Progress report

The interrelationships of the pancreatic enzymes

The quantitative interrelationships between the different functional types of pancreatic enzymes has been the subject of much experimental investigation, but the results are conflicting and the inferences remain controversial. Recent improvements in techniques have provided new and more reliable means of measuring enzyme activities and it therefore seems pertinent to review the current state of knowledge about the factors which determine selective production of the pancreatic enzymes.

Pavlov¹, quoting the studies of his pupil Walther in dogs, described rapid 'adaptive' changes in the amounts of individual pancreatic enzymes secreted in response to changes in the predominant type of food in a single meal. Later studies supported Pavlov's hypothesis that the production of individual enzymes, or groups of enzymes, could be selectively modified, but changed the emphasis by indicating that adaptation of pancreatic enzyme secretion might require prolonged dietary modification, for periods ranging from hours to weeks^{2,3,4}.

The converse hypothesis, that pancreatic enzymes were secreted 'in parallel', was proposed during the early years of the present century by Babkin^{5,6} on the basis of experiments in dogs with pancreatic fistulae and was later supported by studies of pancreatic secretion in other species, including man.

In order to disentangle the current state of the evidence for the two conflicting hypotheses, the interrelationships between the pancreatic enzymes within the pancreatic-secreting cells, in the secreted juice, and within the small intestinal lumen are considered separately, since different factors determine the relative increase or decrease of individual enzymes or groups of enzymes in the three compartments.

Interrelationships of Enzymes within the Pancreatic Acinar Cells

While the original hypotheses of 'dissociated' or 'parallel' secretion of enzymes referred to the enzyme content of pancreatic juice, the interrelationships of the enzymes within the pancreatic acinar cells has been studied because the enzyme content of the zymogen granules has been considered to represent 'preformed' pancreatic juice³.

SUBCELLULAR DISTRIBUTION OF PANCREATIC ENZYMES

Using immunological staining techniques, Marshall⁷ found uniform intracellular distribution of chymotrypsinogen and procarboxypeptidase in the pancreas. Keller, Cohen, and Neurath⁸ also demonstrated uniform incorporation of radioactively labelled arginine into trypsinogen and chymotrypsinogen at all stages of synthesis and storage, although incorporation of

the amino acid into ribonuclease was less. Subsequently, Yasuda and Coons⁹ reported that the intracellular localization of amylase in cells from pig and beef pancreas differed from that of trypsinogen and chymotrypsinogen, while Rothman¹⁰ described the non-parallel distribution of trypsinogen and chymotrypsinogen in the soluble fraction of homogenates from the acinar cells of the rabbit pancreas. Rothman¹⁰ concluded that the two closely related enzymes were segregated and stored in different granules or that there were true differences in the ratios of the concentrations of the two enzymes within the cytoplasm of the pancreatic cells. The most recent studies¹¹ have shown enzymatic homogeneity of the zymogen granules and other components of rat pancreas, even when the total intracellular content of one enzyme (amylase) has been markedly lowered by decreasing the lysine content of the diet.

Evidence about the homogeneity or heterogeneity of the subcellular distribution of the pancreatic enzymes is therefore controversial, partly perhaps because the immunological and physicochemical techniques which have been used are not sufficiently reliable or specific.

INTRACELLULAR INTERRELATIONSHIPS OF PANCREATIC ENZYMES

The strongest evidence for dissociation between different classes of pancreatic enzymes is provided by the differences in the intracellular content of enzymes of different species and by the apparent modification of the intracellular enzymes during experimental manipulation of the diet.

(a) Marked differences have been found in the relative proportions of the different enzymes in the homogenates of pancreas from different species¹² and attributed, in part, to dietary differences between the species. (b) Adaptation of the enzyme content of the pancreas to the predominant constituent of a diet fed to rats for a period of weeks was noted by Grossman *et al*². A diet containing much carbohydrate was shown to result in a high pancreatic content of amylase, while a high pancreatic content of proteases was found in rats fed a high protein diet. These findings were confirmed and extended by similar or related dietary manipulations in rats^{13,14,15,16,17}.

