Acta Anatomica

International Archives of Anatomy, Histology, Embryology and Cytology
Archives Internationales d'Anatomie, d'Histologie, d'Embryologie et de Cytologie
Internationales Archiv für Anatomie, Histologie, Embryologie und Zellforschung

Condiderunt: G. GLIMSTEDT, T. PÉTERFI †, G. WOLF-HEIDEGGER

Supplementum 23 = 1 ad Vol. XXV (1955)

From the Department of Anatomy, University of Aarhus, Denmark

THE CYTOPLASMIC BASOPHILIC SUBSTANCE OF THE EXOCRINE PANCREATIC CELLS

By V. ORAM

BASEL (Schweiz) S. KARGER NEW YORK

Printed in Switzerland

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Denne afhandling er af det matematisk-naturvidenskabelige fakultet ved Københavns Universitet antaget til offentligt at forsvares for den filosofiske doktorgrad.

København, den 22. marts 1955.

JANNIK BJERRUM
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PREFACE

The experimental studies presented here were carried out during the years 1950–1953 in the Department of Anatomy, University of Aarhus (Professor LÁRUS EINARSON).

I wish to express my sincerest thanks to Professor LÁRUS EINARSON for the privilege of working in the Department of Anatomy. In Professor EINARSON I met a highly inspiring scientific enthusiasm combined with a profound sense of criticism, which has been of fundamental importance in my work. In addition, Professor EINARSON took an unfailing interest in the problems related to the present studies and provided the best possible working conditions to me in his department. I owe a great debt of gratitude to Professor LÁRUS EINARSON.

I am very grateful to Mrs. ESTHER CLAUSEN, née NATHANSEN, for her skilful assistance in the work with the experimental animals and in the preparation of the histological specimens.

Miss ANNE MARIE JENSEN has been a very patient secretary, who with outstanding ability has helped me in typing the manuscript, for which I am greatly indebted to her.

I wish to express my special appreciation to Mr. EINAR HANSEN, who has been of invaluable assistance to me in the solution of technical problems and in making the photomicrographs.

In this place I also wish to extend my heartfelt thanks to J. FABRICIUS-MØLLER, M. D., Senior Surgeon of the County Hospital, Aarhus, for his encouragement and stimulating support during the present studies. In a close collaboration extending over many years he has been my teacher in matters of both science and style in writing.

Finally, I owe a deep debt of gratitude to my wife, KAMMA WINTHER ORAM, and to my daughter, VIBEKE MARY-ANN ORAM, who both with great patience sacrificed family life on the altar of science during the various phases of the preparation of this monograph.

May, 1955.

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INTRODUCTION

Modern research has shown that the distribution of the nucleoproteins in animal organisms is wider and their significance greater than has previously been assumed. During recent years, the structural and functional importance of the nucleoproteins has been the subject of intensive chemical and cytochemical studies. It has been disclosed that nucleoproteins are present in all organic structures, particularly in tissues in which an intense formation of new cytoplasmic proteins and enzymes occurs (growing tissue, whether embryonal or neoplastic, regenerating nerve cells, epithelial cells in various organs). It must therefore be assumed that the nucleic acids play an active role in the synthesis of protein and formation of enzymes in the cells. During this process there seems to be a close co-operation, a continuous metabolic turnover, between the nucleic acids of the nuclear chromatin, nucleolus and cytoplasm.

In the exocrine pancreatic cells nucleoproteins or nucleic acids have been demonstrated in the nucleus and the basal portion of the cytoplasm. However, an accurate evaluation of the distribution and functional variation of these substances has not yet been carried out to a sufficient extent. So the purpose of the present monograph is to report the results of a cytomorphological study of the nucleoproteins in the exocrine pancreatic cells, especially as they appear in the cytoplasm (the so-called basophilic substance), and of their quantitative functional changes. With this end in view, EINARSON's specific gallocyanin-chromalum staining method was used in combination with densitometric measurements.

CHAPTER I

PANCREAS

Morphology.

