The Histochemical Demonstration of Secretory Capillaries in the Pancreas with the Aid of Substrate Specific Phosphatases.*

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Staining of the bile canaliculi in the liver of various mammalian species with histochemical techniques for adenosinetriphosphatase and less regularly 5-nucleotidase at pH 7.2 has been previously reported (1–3). When such techniques were applied to the pancreas, marked enzymatic activity was exhibited by the initial portions of the excretory duct system within the centrally located acinar lumens and the lateral intercellular recesses which extend between neighboring acinar cells. These structures constitute the so called secretory capillaries (*Sekretionskapillaren*) which can be visualized only with great difficulty with conventional staining techniques.

Methods

Rats, rabbits, and guinea pigs were used. The pancreas of freshly sacrificed animals was fixed overnight in cold Baker's formalin and frozen sections were cut at 6 micron thickness. For the demonstration of adenosinetriphosphatase, free floating sections were incubated in a substrate mixture containing 20 ml. of a 125 mg. per cent aqueous solution of adenosinetriphosphate (ATP), 22 ml. of 0.2 M Tris-maleate buffer at pH 7.2, 3 ml. of 2 per cent lead nitrate, and 5 ml. of M/10 magnesium sulfate, as previously described (1). For the demonstration of 5-nucleotidase, adenosinetriphosphate was replaced by an equal amount of muscle adenylic acid. Sections were also tested for the presence of non-specific phosphatase at pH 7.2. Following incubation, the sections were washed in tap water and immersed in a dilute solution of ammonium sulfide for several minutes. The sites of enzymatic activity were revealed by deposits of black lead sulfide. In addition, sections were stained for non-specific alkaline phosphatase.

OBSERVATIONS

With the technique for the demonstration of adenosinetriphosphatase, the secretory capillaries of all three species reacted positively. In the rat, optimal staining was observed within 5 minutes of incubation, in the rabbit after 15, and in the guinea pig after 30 minutes. Longer incubation periods led to such intense staining that cellular details were obscured. Within the acini, the initial units of the excretory ductal system could be clearly recognized. From the larger centrally located secretory capillaries, thin branches penetrated to varying lengths between single acinar cells (Fig. 1). In sections incubated for a short time, some capillaries revealed a distinct lumen (Fig. 2), but in most instances the secretory capillaries appeared as solid structures without recognizable lumens due to the dense deposits of lead sulfide. The zymogen granules, easily recognized by their strong light refraction, gave a negative to faintly positive reaction. Otherwise, the cytoplasm of acinar cells and of the larger ducts did not react.

With the technique for 5-nucleotidase, the secretory capillaries did not stain in sections from the pancreas of the rabbit and the guinea pig. In order to obtain optimal results in the rat, it was necessary to incubate the tissue sections for 30 minutes in the substrate. With glycerophosphate as substrate at a pH of 7.2, the secretory capillaries did not stain in the rat or guinea pig. A spotty reaction was observed in the rabbit pancreas.

In addition to secretory capillaries, other structures of the pancreas were also stained to varying degrees. The blood capillaries reacted strongly in the ATPase technique in all three species, but only in the guinea pig consistent 5-nucleotidase activity was seen. Strong ATPase activity was found also in the walls of arteries and veins and connective tissue in rat and guinea pig and in the intima and adventitia of larger arteries of the rabbit. Langerhans' islands gave a positive reaction for ATPase in the guinea pig, and for 5-nucleotidase in the rat. Non-specific phosphatase activity was seen in the Langerhans' islands of the rat and rabbit after incubation had been prolonged to 1 to 3 hours. In these preparations, zymogen granules were also stained in the acinar cells of the pancreas of the rat and guinea pig.

With the technique for non-specific alkaline phosphatase only blood capillaries and connective tissue surrounding larger arteries gave a positive staining reaction in all three species examined.

COMMENT

The secretory capillaries of the pancreas have been extensively discussed and convincingly documented by Zimmerman (4). They are found only between the acinar cells, but not within them.

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The lateral branches of the secretory capillaries are bordered by the surfaces of the secreting cells and thus have obviously a similar spatial relationship to the acinar cells as have the bile canaliculi to the liver cells; the latter have no walls of their own, but are, in effect, intercellular spaces bounded by the cell membranes of adjacent liver cells (5). Essner, Novikoff, and Masek (6) were able to apply histochemical techniques for adenosinetriphosphatase and 5-nucleotidase in electron microscopic studies of rat liver. Deposition of lead salts indicating the site of enzymatic activity occurred primarily in the cellular membranes of the liver cells where they fold to form the microvilli of the bile canaliculi.

The enzyme stains reveal a striking similarity between the liver and pancreas with regard to the canaliculi located between the secretory cells. It seems justified to assume that the concentration of substrate-specific phosphatases, particularly of adenosinetriphosphatase in plasmacellular membranes, might indicate a specific function in the mechanism which transports bile and pancreatic juice from the place of formation in the secretory cells into the excretory channels.

Hokin and Hokin (7–9) have indeed discovered a phosphate turnover in the pancreas during the discharge of zymogen granules. These investigators have concluded that the activation of the splitting and resynthesis of the glycerophosphate bond in the phospholipides is a primary stage in the process of active extrusion of enzymes from the acinar cell.