Dissociation in the pancreatic content of enzymes was also obtained experimentally by the addition of specific amino acids to, or deletion from, the diet of rats. Thus, Magee and Hong¹³ found that supplements of methionine increased the pancreatic content of lipase and proteases, while phenylalanine and isoleucine increased proteases only. Véghelyi and Kemény¹⁸ and Lyman and Wilcox^{19,20} showed that the methionine- and leucine-deficient diets decreased the pancreatic content of lipase and proteases much more than amylase in rats, while Christophe²¹ and Vandermeers, Khayat, Raché, and Christophe²² noted that lysine deficiency in rats most markedly reduced the pancreatic content of amylase and chymotrypsinogen.

The investigation of the dissociation of enzymes in the rat pancreas was greatly refined by Desnuelle and his colleagues³, who studied not only the pancreatic content of enzymes in response to dietary modifications, but also measured the rates of synthesis of the specific enzymes. A starch-rich diet greatly increased the synthesis of amylase and decreased the synthesis of chymotrypsinogen^{23,24,25}, while a casein-rich diet resulted in greater synthesis of chymotrypsinogen than of amylase²⁶. The response to a protein-rich diet was shown to be more complex when Snook²⁷ reported that casein increased the synthesis of amylase more than of trypsinogen or chymotrypsinogen while more amino acids were diverted to chymotrypsinogen than to amylase

when whole egg protein was fed. Similarly, a diet containing gluten decreased the incorporation of radioactively labelled leucine into amylase and increased incorporation into lipase, chymotrypsinogen, and trypsinogen¹²². Subsequent studies showed that dietary glucose, in the form of starch, sucrose, or glucose, most markedly increased the intracellular levels of amylase and lowered lipase, while unsaturated fatty acids were the most efficient means of increasing the levels of lipase⁴.

(c) Dissociation between the pancreatic enzymes has also resulted from the experimental feeding of trypsin inhibitors. Salman, Dal Borgo, Pubols, and McGinnis²⁸ found that in chicks fed raw soybean meal (containing soybean trypsin inhibitor) the rate of synthesis of amylase was depressed, while the pancreatic synthesis and content of chymotrypsinogen and trypsinogen were increased. Similarly, Konijn, Birk, and Guggenheim^{29,30} showed that in rats a diet containing soybean flour was associated with increased pancreatic synthesis of trypsinogen and chymotrypsinogen and depressed synthesis of amylase. On the other hand, Magee and Hong³¹ had previously noted increased production of amylase on feeding soybean flour to dogs, while Rothman and Wells³² showed that feeding egg-white trypsin inhibitor to rats increased the pancreatic content of chymotrypsinogen and amylase, without increasing trypsinogen. The matter was partly clarified by Geratz and Hurt³³, who reported that the trypsin inhibitor p-aminobenzamidine increased the pancreatic content of amylase, together with trypsinogen and chymotrypsinogen, in rats during the first four weeks of dietary supplementation, but specifically reduced the amylase content of the pancreas thereafter.

Dietary modification of the pancreatic content of enzymes has been less often described in species other than rats. In dogs, administration of soybean flour resulted in an increased pancreatic content of trypsin and lipase, while chymotrypsinogen concentration remained unchanged³⁴. However, the matter has been confused by a later report that the trypsinogen content of the pancreas is lowered by the soybean diet, while chymotrypsinogen and lipase concentrations remain normal³⁵.

MECHANISMS OF DISSOCIATION OF ENZYME CONTENT OF THE PANCREAS

Changes in the intracellular content of the different classes of pancreatic enzymes depend on alterations in the rates of synthesis and intracellular transport: selective differences in rates of secretion or different rates of intracellular degradation. Overall changes in the ratios of enzymes in the pancreas could result from uniform modification of the ratios within all the pancreatic acinar cells or, less likely, from selective stimulation of a hypothetical population of cells geared to the manufacture of specific enzymes.