The pancreas is an elongated organ, which from the duodenum extends obliquely upwards behind the stomach towards the spleen across the first and second lumbar vertebrae. The organ is divided into a head with an uncinate process, a body and a tail. In man, the pancreas lies retroperitoneally, firmly attached to the posterior abdominal wall. In the dog, the pancreas is appreciably more mobile, particularly the head, which lies within the mesentery of the duodenum. Furthermore, the uncinate process is well developed and follows the descending portion of the duodenum. Due to these anatomical differences, operations on the pancreas are easier in the dog than in man. In the dog, the pancreas has usually two or three ducts, which may communicate with each other, and which all, whether separate or conjoined, open into the duodenum.

The blood supply to the pancreas is very ample and takes place through the arteries of the stomach and the spleen. The afferent vessels end in a finely ramified capillary network surrounding the acini.

The pancreas is abundantly innervated both from the vagus nerves and the splanchnic nerves, but our knowledge of its nerve supply is as yet rather incomplete. The sympathetic fibres pass, either synaptically interrupted or uninterrupted, the coeliac ganglion and reach the gland along the arteries. The parasympathetic fibres, which either run along the lesser curvature of the stomach and cross the pylorus or pass through the hepatic plexus and reach the pancreas via the pylorus, end in ganglia in the gland itself, from which postganglionic unmyelinated fibres continue to the gland cells.

Microscopically, the pancreas consists of groups of cells (10–50) arranged in spherical or ovoid acini, which in the closely packed tissue assume a polygonal appearance. Groups of acini likewise form polygonal lobules, which are separated by a sparse, loose connective tissue. Several lobules together form lobes, which are separated by more abundant amounts of connective tissue. The lobes may be isolated by dissection. The interlobular ducts, which run within the connective tissue, give off intralobular branches, usually at right angles, which ramify profusely, each ramification ending in its own acinus. Blood vessels, lymphatics and nerves follow the ducts. In addition, each lobule contains the islets of Langerhans scattered among the acini. Thus, the pancreas is a compound, tubulo-acinous gland.

The acinous cells are pyramidal with the apices directed towards the lumen, which is very fine. They are large cells with a well-developed, centrally placed nucleus and one or more nucleoli and abundant cytoplasm containing strongly light-refractive, fuchsinophilic or acidophilic zymogen granules; they are protein gels giving an intense Millon

reaction (GAGE 1945, BENSLEY and BENSLEY 1947). The granules, which are readily stainable, vary in number and position with the functional activity of the cell, and tend to be more abundant in the luminal or apical region of the cell. When very abundant, the granules displace the nucleus towards the base of the cell, but there is usually a basal zone which is free of granules. In this free basal zone there is a finely granular, chromophilic material which stains intensely with basic dyes. With regard to chemistry and stainability, this material is considered to be identical with the Nissl substance of the nerve cells (BENSLEY and GERSH 1933, COWDRY 1946) and with similar substances in other gland cells, such as the chief cells of the stomach and the serous cells of the parotid gland. The large pancreatic ducts are lined with a columnar epithelium composed of cells with a faintly granular cytoplasm staining with mucicarmine. The intermediate ducts are lined with a low columnar or cuboidal epithelium. The intralobular ducts are made up of flattened cells with a relatively sparse cytoplasm. These cells extend into the acini in the form of the so-called »centroacinous cells«. It is as yet obscure whether or not they form a complete lining of the lumen of the acinus (RIES 1935, HEWER 1937, THOMAS 1950).

Pancreatic Juice.

The amount of secreted pancreatic juice varies from individual to individual and with the diet. In dogs, the secretion amounts to 17–22 ml per kg per day (BABKIN 1950). Pancreatic juice is usually colourless, odourless and alkaline, tasting strongly of sodium bicarbonate, with a pH ranging from 8.0 to 8.3. Its specific gravity ranges from 1.007 to 1.042 and varies directly with the protein content. The osmotic activity of pancreatic juice is identical with that of the blood.

The high bicarbonate content is a characteristic chemical feature of pancreatic juice. The amounts of bicarbonate and chloride vary reciprocally so that the sum of the two is constant and equal to the total base of the blood. The bases are Na, K and Ca. The secretion contains a total amount of these bases equal to that of the blood (INGRAHAM and VISSCHER 1939).

