5-HYDROXYTRYPTAMINE IN RAT PANCREATIC ACINAR CELLS

by

Edward Wing-Tung Yu

A thesis submitted to the
Faculty of Graduate Studies and Research
in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

July 1983

Department of Pharmacology and Therapeutics
McGill University
Montreal, Canada

ABSTRACT

In order to determine the role of biogenic monoamines in the control of pancreatic secretion, the metabolism and disposition of 5-hydroxytryptamine (5-HT) and dopamine in rat exocrine pancreas was studied. Pancreatic acinar cells contained 5-HT which was located exclusively in the zymogen granule fraction. Aromatic amino acid decarboxylase activity was located exclusively in the cytosol of acinar cells and was substrate specific for the L-isomers of hydroxylated aromatic amino acids 3,4-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (L-5-HTP). Each substrate competitively inhibited the decarboxylation of the other. When incubated with [^{14}C] 5-HT dispersed acinar cells took up the amine and concentrated it in zymogen granules. These cells also took up [^{14}C] 5-HTP, decarboxylated it and stored the [^{14}C] 5-HT produced in zymogen granules. 5-HTP decarboxylation and 5-HT concentration into zymogen granules occured in the pancreas, but not in the parotid gland. When pancreatic acinar cells pre-labelled with [^{14}C] 5-HT and [^{3}H] Leucine were stimulated with caerulein, there was a synchronous increase in secretion of amylase activity, [^{14}C] 5-HT and [^{3}H] protein. Pancreatic acinar cells took up L-DOPA, decarboxylated it but the dopamine was not retained by the granules and dopamine secretion from the cells incubated with caerulein could not be demonstrated. The results indicate that 1). in the acinar cell of rat pancreas 5-HT is a normal component of zymogen granule; 2). pancreatic acinar cells possess a single aromatic amino acid decarboxylase specific for DOPA and 5-HTP; 3). zymogen granule 5-HT and zymogen granule protein are released together when the cells are stimulated to secrete protein.

The work of the present thesis has been submitted for publication:

Stern,L., Tenenhouse,A., Yu,E.W.T. (1983) Uptake, storage and secretion of 5-hydroxytryptamine and its amino acid precursor by dispersed rat pancreas acinar cells. J.Physiology London (in press).

Yu,E.W.T., Stern,L., Tenenhouse,A. (1983) Aromatic amino acid decarboxylation in dispersed rat pancreas acinar cells. (Submitted to Pharmacology).

RÉSUMÉ

Afin de déterminer le rôle des amines dans le contrôle des sécrétions pancréatiques, nous avons étudié le métabolisme de la 5-hydroxytryptamine (5-HT) et de la dopamine dans le pancréas exocrine de rat. Les cellules des acini pancréatiques contiennent de la 5-HT localisée spécifiquement dans les granules à zymogène. Une décarboxylase, agissant sur les acides aminés aromatiques, a été localiseé exclusivement dans le cytoplasme des cellules des acini; les acides aminés aromatiques hydroxylés 3,4-dihydroxyphenylalanine (L-DOPA) et 5-hydroxytryptophan (L-5-HTP) sont des substrats spécifiques pour cet enzyme. Chaque substrat inhibe de manière compétitive la décarboxylation de l'autre. Lorsque des cellules des acini pancréatiques sont mises en suspension et incubées avec de la [^{14}C] 5-HT, elles pompent les amines et les concentrent dans les granules à zymogène. Ces cellules pompent également la [^{14}C] 5-HTP et après décarboxylation, la stockent sous forme de [^{14}C] 5-HT dans les granules à zymogène. La décarboxylation de la 5-HTP et la concentration de la 5-HT dans les granules à zymogène se font dans le pancréas mais pas dans la parotide.

Lorsque des cellules d'acini pancréatiques sont marquées à la [^{14}C] 5-HT et à la [^{3}H] leucine puis stimulées par la caeruléine, il y a une augmentation parallèle de la sécrétion de l'activité amylase, ainsi que de la sécrétion de la [^{14}C] 5-HT et des [^{3}H] protéines.

Les cellules des acini pancréatiques sont capables de pomper la DOPA et de la décarboxyler mais la dopamine n'est pas concentrée dans les granules et la sécrétion de dopamine à partir de cellules incubées avec de la caeruléine n'a pas pu être démontrée.

Ces résultats indiquent

1) dans les cellules des acini du pancréas de rat, la 5-HT est un composé normal des granules à zymogène

2) les cellules des acini pancréatiques possèdent une seule décarboxylase qui agit sur les acides aminés aromatiques et la DOPA ainsi que la 5-HTP sont ses substrats spécifiques

3) la 5-HT granulaire est libérée en même temps que les autres protéines granulaires quand les cellules sont stimulées.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to thank:

my parents for their support and expectation.

Drs. John Ruedy and Richard Ogilvie for the opportunity of pursuing my studies in this department.

Dr. Alan Tenenhouse, my supervisor, for his guidance, patience, enthusiasm and encouragement.

Drs. Brian Collier and Margaret Warner for their advice, helpful suggestions and support during the preparation of this thesis.

Dr. Margaret Warner for her careful proof-reading of this thesis.

Dr. Seymour Heisler for his advice in preparations of dispersed cells and cell column.

Ms. Ligia Stern for her collaboration and skilled technical assistance during the course of this work.

Dr. Marie-France Bader for translation of the abstract.

Ms. Ophelia Kan for the expert typing.

Charles Peterfy and Nancy Bradley for the preparation of the figures and Mike Masella for the preparation of the prints.

Kenny, Mario and Sharon for their welcome friendship.

Dalia, Rhoda, Chuck, Julio, Terry, Thomas, Andy, Julie, Rahul, Hernani and others who have made these many years of preparation of this thesis enjoyable.

Medical Research Council of Canada, Le Fonds Formation de Chercheurs et Action Concertée du Quebec and J.W. McConnell Foundation for their financial support.

LIST OF ABBREVIATIONS

5-HTP	5-hydroxytryptophan
DOPA	3,4-dihydroxyphenylalanine
5-HT	serotonin, 5-hydroxytryptamine
DA	dopamine, 3,4-dihydroxyphenylethylamine
Cyclic AMP, cAMP	cyclic adenosine-3',5'-monophosphate
Dibutyryl cAMP	N^6,O^2-dibutyryl cyclic adenosine-3',5'-monophosphate
Cyclic GMP, cGMP	cyclic guanosine-3',5'-monophosphate
Dibutyryl cGMP	N^6,O^2-dibutyryl cyclic guanosine-3',5'-monophosphate
V_{max}	maximum initial velocity
Km	affinity constant
IC_{50}	concentration of antagonist causing half-maximal inhibition
EC_{50}	concentration of agonist causing half-maximal activation
Z.G.	zymogen granules
Homog.	homogenates
et al.	and others
DNA	deoxyribonucleic acid
TCA	trichloroacetic acid
Ci	Curie
dpm	disintegration per minute

rpm	revolution per minute
nm	nanometer
g, gm	grams
min.	minutes
X g	acceleration of gravity
l	liter
M	Molar
Prefixes:m-	milli
μ-	micro
p-	pico

LIST OF FIGURES

LIST OF TABLES

SECTION ONE

INTRODUCTION

1.1 EXOCRINE PANCREAS - FUNCTION AND ANATOMY

The principal function of the exocrine pancreas is to synthesize and store digestive enzymes and, upon appropriate stimulation, to secrete these enzymes in an electrolyte solution which provides the optimal pH and ionic strength for their activity within the duodenum.

Functionally, the exocrine pancreas can be divided into two types of cells, namely, those which synthesize and secrete digestive enzymes (acinar cells) and those which secrete water and electrolytes (ductal and centroacinar cells).

The acinar cells are characterized by the presence of massive amounts of rough endoplasmic reticulum, typical of cells responsible for protein secretion. The cell nucleus occupies a central or basal location and is surrounded basally and laterally by rough-surfaced endoplasmic reticulum. The rough endoplasmic reticulum is arranged in a series of more or less parallel, convoluted and interconnected saccules or cisternae. In the apex of the cells are numerous spherical, mature storage granules (zymogen granules). Immature zymogen granules or condensing vacuoles and the Golgi complex lie between mature zymogen granule and the nucleus. At the luminal surface, the apical plasmalemma of the cell is provided with numerous microvilli which protrude into the duct lumen. Morphometric studies of Bolender (1974) have revealed that in resting acinar cells, the cytoplasmic matrix represents 54% of the cell volume, the rough endoplasmic reticulum 22%, nuclei 8.4%, mitochondria 8.1%, zymogen granules 6.4% and condensing vacuoles 0.7%.

Centroacinar cells and duct cells are characterized by the absence

of distinctive cytologic specialization, and both are considered to be part of the pancreatic duct system. Centroacinar cells are connected to the contiguous acinar cells by luminal junctional complexes similar to those which connect acinar cells to each other.

1.2 ACINAR CELLS - SECRETION OF PROTEINS

The secretory process in pancreatic acinar cells can be divided into six major steps: a) polypeptide synthesis on the ribosomes attached to the membranes of endoplasmic reticulum; b) transfer of the polypeptide through the membranes of the rough endoplasmic reticulum into the cisternal space; c) intracellular transport of newly synthesized proteins to the Golgi complex; d) concentration of proteins in the condensing vacuoles of the Golgi complex; e) migration of zymogen granule towards the apex of the cell where they are stored; and f) discharge of the granule contents into the acinar lumen by exocytosis.

1.2.1 Intracellular Transport

Details of protein synthesis and segregation have been reviewed extensively (see Case 1978; Scheele 1980;1982). Proteins that are destined to be secreted have been shown to be synthesized on ribosomes that are bound to the endoplasmic reticulum while proteins destined to remain in the cystol are synthesized on free ribosomes (Redman and Sabatini 1966; Redman 1969; Hicks et al., 1969). The newly synthesized secretory peptides are then translocated across the endoplasmic reticulum

membrane into the cisternae and are subsequently transported from the cisternal space of the endoplasmic reticulum to secretory granules for secretion (Blobel and Dobberstein 1975a;b; Case 1978; Scheele 1980;1982). The secretory pathway for exportable proteins in the exocrine pancreas is from endoplasmic reticulum to Golgi apparatus to zymogen granules.

The classic electron microscope autoradiographic studies of Jamieson and Palade (1967a; 1971a;b) have showed that proteins, pulse labelled with [^{3}H]-Leucine, first appeared in the rough endoplasmic reticulum. After 17 minutes of chase with a complete mixture of [^{1}H]-amino acids, the autoradiographic grains were associated with the Golgi complex. After 37 minutes of chase the grains were associated largely with the condensing vacuoles located on the trans side of the Golgi complex. After 57 minutes of chase, most of the grains were observed in the apical portion of the cell in association with mature zymogen granules. In agreement with the autoradiographic data, subcellular fractionation studies (Jamieson and Palade 1967a; 1968a;b; Scheele et al., 1978) showed that the specific radioactivity of protein in the rough microsomal fraction (representing the rough endoplasmic reticulum) was maximal immediately after the pulse exposure to [^{14}C]-Leucine. The labelled proteins were then transfered to the smooth microsomal fraction (representing the peripheral Golgi complex) and finally concentrated in the zymogen granule fractions. Together, the radioautographic and subcellular fractionation studies demonstrate that newly synthesized proteins from the rough endoplasmic reticulum are transfered successively to the Golgi

complex and from there to condensing vacuoles and zymogen granules.

Immunocytochemical procedures at the level of the electron microscope have been used for qualitative and quantitative localization of secretory proteins in exocrine pancreas acinar cells (Kraehenbuhl et al., 1977). Nine pancreatic secretory proteins, including amylase, were localized within the cisternae of rough endoplasmic reticulum, Golgi cisternae, condensing vacuoles and zymogen granules (Bendayan et al., 1980). Quantitative evaluation showed that secretory proteins were progressively concentrated during their movement along the rough endoplasmic reticulum through the Golgi complex to the granule (Bendayan et al., 1980). In addition, studies by Bendayan and co-workers (1980) using immunocytochemical techniques could not detect secretory proteins in the cell cytosol. This latter finding provides evidence that secretory proteins are confined within membrane bound compartments and, under physiological conditions, are not free in the cytoplasm.

Although the route of secretion is well defined, the mechanism responsible for the movement of secretory macromolecules through the various cellular compartments is still controversial. Intracellular transport of exportable protein is not dependent upon the maintained synthesis and segregation into the rough endoplasmic reticulum cisternae (Morimoto et al., 1967; Jamieson and Palade, 1968a; Singh et al., 1973). Transport through the cisternae of rough endoplasmic reticulum does not require energy and presumably occurs by passive flow (Jamieson and Palade, 1968a).

Jamieson and Palade (1967a;b) suggested that secretory proteins are

transported from rough endoplasmic reticulum to Golgi via discrete vesicles which shuttled back and forth between the two compartments. Rambourg et al. (1974) have suggested an alternative mechanism for such a transport. They hypothesized that convoluted tubules directly connect the rough endoplasmic reticulum to the Golgi cisternae. The vesicles described by Jamieson and Palade (1967) may represent the cross section of such convoluted tubules. Direct evidence in favour of any one of the above mechanisms has not been provided.

Proteins in the Golgi cisternae are transported through the Golgi apparatus, perhaps in a manner similar to transport through rough endoplasmic reticulum cisternae, and reach the mature face of the Golgi cisternae (Völkl et al., 1976). The subsequent step in the secretory pathway is the transport of protein from the Golgi complex to condensing vacuoles. Such transport has been reported to require energy and does not depend on continuous protein synthesis. When energy supply is blocked by inhibitors of respiration or oxidative phosphorylation, formation of condensing vacuoles does not occur (Morré, 1977; Jamieson and Palade, 1968a; 197la;b).

Condensing vacuoles are generally assumed to arise from the inner Golgi cisternae in the pancreas (Palade, 1975; Völkl et al., 1976). However, membranes of zymogen granule and Golgi cisternae differ greatly in enzymic activity (Meldolesi et al., 1971; Ronzio, 1973a;b). It has been proposed that at their margins, the inner Golgi cisternae manufacture vesicles, the membranes of which are characteristic of zymogen granule membrane, and that after these vesicles have reached a

given size, they break free to form condensing vacuoles (Case, 1978).

An alternative view of the origin of condensing vacuoles has been proposed as a result of studies using electron microscopic enzyme cytochemistry. By this technique, thiamine pyrophosphatase appeared concentrated in the inner Golgi cisternae. Novikoff (1976) described a region of smooth endoplasmic reticulum adjacent to the inner Golgi cisternae which is rich in acid phosphatase activity but lacks thiamine pyrophosphatase activity. According to his studies condensing vacuoles of exocrine pancreatic acinar cells show acid phosphatase but not thiamine pyrophosphatase activity, suggesting that condensing vacuoles represent expanded cisternal portion of Golgi-associated smooth endoplasmic reticulum involved in lysosome function (GERL). Secretory proteins are routed to GERL via the Golgi cisternae (Novikoff and Novikoff, 1977; Hand and Oliver, 1977), and from GERL to condensing vacuoles.

In summary, although the relationship between rough endoplasmic reticulum, Golgi complex and condensing vacuoles remain uncertain, it is known that secretory proteins synthesized in rough endoplasmic reticulum are transfered from rough endoplasmic reticulum to Golgi and ultimately appear in condensing vacuoles.

1.2.2 Storage

The earliest form of storage granule is the condensing vacuole (immature zymogen granules). Proteins stored in condensing vacuoles are progressively concentrated and the density of these organelles progressively increases to ultimately become that of zymogen granules.

The high density vacuoles formed from this granule maturation process are "mature" zymogen granules (Caro and Palade, 1964; Jamieson and Palade, 1967b; Sesso et al., 1980). The concentration process does not require energy (Jamieson and Palade, 1971a) nor does it depend on continued protein synthesis (Jamieson and Palade, 1968a). It probably involves a passive and progressive aggregation of proteins with the formation of osmotically inert complexes, thereby reducing osmotic activity within the granules and causing water to flow out (Case, 1978).

It has been suggested that ionic interaction plays a predominant role in the aggregation process since sulfated polyanions and divalent cations are present in significant concentration in these organelles (Case, 1978; Reggio and Dagorn, 1980; Scheele, 1982). Isolated zymogen granules are known to be unstable in solutions of alkaline pH (granule will lyse when the pH of the suspension medium is raised above 7.2) and high ionic strength (5×10^{-2} M sodium chloride or potassium chloride solution) (Hokin 1955; Jamieson and Palade, 1971a; Meldolesi et al., 1971a; Vandermeers-Piret et al., 1971; Rothman, 1971; Fast, 1974).

It has been postulated by Tartakoff et al., (1974) that sulfated polyanions (proteoglycans) play a role in the protein concentration process in zymogen granules, presumably by ionic interaction with cationic pancreatic secretory proteins. Experimental evidence in favour of such interactions was obtained by the addition of small amounts of either chondroitin sulfate or glycosaminoglycans isolated from pancreatic zymogen granules to chymotrypsinogen. This caused the protein to precipitate (Reggio and Palade, 1978). The resulting aggregates, like

zymogen granules, were insensitive to osmolarity, but sensitive to both ionic strength and pH (Scheele, 1982). However, glycosaminoglycans in the pancreas are present at concentrations of less than 0.5 nmole/mg of zymogen granule protein, and this concentration is thought to be insufficient to participate in charge neutralization of the secretory proteins (Kronquist et al., 1977). It remains possible that interactions between proteoglycans and secretory proteins act only as a triggering mechanism for the condensation of secretory protein. Interactions between the secretory proteins themselves have also been detected. Such interactions can induce precipitation in vitro at low pH and in vivo when transport to Golgi complex is slowed down by starvation (Palade, 1956), or blocked by metabolic inhibitors (Kern and Kern, 1969 Kern et al., 1979). However, when glycosylaminoglycans are present, they always coprecipitate with the protein aggregates (Reggio and Dagorn, 1980).

The divalent cations Ca^{+2} and Mg^{+2} are also components of pancreatic zymogen granules and it has been suggested that they act as bridges between adjacent protein molecules to facilitate charge neutralization and protein condensation (Case, 1978). The large amount of Ca^{+2} (about 36 nmoles/mg protein - Ceccarelli et al., 1975; Clemente and Meldolesi, 1975) present in zymogen granules is more than that required for association with proteins, and may be involved in stabilizing the zymogen granule architecture (Clemente and Meldolesi, 1975). Furthermore, both polyanions and Ca^{+2} are thought to be incorporated into zymogen granules at the level of the Golgi complex and to be packaged along with secretory proteins (Riggo and Palade, 1978;

Ceccarelli et al., 1975). Their functions in the protein condensation process await further investigation.

The enzyme composition of zymogen granules has been studied in several species (Scheele, 1982), and the weight of evidence suggests that zymogen granules form a homogeneous population. Several studies have been reported in which the composition of proteins contained within purified zymogen granule fractions were compared with that secreted by the pancreas in response to stimulation (Greene et al., 1963; Palla, 1970; Tartakoff et al., 1975; Castle et al., 1975). In each case, the relative proportions of the various proteins in the purified zymogen granules was the same as that in the secretory fluid. Furthermore, immunocytochemical studies by Bendayan et al. (1980) showed that secretory proteins are not free in the cytoplasm, but are confined within membrane bound compartments. Using immunofluorescent antibody techniques, amylase together with eight other secretory proteins (Kraehenbuhl et al., 1977; Bendayan et al., 1980) have been found in all acinar cells and zymogen granules.

1.2.3 Secretion

1.2.3.1 Acetylcholine and Pancreozymin

Preganglionic parasympathetic fibers are scattered throughout the pancreas and pass directly to the acini so that the direct nerve endings on the acinar cells are principally cholinergic. Although adrenergic innervation reaches the pancreas through the splanchnic nerves, the

adrenergic fibers are mainly distributed to the blood vessels (Pascal and Vaysse, 1976; Tiscornia, 1976). Acetylcholine released from cholinergic nerve terminals interacts with muscarinic receptors on the acinar cells, leading to an increase in cytoplasmic Ca^{+2} and the secretion of pancreatic enzymes (O'Doherty and Stark, 1982; Petersen, 1982). Cholinergic stimulation of acinar cells also leads to glucose uptake and increased cyclic GMP (not cyclic AMP) accumulation. This transient increase in cyclic GMP concentration in acinar cell is not correlated with enzyme secretion. Its role is not known (Petersen, 1982).

The major physiological secretagogues for pancreatic enzyme secretion is peptide hormone pancreozymin. Distension of the duodenum by food causes the release of pancreozymin which causes gall bladder contraction and stimulates pancreatic enzyme secretion (Ivy and Oldberg, 1928; Harper and Raper, 1943). In the pancreas, pancreozymin acts primarily on acinar cells (review see Wormsley, 1979) where its action is mediated through specific binding sites or receptors (Milutinović et al., 1977; Jensen and Gardner, 1981; Williams et al., 1981). Each acinar cell has been estimated to possess 9,000 specific binding sites for pancreozymin, which on the basis of affinity studies are divided into two classes. Occupancy of high affinity pancreozymin binding sites correlates with $^{45}Ca^{+2}$ outflux and amylase release (Williams and co-workers, 1981; Jensen and Gardner, 1981). The biological significance of pancreozymin binding to the low affinity binding sites has not yet been determined. There are reports suggesting that the stimulation of glucose transport in dispersed pancreatic acini by pancreozymin correlates with the occupancy

of the lower affinity binding sites (Korc et al., 1979; Williams and co-workers, 1981).

Caerulein, a decapeptide originally isolated from the skin of the Australian hylid frog Hyla Caerulein (Anastasi et al., 1968), binds specifically to the high affinity pancreozymin binding site (May et al., 1978). It shares a common C-terminal pentapeptide amide sequence with pancreozymin. Studies indicate that binding of pancreozymin to pancreas acini can be inhibited by caerulein and conversely that the specific binding of caerulein to pancreatic plasma membrane can also be displaced by pancreozymin octapeptide (the 8 amino acids that are necessary for full biological activity of pancreozymin)(Milutinovič and co-workers, 1977; Jensen and Gardner, 1981). Caerulein is a potent pancreozymin-like secretagogue. It has a EC_{50} approximately seven times lower than pancreozymin. The action of both pancreozymin and caerulein in increasing enzyme secretion is mediated by an increase in intracellular Ca^{+2} concentration (review see Schulz and Stolze, 1980). The effect of pancreozymin peptides on acinar membrane depolarization, ions fluxes, cyclic GMP accumulation and glucose uptake into acinar cells are similar to the effects of cholinergic agonists, however, the two groups of stimulants act on different types of receptor sites. The actions of acetylcholine can be blocked by atropine in concentrations that have no effect on responses to pancreozymin peptides, whereas the actions of pancreozymin peptides can be blocked by dibutyryl cyclic GMP in concentration that have no effect on acetylcholine-evoked responses (Petersen, 1982).

Binding sites other than specific pancreozymin receptors which are thought to be involved in stimulating enzyme secretion from acinar cells, have also been reported (Jensen and Gardner, 1981; Schulz and Stolze, 1980). The physiological significance of those non-pancreozymin binding sites in pancreatic enzyme secretion has not been determined.

1.2.3.2 Calcium and enzyme secretion

Calcium is essential for secretion of digestive enzymes from the exocrine pancreas. In the resting mammalian cell, the cytoplasmic calcium ion concentration ranges between $5X10^{-8}M$ to $3X10^{-7}M$ and the extracellular fluid calcium ion concentration is between $10^{-4}M$ and $10^{-3}M$ (Borle, 1981). The total intracellular calcium, if all of it was evenly distributed and ionized, would range from 1M to $10^{-1}M$. Most of the calcium inside the cell, however, is in a non-ionized form, probably as calcium phosphate complex (review see Case, 1978; Borle, 1981).

The calcium necessary for secretion is probably derived from the store in one or more of the following: plasma membrane, endoplasmic reticulum and mitochondria (Schulz and Stolze, 1980; Dormer and Williams, 1981; Ponnappa et al., 1981). Although a large proportion of the Ca^{+2} is stored in zymogen granules (Introduction section 1.2.2), this source of calcium does not contribute to enzyme secretion.

The relative importance of various calcium sources in the regulation of the Ca^{+2} concentration in the cytosol is different in different cell types. In skeletal muscle which possesses a limited number of mitochondria, intracellular calcium movement is largely controlled by the

endoplasmic reticulum (i.e. sarcoplasmic reticulum) (Endo, 1977). In liver and kidney the mitochondria are probably more important in regulating cytoplasmic Ca^{+2} concentration (Borle, 1981). In pancreas, both mitochondria and endoplasmic reticulum seem to be involved in "buffering" cytosolic Ca^{+2} concentration (Schulz et al., 1981).

There is evidence suggesting that the initial effect of secretagogues is to release Ca^{+2} from intracellular storage sites until the concentration of cytosolic Ca^{+2} reaches that required to trigger enzyme secretion. The secretory response of these stimulants is then maintained by Ca^{+2} entry from the extracellular space. When secretion is terminated, Ca^{+2} entry from the extracellular space ceases and cytoplasmic Ca^{+2} concentration is restored primarily by the Ca^{+2} buffering capacity of mitochondria and endoplasmic reticulum (Schulz et al., 1981; Gardner, 1979; Renckens et al., 1978; Petersen and Ueda, 1976). Different classes of secretagogues may utilize different intracellular Ca^{+2} storage sites for enzyme secretion and the effect to extracellular Ca^{+2} deprivation on enzyme secretion will also be different with different secretagogues (Argent et al., 1982a).

The role of Ca^{+2} in triggering exocytosis is not clear. Several possibilities for Ca^{+2} involvement in enzyme discharge have been proposed. Dean (1974, 1975) and Dean and Matthew (1975) suggested that Ca^{+2} acts directly to mediate the fusion of the granule membrane with the plasma membrane leading to enzyme discharge. According to these authors many secretory granules, including those of the exocrine pancreas, are negatively charged. Since the inner surface of the plasma membrane is also negatively charged, electrostatic repulsion tends to keep the

granule and plasma membrane apart. Ca^{2+} by binding to both granules and plasma membranes, can promote adhesion, facilitate membrane fusion and lead to discharge of secretory product. Other workers have suggested that Ca^{+2} may act through a specific binding protein in the plasma or vesicle membrane (Zimmeberg et al., 1980). Studies from Creutz et al (1978, 1979) and Pollard and co-workers (1980)indicate that a protein, "synexin", is required to initiate in vitro granule aggregation. Pollard and co-workers (1980) suggested that Ca^{+2}, interacts first with "synexin" and that the "activated synexin" was responsible for fusion of granule to plasma membrane.

1.3 DUCT CELL - SECRETION OF ELECTROLYTE SOLUTION

1.3.1 Release of Secretin

The principal stimulus for pancreatic secretion of electrolytes is secretin. In 1902 Bayliss and Starling demonstrated that hydrochloric acid perfused into denervated small intestine would rapidly produce secretion of alkaline pancreatic juices. It was postulated that such an effect must be mediated via a blood-borne messenger, released from the intestine. Purification of porcine secretin, was accomplished in 1961 by Jorpes and Mutt who subsequently (1968) elucidated the complete amino acid sequence. Secretin is a peptide hormone localized primarily in the duodenum (Hubel, 1972).

The strongest releaser of secretin is H^{+}. It has been reported that

above pH 4.5 secretin is not released in amounts sufficient to stimulate pancreatic secretion (Chey and Konturek, 1982). Continuous acidification of duodenal mucosa diminishes mucosal secretin content. Once the release of secretin has begun, continued stimulation is pH-dependent, and raising the pH above 4.5 will suppress further release (Meyer and Grossman, 1972). There is a closed-loop relation between secretin and bicarbonate. Secretin stimulates pancreatic secretion of bicarbonate into the duodenum; the bicarbonate neutralizes the H^+ from the stomach, raises the pH and eventually halts release of secretin.

Secretin is also released in response to food but this is secondary to gastric acid secretion. Schaffalitzky de Muckadell and Fahrenkrug (1978) reported that in man the release of secretin after a solid meal was associated with the fall in duodenal pH. Such a relationship between duodenal pH and secretin release has been demonstrated in dogs (Kim et al., 1979; Chey and Konturek, 1982). When a liver extract meal adjusted to various pH levels was introduced into canine stomach, the increments in plasma secretin levels correlated with the pH of the liver extract meal and pancreatic bicarbonate outputs.

Further evidence for the role of gastric acid in the stimulation of secretin release was obtained from the studies on the effects of antacids (Chey et al., 1978) and the H_2 receptor antagonist, cimetidine (Schaffalitzky de Muckadell and Fahrenkrug, 1978; Kim et al., 1979). Both these agents abolish the postprandial rise in plasma secretin level. Greenberg (1981) suggested that in man the ingestion of food is associated with increased circulating concentration of plasma secretin. These

increments in plasma secretin are determined not by H^+ ion concentration, but rather by the load of acid delivered to the duodenum and the length of intestine acidified.

In addition to acid, it has been shown that infusion of bile salts (Osnes et al., 1978) and sodium oleate (Faichney et al., 1979) into the duodenum can increase the plasma concentration of secretin. At present, no gut hormones or peptides have been shown to stimulate the release of secretin, nor is secretin release influenced by cholinergic mechanisms (Sum et al., 1969; Konturek et al., 1974).

1.3.2 Action of Secretin

The primary action of secretin is to stimulate secretion of H_2O and bicarbonate via activation of the adenylate cyclase - cAMP system of pancreatic duct cells (Case, 1979; Sarles, 1977; Greenwell, 1975). The involvement of cAMP as a mediator of the secretin effect was suggested by Case et al., (1972). In their studies secretin was either injected into anaesthetized cats intravenously, or included in the perfusion medium in an isolated saline-perfused preparation of the cat's pancreas. In response to secretin the pancreas cAMP concentration rose within 30 seconds and secretion of bicarbonate began only after 45 seconds of secretin administration. When stimulus was withdrawn, the cAMP concentration decreased as secretion declined. Similar studies from Domschke and co-workers (1975) also showed that, in dogs, a correlation exists between tissue cAMP levels and pancreatic bicarbonate output in the pancreatic juice after stimulation by exogenous secretin.

Dibutyryl cAMP, an analogue of cAMP, was reported to elicit a pancreatic secretion which has a water and electrolyte composition similar to that secreted in response to secretin (Case and Scratcherd, 1972). These authors also reported that compounds such as theophylline, theobromine and caffeine which inhibit the destruction of cAMP by phosphodiesterase, potentiate the action of secretin.

The role of cAMP in water and bicarbonate secretion has also been studied by using cholera toxin, which is known to activate adenyl cyclase in several tissues (Field et al., 1972; Al-Awqati et al., 1973). Smith and Case (1975) showed that cholera toxin evoked a secretory response. The composition of the secretion stimulated by cholera toxin resembled that evoked by secretin. According to Smith and Case (1975), cholera toxin action was accompanied by a parallel increase in tissue cAMP concentration and was potentiated by theophylline.

Thus, the above studies demonstrate that electrolyte secretion in the exocrine pancreas is mediated by intracellular cAMP. The mechanism by which an increase in tissue cAMP concentration mediates the transport events responsible for electrolyte secretion remains unknown.

1.3.3 Secretin - stimulated Secretion

Secretin-stimulated pancreatic juice is an alkaline solution rich in bicarbonate. In all animal species that have been studied, the major cation in pancreatic electrolyte secretion is Na^+. Its concentration is about 155 mM, approximately 10 mM greater than that in the plasma. K^+ is secreted at about the same concentration as plasma. The

concentration of both cations are constant and independent of secretory rate. The major anions are bicarbonate and Cl^- and their concentrations depend on flow rate. Micropuncture and micro-cannulation studies of the ductal system indicate that when the flow rate increases, bicarbonate concentration increases and Cl^- concentration decreases; the reverse is observed when the flow rate decreases (Case et al., 1970; Mangos and McSherry, 1971; Swanson and Solomon, 1975; Caflisch et al., 1980). The concentration of bicarbonate in secretin-stimulated secretion fluid is species dependent. In intermittent feeders such as cat, dog, pig and man, the gland is sensitive to secretin and the bicarbonate concentration of the resulting secretion reaches a maximum of approximately 145 mM; in continuous feeders such as rat, sheep, cow and rabbit, the gland responds poorly to secretin with a maximal bicarbonate concentration of approximately 80 mM in secretin-stimulated fluid. Cl^- concentration, in all species, decreases with increased secretory rate in a reciprocal fashion with bicarbonate so that the sum of the two anions remains constant and approximately equal to the sum of Na^+ and K^+ at all secretory rates (review see Case et al., 1980).

1.4 DA, 5-HT AND THE EXOCRINE PANCREAS

1.4.1 Amine Precursor Uptake and Decarboxylation

In 1968, Pearse introduced the term APUD (Amine Precursor Uptake and Decarboxylation) to designate a system of cells, found in several

organs which are able to produce and store polypeptides (hormones) and biogenic monoamines together in cytoplasmic granules. These cells take up L-DOPA and L-5-HTP and decarboxylate them to DA and 5-HT, respectively (review see Sundler et al., 1980).

Pearse and Takor Takor (1979) have classified the cells of the APUD series as central and peripheral. The central division contains the neuroendocrine and endocrine cells of the hypothalamo-pituitary axis and pineal gland, and the peripheral division contains all the APUD cells outside these regions. The majority of the APUD cells in the periphery are the gastroenteropancreatic endocrine cells.

Most of the gastroenteropancreatic endocrine cells contain neither catecholamines nor 5-hydroxytryptamine in histochemically demonstratable amounts. They have one common characteristic, namely, they are capable of taking up either L-DOPA or L-5-HTP and converting them to their corresponding amines, which are then stored in the cytoplasmic granules (Håkanson et al., 1967).

In neuroendocrine cells of the APUD system, the enzyme that is responsible for converting L-5-HTP to 5-HT and L-DOPA to DA respectiviely is L-aromatic amino acid decarboxylase [EC 4.1.1.28] (Pearse, 1976)(Fig 1). In gastroenteropancreatic endocrine cells, histochemical investigation indicates a close correlation between L-aromatic amino acid decarboxylase activity and the number of fluorescent cells that can be produced by treatment of the animal with L-5-HTP or L-DOPA (Håkanson et al., 1970).

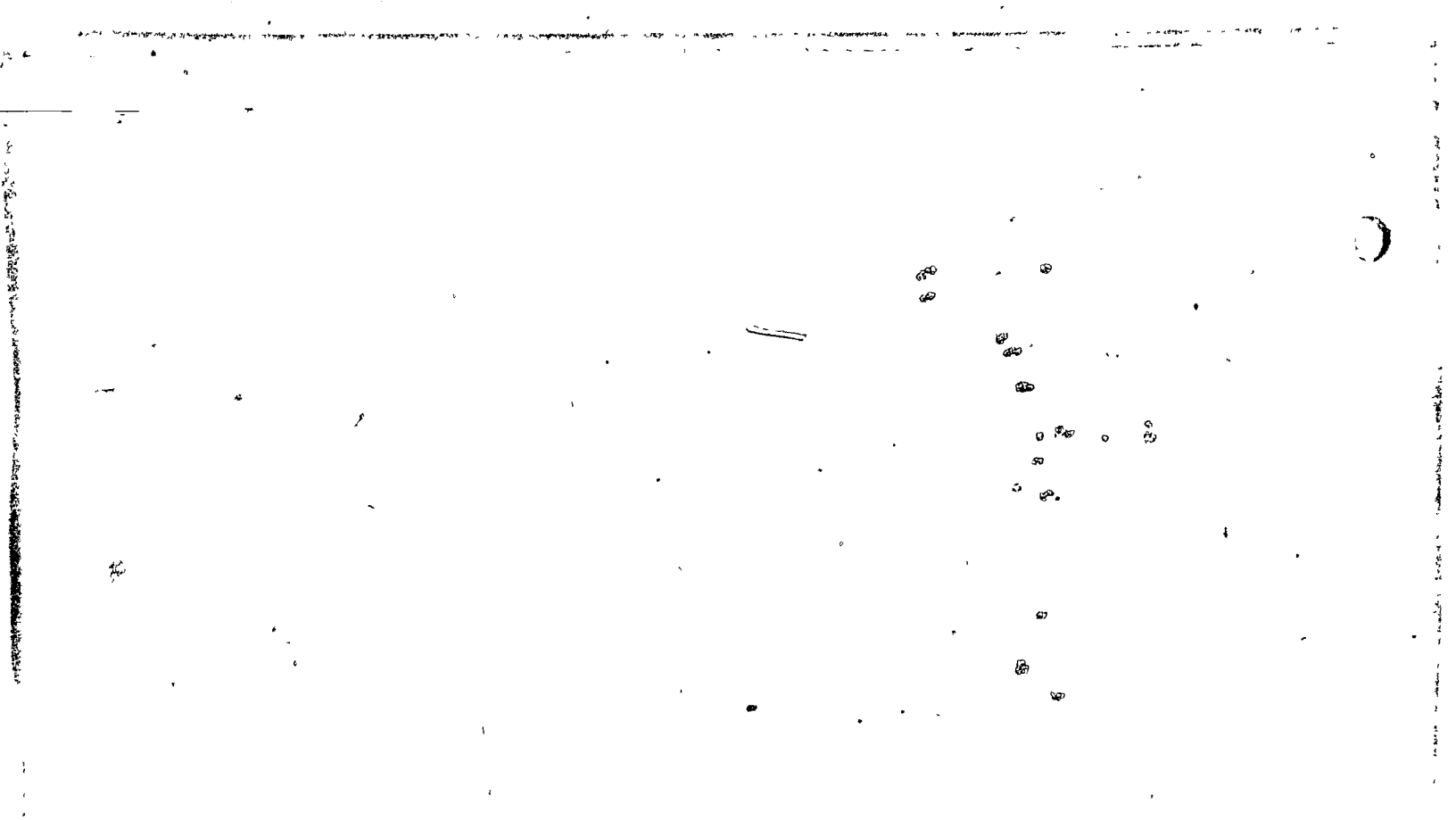
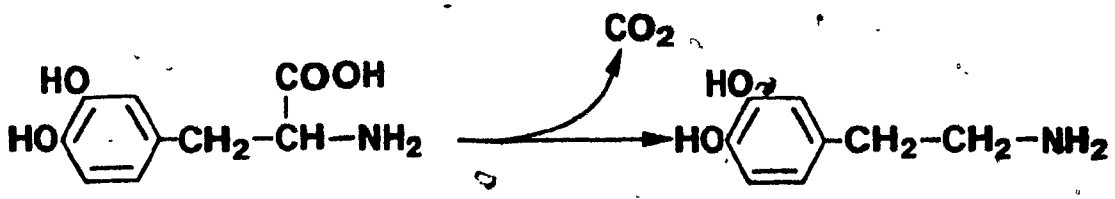


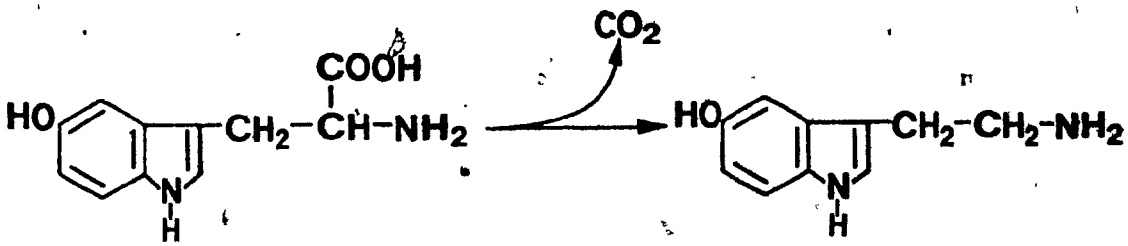
FIGURE 1 The decarboxylations of (a) DOPA to DA; (b) 5-HTP to 5-HT

(a)



(DOPA) (DA)

(b)



(5-HTP) (5-HT)

1.4.2 L-DOPA and L-5-HTP Metabolism in Exocrine Pancreas

1.4.2.1 In vivo studies of biogenic monoamine synthesis and storage

The exocrine pancreas, like the cells of APUD system, is able to take up L-5-HTP and L-DOPA, decarboxylate them to form 5-HT and DA respectively and store the monoamine(s) in zymogen granules.

