ISOLATION OF THE ZYMOGEN GRANULES OF DOG PANCREAS AND A STUDY OF THEIR PROPERTIES*

by

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The presence of zymogen granules in the acinar cells of the pancreas, as well as in the cells of other digestive glands, has been known since the classical histological studies of HEIDENHAIN[1,2], LANGLEY[3,4] and KUHNE AND LEA[5]. These granules have been observed in fixed tissues as well as in living unstained cells. They are the storage sites for the digestive enzymes, disappearing during enzyme secretion and reaccumulating as new enzyme is formed.

In the present investigation pure preparations of zymogen granules have been isolated from dog pancreas by differential centrifugation, and their chemical and enzymic properties have been studied. The zymogen granules sediment as a white layer at 1000 × *g* in 0.25 *M* sucrose. They are rich in amylase, lipase and protease—the three chief enzymic activities of pancreatic secretion. They contain only traces of ribonucleic acid and phospholipid. They retain their morphological structure for long periods when stored in isotonic sucrose at 0° C at a pH between 5.0 and 6.0, but if the pH is raised to 7.2 or above they completely dissolve within a few seconds.

EXPERIMENTAL

Preparation of pancreas homogenates

Pancreases were obtained from dogs which had been fasted for 24 hours. These animals were used in connection with other experiments and had been anaesthetized with pentobarbital or ether before sacrificing. The pancreas was removed immediately after killing (usually by exsanguination) and was either fractionated without delay or was stored in isotonic saline at 0° C for 24 to 48 hours. The tissue was minced and then homogenized with four volumes of 0.25 *M* sucrose in a POTTER-ELVEHJEM[6] all-glass homogenizer. The various cell fractions were isolated by differential centrifugation as indicated below. All operations were carried at 0° C.

Enzyme assays

The homogenates or cell fractions were stored as suspensions in 0.25 *M* sucrose at 0° C and diluted with water just before assay. KHESIN[7] reported that full activity of amylase is not obtained in the isolated particulate matter from homogenates of pigeon pancreas unless the suspensions of particulate material are shaken with *iso*butanol. This could not be confirmed for fractions from dog pancreas; in fact, shaking with *iso*butanol produced a 25% decrease in amylase activity. In view of the fact that the zymogen granules of dog pancreas completely dissolve at the pH of the assay medium (see below), there is obviously no need to extract the enzymes from the granules.

Amylase was assayed by a modified method[8] of SMITH AND ROE[9]; the units are those of SMITH AND ROE. Lipase was assayed by a modification[10] of the method of RONA AND LASNITZKY[11];

* This work was aided by a personal grant from the Blanche Hutchison Fund of McGill University and by a research grant awarded to Dr. K. I. MELVILLE, Department of Pharmacology, McGill University, from the Eli Lilly company.

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the units were defined in a previous paper[10]. Best results were obtained with this enzyme if the assays were performed the day of homogenization of the tissue, and only such results are included. Protease was assayed by a modification of the method of KUNITZ[12] as follows: Appropriate aliquots of the suspensions of zymogen granules or homogenates were made up to 5.0 ml with 0.0025 *M* HCl. Two such samples were prepared. To one was added 3.6 μg of crystalline trypsin (with an equal weight of $MgSO_4$) in 0.2 ml of 0.0025 *M* HCl. The crystalline trypsin was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. The diluted enzyme suspensions were allowed to stand at 4° C until full proteolytic activity was reached. This usually required less than 24 hours. The crystalline trypsin was added to activate the trypsinogen and chymotrypsinogen, but it was found that without addition of crystalline trypsin full proteolytic activity was reached just as rapidly. The substrate consisted of a 1% solution of "highest purity" casein (Special Chemicals Company, Highland Park, Ill., U.S.A.) in 0.1 *M* phosphate buffer, pH 7.6. Test tubes containing 1.0 ml samples of substrate solution were placed in a water bath at 37° C and at 1 minute intervals 1.0 ml of diluted enzyme was added to appropriate tubes. Incubation was for 15 minutes at 37° C. The reaction was stopped by addition of 3.0 ml of trichloracetic acid at 1 min intervals. After standing for 1 hour the mixture was centrifuged, and the optical density of the supernatant was read at a wavelength of 280 mμ in a Beckman Model DU Spectrophotometer. The readings were corrected for blanks which were prepared by adding 3.0 ml of 5% trichloracetic acid to 1.0 ml of substrate, followed by addition of 1.0 ml of enzyme. Various dilutions of crystalline trypsin were assayed and from the data a standard curve was constructed. The units of protease activity are expressed as μg of "trypsin equivalents".