The protein concentration varies with the secretory activity, and is estimated to range from 0.1 to 10.0 per cent of albumin and globulin. In 24 hours, the pancreas produces protein in quantities amounting to 10–20 per cent of the dry weight of the gland (CASPERSSON, HYDÉN and AQUILONIUS 1941, MIRSKY 1943). Electrophoretic analysis of the secretion (in a sodium bicarbonate buffer at pH 8.2) revealed four components, presumably corresponding to a similar number of enzymes (MUNRO and THOMAS 1945). A fifth component was also disclosed and is presumably identical with mucin.

Pancreatic juice contains proteolytic, amylolytic and lipolytic enzymes. The proteolytic enzymes are trypsin, trypsinogen, chymotrypsin, chymotrypsinogen and peptidase, which are all proteins. The lipolytic enzyme is lipase. The amylolytic enzymes are amylase, and perhaps, maltase, lactase and saccharase.

The enzyme concentration of pancreatic juice depends on the composition of the diet (GROSSMANN, GREENGARD and IVY 1942–43) and on the stimulus used to provoke secretion. Functionally, the pancreas thus possesses a certain adaptability since, as already pointed out, the *total* enzyme concentration of the secretion varies with the diet, whereas its quantitative enzymatic composition is constant; the juice undergoes quantitative, but not qualitative changes (LEBRETON and MOCOROA 1931, BABKIN, HEBB and SERGEYEVA 1939). The enzyme production is the end result of a synthesis of new proteins.

Pancreatic Stimuli.

Early experiments (HEIDENHAIN 1868, 1875) showed that the secretion of fluid and the secretion of enzymes by the pancreas occur independently of each other. HEIDENHAIN called the stimuli that provoked secretion of water and salts »secretory stimuli«, whereas those resulting in secretion of enzymes were termed »trophic stimuli«. Since the two terms are not very fortunate, BABKIN (1950) suggested that »hydrelatic« should be substituted for »secretory« and »ecbolic« for »trophic«.

HEIDENHAIN (1883) believed that pancreatic secretion in the dog was intermittent and only occurred following stimulation, but later BABKIN and ISHIKAWA (1912) showed that continuous secretion is likely to be the rule, and that the apparent intermittent secretion may be due to a pressing out of juice from the pancreas by periodical contractions of the ducts. In man, the secretion is continuous.

The secretion may be stimulated in several ways:–

1. *The act of eating* as such serves as a stimulus to increase the secretion of enzymes 1–1½ minutes after feeding, as was shown by PAVLOV in experiments with sham feeding (cited by BABKIN 1950). Vagotomy or injection of atropine abolishes this response.

2. *Distilled water* introduced into the upper part of the intestinal tract gives a secretion with a relatively high enzyme content as shown by DAMASKIN (cited by BABKIN 1950) and by CRIDER and THOMAS (1940).

3. *Acid* is the most powerful excitant of pancreatic secretion (SERGEYEVA 1938, BABKIN 1950). Several acids (hydrochloric, phosphoric, lactic, acetic) are active. The effective stimulus is the hydrogen ion (POPIELSKI 1919), which liberates a hormone (*secretin*) in the intestinal mucosa (BAYLISS and STARLING 1902). The hormone is carried by the blood stream to the pancreas, where it provokes an abundant amount of secretion rich in enzymes. Purified secretin causes an appreciable increase in the volume, whereas the enzyme content remains at a constant low level (WANG, GROSSMANN and IVY 1948). Another hormone, *pancreozymin*, which is isolated as a fraction in the purification of secretin, is supposed to be responsible for the increased secretion of enzymes (HARPER and RAPER 1943.) The effect of acid is not abolished, but is somewhat diminished by section of the vagus and splanchnic nerves or by administration of atropine.

4. *Products of protein digestion* (peptones, proteoses and some amino acids) increase the secretion of enzymes. The effect cannot be provoked in vagotomized animals, at least not during the first 24 hours after the operation (RAMSAY, THOMAS and CRIDER 1943).

5. *Fats, fatty acids and soaps* stimulate the secretion of enzymes, when they are introduced into the upper part of the intestinal tract. An appreciable diminution in the effect occurs after administration of atropine (BABKIN 1950). The effective stimulus is not secretin, but is supposed to be another secretagogue called »*saprocrinin*«.