Alm et al., (1967, 1969), using histofluorescent techniques, found that when a large dose (40-100 mg/kg) of L-DOPA was injected into mice or rats, a specific DA fluorescence appeared in zymogen granules of pancreatic acinar cells. The specific DA fluorescence was enhanced when animals were pretreated with a monoamine oxidase inhibitor and did not appear when animals were pretreated with an aromatic amino acid decarboxylase inhibitor. Dopamine fluorescence did not occur when the animals were given D-DOPA. These authors suggested that L-DOPA was taken up by the exocrine pancreas, decarboxylated by L-aromatic amino acid decarboxylase to form DA which was stored in zymogen granules.

Gershon and Ross (1966a;b) demonstrated that mouse pancreas was able to concentrate a large proportion of radioactivity after intravenous administration of [^{3}H]-5-HTP. According to these authors, approximately 50% of the total radioactivity concentrated in the pancreas was 5-HT. With the technique of counting silver grains, no change of 5-HT could be detected for 4 hours. Electron

microscope autoradiography studies by Alm et al. (1972) showed that mouse pancreatic acinar cells took up L-5-HTP, decarboxylated the amino acid to 5-HT and stored the amine in zymogen granules. The granular autoradiographic grains (representing labelled 5-HT) were not seen when decarboxylase inhibitor was injected into the mouse before the administration of labelled amino acid. These observations, together with those of Alm et al., (1967; 1969) and Gershon and Ross (1966a;b), were confirmed by the histochemical fluorescence studies of Mori et al (1979a;b). These workers, after administering a large dose of L-DOPA and/or 5-HTP to animals, observed that the turnover of DA in acinar cells was faster than that of 5-HT and further that DA metabolism in rat exocrine pancreas was different from that of 5-HT. The role of the amines in zymogen granule is not known.

Although previous literature has provided information on 5-HTP and DOPA uptake by pancreatic acinar cells, neither the subcellular localization of DA or 5-HT in acinar cells, nor the secretion of monoamines with pancreatic enzymes has been examined.

1.4.2.2 Biogenic Monoamines and Pancreatic Secretion

A relationship between biogenic monoamine metabolism and exocrine pancreatic secretion was suggested when Greengard et al., (1942) reported that several biogenic monoamines including "oxytyramine" (DA) could stimulate the secretion of canine pancreatic juice.

The effect of DA on secretion was further examined in isolated blood-perfused pancreas. DA was found to have a secretin like effect in that it stimulated secretion of pancreatic juice containing a high concentration of bicarbonate (Hashimoto et al., 1971; Furuta et al., 1972). This secretin-like effect of DA was mediated by a specific DA receptor and its activity could be abolished by haloperidol. L-DOPA, the immediate precursor of DA, was also reported to induce pancreatic secretion similar to that evoked by secretin when infused into the dog (Hashimoto et al., 1971; Bastie et al., 1977; Satoh et al., 1980). Studies of the effect of L-DOPA on pancreatic secretion indicate that the action of L-DOPA is mediated by DA formed by decarboxylation of the amino acid since this effect of L-DOPA was inhibited by a DOPA decarboxylase inhibitor. Furthermore, when the DA content of the pancreas was measured, it was found to be increased during treatment with L-DOPA (Furuta et al., 1973;1974).

It was suggested that canine pancreatic acinar cells take up L-DOPA, decarboxylate it to DA which then stimulates a bicarbonate-enriched pancreatic juice secretion (Furuta et al., 1973; Bastie et al., 1977). The mechanism by which DA receptor activation leads to stimulation of bicarbonate secretion is unknown. A recent report suggested that cAMP accumulation might be involved in DA-receptor activation in canine exocrine pancreas (Vaysse et al., 1982).

The stimulation by DA of bicarbonate-rich pancreatic juice is

species dependent. It occurs in the dog, but not in cats and rabbits. Moreover, the rate of pancreatic seeretion in the latter animals does not respond to other catecholamines such as adrenaline, noradrenaline and isoprenaline (Hashimoto et al., 1977). In rats, beta-adrenoceptors, rather than DA receptors, are present in the pancreas. Stimulation of the beta-adrenoceptor by isoprenaline causes secretion similar to that evoked by secretin (Furuta et al., 1978).

The above findings on catecholamine stimulated secretion in the exocrine pancreas suggest that catecholamines may be secretagogues or may modify the effect of secretagogues on pancreatic electrolyte secretion.

1.5 STATEMENT OF THE PROBLEM

The pancreas secretes a fluid composed of a mixture of digestive enzymes, water and electrolytes, the relative proportions of which are controlled by both secretin and pancreozymin (see Introduction sections 1.2.3 and 1.3). The regulation of pancreatic secretion by these two hormones requires a precise integration between enzyme secreting cells (acinar cells) and water and electrolyte secreting cells (centroacinar/duct cells) since the highly viscous enzymes packaged in zymogen granules must be solubilized in the acinus and the duct lumen by electrolyte secretion (Sarles, 1977). It seems, therefore, that an intimate interaction between acinar cells and centroacinar/duct cells is required to ensure the optimum composition and rate of flow of pancreatic fluid. The integration between these two types of cells would be carried out by a "communicator" and it is the purpose of the present thesis to test whether biogenic monoamine(s) could serve this function. This communicator must possess the following properties:

(I) acinar cells must synthesize (and/or take up from their environment) such a substance.

(II) the communicator must be packaged with the contents of the zymogen granules.

(III) the communicator must be secreted with the contents of the zymogen granules.

(IV) the communicator must interact with centroacinar and duct cells. This interaction must result in an alteration of the ionic

composition and volume of pancreatic juice.

Current evidence suggests that biogenic monoamines are good candidates in that:

(i) in neuroendocrine cells, peptide hormones are stored with a biogenic monoamine (see Introduction section 1.4.1).

(ii) biogenic monoamines are capable of producing a secretin-like effect in pancreatic secretion at least in some species (see Introduction section 1.4.2.2).

SECTION TWO

METHODS AND MATERIALS

2.01 PREPARATION OF DISPERSED ACINAR CELLS

In addition to the cells of exocrine system, the pancreas contains many cell types including cells of the Islets of Langerhans, connective tissue, blood and blood vessel endothelium . To eliminate any contribution made by these other cell types, our studies were done in dispersed acinar cells.

In order to minimize the contribution of biogenic monoamines from cells other than acinar cells, the rat was used as the source of exocrine pancreas. This is because of all common laboratory animals, rat pancreatic islet cells contain the lowest quantities of biogenic monoamines (Falck and Hellman, 1963; Cegrell, 1968; Lundquist et al., 1975; Glyfe, 1977).

Dispersed acinar cells were prepared as described by Amsterdam and Jamieson (1974) and modified by Chauvelot et al. (1980). The incubation media A, B and C for cell isolation are described in the Appendix. Pancreata were removed from Wistar rats (125-150 gm.), and cleaned free of adhering connective tissue. Collagenase and hyaluronidase (dissolved in solution A were injected into the pancreatic interstitium and the pancreata digested in the enzyme solution for 15 minutes. The tissue was then washed and incubated in solution B for 4 minutes. This washing and incubation in solution B was repeated twice. The tissue was washed twice in solution A and then incubated with collagenase (dissolved in solution C) for 25-30 minutes. The collagenase digestion was terminated by diluting the digestion medium with solution C and the acinar cells were mechanically detached from undigested pancreatic tissues. The

digested tissues were filtered through a nylon filter (pore size 64 micron) and acinar cells were collected by centrifuging the filtrate through solution C containing 4% [w/v] BSA. Approximately 75-100 X 10^6 cells per pancreas were collected. The cell preparations were examined by electron microscopy, and found to contain only exocrine acinar cells.

The viability and function of the isolated cells were tested as follow:

(a) Exclusion of Trypan Blue

200 µl Trypan Blue (0.5% in 0.25% saline) was mixed with 750 µl. 4% [w/v] BSA in solution C [see appendix] and 50 µl. cell suspension. Cells were examined under light microscope to estimate the amount of deformed and destroyed cells.

(b) Amylase secretion

10^7 cells were mixed with Biogel P4 in a PD-10 column and superfused with perfusion buffer (section 2.05). Caerulein (10^{-7}M) was used as secretagogue, and amylase activity was assayed as described in section 2.09.2. There was approximately a 4 fold increase in amylase release upon stimulation with caerulein (Fig. 26, 28).

The cell preparation was used only if more than 95% of the cells were viable as measured by Trypan Blue exclusion and experimental results were accepted only if after the experiment more than 90% of the cells were viable. All glasswares used for the preparation and incubation of cells were siliconized using Prosil-28.

2.02 INCUBATION OF DISPERSED ACINAR CELLS

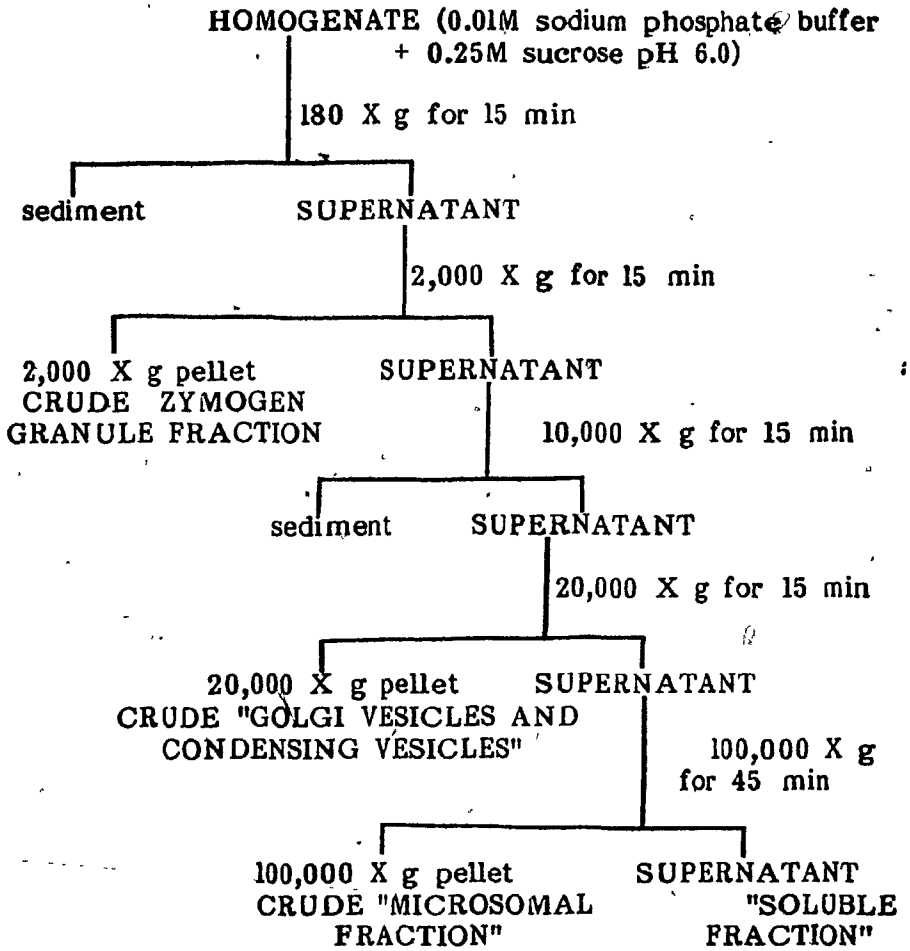
Dispersed acinar cells (10^8) were incubated in 5.0 ml. incubation buffer I [see appendix]. The reaction was started with the addition of radiolabelled substrate. All incubations were done at 37°C and gased with 95% O_2/5% CO_2. After incubation, the cells were separated from the incubation medium by centrifugation at 50 X g for 5 minutes and resuspended in homogenization buffer (see appendix) for subcellular fractionation studies (section 2.3).

In pulse-chase experiments dispersed acinar cells were incubated for 5 minutes or 15 minutes in incubation buffer I modified by the removal of leucine (incubation buffer II) and containing L-[^{3}H] Leucine (50-60 Ci/mMole; 1.0 μCi/ml) and either DL-[^{14}C] 5-HTP or L-[^{14}C] DOPA at concentrations specified in figure legends. After the pulse-incubation, cells were separated from medium by centrifugation at 50 X g for 5 minutes, washed in a large excess of incubation buffer II containing 1.0 mM non-radioactive counterparts of the radiolabelled compounds used in the chase-incubation. The cells were then resuspended in this buffer for the pulse-incubation. The chase-incubation was terminated by separating cells from incubation medium as described above. The cells were washed with homogenization buffer twice and then homogenized for subcellular fractionation (section 2.03).

2.03 SUBCELLULAR FRACTIONATION OF ACINAR CELLS

The general scheme for the isolation of the zymogen granule fraction, 20,000 X g pellet and 100,000 X g pellet from acinar cell homogenate is described in Fig. 2. The scheme is taken from the methods of Jamieson and Palade (1967a), Rothman (1970) and Fast (1974).

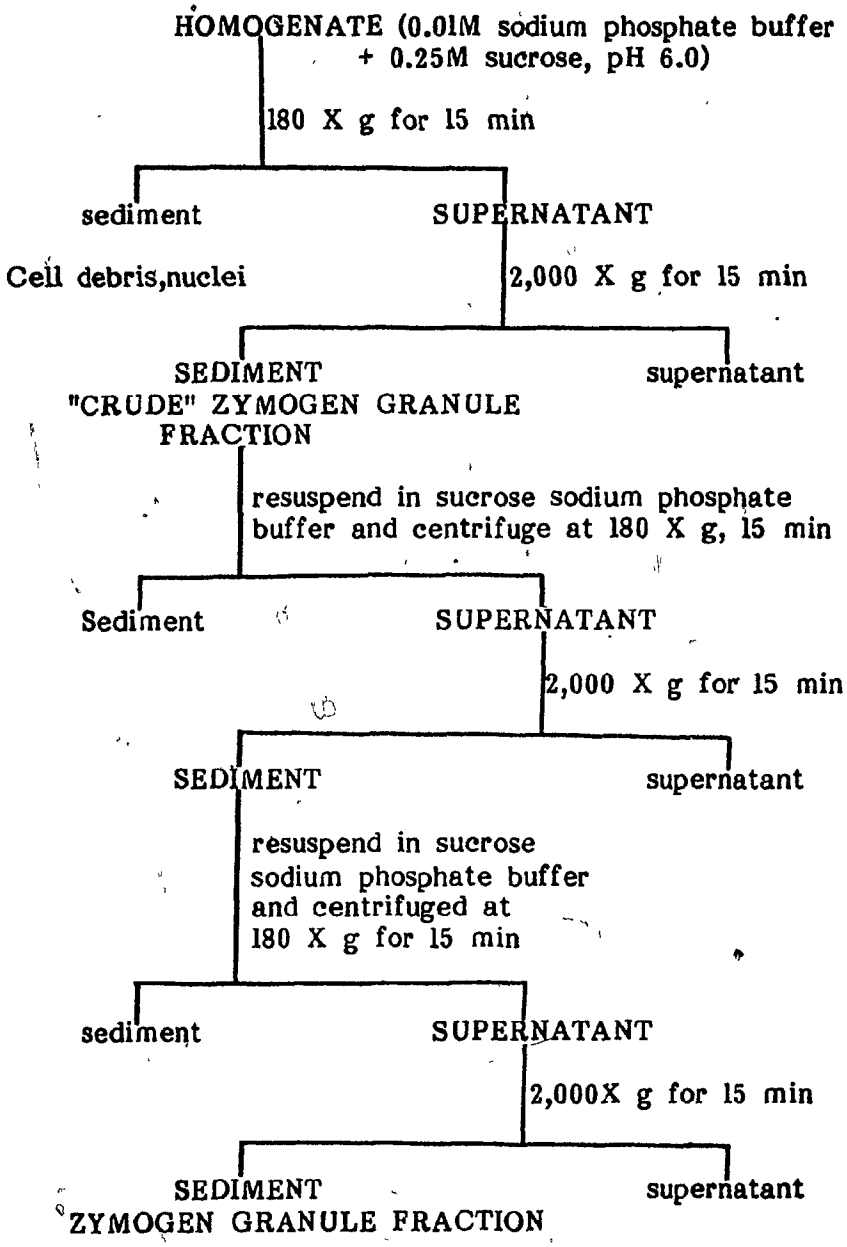
FIGURE 2 A general centrifugation scheme for subcellular fractionation studies. This procedure was adapted from those described by Tartakoff and Jamieson (1974) and Fast (1974).


HOMOGENATE (0.01M sodium phosphate buffer + 0.25M sucrose pH 6.0)
180 X g for 15 min
sediment
SUPERNATANT
2,000 X g for 15 min
2,000 X g pellet CRUDE ZYMOGEN GRANULE FRACTION
SUPERNATANT
10,000 X g for 15 min
sediment
SUPERNATANT
20,000 X g for 15 min
20,000 X g pellet CRUDE "GOLGI VESICLES AND CONDENSING VESICLES"
SUPERNATANT
100,000 X g for 45 min
100,000 X g pellet CRUDE "MICROSOMAL FRACTION"
SUPERNATANT "SOLUBLE FRACTION"

2.03.1 Isolation of Zymogen Granules

Acinar cells were homogenized in a Potter-Elvehgem homogenizer. The cell debris was removed by centrifugation at 180 X g for 15 minutes. The fraction containing the zymogen granules was collected by centrifugation at 2,000 X g for 15 minutes. The granules were further purified by repeating the 180 X g and 2,000 X g centrifugation steps (Fig. 3). Based on amylase activity, the recovery of zymogen granules ranged from 20-25% of that present in the homogenate. The contamination of this fraction by nuclei (DNA assay, section 2.09.5) ranged from 3-6%; by mitochondria (succinic dehydrogenase assay, section 2.09.4), 1-4%; by cytosol (lactic acid dehydrogenase assay, section 2.09.3) 0.1-0.3% of that in cell homogenate.

FIGURE 3 A centrifugation scheme for isolation of zymogen granule fraction from a homogenate of rat pancreas. This procedure was adapted from that described by Fast (1974). Details of the method are given in the text.


HOMOGENATE (0.01M sodium phosphate buffer + 0.25M sucrose, pH 6.0)
180 X g for 15 min
sediment
Cell debris,nuclei
SUPERNATANT
2,000 X g for 15 min
SEDIMENT
"CRUDE" ZYMOGEN GRANULE FRACTION
supernatant
resuspend in sucrose sodium phosphate buffer and centrifuge at 180 X g, 15 min
Sediment
SUPERNATANT
2,000 X g for 15 min
SEDIMENT
supernatant
resuspend in sucrose sodium phosphate buffer and centrifuged at 180 X g for 15 min
sediment
SUPERNATANT
2,000X g for 15 min
SEDIMENT
ZYMOGEN GRANULE FRACTION
supernatant

2.03.2 Purification of Zymogen Granule Fraction

Zymogen granules prepared as described in section 2.03.1 were purified by resuspending them in 60% Percoll and centrifuging this mixture at 80,000 X g for 45 minutes. In the continuous density gradient generated by this procedure, zymogen granules, containing less than 0.01% of total homogenate DNA and lactate dehydrogenase activity accumulated at a density of 1.142. Based on amylase activity, the recovery of zymogen granules from the Percoll gradient ranged from 10% to 17% of that present in the cell homogenate. The recovery of mitochondria in this fraction, based on succinic dehydrogenase activity, was consistently less than 0.2% of that in the cell homogenate. The zymogen granule layer was collected, diluted with 10 volumes of homogenization buffer and centrifuged at 2,000 X g for 15 minutes to remove the Percoll. This wash procedure was repeated once and the resultant purified zymogen granules suspended in this buffer.

In the same continuous density gradient generated by centrifuging the 60% Percoll, a second zymogen granule fraction was observed at a density of 1.108. Based on amylase activity recovery of zymogen granules from this fraction of the Percoll gradient is 4-6% of that in the cell homogenate. The purity of this "low density" zymogen granule fraction has not been assessed.

2.03.3 Isolation of 20,000 X g Pellet and 100,000 X g Pellet

Other particulate fractions were collected by centrifugation of the 10,000 X g supernatant (Fig. 2). Centrifugation at 20,000 X g for 15

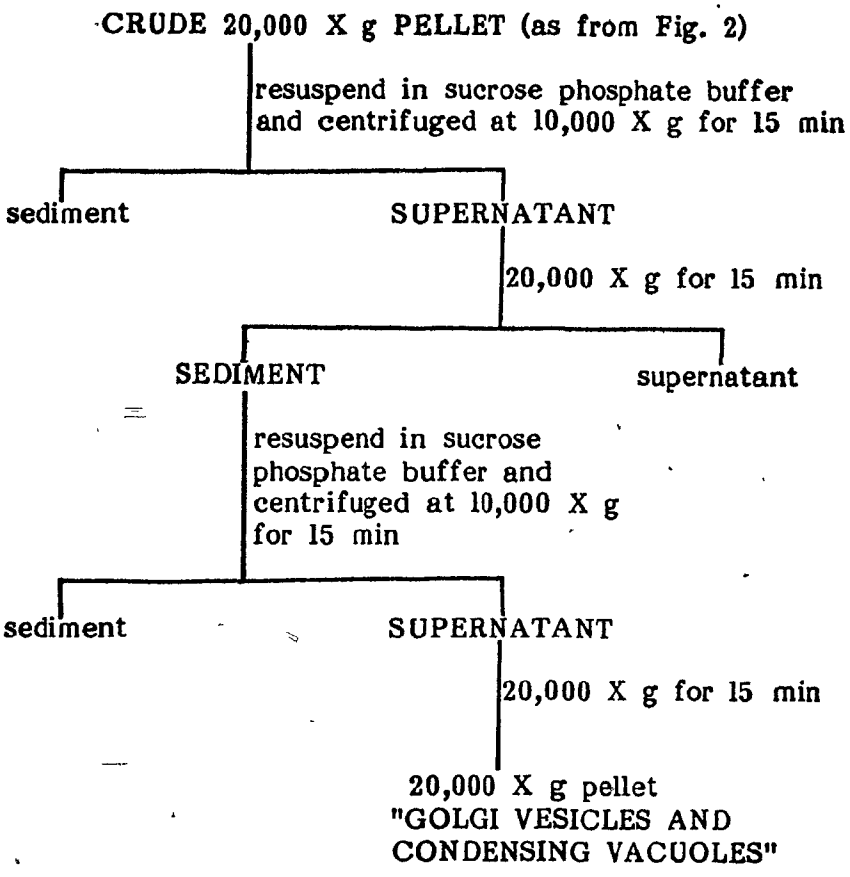
minutes produced a pellet enriched in Golgi vesicles and condensing vacuoles. The 20,000 X g supernatant was centrifuged at 100,000 X g for 60 minutes to produce a microsomal pellet. The 20,000 X g pellet was further purified by repeating the 10,000 X g and 20,000 X g centrifugation (Fig. 4A). The 100,000 X g pellet was also further purified by repeating the 20,000 X g and 100,000 X g centrifugation (Fig. 4B).

FIGURE 4 A centrifugation scheme for washing:

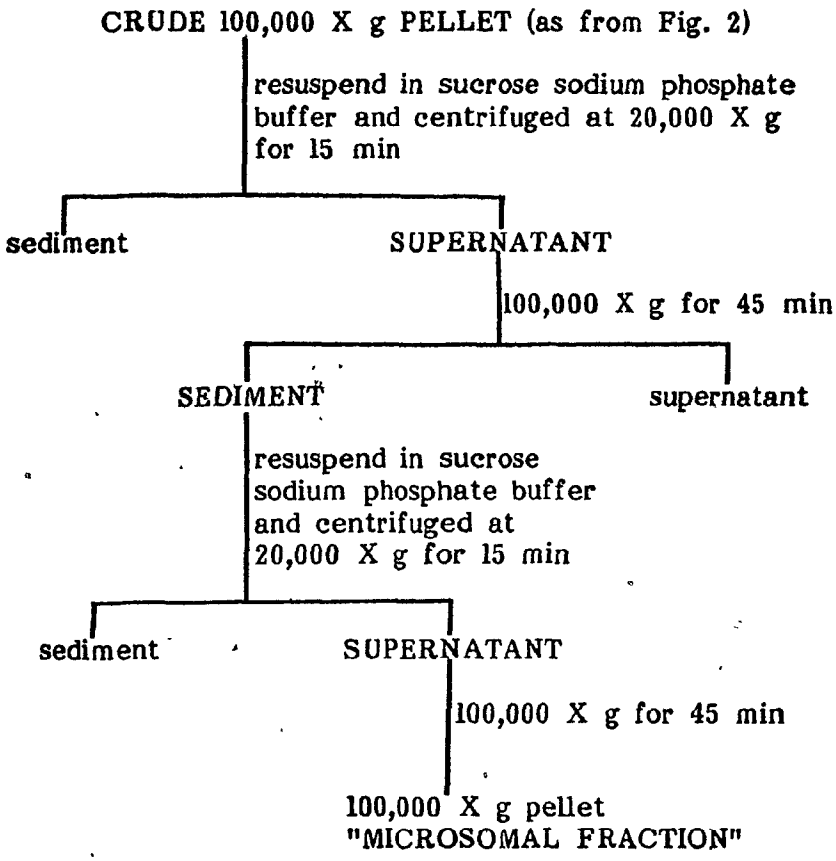
(A) 20,000 X g pellet, and

(B) 100,000 X g pellet

(A)


CRUDE 20,000 X g PELLET (as from Fig. 2)
resuspend in sucrose phosphate buffer and centrifuged at 10,000 X g for 15 min
sediment
SUPERNATANT
20,000 X g for 15 min
SEDIMENT
supernatant
resuspend in sucrose phosphate buffer and centrifuged at 10,000 X g for 15 min
sediment
SUPERNATANT
20,000 X g for 15 min
20,000 X g pellet
"GOLGI VESICLES AND CONDENSING VACUOLES"

(B)


CRUDE 100,000 X g PELLET (as from Fig. 2)
resuspend in sucrose sodium phosphate buffer and centrifuged at 20,000 X g for 15 min
sediment
SUPERNATANT
100,000 X g for 45 min
SEDIMENT
supernatant
resuspend in sucrose sodium phosphate buffer and centrifuged at 20,000 X g for 15 min
sediment
SUPERNATANT
100,000 X g for 45 min
100,000 X g pellet
"MICROSOMAL FRACTION"

2.04 INCORPORATION OF LABELLED 5-HT AND LABELLED PROTEIN INTO SUBCELLULAR COMPONENTS

Labelled 5-HT was measured in aliquots of the 180 X g supernatant, the purified zymogen granules, the 20,000 X g pellet and the 100,000 X g pellet as described in section 2.06. [^{3}H]protein was measured in aliquots of the subcellular fractions as described by Fast (1974). The protein was precipitated with cold trichloroacetic acid (TCA; 5% [w/v], final concentration). The precipitate was collected by centrifugation, dissolved in 0.15M NaOH and reprecipitated with TCA. This procedure was repeated three times. The final TCA precipitate was then dissolved in 0.15M NaOH and an aliquot was taken for measurement of radioactivity by liquid scintillation spectrometry as described below (section 2.10).

2.05 SECRETION FROM PRE-LABELLED DISPERSED ACINAR CELLS

Dispersed acinar cells were incubated for 60 minutes in incubation buffer II (section 2.02) to which had been added L-[^{3}H]leucine (50-60 Ci/mMole; 1μCi/ml) and either 1.0 mM L-[^{14}C]DOPA (4.0 mCi/mMole) or 1.0 mM L-[^{14}C]5-HTP (2.0 mCi/mMole) and soybean trypsin inhibitor 120 μg./ml. After 60 minutes incubation, the cells were separated from the medium by centrifugation, washed three times in incubation buffer II free of radiolabelled compounds but containing 1.0 mM L-[^{1}H]leucine, and 1.0 mM L-[^{12}C]DOPA or L-[^{12}C]5-HTP and incubated in the same buffer for 60 minutes. At the end of this chase-incubation, the cells were

washed in perfusion buffer (incubation buffer I, see section 2.02, modified by reducing the BSA to 0.001% [w/v]). The cells were then packed into a 1.5 cm X 8.0 cm column (PD-10) as described by Guderley and Heisler (1980).

Approximately 2.5 ml. of Biogel-P4 slurry (preswollen in 0.9% NaCl) was washed three times in perfusion buffer (above) and mixed with 2 X 10^7 cells that were suspended in 1.0 ml. perfusion buffer. The mixture was poured into the PD-10 column.

The entire cell column system was maintained at 37°C and the perfusion medium was continuously gased with 95% O_2/5% CO_2 to maintain the pH at 7.4 . The cell column was perfused at a rate of 40 ml/hr. and eluate fractions were collected at 6 minute intervals. After an equilibration perfusion period of between 2-2.5 hours, secretagogue, dissolved in perfusion buffer, was administered for 30 minutes. A 30 minute perfusion with buffer alone separated periods of perfusion with secretagogues. After termination of the perfusion, the viability of the cells was re-examined by Trypan Blue exclusion and the results of the experiment were used only if more than 90% of the cells were viable.

In aliquots of perfusate fractions âmylase activity was assayed as described in section 2.09.2; [^{3}H]protein was measured by adding BSA as protein carrier and proceeding as described in section 2.04; [^{14}C] 5-HT or [^{14}C] DA was measured as described in section 2.06.

2.06 EXTRACTION AND SEPARATION OF RADIOLABELLED BIOGENIC MONOAMINES AND THEIR PRECURSOR AMINO ACIDS

To extract biogenic monoamines, cold ($4^{o}C$) 0.4 M $HClO_4$ with ascorbic acid (1.0 g./ml.) was added to dispersed acinar cells, (section 2.02), subcellular fractions (section 2.03) or aliquots of the perfusate fraction (section 2.05). Precipitated proteins were centrifuged at 12,000 X g for 10 minutes at $4^{o}C$. The supernatant was collected, the pH adjusted to 2.0 with 2M KOH and the $KClO_4$ precipitate formed was removed by centrifugation at 14,000 X g for 10 minutes. The resulting supernatant was applied to a 0.5 X 8.0 cm column of Dowex 50WX4 (400 mesh).

The resin (500 gm.) of Dowex 50WX4 (400 mesh, H^+ form) was prepared as described by Atack (1977). It was washed successively in the following solutions: a) 2 liters of 2M NaOH + 1% [w/v] EDTA; b) 1-2 liters of deionized distilled water until a pH of 7.0 was attained; c) 1 liter of 2.4M HCl + 60% [w/v] ethanol; d) 2 liters of 2M HCl; e) 2 liters of H_2O. The resin was stored in deionized distilled water at pH 7.0.

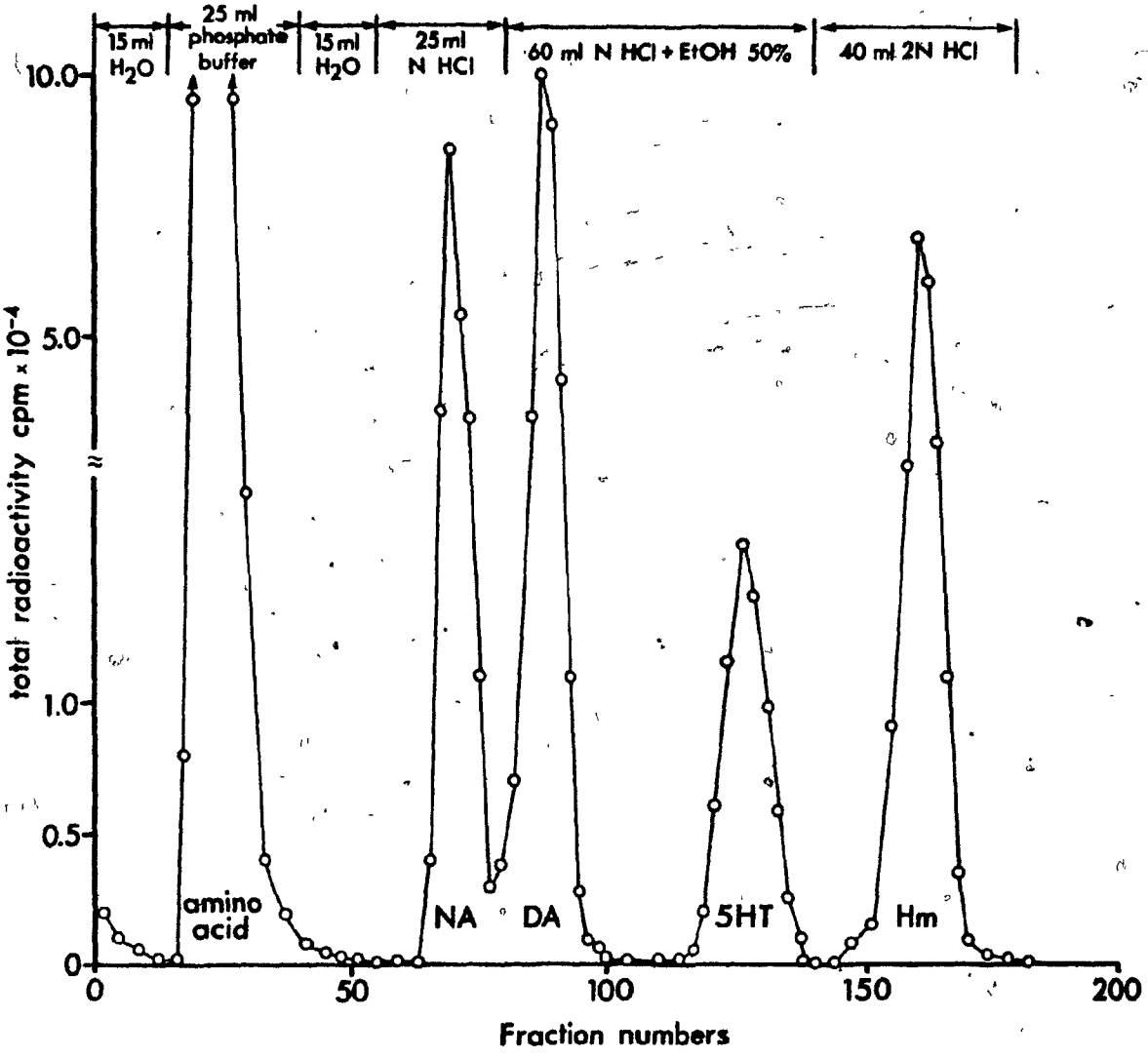
Before the samples were applied, the columns were washed with 20 ml. H_2O, pH 7.0, followed by 20 ml. 0.1M sodium phosphate buffer containing 0.1% EDTA, pH 6.5 . The columns were covered with aluminum foil to exclude light and were run at room temperature at a flow rate of 0.25 ml./min. After the sample was applied, the column was washed with 15 ml. water and eluted with successive washes as follows: 25 ml. sodium phosphate (0.1M, pH 6.5) containing 0.1% [w/v] EDTA; 15

ml. water; 25 ml. 1.0M HCl; 60 ml. of 1.0M HCl in 50% [v/v] ethanol; and 40 ml. of 2M HCl. One ml. fractions of effluent were collected and the radioactivity in each sample measured by liquid scintillation spectrometry (section 2.10). The amino acids (L-DOPA and L-5-HTP, as well as Trp., Tyr., His., and Leu.) were eluted with the sodium phosphate buffer (above), noradrenaline (and adrenaline) eluted with 1M HCl, dopamine and 5-HT both eluted with ethanolic HCl, and histamine was eluted with 2M HCl. The amino acid precursors and their respective amines were sufficiently well separated from each other to be easily identified and precisely quantitated. The elution profile is illustrated in Fig. 5. Using radiolabelled standards, it was determined that recovery from the column was: amino acids (L-DOPA and 5-HTP), 98%; noradrenaline, 95%; dopamine, 99%; 5-HT, 99%; histamine, 83%. The amino acids and biogenic monoamines, after column elution, were identified with authentic standards by thin layer chromatography (silica gel 60F-254) in solvent system - butanol:acetic acid:water (25:4:10 [v/v/v]).

