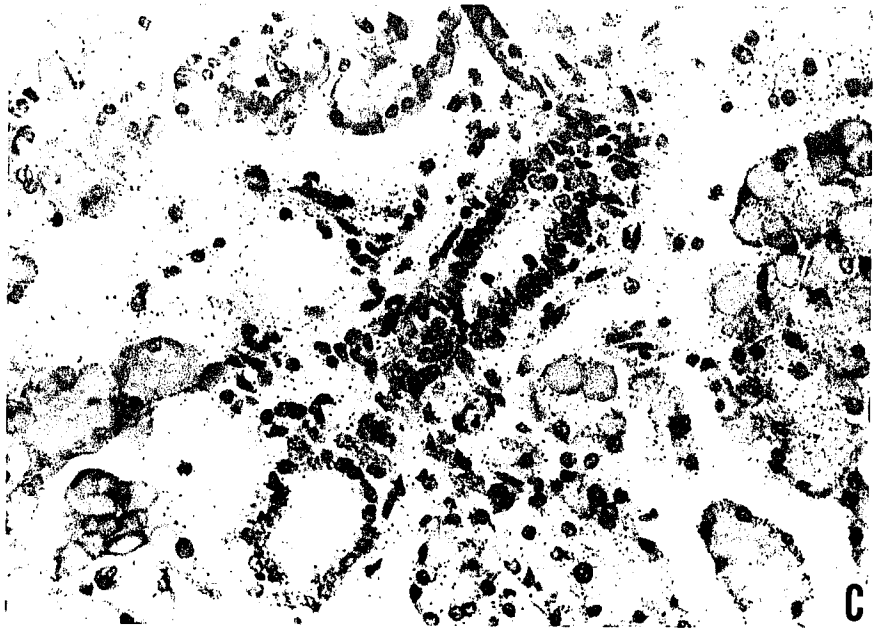

C

Figure 17. The uptake of ^{3}H-5HT was not affected by the incubation of tissue in metergoline, a serotonin post synaptic receptor blocking agent. The labelling in radioautographs was similar to untreated tissue. Radioautographic demonstration of labelling within an islet of a pancreatic slice treated with metergoline (10^{-6}M) during ^{3}H-5HT uptake, revealed dense aggregates in the connective tissue spaces (arrows), which was identical to the pattern seen without metergoline (Fig. 8). Final magnification 1800x. Cresyl violet counterstain.

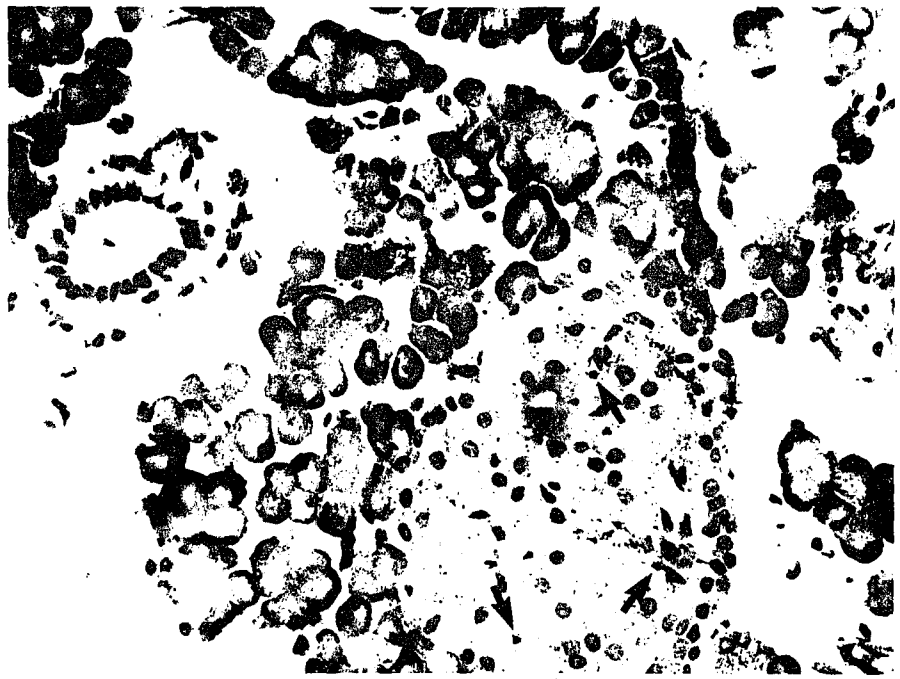
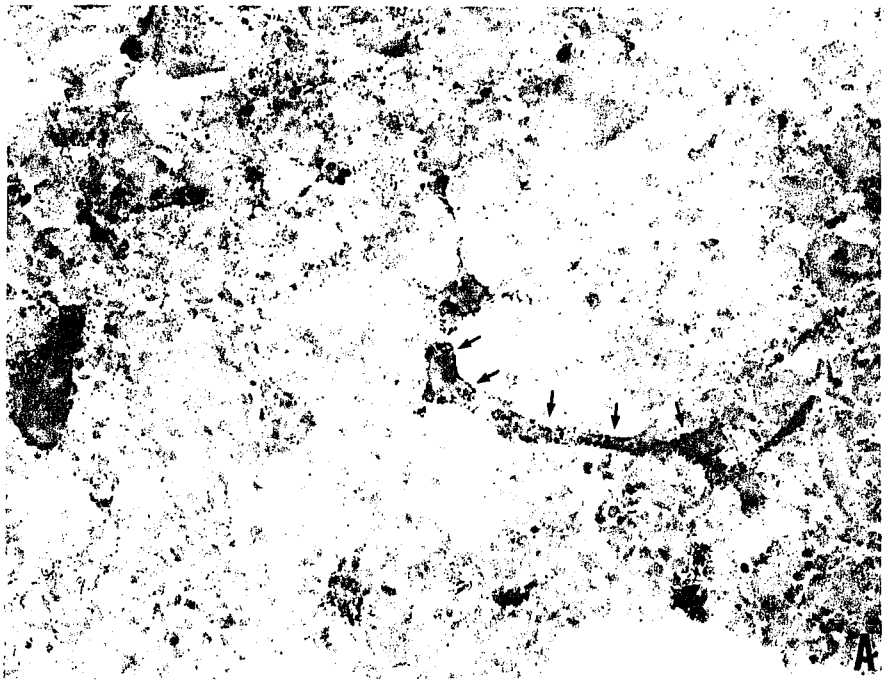
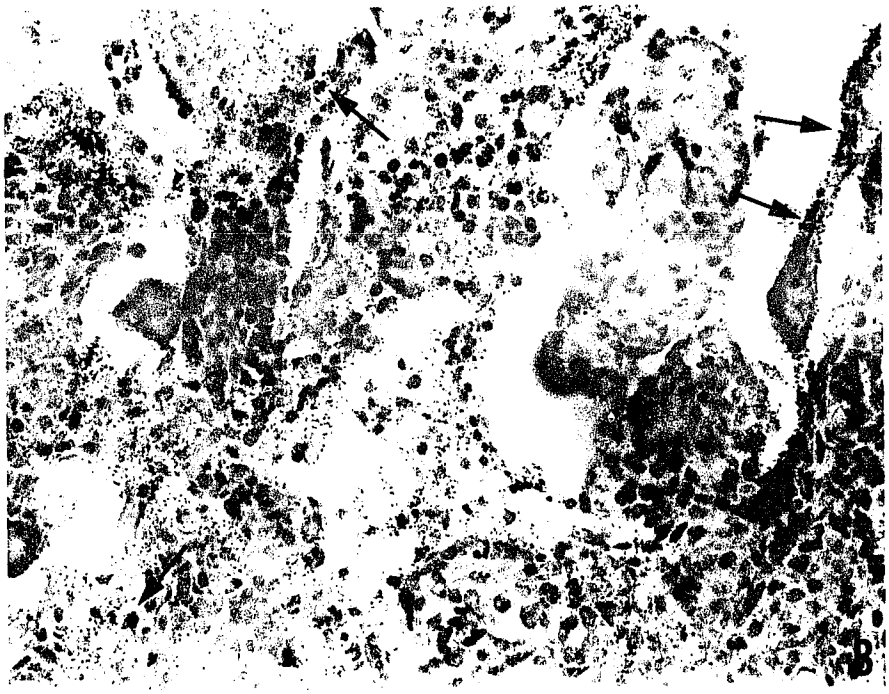


Figure 18. Radioautographs of fetal tissue after incubation with ^{3}H-5HT. 18 day fetal tissue (A) demonstrated dense aggregates of silver grains primarily around blood vessels (arrows). 20 (B) and 22 (C) day tissue showed a similar pattern, though not as pronounced, and demonstrated aggregates of grains in the interstitial spaces (arrows). Final magnification 1000x. Cresyl violet counterstain.




B


C

Figure 19. Radioautograph of pancreatic slice from an 18 day fetus after uptake of ^{3}H-5HT. Interestingly, cells were found to be heavily labelled (arrows). These cells resemble those described in the gut as serotonergic cell precursors. It is possible that a similar role might be played by these pancreatic cells. Cell at arrowhead probably represents mast cell (Epon embedding; Final magnification 1800x. Toluidine blue counterstain).

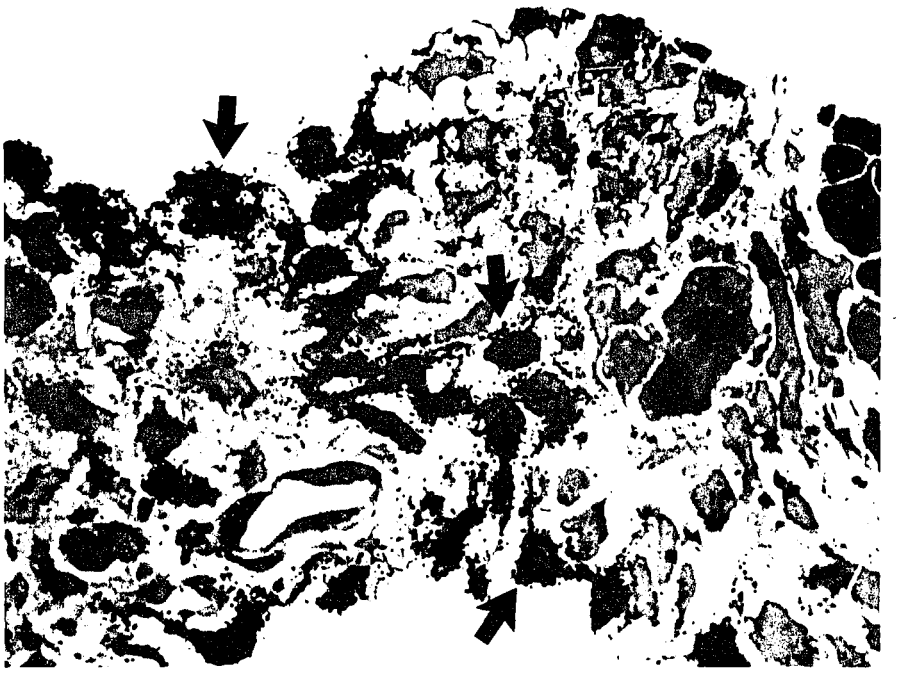


Figure 20. Radioautograph of 18 day fetal tissue after uptake of ^{3}H-5HT. Platelets (arrows), which are known to take up serotonin at the concentration used in this experiment, were demonstrated to be heavily labelled (Epon embedding; Final magnification 1800x. Toluidine blue counterstain).

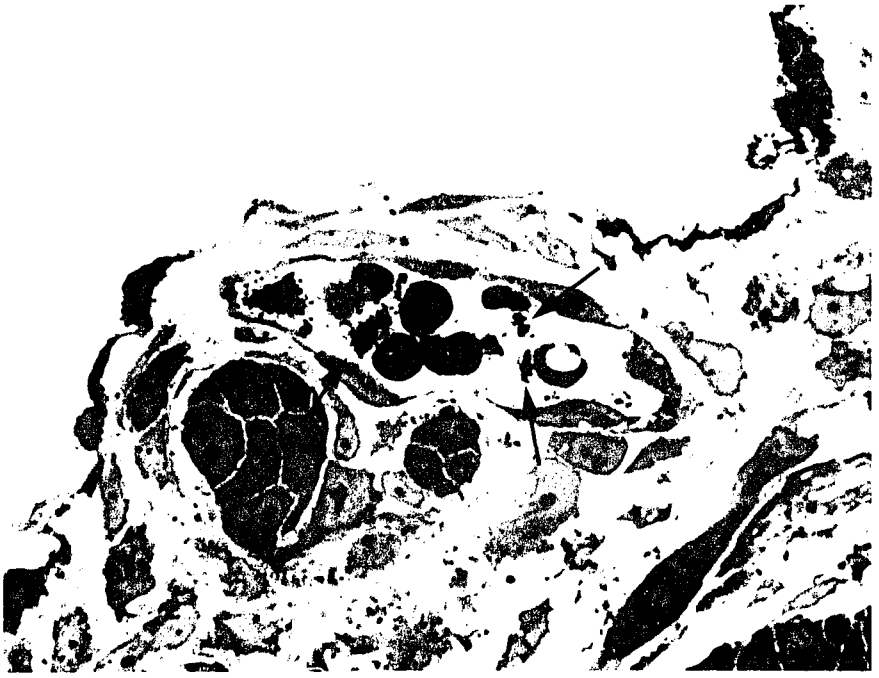


Figure 21. Radioautograph of 18 day fetal rat pancreata after 4 days in organ culture and uptake of ^{3}H-5HT. The pattern of dense silver grain aggregates (arrows) was similar to that seen in all uncultured fetal and adult tissue examined. It is postulated therefore that the cell bodies of the pancreatic serotonergic nerves are intrapancreatic. Final magnification 1000x. Cresyl violet counterstain.