6. *Carbohydrates* introduced into the intestinal tract also seem to increase the secretion of enzymes, but the exact mechanism of this stimulation is as yet unknown (HEBB 1937, THOMAS and CRIDER 1946).

7. *Bile* as such has not with certainty been demonstrated to be capable of increasing pancreatic secretion, but it seems to be active in increasing the absorption of secretin (MELLANBY 1926, THOMAS and CRIDER 1941).

8. *Other pancreatic stimuli* are alcohol, ether, chloral hydrate, mustard oil, pepper extract, formaldehyde and magnesium sulphate, etc. (BABKIN 1950). Stimulation by ether or magnesium sulphate has been used in human subjects for clinical analysis of pancreatic function.

9. *Stimulative effect of various drugs:* Drugs raising or lowering the blood pressure (histamine, methyl, etc.) enhance the secretory effect of other stimuli, such as secretin, but are inactive if the small intestine has been removed (THOMAS 1950). *Acetylcholine, choline, physostigmine* and *pilocarpine*, given in appropriate doses, act as ecbolic stimuli (RIES 1935). However, the volume of the secretion is but slightly increased, for which reason a combination with, e.g., secretin, is necessary to give a distinct quantitative increase in the secretion (BABKIN, HEBB and SERGEYEVA 1939, COMFORT and OSTERBERG 1940). *Atropine* given in doses of 0.2–0.3 mg/kg abolishes the secretory effect of vagus stimulation in dogs. In very large doses, possibly in combination with pepton, milk or cream, atropine is reported to give a distinctly increased volume of pancreatic secretion (THOMAS and CRIDER 1946). In experimental animals, fairly large doses of atropine have only a depressive effect on pancreatic secretion. *Epinephrine* and other sympathomimetic drugs generally inhibit pancreatic secretion. This effect is presumably due, at least in part, to the diminished blood flow through the pancreas (EDMUNDS 1911, SERGEYEVA 1932).

10. *Nervous stimuli:* HEIDENHAIN (1883) was the first to show that the pancreas is supplied by secretory nerves. Later, PAVLOV (1893), BUCHSTAB (1904) and others (cited by BABKIN 1950) showed that the secretory fibres run in the vagus and splanchnic nerves. Faradic *stimulation of the vagus* causes a greatly increased and enzyme-rich pancreatic

secretion. Concurrent secretin stimulation enhances the vagus effect considerably, for which reason a synergism is assumed to exist between these two stimuli. It is now considered likely that the vagus nerve has two secretory effects, viz. one enhancing the effect of other secretory stimuli, and another increasing the secretion independently of other stimuli. *Stimulation of the splanchnic nerves* has the same physiological and histological effects as stimulation of the vagus nerves. Painting with nicotine of the coeliac ganglion does not interrupt the secretory fibres (SERGEYEVA 1932, BABKIN, HEBB and SERGEYEVA 1939.) On the other hand, injection of atropine abolishes the effect of splanchnic stimulation. Correlated with the demonstration of acetylcholine in the venous blood after splanchnic stimulation (BABKIN, HEBB and SERGEYEVA 1939), this shows that the secretory fibres are cholinergic. In addition to cholinergic secretory fibres, KUNTZ and RICHINS (1949) found adrenergic fibres in the splanchnic nerve inhibiting secretion. The latter fibres act through vasoconstriction. Thus, PAVLOV (1893) and BABKIN (1924) (cited by THOMAS 1950) showed that the secretory activity of the pancreas is influenced by changes in its blood supply. Decreased blood flow diminishes secretion, while increased blood flow augments secretory activity. These influences are presumed to occur through the splanchnic and vagus nerves, respectively. So the secretory stimulative effect of these nerves is due not only to purely secretory components but also, in part, to components causing changes in the blood flow through the gland. At the present time, it is believed that the nervous regulation of pancreatic secretion has not only a *cerebral* phase, which via long reflex paths passes through the vagus and splanchnic nerves, but presumably also an *intestinal* phase via short intestinopancreatic reflex paths. However, the latter has not yet been demonstrated morphologically (THOMAS 1950).

Mechanism of Secretion.