FIGURE 5 The elution pattern of amino acids and amines from DOWEX 50W X 4 column

Radiolabelled standards: L-[^{3}H] Leucine; L-[^{14}C] Tyr; L-[^{14}C] DOPA; DL-[^{14}C] 5-HTP; [^{3}H] NA; [^{3}H] Ad; [^{3}H] DA; [^{3}H] 5-HT and [^{3}H] Hm were chromatographed on a 0.5 X 8.0 cm DOWEX 50WX4 column at a flow rate of 0.25 ml/min. Fraction volume was 1.0 ml. Details for elution of the amino acids and amines are given in the text. The recovery from the column was: amino acids (L-DOPA and 5-HTP) 98%; NA, 95%; DA, 99%; 5-HT, 99%; Hm, 83%.

NA = noradrenaline
DA = dopamine
5-HT = 5-hydroxytryptamine
Hm = histamine


15 ml H2O
25 ml phosphate buffer
15 ml H2O
25 ml N HCl
60 ml N HCl + EtOH 50%
40 ml 2N HCl
10.0
5.0
1.0
0.5
0
total radioactivity cpm x 10-4
amino acid
NA
DA
5HT
Hm
0
50
100
150
200
Fraction numbers

2.07 FLUORIMETRIC ASSAY FOR 5-HT

Tissues (dispersed acinar cells, isolated zymogen granules and rat brain) were homogenized with a Polytron Homogenizer (Brinkman Co.) at top speed in 0.4M perchloric acid (5-7 ml. per gm. tissue) for 30 seconds at 4°C. Authentic 5-HT was added as an internal standard to the homogenate for calculation of recovery of the monoamine. After the addition of 0.01 ml. per ml. 5% [w/v] $Na_2S_2O_5$ homogenate and 0.02 ml. 10% [w/v] EDTA per ml. homogenate, the homogenate was centrifuged at 14,000 X g for 10 minutes at 4°C, the supernatant was collected, and the pH was adjusted to 2.0 with 2.5M K_2CO_3. The resulting $KClO_4$ precipitate was removed by centrifugation at 14,000 X g for 10 minutes. The supernatant was then subjected to column chromatography on Dowex 50WX4 as described in section 2.06. The 5-HT was eluted with 1.0M HCl in 50% [v/v] ethanol and was assayed by the method described by Atack and Lindqvist (1973) as follow:

Sample preparation - to 1.0 ml. aliquots of column eluate was added 0.1 ml. of each of 0.6% [w/v] cysteine-HCl and 0.2% [w/v] $K_3[Fe(CN)_6]$, 1.2 ml. of Ultrex (Baker Chemical Co.) purified 12M HCl (Fe^{+3} ion free) and 0.05 ml. of 0.3% [w/v] orthophthaldialdehyde [OPT] (dissolved in absolute methanol).

Oxidized sample blank preparation - to 1.0 ml. aliquots of column eluate was added 0.2 ml. of purified 12M HCl and 0.05 ml. 0.2% [w/v] $K_3[Fe(CN)_6]$. After 10 minutes on ice, 0.05 ml. 6% [w/v] cysteine-HCl, 1.0 ml. of purified 12M HCl and 0.05 ml. 3% [w/v] OPT were added to the oxidized sample blank.

Both sample and oxidized sample blank were incubated at 78°C for 20 minutes, cooled to room temperature and the fluorescence of the OPT-5-HT condensate was measured in an Aminco SPT-125 spectrophotflurorometer at 360 nm (excitation)/480 nm (emission). The fluorescence of OPT-5-HT condensate in the sample was obtained by subtracting that of the sample from that of the oxidized sample blank. 5-HT recovery was calculated as follows:

$$\% \text{ recovery of 5-HT} = \frac{\left[\text{the fluorescence of sample with internal standard}\right] - \left[\text{the fluorescence of sample alone}\right]}{\left[\text{the fluorescence of external standard}\right]} \times 100$$

The fluorimetric assay is linear for 5-HT from 10 to 500 ng.

2.08 H.P.L.C.-ELECTROCHEMICAL DETECTOR METHOD OF MEASURING BIOGENIC MONOAMINES

A preliminary study to determine if the exocrine pancreas contained endogenous DA and/or 5-HT was done with the assistance of Dr. S. Young at the Allan Memorial Hospital, McGill University.

The method of biogenic monoamine separation by H.P.L.C (high performance liquid chromatography) and quantitation with an electrochemical detecting system was adapted from that described by Anderson and Purdy (1979); Moyer (1978); Felice et al. (1978) and Ikenoya and co-workers (1978).

Tissue or dispersed cells (0.2-0.4 gm. wet weight) were homogenized

in cold (4°C) 0.1M HCl (3 ml.) with 1 mg/ml. ascorbic acid (0.3 ml.). Epinine was added to the homogenate as internal standard for peak height calibration and authentic monoamines (5-HT,DA) were also added for calculation of recovery. The homogenate was then mixed with perchloric acid (0.3 ml.) and the precipitated protein was removed by centrifugation at 10,000 X g for 15 minutes. To the supernatant was added 1M KOH to adjust the pH to 4.0. The resulting $KClO_4$ precipitate was removed by centrifugation at 14,000 X g for 15 minutes and the supernatant was applied to an Amberlite CG50 8.0 cm X 0.5 cm column (prewashed with 1M acetate buffer). The Amberlite column was then washed with water (6 ml.) and the amines eluted with 1.2M HCl (4 ml.).

Biogenic monoamines were separated on an ion-paired reverse phase column under the following chromatographic conditions - stationary phase: 30 cm X 3.9 mm Bonda pack C_{18} (Water associates); mobile phase: 0.07M sodium phosphate buffer with 0.1 mM EDTA, 0.25 mM sodium octylsulfate and 8% [v/v] methanol, pH 5.0 at a flow rate of 2.0 ml./min and ambient temperature. The detection system consisted of carbon paste electrode with potential 0.72V vs Ag/AgCl reference electrode (Ikenoya et al., 1978).

The ratio of the peak height for a biogenic monoamine to that of the internal standard (epinine) was calculated for each sample and the recovery of the monoamine was calculated as follows:

$$\text{recovery of biogenic monoamine} = \frac{\left[\frac{\text{peak height sample + standard}}{\text{peak height epinine}}\right] - \left[\frac{\text{peak height sample}}{\text{peak height epinine}}\right]}{\left[\frac{\text{peak height standard}}{\text{peak height epinine}}\right]}$$

The assay is linear for DA between 20 - 100 pg and 5-HT between 50 pg - 2 ng.

Although this method was considerably more sensitive than the fluorimetric method it was not reliable or reproducible because of the inherent instability of the electrode. For this reason, most amine determinations described in this thesis were done with the fluorimetric technique.

2.09 ASSAY FOR AMINO ACID DECARBOXYLASE ACTIVITY, AMYLASE ACTIVITY, SDH ACTIVITY, LDH ACTIVITY, DNA AND PROTEIN

2.09.1 Assay for Amino Acid Decarboxylase Activity

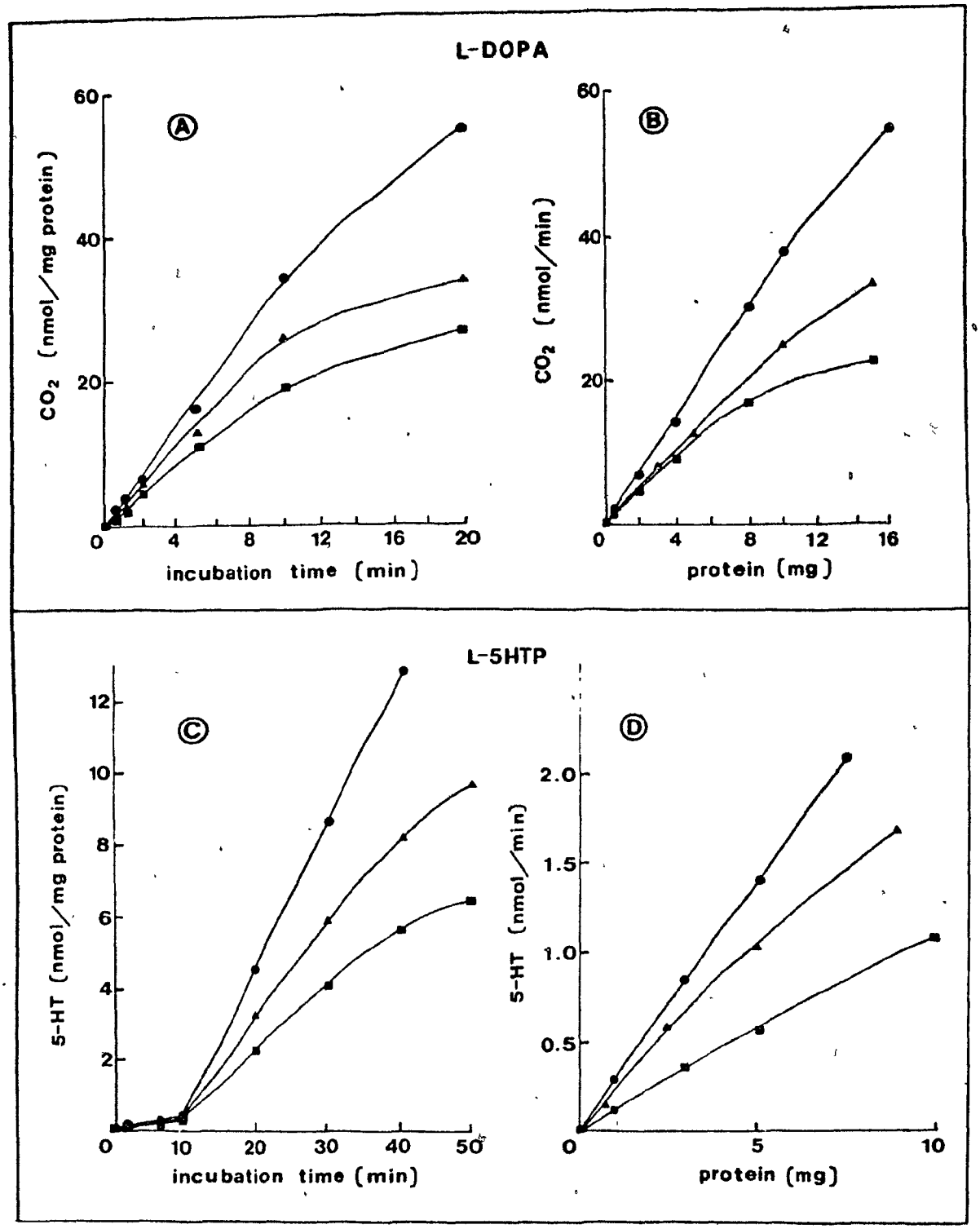
Dispersed acinar cells were homogenized in 0.01M sodium phosphate buffer containing 0.25M sucrose and soybean trypsin inhibitor 12 mg./100 ml., pH 7.0. The 100,000 X g supernatant was prepared according to the general scheme described in section 2.03.

Amino acid decarboxylase activity was measured by [^{14}C] carbon dioxide released from L-[^{14}C-carboxyl] amino acids by the action of the decarboxylase. The procedure was adapted from those described by Rhoads and Udenfriend (1968), Ellenbogen et al. (1969) and Christenson et al. (1970). Samples were incubated at 37^{o}C in 2 ml. isotonic sucrose with 10 mM sodium phosphate buffer (above buffer pH 7.0) in Erlenmeyer flasks equipped with center wells in which was placed a piece of filter paper saturated with hyamine hydroxide. The assay reaction was started

with the addition of L-[^{14}C]-amino acids and was terminated by injecting 0.4 ml. 50% TCA into the main compartment of the incubation flask. After addition of TCA, the incubation was continued at 37°C for 10 minute in order to insure complete CO_2 absorption. Prolongation of the post incubation period did not result in further CO_2 absorption. After the 10 minute post incubation time the filter was removed from the center well and the radioactivity measured by liquid scintillation spectrometry. The rate of decarboxylation activity is linear up to 10 minutes incubation (Fig. 6A) and 8 mg protein (Fig. 6B) at DOPA concentration 10^{-5}M to 10^{-3}M. For 5-HTP the rate is linear from 10 to 40 minutes incubation (Fig. 6C) and 5 mg protein (Fig. 6D) at 5-HTP concentration 10^{-5}M to 10^{-3}M.

FIGURE 6 DOPA and 5-HTP decarboxylation as a function of incubation time and protein cencentration

The 100,000 X g supernatant was isolated from dispersed acinar cells. DOPA and 5-HTP decarboxylase activity was measured as a function of incubation time (FIG 6A, C) and protein concentration (FIG 6B, D) at different concentations of L-[^{14}C-carboxyl] DOPA and L-[^{14}C] 5-HTP (10^{-5}M ■——■ ; 10^{-4}M ▲——▲; 10^{-3}M ●——●). The rate of DOPA decarboxylation was assessed by measuring the rate of CO_2 evolution and the rate of 5-HT production was used as a measure of the rate of 5-HTP decarboxylation. Each point is the mean of two experiments.


L-DOPA
A
B
CO_2 (nmol/mg protein)
CO_2 (nmol/min)
incubation time (min)
protein (mg)
L-5HTP
C
D
5-HT (nmol/mg protein)
5-HT (nmol/min)
incubation time (min)
protein (mg)

2.09.2 Amylase Activity Assay

Amylase activity was assayed with the Phadebas Amylase Test kit based on the method developed by Ceska et al. (1969). In this method, starch, which is the substrate for the enzyme, is coupled with a blue dye. As the starch is digested, dye is solubilized and the rate of release of dye is measured with a spectrophotometer.

Phadebas pills were dispersed in distilled water (one pill per 10 ml. distilled water), mixed thoroughly and transferred (1.0 ml.) to the reaction tubes. The reaction tubes were then preincubated for 5 minutes at 37°C. To the reaction tubes, aliquots of 50 µl. samples were added, mixed and incubated for exactly 15 minutes. The reaction was terminated by addition of 250 µl. of 5M NaOH and the reaction tubes were centrifuged at 10,000 X g for 10 minutes to pellet the undigested starch-dye complex. The solubilized dye quantitated by measuring its absorbance at 620 nm.

Amylase activity was expressed in International Unit (I.U.). One International Unit of amylase activity is defined by the Commission on Clinical Enzyme Units of the International Union of Biochemistry as the amount of enzyme that will catalyze the hydrolysis of 1 µmole of glucosidic linkages per minute at 37°C.

2.09.3 Lactate Dehydrogenase Assay

Lactate dehydrogenase activity was assayed according to the conditions described by Kornberg (1955) and modified by Fast (1974). A sample of zymogen granule fraction (0.1 ml.) was added to the

cuvette containing 0.1 ml. sodium pyruvate (0.01M), 0.1 ml. NADH (0.002M, pH 8.0) and 2.7 ml. sodium phosphate buffer (0.03M, pH 7.4). Lactate dehydrogenase activity was assayed by measuring the rate of oxidation of NADH. The change in absorbancy at 340 nm was recorded for 3 minutes at 25°C. A unit of activity is defined as that which caused an initial rate of oxidation of one micromole NADH per minute under conditions described above.

2.09.4 Succinic Dehydrogenase Assay

Succinic dehydrogenase activity was assayed under aerobic conditions as described by Bonner (1955) and modified by Maeno et al. (1971). The reaction mixture (total volume 1.0 ml.) contained 100 μmoles of sodium phosphate buffer, pH 7.2, 10 μmoles of sodium cyanide, 0.14 μmole of 2,6-dichloroindophenol, 26 μmoles of sodium succinate, and the sample (50 μg.-150 μg. protein). Succinic dehydrogenase activity was assayed by following the decrease in optical density at 600 nm. The change in optical density was recorded for 3 minutes at 25°C. A unit of activity is defined as that which caused an initial rate of reduction of one nmole of 2,6-dichloroindophenol per minute under conditions described above.

2.09.5 DNA Determination

The DNA content of cell homogenates and isolated zymogen granule fractions were determined by the method of Burton (1956).

The samples were homogenized in TCA (final TCA concentration 5% [w/v]), heated for 30 minutes at 90°C, cooled to room temperature and centrifuged at 10,000 X g for 15 minutes to sediment the precipitated protein. The supernatants were kept for DNA assay.

To 1.0 ml. of the TCA extract was added 1.0 ml. of the diphenylamine reagent (2 gm. diphenylamine in 45 ml. glacial acetic acid and 5 ml. concentrated H_2SO_4). The mixture was heated at 90°C for 60 minutes, cooled to 25°C and the intensity of the colour that developed was measured at 600 nm. Calf thymus DNA was used as the standard in a concentration range of 50-300 µg./ml.

2.09.6 Protein Determination

Protein concentrations of the various subcellular fractions were determined by the method of Lowry et al. (1951) with BSA as a standard.

2.10 DETERMINATION OF RADIOACTIVITY

Radioactivity was determined by liquid scintillation spectrometry using Formula-950A as the solvent system. Samples were corrected for quenching by using internal standards. [^{14}C] isotope was determined with close to 70% efficiency and [^{3}H] isotope was about 30% efficiency. In double-label counting, external standards were used to determine the correction factor for [^{14}C] spill into the [^{3}H] channel and to eliminate [^{3}H] spill into [^{14}C]

channel. The efficiency of counting of [^{3}H] was about 15% and [^{14}C] was about 30%.

2.11 STATISTICAL ANALYSIS

All results were expressed as mean ± S.E. or else will be specified in figure legends.

Wilcoxon signed rank-test was used for evaluating the result in experiments involving the measurement of 5-HT in acinar cells compared to that in zymogen granules.

Kruskal-Wallis multiple comparison test was used to assess any statistical difference between [^{3}H]/[^{14}C] ratio obtained from zymogen granules fractions, and secretory product from the first stimulation and secretory product from the second stimulation by caerulein.

2.12 MATERIALS

From New England Nuclear, 575 Albany St., Boston, Mass. 02118, U.S.A.

DL-5-hydroxy [2-^{14}C] tryptophan	S.A. 59.2 mCi/mmole
DL-[side chain 3-^{14}C] tryptophan	S.A. 0.05 mCi/0.2mg
L-[^{14}C(U)] -leucine	S.A. 342 mCi/mmole
L-[4.5 ^{3}H(N)] -leucine	S.A. 52.2 Ci/mmole
L-[^{14}C(U)] -tyrosine	S.A. 450 mCi/mmole
L-[3-^{3}H] -histidine	S.A. 300 mCi/mmole
[^{3}H-(G)] -tyramine	S.A. 5 μCi/mmole

From Amersham Corp. 505 Iroquois Shore Rd. Oakville, Ontario, Canada

L-3,4-dihydroxyphenyl [3-^{14}C] alanine	S.A. 6.7 mCi/mmole
L-3,4-dihydroxyphenyl [1-^{14}C] alanine	S.A. 7.9 mCi/mmole
L-[carboxyl-^{14}C] tyrosine	S.A. 5.7 mCi/mmole
DL-5-hydroxy [G-^{3}H] tryptophan	S.A. 5.0 mCi/mmole
L-[carboxyl-^{14}C] histidine	S.A. 50 mCi/mmole
L-[carboxyl-^{14}C] tryptophan	S.A. 55 mCi/mmole

From Sigm a Chemical Co. P.O. Box 14508, St. Louis, MD 63178, U.S.A.

5-hydroxy-tryptophan(5-HTP)

5-hydroxy-tryptamine hydrochloride(5-HT)

D-beta-3,4-dihydroxyphenylalanine(D-DOPA)

L-beta-3,4-dihydroxyphenylalanine(L-DOPA)

L-tryptophan

L-tyrosine

L-histidine

L-cysteine

Soybean trypsin inhibitor (STI)

Orthophthaldialdehyde(OPT)

Potassium ferricyanide ($K_3[Fe(CN)_6]$)

Collagenase

Bovine serum albumin(BSA)

Diphenylamine

2,6-dichloroindophenol

Sodium cyanide

Sodium succinate

Nicotinamide adenine dinucleotide (NADH)

N-Methyldopamine (Epinine)

From Bio-Rad Laboratories, 32nd Griffin, Richmond, Calif.

Bio-Gel P-4 100-200 mesh

Dowex 50WX4 200-400 mesh, H^+ form

From Pharmacia (Canada) Ltd. 2044 St. Regis Blvd.,
Dorval, Quebec

Phadebas Amylase Test Kit

Sephadex G-25M column PD-10

Percoll

From Boehringer-Mannheim, St. Laurent, Quebec, Canada

Hyaluronidase

From Sandev Ltd., Gilston Park, Harlow

m-hydroxy-p-bromobenzyl-oxyamine (NSD-1055)

From Regis Chemical Co.

Pargyline hydrochloride

From Grand Island Biological Company of Canada, Ltd.

MEM amino acids solution (50X)

From BDH Chemicals, Montreal, Canada

Sodium metabisulphite

Deoxyribonucleic acid, sodium salt

Amberlite CG-50

From PCR Research Chemical Inc. Gainesville, Florida

Prosil - 28

From Eastman Kodak Co., Rochester, N.Y. 14650, U.S.A.

Sodium octylsulfate

From Brinkmann, Rexdal Ontario

Pre-coated TLC plates silica GEL 60F-254

All other chemicals used were obtained from Baker Analyzed Quality and Fisher Scientific Co.

APPENDIX

(a) Incubation media for pancreatic acinar cell isolation:

Solution A contains: NaCl, 118.5 mM; KCl, 4.7 mM; KH_2PO_4, 1.2 mM; Mg_2SO_4, 1.2 mM; $CaCl_2$, 0.1 mM; $NaHCO_3$, 23.7 mM; soybean trypsin inhibitor, 12 mg./100 ml.; D-glucose, 15 mM; pH 7.4

Solution B contains: NaCl, 118.5 mM; KCl, 4.7 mM; KH_2PO_4, 1.2 mM; EDTA, 2 mM; $NaHCO_3$, 23.7 mM; soybean trypsin inhibitor, 12 mg/100 ml.; D-glucose, 15 mM; pH 7.4

Solution C contains: same as solution A with the addition of complete amino acid supplement solution [see (b)], 2 ml./100 ml. and 1% [w/v] BSA, pH 7.4

(b) Complete Amino Acid Supplement Solution:

Amino acids	Concentrations in mM
L-Alanine	15.60
L-Arginine	4.62
L-Aspartic acid	12.10
L-Cysteine $HCl.H_2O$	0.18
L-Glutamate (Na).H_2O	3.74
L-Glutamine	10.00
Glycine	9.61
L-Histidine	1.35

L-Isoleucine	6.56
L-leucine	10.90
L-Lysine HCl	7.02
L-Methionine	2.61
L-Phenylalanine	4.92
L-Proline	6.34
L-Serine	7.50
L-Threonine	6.02
L-Tryptophan	0.90
L-Tyrosine	1.50
L-Valine	8.70

(c) Incubation buffer I contains: NaCl, 118.5mM; KCl, 5.9 mM; $CaCl_2$, 1.0 mM; $NaHCO_3$, 23.7 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM; D-glucose, 15 mM; 1% [w/v] BSA; pargyline, 0.1 mM; ascorbic acid, 1 µg/ml. and complete amino acid solution, 2 ml./100 ml.

(d) Homogenization buffer contains: Sucrose, 0.25M; pargyline, 0.1 mM; NSD-1055, 0.1 mM; ascorbic acid, 1.0 µg./ml. and sodium phosphate buffer, 0.01M, pH 6.0

SECTION THREE

RESULTS

It is known that islet cells contain varying amounts of biogenic monoamines, particularly DA and 5-HT (Cegrell, 1968; Pearse and Takor Takor, 1979). To study the metabolism of DA and 5-HT in exocrine pancreas, it is therefore imperative that the acinar cell preparation be free of contaminating islet tissue capable of accumulating and subsequently releasing biogenic monoamines. This problem was approached in several ways: Firstly, as indicated in Methods the rat was chosen as the source of acinar cells because in this animal pancreatic islet cells contain little, if any, demonstrable 5-HT and DA (Falck and Hellman, 1963; Cegrell, 1968; Lundquist et al., 1975; Glyfe,1977); secondly, acinar cell preparations were examined by electron microscopy, and in the preparations used there were no identifiable cells other than exocrine acinar cells; finally, the Percoll gradient used to purify zymogen granules (described in Method section 2.03.2) would easily separate zymogen granules (density 1.108 - 1.142) from the denser insulin containing granules (density 1.208 -1.258; Lazarow et al., 1964; Randall and Shaw, 1964). Thus, it is concluded that the acinar cell and zymogen granule preparations used in the experiments reported in this thesis were free of islet cell contamination.

3.1 THE PRESENCE OF ENDOGENOUS 5-HT IN EXOCRINE PANCREAS

H.P.L.C. coupled with an electrochemical detector was used in the initial efforts to detect DA and 5-HT in rat pancreas. In the pancreas, 5-HT was found but there was no detectable DA. The 5-HT concentration in pancreas was found to be 0.6 nmoles/gm wet weight and in brain 2.9 nmoles/gm wet weight.

Using the fluorometric assay, the 5-HT content of whole rat brain, isolated pancreatic acinar cells and purified zymogen granules was measured in 8 separate experiments (Table 1). Acinar cells contained 10.86 $\pm$ 2.52 pg/unit of amylase; approximately 0.33nmoles/gm wet weight whole pancreas. Whole brain was found to contain 334.55 $\pm$ 7.51 ng/gm wet weight (1.9 nmoles/gm tissue wet weight) which is approximately 6 times the amount of 5-HT found in pancreatic acinar cells. Zymogen granules purified from isolated acinar cells contained 10.7 $\pm$ 3.06 pg/unit of amylase and there is no statistical difference ($p > 0.01$), in 5-HT pg/unit amylase activity, between acinar cells and zymogen granules. since 95% of the total acinar cell amylase activity is in zymogen granules (Bendayan, unpublished observation), thus the present result suggests that the 5-HT in isolated acinar cells was located exclusively in the zymogen granules.

TABLE 1 5-HT IN RAT BRAIN, PANCREATIC ACINAR CELLS AND ZYMOGEN GRANULES FROM PANCREATIC ACINAR CELLS

Experiment Number	Brain ng/ g wet weight	Acinar Cells pg/unit amylase	Zymogen Granules pg/unit amylase
1	232.70	7.94	5.34
2	309.88	10.60	10.65
3	304.34	10.60	10.66
4	335.80	11.98	15.53
5	331.80	8.48	9.47
6	397.00	8.70	8.90
7	470.22	13.84	13.58
8	294.68	14.76	11.45
Mean ± S.D.	334.55 ± 71.51	10.86 ± 2.52	10.70 ±3.06

5-HT was extracted and measured according to the Methods as described in sections 2.06 and 2.07.

Statistical analysis (*$p > 0.01$)

*p Wilcoxon signed rank-test.

3.2 CAN ACINAR CELLS ACCUMULATE 5-HT AND 5-HTP?

The presence of endogenous 5-HT in acinar cell zymogen granules prompted the investigation of how 5-HT was incorporated into these organelles. As illustrated in Fig. 7A, 5-HT was rapidly taken up by dispersed pancreatic acinar cells. Although the rate of uptake decreased with time, the intracellular 5-HT concentration continued to increase even after 2 hours of incubation. The amine rapidly accumulated in zymogen granules; after 40-60 minutes of incubation, these granules, which make up only 12.4% of the rat acinar cell volume (Nevalainen, 1970), contained 45% of the intracellular 5-HT.

Acinar cells also rapidly accumulated 5-HT when incubated with 5-HTP as illustrated in Fig. 7B. The 5-HTP was taken up, decarboxylated and the resultant 5-HT was concentrated in the zymogen granule fraction of the cells. At any time during the incubation, more than 98% of the radioactivity recovered from zymogen granules was identified as 5-HT; 5-HTP or other metabolites were never detected in this subcellular fraction. The proportion of the total cell radioactivity which was 5-HT varied from 22.7% after 30 minutes incubation to 71% after 90 minutes. Again the accumulation of 5-HT in the zymogen granule fraction was 3 to 5 times what would be predicted if the amine distributed randomly throughout the cell volume; after 60 minutes incubation, 45% of the total cell radioactivity was 5-HT and of that total more than 50% was in zymogen granules. The present results indicate that 5-HT can be taken up by acinar cells and incorporated into zymogen granules. When 5-HTP is used as a substrate, the rate of incorporation of 5-HT into the

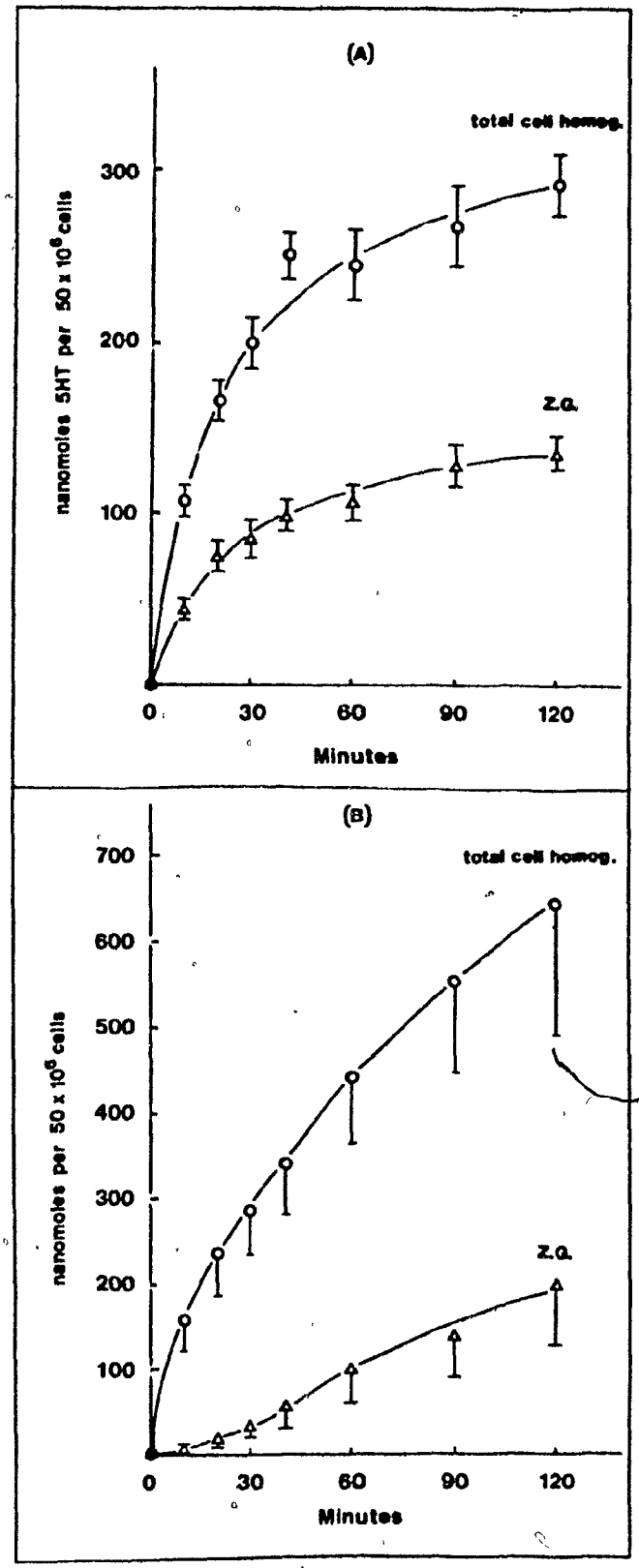
zymogen granules is determined by the rate of 5-HT synthesis.

FIGURE 7 (A) Time course of accumulation of 5-HT by dispersed acinar cells

Acinar cells (50 X 10^6) were incubated with [^{14}C] 5-HT (10^{-3} M) for 2 hours in incubation buffer I. (O) shows the total cell homogenate 5-HT and (Δ) shows the isolated zymogen granule 5-HT. Each point on the graph represents the mean ± S.E.M. of 5 experiments.

(B) Time course of accumultion of 5-HTP and 5-HT by dispersed acinar cells and 5-HT by isolated zymogen granules

Acinar cells (50 X 10^6) were incubated with L-[^{14}C] 5-HTP (10^{-3} M) for 2 hours in incubation buffer I. (O) shows the total cell homogenate 5-HTP and 5-HT, and (Δ) shows the isolated zymogen granule 5-HT. Each point on the graph represents the mean ± S.E.M. of 5 experiments.


(A)
total cell homog.
Z.G.
nanomoles 5HT per 50 x 10⁶ cells
300
200
100
0
30
60
90
120
Minutes
(B)
total cell homog.
Z.G.
nanomoles per 50 x 10⁶ cells
700
600
500
400
300
200
100
0
30
60
90
120
Minutes

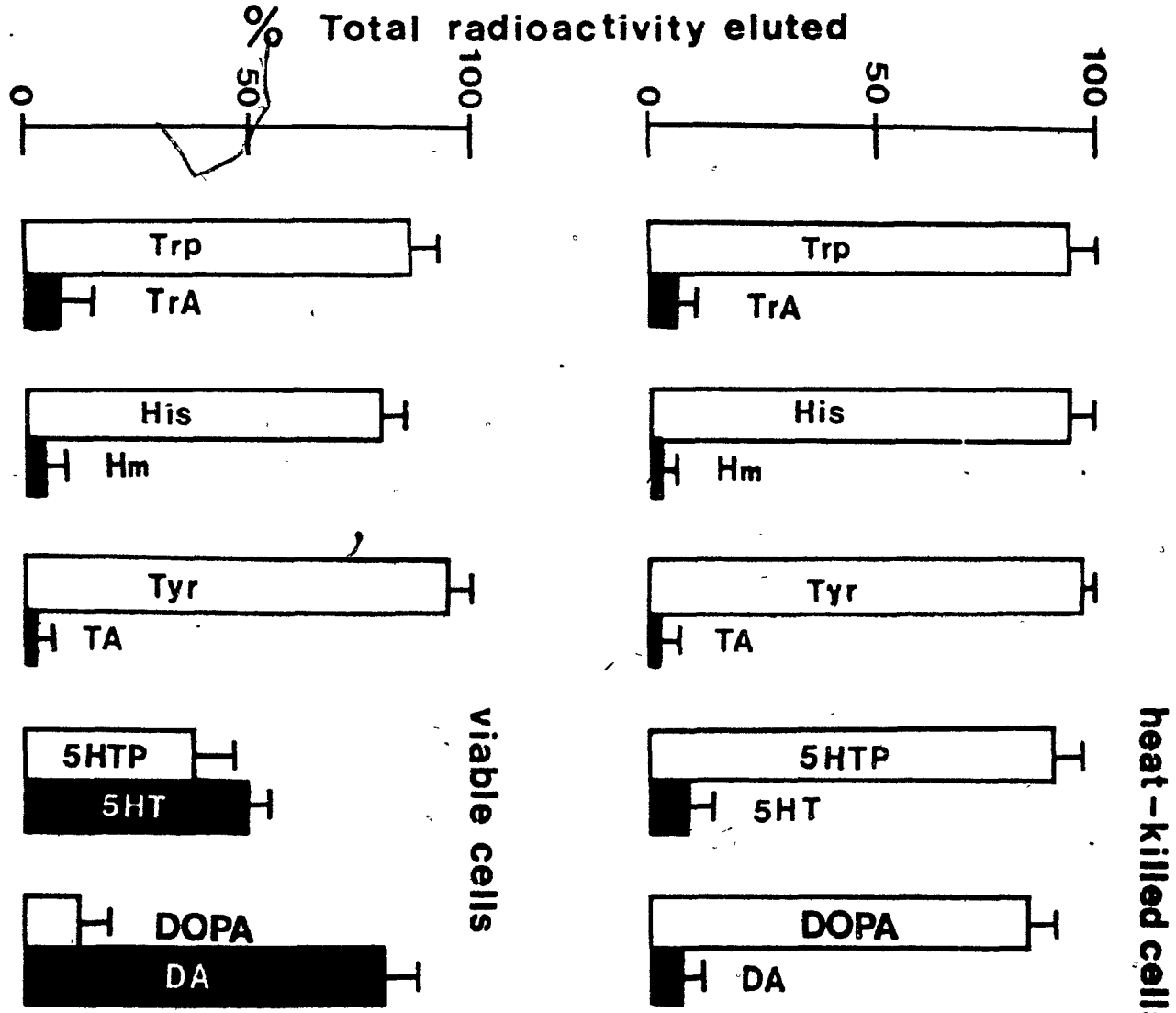
3.3 DOPA/5-HTP DECARBOXYLASE IN EXOCRINE PANCREAS

3.3.1 Amino Acid Decarboxylation in Acinar Cells

Dissociated acinar cells were incubated with labelled amine precursors: L-Tyrosine (L-Tyr.), L-Tryptophan (L-Trp.), L-Histidine (L-His.), L-DOPA and DL-5-HTP for 30 minutes and the accumulation of decarboxylation products were measured as described in Methods. As illustrated in Fig. 8, only DL-5-HTP and L-DOPA were decarboxylated to form 5-HT and DA respectively. During the 30 minutes incubation, approximately 70% of the L-DOPA was converted to DA and 50% of DL-5-HTP was converted to 5-HT. There was no detectable conversion of DA to noradrenaline or adrenaline. When Tyrosine was used as a substrate, there was neither formation of Tyramine nor dopamine. Similarly, Tryptophan was not converted to Tryptamine or 5-HT.