All enzyme assays were performed in duplicate.

Chemical analysis

For determination of ribonucleic acid and phospholipid, 20% trichloracetic acid was added to isotonic sucrose suspensions of the various fractions to give a final concentration of trichloracetic acid of 5 to 10%. The precipitate was centrifuged and washed twice with 5% trichloracetic acid. The phospholipids were extracted with two 3.0 ml portions of 95% ethanol at room temperature and with two 3.0 ml portions of 3:1 ethanol-ether at about 60° C. The combined lipid extracts were reduced in volume to about 0.5 ml by warming in a water bath at 55° C under a stream of nitrogen. 5.0 ml of chloroform was then added and the insoluble matter was removed by filtration. Two additional washings with 5.0 ml of chloroform were also passed through the filter paper. The combined chloroform extracts were made up to a given volume; aliquots were digested and phosphorus was estimated by a modified method of FISKE AND SUBBAROW[13]. The phospholipid content is expressed as μg phospholipid-P per mg N. In initial studies of this investigation, the lipid extracts were taken to dryness *in vacuo* and reextracted with chloroform. Under these conditions there was a considerable loss of phospholipid. Therefore, care was taken in subsequent experiments to avoid evaporating the initial lipid extract to complete dryness.

Ribonucleic acid was determined on the lipid-free residues according to the method of SCHNEIDER[14]. Purine bound ribose was estimated by the method of MEJBAUM[15]. On the basis of the purine content of ribonucleic acid from pig pancreas[16] a figure of 3.35 was calculated for the ratio of micrograms of purine-bound ribose to micrograms of total phosphorus. The ribonucleic acid content is expressed as μg of ribonucleic acid phosphorus per mg nitrogen.

Total nitrogen was determined by the method of UMBREIT, BURRIS AND STAUFFER[17]. All ammonia estimations were performed in duplicate; the duplicates usually agreed within less than 1%.

RESULTS

Isolation of the zymogen granules of the pancreas

Since it was not known at the outset at which centrifugal force the zymogen granules would sediment, the following procedure was first employed: The homogenate was centrifuged for 10 minutes at 600 × *g*. The residue obtained consisted of red blood cells at the bottom, followed by a tannish layer consisting of connective tissue, then a thin pinkish layer which was presumably nuclei and lastly a loosely packed white layer. The supernatant was then centrifuged for 10 minutes at 8,500 × *g*. Three layers were obtained. At the bottom was a firmly packed white layer. This was followed by a firmly packed tan layer with a loosely packed light pink layer on top. It was suspected that the white layer might contain the zymogen granules, the tannish layer, the mitochondria and the pinkish layer, the microsomes. The pinkish

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layer was easily washed away by swirling several times with 0.25 *M* sucrose. The remaining material was resuspended by homogenization and submitted to various centrifugal forces in an attempt to obtain only the white material. This was achieved by centrifuging at 1000 × *g*. Under these conditions a firmly packed white layer was obtained with an overlying loosely packed light tan layer, which could easily be washed away with sucrose without any considerable loss of white material.

In routine experiments the white material was isolated as follows: The homogenate was centrifuged at 600 × *g* for 10 minutes. The supernatant was carefully decanted to avoid inclusion of nuclei and was then centrifuged at 1000 × *g* for 10 minutes. The loosely packed light tan layer was separated from the firmly packed white layer as described above, and the former was resuspended in 0.25 *M* sucrose by homogenization and recentrifuged at 1000 × *g* for 10 minutes. Only the white layer was obtained. This white material was suspended in sucrose and centrifuged at 1000 × *g* two additional times, then finally suspended in 5 to 10 ml of 0.25 *M* sucrose and stored at 0° C.

