CHARACTERIZATION OF DUCTS ISOLATED FROM THE PANCREAS OF THE RAT

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ABSTRACT

Rat pancreases were minced and treated with collagenase or collagenase supplemented with chymotrypsin to yield a mixture of ducts, islets, acinar cell clusters, blood vessels, and nerves. Histologically and ultrastructurally, the isolated tissues resembled their *in situ* counterparts in most respects, the major difference being the destruction of the basement membranes (basal laminae). Ducts ranging in size from the common bile/main pancreatic duct to the intercalated ducts were identified in the digest, although interlobular ducts were most frequently observed. Acinar tissue fragments were separated from nonacinar structures either by flotation through discontinuous gradients of Ficoll or by sieving, the latter technique being the more efficient. Common bile/main ducts, interlobular ducts, and blood vessels were selected manually from the nonacinar fractions. Biochemical analyses showed that the entire nonacinar fraction, as well as isolated ducts and blood vessels, contained larger alkaline phosphatase, carbonic anhydrase, and Mg-ATPase specific activities than acinar tissue, whereas acinar tissue contained larger γ-glutamyltranspeptidase and amylase activities. However, >63% of the total recovered activity of each enzyme was associated with the acinar tissue. Both the association of the majority of each of these enzyme activities with the acinar tissue and the similarity in specific activities associated with ducts and blood vessels indicate that none of the enzymes tested is a unique marker for interlobular and larger ducts of the pancreas of the rat.

Pancreatic ducts comprise a simple cuboidal to columnar epithelium (12, 16, 21) and a layer of connective tissue. The ducts serve as a conduit for the movement of exocrine enzymes from the acinar cells to the duodenum and they also produce at least part of the secretin-stimulated bicarbonate-rich pancreatic fluid secretion (9, 44). The role of the ducts in fluid/electrolyte secretion has been supported by ultrastructural (24) and micropuncture (29, 43) studies and by studies of the relatively unimpaired fluid/electrolyte secretion in rats whose acinar cells have been destroyed (14).

Carbonic anhydrase in the dog (4), (Na+K)-ATPase in the rabbit (40, 48), and HCO_3-ATPase in the dog and cat (45) have been implicated in pancreatic fluid secretion by experiments in which specific inhibitors (acetazolamide, ouabain, and thiocyanate, respectively) reduced fluid secretion by the intact pancreas. Histochemical studies have demonstrated the presence in the duct epithelium

J. CELL BIOLOGY © The Rockefeller University Press · 0021-9525/80/04/0122/14 $1.00
Volume 85 April 1980 122–135

of carbonic anhydrase in the rat (10) and mouse (6), HCO_3-ATPase in the dog (26), γ-glutamyltranspeptidase in the rat (18), and alkaline phosphatase in the rat and other species (19), although more recent studies with the rat[1] (7) show that alkaline phosphatase actually is localized in the connective tissue adjacent to the duct epithelium.

A better understanding of the biochemical and physiological properties of pancreatic ducts is dependent upon studies either on duct epithelial cells or on isolated ducts, because ducts compose only a small portion of the volume of the pancreas (5). The isolation and culture of bovine duct epithelial cells has been reported (47). The isolation from collagenase digests of duct cells from the rat (42) and ductule fragments from the rat (15) and mouse (20) has been reported in abstract form. Pancreatic ducts have been obtained by dissection from the rat (46), cat (52), and cow (27) and have been analyzed for their content of various enzymes. The ultrastructural characteristics of the isolated bovine ducts have been defined (22). However, in only one of these studies (52) were ducts smaller than the main pancreatic duct obtained. Another method for isolating pancreatic ducts is described in this paper. This procedure involves partial enzymatic digestion of the pancreas in order to free the ducts from surrounding tissues, flotation through a Ficoll gradient or sieving to separate duct fragments from acinar tissue, and manual removal of the ducts. This procedure is an improvement over dissection in that it permits isolation of the smaller pancreatic ducts.