FIGURE 8 The conversion of L-amino acids into their corresponding amines in pancreatic acinar cells

Acinar cells (50 X 10^6) were incubated with various L-amino acids: L-[^{14}C] Tryptophan (2.8 X 10^{-5}M, 45 mCi/mmole); L-[^{3}H] Histidine (2.7 X 10^{-5} M, 300 mCi/mmole); L-p-[^{14}C] Tyrosine (2.6 X 10^{-4}M, 450 mCi/mmole); DL-[^{14}C] 5-HTP (1.8 X 10^{-5}M, 59.2 mCi/mmole)and L-[^{14}C] DOPA (3.8 X 10^{-5}M, 6.7 mCi/mmole) for a period of 30 minutes in incubation buffer I. Heat-killed acinar cells, upper panel; viable cells, lower panel. At the end of the incubation, the amino acids and the accumulated decarboxylation products were separated by DOWEX 50WX4 column as described in Methods. The results are expressed as % of total radioactivity eluted from the column as labelled amino acids and labelled amines. Each bar represents the mean $\pm$ S.E.M. of 4 experiments.


% Total radioactivity eluted
0
50
100
Trp
TrA
His
Hm
Tyr
TA
5HTP
5HT
DOPA
DA
viable cells
heat-killed cells

3.3.2 Characteristics of DOPA/5-HTP Decarboxylase

3.3.2.1 Subcellular distribution

DOPA/5-HTP decarboxylase activity was assessed in the following subcellular fractions: zymogen granule fraction, 10,000 X g supernatant and pellet, 20,000 X g supernatant and pellet, 100,000 X g supernatant and pellet. DOPA decarboxylase activity was assessed by measuring the amount of radiolabelled CO_2 produced from carboxyl-labelled DOPA while 5-HTP decarboxylase was assessed by measuring the production of 5-HT. Fig. 9A and 9B illustrate that both DOPA decarboxylase and 5-HTP decarboxylase are located exclusively in the 100,000 X g supernatant, i.e. the cytosol fraction. The decarboxylation reaction exhibited no requirement for added pyridoxal-5-phosphate (Table 2), a co-factor for decarboxylation of several amino acids (Lehninger, 1979).

FIGURE 9 Distribution of DOPA decarboxylase (9A) and 5-HTP decarboxylase (9B) in subcellular fractions of acinar cells

The 100,000 X g supernatant (sup.); 100,000 X g pellet; 20,000 X g supernatant; zymogen granule fraction and 400 X g supernatant were isolated from dispersed acinar cells by differential centrifugation (see Methods section 2.03). Each subcellular fraction was incubated with (A) 1 mM L-[^{14}C-carboxyl] DOPA for 10 minutes, (B) 0.1 mM L-[^{14}C] 5-HTP for 30 minutes in isotonic sucrose containing 10 mM sodium phosphate pH 7.0 (see Methods 2.09.1) at 37°C. For DOPA decarboxylation the evolution of carbon dioxide was measured and for 5-HTP decarboxylation, 5-HT was analysed after separating 5-HTP from 5-HT in DOWEX 50WX4 column. Each point is the mean of 3 experiments.

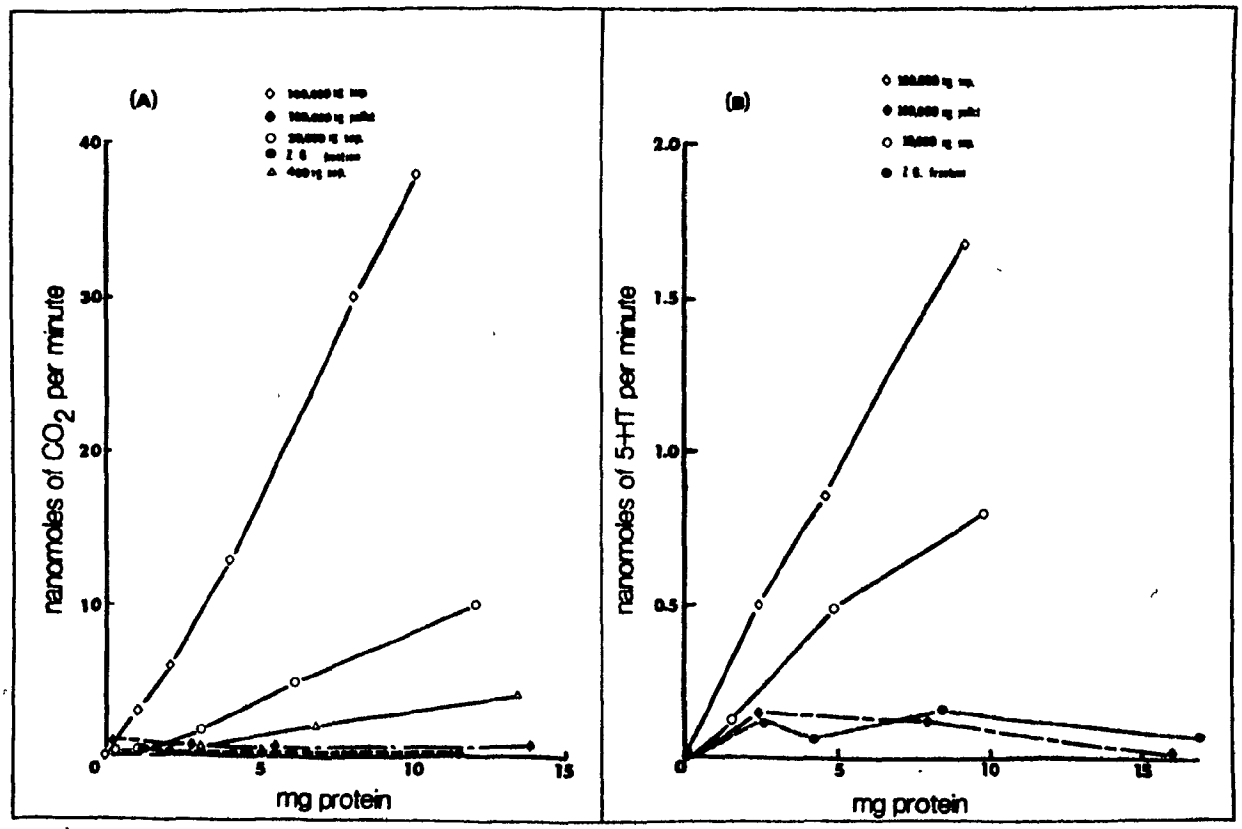

(A)
nanomoles of CO_2 per minute
40
30
20
10
0
5
10
15
mg protein
(B)
nanomoles of 5-HT per minute
2.0
1.5
1.0
0.5
0
5
10
15
mg protein

TABLE 2 THE EFFECT OF EXOGENOUS PYRIDOXAL-5-PHOSPHATE ON THE DECARBOXYLASE ACTIVITY OF THE 100,000 X G SUPERNATANT FROM HOMOGENATES OF DISPERSED RAT PANCREATIC ACINAR CELLS

	SUBSTRATE	
	DOPA	5-HTP
Pyridoxal-5-P concentration	CO_2 nmole/ mg protein/min	5-HT nmole/ mg protein/min
0 μM	2.800 ± 0.017	0.278 ± 0.014
0.1 μM	2.780 ± 0.171	0.288 ± 0.010
1.0 μM	2.811 ± 0.056	0.264 ± 0.023
10 μM	2.710 ± 0.243	0.296 ± 0.026
100 μM	2.812 ± 0.151	0.270 ± 0.020

The 100,000 X g supernatant fraction was incubated with L-[^{14}C-carboxyl] DOPA (10^{-4}M) and L-[^{14}C] 5-HTP (10^{-4}M) in buffer containing isotonic sucrose with 10 mM sodium phosphate pH 7.0 at 37°C. The incubation time was 2 minutes when DOPA was the substrate and 30 minutes when 5-HTP was the substrate. The rate of DOPA decarboxylation was assessed by measuring the evolution of carbon dioxide while 5-HTP decarboxylation was measured by the rate of production of 5-HT.

Mean ± S.E.M. n=4

3.3.2.2 Substrate specificity

Substrate specificity of the decarboxylase in the 100,000 X g supernatant of acinar cells was examined with the amino acids L-DOPA, L-5-HTP, L-His., L-Tyr., and L-Trp., in the concentration range from 10^{-6}M to 10^{-3}M. Decarboxylation was assayed by the evolution of CO_2 from carboxylabelled substrates except in the case of 5-HTP where 5-HT was measured directly. Fig. 10 and 11 show that only 5-HTP and L-DOPA were substrates for the soluble decarboxylase at all substrate concentrations tested (Fig. 10) and at pH 6.0 - 8.5 (Fig. 11). The rate of L-DOPA decarboxylation was maximal at pH 7.0 (3.1 $\pm$ 0.3 nmoles/mg protein/min); that of 5-HTP had a broad pH maximum from pH 7.0 to pH 8.5 (0.29 $\pm$ 0.03 nmoles/mg protein/min).

To investigate the substrate stereospecificity, dispersed acinar cells incubated with 10^{-5}M L-[^{14}C] DOPA were exposed to increasing concentration of L-[^{12}C] DOPA or D-[^{12}C] DOPA. As illustrated in Fig. 12, increasing the D-[^{12}C] DOPA concentration from 10^{-4} to 10^{-3}M had no effect on the production of [^{14}C] DA but as the concentration of L-[^{12}C] DOPA increased, the production of [^{14}C] DA decreased as a direct function of the decreasing specific activity of the L-[^{14}C] DOPA concentration. As illustrated in Table 3 when the 100,000 X g supernatant from dispersed acinar cell homogenate was used as the enzyme source, the rate of decarboxylation of 10^{-5}M L-[^{14}C] DOPA was unaffected by D-[^{12}C] DOPA until the D-DOPA concentration reached 10^{-3}M. These results show that the L-isomer is preferred by the decarboxylase by a factor of at least 100/1.

The apparent V_{max}, Km (Fig. 13) and IC_{50} (Fig. 14) were estimated for both DOPA and 5-HTP decarboxylation. The apparent V_{max} of DA production from DOPA is 2.5 nmoles/mg protein/min. which is 8 fold higher than the corresponding values (0.3 nmoles/mg protein/min.) for the production of 5-HT from 5-HTP. The apparent Km for 5-HTP decarboxylation is 2.9×10^{-5}M, which is similar to the apparent Km for DOPA decarboxylation (4.8×10^{-5}M). Both 5-HTP and DOPA decarboxylation were completely inhibited by 10^{-7} to 10^{-6}M NSD-1055 (Aures et al., 1970) (Fig. 14). The IC_{50}, are within the same order of magnitude for both substrates (DOPA, $IC_{50} = 7.5 \times 10^{-9}$M; 5-HTP, $IC_{50} = 4.0 \times 10^{-9}$M).

FIGURE 10 The decarboxylation of various amino acid substrates in the 100,000 X g supernatant from dispersed acinar cells

The 100,000 X g supernatant fraction was isolated from acinar cells by differential centrifugation and was incubated in isotonic sucrose containing 10 mM sodium phosphate buffer pH 7.0 with L-[^{3}H-carboxyl] His; L-[^{3}H-carboxyl] Trp; L-[^{14}C-carboxyl] Tyr; L-[^{14}C-carboxyl] DOPA and L-[^{14}C] 5-HTP at concentrations shown in the figure. When His, Trp, Tyr, and DOPA were substrates, incubation time was 2 minutes and carbon dioxide evolution was measured; when 5-HTP was substrate, incubation time was 30 minutes and 5-HT production was measured. Each point is the mean of 3 experiments.

His.
Trp.
Tyr.
5-HTP
DOPA
2.4
1.6
0.8
0
0.2
0.1
0
10^{-6}
10^{-5}
10^{-4}
10^{-3}
[L-Amino Acid], M
nanomoles of CO_2 per mg protein per minute
nanomoles of 5-HT per mg protein per minute

FIGURE 11 The effect of pH on the decarboxylation of various amino acid substrates in the 100,000 X g supernatant isolated from dispersed acinar cells

The 100,000 X g supernatant fraction isolated from acinar cells was incubated for 10 minutes with 0.1 mM amino acids L-DOPA, L-His, L-Trp, L-Tyr, and for 30 minutes with L-5-HTP in isotonic sucrose with 10 mM sodium phosphate at the indicated pH. The rate of carbon dioxide evolution was measured for L-DOPA, L-His, L-Tyr, and L-Trp decarboxylation and the rate of 5-HT production was measured for 5-HTP decarboxylation. Each point in the graph represents mean $\pm$ S.E.M. of 4 separate experiments.

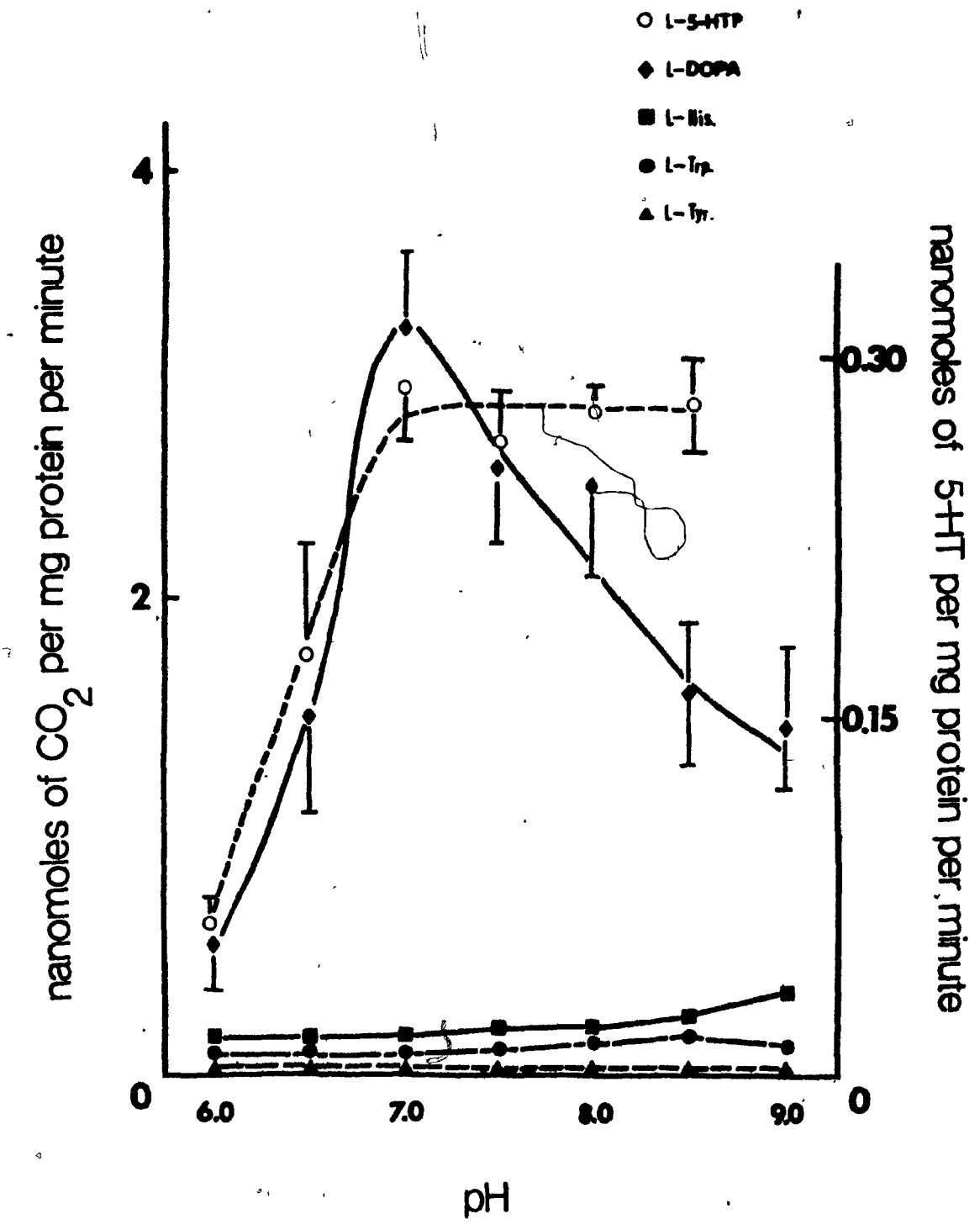

O L-5-HTP
◆ L-DOPA
■ L-His.
● L-Trp.
▲ L-Tyr.
nanomoles of CO_2 per mg protein per minute
nanomoles of 5-HT per mg protein per minute
4
2
0
0.30
0.15
0
6.0
7.0
8.0
9.0
pH

FIGURE 12 The effect of D-DOPA on the synthesis of dopamine from L-[^{14}C] DOPA by isolated pancreatic acinar cells

Dispersed acinar cells (50 X 10^6) were incubated for 3 minute in incubation buffer I at a constant concentration of L-[^{14}C] DOPA (10^{-5}M, 6.7 mCi/mmole) with increasing concentrations of D-[^{12}C] DOPA ($\triangle$) or L-[^{12}C] DOPA (O). Each point is the mean of 3 experiments.

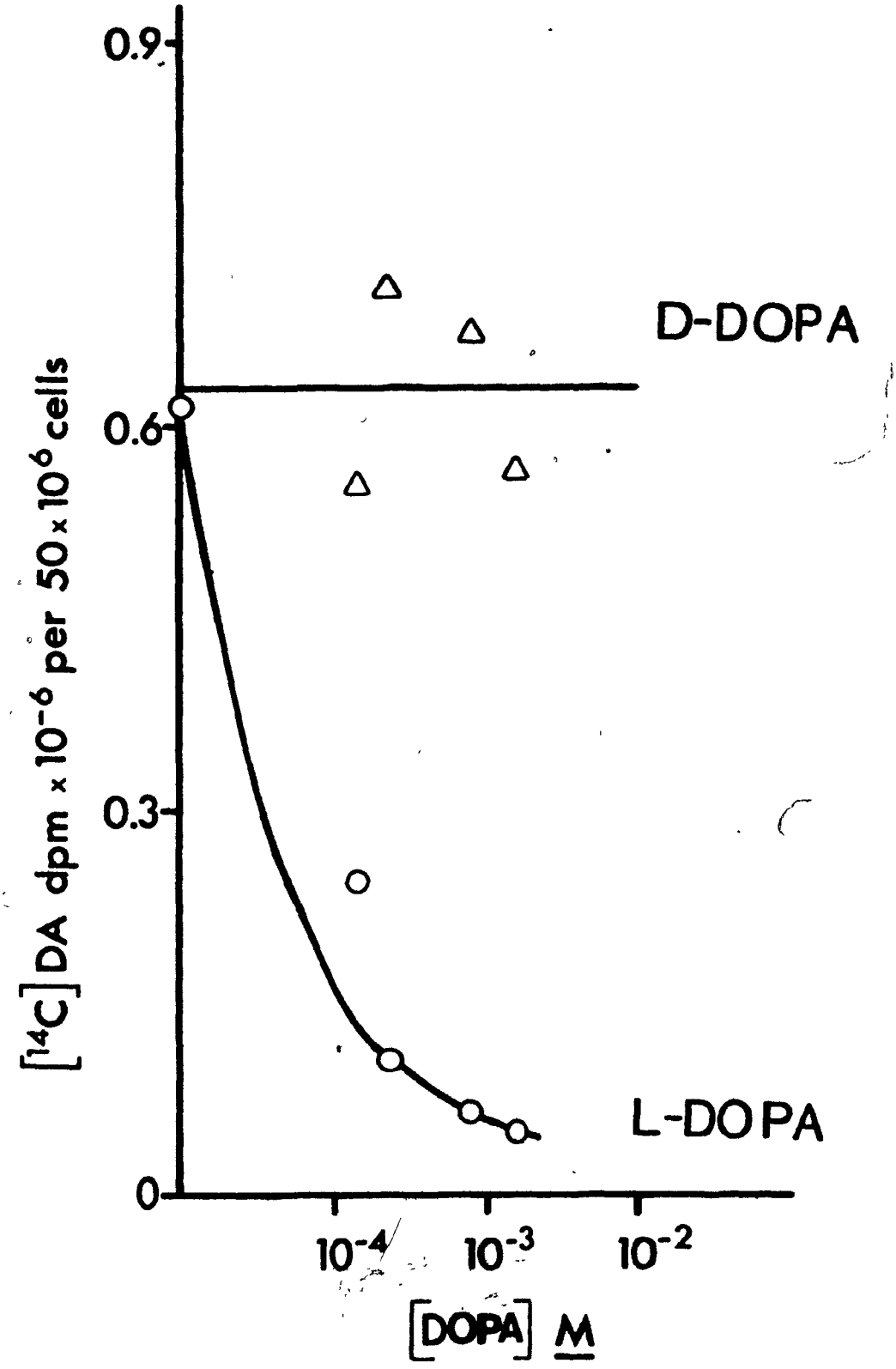

0.9
0.6
0.3
0
D-DOPA
L-DOPA
[14C]DA dpm x 10-6 per 50 x 10^6 cells
10-4
10-3
10-2
[DOPA] M

TABLE 3 THE EFFECT OF D-DOPA ON L-DOPA DECARBOXYLATION OF THE 100,000 X g SUPERNATANT FROM HOMOGENATES OF DISPERSED RAT PANCREATIC ACINAR CELLS

L-DOPA concentration	D-DOPA concentration	CO_2 (nmoles/mg protein/min)
10 μM	0 μM	0.386 ± 0.020
10 μM	0.1 μM	0.398 ± 0.020
10 μM	1 μM	0.348 ± 0.018
10 μM	10 μM	0.363 ± 0.013
10 μM	100 μM	0.385 ± 0.011
10 μM	1 mM	0.157 ± 0.059

The 100,000 X g supernatant fraction was incubated for 3 minutes with L-[^{14}C-carboxyl] DOPA (10^{-5}M) and increasing concentrations of D-[^{12}C] DOPA in isotonic sucrose with 10 mM sodium phosphate, pH 7.0 at 37°C. DOPA decarboxylation was assessed by measuring the rate of production of carbon dioxide.

Mean ± S.E.M. n=4

FIGURE 13 Lineweaver-Burk plots of DOPA and 5-HTP decarboxylase activity

(A) L-5-HTP or (B) L-DOPA was added to the 100,000 X g supernatants isolated from acinar cells. The incubation medium was isotonic sucrose with 10 mM sodium phosphate buffer pH 7.0 (Methods section 2.09.1) and the incubation time was 30 minutes for 5-HTP and 10 minutes for DOPA. The rate of DOPA decarboxylation was expressed as nmoles CO_2/mg protein/min and for 5-HTP was nmoles of 5-HT/mg protein/min. Each experiment is mean $\pm$ S.E. of 4 experiments.

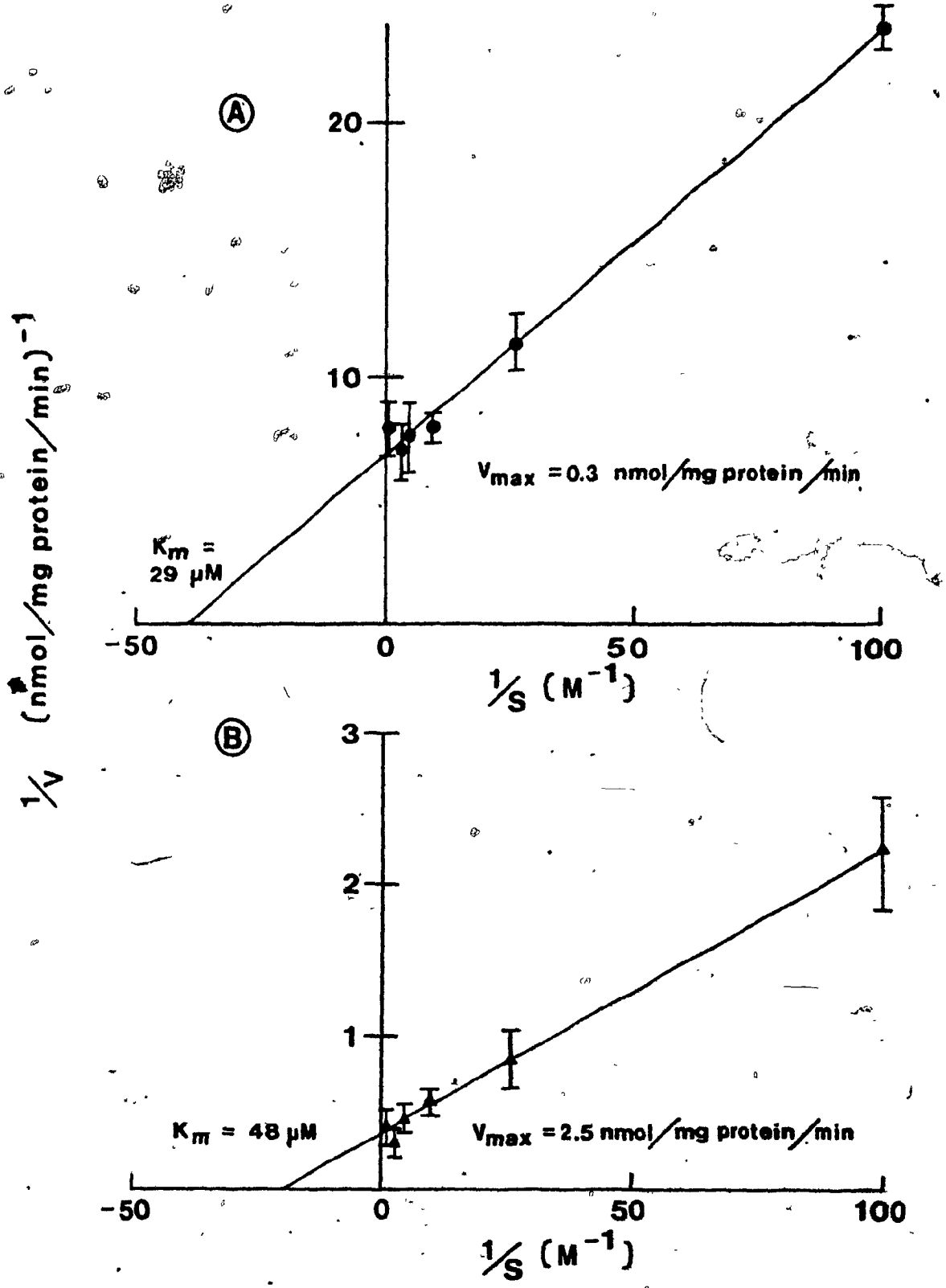
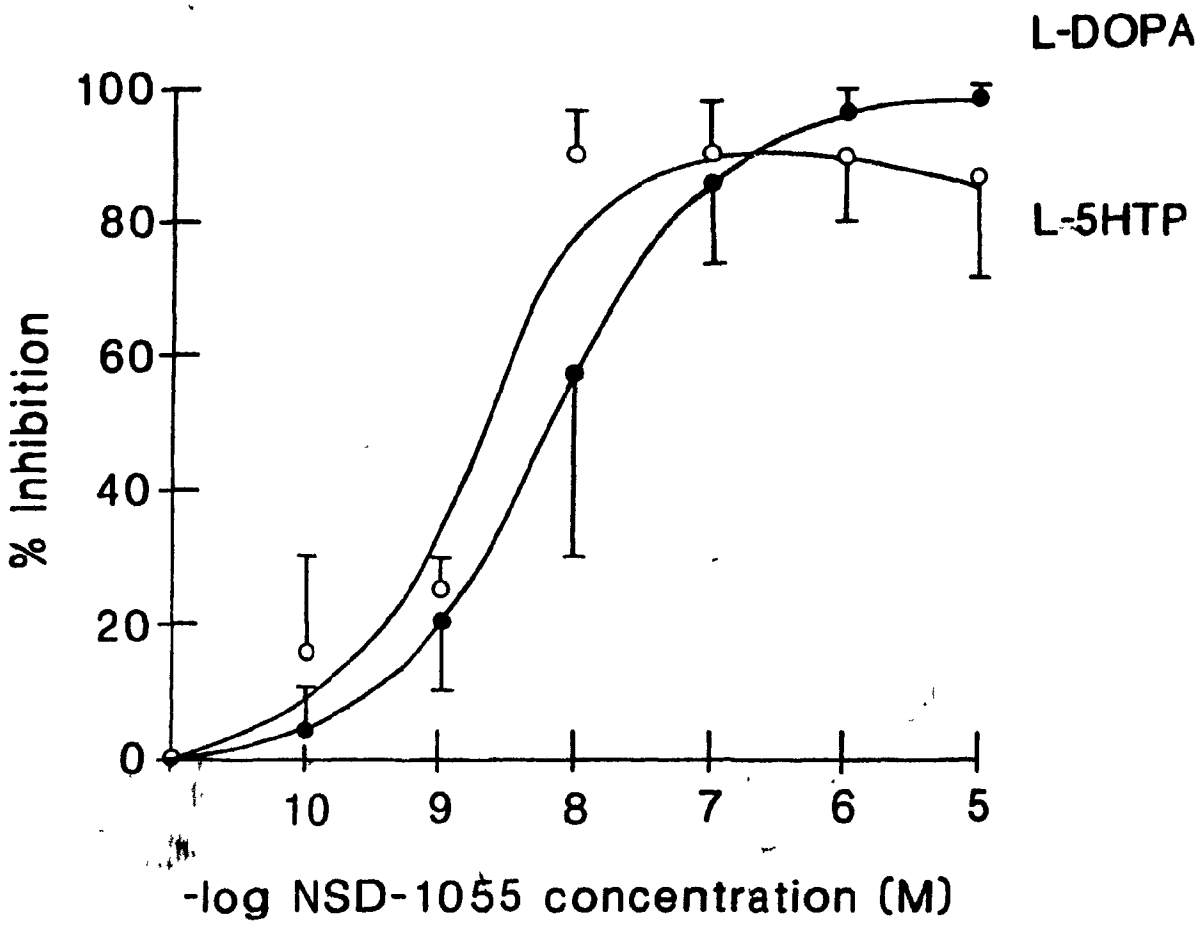

A
20
10
V_{max} = 0.3 nmol/mg protein/min
K_m = 29 μM
-50
0
50
100
$\frac{1}{S}$ (M^{-1})
$\frac{1}{V}$ (nmol/mg protein/min)$^{-1}$
B
3
2
1
K_m = 48 μM
V_{max} = 2.5 nmol/mg protein/min
-50
0
50
100
$\frac{1}{S}$ (M^{-1})

FIGURE 14 Inhibition of DOPA and 5-HTP decarboxylation by NSD-1055

The 100,000 X g supernatant isolated from dispersed acinar cells was incubated in isotonic sucrose containing 10 mM sodium phosphate buffer pH 7.0 with either L-DOPA (10^{-5}M) or L-5-HTP (10^{-5}M) in the presence of increasing concentrations of NSD-1055. The incubation time was 30 minutes for 5-HTP and 10 minutes for DOPA. The rate of DOPA decarboxylation was assessed by measuring the rate of CO_2 production and 5-HTP decarboxylation by measuring 5-HT production. The results are expressed as percent (%) inhibition versus NSD-1055 concentration. Each point represents mean $\pm$ S.E.M. of 4 experiments.


L-DOPA
L-5HTP
100
80
60
40
20
0
% Inhibition
10
9
8
7
6
5
-log NSD-1055 concentration (M)

3.3.2.3 Competitive substrate inhibition studies

Although both 5-HTP and DOPA decarboxylation occur in the soluble fraction of the cells, it remained to be determined whether a single enzyme or two distinct enzymes catalyze the two reactions. Competition experiments between L-DOPA and L-5-HTP, which are known substrates and L-Tyr., which is not a substrate, were undertaken in both acinar cells and 100,000 X g supernatant. Fig. 15 shows the effect of L-Tyr. and L-5-HTP on [^{14}C] DA synthesis from L-[^{14}C] DOPA (7.5×10^{-5}M) in dispersed cells. L-Tyr., an amino acid that was not decarboxylated in the acinar cells, had no effect on [^{14}C] DA synthesis over all concentrations tested (10^{-5}M to 10^{-3}M). L-5-HTP, an amino acid that was decarboxylated in these cells, at a concentration of 7×10^{-5}M reduced the rate of [^{14}C] DA production by 50%. At 10^{-3}M, L-5-HTP completely abolished [^{14}C] DA production. Similar observations were made with the 100,000 X g supernatant. Fig. 16 shows the effect of L-Tyr., and L-5-HTP on CO_2 evolution from L-[^{14}C-carboxyl] DOPA (10^{-4}M). L-5-HTP at a concentration of approximately 3×10^{-4}M inhibited dopamine synthesis by 50% while 10^{-4}M Tyr. did not change the rate of CO_2 production from L-DOPA. In a similar fashion, the rate of 5-HT synthesis was decreased by L-DOPA (Fig. 17). The rate of decarboxylation of 10^{-4}M L-5-HTP was inhibited 50% by 10^{-4}M L-DOPA. Tyr. did not change the rate of 5-HT synthesis until the Tyr. concentration reached 10^{-3}M.

The present results suggest that both L-DOPA and L-5-HTP compete for the same catalytic site on the decarboxylase and both L-DOPA and

L-5-HTP have similar affinities for the enzyme.

FIGURE 15 The effect of L-Tyrosine and L-5-HTP on [^{14}C] DA synthesis from L-[^{14}C] DOPA in isolated pancreatic acinar cells

Dispersed acinar cells (20 X 10^6) were incubated for 3 minutes in incubation buffer I at L-[^{14}C] DOPA concentration of 7.5 X 10^{-5}M, (6.7 mCi/mmole) with increasing concentrations of L-Tyr. and L-5-HTP. [^{14}C] DA production in the presence of increasing concentrations of L-5-HTP or L-Tyr. is illustrated in the lower panel and the recovered [^{14}C] DOPA is shown in the upper panel. The insert shows the % inhibition of DA synthesis with increasing concentration of L-5-HTP. Each point represents mean of 3 experiments.

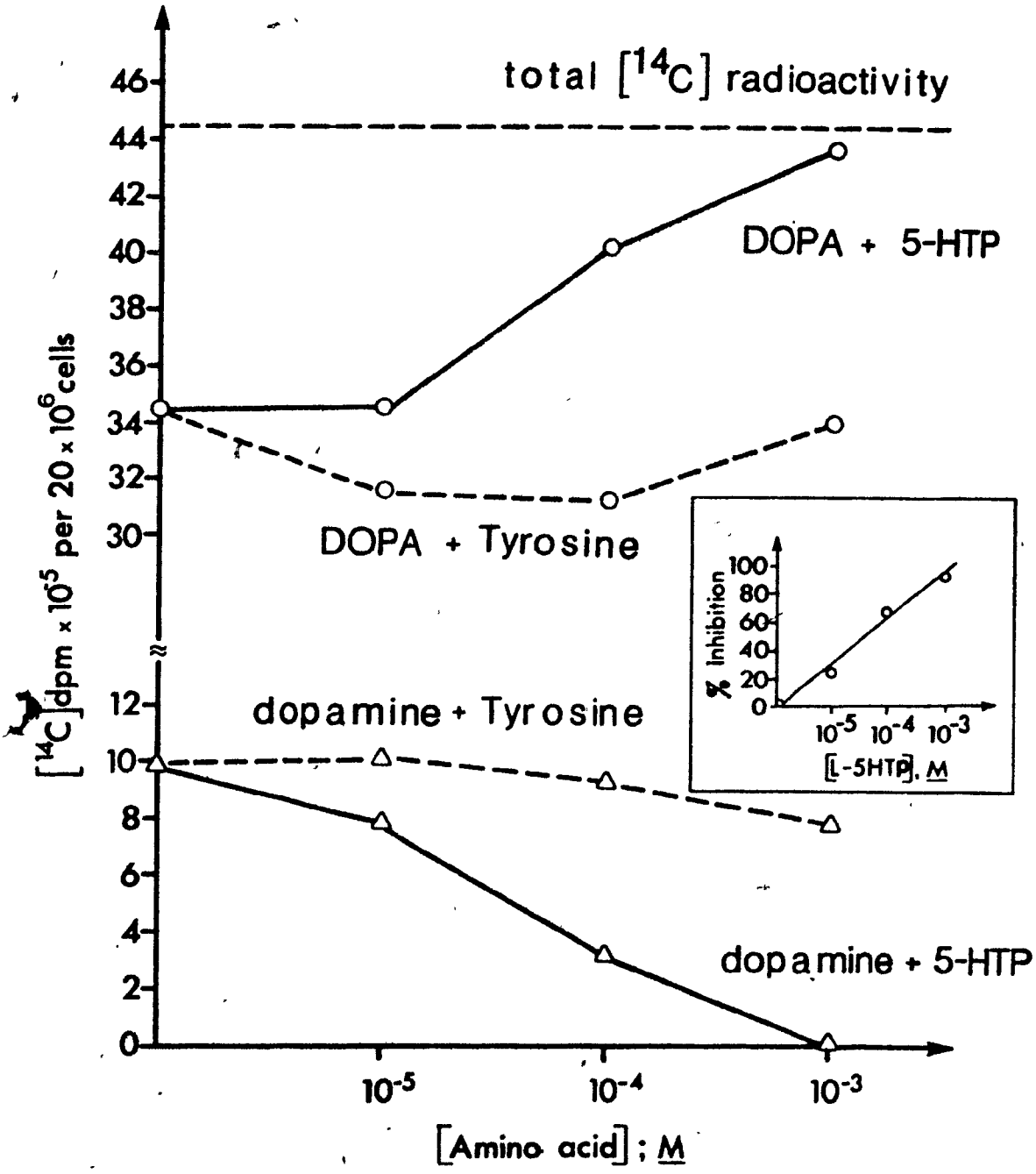

total [14C] radioactivity
DOPA + 5-HTP
DOPA + Tyrosine
dopamine + Tyrosine
dopamine + 5-HTP
[14C]dpm x 10^5 per 20 x 10^6 cells
[Amino acid] ; M
% Inhibition
[L-5HTP], M

FIGURE 16 The effect of L-Tyrosine and L-5-HTP on L-[^{14}C] DOPA decarboxylation in the 100,000 X g supernatant from acinar cells

The 100,000 X g supernatant fraction isolated from acinar cells was incubated for 10 minutes in isotonic sucrose with 10 mM sodium phosphate pH 7.0 at a L-[^{14}C-carboxyl] DOPA concentration of 10^{-4}M with increasing concentration of L-Tyr. (▲) or L-5-HTP (●). The rate of DOPA decarboxylation was expressed as the rate of CO_2 production. The inset shows the % inhibition of DA synthesis versus L-5-HTP concentrations. Each point in the graph is mean ± S.E.M. of 4 experiments.