Microscopic identification of the zymogen granules

Examination of suspensions of the white material in the microscope revealed highly refractile bodies in Brownian motion. They ranged in diameter from about 0.5 to 1.5 microns. They were light green in colour. Depending on the depth of focus they appeared as hollow or as solid spheres (Fig. 1). There appeared to be very little contamination with extraneous particulate matter. The granules did not stain with 1:10,000 Janus green nor with 1:10,000 neutral red. Zymogen granules do not stain *intra vitam* with either of these dyes (HIRSCH[18]). The granules remained intact in 95% ethanol, although most of them agglutinated. When concentrated suspensions

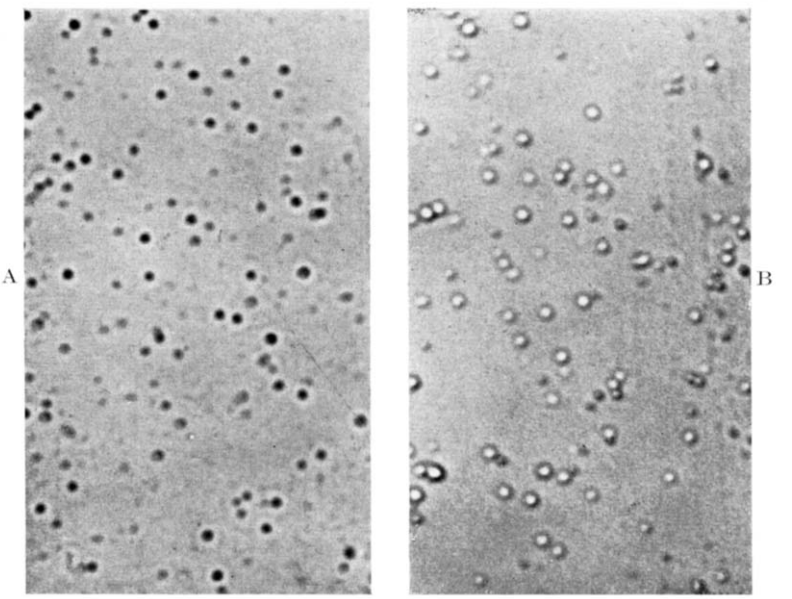

Fig. 1. Photomicrograph of a suspension of zymogen granules in 0.25 *M* sucrose. A and B show the different appearances of zymogen granules at different depths of focus. Because of the thickness of the suspension, not all of the zymogen granules are in focus. (Magnification 2,600 ×.)

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of the zymogen granules were diluted with water the gross turbidity was about the same as when the granules were similarly diluted with 0.25 *M* sucrose, but microscopic examination revealed only masses of amorphous material. This suggested that the granules had been lysed but that most of their substance remained insoluble in water. Freezing of suspensions of the granules in isotonic sucrose did not appreciably alter their morphological appearance but caused them to agglutinate.

From their morphological appearance, staining properties and size, it seemed very probable that the large granules comprising the white layer which sedimented at 1000 × *g* were zymogen granules, and they will be referred to as such in the following sections.

Enzyme content of the zymogen granules

In Table I are shown the specific activities (activities per mg N) of amylase, lipase and protease in the zymogen granules and in whole homogenates of the pancreases of five dogs. Only those amylase and lipase assays which were performed

TABLE I

ACTIVITIES OF AMYLASE, LIPASE AND PROTEASE IN ZYMOGEN GRANULES AND HOMOGENATES OF DOG PANCREAS

Enzyme activity per mg N.

Dog. No.	*Amylase*		*Lipase*		*Protease*	
	Z.G.	*H.*	*Z.G.*	*H.*	*Z.G.*	*H.*
1	1885	712				
2			1490	754		
3	1788	678				
4	1980	1015	1520	831	3250	48
5					3030	18

Z.G.: zymogen granules. H: homogenates. All of the above assays were performed the day of preparation except those for protease. The enzyme activities are defined in the Experimental Section.

the day the tissue was homogenized and fractionated are included. The specific activities of amylase and lipase in the zymogen granules were 2 to 2.5 times greater than the respective activities in the homogenates. The protease activity in the granules was 700 to 1700 times that of the homogenate. The very low protease activity of the whole homogenate relative to that of the zymogen granules was probably due to the presence of trypsin inhibitor in the pancreas (NORTHROP, KUNITZ AND HERRIOTT[19]). The trypsin inhibitor is presumably present in some component of the cell other than the zymogen granules.

The specific activities of the enzymes appeared to vary more in the homogenates of different pancreases than in the zymogen granules. These findings suggest that the enzymes are present in the zymogen granules in fairly constant proportions, the variations in specific activities of the homogenates being due to varying numbers of zymogen granules in different pancreases.