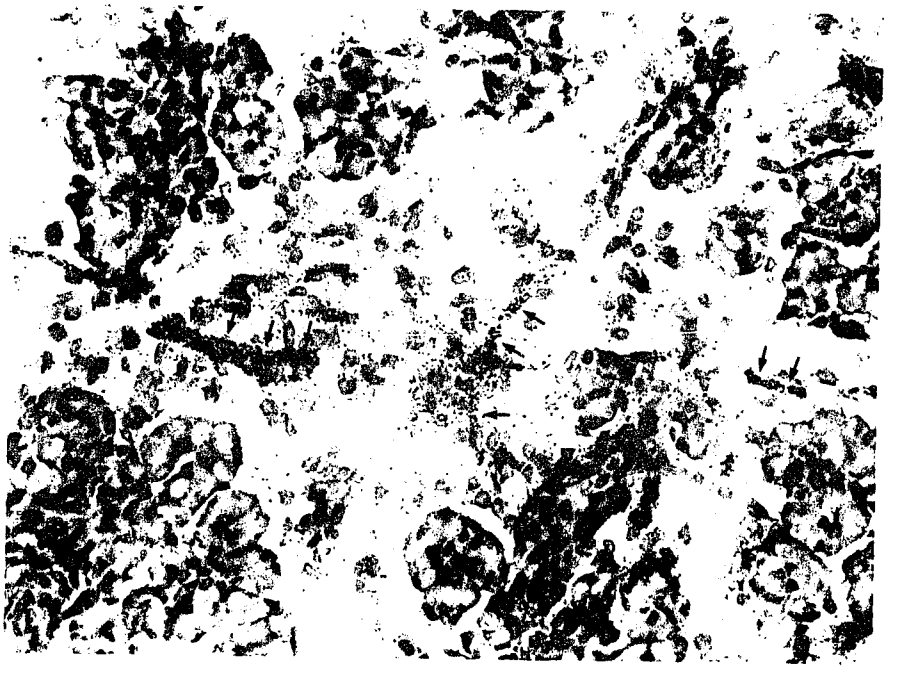
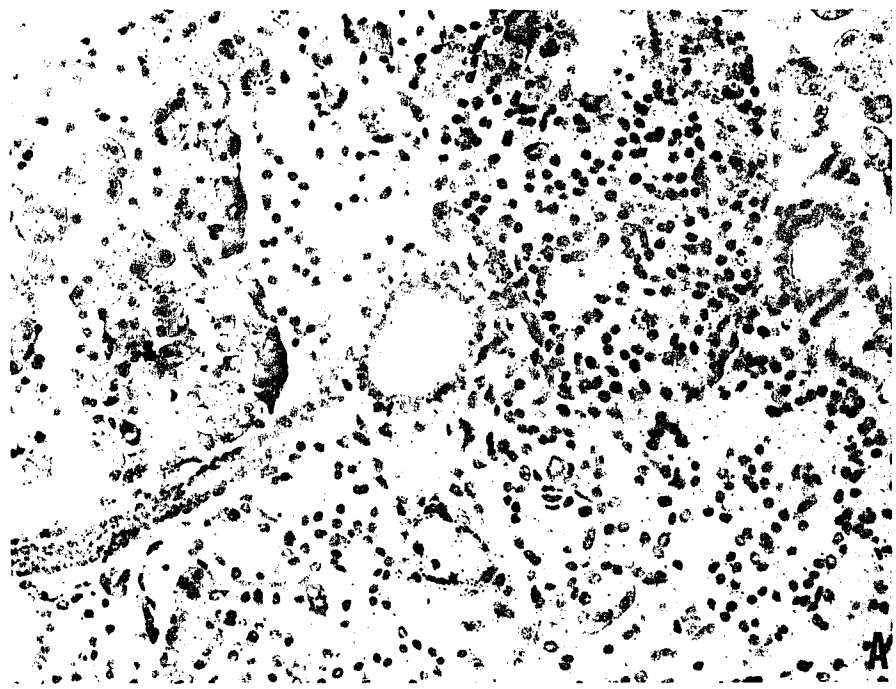


Figure 22. Radioautograph from tissue perifused with veratridine (10^{-5}M) after uptake of ^{3}H-5HT. A = The characteristic dense silver grain aggregates were almost completely absent from the connective tissue spaces. B = Control tissue, incubated with ^{3}H-5HT and allowed to perifuse for the same time as that exposed to veratridine, demonstrated the expected labelling (arrows). Therefore, nerve depolarization stimulated by veratridine administration resulted in an almost complete removal of newly uptaken serotonin. Final magnification 1000x. Cresyl violet counterstain.



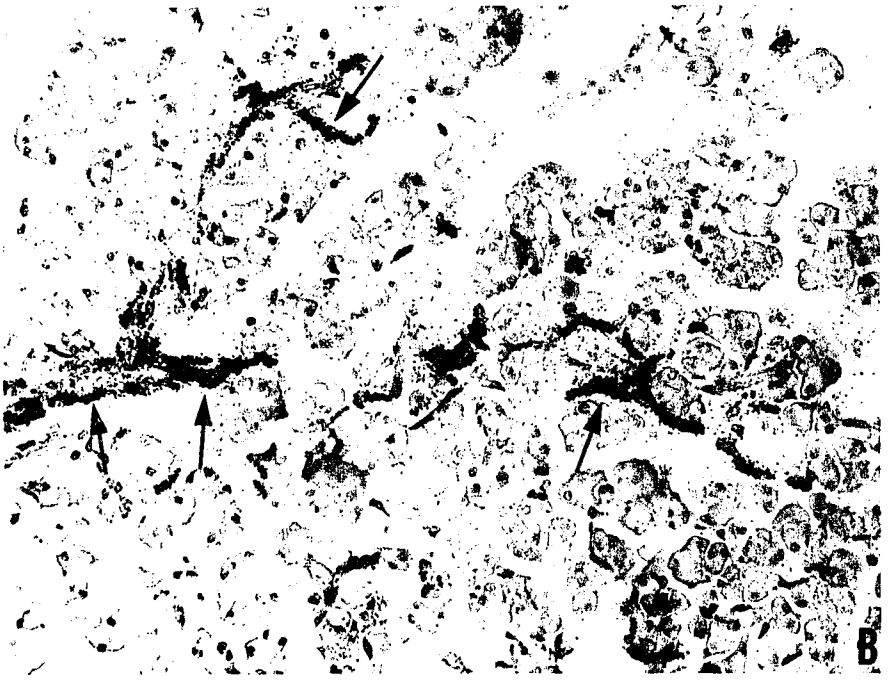
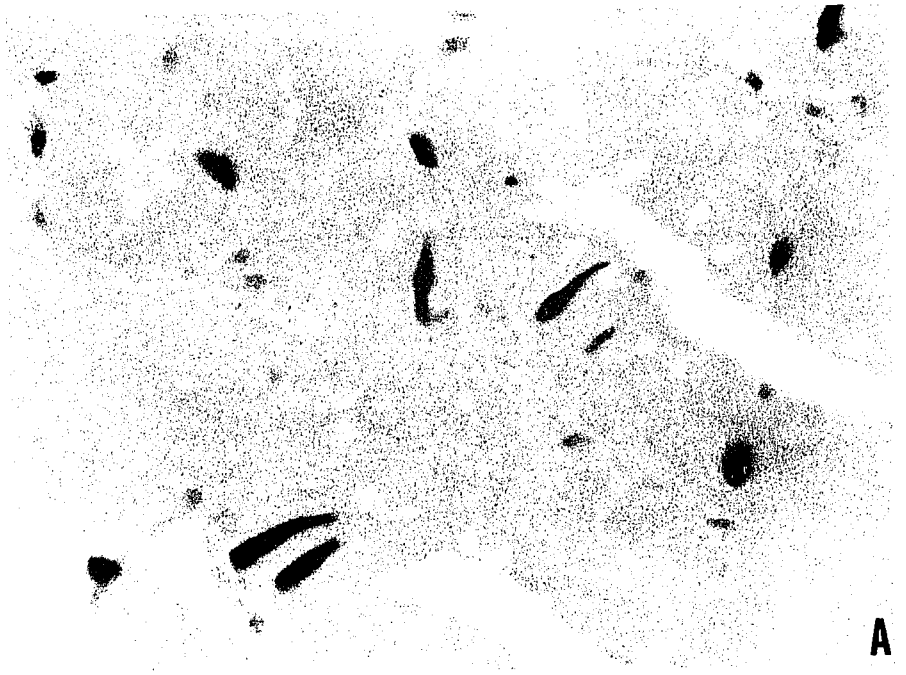

B

Figure 23. Immunocytochemical localization of serotonin in the rat duodenum after pretreatment of animal with pargyline and tryptophan. A = Enterochromaffin cells were clearly stained. Generally, labelling of these cells was more characteristic at their basilar region, which is where the serotonin containing secretory granules are known to reside. B = Tissue treated with a serotonin antibody that was incubated with excess serotonin served as a specificity control; staining was completely abolished by this absorption. Final magnification 1000x.



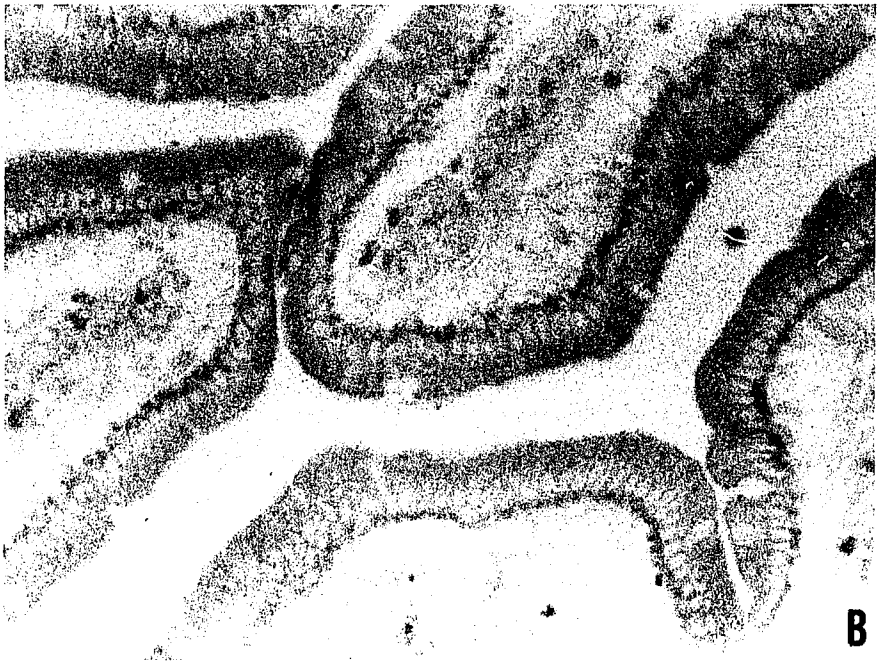
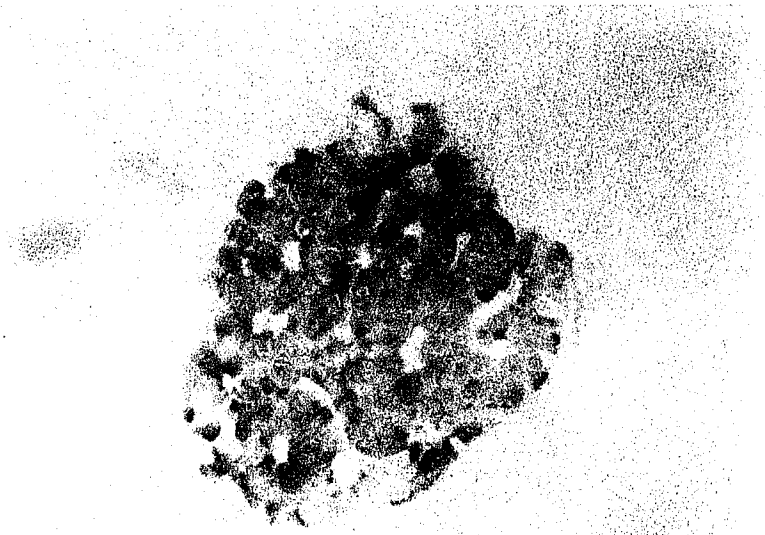
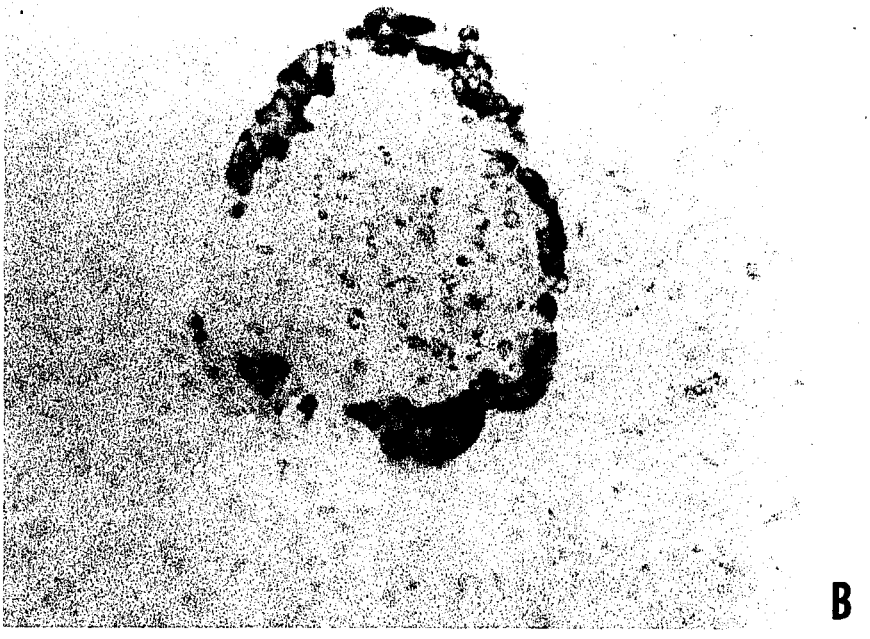

B

Figure 24. Immunocytochemical localization of serotonin in pancreatic slices after pretreatment in vivo with pargyline and tryptophan. There was positive staining of an occasional mast cell (arrow). These cells are thought to produce serotonin and secrete it under various conditions. No staining of nerve fibers or cells in the pancreas could be demonstrated. Final magnification 1000x.