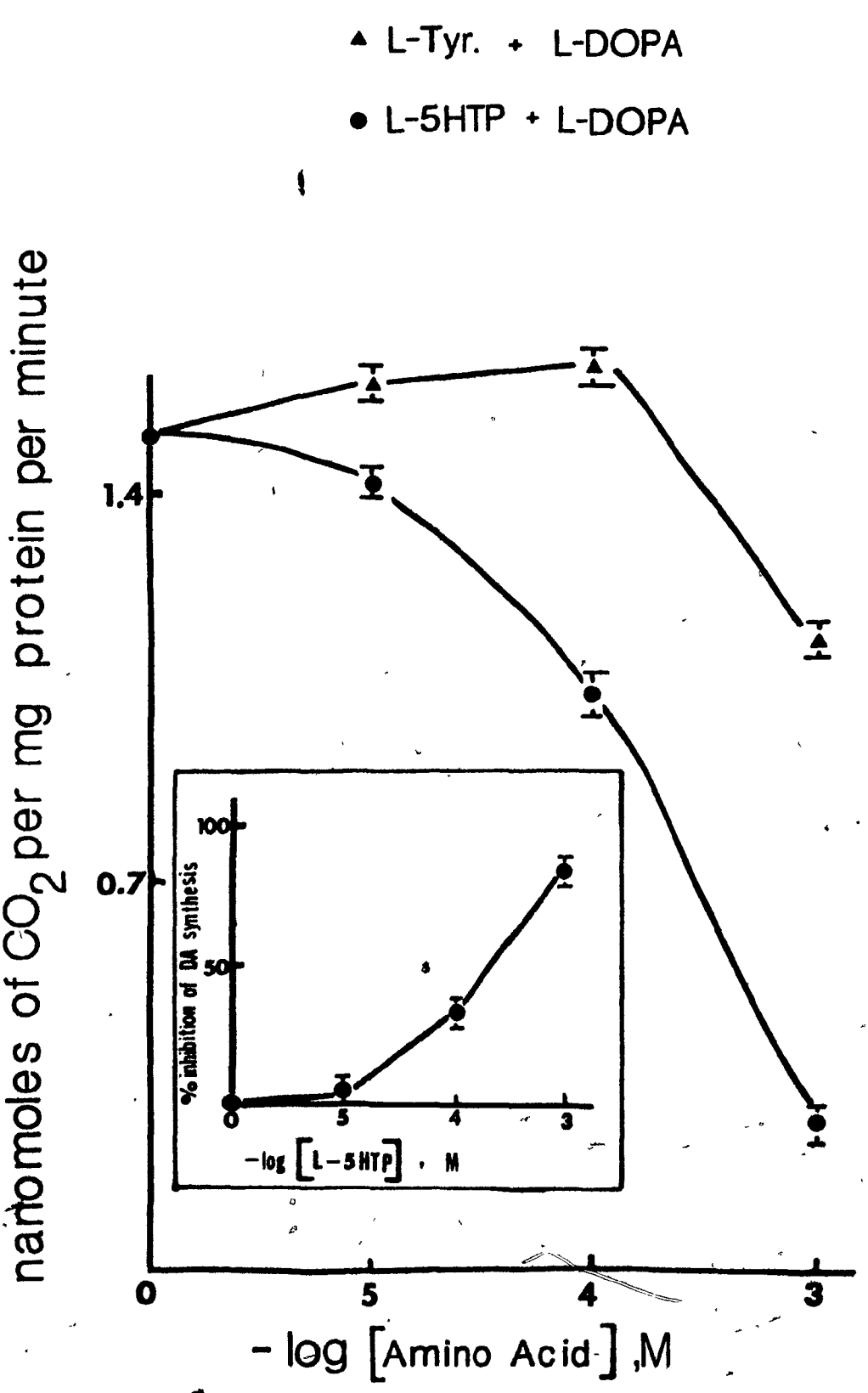
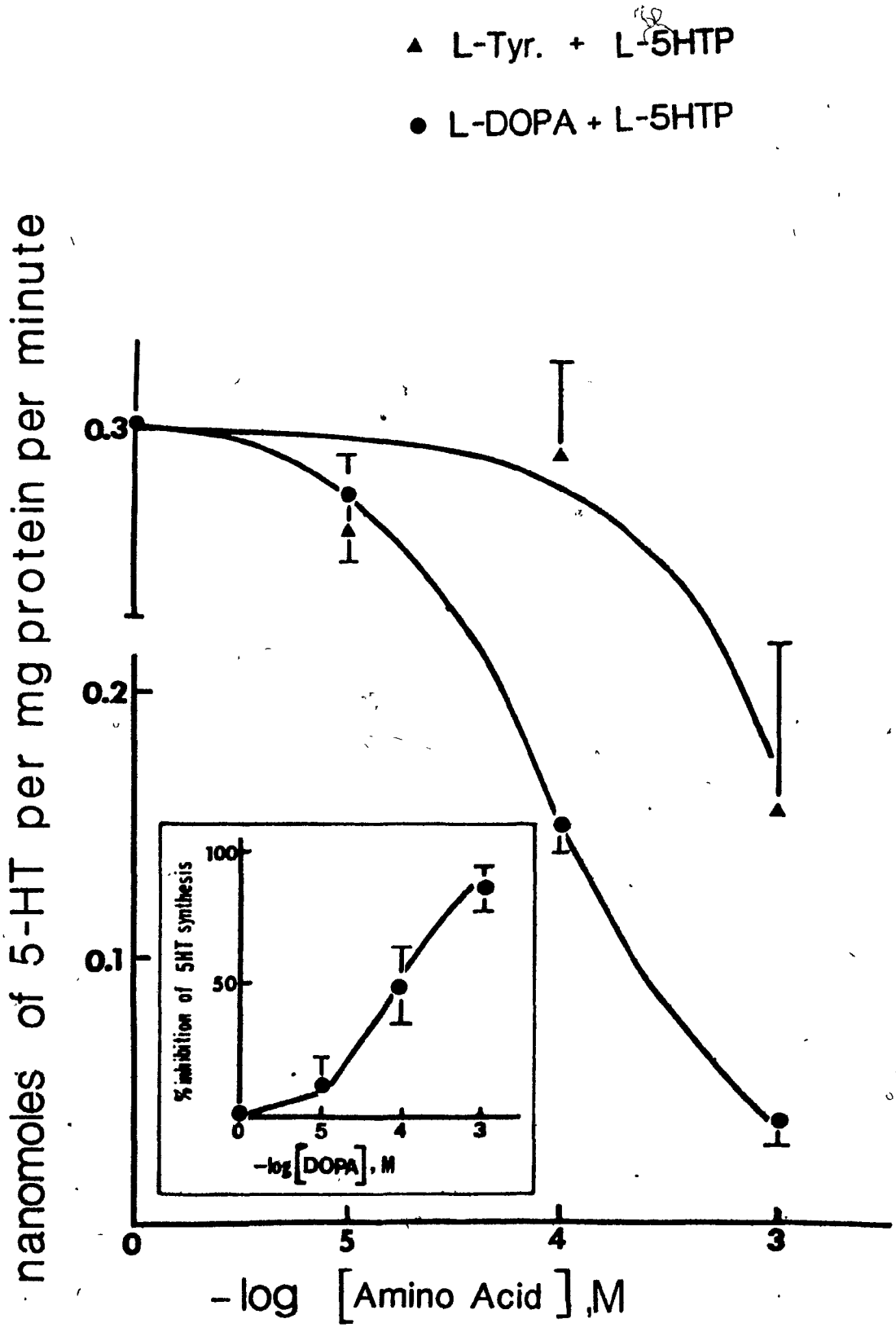

▲ L-Tyr. + L-DOPA
● L-5HTP + L-DOPA
nanomoles of CO_2 per mg protein per minute
1.4
0.7
0
5
4
3
- log [Amino Acid] ,M
% inhibition of DA synthesis
100
50
0
5
4
3
-log [L-5HTP] , M

FIGURE 17 The effect of L-Tyrosine and L-DOPA on L-[^{14}C] 5-HTP decarboxylation in the 100,000 X g supernatant from acinar cells

The 100,000 X g supernatant fraction isolated from acinar cells was incubated for 30 minutes in isotonic sucrose containing 10 mM sodium phosphate pH 7.0 with L-[^{14}C] 5-HTP, 10^{-4}M and with increasing concentrations of L-DOPA (●) or L-Tyr (▲). The rate of L-5-HTP decarboxylation was expressed as the rate of 5-HT production. The inset shows the % inhibition of 5-HT synthesis versus L-DOPA concentrations. Each point in the graph is mean ± S.E.M. of 4 experiments.


▲ L-Tyr. + L-5HTP
● L-DOPA + L-5HTP
nanomoles of 5-HT per mg protein per minute
0.3
0.2
0.1
0
5
4
3
- log [Amino Acid], M
% inhibition of 5HT synthesis
100
50
0
5
4
3
-log[DOPA], M

3.4 AMINE ACCUMULATION IN ZYMOGEN GRANULES OF EXOCRINE PANCREAS

Biogenic monoamines synthesized in the cytosol of pancreatic acinar cells are thought to be stored and to be released from zymogen granules mainly, if not exclusively, by secretion into pancreatic ducts (Mori et al., 1979a;b). Incorporation of 5-HT into zymogen granules was further investigated.

3.4.1 Stability of 5-HT in Zymogen Granule

As illustrated by the results in section 3.3, pancreatic acinar cells can decarboxylate both L-DOPA and L-5-HTP to form DA and 5-HT respectively. The incorporation of DA and 5-HT into zymogen granules was studied using the pulse-chase protocol described in "Methods section 2.02". At various times during the chase incubation, samples of acinar cells were taken, the zymogen granules were isolated and their amine content measured. Figure 18A illustrates that both 5-HT and DA are present in zymogen granules as early as 15 minutes after the start of the pulse incubation. More than 98% of the radioactivity was identified as the amines; amine precursors and amine metabolites were not found in the zymogen granules. Of the two amines, only 5-HT was stable within the granule fraction. There was no detectable decrease of 5-HT concentration in the granule fraction during the 60 minutes incubation period. On the other hand, the DA concentration in the granule fraction began to decrease after 20 minutes of incubation. After 90 minutes incubation, approximately 50% of the DA had disappeared from the

zymogen granule fraction. This result is consistent with the results from the initial experiments that were done with pancreatic tissue slices, Fig. 18 B,C in which the tissue slices were incubated with DL-[^{14}C] 5-HTP (Fig. 18B) L-[^{14}C] DOPA (Fig. 18C) under the same experimental conditions described above for a period of 2 hours. 5-HT once incorporated remained in the granule fraction for the 2 hour period whereas DA dissociated from the granule fraction so that after 2 hours approximately 2/3 of the DA present at the start of the chase incubation was lost.

5-HT in the zymogen granules does not seem to exchange with exogenous 5-HT. This is shown in the experiments in which [^{12}C] 5-HT (10^{-3}M) was added to the incubation medium 30 minutes after the pulse of L-[^{14}C] 5-HTP (10^{-3}M). As illustrated in Fig. 19, the ratio of [^{14}C] 5-HT to zymogen granule protein was not altered by the presence of added [^{12}C] 5-HT indicating that the zymogen granule 5-HT was in a non-exchangeable state.

The co-existence of 5-HT and amylase in zymogen granules was confirmed using density gradient centrifugation. Experiments were done using acinar cells (100 X 10^6) that were prelabelled with L-[^{14}C] 5-HTP (10^{-3}M, 1.5 mCi/nmole) for one hour, and chase-incubated with [^{12}C] 5-HTP (10^{-3}M) for one hour. The zymogen granule fraction was isolated and purified by passing it through a 60% continuous Percoll gradient. Pelletable amylase activity was measured as an index of zymogen granule recovery. A zymogen granule fraction with a density of 1.142 (mature zymogen granule fraction) was recovered from the

Percoll (fraction 2 to 6) gradient as shown in Fig. 20A. Fractions number 18 to 23 represented the immature granule fraction (density 1.108). As illustrated, the pelletable [^{14}C]5-HT is also restricted to these two locations of the gradient. The mature granule fraction was collected, layered on a discontinuous sucrose gradient (10-60% w/w) and centrifuged (Fig. 20B). Both amylase and [^{14}C] 5-HT are in pellets recovered from fractions number 4 and 5 of the sucrose gradient. Approximately half of the total [^{14}C] 5-HT and amylase activity are recovered from the sucrose gradient. The proportion of [^{14}C] 5-HT and amylase lost are always the same. Again [^{14}C]-5-HT and amylase activity are not separable. Fractions number 12 and 13 are the soluble protein fractions. The present result provides supporting evidence that 5-HT and the enzyme contents of zymogen granules are contained within the same subcellular particle.

FIGURE 18 Time course of incorporation of DA and 5-HT into zymogen granules of pancreatic acinar cells (A) and tissue slices (B and C)

(A) Acinar cells (50 X 10^6) were pulse-incubated with L-[^{14}C] DOPA (10^{-5}M) or L-[^{14}C] 5-HTP (10^{-5}M) for 15 minutes in incubation buffer II and chase-incubated with incubation buffer I for varying time periods up to 90 minutes. At the end of the chase incubation zymogen granules were isolated and the amines were separated from amino acids on DOWEX 50WX4 columns (see Methods section 2.06). The results are expressed as nmoles 5-HT/mg Z.G. protein (O) and nmoles of DA/mg Z.G. protein (●). Each point is the mean ± S.E.M. of 5 experiments.

(B and C) Pancreatic tissue slices were pulse-incubated with [^{3}H] Leucine (1.8 X 10^{-8}M, 56.5 Ci/mmole) and either (B) DL-[^{14}C] 5-HTP (1.7 X 10^{-5}M, 59.2 mCi/mmole) or (C) L-[^{14}C] DOPA (7.5 X 10^{-5}M, 6.7 mCi/mmole) in incubation buffer II for 15 minutes and chase-incubated in incubation buffer I for different time periods up to 2 hours. At the end of the chase-incubation zymogen granules were isolated and the amines were separated from amino acids as described in (A). The results are expressed as [^{14}C] DA (dpm X 10^{-3} per mg zymogen granule protein); [^{14}C] 5-HT (dpm X 10^{-3} per mg zymogen granule protein); and [^{3}H] protein (dpm X 10^{-3} per mg zymogen granule protein). Each point is the mean of 3 experiments.

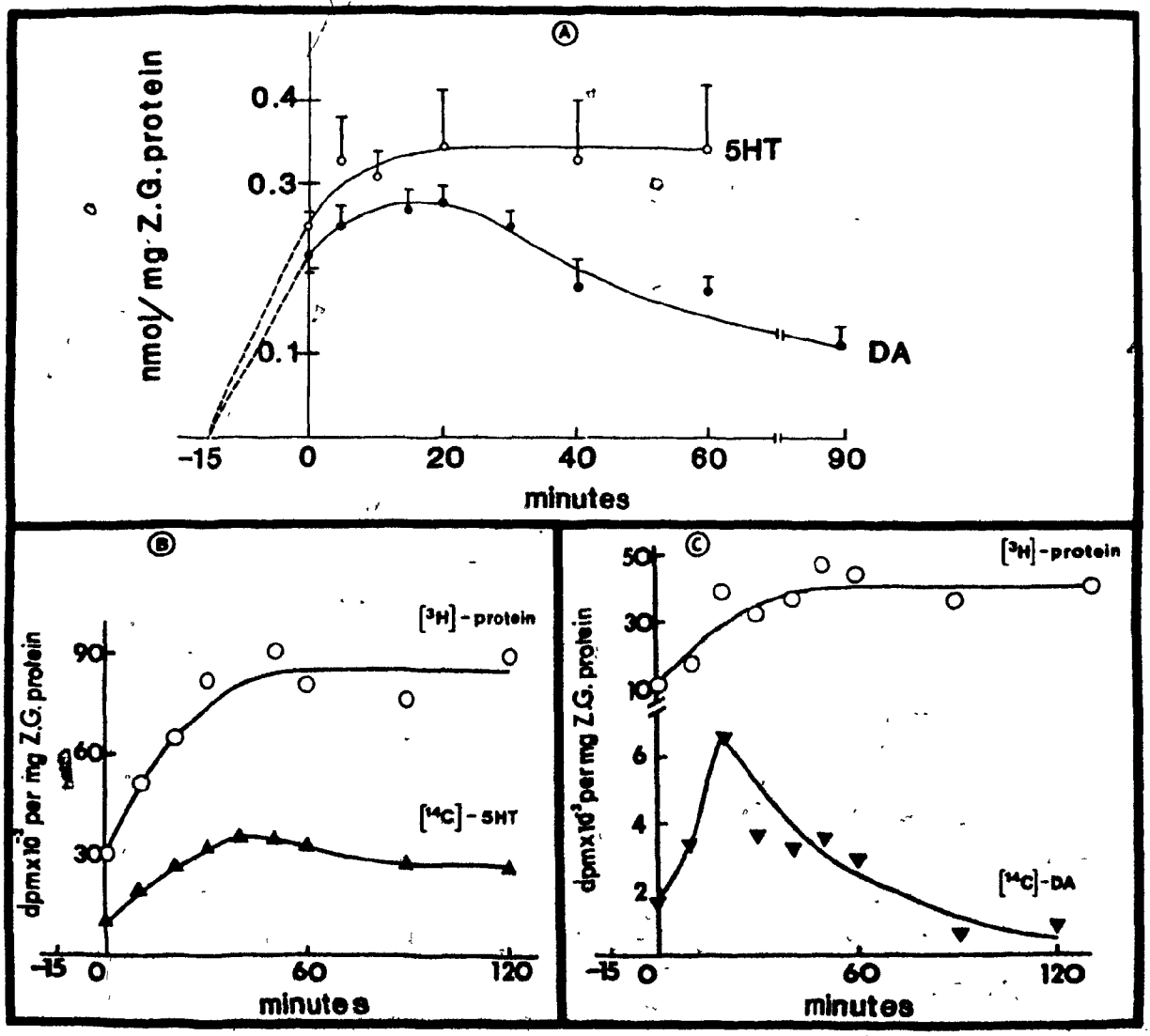

A
nmol/mg Z.G. protein
0.4
0.3
0.1
5HT
DA
-15
0
20
40
60
90
minutes
B
dpmx10³ per mg Z.G. protein
[³H]-protein
[¹⁴C]-5HT
90
60
30
-15
0
60
120
minutes
C
dpmx10³ per mg Z.G. protein
[³H]-protein
[¹⁴C]-DA
50
30
10
6
4
2
-15
0
60
120
minutes

FIGURE 19 The stability of zymogen granules [^{14}C] 5-HT in the presence of [^{12}C] 5-HT in acinar cells

Dispersed acinar cells (50 X 10^6) were pulse-incubated with L-[^{14}C] 5-HTP (10^{-5}M) in incubation buffer II for 15 minutes. This was followed by a chase-incubation of 75 minutes either in the absence (O) or presence (●) of [^{12}C] 5-HT. The results are expressed as [^{14}C] 5-HT accumulated per mg zymogen granule protein versus chase-incubation time. Each point represents mean $\pm$ S.E.M. of 5 experiments.

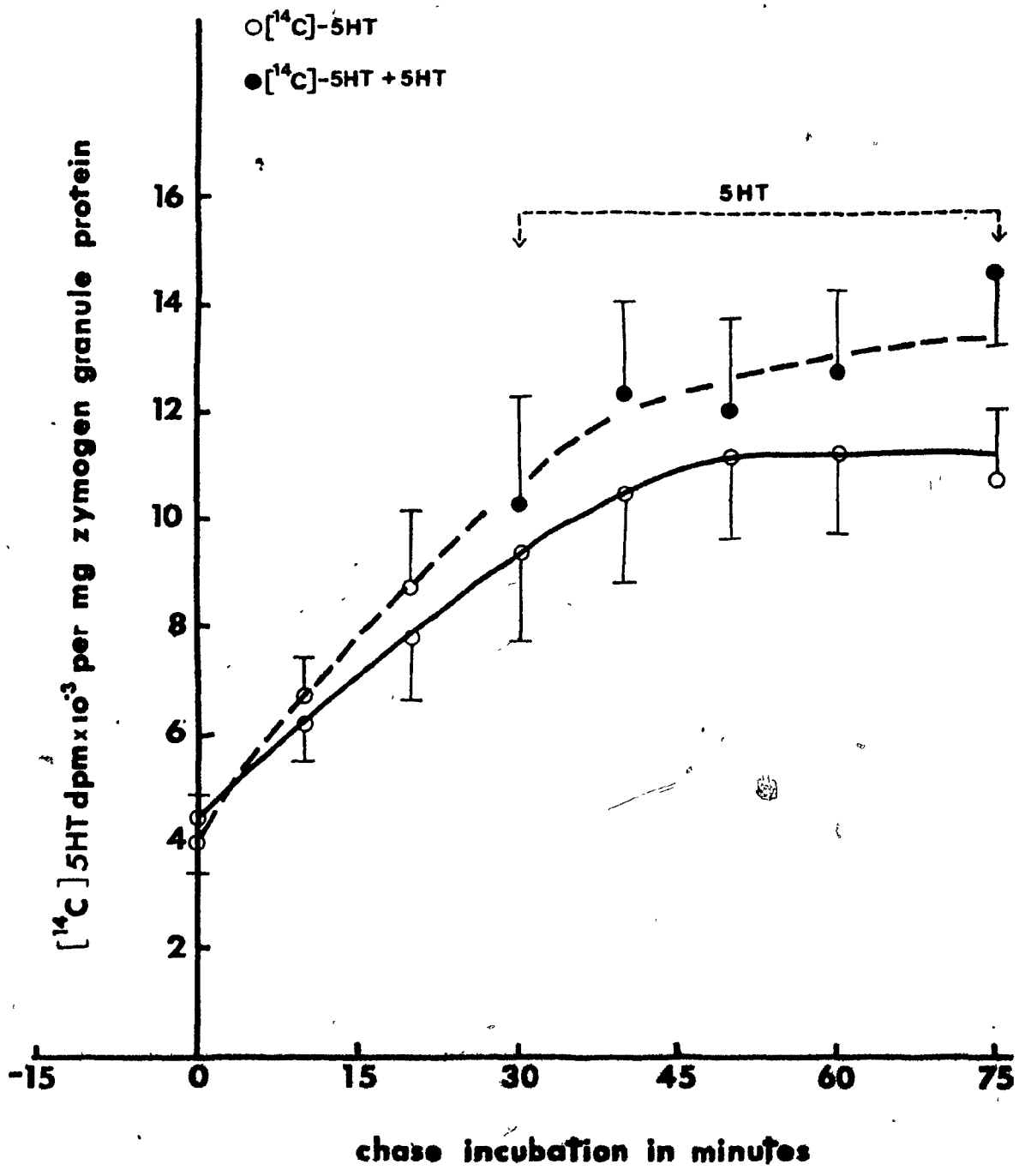
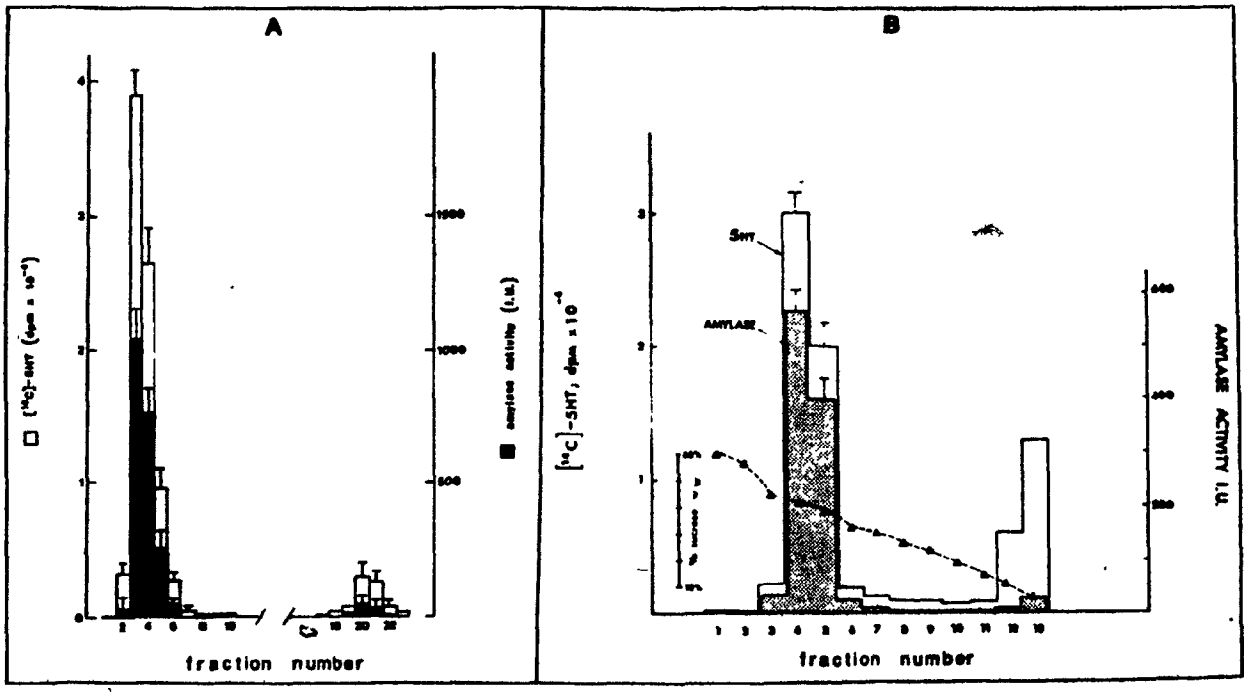

○[14C]-5HT
●[14C]-5HT + 5HT
5HT
[14C]5HT dpm x 10-3 per mg zymogen granule protein
16
14
12
10
8
6
4
2
-15
0
15
30
45
60
75
chase incubation in minutes

FIGURE 20 The [^{14}C] 5-HT and amylase content of zymogen granule fractions prepared by density gradient centrifugation

Dispersed acinar cells (100 X 10^6) were pulse-incubated with L-[^{14}C] 5-HTP (1 mM, 1.5 mCi/mmole) in incubation buffer I for 60 minutes. This was followed by a chase-incubation period of 60 minutes in the presence of 1 mM L-[^{12}C] 5-HTP. The crude zymogen granule fraction was isolated and centrifuged through a 60% Percoll continuous density gradient as described in Methods. In this Percoll density gradient the crude zymogen granule fraction was separated into a high density (density=1.142) (number 2-6) and a low density (density=1.108) granule fraction (number 18-23) as shown in (A). Amylase activity (an index for zymogen granule recovery) and [^{14}C] 5-HT were measured in each fraction. The components of high density (density=1.142) granule fraction were further resolved on a discontinuous sucrose density gradient (10-60% w/w). As illustrated in (B) [^{14}C] 5-HT and amylase are found in fraction 4,5. This sucrose density gradient separates the zymogen granule fraction (4,5) from mitochondria (6,7) microsomal fraction (8-10) and soluble protein fraction (12-13). (▲) shows the % sucrose in each fraction.


A
B
fraction number
fraction number
5HT
AMYLASE
AMYLASE ACTIVITY I.U.

3.4.2 Accumulation of 5-HT and Labelled Protein by Zymogen Granules

In order to compare the kinetics of incorporation of 5-HT and protein into zymogen granules, a time course of 5-HT and and [^{3}H]protein accumulation into the granules was measured.

Fig. 21 shows the accumulation of [^{14}C]5-HT and [^{3}H]protein into zymogen granules after a 15 minute pulse with L-[^{14}C]5-HTP (10^{-3}M) and [^{3}H]Leucine (1.7×10^{-8}M; 59.8 Ci/mmole). Both [^{3}H]protein and [^{14}C]5-HT were taken up into the granules, reached a plateau at 40 to 60 minutes and remained constant for the duration of the incubation, i.e. [^{14}C]5-HT in the granules was as stable as the [^{3}H]protein for at least 75 minutes of incubation. There were no amine precursors or amine metabolites found in the zymogen granule fractions. The amount of 5-HT incorporated into zymogen granules at the end of the 60 minute incubation (6 nmoles/mg zymogen granule protein) represents 45-50% of the total labelled 5-HT present in the cells at that time.

It is possible using 60% Percoll gradient centrifugation to separate the "crude" zymogen granule preparation (see Methods section 2.03) into low (1.108) and high (1.142) density granule fractions. This was done with zymogen granules prepared from acinar cells after 5 and 60 minutes chase incubation as described above. The high density fraction is the mature granule fraction whereas the low density fraction contains immature zymogen granules (condensing vacuoles) (review see Case, 1978). Mature zymogen granules are formed from condensing vacuoles through a process involving the progressive aggregation of protein within the condensing vacuoles (see Introduction section 1.2.2).

Fig. 22A shows the distribution of zymogen granule [^{3}H] protein between the high density and low density granule fractions. 5 minutes after the pulse, 68 ± 6% of the [^{3}H] protein was found in the low density zymogen granule fraction and 25 ± 3% in the high density granule fraction. After 60 minutes chase-incubation, only 30 ± 9% of the [^{3}H] protein remained in the low density granule fraction whereas the high density granule fraction now contained 56 ± 6% of the [^{3}H] protein. An estimated 31% of the crude zymogen granule [^{3}H] protein moved from the low density granule fraction to the high density granule fraction during the chase incubation. Similar experiments were done with 5-HT incorporation into zymogen granules, as illustrated in Fig. 22B. At 5 minutes after the pulse, 67 ± 3% of the total crude zymogen granule [^{14}C] 5-HT was found in the low density granule fraction which decreased to 33 ± 6% after the 60 minutes incubation. On the other hand, 23 ± 3% of total crude zymogen granule [^{14}C] 5-HT was found in high density granule fraction at 5 minutes after the pulse and this increased to 56 ± 3% after the 60 minutes incubation. Similar to the [^{3}H] protein an estimated 33% of the crude zymogen granule [^{14}C] 5-HT moved to the high density granule fraction from the low density granule fraction during the chase-incubation.

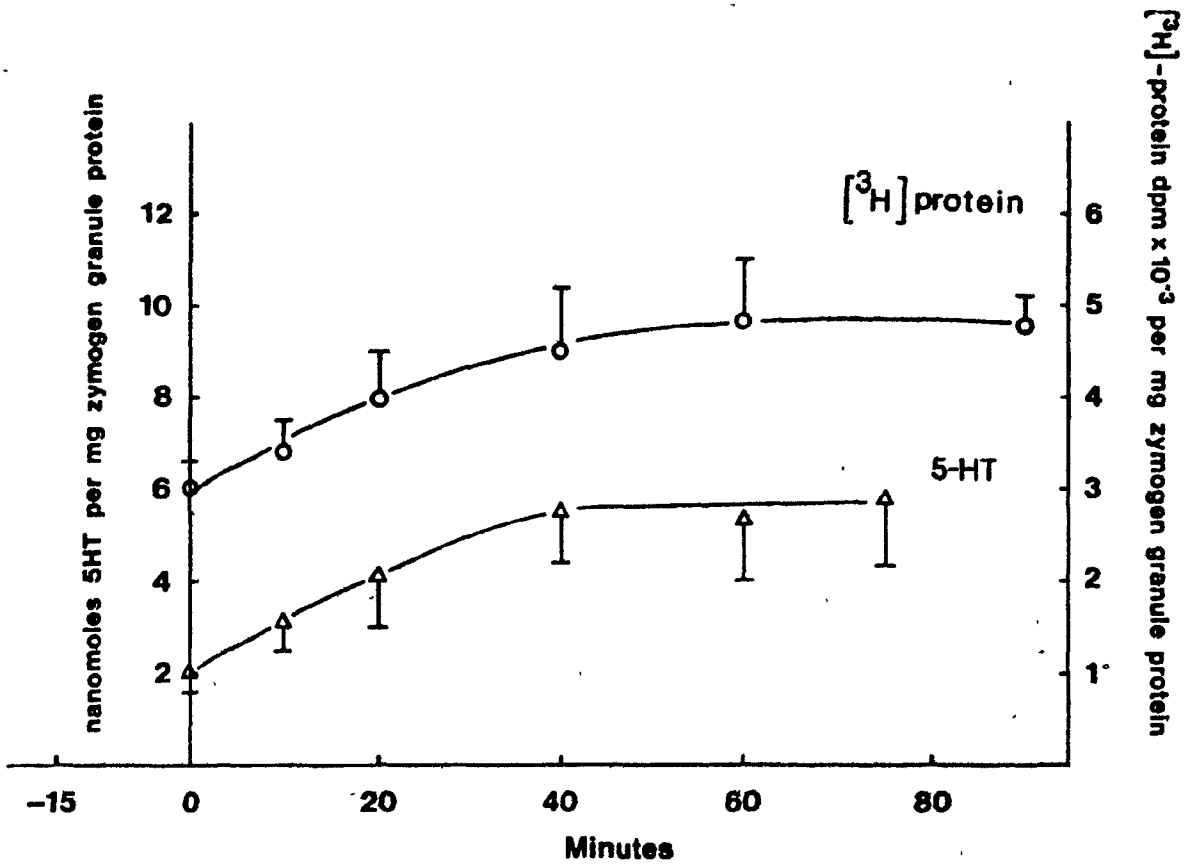
To further study the kinetics of incorporation of 5-HT and labelled protein into zymogen granules, acinar cells were pulsed with [^{14}C] 5-HT (10^{-4}M) and [^{3}H] leucine (1.7×10^{-8}M) for 5 minutes and chase-incubated in the presence of [^{12}C] 5-HT (10^{-3}M) and [^{1}H] leucine (10^{-3}M). Samples were collected at various times during the 60 minutes chase-incubation

period, the cells were homogenized and fractionated into the 100,000 X g pellet (microsomal); 20,000 X g pellet (Golgi vesicles and condensing vacuoles) and mature zymogen granule fraction (density 1.142). [^{3}H] protein and [^{14}C] 5-HT incorporation into these fractions was measured. The results are illustrated in Fig. 23A,B. In both the 20,000 X g and 100,000 X g pellets, [^{3}H] protein concentration was greatest immediately after the pulse incubation and declined throughout the duration of the incubation (Fig. 23A). In the zymogen granule fraction, the [^{3}H] protein concentration was minimal immediately after the pulse incubation. The concentration increased to a maximum at 30 minutes and remained constant thereafter. An increase in [^{3}H] protein concentration in the zymogen granule fraction is observed with a concomitant decrease in [^{3}H] protein concentration of the 20,000 X g and 100,000 X g pellets, a situation compatible with a product/precursor relationship. 5-HT incorporation into the zymogen granule fraction (Fig. 23B) follows the same pattern as that of [^{3}H] protein: immediatedly after pulse incubation the 5-HT concentration (2.0 $\pm$ 3 nmoles/mg protein; 1.5 $\pm$ 0.3 nmoles/mg protein) was greatest in the 20,000 X g and 100,000 X g pellet respectively and declined with further incubation. The 5-HT concentration in the zymogen granule fraction, at this time, was minimal (0.6 $\pm$ 0.2 nmoles/mg protein). After 60 minute chase-incubation the 5-HT concentration in the zymogen granules increased to 2.5 $\pm$ 0.5 nmoles/mg protein, with a concomitant decrease in 5-HT concentration (1.0 $\pm$ 0.2 nmoles/mg protein; 0.5 $\pm$ 0.1 nmoles/mg protein) in the 20,000 X g and the 100,000 X g pellet respectively. It appears from the results

illustrated in Fig. 22A,B and 23A,B that 5-HT and secretory protein are incorporated together at an early stage of zymogen granule formation and remain within the granule as it matures.

FIGURE 21 Time course of incorporation of [^{3}H]protein and 5-HT into zymogen granules of dispersed acinar cells

Dispersed acinar cells (50 X 10^6) were pulse-incubated with L-[^{14}C]5-HTP (1 mM) and L-[^{3}H]Leucine (3 X 10^{-8}M; 59.8 Ci/mmole) incubation buffer II for 15 minutes and chase-incubated in incubation buffer I containing 1 mM [^{3}H]Leucine and 1 mM [^{12}C]5-HTP for 90 minutes. (Δ) indicates nmoles of 5-HT per mg zymogen granule protein and (O) represents [^{3}H]protein in dpm X 10^{-3} per mg zymogen protein. Each point is the mean ± S.E.M. of 5 experiments.


nanomoles 5HT per mg zymogen granule protein
[3H] protein
5-HT
[3H]-protein dpm x 10-3 per mg zymogen granule protein
12
10
8
6
4
2
6
5
4
3
2
1
-15
0
20
40
60
80
Minutes

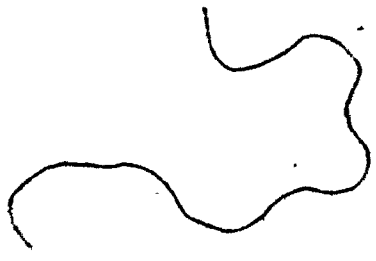


FIGURE 22 Time course of incorporation of (A) [^{3}H]protein and (B) 5-HT into low density and high density zymogen granule fractions

Dispersed acinar cells (50 X 10^{6}) were pulse-incubated with (A) L-[^{3}H] Leucine (1.7 X 10^{-8}M, 52.2 Ci/mmole) for 3 minutes and (B) L-[^{14}C] 5-HTP (10^{-5}M) for 15 minutes in incubation buffer II and chase-incubated in incubation buffer I containing 1 mM [^{1}H] Leucine and 1 mM [^{12}C] 5-HTP for varying time periods up to 60 minutes. Immediately after the chase-incubation a crude zymogen granule fraction (see Methods section 2.03) was isolated and was separated into low density (density=1.108, open bars), and high density (density=1.142, hatched bars) zymogen granule fractions. Each bar represents the % of total [^{3}H]protein or [^{14}C] 5-HT distributed into low and high density zymogen granule fractions and are the mean $\pm$ S.E.M. of 4 experiments.