The lipase activity in the zymogen granules appeared to fall on storage in the refrigerator. The amylase activity showed no decrease for at least 24 hours, and the protease was quite stable for at least a week.

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Of the various enzyme activities assayed the amylase activity should be regarded as the most reliable. This enzyme can be assayed with greatest precision, it appears to be more stable than lipase, and it does not require activation as do the proteases nor is its assay complicated by the presence of inhibitors.

It is of interest that the specific activities of amylase and lipase in the zymogen granules were only 2 to 2.5 times the respective activities in the homogenate. Assuming that the digestive enzymes are contained almost exclusively in the zymogen granules this finding means that the zymogen granules account for 40 to 50% of the total nitrogen of the pancreas of a 24 hour fasted dog. This is quite compatible with histological studies of the acinar cells of the pancreas of the fasted dog in which the zymogen granules appear to occupy well over half of the cellular volume (BABKIN, RUBASHKIN AND SAVICH[20]). On a volume basis the zymogen granules would also be expected to contain more nitrogen than the cell sap, which probably comprises the largest proportion of the remaining cell volume.

The phospholipid and ribonucleic acid content of the zymogen granules and other cellular components

In Table II are shown the concentrations of ribonucleic acid, phospholipid and amylase in the zymogen granules as well as in other fractions of a pancreas homogenate; *i.e.* those fractions sedimenting by centrifugation at 600 × *g*, 2,000 × *g*, 8,500 × *g* and 25,000 × *g* in 0.25 *M* sucrose as well as the remaining soluble material.

TABLE II

CONCENTRATIONS OF PHOSPHOLIPID, RIBONUCLEIC ACID AND AMYLASE IN VARIOUS FRACTIONS OF DOG PANCREAS

Fraction	*μg Phospholipid-P per mg N*	*μg Ribonucleic acid-P per mg N*	*Amylase activity per mg N*
Nuclei	29	39	1390
Zymogen granules	7	3	1730
Zymogen granules and mitochondria	25	5	910
Mitochondria	53	37	520
Microsomes	66	112	23
Soluble	11	38	1060

All centrifugations were for 10 minutes except that at 25,000 × *g*, which was for 60 minutes.

All centrifugations were for 10 minutes except that at 25,000 × *g* which was for 60 min. The fractions sedimenting at 600 × *g*, 8,500 × *g* and 25,000 × *g* will be referred to respectively as the nuclear, mitochondrial and microsomal fractions for convenience, although it is realized that these fractions are by no means homogeneous. The fraction sedimenting at 2,000 × *g* contained zymogen granules and mitochondria. With the exception of the microsomal fraction, all fractions were washed at least once by resuspension in isotonic sucrose and centrifugation at the indicated speed for that fraction.

The ribonucleic acid content of the zymogen granule fraction was very low, being only 3 μg ribonucleic acid-P per mg N. This is only 2.5% of the ribonucleic acid content of the microsomal fraction. The traces of ribonucleic acid in the zymogen granule fraction could well be due to contamination.

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Earlier studies have shown that when enzyme secretion is stimulated by acetylcholine there is an increased turnover of phosphoryl units in the phospholipids of the pancreas (HOKIN AND HOKIN[21,22]). The possibility was considered that the stimulation of this turnover might take place in the zymogen granules. The data in Table II make this possibility very unlikely: the phospholipid content of the zymogen granule fraction was very low, being 7 to 9 μg of phospholipid-P per mg N.

The question arose as to whether the small quantity of phospholipid in the zymogen granule fraction might be due to impurities. To throw some light on this problem a dog was anaesthetized, the pancreatic duct was cannulated and the pancreatic juice was collected after intravenous injection of pilocarpine (1 mg per kg). No phospholipid was detected in the pancreatic juice. If the pancreatic juice would have contained one-fiftieth as much phospholipid per mg N as the zymogen granule fraction this phospholipid would have been detectable. If it is assumed that all of the material in the zymogen granule is secreted, the failure to find phospholipid in the pancreatic juice may be taken as evidence that the zymogen granules contain no phospholipid. This is compatible with the earlier observation that there is no decrease in the phospholipid content of the pancreas during enzyme secretion[22]. Reabsorption of the phospholipid or its hydrolysis products seems unlikely in view of the fact that the turnover of the whole phospholipid molecule, as measured by the incorporation of glycerol-1-^{14}C, is not increased during enzyme secretion[22].