Figure 25. Immunocytochemical localization of serotonin in islets from rats injected with 5-hydroxytryptophan, the immediate biosynthetic precursor of serotonin. A = Cells in the center of the islet stain positively for serotonin suggesting that B-cells convert 5-HTP to 5-HT. B = Analysis of the pattern of glucagon positive alpha cells in an adjacent section further suggests that serotonin positivity is localized primarily in B cells. Final magnification 1800x.



A



B

DISCUSSION

The data presented above support the following hypotheses: 1) There are 5-HT nerve fibers in the rodent pancreas; 2) These fibers resemble, in their biochemical and pharmacological properties, the 5-HT fibers in the brain and gut; 3) The cell bodies of these fibers are, totally or in part, contained within the parenchyma of the pancreas; and 4) the fibers are physiologically responsive and can be ablated in vitro, which may provide a mechanism by which their role in pancreatic secretion (exocrine, endocrine, or both) can be evaluated.

The uptake of radiolabelled serotonin, as demonstrated by radioautography, has been used extensively to study the distribution of serotonergic nerves in the brain and gut. In order to determine whether such nerves also exist in the pancreas, a similar procedure was employed. When pancreatic fragments were incubated with ^{3}H-serotonin, they demonstrated the ability to accumulate the amine. Maximal accumulation was found to occur after an incubation of 15 minutes (Figure 2). When a linear regression was performed on the log of the uptake values, a straight line with a highly significant correlation coefficient was obtained, suggesting that the uptake follows an exponential course. Blackburn et al. (1967) demonstrated a similar time course for the ^{14}C-5HT uptake into rat brain slices. Similarly, Azmitia and Marovitz (1980) demonstrated maximal serotonin uptake after a 15 minute incubation of rat hippocampal slices.

The uptake system for serotonin in the pancreas was found to be saturable (Figure 3). Radioautographically, the dense aggregates of silver grains in the connective tissue spaces, and not the diffuse grains over cells, were found to represent the saturable, high affinity component of the uptake (Figure 11). The radioautographic pattern of high affinity uptake (Figures 7, 8) was consistent with the distribution in nerve fibers. Autonomic nerves are known to enter the gland along the pancreatic vasculature, from which they branch off into the connective tissue to innervate the exocrine and endocrine cells (Lenninger, 1974). The demonstration of silver grain aggregates adjacent to these cells suggests their innervation by serotonergic nerves. Platelets are known to possess an uptake system for serotonin with similar kinetics to the pancreatic uptake (Lingjearde, 1979). Platelets were an unlikely source of the aggregates in the pancreas. First, the vast majority of the aggregates were clearly extravascular and second, perfusion of the pancreas with saline prior to uptake did not affect the labelling (Figure 10). Some of the silver grain aggregates were demonstrated in the outer wall of pancreatic arteries, arterioles and venules (Figure 9), suggesting a possible vasomotor function of these nerves. Such a role for serotonin on the mesenteric blood flow in the gut has been suggested (Fahrenkrug, 1979).

The radioautographic pattern of silver grain aggregates in the pancreas resembled that seen in the hippocampus (Azmitia

and Marovitz, 1980) and spinal cord (Segu and Calas, 1978), after incubation with ^{3}H-5HT. In the latter study, such aggregates were demonstrated by electron microscopic radioautography to correspond to nerve axons and terminals. It would be expected that the same correlation exists in the pancreas. While ultrastructural radioautography was not obtained of preparations of the rat pancreas, it has been performed in that of the bat, where incubation of slices of bat pancreas with ^{3}H-5HT revealed a highly significant localization of silver grains over nerve fibers (Nunez et al., 1980). It is interesting to note that these authors also demonstrated a moderate uptake of serotonin by the islet alpha cells.

The kinetics of the saturable, high affinity pancreatic serotonin uptake was determined by incubating tissue fragments with varying concentrations of ^{3}H-5HT, in the presence or absence of unlabelled 5-HT (10^{-5}M). An apparent Km value of 8.75×10^{-7}M, and Vmax of 873 pmoles/gram were obtained (Figure 4). The Km values for specific serotonin uptake by brain and gut serotonergic nerves were found to be 2×10^{-7}M (Azmitia and Marovitz, 1980) and 7.4×10^{-7}M (Gershon and Dreyfus, 1977), respectively. Since high concentrations of ^{3}H-5HT are taken up by the low affinity uptake system, it was not desirable to work with concentrations much above these values. In addition to cellular uptake, there was probably an uptake of low affinity into non-serotonergic

nerves. Shaskan and Snyder (1970) demonstrated that serotonin uptake into adrenergic nerves in the brain occurs at concentrations above 1 x 10^{-5}M. Thus, concentrations of ^{3}H-5HT for use in localizing pancreatic serotonergic nerves were kept significantly below that concentration.

It was demonstrated by Hellman et al. (1972) and recently confirmed by Lindstrom et al. (1980), that isolated islets from obese hyperglycemic mice possess two uptake systems for serotonin. The low affinity uptake was speculated to be into islet cells, and exhibited a Km of 1 x 10^{-2}M, and a Vmax of 1 mole/Kg dry islet/hour. The high affinity uptake exhibited a Km of 10^{-6}M. The authors speculated that the high affinity uptake might represent uptake by nerve fibers. The evidence, presented here, of silver grain aggregates between islet cells after uptake of ^{3}H-5HT (Figure 8), as well as the demonstration that isolated islets possess intact nerve terminals (Quansah et al., 1981), further support this hypothesis. Since Feldman and Chapman (1974) demonstrated that islet cell uptake of norepinephrine may represent a means of inactivation of the amine, it is possible that serotonin, released from nerve terminals in the islets, may similarly be utilized by these cells (possibly alpha cells in the bat), accounting for their possession of an uptake system.

As a measure of the variability, sixty values for total and nonspecific uptake from sixteen separate experiments done over two years were analyzed statistically to determine mean,

standard deviation and range. For pancreatic slices treated with ^{3}H-5HT (9×10^{-8}M), the mean value for specific uptake was 78 pmoles/gram, with a standard deviation of 10.6 (13.6%). The range of values for total uptake was 151.5 to 290.8, and the range for nonspecific uptake was 93.1 to 208.2. The variability in the uptake (although small), may have been due to the variation in the purity of the isotope from 90 to 98% over two years. In addition, though intraglandular uptake was found to be uniform (see below), individual samples in any given experiment may not be. Intra-uptake variability was generally small (see SEM in Tables), except in fetal tissue, where standard errors were considerably higher. The difficulty in handling this tissue, due to its consistency, was probably responsible for this variability.

It has been shown that ^{3}H-5HT accumulation into synaptosomes from the rat hypothalamus and hippocampus was markedly reduced by norepinephrine only when concentrations of ^{3}H-5HT exceeded 1×10^{-5}M; norepinephrine had no effect at lower concentrations of ^{3}H-5HT (Shaskan and Snyder, 1970; Azmitia and Marovitz, 1980). This suggests that ^{3}H-5HT, when present in high concentrations, can be taken up nonspecifically by adrenergic nerves. Incubation of pancreatic fragments with ^{3}H-5HT in the presence of norepinephrine (10^{-5}M) did not result in a reduction in uptake (Table 1; Figure 12). This suggests that the pancreatic noradrenergic nerve fibers do not contribute to the serotonin

uptake at the concentration of ^{3}H-5HT used in this experiment. A similar finding was reported by Dreyfus et al. (1977b) who found that specific uptake of serotonin into neurons of the myenteric plexus of the guinea pig was not inhibited by norepinephrine up to a tenfold greater concentration than that of 5-HT.

The specific uptake of serotonin was compared between male and female rats, and the variation in uptake in different areas of the gland was also investigated. No significant difference based on sex was obtained (Table 2). Similarly, there was no intrapancreatic variation in uptake (Table 3). This latter finding suggests a uniform distribution of pancreatic serotonergic fibers. Since there is an unequal distribution of islets in the rat pancreas, with more of the islets in the splenic region (Bensley, 1911), if the serotonergic nerves were involved solely in the regulation of islet cell secretion, a more polarized distribution would have been expected. The deliniation of the role or roles of the pancreatic 5-HT fibers in the regulation of the secretion of one or more of the pancreatic hormones must await further experimentation.

Uptake of ^{3}H-serotonin by pancreatic fragments from rabbits, hamsters and monkeys were compared. Of all species examined, uptake by the rabbit pancreas was found to be greatest (Table 4). Hamster uptake was similar to that seen in the rat. Radioautographically, the specific uptake sites were similar in all three species (Figure 14). Analysis of the

uptake in monkey pancreatic fragments required that they be incubated in a higher concentration of ^{3}H-5HT (1 x 10^{-7}M). Uptake at this concentration was found to be comparable to that seen in the rat and hamster at 8.3 x 10^{-8}M. Assuming a concentration dependent linear uptake of serotonin in the monkey pancreas (which was demonstrated in the rat), this represents a smaller degree of uptake. Radioautography of monkey fragments after uptake again demonstrated the same pattern of labelling.

Since the presence of serotonergic nerves necessitates the presence of the biosynthetic machinery for the synthesis of neurotransmitter, pancreatic homogenates were assayed for tryptophan hydroxylase, the rate limiting enzyme in serotonin biosynthesis from tryptophan. Pancreatic homogenates demonstrated a small quantity of enzyme activity (Table 12). This activity was approximately 20% of that demonstrated in the hippocampus using a similar technique (Azmitia, 1973). Two specificity controls were used in this experiment. PCPA (parachlorophenylalanine), which has been demonstrated to inhibit both tryptophan and phenylalanine hydroxylase (Koe and Weissman, 1966), significantly reduced the activity in the pancreas. To rule out phenylalanine hydroxylase as the source of the activity, it was necessary to incubate homogenates with phenylalanine. The affinity of phenylalanine hydroxylase for phenylalanine is 20x that of tryptophan (Ichiyama et al., 1968). Such treatment should have resulted in a marked

decrease in activity if the enzyme were phenylalanine hydroxylase. No such decrease was demonstrated.

If the specific uptake of serotonin demonstrated by the pancreas is indeed into serotonergic nerves, as the hypothesis states, it would be expected that drugs which interfere with the uptake into known 5-HT nerves would have similar effects on the pancreatic uptake. Fluoxetine has been demonstrated to be a competitive inhibitor of serotonin uptake into synaptosomes from the rat brain (Fuller et al., 1975), as well as into myenteric plexus serotonergic nerves (Gershon and Jonakait, 1979). The ED_{50} for fluoxetine in the gut was 1.7×10^{-6}M. Using a concentration of 10^{-5}M in vitro, following a previous dose administered in vivo, fluoxetine was demonstrated to completely abolish the specific serotonin uptake (Table 5; Figure 15).

PCA (parachloroamphetamine) is a drug which has specific neurotoxic effects on brain serotonergic nerves, lowering brain serotonin levels (Fuller et al., 1979) and tryptophan hydroxylase activity (Sanders-Bush et al., 1975) for 4 months following a single injection of 10 mg/Kg. When animals were injected with PCA (20 mg/Kg i.p.) for five days, however, the specific uptake of serotonin in the pancreas was preserved (Table 6). In vitro studies with PCA on brain tissue have demonstrated an ED_{50} for ^{3}H-5HT uptake inhibition of 7.5×10^{-5}M (Hwang and van Woert, 1980). When pancreatic fragments were incubated with 5×10^{-4}M PCA, specific

uptake of serotonin was eliminated (Table 7; Figure 16b). Evidence has been presented suggesting that PCA is taken up by brain serotonergic terminals by the same mechanism as that of serotonin. Thus, the in vitro inhibition of specific serotonin uptake may represent a competition phenomenon (Silverman and Ho, 1979). To determine whether PCA has a destructive effect on the pancreatic nerves, tissue fragments were preincubated with PCA for 30 minutes, then washed well with buffer and incubated with ^{3}H-5HT. When this was done, no specific uptake of serotonin was demonstrated (Table 8; Figure 16c). It therefore appears that while PCA may compete with serotonin for uptake, it also has a destructive effect on the pancreatic uptake mechanism, and probably other functions of the nerve terminal as well.