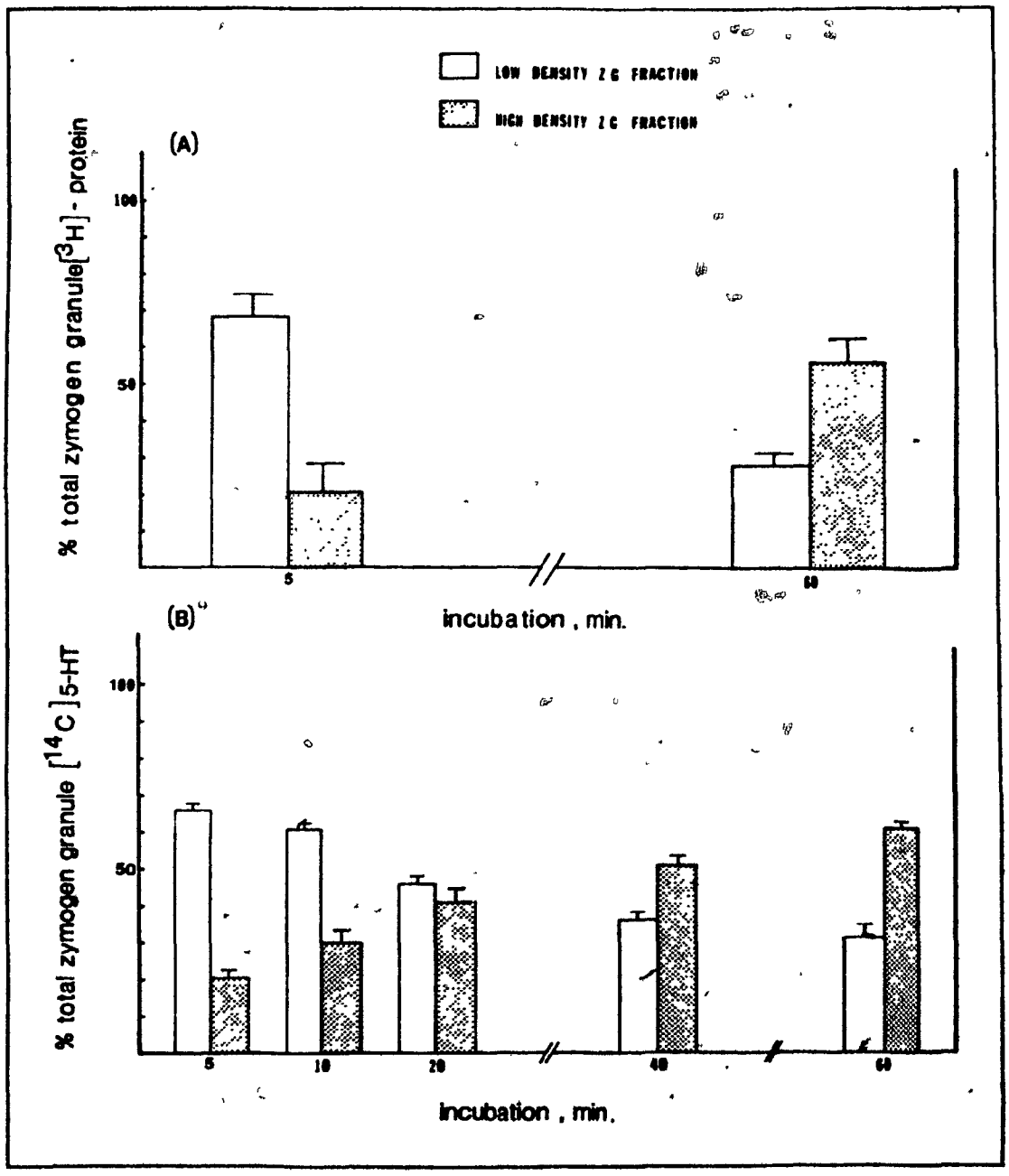
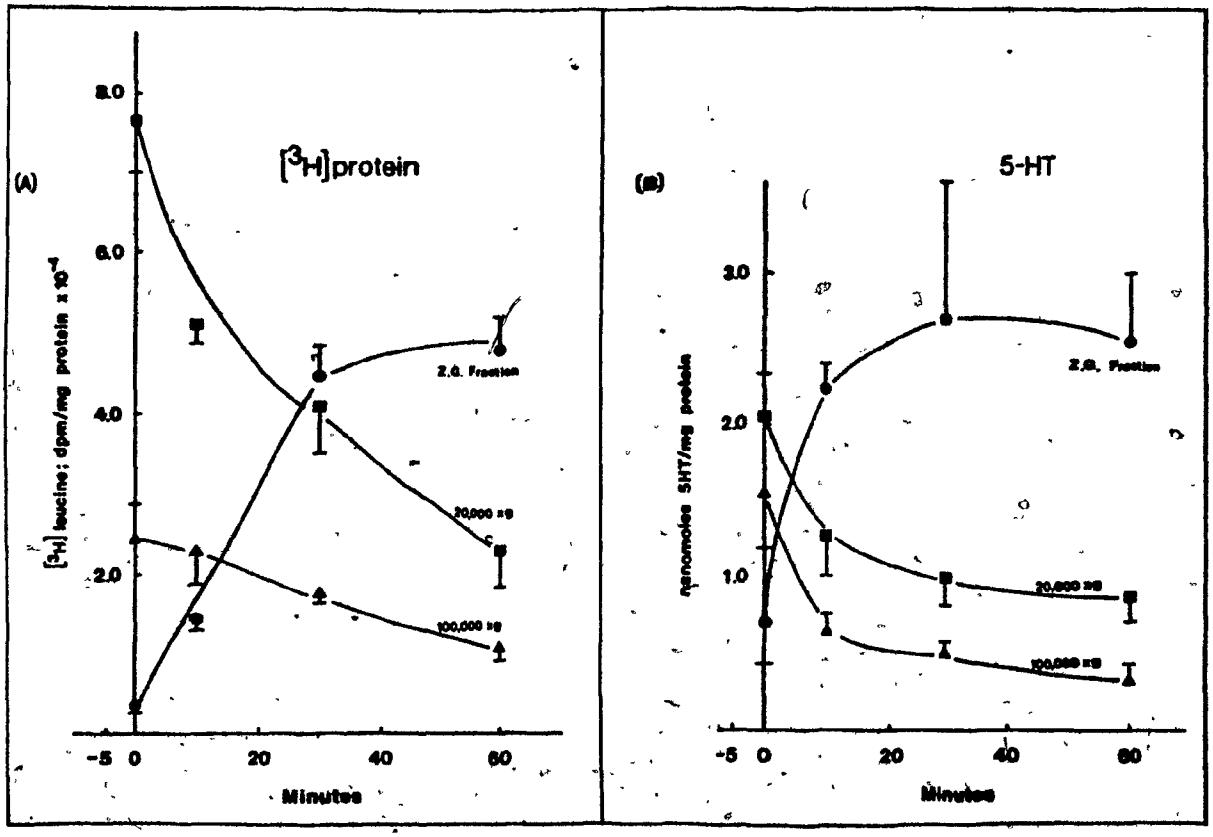

LOW DENSITY ZG FRACTION
HIGH DENSITY ZG FRACTION
(A)
% total zymogen granule[3H]- protein
100
50
5
60
incubation , min.
(B)
% total zymogen granule [14C]5-HT
100
50
5
10
20
40
60
incubation , min.

FIGURE 23 Time course of intracellular distribution of (A) [^{3}H]protein (B) [^{14}C]5-HT in zymogen granules, 20,000 X g and 100,000 X g pellets

Dispersed acinar cells (50 X 10^{6}) were pulse-incubated with L-[^{3}H]Leucine (3.34 X 10^{-8}M; 59.8 Ci/mmole) (A) and [^{14}C]5-HT (10^{-4}M) (B) in incubation buffer II for 5 minutes and chase-incubated in incubation buffer I for varying time periods up to 60 minutes. Zymogen granule (density=1.142) fraction (- ●), 20,000 X g pellet (■) and 100,000 X g pellet (▲) were isolated as described in Methods section 2.03 and assessed for their content of [^{3}H]protein (dpm/mg protein), and as 5-HT (nmole/mg protein). Each point represents mean ± S.E.M. of 4 experiments.


(A)
[3H]protein
[3H] leucine; dpm/mg protein x 10-4
8.0
6.0
4.0
2.0
2.0. Fraction
20,000 xg
100,000 xg
-5
0
20
40
60
Minutes
(B)
5-HT
nanomoles 5HT/mg protein
3.0
2.0
1.0
2.0. Fraction
20,000 xg
100,000 xg
-5
0
20
40
60
Minutes

3.4.3 Comparison of 5-HTP Decarboxylation and 5-HT Accumulation Into Zymogen Granules in Pancreas and Parotid

5-HTP decarboxylation in parotid gland was investigated. Parotid gland pancreatic acinar cells were incubated with DL-[^{14}C] 5-HTP (1.8×10^{-5}M, 59.2 mCi/mmole) for a period of 30 minutes in incubation buffer I and 5-HT production was measured. As illustrated in Fig. 24, 50% of DL-5-HTP was converted to 5-HT in pancreas whereas in parotid there was no conversion of 5-HTP to 5-HT. Under similar experimental conditions DOPA was not decarboxylated to DA in parotid gland.

To investigate whether the observed accumulation of 5-HT by zymogen granules of the pancreas also occurs with zymogen granules of the parotid gland, minced pancreatic and parotid tissue were pulse incubated separately with [^{14}C] 5-HT (10^{-4}M) and [^{3}H] leucine (3×10^{-8}M) for 5 minutes, then chased with [^{1}H] leucine only. [^{3}H] protein and [^{14}C] 5-HT incorporation into zymogen granule fraction was measured. The results are shown in Fig. 25. At 0 time after the pulse [^{3}H] protein incorporation into zymogen granules was 0.10 ± 0.09 dpm $\times 10^{2}$/unit of amylase for pancreas and 1.0 ± 0.1 dpm /unit of amylase for parotid. At 60 minutes after the pulse [^{3}H] protein incorporation into zymogen granules was 4.96 ± 0.25 dpm $\times 10^{2}$ /unit of amylase for pancreas (approximately 0.78 dpm $\times 10^{5}$ /mg zymogen granule protein) and 3.75 ± 0.38 dpm $\times 10^{2}$ /unit of amylase for parotid (approximately 1.15 dpm $\times 10^{5}$ /mg zymogen granule protein). During the same period of time, 16.4 pmoles 5-HT/unit of amylase (approximately 3 nmoles/mg zymogen granule protein) was accumulated by pancreas zymogen granules, but no

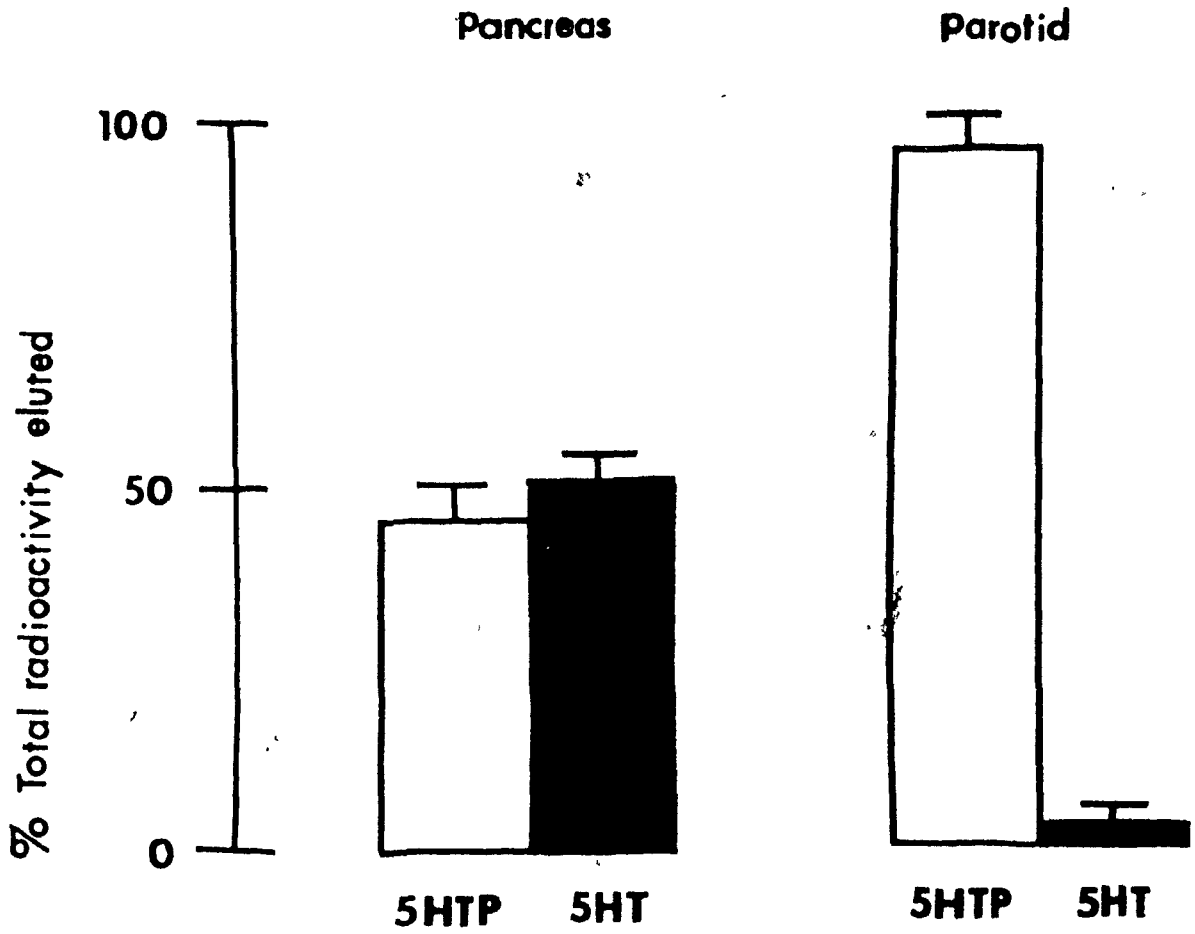
accumulation of 5-HT in zymogen granules of parotid gland was detected. The amount of 5-HT present in zymogen granules after the 60 minute incubation period was approximately 25-30% of the total 5-HT (75 pmoles/unit of amylase) taken up by pancreatic tissue. For parotid gland, the amount of 5-HT (0.35 pmoles/unit of amylase about 0.11 nmoles/mg zymogen granule protein) associated with the zymogen granule fraction 60 minutes after pulse represented approximately 2.4% of the total 5-HT (15 pmoles/ unit of amylase) taken up by the tissue.

There was no detectable dopamine or L-DOPA accumulation in parotid tissue zymogen granules when that tissue was incubated with [^{14}C] DA or L-[^{14}C] DOPA. It must be concluded therefore, that rat parotid gland zymogen granules do not accumulate either 5-HT or DA.

FIGURE 24 Comparison of 5-HTP decarboxylase activity in pancreatic and parotid gland acinar cells

Acinar cells (50×10^6) from pancreas or parotid gland* were incubated with DL-[^{14}C] 5-HTP (1.8×10^{-5}M; 59.2 mCi/mmole) for a period of 30 minutes in incubation buffer I and 5-HT production was measured. Each bar represents mean $\pm$ S.E.M. of 4 experiments.

*Parotid acinar cells were isolated according to Peterfy and Tenenhouse (1982).


Pancreas
Parotid
100
50
0
% Total radioactivity eluted
5HTP
5HT
5HTP
5HT

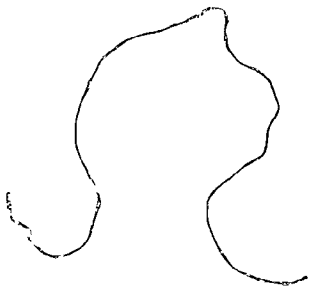
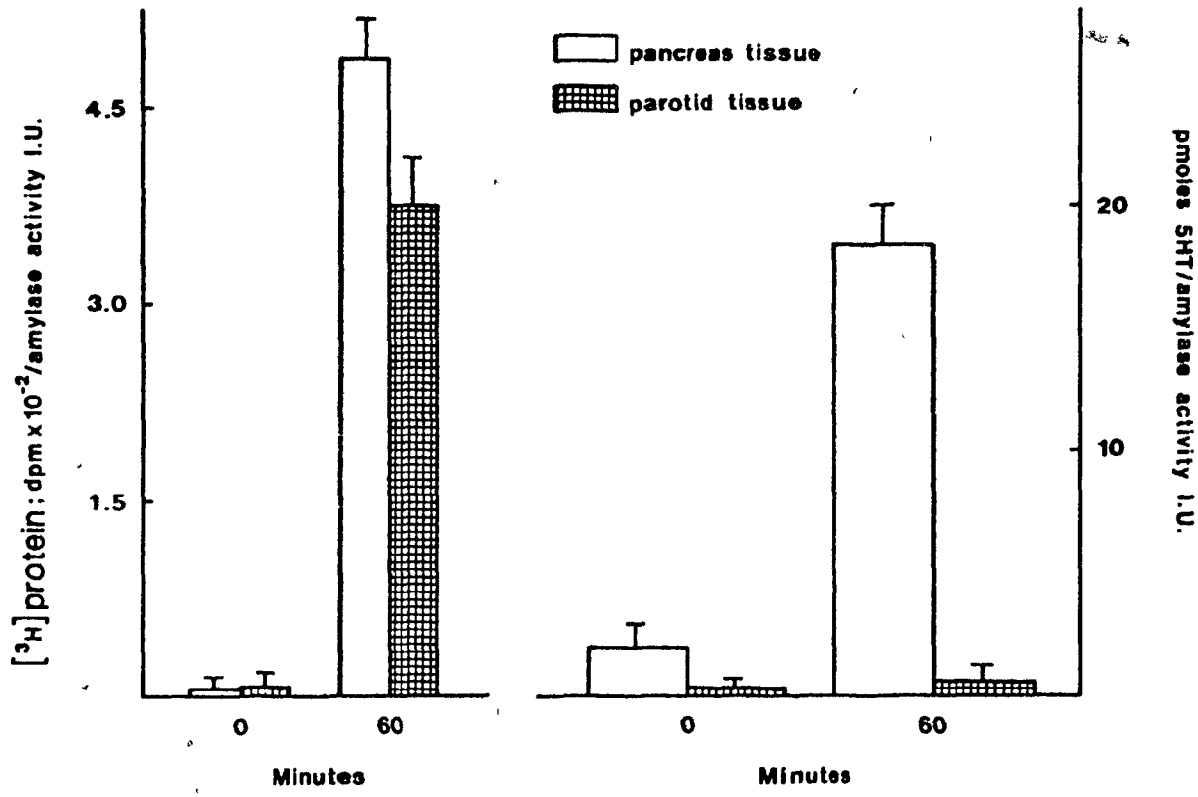


FIGURE 25 Comparison of the incorporation of 5-HT into zymogen granule fractions from parotid gland and pancreas.

Pancreatic and parotid tissue slices were pulse-incubated with L-[^{14}C] 5-HTP (0.1 mM) and L-[^{3}H] Leucine (3 X 10^{-8}M; 59.8 Ci/mmole) in incubation buffer II for 5 minutes and chase-incubated in incubation buffer I for 60 minutes. The bars in the left hand panel represent [^{3}H] protein incorporation into the zymogen granule fraction per unit of amylase activity and the bars in the right hand panel represent picomoles of 5-HT incorporated into zymogen granule per unit of amylase activity. The open bars indicate pancreatic tissues and hatched bars represent parotid tissues. Each bar is mean $\pm$ S.E.M. of 4 experiments. Parotid zymogen granule fraction were isolated and purified according to Fast (1974).


pancreas tissue
parotid tissue
[3H]protein: dpm x 10-2/amylase activity I.U.
4.5
3.0
1.5
0
60
Minutes
0
60
Minutes
20
10
pmoles 5HT/amylase activity I.U.

3.5 SECRETION OF BIOGENIC MONOAMINES WITH LABELLED PROTEIN AND AMYLASE ACTIVITY IN RESPONSE TO CAERULEIN STIMULATION

Experiments described below employed the cell column perfusion system developed by Guderley and Heisler (1980) to test whether the 5-HT incorporated into pancreatic zymogen granules *in vitro* would be released along with other zymogen granule contents when the acinar cells were stimulated with a secretagogue. The cell column perfusion system has the advantage over a static incubation system of not only quickly removing secreted proteolytic products from the cell vicinity and preventing cellular damage, but it also prevents the re-uptake of the secreted amines.

When pancreatic acinar cells, prelabelled with [^{3}H] leucine and L-[^{14}C] 5-HTP were stimulated with caerulein, amylase activity, [^{3}H] protein and [^{14}C] 5-HT were secreted as illustrated in Fig. 26. The secretion of all three occurred synchronously. The secreted material represented 8.6 $\pm$ 0.2%, 8.1 $\pm$ 0.7% and 6.1 $\pm$ 0.3% of the total [^{14}C] 5-HT, [^{3}H] protein and amylase activity respectively (Table 4) in dispersed acinar cells. When the secretagogue was removed, the release of all three secretion products declined together. A second exposure to the secretagogue resulted in an identical pattern of secretion. The secreted products again represent 8.4 $\pm$ 0.3%, 9.3 $\pm$ 0.9% and 6.7 $\pm$ 0.4% of the total [^{14}C] 5-HT, [^{3}H] protein and amylase activity respectively in the cells (Table 4). Greater than 90% of the secreted [^{14}C] was recoverable as [^{14}C] 5-HT with only a trace of [^{14}C] 5-HTP being detected (Fig. 26 inset).

In order to determine whether the [^{14}C] 5-HT recovered in the secretory product came exclusively from zymogen granules, the following experiment was done. Dispersed acinar cells were prelabelled with [^{14}C] 5-HTP and [^{3}H] leucine as described above. Zymogen granules were isolated from one half the cells and the [^{14}C] 5-HT and [^{3}H] protein content of these organelles measured. The remainder of the cells were exposed to caerulein as described above and the rate of secretion of [^{14}C] 5-HT and [^{3}H] protein measured. As illustrated in Fig. 27, there is no significant difference ($p>0.05$) between the ratio of [^{3}H] protein to [^{14}C] 5-HT extracted from the isolated zymogen granules (14.69 ± 0.87) and the ratio of [^{3}H] protein to [^{14}C] 5-HT in the secretory product during both periods of secretion (S_1, 13.03 ± 2.26; S_2, 14.51 ± 2.48).

Since DA does not accumulate in zymogen granules, it was not surprising to find that after incubation of the cells with L-[^{14}C] DOPA and measuring products released after exposure to caerulein (as described above), no release of DA was detected (Fig. 28, Table 5).

FIGURE 26 Secretion of [^{14}C] 5-HT, [^{3}H] protein and amylase from dispersed acinar cells

Dispersed acinar cells (20 X 10^{6}) were pulse-incubated with L-[^{14}C] 5-HTP (10^{-3}M, 2.0 mCi/mmole) and L-[^{3}H] Leucine (3 X 10^{-8}M, 52.2 Ci/mmole) in incubation buffer II for 45 minutes and chase-incubated in incubation buffer I containing 1.0 mM [^{1}H] Leucine and 1.0 mM [^{12}C] 5-HTP for 60 minutes. At the end of the chase-incubation period acinar cells were washed 3 times in perfusion buffer (incubation buffer I modified by reducing 1% to 0.001% [w/v] BSA). The acinar cells were packed into a Pharmacia PD-10 column as described in Methods section 2.05. The column was perfused at the rate of 40 ml/hr and eluate fractions were collected at 6 minute intervals. The entire system was maintained at 37°C. After an equilibration period of between 120 minutes and 180 minutes, caerulein, was added to the perfusate. S_1 represents stimulation for 30 minutes by 10^{-7}M caerulein and S_2 a second period of stimulation with 10^{-6}M caerulein. Amylase activity in perfusate fractions are expressed as I.U. per fraction (O---O); [^{3}H] protein as dpm X 10^{-3} per fraction (●——●); and [^{14}C] 5-HT as dpm X 10^{-3} per fraction (■- -■). The inset shows the composition of materials secreted after separation of 5-HT from 5-HTP by DOWEX 50WX4 column (see Methods section 2.06). Filled bars are 5-HTP and opened columns are 5-HT.

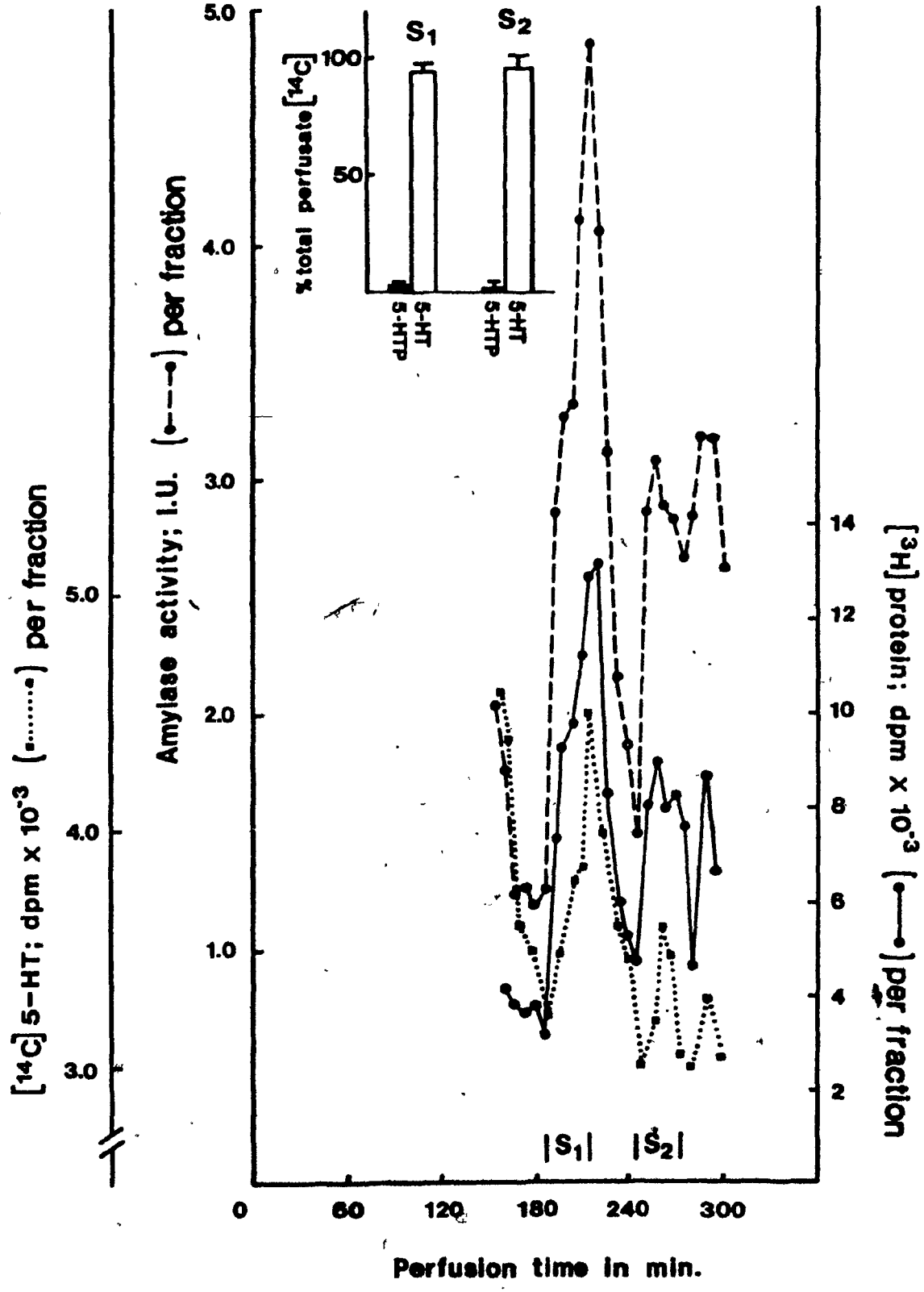

[14C]5-HT; dpm x 10-3 (.....•) per fraction
Amylase activity; I.U. (•——•) per fraction
[3H] protein; dpm x 10-3 (•——•) per fraction
% total perfusate [14C]
S1
S2
5-HT
5-HTP
|S1|
|S2|
Perfusion time in min.

TABLE 4. SECRETION OF [^{14}C] 5-HT WITH AMYLASE ACTIVTY AND [^{3}H] PROTEIN FROM DISPERSED ACINAR CELLS

Amine Precursor	Substance secreted	S_1 % of total	S_2 % of total
	Amylase	6.1 ± 0.3	6.7 ± 0.4
L-5-HTP	[^{3}H] protein	8.1 ± 0.7	9.3 ± 0.9
	[^{14}C] 5-HT	8.6 ± 0.2	8.4 ± 0.3

[^{14}C] 5-HT, amylase activity and [^{3}H] protein present in the secretory products during S_1 and S_2 stimulations (from Figure 26) are expressed as percent of the totals present in the zymogen granule fractions before the acinar cells were exposed to caerulein (see Methods). Mean ± S.E.M. n=4

FIGURE 27 Comparison of the ratio of [^{3}H] protein/[^{14}C] 5-HT in isolated zymogen granules and in the secretory product of acinar cells

Dispersed acinar cells were prelabelled with L-[^{14}C] 5-HTP and L-[^{3}H] Leucine as described in Figure 26. Zymogen granules were isolated from one half the cells and the [^{14}C] 5-HT and [^{3}H] protein content of the zymogen granules measured. The remainder of the cells were exposed to caerulein as described in Methods. The hatched bars represent the ratio of [^{14}C] 5-HT/[^{3}H] protein in the zymogen granules. The open bars represent the ratio of [^{14}C] 5-HT/[^{3}H] protein in the secretory products after the first and second stimulations. Statistical analysis (*$p > 0.05$). Each bar is mean $\pm$ S.E.M. of 4 experiments.

* Kruskal-Wallis multiple comparison test

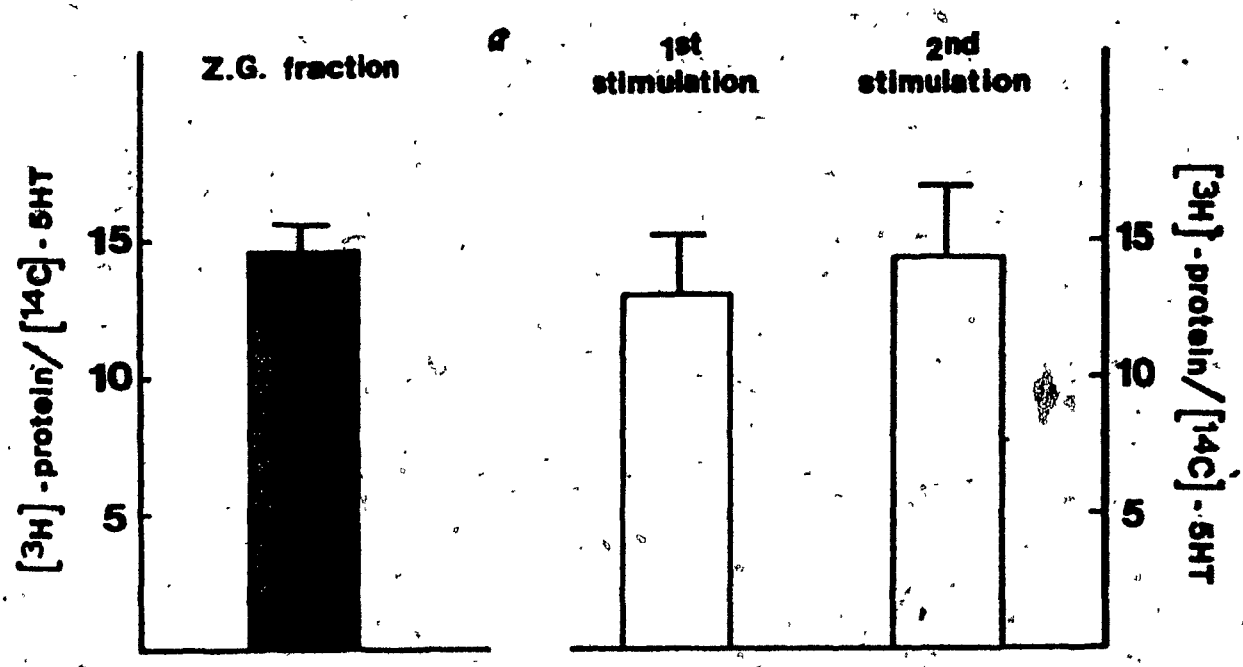

Z.G. fraction
1st stimulation
2nd stimulation
[3H] - protein / [14C] - 5HT
15
10
5
[3H] - protein / [14C] - 5HT
15
10
5

FIGURE 28 Secretion of [^{14}C] DA, [^{3}H] protein and amylase from dispersed acinar cells

Acinar cells (20 X 10^{6}) were pulse-incubated with L-[^{14}C] DOPA (10^{-3}M, 4.0 mCi/mmole) and L-[^{3}H] Leucine (3 X 10^{-8}M, 52.2 Ci/mmole) in similar experimental conditions as described in Figure 26. Amylase activity in the perfusate is expressed as I.U. per fraction (O- - -O); [^{3}H] protein is expressed as dpm X 10^{-3} per fraction (●——●). Upon chromatography on DOWEX 50WX4 90% of the [^{14}C] secreted during S_1 and S_2 was found in the DA fraction. [^{14}C] DA is expressed as dpm X 10^{-3} per fraction (■- -■).

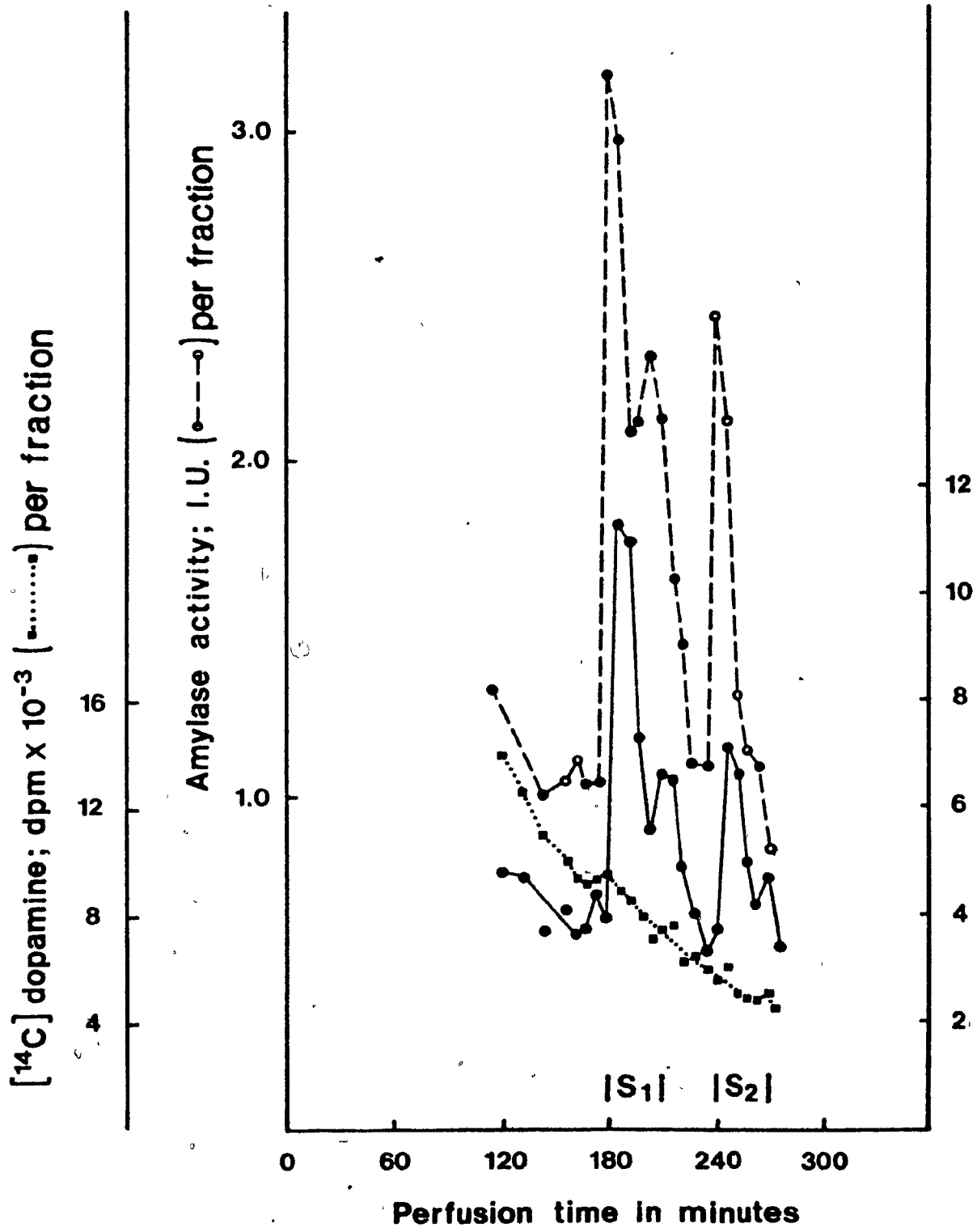

[14C] dopamine; dpm x 10-3 (▪.....▪) per fraction
Amylase activity; I.U. (•——•) per fraction
3.0
2.0
1.0
16
12
8
4
12
10
8
6
4
2
|S1|
|S2|
0
60
120
180
240
300
Perfusion time in minutes

TABLE 5 SECRETION OF [^{14}C] DA, [^{3}H] PROTEIN AND AMYLASE FROM dispersed acinar cells

Amine Precursor	Substance secreted	S_1 % of total	S_2 % of total
	amylase	6.3 ± 0.3	5.9 ± 0.6
L-DOPA	[^{3}H] protein	7.3 ± 0.5	7.1 ± 0.2
	[^{14}C] DA	0	0

Since [^{14}C] DA is not secreted with amylase activity and [^{3}H] protein as shown in Figure 28, only amylase activity and [^{3}H] protein in secretory products during S_1 and S_2 stimulation were expressed as percent in the zymogen granule fractions. Mean ± S.E.M. n=4

SECTION FOUR

DISCUSSION

Early studies of the uptake and storage of biogenic monoamines and their precursors demonstrated that both dopamine and 5-HT were concentrated within acinar cells of the rat exocrine pancreas following administration of large doses of L-DOPA and 5-HTP respectively (Alm et al., 1967; 1969; Gershon and Ross, 1966a;b). Histofluorescence studies further suggested that the amines were concentrated within zymogen granules of these cells and were eventually secreted, with other zymogen granule contents into pancreatic duct lumina (Alm et al., 1972; Mori et al., 1979a;b).