The results of measurements of incorporation of amino acids indicate that selective changes in the rates of synthesis of specific pancreatic enzymes are associated with, and probably mainly responsible for, the observed changes in the intracellular content of the pancreatic enzymes⁴. The specific modifications of the biosynthetic mechanisms of the pancreatic acinar cells are thought to depend on an alimentary stimulus evoked by a selectively enriched diet²⁵ and may be mediated by a humoral factor³⁶. It is not yet clear whether the increased synthesis of enzymes is dependent on additional ('by-pass') synthetic and transport pathways¹⁰ or not¹¹ to complement the usual mechanisms of intracellular transport, storage, and secretion

represented by the processes involved in the formation and discharge of the zymogen granules.

Evidence for selective changes in the secretion of enzymes is presented below. No information is available to assess whether differential intracellular inactivation or degradation of pancreatic enzymes occurs. In the fasting state, however, it seems that intracellular degradation of rat pancreatic enzymes is slight³⁷.

Early during the study of the changes in the enzyme content of rat pancreases associated with changes in diet it was shown that the amylase content of the pancreas increased selectively when glucose was substituted for starch in a high carbohydrate diet^{36,14,38,4}. Further investigation showed that insulin depressed the pancreatic content of amylase^{36,17} and pancreatic amylase content was also shown to be markedly reduced in rats made diabetic with alloxan^{39,40}. The reduced synthesis of amylase in the diabetic rats reverted to normal with insulin³⁹, suggesting that pancreatic cellular levels of glucose might be important in regulating the synthesis of amylase.

The changes in synthesis of amylase brought about by changes in the levels of glucose were interpreted as indicating that the pancreas responded to information from the products of digestion, rather than to the ingested food materials themselves^{15,16}. However, the role of amino acids in mediating the effects of protein-rich diets has been more difficult to interpret. While Ben Abdeljlil³⁸ stated that amino acid mixtures could mimic the effects of protein by producing a selective increase in the synthesis and secretion of pancreatic proteases, other studies had shown that casein hydrolysate selectively depressed the pancreatic content of trypsin^{36,13,14}. It was therefore suggested that nervous factors might be involved in the regulation of the pancreatic content of trypsin³⁶ or, alternatively, that the presence of protein in the small intestine released two hormones, one of which increased the production of proteases and lipase, while the other was involved with amylase³¹. The lowered pancreatic content of proteases was attributed to failure of amino acid mixtures to effect the release of the specific activating hormone¹³.

It seems, therefore, that very little is known about the mechanisms whereby adaptation to specific diets is brought about. Knowledge about the transmission of information to the pancreatic cells is scanty and ignorance about the modification of intracellular processes is complete.

Interrelationships of Enzymes in Pancreatic Juice

Babkin's hypothesis of 'parallel' secretion of pancreatic enzymes has received support from studies in rabbits, in response to injection of pilocarpine⁴¹; in dogs, in response to injections of secretin^{42,43}; and in healthy human subjects in response to secretin^{44,45,46,47}, to cholecystokinin-pancreozymin (CCK-PZ)^{46,47,48}, and to pilocarpine⁴⁹. Pancreatic disease was found to result in parallel decrease in the secretion of amylase, lipase, and proteases^{46,50}.

The many investigations supporting Babkin's hypothesis are, however, far outnumbered by the studies which imply dissociation between the enzymes.

TECHNICAL DISSOCIATION

The original observations suggesting enzyme dissociation¹ were soon recog-

nized to be technical artefacts^{5,42}. It was shown that proteolytic activity could not be detected in pancreatic juice because the secreted zymogens, or precursors, of the proteases were enzymatically inactive and required activation by enterokinase (enteropeptidase) from the duodenal mucosa. Similarly, the activity of lipase in pancreatic juice might be apparently low both because lipase required activation by bile salts (and co-lipase^{51,52}) for full activity and because lipase was very unstable and liable to rapid and irreversible inactivation.

An additional problem in interpreting the many published reports is provided by the spontaneous fluctuations in the patterns of the enzymes secreted in the pancreatic juice of dogs⁵³ and rats³ during the administration of constant diets. While Guth, Komarov, Shay, and Style⁵³ found no regularity in the fluctuations, Desnuelle *et al*³ noted that the activity of amylase decreased during the course of days, while the content of lipase and chymotrypsin increased.