The effects of the neurotoxin 5,7DHT (5,7 dihydroxytryptamine) on the pancreatic uptake were also investigated. This drug has been demonstrated to have destructive effects on serotonergic and adrenergic nerves (Breese and Muller, 1978). Since adrenergic nerves probably do not contribute to the specific uptake of ^{3}H-5HT in the pancreas (see above), the destruction of adrenergic nerves by the drug should have been of little consequence. In addition, pargyline, an MAO inhibitor present in the uptake buffer, was shown to antagonize the effects of 5,7DHT on brain adrenergic nerves, without affecting its action on serotonergic fibers (Breese and Muller, 1978). When pancreatic fragments were

incubated with 5,7DHT (5×10^{-4}M) together with ^{3}H-5HT, specific uptake was again abolished, while nonspecific cellular uptake appeared normal (Table 7). Since long term incubation with 5,7DHT prior to ^{3}H-5HT administration was not performed, the apparent destructive effect of the drug on the uptake mechanism may have resulted from competition with serotonin. Tissue incubated with PCA and 5,7DHT, together with ^{3}H-5HT, did not demonstrate specific uptake of ^{3}H-5HT when concentrations of 10^{-4} and 10^{-5}M were used, but a significant degree of specific uptake remained with a concentration of 10^{-6}M.

Incubation of tissue with metergoline did not significantly reduce the specific uptake of ^{3}H-5HT (Table 9; Figure 17). Since metergoline was demonstrated to antagonize serotonin binding in various organs (Beretta et al., 1965; Sastry and Phillis, 1977), it appears that the binding of serotonin to its receptor is not responsible for the uptake seen in the pancreas. The absence of dense silver grain aggregates over vascular smooth muscle cells, which are known to possess serotonin receptors, further substantiates this claim.

In addition to adult tissue, fetal rat pancreata were analyzed for the presence of a specific serotonin uptake system. Since neurons were found to colonize the rat pancreas soon after formation of the pancreatic bud at 11 days gestation (Pictet et al., 1976), it seemed likely that if the hypothesis

that the uptake is into nerves is correct, specific uptake would be present by 18 days of gestation. Indeed, specific uptake was demonstrated at all three of the gestational ages tested (Table 10), and radioautographic localization was similar to that seen in the adult, with dense silver grain aggregates in the connective spaces (Figure 18). The specific uptake by the fetal pancreata was greater than that seen in the adult, suggesting that the number of uptake sites per unit weight is greater in the fetus.

If serotonin acts as a neurotransmitter in the pancreas, its release upon nerve stimulation should be demonstrable. This question was approached in two ways. After specific uptake of ^{3}H-5HT by pancreatic fragments, the release of labelled serotonin in response to fenfluramine was determined. Fenfluramine has been demonstrated to be a potent stimulator, in a dose dependent manner, of ^{3}H-5HT release from the cerebral cortex and spinal cord (Fuxe et al., 1975). The ED_{50} for ^{3}H-5HT release by fenfluramine was demonstrated to be 3.5×10^{-5}M (Hwang and van Woert, 1980). Using a similar concentration, tissue perfused with fenfluramine was demonstrated to release radioactivity at a significantly higher rate for a longer period of time than either tissue perifused without fenfluramine, or tissue preincubated in the presence of 5-HT (10^{-5}M) and treated with fenfluramine (Figure 5). The absolute number of counts in the experimental tissue after perifusion was found to be

similar to that of untreated tissue (Table 13). Since tissue preincubated with excess, unlabelled serotonin and perifused with fenfluramine also showed a slightly lower number of counts than untreated tissue, a small proportion of the nonspecific uptake may also be released by the drug (Table 13).

Pancreatic fragments after uptake were similarly perifused with veratridine. This drug has been demonstrated to nonspecifically stimulate release of neurotransmitters from nerve terminals, by opening sodium channels in the nerve axon (Ulbricht, 1967; Pollard and Pappas, 1979). Such treatment acutely stimulated the release of radioactivity, whereas no stimulation was observed from tissue treated with ^{3}H-5HT in the presence of unlabelled 5-HT (10^{-5}M) (Figure 6). Perifusion data from this and the above experiment were expressed as percent of baseline, since the absolute number of counts at any given time point during the perifusion were not always the same in any given group. Analysis of the experimental tissue revealed a significant loss of radioactivity to near nonspecific levels (Table 14). Though veratridine has also been demonstrated to release the contents of islet alpha and beta cells (Kawazu et al., 1981), uptake of serotonin by the islet cells represents low affinity uptake. Radioautography of experimental tissue demonstrated an almost complete absence of silver grain aggregates, while the diffuse grains were intact (Figure 22). Thus, the high affinity uptake sites in the pancreas have the ability to depolarize and

release their contents. This evidence, together with the demonstration of a serotonin releasing effect of fenfluramine, further substantiates the hypothesis that the pancreas is innervated by 5-HT nerves.

The specific uptake of ^{3}H-5HT was preserved in pancreata cultured alone or with duodenum (Table 11; Figure 21). This supports the hypothesis that the location of the pancreatic cell bodies is intraglandular. Further evidence for a pancreatic location for the cell bodies of the serotonergic nerves comes from radioautographic data on plastic sections from 18 day fetal rats. In such sections, a group of cells were found to be specifically labelled (Figure 19). These labelled cells were primitive in appearance and displayed no characterizing features that permitted their morphological identification. Similar cells, which also took up ^{3}H-5HT in the developing chick duodenum, were found to represent the precursors of myenteric plexus serotonergic nerves (Epstein et al., 1980; Gershon et al., 1980). It is possible that a similar role might be played by these cells in the development of pancreatic serotonergic nerves. Eventually, in the developing chick, these cells were found to lose their ability to accumulate serotonin. A similar loss of uptake ability by the cells in the pancreas could account for the lack of demonstrable specific high affinity uptake by nerve cell bodies in adult tissue.

Serotonin immunocytochemistry was performed on pargyline

and tryptophan treated rats, in an attempt to demonstrate the presence of endogenous serotonin in pancreatic nerves and/or cell bodies. Unfortunately, under three separate fixation conditions, only pancreatic mast cells and gut mast and enterochromaffin cells were positively stained. Even when tissue was incubated with unlabelled serotonin (10^{-7}M) prior to immunocytochemistry, no staining of the high affinity uptake sites was observed. Indeed, while gut enterochromaffin cells stained, staining of myenteric plexus serotonergic nerves was not observed. This may indicate that the serotonin within these fibers is present at a concentration below the sensitivity of the antibody. It should be emphasized that a lack of staining is impossible to interpret. Since the presence of endogenous serotonin in nerve fibers is an essential requirement for neurotransmitter status, more work needs to be done to perfect this technique in order to solidify the hypothesis. Since they lack tryptophan hydroxylase, no islet cells were found to be stained after pargyline and tryptophan administration. However, when the serotonin precursor 5-HTP was given, staining of the islets (primarily beta cells) was observed. This was expected since these cells contain the nonspecific aromatic amino acid decarboxylase enzyme necessary for the conversion (Pearse and Takor, 1976). Since the amount of circulating 5-HTP is negligible, the demonstration of a high concentration of serotonin in isolated islets (Quansah et al., 1981) probably represents neuronal serotonin.

The role of the pancreatic serotonergic fibers in the regulation of pancreatic secretion is unknown. Studies by other investigators on the effects of exogenous 5-HT on insulin secretion have suggested that 5-HT is inhibitory, at concentrations of 10^{-4}M and greater. Assuming that serotonin, released from these fibers in the islets, can reach such concentrations, it is possible that the 5-HT fibers may have an inhibitory effect on insulin secretion. Since the administration of serotonin receptor antagonists to Type II non-insulin dependent diabetics increased their glucose tolerance (Feldman et al., 1972), it is possible that an abnormality in these nerves may play a role in the development of the disease. It has been speculated that catecholaminergic nerves may also be involved in the pathogenesis of Type II diabetes.

Fujita and Kobayashi (1979) were the first to propose that nerves may release their secretion products into the capillary blood of the islet. If this neurosecretion actually occurs, then serotonin so released may be transported via the insulo-acinar portal vessels to the exocrine tissue in high concentrations. The transport of insulin by these vessels has been implicated in the enhancement of CCK-PZ secretion (Fujita and Murakami, 1973). Since serotonin has been demonstrated to reduce basal pancreatic bicarbonate secretion in dogs (Nakano, 1968), the neurosecretion of serotonin may similarly have a physiological effect on the exocrine pancreas. Serotonin,

released from nerve terminals at the base of exocrine acinar cells, would also be a means of regulation of secretion.

A role for serotonin in the regulation of pancreatic blood flow can also be hypothesized. Vasoconstriction of islet arterioles, which are usually located within the non-beta cell portion of the islet (Larsson, 1980), for example, may be a method of shunting blood away from the insulin secreting cells during times of stress. Such vasoconstriction would also result in reduced exocrine blood flow, which would also be beneficial at these times.

LITERATURE CITED

Adrian, T. E., S. R. Bloom, H. S. Besterman, A. J. Barnes, T. J. C. Cooke, R. C. G. Russell, and R. G. Faber 1977 Mechanisms of pancreatic polypeptide release in man. Lancet, 1:161-163.

Aghajanian, G. K. 1972 Influence of drugs on the firing of serotonin-containing neurons in the brain. Fed. Proc. Fed. Am. Soc. Exp. Biol., 31:91-96.

Aleyassine, H., and S. H. Lee 1971 Inhibition by hydrazine, phenelzine and pargyline of insulin release from rat pancreas. Endocrinology, 89:125-129.

Alm, P., J. Alumets, R. Hakanson, C. Owman, N. D. Sjoberg, F. Sundler, and B. Walles 1980 Origin and distribution of VIP (vasoactive intestinal polypeptide)- nerves in the genito-urinary tract. Cell Tiss. Res., 205:337-347.

Azmitia, E. C. 1973 "Rapid alterations in rat brain trytophan hydroxylase activity: Effects of adrenal steroids, drugs and stressors." Ph.D. dissertation, The Rockefeller University, N. Y.

Azmitia, E. C. 1978 In: Handbook of Psychopharmacology, Vol. 9. Iversen, L. L., S. D. Iversen, and S. Snyder, eds. Plenum Press, New York, pp. 233-314.

Azmitia, E. C., and L. C. A. Conrad 1976 Temporal effects of fornix transection on brain tryptophan hydroxylase activity and plasma corticosterone levels. Neuroendocrinology, 21:338-349.

Azmitia, E. C., and B. S. McEwen 1976 Early response of rat brain tryptophan hydroxylase activity to cyclohexamide, puromycin and corticosterone. J. Neurochem., 27:773-778.

Azmitia, E. C., and W. F. Marovitz 1980 In vitro hippocampal uptake of tritiated serotonin (^{3}H-5HT). A morphological, biochemical, and pharmacological approach to specificity. J. Histochem. Cytochem., 28:636-644.

Baumgarten, H. G., A. F. Holstein, and C. Owman 1970 Auerbach's plexus of mammals and man: Electron microscopic identification of three different types of neuronal processes in myenteric ganglia of the large intestine from Rhesus monkeys, guinea pigs and man. Z. Zellforsch., 106:376-397.

Baumgartner, H. R., and G. V. R. Born 1968 Effect of 5-hydroxytryptamine on platelet aggregation. Nature, 218:137-141.

Becker, H. D., H. W. Borger, and A. Schafmayer 1979 Effect of vagotomy on gastrointestinal hormones. World J. Surg., 3:615-622.

Benditt, E. P., R. L. Wong, M. Arase, and E. Roeper 1955 5-hydroxytryptamine in mast cells. Proc. Soc. Exp. Biol., 90:303-304.

Bennett, W. F., J. S. Belville, and G. Lynch 1980 The relationship between extracellular pH and control of blood platelet serotonin secretion. Mol. Pharmacol., 17:356-361.

Bensley, R. R. 1911 Studies on the pancreas of the guinea pig. Amer. J. Anat., 12:297-387.

Beretta, C., A. H. Glasser, M. B. Nobili, and R. Silvestri 1965 Antagonism of 5-hydroxytryptamine-induced bronchospasm in guinea pigs by 8B-carbobenzyloxy-aminomethyl -1,6-dimethyl-10A-ergoline. J. Pharm. Pharmacol., 17:423-428.

Bjorklund, A., A. Nobin, and U. Stenevi 1973 Regeneration of central serotonin neurons after axonal degeneration induced by 5,6- dihydroxytryptamine. Brain Res., 50:214-220.

Blackburn, K. J., P. C. French, and R. J. Merrills 1967 5-hydroxytryptamine uptake by rat brain in vitro. Life Sciences, 6:1653-1663.

Bloom, S. R., A. V. Edwards, and N. J. A. Vaughan 1973 Role of sympathetic innervation in the control of plasma glucagon concentration in the calf. J. Physiol., 233:457-466.

Boullin, D. J. 1964 Observations on the significance of 5-hydroxytryptamine in relation to the peristaltic reflex of the rat. Brit. J. Pharmacol., 23:14.

Breese, G. R., and R. A. Mueller 1978 Alterations in the neurotoxicity of 5,7-dihydroxytryptamine by pharmacologic agents in adult and developing rats. Ann. N. Y. Acad. Sci., 305:160-174.

Bremer, F. 1977 Cerebral hypnogenic centers. Ann. Neurol., 2:1-6.

Brinn, J. E. 1975 Pancreatic islet cytology in Octaluridae(Teleostei). Cell Tissue Res., 162:357-365.