The distribution of ribonucleic acid in the nuclear, mitochondrial, microsomal and soluble fractions is similar to that reported for liver (HOGEBOOM, SCHNEIDER AND PALLADE[23]), except that the quantity of ribonucleic acid per mg N in each of the fractions from pancreas was more than double that of liver. This is to be expected from the very high ribonucleic acid content of the pancreas. There were appreciable quantities of phospholipid in the nuclear, mitochondrial and microsomal fractions, the largest amount being in the microsomal fraction. In addition to microsomes, the microsomal fraction probably contains Golgi bodies and fragments of the "endoplasmic reticulum" of PORTER[24]. Both of these latter structures are very prominent in the pancreas. From their staining properties the Golgi bodies have long been thought to be rich in phospholipid; nothing is known about the chemical composition of the "endoplasmic reticulum".

It will be noted that of all the cell fractions isolated, the zymogen granules contained the highest amylase activity per mg N. This activity remained constant on repeated washings of the zymogen granules in isotonic sucrose. The specific activity of amylase was high in several other fractions, being particularly high in the fraction sedimenting at 600 $\times$ g and in the soluble fraction. Microscopic examination of the fraction sedimenting at 600 $\times$ g revealed that the major visible element of this fraction was zymogen granules. With regard to the high content of amylase in the soluble fraction, it is likely that homogenization disrupts a large number of zymogen granules releasing the amylase into solution. It should also be borne in mind that zymogen granules are of a wide range of sizes. Because of this, they would distribute themselves in various fractions. In fact, judging from the distribution of amylase the only particulate fraction which appeared to be free of zymogen granules was that fraction sedimenting at 25,000 $\times$ g.

Although most of the fractions were highly contaminated with zymogen granules the converse was not true; the very low ribonucleic acid content of the zymogen

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granule fraction indicates that the zymogen granules were not contaminated to any appreciable extent by other cellular components.

Solubilization of the zymogen granules by alteration in pH

During a routine enzyme assay it was found that when a turbid suspension of lysed zymogen granules was added to the substrate solution the turbidity was immediately lost. Further investigations revealed that this was an effect of pH. The pH of suspensions of zymogen granules in isotonic sucrose was between 5.5 and 6.0. If the pH of these suspensions was raised to 7.2 or above the zymogen granules were dissolved within a few seconds, leaving a clear or almost clear solution.

The quantity of zymogen granules could be estimated turbidimetrically by measuring the optical density of suspensions in a colorimeter with a 650 mμ filter. Fig. 2 shows the effect of altering the pH on the turbidity of a suspension of zymogen granules in isotonic sucrose. It can be seen that the zymogen granules exhibited optimum stability at pH 5.5. At pH's between 6.0 and 7.2 they were partially solubilized, the solubilization increasing until it reached a maximum at pH 7.2. At pH 7.2 and above the optical density was only 5% of maximum. This residual optical density is probably due to impurities. Solubilization of the zymogen granules was less sharp on the acid side of pH 5.5, the optical density of the suspensions at pH 3.8 being more than half of maximum. At those pH's where solubilization was only partial, the optical density remained constant for long periods. This shows that the solubilizing effect of alkali or acid was not due to the activation of an enzyme but was a physical phenomenon. The stability of the zymogen granules at pH 5.0 to 6.0 could be due to the presence of structural proteins within the granules which have isoelectric points in the region of pH 5.0 to 6.0. But this seems rather unlikely in view of the fact that when the pH of a solubilized suspension of granules was returned to between 5.0 and 6.0 no reprecipitation occurred.