Technical artefacts and difficulties in measuring the activities of the pancreatic enzymes have vitiated many studies during the past seven decades and continue to do so.

DEVELOPMENTAL DISSOCIATION

Pancreatic secretion of enzymes in childhood has not yet been studied satisfactorily, but enough information is available to show that the secretion of amylase is impaired during the first three to six months of life, compared with the secretion of trypsin^{54,55,56,57,58,59,60}. Similarly, in piglets, the amylolytic activity of the pancreas is low after birth and then increases rapidly, perhaps due to the increased content of starch in the diet⁶¹. In rats, developmental alterations in enzymic interrelationships have also been noted and attributed partly to dietary changes during weaning and partly to hormonal influences⁶². Non-parallel change in the development of pancreatic enzymes has also been noted in chicks⁶³.

INHERITED DISSOCIATION

The relative proportions of the different pancreatic enzymes varies markedly between species. While rat pancreatic juice contains more amylase than trypsin, the relationship is reversed in the pancreatic juice of cows and pigs⁶⁴.

In man, hereditary deficiency of single enzymes has been postulated occasionally but never satisfactorily proven. In children with 'lipase deficiency'^{65,66,67} all other estimated enzymes were defectively secreted, as was bicarbonate. Since abnormal lipase secretion has been considered the predominant characteristic of adult chronic pancreatitis^{68,47,58}, the reports of 'inherited' selective defects in lipase secretion cannot be accepted as proven. Isolated 'trypsinogen deficiency'⁶⁹ was associated with gross abnormalities of secretion of bicarbonate and of the other pancreatic enzymes in response to CCK-PZ, although a later paper demonstrated apparently normal secretion of amylase and lipase but did not mention bicarbonate secretion⁷⁰. Similarly, Morris and Fisher⁷¹ described 'trypsinogen deficiency disease' with allegedly normal secretion of chymotrypsin and carboxypeptidase, although the bicarbonate concentration only reached values of 17 m-equiv/l. Moreover, no patient with isolated 'trypsinogen deficiency' has been tested for levels of duodenal enterokinase activity. Hereditary deficiency of amylase secretion has been

postulated by Lagerlöf, Schütz, and Holmer⁷² on the basis of abnormally low ratios of amylase to trypsin and amylase to lipase in response to hormonal stimulation of the pancreas. No further details are available.

DISSOCIATION OF ENZYME SECRETION IN HEALTHY HUMAN SUBJECTS

Sarles, Bauer, and Prezlin^{73,74} noted that during continuous intravenous infusion of secretin, lipase disappeared from the duodenal contents, while trypsin and chymotrypsin were secreted in constant relative proportions and amylase varied independently of both lipase and proteases. Sarles *et al*⁷⁴ also showed that sham feeding markedly increased the content of lipase in the duodenal aspirate, with lesser secretion of trypsin and chymotrypsin and no increase in the output of amylase. On the other hand, injections of CCK-PZ evoked a parallel increase in the output of all four enzymes into the duodenum.

Subsequently, Lagerlöf *et al*⁷² noted poor correlation between the outputs of trypsin and amylase during stimulation with secretin, with a six-fold range of variation. In a later report⁷⁵ the ratio of amylase to trypsin was shown to increase during continuous infusion of CCK-PZ and secretin, because trypsin output decreased more than amylase.

Ribet, Pascal, and Vaysse⁷⁶ noted good correlation between different pancreatic enzymes during stimulation with CCK-PZ, but recorded an increase in the ratio of trypsin and chymotrypsin to lipase when CCK-PZ was added to secretin. The proteases were secreted in parallel, under all experimental conditions.

Goldberg and Wormsley⁷⁷ noted significant alterations in the ratio of proteases to amylase with changes in the secretory stimulant and subsequently also noted changes in the ratio of trypsin to chymotrypsin with changes in the mode of pancreatic stimulation⁷⁸.

DISSOCIATION OF ENZYMES IN PANCREATIC DISEASE

Dissociated enzyme secretion has been repeatedly recorded in patients with pancreatic disease, but the reported characteristics and attributed significance of the dissociations are so contradictory, confused, and controversial that no clear picture is available at present.