Britton, S. W. 1925 Studies on the condition of activity in endocrine glands: XVII. The nervous control of insulin secretion. Am. J. Physiol., 74:291-308.

Brodie, B. B., and P. A. Shore 1956 A concept for a role of serotonin and norepinephrine as chemical mediators in the brain. Ann. N. Y. Acad. Sci., 66:631-642.

Brodows, R. G., F. Xavier Pi-Sunyen, and R. G. Campbell 1974 Insulin secretion in adrenergic insufficiency in man. J. Clin. End. Metab., 38:1103-1108.

Brown, M., J. Villarreal, and W. Vale 1976 Neurotensin and substance P: Effect on plasma insulin and glucagon levels. Metabolism, 25:1459-1461.

Bruni, J. F., W. B. Watkins, and S. S. C. Yen 1979 B-endorphin in the human pancreas. J. Clinical End. Metab., 49:649-651.

Bryce, G. F., and J. H. Jacoby 1978 A comparative study of several serotonin receptor antagonists on basal and stimulus induced release of insulin and glucagon in the intact rat. Life Sciences, 22:2215-2224.

Buchanan, K. D., J. E. Vance, K. Denstle, and R. H. Williams 1969 Effect of blood glucose on glucagon secretion in anesthetized dogs. Diabetes, 18:11-18.

Buchanan, K. D. 1977 Glucagon. In: Insulin and Metabolism. Bajaj, J. S., editor. Williams and Wilkins Company, Baltimore, p233.

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

Bulbring, E., and M. D. Gershon 1967 5-hydroxytyptamine participation in the vagal inhibitory innervation of the stomach. J. Physiol., 192:823-846.

Burr, I. M., L. Balant, and W. Stauffacher 1971 Adrenergic modification of glucose induced biphasic insulin release from perfused rat pancreas. Eur. J. Clin. Invest., 1:216-224.

Cajal, S. R. Y., et C. Sala 1891 Termination de los nervios y tubos glandulares del pancreas de los vertebrados. Trab. del Labor. de Histol. de la Facultad de Medicini de Barcelona.

Campenhout, E. van 1927 Contribution a l'etude de l'histogenese du pancreas chez quelques mammiferes. Les complexes sympathetico-insulaires. Arch Biol. (Liege) 37:121-171.

Carlsson, A., M. Lindqvist, T. Magnusson, and C. Atack 1973 Effect of acute transection on the synthesis and turnover of 5-HT in the rat spinal cord. Naunyn-Schmiedebergs Arch. Pharmacol., 277:1-12.

Cataland, S., and T. M. O'Dorisio 1980 Pathology of gastric inhibitory polypeptide. Front. Hormone Res., 7:145-154.

Cegrell, L. 1968 The ocurrence of biogenic monoamines in mammalian endocrine pancreas. Acta. Physiol. Scand., 314:1-60.

Chance, R. E. 1972 Discussion of presentations. Diabetes, 21(suppl. 2):536.

Chen, M., and D. Porte 1976 The effect of rate and dose of glucose infusion on the acute insulin response in man. J. Clin. End. Metab., 42:1168-1175.

Colwell, J. A. 1969 Inhibition of insulin secretion by catecholamines in pheochromocytoma. Ann. Int. Med., 71:

Conlon, J., V. Harris, and P. Raskin 1978 Somatostatin-like immunoreactivity in human plasma. Clin. Res., 26:789A.

Cook, R. D., and G. Burnstock 1976 The ultrastructure of Auerbach's plexus in the guinea pig. I. Neuronal elements. J. Neurocytology, 5:171-194.

Coore, H. G., and P. J. Randle 1964 Regulation of insulin secretion studied with pieces of rabbit pancreas incubated in vitro. Biochem. J., 93:66-78.

Coppen, A., and K. Wood 1979 Etudes cliniques et biologiques du L-tryptophan dans la depression. Encephale, 5:627-632.

Coujard, R., and C. Theret 1952 Les reactions insulaires du pancreas par agression sur le sympathique peripherique. C. R. Soc. Biol., 146:1883-1886.

Coupland, R. E. 1958 The innervation of pancreas of the rat, cat and rabbit as revealed by the cholinesterase technique. J. Anatomy, 92:143-149.

Creutzfeldt, W. 1979 The incretin concept today. Diabetologia, 16:75-85.

Curry, D. C., L. Bennett, G. M. Grodsky 1968 Dynamics of insulin secretion by the perfused rat pancreas. Endocrinology, 83:572-584.

Dahlstrom, A., and K. Fuxe 1965 Evidence for the existence of monoamine neurons in the central nervous system. II. Experimentally induced changes in the intraneuronal amine levels of bulbospinal neuron systems. Acta Physiol. Scand., 64:1-36.

de Bold, M. L., and S. A. Bencosme 1975 Inhibition of the first phase of glucose mediated insulin release by serotonin in an in situ perfused pancreas preparation. Fed. Proc., 42:253.

de la Torre, J. C. 1972 Dynamics of Brain Monoamines. Plenum Press, N. Y., pg. 113.

deLeiva, A., R. J. Tanenberg, G. Anderson, B. Greenberg, B. Senske, and F. C. Goetz 1978 Serotoninergic activation and inhibition: effects on carbohydrate tolerance and plasma insulin and glucagon. Metabolism, 27:511-520.

Descarries, L., A. Beaudet, and K. C. Watkins 1975 Serotonin nerve terminals in adult rat neocortex. Brain Res., 100:563-588.

Dolais-Kitabgi, J., P. Kitabgi, P. Brazeau, and P. Freychet 1979 Effect of neurotensin on insulin, glucagon, and somatostatin release from isolated pancreatic islets. Endocrinology, 105:256-260.

Drakontides, A. B., and M. D. Gershon 1968 5-Hydroxytryptamine receptors in the mouse duodenum. Brit. J. Pharmacol., 33:480-492.

Dreyfus, C. F., M. B. Bornstein, and M. D. Gershon 1977a Synthesis of serotonin by neurons of the myenteric plexus in situ and in organotypic tissue culture. Brain Research, 128:125-139.

Dreyfus, C. F., D. L. Sherman, and M. D. Gershon 1977b Uptake of serotonin by intrinsic neurons of the myenteric plexus grown in organotypic tissue culture. Brain Res., 128:109-123.

Duckworth, W. C., and A. E. Kitabchi 1981 Insulin metabolism and degradation. Endocrine Reviews 2:210-233.

Dupre, J., D. J. Chisholm, T. J. McDonald, and A. Rabinovitch 1975 Effects of secretin on insulin secretion and glucose tolerance. Can. J. Physiol. Pharmacol., 53:1115-1121.

Efendic, S., R. Luft, and E. Cerasi 1978 Quantitative determination of the interaction between epinephrine and various insulin releasers in man. Diabetes, 27:219-326.

Ekholm, R., L. E. Ericson, and I. Lundquist 1971 Monoamines in the pancreatic islets of the mouse. Subcellular localization of 5-HT by E.M. autoradiography. Diabetologia, 7:339-348.

Epstein, M. L., D. Sherman, and M. D. Gershon 1980 Development of serotonergic neurons in the chick duodenum. Dev. Biol., 77:22-40.

Erlandsen, S. L., O. D. Hegre, J. A. Parsons, R. C. McEvoy, and R. P. Elde 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.

Erspamer, V. 1955 Observation on the fate of indolealkylamines in the organism. J. Physiol., 127:118-133.

Fahrenkrug, J. 1979 Vasoactive intestinal polypeptide: Measurement, distribution, and putative neurotransmitter function. Digestion, 19:149-169.

Fajans, S. S., J. C. Floyd, R. F. Knopf, and J. W. Conn 1967 Effects of amino acids and proteins on insulin secretion in man. Rec. Prog. Horm. Res., 23:617-662.

Falck, B., N. A. Hillarp, G. Thieme, and A. Torp 1962 Fluorescence of catecholamines anb related compounds condensed with formaldehyde. J. Histochem. Cytochem., 10:348-354.

Falck, B., and B. Hellman 1963 Evidence for the presence of biogenic amines in pancreatic islets. Experientia, 19:139-141.

Faud, L., E. Coll-Garcia, B. van der Dijs, et al. 1975 The effect of serotonin (5-HT) on insulin secretion. Acta Physiol. Latinoam., 25:339-346.

Feldman, J. M. 1979 Species variation in the islets of Langerhans. Diabetologia, 16:1-4.

Feldman, J. M., and B. Chapman 1975a Characterization of pancreatic islet monoamine oxidase. Metabolism, 24:591-588.

Feldman, J. M., and B. Chapman 1975b Monoamine oxidase inhibitors: Nature of their interaction with rabbit pancreatic islets to alter insulin secretion. Diabetologia, 11:487-494.

Feldman, J. M., B. A. Chapman, and J. W. Plank 1974 Monoamine oxidase inhibition: Possible mechansim for cyproheptadine-induced alterations in insulin secretion. Life Sciences, 15:1121-1134.

Feldman, J. M., and H. E. Lebovitz 1970 Mechanism of epinephrine and serotonin inhibition of insulin release in the golden hamster in vitro. Diabetes, 19:480-486.

Feldman, J. M., K. E. Quickel, and H. E. Lebovitz 1972 Potentiation of insulin secretion in vitro by 5-HT antagonsits. Diabetes, 21:779-788.

Fernex, M. 1968 The Mast Cell System. It's Relationship to Atherosclerosis, Fibrosis, and Encephalitis. The Williams and Wilkins Co., Baltimore, MD., pg. 16.

Findlay, D. M., S. Omond, F. P. Alford, and D. J. Chisholm 1979 Hyperglycemia and glucagon suppression. Possible importance of the vagus and enteric humoral factors. J. Clin. Endocrinol. Metab., 48:13-16.

Findlay, J. A., J. R. Grill, J. D. Lever, P. J. Randle, and T. L. B. Spriggs 1969 Increased insulin output following stimulation of the vagal supply to the perfused rabbit pancreas. J. Anat., 104:580.

Floyd, J. C., Jr. 1979 Human pancreatic polypeptide. Clinics End. Metab., 8:379-399.

Floyd, J. C., Jr., S. S. Fajans, S. Pek, R. E. Chance 1977 A newly recognized pancreatic polypeptide; plasma levels in health and disease. Recent Prog. Horm. Res., 33:519-570.

Floyd, J. C. Jr., S. S. Fajans, and S. Pek 1978 Physiological regulation of plasma levels of PP in man. In: Gut Hormones. Bloom, S. R., ed. Edinburgh, Churchill and Livingstone, England, pp. 247-253.

Forssman, W. G., and J. Greenberg 1977 Innervation of the endocrine pancreas in primates. In: Peripheral Neuroendocrine Interactions. Coupland, R. E., and W. G. Forssman, eds., Springer-Verlag, Berlin, pp. 124-133.

Forssman, W. G., and J. Greenberg 1978 Innervation of the endocrine pancreas in primates. In: Peripheral Neuroendocrine Interaction, Coupland, R. E., and W. G. Forssman, eds., Springer-Verlag, N. Y., pp. 124-133.

Frankfurt, M., J. M. Lauder, and E. C. Azmitia 1981 The immunocytochemical localization of serotonergic neurons in the rat hypothalamus. Neuroscience Letters, 24:227-232.

Frohman, L. A., and L. L. Bernardis 1971 Effect of hypothalamic stimulation on plasma glucose, insulin, and glucagon levels. Am. J. Physiol., 221:1596-1603.

Fujita, T. 1959 Histological studies on the neuro-insular complex in the pancreas of some mammals. Z. Zellforsch., 50:94-109.

Fujita, T. and T. Murakami 1973 Microcirculation of monkey pancreas with special reference to the insulo-acinar portal system. A scanning electron microscope study of vascular casts. Arch. Histol. Jap., 35:255-263.

Fujita, T. and S. Kobayashi 1979 Proposal of a neurosecretory system in the pancreas. An electron microscope study in the dog. Arch. Histol. Jap., 42:277-295.

Fujita, T., Y. Yanatori, and T. Murakami 1976 Insulo-acinar axis, it's vascular basis and it's functional and morphological changes caused by CCK-PZ and cerulein. In: Endocrine Gut and Pancreas. Fujita, T., ed., Plenum Press, N. Y., pp. 156.

Fuller, R. W., K. W. Perry, and B. B. Molloy 1975 Effect of an uptake inhibitor on serotonin metabolism in rat brain: studies with 3-(p trifluoromethylphenoxy) -N- methyl -3-phenylpropylamine (Lilly 110140). Life Sciences, 15:1161-1171.

Fuller, R. W., D. B. Meyers, W. R. Gibson, and H. D. Snoddy 1979 Depletion of brain serotonin by chronic administration of p-chloroamphetamine orally to rats and dogs. Tox. Applied Pharmacol., 48:369-374.

Gaddum, J. H. 1953 Antagonism between lysergic acid diethylamide and 5-hydroxytryptamine. J. Physiol., 121:15P.

Gagliardino, J. J., L. M. Zieher, F. C. Iturriza, R. E. Hernandez, and R. R. Rodriguez 1971 Insulin release and glucose changes induced by serotonin. Horm. Metab. Res., 3:145-150.

Garattini, S., and L. Valzelli 1965 Serotonin. Elsevier, Amsterdam, pg.44.

Gardner, E., D. J. Gray, and R. O'Rahilly 1975 Anatomy. W. B. Saunders Co., PA, pg. 401.