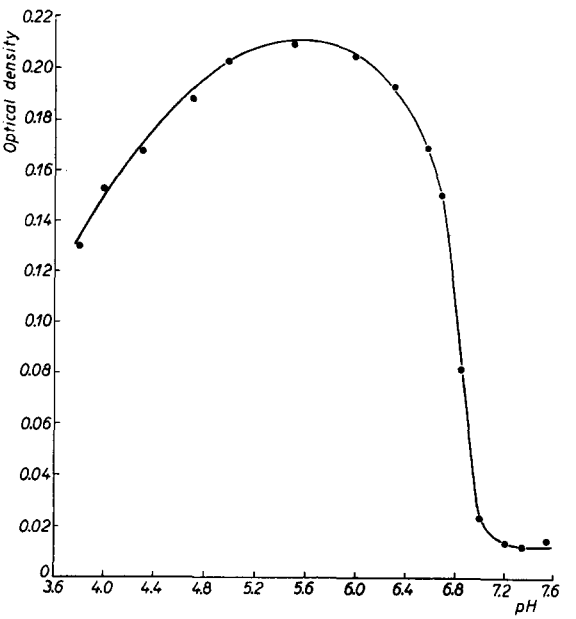

Fig. 2. The solubilization of the zymogen granules at various pH's. The suspension of zymogen granules used in this experiment contained 23.2 μg N per ml. 0.005 *M* phosphate buffer was used for pH's between 5.8 and 7.6. 0.005 *M* acetate buffer was used between pH 3.8 and 5.7. The pH's were determined with a glass electrode in the Beckman pH meter, model G. The optical density was measured in a photoelectric colorimeter with a 650 mu filter.

One interesting implication of these studies is that if the same pH dependence for optimum stability exists within the cell as was found in suspensions of isolated zymogen granules, then the pH of the cell, at least in the vicinity of the zymogen granules, must be in the neighborhood of 5.0 to 6.0. This pH is lower than is usually

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thought to exist within cells. But it is well known that when most tissues are homogenized in the cold the pH is found to be in this range immediately after homogenization—before sufficient time could elapse for much autolysis to take place. The "pH of the cell" is however a term of limited significance—the cell is a highly heterogeneous system, and it is probable that the pH is different in different parts.

After lysis of the granules in water over two-thirds of the lipase and over three-quarters of the trypsin remained bound to the insoluble material. However, over 95% of the amylase was liberated into solution. About 25% of the total nitrogen was released into solution. Freezing of suspensions of zymogen granules in isotonic sucrose released about 15% of the amylase into solution. The fact that a large proportion of the lipase and protease remained bound to the insoluble material after lysis shows that these enzymes are not retained in simple solution within the zymogen granules, but probably exist in the form of insoluble complexes with matrix proteins. It is likely that all of the enzymes are held in such complexes; the complex between amylase and matrix protein would, in this case, be very labile. The solubilizing effect of altering the pH to the acid or alkaline side of pH 5.5 could be due to a dissociation of the complex between enzyme and matrix protein.

DISCUSSION

The fact that the zymogen granule fraction contained very small amounts of ribonucleic acid and phospholipid is of interest. This is in contrast to other particulate fractions such as mitochondria and microsomes. The small amounts of phospholipid and ribonucleic acid which were found in the zymogen granule fraction might be due to contamination. It appears that the zymogen granules consist almost entirely of protein. This is of course in keeping with their function as storage sites for the digestive enzymes. CLAUDE[25], in one of the earliest attempts to isolate subcellular particles by differential centrifugation, claimed to have isolated the zymogen granules of the pancreas. He stated that these granules contained 20% lipid, mainly phospholipid. In view of the conditions used (sedimentation at 2,000 $\times$ *g* in 0.85% NaCl), his fraction no doubt contained many mitochondria, which could account for the high lipid content of the fraction. KHESIN[7] isolated a "large granule" fraction from homogenates of pigeon pancreas by centrifuging at "12,000 to 15,000 r.p.m." in 0.25 *M* sucrose plus Ringer-bicarbonate for 10 minutes. This fraction would contain most of the mitochondria and probably a considerable quantity of the microsomes. It seems that a fairly large proportion of the zymogen granules were not solubilized under KHESIN's conditions, and were present in the "large granule" fraction, since this fraction contained about 30 to 60% of the total amylase. This suggests that zymogen granules of pigeon pancreas may be more stable at pH's above 5.0 to 6.0 than those from dog pancreas.

The digestive enzymes or zymogens are present in pancreatic juice in the soluble form; no zymogen granules are ever seen. It is thus clear that at some stage in the secretory process the zymogen granules are dissolved. Whether the zymogen granules are extruded intact, dissolving in the pancreatic juice, or whether they are solubilized within the cell prior to the secretion of the enzymes is not certain. There is evidence that both mechanisms may operate (see KUHNE AND LEA[5], BABKIN *et al.*[20], HORNING[26], COVELL[27]). If the zymogen granules are extruded intact it is quite clear from their

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solubility at higher pH's that they would rapidly dissolve in the alkaline pancreatic juice. It is also possible that in the process of secreting an alkaline juice the pH of the acinar cell rises so as to solubilize the granules intracellularly. One argument against this latter possibility, however, is the fact that secretin is a much more potent stimulant of alkali secretion than is acetylcholine; yet secretin is much less effective than acetylcholine in stimulating enzyme secretion (see BABKIN[27]).