Lagerlöf^{79,45} reported that secretion of amylase was more severely impaired than trypsin in patients with acute inflammation of the pancreas. The severe impairment of amylase secretion was subsequently confirmed and regarded as the most sensitive indication of disturbed pancreatic function^{80,46,48}. On the other hand, the secretion of amylase remained normal, or was less severely impaired than secretion of lipase or of proteolytic enzymes in pancreatic disease in a number of studies^{81,68,47,82,77}. It has also been claimed that lipase secretion is most severely impaired in pancreatic disease^{18,68,47} or dysfunction^{83,84,85} or that secretion of trypsin⁸² or of chymotrypsin⁴⁸ is specifically severely diminished. Lipase secretion appears to be most severely affected in children with pancreatic exocrine insufficiency due to conditions other than cystic fibrosis⁵⁸ although Barbezat and Hansen⁸⁶ found severe impairment of chymotrypsin secretion in children with malnutrition.

The discrepancies among published reports are partly due to the very marked scatter of ratios between the different enzymes in normal subjects^{87,88,77} and, indeed, it has been claimed that the best correlations between enzymes are observed when pancreatic function is markedly impaired⁸⁸.

DISSOCIATION PRODUCED BY DIET

The finding that the enzyme content of the pancreas adapts to the composition of the diet has been extended in rats to show that a diet rich in starch increases not only the pancreatic content, but also the pancreatic output of amylase, while a casein-rich diet increases the levels of proteolytic enzymes both in the pancreas and in pancreatic juice^{15,16}. Similarly, in cockerels, a protein-rich diet resulted in increased proteolytic activity of the pancreatic juice, while a fat-rich diet was associated with increased lipase activity and carbohydrate with increased amylase activity in the pancreatic secretions⁸⁹. The matter is not entirely clear, however, since it has been noted in pigs that increase in the protein content of the diet is associated with increased secretion of chymotrypsinogen, although the trypsin content of the pancreatic juice remains constant⁹⁰.

The importance of the addition of specific amino acids to the diet was studied in dogs by Magee and Hong³¹, who reported that isoleucine, threonine, and lysine increased the pancreatic secretion of amylase, while methionine increased the secretion of proteases, but not of amylase.

Wohlgemuth⁹¹ and Michelson⁹² studied the effect of diet on the secretion of enzymes in man. They noted that there was no evidence of immediate adaptation of the secretion of pancreatic enzymes to the predominant food in the meal. On the other hand, Michelson⁹² noted that prolonged feeding of a diet containing much protein and fat was associated with increased secretion of 'trypsin' compared with the response to a diet rich in fat alone. Subsequently Veghlyi⁴⁹ denied that dietary manipulation influenced the parallel secretion of pancreatic enzymes in children.

MECHANISMS OF DISSOCIATION OF ENZYME SECRETION

Differences in the ratios of the pancreatic enzymes in pancreatic juice are due to changes in the relative amounts of enzymes available for secretion in the pancreatic acinar cells; to differences in the relative rates of transport from the cells, if transport is selective; or to changes undergone by the enzymes in the pancreatic juice after secretion. Postsecretory modification of pancreatic enzymes must be kept in mind, particularly in interpreting the results of human studies, since the enzymes have usually been aspirated from the duodenum.

Changes in the interrelationships of secreted pancreatic enzymes appear to be due to selective changes in the rates of synthesis of individual enzymes or groups of enzymes, as demonstrated in rats during dietary manipulations^{23,24,26}. An increased production of enzyme molecules results in increased secretion of those enzymes if the mechanisms of intracellular transport and of cellular secretion remain unchanged¹¹.