Garthwaite, T. L., and T. C. Hagen 1979 Evidence that serotonin stimulates a prolactin-releasing factor in the rat. Neuroendocrinology, 29:215-220.

Gentes, M. 1902 Note sur les terminaisons nerveuses des ilots de Langerhans du pancreas. C. R. Soc. Biol. (Paris), 54:202-203.

Gerich, J. E. 1981 Somatostatin. In: Diabetes Mellitus. Brownlee, M., ed. Garland Press, N. Y., pp. 230-257.

Gerich, J., M. Charles, G. Grodsky 1974a Characterization of the effects of arginine and glucose on glucagon and insulin release from the perfused rat pancreas. J. Clin. Invest., 54:833-841.

Gerich, J. E., M. Langlois, C. Noacco, V. Schneider, and P. H. Forsham 1974b Adrenergic modulation of pancreatic glucagon secretion in man. J. Clin. Invest., 53:1441-1446.

Gerich, J. E., M. A. Charles, and G. M. Grodsky 1976a Regulation of pancreatic insulin and glucagon secretion. Ann. Rev. Physiol., 38:353-388.

Gerich, J. E., M. Lorenzi, E. Tsalikian, and J. H. Karam 1976b Studies on the mechanism of epinephrine induced hyperglycemia in man. Diabetes, 25:65-72.

Gershon, M. D. 1967 Effects of tetrodotoxin on innervated smooth muscle preparations. Brit. J. Pharmacol., 29:259-279.

Gershon, M. D., and R. F. Altman 1971 An analysis of the uptake of 5-hydroxytryptamine by the myenteric plexus of the small intestine of the guinea pig. J. Pharmacol. Exp. Ther., 179:29-41.

Gershon, M. D., A. B. Drakontides, and L. L. Ross 1965 serotonin: Synthesis and release from the myenteric plexus of the mouse intestine. Science, 149:197-199.

Gershon, M. D., and C. F. Dreyfus 1977 Serotonergic neurons in the mammalian gut. In: Nerves and the Gut. Brooks, F. P., and P. W. Evers, eds. Charles B. Slack, Inc, Illinois.

Gershon, M. D., and C. F. Dreyfus 1980 Stimulation of tryptophan uptake into enteric neurons by 5-hydroxytrptamine; a novel form of neuromodulation. Brain Res., 184:229-233.

Gershon, M. D., C. F. Dreyfus, V. M. Pickel, T. H. Joh, and D. J. Reis 1977 Serotonergic neurons in the peripheral nervous sytem: Identification in gut by immunohistochemical localization of tryptophan hydroxylase. Proc. Natl. Acad. Sci., 74:3086-3089.

Gershon, M. D., M. L. Epstein, and L. Hegstrand 1980 Colonization of the chick gut by progenitors of enteric serotonergic neurons: Distribution, differentiation, and maturation within the gut. Dev. Biol., 77:41-51.

Gershon, M. D., and G. M. Jonakait 1979 Uptake and release of 5-hydroxytryptamine by enteric 5-hydroxytryptaminergic neurons: effects of fluoxetine (Lilly

110140) and chlorimipramine. Br. J. Pharmacol., 66:7-9.

Gershon, M. D., and L. L. Ross 1966 Radioisotopic studies of the binding, exchange and distribution of 5-hydroxytryptamine synthesized from its radioactive precursor. J. Physiol., 186:451-476.

Gershon, M. D., D. L. Sherman, and C. F. Dreyfus 1980 Effects of indolic neurotoxins on enteric serotonergic neurons. J. Comp. Neurol., 190:581-596.

Goodman, H. M. 1974 The pancreas and regulation of metabolism. In: Medical Physiology, Mountcastle, J. B., ed. C. V. Mosby Co., St. Louis.

Goodrich, J. T., P. Bernd, D. Sherman, and M. D. Gershon 1980 Phylogeny of enteric serotonergic neurons. J. Comp. Neurology, 190:15-28.

Green, A. R., and D. G. Grahame-Smith 1975 5-hydroxytryptamine and other indoles in the central nervous system. In: Handbook of Psychopharmacology, Vol. 3, L. L. Iversen, S. D. Iversen, and S. H. Snyder, eds., Plenum Press, N. Y., pp. 246-269.

Greenberg, G. R., R. F. McCloy, V. S. Chadwick, T. E. Adrian, J. H. Baron, and S. R. Bloom 1979 Effect of bovine pancreatic polypeptide on basal pancreata and biliary outputs in man. Am. J. Dig. Disease, 24:11-14.

Grodsky, G. M. 1972 A threshold distribution hypothesis for packet storage of insulin and it's mathematical modeling. J. Clin. Invest., 51:2047-2059.

Grodsky, G. M., R. Fanska, and L. West 1974 Anomeric specificity of a glucose-stimulated insulin release: Evidence for a glucoreceptor? Science, 186:536-538.

Grube, D., V. Maier, S. Raptis, and W. Schlegel 1978a Immunoreactivity of the endocrine pancreas. Evidence for the presence of cholecystokinin-pancreomyzin within the A-cell. Histochemistry, 56:13-35.

Grube, D., K. H. Voigt, and E. Weber 1978b Pancreatic glucagon cells contain endorphin like immunoreactivity. Histochemistry, 59:75-79.

Gylfe, E. 1980 Effects of metabolic inhibitors on the efflux of 5-hydroxytryptamine from pancreatic B cells. Acta Physiol. Scand., 109:155-161.

Gylfe, E., B. Hellman, J. Sehlin, and I. B. Taljeda 1973 Amino acid conversion into 5-HT in pancreatic beta cell.

Endocrinology, 93:932-937.

Hagen, E. 1956 Uber die Innervation der exkretorischen Drusen und der Langerhansschen Inseln des Pankreas bein Hund. Z. Zellforsch., 43:486-500.

Hakanson, R., J. Alumets, and F. Sundler 1978 GH-like immunoreactivity in gastrinoma and in endocrine cells of gut and pancreas. Scand. J. Gastroenterology, 13:75.

Hakanson, R., G. Liedberg, and I. Lundquist 1971 Effect of vagal denervation on insulin release after oral and intravenous glucose. Experientia, 27:460-461.

Hamlin, K. E., and F. E. Fisher 1951 Synthesis of 5-hydroxytrptamine. J. Amer. Chem. Soc., 73:5007-5008.

Hardebo, J. E., L. Edvinsson, C. Owman, N. A. Svendgaard 1978 Potentiation and antagonism of serotonin effects on intracranial and extracranial vessels. possible implication in migraine. Neurology, 28:64-70.

Harvey, W. D., G. R. Faloona, and R. H. Unger 1974 The effect of adrenergic blockade on exercize-induced hyperglucagonemia. Endocrinology, 94:1254-1258.

Hegre, O. D., R. C. McEvoy, V. Bachelder, and A. Lazarow 1972 Organ culture of fetal rat pancreas: Quantitative analysis by linear scanning of islet and other tissue components. In Vitro, 7:366-376.

Hellman, B., A. Lernmark, J. Sehlin, and I. Taljedal 1972 Transport and storage of 5-hydroxytryptamine in pancreatic B-cells. Biochem. Pharmacol., 21:695-706.

Henderson, J. R., D. B. Jeffreys, R. H. Jones, and D. Stanley 1976 The effect of atropine on the insulin release caused by oral and intravenous glucose in human subjects. Acta Endocrinol., 83:772-780.

Honey, R. N., and G. C. Weir 1980 Acetylcholine stimulates insulin, glucagon, and somatostatin release in the perfused chicken pancreas. Endo., 107:1065-1068.

Howell, S. L., and W. Montague 1974 Regulation of guanylate cyclase in guinea pig islets of Langerhans. Biochem. J., 142:379-384.

Hwang, E. C., and van Woert 1980 Comparative effects of various serotonin releasing agents in mice. Biochem. Phar., 29:3163-3167.

Ichiyama, A., S. Nakamura, Y. Nishizuka, and O.

Hayaishi 1968 Tryptophan - 5 - hydroxylase in mammalian brain. Adv. Pharmacol., 6A:5-17.

Ipp, E., R. Dobbs, V. Harris, et al 1977 The effect of gastrin, gastric inhibitory polypeptide, secretin, and the octapeptide of cholecystokinin upon immunoreactive somatostatin release by the perfused canine pancreas. J. Clin. Invest., 60:1216-1219.

Ipp, E., R. Dobbs, and R. H. Unger 1978a Morphine and B-endorphin influence the secretion of the endocrine pancreas. Nature, 276:190-191.

Ipp, E., R. Dobbs, and R. Unger 1978b Vasoactive intestinal peptide stimulates pancreatic somatostatin release. FEBS Lett., 90:76-78.

Ipp, E., and R. H. Unger 1979 Bombesin stimulates the release of insulin and glucagon but not pancreatic somatostatin, from the isolated perfused dog pancreas. Endo. Res. Com., 6:37-42.

Iversen, L. L. 1967 The catecholamines. Nature, 214:8-14.

Iversen, J. 1973 Adrenergic receptors and the secretion of glucagon and insulin from the isolated perfused canine pancreas. J. Clin. Invest., 52:2102-2116.

Iversen, J., and K. Hermansen 1977 Calcium, glucose, and glucagon release. Diabetologia, 13:297-303.

Iversen, J., and D. W. Miles 1971 Evidence for a feedback inhibition of insulin on insulin secretion in the isolated perfused canine pancreas. Diabetes, 20:1-9.

Jaime-Etcheverry, G., and L. M. Zieher 1968 Electron microscopic cytochemistry of 5-hydroxytryptamine (5-HT) in the beta cells of guinea pig endocrine pancreas. Endocrinology, 83:917-923.

Jarrousse, C. L., and G. Rosselin 1975 Interaction of amino acids and cyclic AMP on the release of insulin and glucagon by newborn rat pancreas. Endocrinology, 96:168-177.

Jordan, D., P. Agem, A. McRae-Degueurce, J. F. Pujol, and R. Mornex 1979 Participation of serotonin in thyrotropin release. II. Evidence for the action of serotonin on the phasic release of thyrotropin. Endocrinology, 105:975-979.

Kajinuma, H., A. Kaneto, and T. Kuzuya 1968 Effects of methacholine on insulin secretion in man. Endocrinology, 82:1384-1388.

Kaneto, A., T. Kaneto, H. Kajinuma, and K. Kosaka 1977 Effect of vasoactive intestinal polypeptide infused intrapancreatically on glucagon and insulin secretion. Metabolism, 26:781-786.

Kaneto, A., T. Kaneto, H. Kajinuma, and K. Kosaka 1978 Effects of substance P and neurotensin infused intrapancreatically on glucagon and insulin secretion. Endocrinology, 102:393-401.

Kaneto, A., K. Kosaka, and K. Nakao 1967 Effects of stimulation of the vagus nerve on insulin secretion. Endocrinology, 80:530-536.

Kaneto, A., E. Miki, and K. Kosaka 1975 Effect of beta and beta2 adrenoreceptor stimulants infused intrapancreatically on glucagon and insulin secretion. Endocrinology, 97:1166-1173.

Kimmel, J. R., H. G. Pollack, and R. L. Hazelwood 1968 Isolation and characterization of chicken insulin. Endo., 83:1323-1330.

Kloppel, G. and G. Bommer 1979 Ultracytochemical calcium distribution in B cells in relation to biphasic glucose-stimulated insulin release by the perfused rat pancreas. Diabetes, 28:585-592.

Kobayashi, S., and T. Fujita 1969 Fine structure of mammalian and avian pancreatic islets with special reference to D cells and nervous elements. Z. Zellforsch., 100:340-363.

Kobayashi, K., K. Yoshida, Y. Tsumura, S. Kagawa, and A. Matsuoka 1980 Studies on glucoreceptor. I. Specific binding of D-glucose to partially purified membrane of rat islets of Langerhans. J. Pharm. Dyn., 3:33-40.

Koe, B. K., and A. Weissman 1966 P-chlorophenylalanine: a specific depletor of brain serotonin. J. Pharm. Exp. Ther., 154:499-516.

Koivusalo, F., and J. Leppaluoto 1977 High TRF immunoreactivity in purified pancreatic extracts of fetal and newborn rats. Life Sciences, 24:1655-1658.

Konturek, S. J., A Pucher, and T. Radecki 1976 Comparison of vasoactive intestinal peptide and secretin in stimulation of pancreatic secretion. J. Physiol., 255:497-509.

Konturek, S. J. 1978 Current concepts of neurohormonal control of pancreatic secretion. Irish J. Med. Sci., 147:1-10.

Kuhn, D. M., M. A. Meyer, and W. Lovenberg 1980 Comparison of tryptophan hydroxylase from a malignant murine mast cell tumor and rat mesencephalic tegmentum. Arch. Biochem. Biophys., 199:355-361.

La Barre, J. 1930 The role of the central nervous system in the control of pancreatic secretion: I. The secretion of insulin during hyperglycemia. Am. J. Physiol., 94:13-16.

Lacy, P. E. 1977 The physiology of insulin release. In: The Diabetic Pancreas. Volk, B. W., and K. F. Wellman, eds. Plenum Press, N. Y., pg. 224.

Larsson, L. 1978 ACTH-like peptides in gastrin and pancreatic polypeptide cells. Scand. J. Gastroenterology, 13:109.

Larsson, L. 1979 Innervation of the pancreas by substance P, enkephalin, vasoactive intestinal polypeptide, and gastrin/CCK immunoreactive nerves. J. Histochem. Cytochem., 27:1283-1284.