MATERIALS AND METHODS

Isolation of Pancreatic Ducts from Collagenase Digests

COLLAGENASE DIGESTION: The procedure for the isolation of ducts was a modification of that used for obtaining pancreatic islets (28, 36). Pancreases from 125–350-g Sprague-Dawley rats were minced with sharp scissors for 2–4 min in a vial containing, for each gram wet weight of pancreas, 6.5 ml of Hanks' balanced salt solution (HBSS) and 3–6 mg/ml of collagenase (type IV, Worthington Biochemical Corp., Freehold, N.J., or in early experiments, type V, Sigma Chemical Co., St. Louis, Mo.). The HBSS was supplemented here and in all other manipulations with 0.02% bovine serum albumin (fraction V, Sigma Chemical Co.) and 0.01% soybean trypsin inhibitor (code SIC, Worthington Biochemical Corp.). If necessary, 0.2 mg/ml of α-chymotrypsin (code CDI, Worthington Biochemical Corp.) was added (2) (see Results). In some experiments, the mincing was done in 25% of the final volume of HBSS. The vial was capped, and the mixture was incubated in a 37°C water bath on a magnetic stirrer for 40 min. The digest was diluted with cold HBSS and centrifuged in 12-ml screw-cap conical glass centrifuge tubes for 30 s at 1,600 rpm in an HN-S centrifuge (Damon/IEC Div., Damon Corp., Needham Heights, Mass.) with a swinging bucket rotor. The supernates were discarded and the pellets washed at least three times in HBSS as described above; a Vortex mixer (Scientific Products Co., Div. American Hospital Supply Corp., McGraw Park, Ill.) was used to resuspend the pellets.

FRACTIONATION ON DISCONTINOUS GRADIENTS: Less dense structures were separated from acinar tissue by flotation through discontinuous gradients of Ficoll. The digest was resuspended with a glass rod in 5.2 ml of HBSS containing 30% (wt/vol) Ficoll (average molecular weight 400,000; Sigma Chemical Co. or Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) for each gram wet weight of pancreas. This suspension was transferred to centrifuge tubes and overlayered with equal volumes of 23.5, 20.8, and 12.2% Ficoll, and finally 1–2 ml of HBSS. In some experiments, the 20.8 and 12.2% layers were omitted. The tubes were centrifuged for 30 min at 10,000 rpm (15,900 *g* max) at 4°C in a JS13 swinging bucket rotor in a Beckman J21C centrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The tissue fragments at the interfaces and in the pellet were collected with a Pasteur pipette and diluted with HBSS, pelleted in the HN-S centrifuge, and resuspended in HBSS by Vortex mixing.

FRACTIONATION ON SIEVES: The fragments produced by digesting about 1 g of pancreas were resuspended in 6 ml of HBSS and were pipetted onto a pre-wetted 60-mesh stainless-steel sieve (pore size, 200 × 300 μm; Bellco Glass, Inc., Vineland, N. J.). After the fluid and small fragments had passed through the sieve, the residue was washed with 20 ml of HBSS, delivered gently from a syringe. The fragments remaining on the sieve were harvested by rinsing the inverted sieve with another 20 ml of HBSS.

Selection of Specific Fragment Types

Using a dissecting microscope and a drawn-out Pasteur pipette, and relying on characteristic gross morphology, we removed nonacinar structures from the appropriate fractions for morphological and/or biochemical studies. Acinar tissue (also containing islets, in the case of material passing through the sieve) was pelleted by centrifugation.

Biochemical Assays

Tissue samples were homogenized in cold water in either a hand-held ground-glass homogenizer or a motor-driven Teflon-glass Potter-Elvehjem homogenizer (A. H. Thomas Co., Philadelphia, Pa.). The ATPase assays were done immediately. If necessary, the samples were frozen at −20°C and the remaining assays were done the following day without significant loss of enzyme activity. We performed the following assays: protein, using bovine serum albumin as a standard (8, 31); alkaline (pH 10) *p*-nitrophenylphosphatase (17); HCO_3-ATPase, using as the measure of activity the difference between activity in the presence of 25 mM $NaHCO_3$ and activity in the presence of 10 mM sodium thiocyanate (45); Mg-ATPase, residual ATPase activity in the presence of 10 mM sodium thiocyanate (45); (Na+K)-ATPase (40); carbonic anhydrase (30, 32); α-amylase (3); and γ-glutamyltranspeptidase (37). The addition of 20 mM glycylglycine to the γ-glutamyltranspeptidase assay (49) increased the specific activities of all the tissues assayed fivefold. A unit of

[1] Mayer, H. K., D. R. G. Holmquist, and S. Githens. Manuscript in preparation.

enzyme activity was defined as the amount of enzyme producing 1 nm of product/min, except for carbonic anhydrase (one unit being the amount of enzyme that decreases the reaction time by one half in a reaction volume of 0.2 ml), and amylase (one unit producing 1 mg of maltose in 1 min). All assays produced product as a linear function of time except carbonic anhydrase, for which this parameter is not meaningful, and the amount of product formed was proportional to the amount of homogenate added to the assay. All assays were scaled down to final volumes of 0.3–0.5 ml for absorbance measurements in a model 24 spectrophotometer (Beckman Instruments, Inc.).