SUMMARY

By the use of histochemical techniques for the demonstration of adenosinetriphosphatase and in the case of the rat also of 5-nucleotidase, a consistent staining reaction of the secretory capillaries (*Sekretionskapillaren*) located within the acini of the pancreas of several species can be accomplished. The similarity of this staining reaction to that given by the bile canaliculi of the liver is striking and suggests a similar function for these enzymes in the transport mechanism between secretory cells and excretory channels in both organs.

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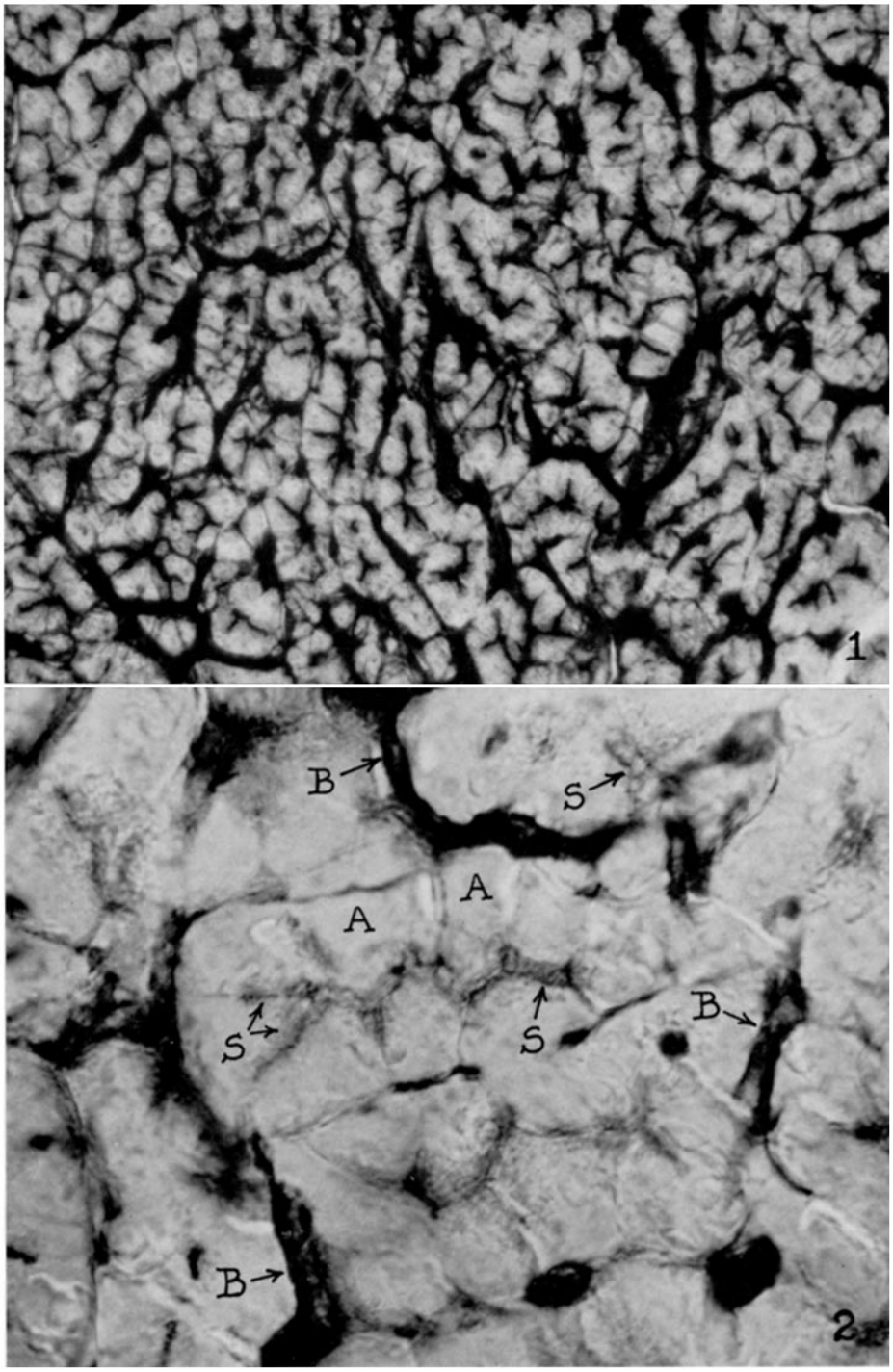
EXPLANATION OF PLATE 76

FIG. 1. Section from the pancreas of a rat stained with the technique for the demonstration of adenosinetriphosphatase. The sites of enzymatic activity appear black. Blood capillaries and secretory capillaries (*Sekretionskapillaren*) stain strongly. The latter appear in most instances as solidly stained, centroacinar located canaliculi from which numerous smaller side branches extend between acinar cells. Incubation time in the substrate 5 minutes. × 450.

FIG. 2. Section from the pancreas of a rat stained with the technique for the demonstration of adenosinetriphosphatase at a higher magnification. The secretory capillaries (*S*) have clearly recognizable lumens. The deposits of lead sulfide outline the walls of the secretory capillaries in this preparation. Acinar cells (*A*) reveal no activity while blood capillaries (*B*) show intense staining. Incubation time in the substrate 3 minutes. × 1150.



(Wachstein and Meisel: Secretory capillaries in pancreas)