Larsson, L. 1980 New aspects on the neural, paracrine and endocrine regulation of islet function. Front. Hormone Res., 7:14-29.

Larsson, L. L., F. Sundler, and R. Hakanson 1976 Pancreatic hormones in the gut and gut hormones in the pancreas. In: Endocrine Gut and Pancreas. Fujita, T., editor., p. 133.

Lebovitz, H. E., and J. M. Feldman 1973 Pancreatic biogenic amines and insulin secretion in health and disease. Fed. Proc., 32:1797-1802.

Leclercq-Meyer, V., J. Marchand, and W. J. Malaisse 1977 The versatile role of calcium in glucagon release. In: Glucagon: It's Role in Physiology and Clinical Medicine. Foa, P.P., J. S. Bajaj, and N. L. Foa, eds. Springer-Verlag, N. Y., pp. 185-213.

Legg, P. G. 1967 The fine structure and innervation of the beta and delta cells in the islets of Langerhans of the cat. Z. Zellforsch., 80:307-321.

Legg, P. G. 1968 Fluorescence studies on neural structures and endocrine cells in the pancreas of the cat. Z. Zellforsch., 88:487-495.

Lenninger, S. 1974 The autonomic innervation of the exocrine pancreas. Med. Clin. N. Amer., 58:1311-1318.

Leppaluoto, J., F. Koivusalo, and R. Kraama 1978

Thyrotropin-releasing factor: Distribution in neural and gastrointestinal tissues. Acta. Physiol. Scand., 104:175-179.

Lernmark, A. 1971 The significance of 5-HT for insulin secretion in the mouse. Horm. Metab. Res., 3:305-309.

Lever, J. D. 1971 The innervation of the endocrine pancreas. Sub. Org. Fun. End. Tissue, 2:839-840.

Lewis, G. P. 1957 5-hydroxytryptamine in the mast cell of the rat. In: 5-Hydroxytryptamine. Pergamon Press, London, pp. 26-31.

Lindkaer, J. S., J. Fahrenkrug, J. J. Holst, O. Vagn Nielsen, and O. B. Schaffalitzky de Muckadell 1978 Secretory effects of vasoactive intestinal polypeptide (VIP) on the isolated perfused porcine pancreas. Am. J. Physiol., 235:E387-E391.

Lindstrom, P., and J. Sehlin 1979 Effects of 5-HTP and 5-HT on Ca++ fluxes in the pancreatic B-cells. Acta Endo., 91:49-50.

Lindstrom, P. 1981 Further studies on 5-hydroxytryptamine transport in pancreatic islets and isolated B-cells. Br. J. Pharmac., 73:385-391.

Lingjearde, O. 1979 Inhibitory effect of ethanol on 5-hydroxytryptamine (serotonin) uptake in human blood platelets in vitro. Acta Phramacol. et. toxicol., 45:394-398.

Lockhart-Ewart, R. B., C. Mok, and J. M. Martin 1976 Neuroendocrine control of insulin secretion. Diabetes, 25:96-100.

Luna, G. L. 1968 Manual of histologic staining methods of the Armed Forces Institute of Pathology. McGraw-Hill Book Co., New York.

Lundquist, I., R. Ekholm, and L. E. Ericson 1971 Monoamines in the pancreatic islets of the mouse. 5-HT as an intracellular modifier of insulin secretion, and the hypoglycemic action of monoamine oxidase inhibitors. Diabetologia, 7:414-422.

Luyckx, A. S., and P. J. Lefebvre 1970 Arguments for a regulation of pancreatic glucagon secretion by circulating plasma free fatty acids (34511). Proc. Soc. Exp. Biol. Med., 133:524-528.

Madson, K. L. 1979 "Quantitative comparison of the perinatal development of the principle cells in the pancreatic

islets from the offspring of normal and diabetic pregnancies in the rat." Ph.D. dissertation, University of Minnesota.

Malaisse, W. J., F. Malaise-Lagae, and D. Mayhem 1967a A possible role for the adenyl cyclase system in insulin secretion. J. Clin. Invest., 46:1724-1734.

Malaisse, W. J., F. Malaisse-Lagae, P. E. Lacy, and P. H. Wright 1967b Insulin secretion by isolated islets in presence of glucose, insulin, and anti-insulin serum. Proc. Soc. Exp. Biol. Med., 124:497-503.

Malaisse, W. J., F. Malaisse-Lagae, P. H. Wright, and J. Ashmore 1967c Effects of adrenergic and cholinergic agents upon insulin secretion in vitro. Endocrinology, 80:975-978.

Marco, J., J. A. Hedo, and M. L. Villanueva 1977 Inhibition of glucagon release by 5-HT in mouse pancreatic islets. Diabetologia, 13:585-588.

Marco, J., J. A. Hedo, and M. L. Villanueva 1978 Control of pancreatic polypeptide secretion by glucose in man. J. Clin. End. Metab., 46:140-145.

Martino, E., A. Lermark, H. Seo, D. F. Steiner, and S. Refetoff 1978 High concentration of thyrotropin-releasing hormone in pancreatic islets. Proc. Natl. Acad. Sci., 75:4265-4267.

Matsuyama, T., and P. P. Foa 1974 Plasma glucose, insulin, pancreatic and entero- glucagon levels in normal and depancreatized dogs. Proc. Soc. Exp. Biol. Med., 147:97-102.

McEvoy, R. C. 1980 Fetal and neonatal rat pancreas in organ culture: Age-related effects of corticosterone on the development of the islet cells. Am. J. Anat., 157:319-327.

McEvoy, R. C., and O. D. Hegre 1977 Morphometric quantitation of the pancreatic insulin, glucagon and somatostatin positive cell populations in normal and alloxan diabetic rats. Diabetes 26:1140-1147.

McEvoy, R. C., K. L. Madson, and R. P. Elde 1977 Stimulation of antiglucagon antibodies in rabbits. Horm. Metab. Res., 9:272-274.

McIntyre, N., C. D. Holdsworth, and D. S. Turner 1964 New interpretation of oral glucose tolerance. Lancet, II:20-21.

Meissner, H. P., and H. Schmelz 1974 Membrane potential of beta cells in pancreatic islets. Pfluegers Arch.,

351:195-206.

Miller, R. E., and J. L. Whittenberger 1968 Effect of vagotomy on venous insulin and glucose concentrations in the conscious monkey. Fed. Proc., 27:565.

Modai, I., A. Rotman, H. Munitz, S. Tjano, and H. Wijsenbeek 1979 Serotonin uptake by blood platelets of acute schizophrenic patients. Psychopharmacology, 64:193-195.

Moody, A. J. 1977 Gastrointestinal glucagon-like immunoreactivity. In: Glucagon: Molecular Physiology, Clinical and Therapeutic Implications. Lefebvre, P. J., and R. H. Unger, eds. Oxford, Pergamon Press, England, pp. 319-341.

Morgan, C. R., and R. T. Lobl 1959 A histochemical study of neuro-insular complexes in the pancreas of the rat. Anat. Rec., 160:231-238.

Morrissey, J. J., M. N. Walker, and W. Lovenberg 1977 The absence of tryptophan hydropxylase activity in blood platelets. Proc. Soc. Exp. Biol. Med., 154:496-499.

Muller, E. 1892 Zur Kenntnis der Ausbreitung und Endigungweise der Magen-, Darm-, und Pankreasnerven. Arch. mikr. Anat. 40:390-409.

Muller, E. E., L. A. Frohman, and D. Cocchi 1973 Drug control of hyperglycemia and inhibition of insulin secretion due to centrally administered 2-deoxy-glucose. Am. J. Physiol., 224:1210-1217.

Muller, W., D. Roche, G. Faloona, and R. Unger 1971 Alanine-glucagon relationships in control of gluconeogenesis. Clin. Res., 19:480.

Nakano, S. 1968 Some physiological observations on the exocrine pancreas. The effects of some agents on pancreatic secretion. Nagoya J. Med. Sci., 31:79-116.

Nakao, K., Y. Nakai, H. Jingami, S. Oki, J. Fukata, and H. Imura 1979 Substantial rise of plasma B-endorphin levels after insulin-induced hypoglycemia in human subjects. J. Clin. End. Metab., 49:838-841.

Nelson, W. C., W. G. Blackard, J. C. Cocchiara, and J. A. Labat 1967 Influence of the vagus nerves on pancreatic insulin secretion. Diabetes, 16:852-857.

Nunez, E. A., P. Gershon, and M. D. Gershon 1980 Serotonin and seasonal variation in the pancreatic structure of bats: Possible presence of serotonergic axons in the gland. Am. J. Anat., 159:347-360.

Oomura, Y., T. Ono, and M. Sugimori 1976 Acetylcholine, an inhibitory transmitter in the rat lateral hypothalamus. Brain Res. Bull., 1:151-153.

Orci, L., A. Pevelet, M. Ravazzoli, F. Malaisse-Lagae, and A. E. Renold 1973 A specialized membrane junction between nerve endings and B cells in islets of Langerhans. Europ. J. Clin. Invest., 3:443-445.

Orci, L., D. Baetons, M. Ravazzola, Y. Stefan, and F. Malaisse-Lagae 1976 Pancreatic polypeptide and glucagon; nonrandom distribution in pancreatic islets. Life Sciences, 19:1811-1816.

Paasonen, M. K., P. D. MacLean, and M. J. Giarman 1957 5-hydroxytryptamine (serotonin, enteramine) content of structures of the limbic system. J. Neurochem., 1:326-333.

Pace, C. S., S. Conant, and P. E. Lacy 1978 Electrical responses of rat islets maintained in culture with varying levels of glucose. Endo., 102:1350-1355.

Pace, C. S., and M. Blaustein 1979 Effects of neurotoxins on pancreatic islets: Elucidation of a possible role for sodium channels in the secretory response. Biochimica et Biophysica Acta, 585:100-106.

Page, I. H. 1968 Serotonin. Year Book Medical Publishers, Chicago, pg. 46.

Palmer, J. P., J. Halter, and P. L. Weiner 1979 Differential effect of isoproterenol on acute glucagon and insulin release in man. Metabolism, 28:237-240.

Parra-Covarrubias, A., I. Rivera-Rodriguez, and A. Almarez-Ugalde 1971 Cephalic phase of insulin release in obese adolescents. Diabetes, 20:800-802.

Parratt, J. R., and G. B. West 1956 Tissue histamine and 5-hydroxytryptamine. J. Physiol., 132:40-41.

Patton, G., E. Ipps, and R. Dobbs 1977 Pancreatic immunoreactive somatostatin release. Proc. Natl. Acad. Sci., 74:2140-2143.

Pearse, A. G. E., and T. T. Takor 1976 Neuroendocrine embryology and the APUD concept. Clin. Endo., Suppl. 5:229S-244S.

Pederson, R. A., and J. C. Brown 1979 Effect of cholecytokinin, secretin, and gastric inhibitory polypetide on insulin release from the isolated perfused rat pancreas. Can. J. Physiol. Pharmacol., 57:1233-1237.

Pensa, A. 1905 Osservazioni sulla distribuzione dei vasi sanguigni e dei nervi nel pancreas. Int. Mschr. Anat. Physiol., 22:90-125.

Pictet, R. L., L. B. Rall, P. Phelps, and W. J. Rutter 1976 Neural crest and origin of the insulin-producing and other GI hormone producing cells. Science, 191:191-192.

Polak, J. M., and S. R. Bloom 1979a The diffuse neuroendocrine system. J. Histochem. Cytochem., 27:1398-1400.

Polak, J. M., and S. R. Bloom 1979b The neuroendocrine design of the gut. Clinics End. Metab., 8:313-330.

Pollard, H. B., and G. D. Pappas 1979 Veratridine-activated release of adenosine- 5'-triphosphate from synaptosomes: evidence for calcium dependency and blockade by tetrodotoxin. Biochem. Biophys. Res. Com., 88:1315-1321.

Pontiroli, A. E., P. Micossi, and P. P. Foa 1978 Effect of serotonin, of its biosynthetic precursors, and of the anti-serotonin agent metergoline on the release of glucagon and insulin from rat pancreas. Horm. Metab. Res., 10:200-203.

Porte, D., Jr. 1967 A receptor mechanism for the inhibition of insulin release by epinephrine in man. J. Clin. Invest., 46:86-94.

Porte, D., Jr., L. Girardier, and J. Seydoux 1973 Neural regulation of insulin secretion in the dog. J. Clin. Invest., 52:210-214.

Porte, D., Jr., and R. P. Robertson 1973 Control of insulin secretion by catecholamines, stress, and the sympathetic nervous system. Fed. Proc., 32:1792-1796.

Porte, D., Jr., P. H. Smith, and J. W. Ensinck 1976 Neurohumoral regulation of the pancreatic islet A and B cells. Metabolism, 25:1453-1456.

Powell, D., and P. Skrabanek 1979 Brain and Gut. Clinics End. Metab., 8:299-312.

Pulido, O. M., S. A. Bencosme, M. L. de Bold, and A. J. de Bold 1978 Intracellular pancreatic B cell serotonin and the dynamics of insulin release. Diabetologia, 15:197-204.

Quansah, F., C. Klatt, and J. M. Feldman 1981 Effect of diabetes mellitus and chemical sympathectomy on tissue monoamine oxidase activity and norepinephrine concentration in the golden hamster. Metabolism, 30:242-247.