While it has been shown that the proportion of enzymes is the same in the pancreatic cells² and in the zymogen granules, as in secreted pancreatic juice^{93,94,95}, some evidence has also been presented that selective transport of enzymes may occur, both within and from the pancreatic cells. Christophe *et al*⁴ reported that amylase was transported across the pancreatic acinar cell more slowly than lipase, but that the rate of secretion of the two enzymes was the same. Rothman⁹⁶ showed that CCK-PZ increased the secretion of trypsin, but not of chymotrypsin, from the isolated rabbit pancreas while methacholine evoked parallel secretion of the two enzymes. In contrast, Robberecht and Christophe⁹⁷ found that CCK-PZ and carbamylcholine both stimulated the

release of amylase and lipase from the perfused fragments of rat pancreas to the same extent and concluded that the parallel secretion of the enzymes denoted homogeneity of the secretory process. Rothman⁹⁶, on the other hand, postulated selective release of enzymes from zymogen granules or from the cytoplasm into the pancreatic ducts. Selective transport could explain the observed changes in the ratio in which such closely related enzymes as trypsinogen and chymotrypsinogen⁹⁸ are secreted under different conditions of stimulation in man⁷⁸, particularly since the synthesis and intracellular transport of the two enzymes appear to be identical⁸. Additional evidence for selective transport of pancreatic enzymes is provided by the studies of Rothman and Wells³² who showed that egg white trypsin inhibitor increased the content of chymotrypsinogen and amylase, but not of trypsin, in rat pancreas. Subsequent stimulation with methacholine greatly increased the secretion of trypsinogen, with lesser effect on the secretion of chymotrypsinogen and no effect at all on the secretion of amylase. Rothman and Wells³² suggest that the digestive needs of an organism may selectively influence not only the synthesis of digestive enzymes, but also the capacity of the acinar cells to transport the enzymes. If selective modification of the specific transport of individual pancreatic enzymes can be confirmed, then the hypothesis that the proportion of different enzymes in pancreatic juice solely reflects the relative rates at which the enzymes are synthesized will have been disproved⁶. It would, of course, remain necessary to determine the relative roles of selective transport and selective synthesis, and their interactions, in determining the interrelationships of the enzymes in the pancreatic secretions. In this connexion, it has been shown that in intact animals stimulation of secretion is associated with, and closely linked to, stimulation of enzyme synthesis^{99,100} although evidence from the pigeon pancreas and from studies *in vitro* of rat pancreas indicates that activation of secretion may occur through processes which differ from those involved in stimulating synthesis of enzymes^{101,99}.

Interrelationships of Pancreatic Enzymes in Intestinal Contents

Marked changes occur in the ratios of the pancreatic enzymes in the luminal contents at different levels of the alimentary tract.

STOMACH

The action of acid, with and without pepsin, on the activities of the pancreatic enzymes has been studied in detail^{102,103}. It has been found that lipase and amylase are rapidly and irreversibly inactivated by low *pH* alone. Trypsin is more stable at low *pH*, but is rapidly and irreversibly inactivated by pepsin in the presence of acid and also becomes inactive in neutral or alkaline solution.

DUODENUM

Marked alterations in the enzyme composition of human duodenal contents have been recorded in healthy subjects and in patients with pancreatic disease, both in response to different modes of direct stimulation of the pancreas with parenteral secretin and CCK-PZ and during the response to meals (see above). In part, the variable duodenal content of individual pancreatic enzymes during the digestion of food is related to the stabilities of the enzymes

in the face of changes in pH. The changing ratios of the pancreatic enzymes in duodenal aspirate during stimulation with hormones have generally been assumed to reflect changes in the composition of human pancreatic secretion. The assumption has not been tested directly, since uncontaminated pancreatic juice from normal human subjects can rarely be obtained and in any case has not been compared with secretion from the same patient aspirated by the usual techniques from the duodenum. The only evidence for the similarity in enzyme composition of pancreatic juice and of duodenal aspirate is indirect, since Robinson, Churchill, and White¹⁰⁴ reported that the order of electrophoretic migration of the enzymes of human pancreatic juice was qualitatively similar to that previously reported in human duodenal aspirate¹⁰⁵. On the other hand, the electrophoretic pattern of canine pancreatic juice in which proteases have been activated is reported to differ strikingly from that of the inactive juice¹⁰⁶.