Pancreatic juice is a product of the acinous cells, because other cells with the commonly known secretory characteristics are absent in the pancreas. The functions of the acinous cells seem to be dissociated, since pancreatic juice under certain conditions mainly contains inorganic material and practically no enzymes, while the opposite may be the case under other conditions (BABKIN 1909, LAGERLÖF 1939). Experiments with radioactive substances have shown that, for instance, the greater part of the bicarbonate content of the juice, originates from the blood. The acinous cells seem to be freely permeable to most cations, but exert a considerable selective action on anions. Only a trace of phosphate is present in the secretion. The chloride concentration is lower, and the bicarbonate concentration higher, than in the blood. It is likely that the acinous cells are freely permeable to the substances which are present in the juice in the same concentration as in the blood (BALL 1941). During the secretory process a considerable increase in the cell metabolism occurs, which is evidenced by increases in the oxygen consumption by more than 50 per cent (GERARD and STILL 1933) and in the secretory pressure in the pancreatic ducts to 22 mm Hg (HARMS 1927).

The production of enzymes involves two distinct processes, viz. (1) synthesis in the cells and (2) discharge of the secretion, sometimes called the process of excretion.

The cellular *synthesis* is artificially divided into several, ill-defined phases, but is presumably a continuous process influenced only by the laws of chemical equilibrium and by the stimulative factors determining the rate of cell metabolism. The histologically visible result of this process is an accumulation of the characteristic zymogen granules. When this process reaches its maximum, the cell is closely packed with granules; the basal zone containing the basophilic substance is narrow and the amount of this substance slight. A resting stage is believed to be reached. The *discharge* of enzymes is the characteristic response to the various aforementioned stimuli and involves the passage of the stored product into the lumen of the acinus and the excretory ducts (excretion). This process, although ultimately dependent on synthesis, is independently controlled, and may by prolonged stimulation (considerable functional stress) proceed so rapidly that not only the granules but also the basophilic substance may disappear completely. The cells have been completely exhausted and are but slowly restituted (Bensley 1911, Davidson 1947, Altmann 1952). Vigorous stimuli are necessary, as the capacity of synthesis of the pancreatic cells is very great (Dolley 1925).

The individual pancreatic acinus constitutes a functional entity, all its cells being at the same functional stage, whereas the various acini may be in different functional stages. By vigorous stimulation the action of the acini may be synchronized and the secretion discharged (Ries 1935). *During the discharge* the granules are reduced in size and are dissolved to a homogeneous mass. The latter process may occur in the cells or in the ducts. Simultaneously with the disappearance of the granules the restitution sets in; the basophilic substance is increased, particularly along the nuclear membrane, and gradually fills up a greater part of the basal zone of the cell and, thus, at this stage constitutes a greater proportion of the cell (Kühne and Lea 1882, Heidenhain 1883, Mouret 1895, Babkin 1909, Dolley 1925, Covell 1928, Bowen 1929, Stormont 1932, Ries 1935, Hewer 1937, Caspersson, Hydén and Aquilonius 1941, Mirsky 1943, Noback and Montagna 1947). Heidenhain (1883) showed that the enzyme content of the secretion varies directly with the content of zymogen granules in the cells, which proves that the granules are actually precursors of the enzymes. Even though the amount of secretion produced by stimulation may vary and be slight, as is seen, for example, after vagus stimulation (i.e., the aqueous phase is slight), the histomorphological changes are invariably pronounced (Sergeyeva 1938).

The synthesis of the granules begins rapidly after the depletion of the cells; as early as 10 minutes later the first granules are seen basally in the cell, from which they migrate apically. The number of granules increases rapidly, and after the lapse of $1\frac{1}{2}$–3 hours the apical zone begins to be filled with granules. In this zone the granules increase in size, pass through several transformative stages and change their reaction to stains; in other words, they undergo »maturation«. After the lapse of $3\frac{1}{2}$ hours the cells are again capable of secreting (Hirsh 1932, Duthie 1934, Bensley 1947). Simultaneously with the increase in the amount of granules, the amount of basophilic substance decreases, i.e., the amount of basophilic substance varies with the cellular activity, and even though it with regard to time is in a certain antagonistic relation to the granules, it participates in their resynthesis (Mouret 1895, Dolley 1925, Bensley 1947, Noback and