These observations suggest that the biogenic monoamines, DA and/or 5-HT may have specific functions in the exocrine pancreas and led us to formulate the hypothesis that monoamines, secreted from acinar cells, interact with centroacinar and duct cells and in so doing cause alterations in the ionic composition and volume of pancreatic juice.

If a biogenic monoamine is functioning as a "communicator" between acinar and duct cells, it should be possible to demonstrate:

1. that acinar cells contain the monoamine under normal conditions and that there are mechanisms for its synthesis from precursors and/or for its uptake from surrounding tissues.
2. that the monoamine is packaged with the contents of the zymogen granules.
3. that the monoamine is secreted along with the contents of the zymogen granules.

The first requirement was to identify and to quantitate the biogenic monoamines present in pancreatic acinar cells in the absence of treatment

of the animal with amine or amine precursor. Thus pancreata were homogenized and various cell fractions screened for the presence of biogenic monoamines. Only 5-HT was found in acinar cells. No dopamine, epinephrine or norepinephrine was detected. Furthermore, all of the 5-HT was located in the zymogen granule fraction of the cell homogenate, that is, it segregated with pelletable amylase. The concentration of endogenous 5-HT in acinar cell zymogen granules was about 11 pgm/unit amylase, or approximately 11 pmoles/mg zymogen granule protein.

When pancreatic acinar cells were incubated with either 5-HT or its precursor 5-HTP, only 5-HT was found in zymogen granules. Subcellular fractionation studies of pancreatic acinar cells demonstrated that DOPA/5-HTP decarboxylase activity is located exclusively in the cytosolic fraction of the cells; there is no evidence from our experiments that the enzyme is associated with any intracelluar organelle. The decarboxylase does not act on D-DOPA and a maximum of 50% of the DL-5-HTP is converted to 5-HT suggesting that the enzyme is stereospecific for the L-isomers applies to both substrates. Conversion of tyrosine to DA or tyramine, of DA to epinephrine or norepinephrine, and of tryptophan to 5-HT or tryptamine does not occur in the exocrine pancreas under any of the experimental conditions used.

The rate of DOPA decarboxylation by the enzyme from the exocrine pancreas is about 8 fold greater than that of 5-HTP. This difference in rate of decarboxylation of the two substrates has been observed with the L-aromatic amino acid decarboxylase activity in several regions of the

brain as well as in peripheral tissues (Rahman et al., 1981). Although there is no requirement for exogenous pyridoxal-5-phosphate, the fact that the enzyme activity is inhibited by NSD-1055 (Aures et al., 1970), a known specific potent inhibitor of pyridoxal-5-phosphate requiring decarboxylases, suggests that the pancreatic acinar cell enzyme also requires this co-enzyme. The substrate preference for L-DOPA and L-5-HTP in exocrine pancreatic acinar cells resembles that of neuroendocrine cells of the APUD system in which L-DOPA and L-5-HTP serve as precursors for DA and 5-HT synthesis respectively. Although the decarboxylation of L-5-HTP and L-DOPA in these APUD cells has not been studied extensively, it is believed that the reaction is catalyzed by a single L-aromatic amino acid decarboxylase (L-AAD) [EC 4.1.1.28] (Pearse, 1976; 1982; Pearse and Takor Takor, 1979).

Mammalian L-AAD was discovered by Holtz et al. (1938) and by Clark et al. (1954) as the enzyme that is responsible for the synthesis of DA and 5-HT. This enzyme exists in various mammalian tissues as well as the central and peripheral nervous systems. Although it has been suggested that L-AAD can decarboxylate a broad range of naturally occurring L-aromatic amino acids, including phenylalanine and histidine (Lovenberg et al., 1962), other workers (Hagen, 1962; Awapara et al., 1964; Corgie and Pacheo, 1975; Srinivasan and Awapara, 1978) have shown that, among all naturally occurring L-aromatic amino acids, only L-DOPA and L-5-HTP are substrates. Srinivasan and Awapara (1978) reported that L-AAD can decarboxylate L-o-tyrosine and L-m-tyrosine (not naturally occurring amino acids) because of the structural features that

they have in common with L-DOPA. Alm et al. (1969), and Gershon and Ross (1966a;b) suggested that the conversion of L-DOPA to DA and of L-5-HTP to 5-HT observed in exocrine pancreas was probably carried out by L-AAD. The present findings indicate that DOPA/5-HTP decarboxylase in pancreatic acinar cells exhibits substrate specificity for L-DOPA and L-5-HTP, stereospecificity for L-isomers and is located exclusively in the cytosolic fraction. These properties of DOPA/5-HTP decarboxylase in exocrine pancreas correspond closely with the general properties of L-AAD [EC 4.1.1.28] reported by previous workers who studied this enzyme in various tissues.

It has been suggested that L-DOPA decarboxylase and 5-HTP decarboxylase are distinct enzymes (Sims et al., 1973). These authors reported that rat brain L-5-HTP decarboxylase and L-DOPA decarboxylase activities exhibited different optima for pH and temperature and had different subcellular distributions. However, as analytical procedures improved, reports from many laboratories using various mammalian tissue preparations (including rat brain) indicated that a single species of L-AAD acts on both L-DOPA and L-5-HTP (Fellman, 1959; Rosengren, 1960; Christenson et al., 1972; Corgier and Pacheco, 1975; Srinivasan and Awapara, 1978). Rosengren (1960) showed that the decarboxylation of L-DOPA by a rabbit kidney extract was competitively inhibited by L-5-HTP and that in the presence of the inhibitors o-tyrosine or caffeic acid, the inhibition constants were the same irrespective of whether L-DOPA or L-5-HTP were used as substrate for the decarboxylase. On the basis of similar inhibition studies with L-DOPA and L-5-HTP, Fellman

(1959) concluded that both substrates are decarboxylated by the same enzyme in extracts of ox adrenal gland. Highly purified L-AAD from pig kidney (Srinivasan and Awapara, 1978) and rat brain (Corgier and Pacheo, 1975) indicated that the decarboxylase activity could not be resolved into separate L-DOPA and L-5-HTP decarboxylase activities.

Although extensive purification of the pancreatic decarboxylase has not been done, the present findings indicate that L-DOPA and L-5-HTP share the same catalytic site; both catalytic activities have the same pH optima, the same subcellular distribution in acinar cells; and L-DOPA and L-5-HTP compete with each other for decarboxylation. When NSD-1055 was tested as an inhibitor of the partially purified pancreas decarboxylase, the inhibition constants were similar (within one order of magnitude) for both substrates, L-DOPA and L-5-HTP.

It has been suggested that structural similarities between L-DOPA and L-5-HTP are the basis for their decarboxylation by the same catalytic site of the enzyme. Hagen and Cohen (1966) have suggested that there are three important points for the attachment of the substrate to L-AAD, the "aromatic site", the "carboxyl site" and the "amino site". Attachment to the "carboxyl" and the "amino" sites is restricted to L-isomers whereas the localization of the aromatic hydroxyl groups in the substrate determines binding to the "aromatic site". That the location of the aromatic hydroxyl group in the substrate is important, is shown by the findings of Srinivasan and Awapara (1978). These authors reported that L-AAD did not decarboxylate phenylalanine, but hydroxylphenylalanine with OH groups in the ortho or meta positions was a substrate. Hagen and

Cohen (1966) suggested that L-5-HTP and L-DOPA share some common spatial structural relationships, thus allowing both substrates to bind to the same catalytic site of the enzyme.

At present, it is not known whether one L-AAD is common to all mammalian organs or whether there are several isozymes. Rahman et al. (1981) have found different DOPA/5-HTP decarboxylation ratios in various central and peripheral tissues and have purified from rat brain two forms of L-AAD with different affinities of L-DOPA and L-5-HTP. The enzymic properties of the decarboxylase of the exocrine pancreas suggests that this enzyme is similar to, or an isozyme of the mammalian L-AAD.

The ability to decarboxylate 5-HTP and DOPA is not universal of all exocrine organs. Rat parotid gland is an exocrine gland that secretes salivary proteins and shares many similarities with exocrine pancreas, i.e. secretory protein accumulation into zymogen granules, zymogen granule formation and secretion of secretory protein by exocytosis (Wallach, 1982; Scheele, 1982). Parotid acinar cells cannot convert either DOPA or 5-HTP into the corresponding amines. After incubation of these cells with the amino acids, only the amino acids were found in the cell homogenates. Thus, the inability of parotid acinar cells to decarboxylate DOPA or 5-HTP is not due to an inability of these cells to take up the amino acids.

In summary, 5-HT was found in exocrine pancreas. It can be taken up or synthesized from 5-HTP by pancreatic acinar cells. Although DA can also be synthesized by acinar cells, endogenous DA was not found in these cells. Thus, 5-HT satisfies the first requirement as the functional

monoamine in rat exocrine pancreas. In the parotid gland neither 5-HT nor DA was found and this tissue did not possess the decarboxylase required for the synthesis of these monoamines. It is clear that these biogenic monoamines are not found in all exocrine organs and therefore they may serve an essential function unique to pancreas.

The second requirement for the putative "communicator" was to demonstrate that it can be accumulated by zymogen granules and packaged with the content of the granules. After pulse-incubation of pancreatic acinar cells with radiolabelled leucine and radiolabelled 5-HTP the kinetics of labelled 5-HT incorporation into zymogen granules was found to be similar to that of labelled protein i.e. 5-HT accumulated into zymogen granules rapidly and remained within the granules. The granular 5-HT did not exchange with extragranular 5-HT. Incorporation of 5-HT into granules other than amylase containing granules is not likely since Percoll and sucrose density gradients did not separate the amylase from 5-HT containing granules. These observations suggest that 5-HT synthesized from 5-HTP in the cell cytosol is sequestered by zymogen granules and segregate with amylase in the granules.

DA was also synthesized from its amino acid precursor by acinar cells and was incorporated into zymogen granules, but there were differences in the kinetics of uptake and storage between DA and 5-HT. The retention time of 5-HT in the zymogen granule fraction was different from that of DA. 5-HT was stable within the zymogen granule for at least 2 hours whereas DA had a half-life of 90 minutes in zymogen granules even though the total cell homogenate DA concentration was

constant throughout. The short retention time of DA by zymogen granules makes it unlikely that DA is stored in zymogen granules for secretion in parallel with secretory proteins. The present observations on the difference in turnover rate of 5-HT and DA in acinar cells zymogen granules is consistent with the findings of Alm et al. (1972); Mori et al. (1979a;b). These authors observed that L-DOPA was metabolized somewhat faster than the L-5-HTP and the turnover rate of granular 5-HT was slower than that of DA in exocrine pancreas.

In addition to the difference in retention time for 5-HT and DA in zymogen granules, the kinetics of 5-HT incorporation into zymogen granules is also different from that of DA. We found that when pancreatic acinar cells were incubated with L-[^{14}C] 5-HTP, the rate of uptake of [^{14}C] 5-HT was linear as a function of L-[^{14}C] 5-HTP concentrations throughout the concentration range tested (10^{-6}M to 10^{-2}M). The rate of accumulation of [^{14}C] 5-HT into zymogen granules plateaued at 10^{-3}M L-[^{14}C] 5-HTP and remained constant with further increase in L-[^{14}C] 5-HTP concentration. In contrast when acinar cells were incubated with L-[^{14}C] DOPA the rate of accumulation of [^{14}C] DA into pancreatic cells and into zymogen granules increased linearly as a function of L-[^{14}C] DOPA concentration up to 10^{-2}M. It appears therefore that the uptake of 5-HT into zymogen granule is a saturable process whereas the uptake of dopamine is not. The specificity of the amine uptake system in zymogen granule has not yet been defined. With regard to its presence and stability within zymogen granules, the present study suggests that 5-HT is a normal constituent of rat pancreas zymogen

granules. On the other hand, the short half-life of DA in zymogen granules, and the observation that no endogenous DA is found suggests that DA is not normally present in zymogen granules.

The maximum concentration of 5-HT in zymogen granules under our experimental conditions was 8 nmoles per mg zymogen protein. This is approximately 700 times higher than that of the endogenous 5-HT concentration. There are two possible explanations for this discrepancy in granular 5-HT concentration. Our studies of 5-HT accumulation into zymogen granules were done in the presence of pargyline, a monoamine oxidase inhibitor. This compound prevents the degradation of 5-HT to 5-hydroxyindole acetic acid thus allowing for the accumulation of greater amounts of 5-HT within the cell compared to the situation without a monoamine oxidase inhibitor. Proportionately larger quantities of 5-HT will also accumulate in the zymogen granules. We believe that this accounts, at least in part for the fact that cells exposed to 5-HT or 5-HTP in vitro accumulated much greater quantities of 5-HT than that found in freshly isolated pancreatic acinar cells. A second factor is that in vitro the cells were exposed to concentrations of substrate which were considerably greater than that seen in vivo. Since the rate of uptake of 5-HT is a function of substrate concentration (10^{-3}M 5-HTP results in maximum granular [5-HT] under our experimental conditions) it follows that the granular [5-HT] will exceed that seen under normal conditions in vivo.

We next attempted to define at what stage of granule development 5-HT was accumulated. As outlined in the Introduction, secretory

proteins in the exocrine pancreas are synthesized by membrane-bound ribosomes, subsequently transported from rough endoplasmic reticulum to Golgi, and ultimately stored in the zymogen granules. Since the site of exportable protein synthesis as well as the route and kinetics of the intracellular transport are well known, some information concerning the cellular disposition of 5-HT can be deduced by comparing the distribution of labelled protein with labelled 5-HT in isolated subcellular fractions. The present experiments demonstrate that 5-HT incorporation into the zymogen granule fraction follows closely the kinetics of pulse-labelled protein incorporation into TCA insoluble zymogen granule protein.

There is no evidence that 5-HT is covalently bound in the primary structure of any protein and therefore it is unlikely that the zymogen granule 5-HT is covalently associated with the granular protein. Furthermore, 98% of granular 5-HT is separated from protein by TCA precipitation. We have no evidence for the incorporation of 5-HTP into protein. In all of our experiments using radiolabelled 5-HTP this amino acid was never detected in TCA precipitable protein. It is therefore concluded that the 5-HT in zymogen granules is free or in non-covalent (e.g. ionic) linkage with other granular contents.

It has been shown that condensing vacuoles are the precursors from which zymogen granules are formed (Jamieson and Palade, 1971; also see Introduction section 1.2.2). Condensing vacuoles and zymogen granules are sedimented in the same fraction and are separated from each other by density gradient centrifugation. The low density zymogen granule fraction (1.108) in the present studies represent the crude fraction of

condensing vacuoles. This was demonstrated by the observation that immediately after pulse-incubation with labelled leucine, maximum specific activity of labelled-protein appeared in the low density granule fraction while in the high density granule fraction (1.142) consisting of relatively pure mature zymogen granules, the specific activity of labelled protein was minimal immediately after the pulse-incubation period. This suggested that the low density granule fraction was indeed newly synthesized granules. The present experiments suggest that labelled proteins appear in the mature zymogen granule fraction from the crude condensing vacuoles fraction and 5-HT followed the kinetics of the pulse-labelled proteins.

Jamieson and Palade (1967a;b) demonstrated that pulse labelled proteins were first associated with the microsomal fraction, and later with the condensing vacuoles fraction and zymogen granules fraction. The present results on kinetics of pulse-labelled proteins are similar to that reported by Jamieson and Palade (1967a;b). Pulse-labelled proteins first appeared in the microsomal fraction and the Golgi vesicles and condensing vacuoles fraction, and were eventually transported to the high density zymogen granules fraction (mature zymogen granules). 5-HT incorporation into high density zymogen granule fraction follows the same kinetics as that of labelled proteins. The present study is the first demonstration of this type of association and provides evidence to confirm the autoradiographic studies by Gershon and Ross (1966) in which a striking similarity was found between the pattern of movement of radioactive 5-HT through the acinar cells and the movement of proteins labelled by

injection of tritiated leucine reported by Caro and Palade (1964).

Although the mechanism by which 5-HT is incorporated into zymogen granules is not known, the present observations suggest that 5-HT and secretory proteins are incorporated together at an early stage of zymogen granule formation and remain within the granule as it matures.

Alm et al., (1972) reported that, after injection of labelled 5-HTP into mice, labelled material was found in the duct lumina of exocrine pancreas. Although these authors were not able to identify the labelled material, they suggested that it was probably 5-HT that had been discharged from the exocrine acinar cells upon the emptying of zymogen granules that stored them. The same conclusion was made by Mori et al. (1979b) when they detected specific 5-HT fluorescence in the pancreatic juice after injection of L-5-HTP into rats. However, evidence for co-secretion of 5-HT with the contents of zymogen granules has not previously been provided. The present study shows that when isolated acinar cells were prelabelled with L-[^{14}C] 5-HTP and L-[^{3}H] leucine and then exposed to the secretagogue, caerulein, [^{14}C] 5-HT and [^{3}H] protein were released together and the ratio of [^{3}H] protein to [^{14}C] 5-HT in the secretory product was identical to that found in the zymogen granules.

Non-specific leakage of 5-HT during caerulein stimulation is unlikely since DA which accumulates in large amount within the acinar cells is not released during caerulein stimulation (see below).

During each period of stimulation with caerulein approximately 8% of the total [^{3}H] protein and [^{14}C] 5-HT, and 6% of the total amylase are released. Since amylase has been found exclusively in zymogen

granules (amylase is an index for zymogen granules) and 99% of the newly synthesized proteins are stored into zymogen granules (Matos-Medina, 1975), these suggest that approximately 75% of the zymogen granules in acinar cells are labelled with [^{3}H] protein under our experimental conditions (one hour "pulse" with [^{3}H] leucine and two hours "chase" with [^{1}H] leucine). The observation that the same proportion (8%) of [^{14}C] 5-HT and [^{3}H] protein are released suggests that the kinetics of [^{14}C] 5-HT incorporation into zymogen granules is the same as that of [^{3}H] protein. This further strengthens our statements that 5-HT and protein are incorporated together into zymogen granules and released together when stimulated by secretagogues.

Under similar experimental conditions, DA was not secreted together with [^{3}H] protein. Thus, it is consistent with the present observation that DA (half life 90 minutes) is not stable with the granular protein. Although L-DOPA is taken up by isolated acinar cells and decarboxylated, and the amine so formed taken up by zymogen granules, its transient appearance and its inability to be released with the granule content raises doubts as to whether DA is a normal element of the zymogen granule content.

The overall sequence of events of 5-HT synthesis, storage and secretion from pancreatic acinar cells is summarized in Fig 29.

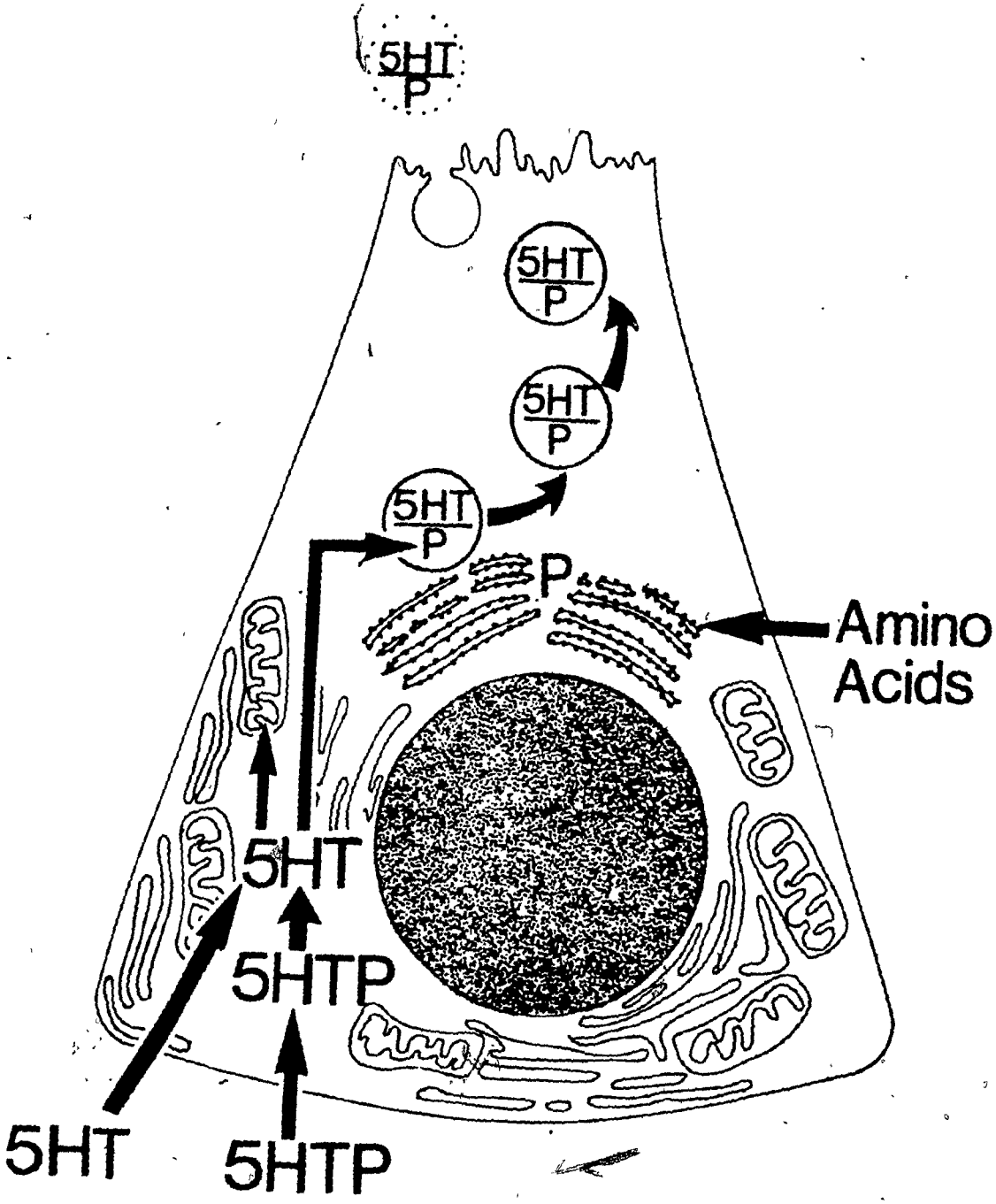
Figure 29 Synthesis, storage and secretion of 5-HT from pancreatic acinar cells

Pancreatic acinar cells take up L-5-HTP from the environment (e.g. blood circulation), decarboxylate it to form 5-HT via a substrate specific L-AAD in the cytoplasm. Acinar cells can also take up 5-HT from the surroundings. 5-HT, sequestered by the cell and/or synthesized from L-5-HTP in the cell cytoplasm, is packaged with the granule proteins. When acinar cells are activated by a secretagogue, 5-HT is released along with the granule contents into the lumen.

5-HTP=5-hydroxy tryptophan

5-HT=5-hydroxytryptamine

p=protein


5HT
P
5HT
P
5HT
P
5HT
P
P
Amino
Acids
5HT
5HTP
5HT
5HTP

In summary 5-HT satisfies the three criteria for the putative "communicator" in our working hypothesis. 5-HT exists in acinar cell zymogen granules; it can be synthesized by acinar cells or taken up from their environment; it is stable within the granules and secreted with the granule contents. Not all biogenic monoamines are able to satisfy these criteria of a putative communicator in exocrine pancreas and not all exocrine organs have specificity for the synthesis, storage and secretion of 5-HT. Thus, 5-HT may have a unique function in exocrine pancreas.

The metabolism and disposition of L-5-HTP and 5-HT in exocrine pancreatic acinar cells are analogous to that in cells of the APUD system described by Pearse (1968) and Pearse and Takor Takor (1979). Acinar cells decarboxylate the amino acid and store the resultant monoamine along with protein(s) in cytoplasmic granules. In the case of cells of the APUD system, the protein is a hormone while in exocrine pancreatic acinar cells the proteins are digestive enzymes or proenzymes. The fact that many peptide hormone-producing APUD cells have the capacity to take up L-DOPA and L-5-HTP, convert them to their respective monoamines, and retain the monoamines in the cytoplasmic granules suggest that monoamines are somehow important for the function of these cells (Sundler et al., 1980). The role of the monoamine in the APUD cells has not yet been defined; similarly, the role of 5-HT in exocrine pancreas is not clear.

As proposed in our working hypothesis 5-HT may act as a paracrine messenger, i.e. the agent that acts "locally per diffusioneum" (Larsson et al., 1979; Larsson, 1979; 1980). The proteins secreted by acinar cells

must be delivered to their sites of action. In the exocrine pancreas, the rate of removal of secreted products (hydrolytic enzymes and mucus) depends primarily on secretion pressure and viscosity of the fluid produced, since there is no propulsion system such as the contractile myoepithelial cell system which exists in some other exocrine organs (Case, 1978). The primary factors in determining the efficiency of delivery of secretory protein from the exocrine pancreas are therefore those properties that determine viscosity such as electrolyte composition, pH, and the volume of fluid which is secreted primarily by centroacinar cells and duct cells. Fluid secretion from acinar cells contributes minimally in this respect (Bundgaard et al., 1981). The major stimulus of fluid secretion from centroacinar/duct cells in the exocrine pancreas is secretin (see Introduction section 1.3). The present studies suggest that 5-HT is secreted with the protein contents of zymogen granules and may function to modulate the ionic composition, pH and/or volume of fluid secreted by centroacinar/duct cells as required for optimum flow of the secretory product from acinar cells.

Although the action of 5-HT on electrolyte transport in exocrine pancreas has not been investigated, this monoamine has a profound effect on intestinal electrolyte secretion. As in the exocrine pancreas, 5-HT in the intestinal tract is stored in cytoplasmic granules of the enterochromaffin cells which also possess the capacity to take up and decarboxylate L-5-HTP (Thompson, 1971; Alumets et al., 1977). The modulation of electrolyte secretion in intestine by 5-HT has been shown to be mediated by Ca^{+2} (Kisloff and Moore, 1976; Donowitz et al., 1979;

Hardcastle et al., 1981; Donowitz et al., 1980).

Furthermore, clinical studies from patients with carcinoid syndrome and diarrhea revealed that there was an increase in jejunal secretion of water, sodium and chloride ions. Therapy with methysergide, a peripheral inhibitor of serotonin action, significantly decreased the diarrhea in these patients. Because patients with carcinoid syndrome have been shown to have increased blood levels of serotonin, it is suggested that 5-HT is a potential intestinal secretagogue (Donowitz and Binder, 1975; Donowitz et al., 1979).

As outlined in the introduction, secretion from the exocrine pancreas involves primarily two types of cells, acinar cells (enzyme secreting cells) and centroacinar/duct cells (water and electrolyte secreting cells). Unlike the pancreas, in the parotid gland, the mechanisms for protein and electrolyte secretion appears to co-exist in one cell type (acinar cells) (Scratcherd and Case, 1973). Epinephrine is a major stimulus for secretion of enzymes (beta-adrenergic) and water and electrolytes (alpha-adrenergic) from the parotid gland. This organ also possesses a myoepithelial system for the encouragement of flow of secretory product. Thus the necessity for a transmitter system in the parotid gland to stimulate water and electrolyte secretion and maintain optimum flow of secretory products is not as great as in exocrine pancreas. The present observation that 5-HT is incorporated into zymogen granules in the exocrine pancreas, and not in parotid gland is consistent with this view.

Besides the above proposed role of 5-HT there are other possible functions that this amine can have. One of these may be in the

packaging of proteins with the zymogen granules. Preliminary experiments from our laboratory indicate that 5-HT at concentrations 10^{-10} to 10^{-5}M affects neither protein synthesis, nor the rate of protein incorporation into mature zymogen granules. The mature zymogen granule is an electron dense, osmotically inert organelle containing large quantities of Ca^{+2} as well as protein and 5-HT. Pancreatic zymogen granules contain approximately 36 nmoles Ca^{+2}/mg protein and it has been proposed that this Ca^{+2} is packaged with exportable protein at some time early in the formation of the zymogen granule (Ceccarelli et al., 1975). The role of Ca^{+2} in the granules is not clear. Secretory proteins are concentrated in the granules in a way which allows the storage, in aggregated form, of large quantities of secretory products within a minimum of granular space. Thus, factors such as charge neutralization, pH, and ionic strength within the granules are important for condensation of secretory protein. It has been suggested that Ca^{+2} is involved in secretory protein condensation and zymogen granule stabilization (Scheele, 1982). Similar to Ca^{+2}, 5-HT was sequestered by zymogen granules during their early formation. 5-HT has a similar retention time in zymogen granules as secretory protein. Unlike DA, 5-HT once it is incorporated, does not dissociate from the zymogen granule, nor is granular 5-HT exchanged with cytosolic 5-HT. It is possible therefore that 5-HT has a role, directly or indirectly through regulating the rate of Ca^{+2} incorporation into granule, and thus in maintaining the stability of zymogen granules.

Another possible role for 5-HT is its direct effect on acinar cells.

At the moment of release, the granule contents must be made sufficiently fluid to flow from the cell into the lumen. The factors that determine "fluidization" of granule content, are not understood. It is known that enzyme secretion is accompanied by neutral fluid secretion when acinar cells are stimulated by secretagogues. The neutral fluid secreted from acinar cells contains Na^+, K^+ and Cl^- at concentrations close to those in plasma (Case et al., 1980; Petersen et al., 1981). 5-HT may be involved in "fluidization" of granule contents by modulating the composition and/or the secretion of neutral fluid by acinar cells.

A role of the exocrine pancreas in excretion of monoamines has been suggested (Mori et al., 1979a;b;c). Such a function seems unlikely for several reasons. These compounds are very rapidly and efficiently metabolized to biologically inactive compounds by several organs including the exocrine pancreas. These metabolites are rapidly excreted in the urine. The finding that dopamine, although taken up by zymogen granules, is rapidly released back into the cytoplasm and is not secreted from the cell indicates that at least for this amine, this is not an excretory pathway. One is forced to conclude that either 5-HT has a special function in the exocrine pancreas or that the postulated excretory function is restricted to 5-HT. The former seems much more likely than the latter.

It has been known for some time that 5-HT has profound effects on water and electrolyte secretion in the intestine (see Donowitz et al., 1979; Hardcastle et al., 1981). The main site of action is the small intestine (Donowitz et al., 1980), therefore, it is possible that the major

role of 5-HT in pancreatic secretion is to modulate water and electrolyte secretion in this organ.

Of all the functions of 5-HT suggested above, the one which proposes a role for 5-HT in modulating water and electrolyte secretion by duct cells allows some interesting predictions to be made about exocrine gland function when there is a defect in the monoamine modulating system. We have suggested that 5-HT plays a role in maintaining fluid viscosity and thereby ensuring that the fluid will flow. Any interference with this system will lead to an increased viscosity and possible decrease or total cessation of flow with duct obstruction. Such a system may not be necessary in all exocrine organs. In the parotid gland, which is a pure serous gland, flow is ensured by myoepithelial cells which surround the acini and propel fluid along the ducts when they contract. This might explain why biogenic monoamines are not present in zymogen granules of the parotid gland. Our thesis leads to the prediction that other salivary glands which do secrete mucous should contain a biogenic monoamine within their secretory granules which is secreted when the other granule contents are secreted. Exocrine glands of the intestine, lung, reproductive tract should also contain biogenic monoamine within their secretory granules.

Cystic fibrosis is an inherited disease of exocrine organs in which the duct systems of these glands become obstructed with very viscous, mucous containing secretions (review see Taussig and Landau, 1976; Davis and DiSant'Agnese,1980). The systems most frequently involved include the exocrine pancreas, mucous glands of the lung and intestine and the

glands of the male reproductive system. There are no good animal models of cystic fibrosis but Martinez et al. (1975a;b) have reported that rats treated chronically with reserpine develop lesions in the pancreas and submandibular glands similar to those found in patients with cystic fibrosis. In the pancreas of the reserpinized rat, there is a reduction in volume and total bicarbonate secreted in response to secretin administration (Perlmutter and Martinez, 1978). Although reserpine may have many pharmacological actions its principle effect is to deplete all stores of catecholamines and 5-HT (Weiner, 1980). Given the fact that no known store of catecholamines or 5-HT is resistent to this effect of reserpine it seems likely that chronic administration of the drug would cause depletion of zymogen granule stores of 5-HT. We would predict therefore that this drug would impair the ability of the exocrine pancreas to make the adjustments in fluid volume, ionic strength and pH required to ensure the flow of pancreatic juice. Inspissation of fluid contents and duct obstruction would ensue. These effects of chronic reserpine administration lend support to our hypothesis and suggest that interference with the "amine" system leads to pathology. They further suggest that if such a modulating system exists in man interference with it leads to morphological and functional changes similar to cystic fibrosis.

SECTION FIVE

SUMMARY AND CLAIMS TO ORIGINAL RESEARCH

1. The endogenous content of 5-HT in acinar cells was found to be 10.86 ± 2.52 ng/unit of amylase. This can be totally accounted for by the 5-HT recovered from the zymogen granule fraction of the cells (10.70 ± 3.06 ng/unit of amylase).
2. Pancreatic acinar cells can decarboxylate L-5-HTP and L-DOPA to form 5-HT and DA. DOPA/5-HTP decarboxylase in acinar cells is located in the cytosolic fraction and is stereospecific for L-isomers. Of the five aromatic amino acids tested, only L-DOPA and L-5HTP are substrates of the decarboxylase.
3. Both L-DOPA and L-5-HTP compete with each other for decarboxylation, and both decarboxylations are inhibited by NSD-1055. The IC_{50}'s for decarboxylation of L-DOPA (7.0×10^{-9}M) and L-5-HTP (3.5×10^{-9}M) under identical conditions differ by less than one order of magnitude.
4. Conversion of DA to NA is not detected in acinar cells, nor was there any detectable decarboxylation of the amino acids L-p-tyrosine, L-tryptophan and L-histidine.
5. When incubated with [^{14}C] 5-HT, dispersed acinar cells take up the amine and concentrate it in zymogen granules. These cells can also take up [^{14}C] 5-HTP, decarboxylate it and store the [^{14}C] 5-HT in zymogen granules. 5-HTP itself is not taken up by the granules.
6. 5-HT within the zymogen granule is not exchangeable with extragranular 5-HT.
7. When tissue slices from parotid gland were incubated with 5-HT the amine was not incorporated into zymogen granule, nor was 5-HTP

decarboxylated to form 5-HT.

8. When dispersed acinar cells, prelabelled with [^{14}C] 5-HT and [^{3}H] Leucine, are stimulated with caerulein, [^{14}C] 5-HT, [^{3}H] protein and amylase activity are secreted synchronously. The ratio of [^{3}H] protein/[^{14}C] 5-HT in zymogen granules and in secretory product are identical.

9. Pancreas acinar cells take up L-DOPA, decarboxylate it and store the dopamine produced in zymogen granules, but the amine is not retained by the granules (half life approximately 90 minutes), and dopamine secretion from cells incubated with caerulein could not be demonstrated.

REFERENCE

Al-Awqati Q., Cameron J.L., Greenough III W.B. (1973) Electrolyte transport in human ileum: effect of purified cholera exotoxin. Am. J. Physiol. s224, 818-823

Alm P., Ehinger B., Flack B. (1967) L-Dopa turnover in mouse pancreas. Life Sci. 6, 913-917

Alm P., Ehinger B., Flack B. (1969) Histochemical studies on the metabolism of L-Dopa and some related substances in the exocrine pancreas. Acta Physiol. Scan. 76, 106-120

Alm P., Ekholm R., Ericson L.E. (1972) Metabolism of L-Dopa and L-5HTP in exocrine pancreas studied with autoradiography in electron microscope. J. Ultrastructure Res. 38, 265-278

Alumets J., Håkanson R., Ingemansson S., Sundler F. (1977) Substance P and 5-HT in granules isolated from intestinal argentaffin carcinoid. Histochemistry 52, 217-222

Amsterdam A., Jamieson J.D. (1974) Studies on dispersed pancreatic exocrin cells. I. Dissociation technique and morphologic characteristics of separated cells. J. Cell Biol. 63, 1037-1056

Anastasi A., Erspamer V., Endean R. (1968) Isolation and amino acid sequence of caerulein, the active decapeptide of the skin of Hyla caerulea. Arch. Biochem. Biophys. 125, 57-68

Anderson G.M., Purdy W.C. (1979) A liquid chromatographic fluorometric system for the analysis of indoles in physiological samples. Anal. Chem. 51, 283-286

Argent B.E., Case R.M., Hirst F.C. (1977) The effects of manganese on amylase secretion and calcium efflux in rat pancreas. J. Physiol. 269, 70-71p

Argent B.E., Case R.M., Hirst F.C. (1982a) The effect of extracellular calcium deprivation on amylase secretion and ^{45}Ca efflux from rat pancreas. J. Physiol. 323, 339-352

Argent B.E., Case R.M., Hirst F.C. (1982b) The effect of manganese, cobalt and calcium on amylase secretion and calcium homeostasis in rat pancreas. J. Physiol. 323, 353-375

Atack C. (1977) Measurement of biogenic amines using cation exchange chromatography and fluorimetric assay. Acta Physiologica Scandinavic Suppl 451, 2-84

Atack C., Linquist M. (1973) Conjoint native and orthophthaldialdehyde - condensate assays for the fluorimetric determination of 5-hydroxyindoles in brain. Naunyn-Schmiedeberg's Arch. Pharmac.