An important difference between the pancreatic enzymes in pancreatic juice and in duodenal contents is due to the activation of the protease precursors in the duodenum. The initial activation is brought about by duodenal enterokinase, which converts trypsinogen to active trypsin by splitting off a terminal hexapeptide. Trypsin then activates the other zymogens (chymotrypsinogen, procarboxypeptidase, proelastase). Defective or absent enterokinase can therefore result in duodenal contents with normal levels of amylase and lipase, but apparent absence of proteases, despite normal secretion of proteases by the pancreas.^{107,108}

SMALL INTESTINE

(a) In addition to altered interrelationships among the pancreatic enzymes due to defective activation of zymogens, converse alterations are brought about by 'autodigestion' under the influence of the activated proteases, particularly trypsin¹⁰³. As a result, the content of pancreatic enzymes decreases during transit through the human small intestine¹⁰⁹, although in the rat the enzyme concentration has been noted to be greater in the lower than in the upper small intestine¹¹⁰. There is, however, marked loss of enzyme activity during colonic transit in the rat²⁷.

There is some controversy about the degree and importance of 'autodigestion' of the pancreatic enzymes which is observed *in vitro*. On the one hand, Khayat and Christophe¹⁰³ quantitated the autodigestion of the different enzymes by each of the proteases. On the other hand, Müller-Wieland and Berndt¹¹¹ did not find any decrease in the activities of trypsin and chymotrypsin after incubation of the purified enzymes, while Goldberg, Campbell, and Roy¹¹² introduced pancreatic enzymes into the human duodenum and recovered most of the activity in the ileal effluent. In order to account for the apparent absence of autodigestion, despite the disappearance of trypsin and chymotrypsin from duodenal contents, Müller-Wieland and Berndt¹¹¹ postulated the presence of protease-inactivating enzymes derived from disrupted lysosomes, originating in desquamated small intestinal epithelial cells. Enzymes of this type had been demonstrated in small intestinal homogenates of rat and pig by Hsu and Tappel¹¹³. Species differences do appear to be involved in the degree and rapidity of inactivation of the pancreatic enzymes in the small intestinal lumen, since Pelot and Grossman¹¹⁰ found rapid loss of activity of pancreatic enzymes introduced into the small intestine of rats.

(b) Factors other than proteolytic inactivation have also been postulated to account for selective alterations in the ratios of the pancreatic enzymes during passage through the small intestine. It has been noted that trypsin disappears most rapidly during incubation of duodenal aspirate¹¹¹ and of ileal effluent¹¹² and, indeed, tryptic activity is less stable than chymotrypsin in human intestinal juice *in vitro*¹¹⁴. Greater instability of trypsin than chymotrypsin during small intestinal transit has been considered to explain the finding that the ratio of trypsin to chymotrypsin in ileal effluent approximates to unity (trypsin to chymotrypsin ratio about 0.67)¹¹⁵ while the ratio of trypsin to chymotrypsin in duodenal aspirate, both basal and stimulated, is about 2.0^{77,78}. The disappearance of trypsin during passage through the colon is even more marked, since the ratio of trypsin to chymotrypsin in faeces is about 0.25^{116,117,48}.

(c) Since the auto-inactivation of trypsin does not appear to be as rapid as the tryptic inactivation of chymotrypsin¹⁰³, interference with the action of trypsin by specific inhibitors has also been postulated¹¹⁴. Homologous trypsin inhibitors¹¹⁸ are known to be present in rat¹¹⁹, dog¹²⁰, and human pancreatic juice^{121,122,123,124,104}. Although the action of the secreted inhibitors is usually 'temporary', due to degradation of the trypsin-inhibitor complexes and digestion of the inhibitor¹²⁵, the secreted inhibitor is specific for trypsin and does not inhibit chymotrypsin and inhibitory activity could therefore account for changes in the ratio of trypsin to chymotrypsin in intestinal contents. Both trypsin and chymotrypsin are also inhibited by inhibitors which differ from the trypsin-specific inhibitor of pancreatic juice. Kunitz and Northrop¹²⁶ found that an inhibitor extracted from bovine pancreatic cells resisted destruction by trypsin and inhibited both trypsin and chymotrypsin. Goldberg *et al*¹²⁷ noted that extracts of human colonic mucosal cells also inhibited both enzymes. Change in the ratio between trypsin and chymotrypsin due to selective inhibition of trypsin during intestinal transit does not, of course, preclude additional inhibition of both enzymes.