ACKNOWLEDGEMENTS

The author wishes to thank Dr. H. SLATIS of the Department of Genetics, McGill University, for kindly taking the photomicrographs.

SUMMARY

The zymogen granules have been isolated from dog pancreas in relatively pure form. They contain very small amounts of ribonucleic acid and phospholipid. On a nitrogen basis they contain 2–2.5 times as much amylase and lipase and 700 to 1700 times as much protease as the whole cell. The disproportionately high protease activity in the zymogen granules is probably due to the presence of trypsin inhibitor in the whole homogenate, but not in the purified zymogen granules. The zymogen granules are stable in isotonic sucrose for long periods at pH's ranging from 5.0 to 6.0, but they are rapidly solubilized if the pH is raised to 7.2 or higher. Solubilization occurs on the acid side of pH 5.0, but is less sharp than solubilization with alkali. Suspension of the granules in water liberates about 95% of the amylase into solution, but most of the lipase and protease remain bound to the insoluble material. Since pancreatic juice is alkaline the solubilization of the zymogen granules at higher pH's can explain how they are dissolved in the pancreatic juice.

RÉSUMÉ

Les granules de zymogènes ont été isolés du pancréas du chien à l'état relativement pur. Ils contiennent de très petites quantités d'acide ribonucléique et de phospholipides. En prenant pour base la teneur en azote, ils contiennent 2–2.5 fois plus d'amylase et de lipase et de 700 à 1700 fois plus de protéase que les cellules entières. La teneur disproportionnée en activité protéasique des granules de zymogènes est probablement due à la présence de l'inhibiteur de la trypsine dans l'homogénat entier et à son absence dans les granules de zymogènes purifiés. Les granules de zymogènes sont stables dans le saccharose isotonique pendant des temps prolongés à des pH compris entre 5.0 et 6.0, mais ils sont rapidement dissous si le pH atteint ou dépasse 7.2. Ils se dissolvent également à des pH inférieurs à 5.0, mais moins rapidement qu'aux pH alcalins. Une suspension de granules dans l'eau libère environ 95% de l'amylase en solution, mais la majeure partie de la lipase et des protéases reste liée aux particules insolubles. Puisque le suc pancréatique est alcalin, la solubilisation des granules de zymogène à des pH élevés peut expliquer comment ils se dissolvent dans le suc pancréatique.

ZUSAMMENFASSUNG

Zymogenpartikel wurden aus Hundepankreas in verhältnissmässiger Reinheit isoliert. Sie enthalten sehr kleine Mengen Ribonukleinsäure und Phospholipide. Auf Grund von Stickstoffberechnungen enthalten sie 2 bis 2.5 mal so viel Amylase und Lipase und 700 bis 1700 mal so viel Protease wie die ganze Zelle. Die unverhältnismässig hohe Proteaseaktivität der Zymogenpartikel ist wahrscheinlich der Gegenwart eines Trypsin-Hemmungsfaktors im ganzen Homogenat zuzuschreiben, welcher in den gereinigten Zymogenpartikeln nicht zugegen ist. Die Zymogenpartikel sind in isotonischer Saccharose in einem pH-Bereich von 5.0 bis 6.0 für lange Zeit stabil, lösen sich jedoch schnell, wenn der pH-Wert bis 7.2 oder höher ansteigt. Die Partikel lösen sich auch, wenn der pH-Wert tiefer sinkt als 5, aber die Wirkung ist weniger krass als auf der alkalischen Seite. Durch Wassersuspendierung der Partikel wird etwa 95% der Amylase in Lösung gebracht, während der grösste Teil der Lipase und Protease an das unlösliche Material gebunden bleibt. Da die Zymogenpartikel bei höheren pH-Werten löslich sind, kann die Alkalinität des Pankreassaftes als Erklärung für ihre Löslichkeit im Pankreassaft dienen.

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Received May 4th, 1955