279, 267-284

Aures D., Håkanson R., Clark W.G. (1970) Histidine decarboxylase and Dopa decarboxylase. In "Handbook of Neurochemistry", Vol 4. Ed. Lajtha A. New York London Plenum Press. p.165-196

Awapara J., Perry T.L., Hanly C., Peck E. (1964) Substrate specificty of Dopa decarboxylase. Clin. Chim. Acta 10, 286-289

Bastie N.J., Vaysse N., Brenac B., Pascal J.P., Ribet A. (1977) effects of catecholamines and their inhibitors on the isolated canine pancreas. Gasteroenterology 72, 719-723

Bayliss W.M., Starling E.H. (1902) The mechanism of pancreatic secretion. J. Physiol. London 28, 325-353

Bendayan M., Roth J., Perrelet A., Orci L. (1980) Quantitative immunocytochemical localization of pancreatic secretory proteins in subcellular compartments of rat acinar cell. J. Histochem. Cytochem. 28, 149-160

Blobel G., Dobberstein B. (1975a) Transfer of proteins across membranes. I. Presence of proteolytically proceed and unprocessed nascent immunoglobulin light chain on membrane-bound ribosomes of murine myeloma. J. Cell Biol. 67, 835-851

Blobel G., Dobberstein B. (1975b) Transfer of proteins across membtanes. II. Reconstitution of functional rough microsomes from heterologous components. J. Cell Biol. 67, 852-862

Bolender R.P. (1974) Stereological analysis of the guinea-pig pancreas. I. Analytical model and quantitative description of nonstimulated pancreatic exocrine cells. J. Cell Biol. 61, 269-287

Bonner N.D. (1955) Succinic dehydrogenase assay. IN "Methods in enzymology". Eds. Colowick S.P., Kaplan N.O. Vol 1. Academic Press, N.Y. p.722

Borle A.B. (1981) Control, modulation and regulation of cell calcium Rev. Physiol. Biochem. Pharmacol. 90, 14-153

Bundgaard M., Møller M., Hedemark Poulsen J. (1981) Localization of sodium pump sites in cat pancreas. J. Physiol. 313, 405-414

Burton K. (1956) A study of the conditions and mechanisms of the diphenylamine secretion for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62, 315-322

Caflisch C.R., Solomon S., Galey W.R. (1980) In situ micropuncture study of pancreatic duct pH. Am. J. Physiol. 238, G263-268

Caro L.G., Palade G.E. (1964) Protein synthesis, storage and discharge in the pancreatic exocrine cell. An autoradiographic study. J. Cell Biol. 20, 473-495

Case R.M. (1978) Synthesis, intracellular transport and discharge of exportable proteins in the pancreatic acinar cell and other cells. Biol. Rev. 53, 211-354

Case R.M. (1979) Pancreatic secretion: cellular aspects. In "Scientific basis of gastroenterology". Eds. Duthie H.L., Wormsley K.G. churchill Livingstone: Edinburgh. p. 163-198

Case R.M., Charlton M., Smith P.A., Scratcherd T. (1980) Electrolyte secretory process in exocrine pancreas and their intracellular control. In "Biology of normal and cancerous exocrine pancreatic cells" INSERM symposium No. 15 Eds. Ribet A., Pradayrol L., Sisini C. Elsevier/North-Holland Biochemical Press, Amsterdam, N.y., Oxford. p.41-54

Case R.M., Johnson M., Scratcherd T., Sherratt H.S.A. (1972) Cyclic adenosine 3',5'-monophosphate concentration in the pancreas following stimulation by secretin, cholecystokinin-pancreozymin and acetylcholine. J. Physiol. 223, 669-684

Case R.M., Scratcherd T. (1972) The actions of dibutyryl cyclic adenosine 3',5'-monophosphate and methyl xanthines and pancreatic exocrine secretion J. Physiol. 223, 649-667

Case R.M., Scratcherd T., Wynne R.D'A. (1970) The origin and secretion of pancreatic juice bicarbonate. J. Physiol. 210, 1-15

Castle J.D., Jamieson J.D., Palade G.E. (1975) Secretion granules of the rabbit parotid gland. Isolation, subfractionation and characterization of the membrane and contents subfractions. J. Cell Biol. 64, 182-210

Ceccarelli B., Clemente F., Meldolesi J. (1975) Secretion of calcium in pancreatic juice. J. Physiol. 245, 617-638

Cegrell L. (1968) Adrenergic nerves and monoamine-containing cells in the mammalian endocrine pancreas. A comparative study. Acta Physiol. Scand. Suppl. 314, 17-23

Ceska M., Hultman E., Ingelman B. (1969) A new method for determinating of alpha-amylase. Experimentia 25, 555-556

Chauvelot L., Heisler S., Huot J., Gagnon D. (1980) Prostaglandins and enzyme secretion from dispersed rat pancreatic acinar cells. Life Sci. 25, 913-920

Chey W.Y., Konturek S.J. (1982) Plasma secretin and pancreatic secretion in response to liver extract meal with varied pH and exogenous secretion in the dog. J. Physiol. 324, 263-272

Chey W.Y., Lee H., Hendricks J.G., Rhodes R.A., Tai H.H. (1978) Plasma secretin concentrations in fasting and postprandial state in man Am. J. Dig. Dis. 23, 981-988

Christenson J.G., Dairman W., Udenfriend S. (1972) On the identity of Dopa decarboxylase and 5-hydroxytryptophan decarboxylase. Proc. Natl. Acad. Sci. USA 69, #2, 343-347

Christenson J.G., Dairman W., Udenfriend S. (1970) Preparation and properties of a homogenous AAAD from frog kidney. Archs. Biochem. Biophys. 141, 356-367

Clark C.T., Weissbach H., Udenfriend S. (1954) 5-hydroxytryptophan decarboxylase: preparation and properties. J. Biol. Chem. 210, 139

Clemente F., Meldolesi J. (1975) Calcium and pancreatic secretion. I. Subcellular distribution of calcium and magnesium in the exocrine pancreas of the guinea pig. J. Cell Biol. 65, 88-102

Corgier M., Pacheco H. (1975) Purification et proprietes de la L-amino acide aromatique decarboxylase (4.1.1.28) du cerveau de rat. Biochimie 57, 1005-1017

Creutz C.E., Pazoles C.J., Pollard H.B. (1978) Identification and purification of an adrenal medullary protein (synexin) that causes calcium-dependent aggregation of isolated chromaffin granules. J. Biol. Chem. 253, 2858-2866

Creutz C.E., Pazoles C.J., Pollard H.B. (1979) Self-association of synexin in the presence of calcium. J. Biol. Chem. 254, 553-558

Davis P.B., Di Sant'Agnese P.A. (1980) A review. Cystic fibrosis at forty - quo vadis. Pediat. Res. 14, 83-87

Dean P.M. (1974) Surface electrostatic-charge measurements on islet and zymogen granules: effect of calcium ions. Diabetologia 10, 427-430

Dean P.M. (1975) Exocytosis modelling: an electrostatic function for calcium in stimulus-secretion coupling. J. Theor. Biol. 54, 289-308

Dean P.M., Matthews E.K. (1975) the London-Van der Waals attraction constant of secretory granules and its significance. J. Theor. Biol. 54, 309-321

Domschke S., Konturlk S.J., Domschke W., Dembinski A., Thor P., Krol R., Demling L. (1975) Cyclic-AMP and pancreatic bicarbonate

secretion in response to secretin in dogs (39123). Proc. Soc. Exp. Biol. Med. 150, 773-779

Donowitz M., Asarkof N., Pike G. (1980) Calcium dependence of serotonin-induced charges in rabbit ileal electrolyte transport. J. Clin. Invest. 66, 341-352

Donowitz M., Binder H.J. (1975) Jejunal fluid and electrolyte secretion in carcinoid syndrome. Am. J. Dig. Dis. 20, 1115-1122

Donowitz M., Charney A.N., Tai Y.H. (1979) A comprehensive picture of serotonin-induced ileal secretion. In "Mechanism of intestinal secretion". Ed. Binder H.J. Alan R. Liss Inc. N.Y. p.217-230

Dormer R.L., Williams J.A. (1981) Secretagogue-induced changes in subcellular Ca^{+2} distribution in isolated pancreatic acini. Am. J. Physiol. 240, G130-140

Ellenbogen L., Marklay E., Taylor R.J. (1969) Inhibition of histidine decarboxylase by benzyl and aliphatic amino oxamines. Biochem. Pharmacol. 18, 683-685

Endo M. (1977) Calcium release from the sarcoplasmic reticulum. Physiological Reviews 57, 71-108

Faichney A., Chey W.Y., Kim M.S. (1979) Release of endogenous secretin by sodium oleate in dog. Clin. Res. 27, 226A

Falck B., Hellman B. (1963) Evidence for the presence of biogenic amines in pancreatic islets. Experientia 19, 139-140

Fast D. (1974) Protein secretion from the rat exocrine pancreas: role of calcium, carbachol and the cyclic nucleotides. Dept. Pharmacol. & Ther. McGill University, Ph.D. thesis.

Felice L.J., Felice J.D., Kissinger P.T. (1978) Determination of catecholamines in rat brain parts by reverse-phase ion-pair liquid chromatography. J. Neurochem. 31, 1461-1465

Fellman J.H. (1959) Purification and properties of adrenal L-dopa decarboxylase. Enzymologia 20, 366-376

Feyster F. (1954) liber die peripheren endokrinen (parakrinen) Driisen des Menschen. wien and Dusseldorf; Wilhelm Maudrich. Quoted from Larsson L.I.(1980)

Field M., Fromm D., Al-Awqati Q., Greenough III W.B. (1972) Effect of cholera enterotoxin on ion transport across isolated ileal mucosa J. Clin. Invest. 51, 796-804

Furuta Y., Hashimoto K., Ishii I.Y., Iwatsuki K. (1974) Modification by drugs of the secretagogues effect of dopamine on the pancreas. Br. J. Pharmacol. 51, 225-230

Furuta Y., Hashimoto K., Iwatsuki K., Takeuchi O. (1973) Effects of enzyme inhibitors of catecholamine metabolism and of haloperidol on the pancreatic secretion induced by L-DOPA and by dopamine in dogs. Br. J. Pharmacol. 47, 77-84

Furuta Y., Hashimoto K., Washizaki M. (1978) beta-adrenoceptor stimulation of exocrine secretion from the rat pancreas. Br. J. Pharmacol. 62, 25-29

Furuta Y., Iwatsuki K., Takeuchi O., Hashimoto K. (1972) Secretin-like activity of dopamine on canine pancreatic secretion. Tokyo J. Exp. Med. 108, 353-360

Gardner J.D. (1979) Regulation of pancreatic exocrine function in vitro. Initial steps in the actions of secretagogues. Ann. Rev. Physiol. 41, 55-66

Gardner J.D., Jackson M.J. (1977) Regulation of amylase release from dispersed acinar cells. J. Physiol. London 270, 439-454

Gardner J.D., Rothman A.J. (1979) Action of cholera toxin on dispersed acini from guinea pig pancreas. Biochim. Biophys. Acta 585, 250-265

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

Gershon M.D., Ross L.L. (1966b) Location of sites of 5-HT storage and metabolism by radioautography. J. Physiol. 186, 477-492

Glyfe E. (1977) Serotonin as a marker for the secretory granules in the pancreatic beta-cells. Acta Physiol. Scand. Suppl. 452, 125-128

Greenberg G.R. (1981) Role of secretin in man. In "Gut Hormones" 2nd edition. Eds. Bloom S.R., Polak J.M., Livingstone C. p.220-227

Greene L.J., Hirs C.H., Palade G.E.(1963) On the protein composition of bovine pancreatic zymogen granules. J. Biol. Chem. 238, 2054-2070

Greengard H., Roback R., Ivy A.C. (1942) The effect of sympathomimetic amines on pancreatic secretion. J. Pharmacol. Exp. Ther. 74, 309-318

Greenwell J.R. (1975) The effects of cholecytokinin- pancreozymin, acetylcholine and secretin on membrane potentials of mouse

pancreatic cells in vitro. Pfluger Arch. 353, 159-170

Guderley H., Heisler S. (1980) A model system for the study of stimulus-enzyme secretion coupling in rat pancreatic acinar cells. Can. J. Physiol. Pharmacol. 58, 965-973

Hagen P. (1962) Observations on the substrate specificity of Dopa decarboxylase from ox adrenal medulla, human phaeochromocytoma and human argentaffinoma. Br. J. Pharmacol. 18, 175-182

Hagen P.B., Cohen L.H. (1966) Biosynthesis of indolealkylamines. Physiological release and transport of 5-hydroxytrptamine. In "Handbook of Experimental Pharmacology". Chapter 5 XIX. Eds. Eichler E., Farah A. Springer-Verlay, Berlin, Heidelbug, N.Y. p.182-221

Håkanson R., Lilja B., Owman C.H. (1967) Properties of a new system of amine-storing cells in the gastric mucosa of the rat. Eur. J. Pharmacol. 1, 189-199

Håkanson R., Owman C.H., Sjöberg N.O., Sporrong B. (1970) Amine mechanisms in enterochromaffin and enterochromaffin-like cells of gastric mucosa in various mammals. Histochemie 21, 189-220

Hand A.R., Oliver C. (1977) Cytochemical studies of GERL and its role in the secretory granule formation in exocrine cells. Histochemical Journal 9, 375-392

Hardcastle J., Hardcastle P.T., Redfern J.S. (1981) Action of 5-hydroxytryptamine on intestinal ion transport in the rat. J. Physiol. 320, 41-55

Harper A.A., Raper H.S. (1943) Pancreozymin, a stimulant of the secretion of pancreatic enzymes in extracts of the small intestine. J. Physiol. 102, 115-125

Hashimoto K., Ogmo K., Furuta Y. (1977) Species difference in the secretory response to dopamine in the pancreas of dogs, cats, rabbits and rats. Arch. Histologicum Japonicum 40, supp. 129-132

Hashimoto K., Satoh S., Takeuchi O. (1971) Effect of dopamine on pancreatic secretion in the dog. Br. J. Pharmacol. 43, 739-746

Hicks S.J., Drysdale J.W., Munro H.N. (1969) Preferential synthesis of ferritin and albumin by different populations of liver polysomes. Science 164, 584-585

Hokin L.E. (1955) Isolation of the zymogen granules of dog pancreas and a study of their properties. Biochim. Biophys. Acta 18, 379-388

Holtz P., Heise R., Lüdtke K. (1938) Fermentativer Abbau von 3,4-dioxyphenylalanine (Dopa) durch niere. Arch. Exp. Pathol. Pharmakol. 191, 87-118

Hubel K.A. (1972) Progress in gastroenterology: secretin. Gastroenterology 62, 318-341

Ikenoya S., Tsuda T., Yamano Y., Yamanishi Y., Yamatsu K., Ohmae M., Kawabe K., Nishino H., Kurahashi T. (1978) Design and characterization of electrochemical detector for high performance liquid chromatography and application to the determination of biogenic amines. Chem. Pharm. Bull. 26, 3530-3539

Ingram V.M. (1972) Biosynthesis of macromolecules. 2nd edition. Benjamin: Menlo Park, California.

Ivy A.C., Oldberg E. (1928) A hormone mechanism for gall bladder contraction and evacuation. Am. J. Physiol. 86, 599-613

Jamieson J.D., Palade G.E. (1967a) Intracellular transport of secretory proteins in the pancreatic exocrine cell. I. Role of the peripheral elements of the Golgi complex. J. Cell Biol. 34, 577-596

Jamieson J.D., Palade G.E. (1967b) Intracellular transport of secretory proteins in the pancreatic exocrine cell. II. transport to condensing vacuoles and zymogen granules. J. Cell Biol. 34, 547-615

Jamieson J.D., Palade G.E. (1968a) Intracellular transport of secretory proteins in the pancreatic exocrine cell. III. Dissociation of intracellular transport from protein synthesis. J. Cell Biol. 39, 580-588

Jamieson J.D., Palade G.E. (1968b) Intracellular transport of secretory proteins in the pancreatic exocrine cell. IV. Metabolic requirements. J. Cell Biol. 39, 589-603

Jamieson J.D., Palade G.E. (1971a) Condensing vacuole conversion and zymogen granule discharge in pancreatic exocrine cells: metabolic studies. J. Cell Biol. 48, 503-522

Jamieson J.D., Palade G.E. (1971b) Synthesis, intracellular transport and discharge of secretory proteins in stimulated pancreatic exocrine cells. J. Cell Biol. 50, 125-158

Jamieson J.D., Palade G.E. (1977) Modulation of secretory proteins in animal cells. In "International Cell Biology, 1976-1977". Eds. Brinkley B.R., Porter K.R. Rockefeller University Press: New York p. 308-319

Jensen R.T., Gardner J.D. (1981) Identification and characterization of

receptors for secretagogues on pancreatic acinar cells. Fed. Proc. 40, #10 2486-2496

Johnson L.R. (1974) Gastrointestinal hormines. In "Gastrointestinal Physiology" Eds. Jacobson E.D., Shanbour L.L. Baltimore, University Park Press. p.1-43

Jorpes J.E. (1968) Memorial lecture: the isolation and chemistry of secretin and cholecystolkinin. Gastroenterology 55, 157-164

Jorpes J.E., Mutt V. (1961) On the biological activity and amino acid composition of secretin Acta Chemica Scandinavica 15 1790-1791

Kern H.F., Bieger W., Völkl A., Rohr G., Adler G. (1979) Regulation of intracellular transport of exportable proteins in the rat exocrine pancreas. In "Secretory Mechanism, Symposium XXXIII, Soc. Expl, Biol." Eds. Hopkins C.R., Duncan C.J. Cambridge University Press p.79-99

Kern H.F., Kern D. (1969) Elektronen mikroskopische untersuchungen uber die wirkung von kobaltchlorid auf das exokrine pankreasge webe des Meerschweinchens virchows Archiv B. Cell Pathology 4, 54-70

Kim M.S., Lee K.Y., Chey W.Y. (1979) Plasma secretin concentration in fasting and postprandial states in dog Am. J. Physiol. 236, E539-544

Kisloff B., Moore E.W. (1976) Effect of serotonin on water and electrolyte transport in the in vivo rabbit small intestine. Gastroenterology 71, 1033-1038

Konturek S.J., Becker H.D., Thompson J.C. (1974) Effect of vagotomy on hormones stimulating pancreatic secretion. Arch. Surg. 108, 704-708

Korc M., Williams J.A., Goldfine I.D. (1979) Stimulation of the glucose transport system in isolated mouse pancreatic acini by cholecystokinin and analogues. J. Biol. Chem. 254, 7624-7629

Kornberg A. (1955) Lactate dehydrogenase assay. In "Methods in enzymology". Eds. Colowick S.P., Kaplan N.O. Vol 1. Academic Press, N.Y. p.441

Kraehenbuhl J.P., Racine L., Jamieson J.D. (1977) Immunocytochemical localization of secretory proteins in bovine pancreatic exocrine cells. J. Cell Biol. 72, 406-423

Kronquist K.E., Elmahdy A., Ronzio R.A. (1977) Synthesis and subcellular distribution of heparan sulfate in the rat exocrine pancreas. Arch. Biochem. Biophys. 182, 188-196

Larsson L.I. (1979) Structure and function of putative paracrine cells. Proceedings of Miroshima symposium on gut hormones. In "Gut, peptides secretion, function and clinical aspects". Eds. Miyoshi A., Grossman M.I.. Elsevier/North Holland Biochemical Press, Tokyo. p.112-119

Larsson L.I. (1980) On the possible existence of multiple endocrine, paracrine and neurocrine messengers in secretory cell systems. Invest. Cell Pathol. 3, 73-85

Larsson L.I., Goltermann N., Nagistris L.D., Rehfeld J.F., Schwartz T.W. (1979) Somatostatin cell processes as pathways for paracrine secretion. Science 205, 1393-1394

Lazarow A., Bauer G.E., Lindall A. (1964) Protein synthesis in islet tissue. In "The structure and metabolism of pancreatic islets". Eds. Brolin S.E., Hellman B., Krutson H. Oxford: Pergamon Press p. 203-211

Lehninger A.L. (1979) Oxidative degradation of amino acids. In "Biochemistry", 2nd edition. Chapter 21. Worth Publish Inc. N.Y. p.559-586

Lovenberg W., Weissbach H., Udenfriend S. (1962) Aromatic L-amino acid decarboxylase. J. Biol. Chem. 237, 89

Lowry O.H., Rosebrough N.J., Farr A.L. Randall R.J. (1951) Protein measurement with the phenol reagent. J. Biol. Chem. 192, 265-275

Lundquist I., Sundler F., Håkanson R., Larsson L.I., Heding L.G. (1975) Differential changes in 5-hydroxytryptamine and insulin content of guinea pig beta-cells. Endocrinology 97, 937-947

Maeno H., Johnson E.M., Greengard P. (1971) Subcellular distribution of adenosine 3',5'-monophosphate dependent protein kinase in rat brain. J. Biol. Chem. 246, 134-142

Mangos J.A., McSherry N.R. (1971) Micropuncture study of excretion of water and electrolyte by the pancreas. Am. J. Physiol. 221, 496-503

Martinez J.R., Adelstein E., Quissel D., Barbero G. (1975a) The chronically reserpinized rat as a possible model for cystic fibrosis: I. Submaxillary gland morphology and ultrastructure. Pediat. Res. 9, 463-469

Martinez J.R., Adshead P., Quissel D., Barbero G. (1975b) The chronically reserpinized rat as a possible model for cystic fibrosis: II. Composition and cilioinhibitory effects of submaxillary saliva. Pediat. Res. 9, 470-475

Matos-Medina A.N. (1975) Synthesis of amylase by rat exocrine pancreas. Ph.D. thesis, McGill University, Dept. Pharmacol. & Therap.

May R.J., Conlon T.P., Erspamer V., Gardner J.D. (1978) Actions of peptides isolated from amphibian skin on pancreatic acinar cells. Am. J. Physiol. 235, E112-118

Meldolesi J., Jamieson J.D., Palade G.E. (1971) Composition of cellular membranes in the pancreas of the guinea pig. III. Enzymatic activities. J. Cell Biol. 49, 150-158

Meldolesi J., Jamieson J.D., Palade G.E. (1971a) Composition of cellular membranes in the pancreas of the guinea pig. I. Isolation of membrane fraction. J. Cell Biol. 49, 109-129

Meyer J.H., Grossman M.I. (1972) Release of secretin and cholecystokinin, gastrointestinal hormones. In "Gastrointestinal Hormones". Eds. Demling Z., Stuttgant, Verlag. International symposium at Edangen, Aug. 71. p.43-55

Milutinović S., Schultz I., Rosselin G., Fasold H. (1977) the interaction of pancreatic secretagogues with pancreatic plasma membranes. In "Hormonal Receptor in Digestive Tract Physiology" INSERM, Symposium No. 5. Eds. Bonfils S., Fonnageot P., Rosselin G. Elsevier/North Holland press. p.213-216

Mori J., Sato H.Y., Hashimoto M., Honda F. (1979b) Amines and the rat exocrine pancreas: II. Effects of receptors blockers on turnover of L-5HTP. Jap. J. Pharmacol. 29, 913-921

Mori J., Sato H.Y., Hashimoto M., Honda F. (1979c) Amines and the rat exocrine pancreas. III. Effects of amines on pancreatic secretion. Jap. J. Pharmacol. 29, 923-933

Mori J., Sato Y., Hashimoto M., Honda F. (1979a) Amines and the rat exocrine pancreas: I. Effects of receptors blockers on turnover of L-Dopa. Jap. J. Pharmacol. 29, 899-911

Morimoto Y., Tashiro Y., Matsura S. (1967) Chase of newly synthesized proteins in guinea-pig pancreas with cycloheximide. Biochim. Biophys. Acta 138, 631-633

Morré D.J. (1977) Membrane differentiation and the control of secretion: A comparison of plant and animal Golgi apparatus. In "International cell biolgy". Eds. Brinkley B.R., Porter K.R. The Rockefeller University Press, N.Y. p. 293-303

Morton D., Parker A., Estrada P., Martinez J.R. (1980) Exocrine pancreatic secretion in rats treated with reserpine after stimulation with pilocarpine, dopamine, and caerulein. Pediat. Res. 14, 18-20

Moyer T.P. (1978) Optimized isocratic conditions for analysis of catecholamines by high performance reversed phase paired-ion chromatography with amperometric detection. J. Chromat. 153, 365-372

Nevalainen T.J. (1970) Effect of pilocarpine stimulation on rat pancreatic acinar cells. An electron microscopic study with morphometric analysis. Acta Pathologica et Microbiologica Scand Suppl 210

Novikoff A.B. (1976) The endoplasmic reticulum: a cytochemist's view (a review). Proc. Natl. Acad. Sci. USA 73, 2781-2787

Novikoff A.B., Novikoff P.M. (1977) Cytochemical contributions to differentiating GERL from Golgi apparatus. Histochemical Journal 9, 525-551

O'Doherty J., Stark R.J. (1982) Stimulation of pancreatic acinar secretion - increase in cytosolic calcium and sodium. Am. J. Physiol. 242, 513-521

Osnes M., Hanssen L.E., Flaten O., Myren J. (1978) exocrine pancreatic secretion and immunoreactive secretin (IRS) release after intraduodenal instillation of bile in man. Gut 19 180-184

Palade G.E. (1956) Intracisternal granules in the exocrine cells of the pancreas. J. Biophys. Biochem. Cyto. 2, 417-422

Palade G.E. (1975) Intracellular aspects of the process of protein synthesis. Science 189, 347-358

Palla J.C. (1970) Docteur d'Etat thesis, University of Provense. (Cited by Tartakoff et al, 1974)

Pascal J.P. Vaysse N. (1976) Action du systeme adrenergique sur le pancreas exocrine. Biologie et Gastroenterologie (Paris) 9, 243-254

Pearse A.G.E. (1968) Common cytochemical and ultrastructural characteristics of cells producing polypeptide hormones (APUD) series and their relevance to thyroid and ultimobranchial C cells and calcitonin. Proc. Royal Soc. London, Ser B. 170, 71-80

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

Pearse A.G.E. (1976) Neurotransmission and the APUD concept. In "Chromaffin, enterochromaffin and related cells". Eds. Coupland R.E., Fujita T. Elsevier, p. 147-154

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

Pearse A.G.E. (1982) Islet cell precursors are neurones. Nature 295, 96-97

Pearse A.G.E., Takor Takor T. (1979) Embryology of the diffuse neuroendocrine system and its relationship to the common peptides. Fed. Proc. 38, 2288-2294

Pekas J.C. (1971) Pancreatic incorporation of ^{65}Zn and Histidine-^{14}C into secreted proteins of the pig. Am. J. Physiol. 220, 799-803

Perlmutter J., Martinez J.R. (1978) The chronically reserpinized rat as a possible model for cystic fibrosis: VII. Alterations in the secretory response to cholecystokinin and to secretin from the pancreas in vivo. Pediat. Res. 12, 188-194

Peterfy P., Tenenhouse A. (1982) Vitamine D receptors in isolated rat parotid gland acinar cells. Biochimica et Biophysica Acta 721, 158-163

Petersen O.H. (1982) Stimulus-excitation coupling in plasma membranes of pancreatic acinar cells. Biochim. Biophys. Acta 694, 163-184

Petersen O.H., Maruyama Y., Grof J., Laugier R., Nishiyama A., Pearson G.T. (1981) Ionic currents across pancreatic acinar cell membranes and their roles in fluid secretion. Phil. Trans. R. Soc. Lond. B. 296, 151-166

Petersen O.M., Ueda N. (1976) Pancreatic acinar cells: the role of calcium in stimulus secretion coupling. J. Physiol. 254, 585-606

Pollard H.B., Pazoles C.J., Creutz C.E., Zinder O. (1980) Role of intracellular proteins in the regulation of calcium action and transmitter release during exocytosis. Monogr. Neural Sci. vol. 7, 106-116

Ponnappa B.C., Dormer R.L., Williams J.A. (1981) Characterization of an ATP-dependent Ca^{+2} uptake system in mouse pancreatic microsomes. Am. J. Physiol. 240, G122-129

Rahman M.K., Nagatsu T., Kato T. (1981) Aromatic L-amino acid decarboxylase activity in central and peripheral tissues and serum of rats with L-Dopa and L-5-hydroxytryptophan as substrates. Biochem. Pharmacol. 30, 645-649

Rambourg A., Clermont Y., Marraud A. (1974) Three-dimensional structure of the osmium impregnated Golgi apparatus as seen in the

high voltage electron microscope. Am J. Anatomy 140, 27-46

Randall S.S., Shaw B. (1964) Distribution of insulin in subcellular fractions of ox pancreas. In "The structure and metabolism of pancreatic islets". Eds. Brolin S.E., Hellman B., Krutson H. Oxford: Pergamon Press p. 237-242

Redman C.M. (1969) The rite of synthesis of serum proteins and ferritin on ribosomes of rat liver. Fed. Proc. 28, 726

Redman C.M., Sabatini D.D. (1966) Vectorial discharge of peptides released by puromycin from attached ribosomes. Proc. Natl. Acad. Sci. USA 56, 608-615

Reggio H., Dagorn J.C. (1980) Packaging of pancreas secretory proteins in the condensing vacuoles of the Golgi compled. In "Biology of normal and cancerous exocrine pancreatic cells" Eds. Ribet C.A., Pradayrol L., Susini C. INSERM symposium No. 15 Elsevier/North-Holland Biomedical Press, Amsterdam, N.Y., Oxford. p.229-244

Reggio H.A., Palade G.E. (1978) Sulfated compounds in the zymogen granules of the guinea pig pancreas J. Cell Biol. 77 288-314

Renckens B.A.M., Schrijen J.J., Swarts H.G.P., DePont J.J.H.H.M., Bonting S.L. (1978) Role of calcium in exocrine pancreatic secretion. IV. Calcium movements in isolated acinar cells of rabbit pancreas. Biochim. Biophys. Acta 544, 338-350

Rhoads R.E., Udenfriend S. (1968) Decarboxylation of alpha-ketoglutarate coupled to collagen proline hydroxylase. Proc. Natl. Acad. Sci. USA 60, 1473-1478

Ronzio R.A. (1973a) Glycoprotein synthesis in the adult rat pancreas. I. Subcellular distributions of uridine diphosphate galactose: glycoprotein galactosyltransferase and thiamine pyrophosphate phosphohydrolase. Biochim. Biophys. Acta 313, 286-295

Ronzio R.A. (1973b) Glycoprotein synthesis in the adult rat pancreas. II. Characterization of golgi-rich fractions. Arch. Biochem. Biophys. 159, 777-784

Rosengren E. (1960) Are dihydroxyphenylalanine decarboxylase and 5-Hydroxytryptophan decarboxylase individual enzymes? Acta Physiol. Scand. 49, 364-369

Rothman S.S. (1970) Intracellular storage of exportable protein in functionally hypertrophied pancreas. Am J. Physiol. 219, 1652-1657

Rothman S.S. (1971) The behaviour of isolated zymogen granules:

pH-dependent release and reassociation of protein. Biochim. Biophys. Acta 241, 567-577

Sarles H. (1977) The exocrine pancreas. In "Gastrointestinal Physiology II". Intenal review of physiology Vol 12. Ed. Crane R.K. University Park Press, Baltimore. p. 173-221

Satoh Y., Satoh H., Honda F. (1980) Dopamine receptor blocking activity of sulpiside in the canine exocrine pancreas. Jap. J. Pharmacol. 30, 689-699

Schaffalitzky de Muckadell O.B., Fahrenkrug J. (1978) Secretion pattern of secretin in man: Regulation by gastric acid. Gut 19, 812-818

Scheele G.A. (1980) Biosynthesis, segregation and secretion of exportable proteins by the exocrine pancreas. Am. J. Physiol. 238, G467-G477

Scheele G.A. (1982) Pancreatic zymogen granules. In "The secretory granule". Eds. Poisner A.M., Trifaro M.J. The secretory process Vol #1. Elsevier Biochemical Press, Amsterdam, N.Y., Oxford. Chapter 6, p.213-246

Scheele G.A., Palade G.E., Tartakoff A.M. (1978) Cell fractionation studies on the guinea pig pancreas. Redistribution of exocrine pancreas during tissue homogenization. J. Cell. Biol. 78, 110-130

Schulz I., Kimma T., Wakasugi H., Hasse W., Kribban A. (1981) Analysis of Ca^{+2} fluxes and Ca^{+2} pools in pancreatic acini. Phil. Trans. R. Soc. Lond. B296, 105-113

Schulz I., Stolze H. (1980) the exocrine pancreas: the role of secretagogues, cyclic nucleotides and calcium in enzyme secretion. Ann. Rev. Physiol. 42, 127-156

Schulz I., Wakasugi H., Stolze H., Kribben A., Haase W. (1981) Analysis of Ca^{+2} fluxes and their relation to enzyme secretion in dispersed pancreatic acinar cells. Fed. Proc. 40, #10 2503-2510

Scratcherd T., Case R.M. (1973) The secretion of electrolytes by the pancreas. Am. J. Clin. Nutr. 26, 326-339

Sesso A., Paula Assis J.E., Kuwajima V.Y., Kachar B. (1980) Freeze-fracture and thin-section study of condensing vacuoles in rat pancreatic acinar cell. Acta Anat. 108, 521-539

Sims K.L., Davis G.A., Bloom F.E. (1973) Activities of 3,4-dihydroxy -L-phenylalanine and 5-hydroxy -L-tryptophan decarboxylases in rat brain: assay characteristics and distribution. J. Neurochem. 20, 449-464

Singh M., Black O., Webster P.D. (1973) Effects of selected drugs on pancreatic macromolecular transport. Gastroenterology 64, 983-991

Smith P.A., Case R.M. (1975) Effect of cholera toxin on cyclic adenosine 3',5'-monophosphate concentration and secretory processes in the exocrine pancreas. Biochim. Biophys. Acta 399 277-290

Srinivasan K., Awapara J. (1978) Substrate specificity and other properties of Dopa decarboxylase from guinea pig kidneys. Biochim. Biophys. Acta 526, 597-604

Sum P.T., Schipper H.L., Presbaw R.M. (1969) Canine gastric and pancreatic secretion during intestinal distention and intestinal perfusion with choline derivatives. Can. J. Physiol. Pharmacol. 47, 115-118

Sundler F., Håkanson R., Loren I., Lundquist I. (1980) Amine storate and function in peptide hormone-producing cells. Invest. Cell Pathol. 3, 87-103

Swanson C.H., Solomon A.K. (1975) Micropuncture analysis of the cellular mechanisms of electrolyte secretion by the in vitro rabbit pancreas. J. Gen. Physiol. 65, 22-45

Tartakoff A., Greene L.J., Palade G.E. (1974) Studies on the guinea pig pancreas. Fractionation and partial characterization of exocrine proteins. J. Biol. Chem. 249, 7420-7431

Tartakoff A.M., Jamieson J.D. (1974) Subcellular fraction of the pancreas. In "Methods in Enzymology" Vol XXXI Biomembrane part A. Eds. Fleischer S., Packer L. Academic Press, N.Y. p. 41-59

Tartakoff A.M., Jamieson J.D., Scheele G.A., Palade G.E. (1975) Studies on the pancreas of the guinea pig. Parallel processing and discharge of exocrine proteins. J. Biol. Chem. 250, 2671-2677

Taussig L.M., Landau L.I. (1976) Cystic fibrosis, in Practice of Pediatrics, edited by Kelley V.C., Harper and Row, New York.

Thompson J.H. (1971) Serotonin and the alimentary tract. Res. Comm. Chem. Path. Pharmacol. 2, 687-781

Tiscornia O.M. (1976) Contrôle nerveux cholinergique du pancreas. Biologie et Gastroenterologie (Paris) 9, 255-275

Trifaró J.M., Duerr A.C. (1976) Isolation and characterization of Golgi-rich fraction from the adrenal medulla. Biochim. Biophys. Acta 421, 153-167

Vandermeers-Piret M.C., Camus J., Rathe J., Vandermeers A., Christophe

J. (1971) Distribution of hydrolases in the rat pancreas: some properties of the zymogen granules. Am J. Physiol. 220, 1037-1045

Vaysse N., Laval J., Senarens C., Estere J.P., Ribet A. (1982) Dopamine-stimulted cyclic AMP and binding of [^{3}H]dopamine in acini from dog pancreas. Biochim. Biophys. Acta 720, 378-383

Völkl A., Bieger W., Kern H.F. (1976) Studies in secretory glycoproteins in the rat exocrine pancreas. I. Fine structure of the Golgi complex and release of glucose-labeled proteins after in vivo stimulation with caerulein. Cell and Tissue Research 175, 227-243

Wallach D. (1982) The secretory granule of the parotid gland. In "The secretory granules". Eds. Poisner A.M., Trifaró M.J. The secretory process Vol #1. Elsevier Biochemical Press, Amsterdam, N.Y. Oxford. Chapter 7, p 247-276

Weiner N. (1980) Drugs that inhibit adrenergic nerves and block adrenergic receptors in "The pharmacological basis of therapeutics", Editors A. Goodman Gilman; L.S. Goodman and A. Gilman. 6th edition, Macmillan Publishing Co., New York, Chapter 9, p 176-210

Williams J.A., Sankaran H., Korc M., Goldfine I.D. (1981) Receptors for cholecystokinin and insulin in isolated pancreatic acini: hormone control of secretion and metabolism. Fed. Proc. 40, #10 2497-2502

Wormsley K.G. (1979) Pancreatic secretion: physiological control. In "Scientific basis of gastroenterology" Eds. Duthie H.L., Wormsley K.G. Churchill Livingstone: Edinburgh, London. p. 199-248

Zimmeberg J., Cohen F.S., Finkelstein A. (1980) Micromolar Ca^{+2} stimulates fusion of lipid vesicles with planar bilayers containing a calcium-binding protein. Science 210, 906-908