Protease inhibitors have been sought in human duodenal contents and not found¹¹¹, perhaps because the trypsin inhibitor is rapidly degraded. Thus, Fritz, Hutzel, Müller, Wiedemann, Stahlheber, Lehnert, and Forell¹²⁸ found that in man amounts of inhibitor sufficient to inhibit 4 to 10% of active trypsin were secreted, but were rapidly destroyed in the duodenum.

(d) Some inactivation of pancreatic enzymes probably occurs as a result of the action of the bacterial flora of the intestine^{116,129,103} although the evidence is controversial^{130,112} and the degree of bacterial degradation may be much reduced by the presence of human small intestinal cells¹¹². It is not known to what extent bacterial inactivation of pancreatic enzymes occurs in man, particularly in the small intestine. Patients have been reported, however, in whom malabsorption has been ascribed to bacterial destruction of normally secreted pancreatic enzymes¹³¹. The degree of bacterial degradation of the pancreatic enzymes was considered analogous to that encountered in conventional, compared with germ-free, rats^{132,131,116}.

(e) In contrast to the factors promoting inactivation of all, or specific, pancreatic enzymes in the intestinal lumen, stabilizing mechanisms have also been postulated. Snook and Meyer¹³³ and Snook¹³⁴ suggested that dietary proteins might render pancreatic enzymes less susceptible to degradation in the small intestine, and subsequently Snook²⁷ considered that the greater stability of amylase in the rat intestine during ingestion of a nitrogen-free

diet might be due to a protective action of starch. The role of intraluminal stabilizing factors is not clear, but an additional degree of protection against degradation may be provided by the adsorption of pancreatic enzymes to small intestinal mucosal cells¹³⁵.

MECHANISMS OF DISSOCIATION OF PANCREATIC ENZYMES IN INTESTINAL CONTENTS

The interrelationships of the pancreatic enzymes in intestinal contents are determined by the enzymes secreted into the duodenum in pancreatic juice and by the fate of the different groups of enzymes within the gut under the conflicting influences of processes such as segregation, adsorption, inhibition, and inactivation. The extent of the 'protective' and 'destructive' processes and their interactions in health and disease has been very little studied, so that it is impossible to interpret the changing interrelationships which have been described, for example, between the trypsin and chymotrypsin in ileal effluent under the influence of diet¹³⁶ or neomycin¹¹².

Conclusion

The predominant impression gained from a study of the current knowledge about the interrelationships of the pancreatic enzymes is one of confusion, controversy, and paucity of data.

The significance of most of the studies carried out before the past decade cannot be assessed because the analytical techniques have been too crude to permit satisfactory inferences from the results of the experimental procedures. The technical problems have not yet been wholly settled.

At present, there seems to be virtually no evidence for 'rapid' adaptation during the course of a single meal, although there may be changes in enzyme interrelationships during the course of secretory activity. The problems of adaptation to longer continued specific changes in the diet have not been satisfactorily resolved, except that studies in rats seem to indicate that apparently 'purposeful' adaptation of pancreatic enzyme synthesis and secretion can be produced by dietary manipulation. The studies need confirmation, elaboration, and explanation, and the results cannot be extrapolated to other species without further investigation. Human studies point to changing interrelationships among the secreted pancreatic enzymes under different conditions of experimental stimulation and perhaps in disease, although these findings also require confirmation and extension. The mechanisms of the observed changes remain virtually unexplained and their significance, both from a physiological and, in man, for a diagnostic point of view, require much further investigation. From a more general point of view, the study of mechanisms controlling the formation and secretion of pancreatic enzymes can be expected to add greatly to the understanding of processes controlling the levels of enzymes in, and secretion from, other tissues, particularly since the enzymes produced by the pancreatic cells are uniquely accessible.

K. G. WORMSLEY and D. M. GOLDBERG
Maryfield Hospital, Dundee
The Royal Hospital, Sheffield

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