Quickel, K. E., J. M. Feldman, and H. E. Lebovitz 1971 Inhibition of insulin secretion by serotonin and dopamine: Species variation. Endocrinology, 89:1295-1302.

Rapport, M. M., A. A. Green, and I. H. Page 1948 Partial purification of vasoconstrictor in beef serum. J. Biol. Chem., 174:735-741.

Rehfeld, J. F. 1971 Effect of gastrin and its C-terminal tetrapeptide on insulin secretion in man. Acta Endocrinologica, 66:169-176.

Reinhard, J. F., J. E. Liebmann, A. J. Schlosberg, and M. A. Moskowitz 1979 Serotonin neurons project to small blood vessels in the brain. Science, 206:85-86.

Richardson, B. P., M. L. McDaniel, and P. E. Lacy 1975 Effects of cyproheptadine on insulin secretion by isolated perifused rat islets. Diabetes, 24:836-841.

Richardson, K. C. 1964 The fine structure of the albino rabbit iris with special reference to the identification of adrenergic and cholinergic nerves and nerve endings in its intrinsic muscles. Amer. J. Anat., 114:173-205.

Richins, C. A. 1945 The innervation of the pancreas. J. Comp. Neurology, 83:223-236.

Ritter, O. 1946 Etude sur les neifs du pancreas exocrine et endocrine. Vjschr. Naturforsch. Ges. Zurich, 91:51-57.

Robertson, R. P., and D. Porte 1973 Adrenergic modulation of basal insulin secretion in man. Diabetes, 22:1-8.

Rossini, A. A., and M. G. Buse 1973 The effect of L-Dopa on insulin release by isolated pancreatic islets of the rat. Horm. Metab. Res., 5:26-28.

Rossini, A. A., M. Berger, J. Shadden, and C. F. Cahill 1975 Beta cell protection to alloxan necrosis by anomers of D-glucose. Science, 183:424.

Rothman, T. D., L. L. Ross, and M. D. Gershon 1976 Separately developing axonal uptake of 5-hydroxytryptamine and norepinephrine in the fetal ileum of the rabbit. Brain Research, 115:437-456.

Rubenstein, A. H. 1981 Biosynthesis of insulin. In: Diabetes Mellitus, Vol. 5, Rifkin, H., and P. Raskin, eds. Robert J. Brady Co., Bowie, Md., pg. 27.

Rubenstein, A. H., D. L. Horwitz, and D. F. Steiner

1975 Proinsulin and insulin biosynthesis. In: Diabetes: It's Physiological and Biochemical Basis. Vallance-Owen, J., ed. University Park Press, Baltimore, Md., pg. 10.

Russell, R. C., R. G. Faber, S. R. Bloom, and M. Hobsley 1974 The effect of vagotomy on gastrin release in response to insulin hyperglycemia. Br. J. Surg., 61:328.

Sakata, K., S. Hayano, and H. A. Sloviter 1963 Effect on blood glucose concentration of changes in the availability of glucose to the brain. Am. J. Physiol., 204:1127-1132.

Samols, E., G. C. Weir, Y. C. Patel, S. W. Loo, and K. H. Gabbay 1977 Autonomic control of SRIF and PP secretion by the isolated perfused canine pancreas. Clin. Res., 25:499A.

Sanders-Bush, E., J. Bushing, and F. Sulser 1975 Long term effects of p-chloroamphetamine and related drugs on central serotonergic transmission. J. Pharmacol. Exp. Ther., 192:33-41.

Sastry, B. S. R., and J. W. Phillis 1977 Metergoline as a selective 5-hydroxytryptamine antagonist in the cerebral cortex. Can. J. Physiol. Pharmacol., 55:130-133.

Schafer, R., and H. Schatz 1979 Stimulation of (pro)-insulin biosynthesis and release by gastric inhibitory polypeptide in isolated islets of rat pancreas. Acta Endo., 91:493-500.

Schauder, P., C. McIntosh, J. Arends, H. Arnold, H. Frerichs, and W. Creutzfeldt 1976 Somatostatin and insulin release from isolated rat pancreatic islets stimulated by glucose. Febs Letters, 68:225-227.

Schauder, P., C. McIntosh, and J. Arends 1977 Somatostatin and insulin release from isolated rat pancreatic islets in response to D-glucose, L-leucicine, alpha-ketoisocaproic acid, or D-glyceraldehyde. Evidence for a regulatory role of adenosine -3',5' cyclic monophosphate. Biochem. Biophys. Res. Com., 75:630-635.

Schultzberg, M., C. F. Dreyfus, M. D. Gershon, T. Hokfelt, R. P. Elde, G. Nilsson, S. Said, and M. Goldstein 1978 VIP-, enkephalin-, substance P-, and somatostatin-like immunoreactivity in neurons intrinsic to the intestine: Immunohistochemical evidence from organotypic tissue cultures. Brain Research, 155:239-248.

Schulz, R., and M. Goldstein 1973 Morphine tolerance and supersensitivity to 5-hydroxytryptamine in the myenteric plexus of the guinea pig. Nature(London), 244:168-170.

Segal, M. 1974 Response of septal nuclei neurons to microiontophoretically administered putative neurotransmitters. Life Sciences, 14:1345-1351.

Segu, L., and A. Calas 1978 The topographic distribution of serotonergic terminals in the spinal cord of the cat: Quantitative radioautographic studies. Brain Res., 153:449-464.

Shaskan, E. G., and S. H. Snyder 1970 Kinetics of serotonin accumulation into slices from rat brain: Relationship to catecholamine uptake. J. Pharmacol. Exp. Ther., 175:404-418.

Shorr, S. S., and F. E. Bloom 1970 Fine structure of islet-cell innervation in the pancreas of normal and alloxan treated rats. Z. Zellforsch., 103:12-25.

Silverman, P. B., and B. T. Ho 1979 P-chloroamphetamine: evaluation of a brain metabolite. Com. Psychopharm., 3:291-294.

Simard, L. C. 1937 Les complexes neuro-insulaire du pancreas humain. Arch Anat. Micr., 33:49-64.

Singh, M., and P. D. Webster 1978 Neurohormonal control of pancreatic secretion. Gastroenterology, 74:294-309.

Smith, P. H. 1975 Structural modification of Schwann cells in the pancreatic islets of the dog. Amer. J. Anat., 144:513-517.

Smith, P. H., and D. Porte 1976 Neuropharmacology of the pancreatic islets. Ann. Rev. Pharm. Tox., 16:269-285.

Smith, B. H., and W. H. Sweet 1978 Monoaminergic regulation of central nervous system function: II. Serotonergic systems. Neurosurg., 3:257-272.

Snyder, S. H., W. J. Logan, J. P. Bennett, and A. Arregui 1973 Amino acids as central nervous transmitters: Biochemical studies. Neuroscience Res., 5:131-157.

Solcia, E., C. Capella, G. Vassallo, and R. Buffa 1975 Endocrine cells of the gastric mucosa. Int. Rev. Cytol., 42:223.

Steinbush, H. W. M. 1981 Distribution of serotonin immunocytochemistry in the central nervous system of the rat - cell bodies and terminals. Neuroscience, 6:557-618.

Sternberger, L. A., P. H. Hardy, J. J. Cuculis, and H. C. Meyer 1970 The unlabelled antibody method of

immunocytochemistry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and it's uses in the identification of spirochetes. J. Histochem. Cytochem., 18:315-333.

Sundler, F., J. Alumets, R. Hakanson, J. Fahrenkrug, and O. B. Schaffalitzky de Muckadell 1978 Peptidergic (VIP) nerves in the pancreas. Histochemistry, 55:173-176.

Szabo, O., and A. J. Szabo 1972 Evidence for an insulin sensitive receptor in the central nervous system. Am. J. Physiol., 223:1349-1353.

Szecowka, J., Sandberg, E., and Efendic, S. 1980 The interaction of vasoactive intestinal polypeptide (VIP), glucose and arginine on the secretion of insulin, glucagon, and somatostatin in the perfused rat pancreas. Diabetologia, 19:137-142.

Tannenbaum, G., A. E. Panera, and H. G. Friesen 1979 Failure of B-endorphin antiserum, naloxine, and naltrexone to alter physiologic growth hormone and insulin secretion. Life Sciences, 25:1983-1990.

Teitelbaun, H. A. 1933 The nature of the thoracic and abdominal distribution of the vagus nerves. Anat. Rec., 55:299-318.

Tiscornia, O. M., J. L. Martinez, and H. Sarles 1974 Some aspects of human and canine macroscopic pancreas innervation. Amer. J. Gastroenterology, 66:353-361.

Tobe, T., F. Izumikawa, and M. Sano 1976 Release mechanism of 5-HT in mammalian gastrointestinal tract - especially, vagal release of 5-HT. In: Endocrine Gut and Pancreas. Elsevier Scientific, Amsterdam, pp. 371-380.

Toyota, T., S. Sato, M. Kredo, K. Abe, and Y. Goto 1975 Secretory regulation of endocrine pancreas: cyclic AMP and glucagon secretion. J. Clin. End. Metab., 41:81-89.

Turtle, J. R., and D. M. Kipnis 1967 An adrenergic receptor mechanism for the control of cyclic 3',5' adenosine monophosphate synthesis in. Biochem. Biophys. Res. Commun., 28:797-802.

Twarog, B. M., and I. H. Page 1953 Serotonin content of some mammalian tissues and urine and a method for its determination. Am. J. Physiol., 175:157-161.

Ulbricht, W. 1967 Kinetics of tetrodotoxin and saxitoxin action at the node of Ranvier. In: Advances in

Cytopharmacology, Vol. 3. Ceccarelli, B., and F. Clementi, eds., Raven Press, N. Y., pp. 363-371.

Unger, R. H., A. Ohneida, I. Valverde, A. M. Eisentraut, and J. Exton 1968 Characterization of the responses of circulating glucagon-like immunoreactivity to intraduodenal and intravenous administration of glucose. J. Clin. Invest., 47:48-65.

Unger, R. H. 1981 Insulin-, glucagon-, somatostatin interactions. In: Diabetes Mellitus, Vol. V. Rifkin, H., and P. Raskin, eds. Robert J. Brady Co., Bowie, Md., pg. 44.

Vialli, M. 1966 Contributi alla conoscenza istochimica del sistema allulare di Leptodactylus ocellate. Acta Histochem. (Jena), 23:163-170.

Walter, R. M., R. J. Dudl, J. P. Palmer, and J. W. Ensinck 1974 The effect of adrenergic blockade on the glucagon responses to starvation and hypoglycemia in man. J. Clin. Invest., 54:1214-1220.

Watari, N. 1968 Fine structure of nervous elements in the pancreas of some vertebrates. Z. Zellforsch., 85:291-314.

Weir, G. C. 1981 Glucagon in normal physiology and diabetes mellitus. In Diabetes Mellitus. M. Brownlee, ed. Garland STPM Press, New York, pg. 207.

Weir, G. C., S. D. Knowlton, and D. B. Martin 1974 Glucagon secretion from the perfused rat pancreas: Studies with glucose and catecholamines. J. Clin. Invest., 54:1403-1412.

Weir, G. C., S. D. Knowlton, and D. B. Martin 1975 Nucleotide and nucleoside stimulation of glucagon secretion. Endocrinology, 97:932-936.

White, A., P. Handler, E. C. Smith, R. L. Hall, and I. R. Lehman 1978 Principles of Biochemistry, Vol. 6. McGraw Hill, N. Y., pg. 1266.

White, M. K., H. B. Hechtman, and D. Shepro 1975 Canine lung uptake of plasma and platelet serotonin. Microvas. Res., 9:230-241.

Wilson, C. J., and P. M. Groves 1979 A simple and rapid section embedding technique for sequential light and electron microscopic examination of individually stained central neurons. J. Neuroscience Methods, 1:383-391.

Wilson, J. P., R. W. Downs, J. M. Feldman, and H. E. Lebovitz 1974 Beta cell monoamines: Further evidence for their role in modulating insulin secretion. Am. J. Physiol.,

227:305-312.

Wold, J. S., D. S. Longnecker, L. J. Fischer 1971 Species dependent pancreatic islet toxicity produced by cyproheptadine: Alterations in beta cell structure and function. Toxicol. Appl. Pharmacol., 19:188-201.

Wolheim, C. B., B. Blondel, A. E. Renold, and G. W. G. Sharp 1976a Stimulatiry and inhibitory effect of cyclic AMP in pancreatic glucagon release from monolayer cultures and the controlling role of calcium. Diabetologia, 12:269-277.

Wolheim, C. B., B. Blondel, A. E. Renold, and G. W. G. Sharp 1976b Calcium induced glucagon release in monolayer culture of the endocrine pancreas. Studies with ionophore A23187. Diabetologia, 12:287-294.

Woods, S. C., and D. Porte 1974 Neural control of the endocrine pancreas. Physiol. Rev., 54:596-618.

Woods, S. C., and D. Porte 1978 The central nervous system, pancreatic hormones, feeding, and obesity. Adv. Metab. Dis., 9:283-312.

Woolley, D. W. 1958 Serotonin receptors. I. Extraction and assay of a substance which renders serotonin fat soluble. Proc. Nat. Acad. Sci., 44:1202-1210.

Woolley, D. W., and E. Shaw 1954 A biochemical and pharmacological suggestion about certain mental disorders. Science, 119:587-589.

Zawalich, W. S. 1979 Intermediary metabolism and insulin secretion from isolated rat islets of Langerhans. Diabetes, 28:252-260.