Histology

Tissue samples from fresh pancreas and tissue fragments after enzyme digestion and fractionation were routinely fixed in Carnoy's solution and transferred to 70% ethanol. The tissue fragments were collected after fixation by low-speed centrifugation, and the excess ethanol was removed. The centrifuge tubes were placed in a 40°C bath for a few seconds, and a small volume of 1% agarose at 40°C was added to each tube with gentle mixing. The tubes were then chilled to solidify the agarose. The agarose plug containing the tissue was processed by routine methods for paraffin sectioning. Staining with neutral red in absolute ethanol before paraffin embedding rendered the tissue fragments visible for further processing. The paraffin blocks were sectioned at 5–7 μm, and sections were stained with hematoxylin-eosin-phloxin.

Electron Microscopy

For electron microscopy, tissue was fixed 2–3 h or overnight in a mixture of cold 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2) (23), followed by a rinse for 10–12 h with three to four changes of cold 0.1 M cacodylate buffer (pH 7.2) containing 0.2 M sucrose. The tissue was then placed in cold 1% osmium tetroxide buffered to pH 7.2 with 0.1 M cacodylate for 1.5–2 h. The pieces of tissue were stained in block overnight in aqueous 0.5% uranyl acetate and then dehydrated in ethanol. All material was flat embedded in a mixture of Epon-Araldite (35). Thin sections were cut on a Reichert OM-U2 ultramicrotome (C. Reichert Optische Werke A. G., Vienna, Austria), stained with lead citrate (38), and viewed in a Philips EM 300 electron microscope.

RESULTS

Digestion of Pancreas and Isolation of Ducts: Important Variables

The success of the two methods for isolating ducts was dependent primarily on the effectiveness of the digestion procedure. Digestion was more effective with younger animals. Sharp scissors and a small volume of fluid during the mincing procedure aided digestion by producing fragments of a smaller size. The addition of α-chymotrypsin improved digestion, although some batches of collagenase did not require this addition. Extensive washing tended to disrupt the tissue further. However, it was important to avoid too extensive digestion and mechanical disruption if a good yield of ducts was to be obtained.

Fractionation of the Digested Pancreas in a Discontinuous Gradient of Ficoll

Flotation of the pancreatic digest through a discontinuous density gradient of Ficoll resulted in a good separation of acinar tissue from such structures as ducts, vessels, nerves, and islets as revealed by examination with a dissecting microscope and confirmed histologically. The acinar tissue either sedimented to the bottom of the tube (P) or floated to the lowest interface (I-1) between the two densest layers of Ficoll. Nonacinar structures were essentially absent in the pellet but were present at interfaces I-1, I-2, I-3, and I-4, with the greatest concentrations at I-2 and I-3. The islets were found primarily at I-3, as reported by others (28). There were no obvious differences in the distributions of ducts, vessels, and nerves among interfaces I-1–I-4.

Fractionation of the Digested Pancreas by Filtration Through Sieves

Passage of the pancreatic digest through a 60-mesh stainless-steel sieve resulted in an excellent and rapid separation of elongate structures from acinar fragments and islets, because the latter tissue types passed readily through the sieve. Fragments of ducts and blood vessels obtained by sieving or Ficoll fractionation measured 860 ± 380 μm (mean ± SD, 52 fragments) in length.

Types of Tissues Identified in the Fractionated Pancreatic Digests

Examination of the nonacinar tissue fragments with a dissecting microscope revealed characteristic structural features that allowed the recognition and selective removal of specific structures for histological, ultrastructural, and biochemical study.

COMMON BILE DUCT-INTRAPANCREATIC SEGMENT: The common bile duct fragments were recognizable as large, yellowish structures with large lumina and thick walls containing numerous translucent spheres (Fig. 1). Occasionally, smaller ducts were attached at right angles to the large duct. Histological examination of these duct fragments revealed a folded, simple columnar epithelium that was frequently disrupted (Fig. 2, isolated; Fig. 3, *in situ*). The translucent spheres were shown to be intact tubular evaginations of the lining epithelium into the surrounding connective tissue, as previously described (34, 53). Occa-

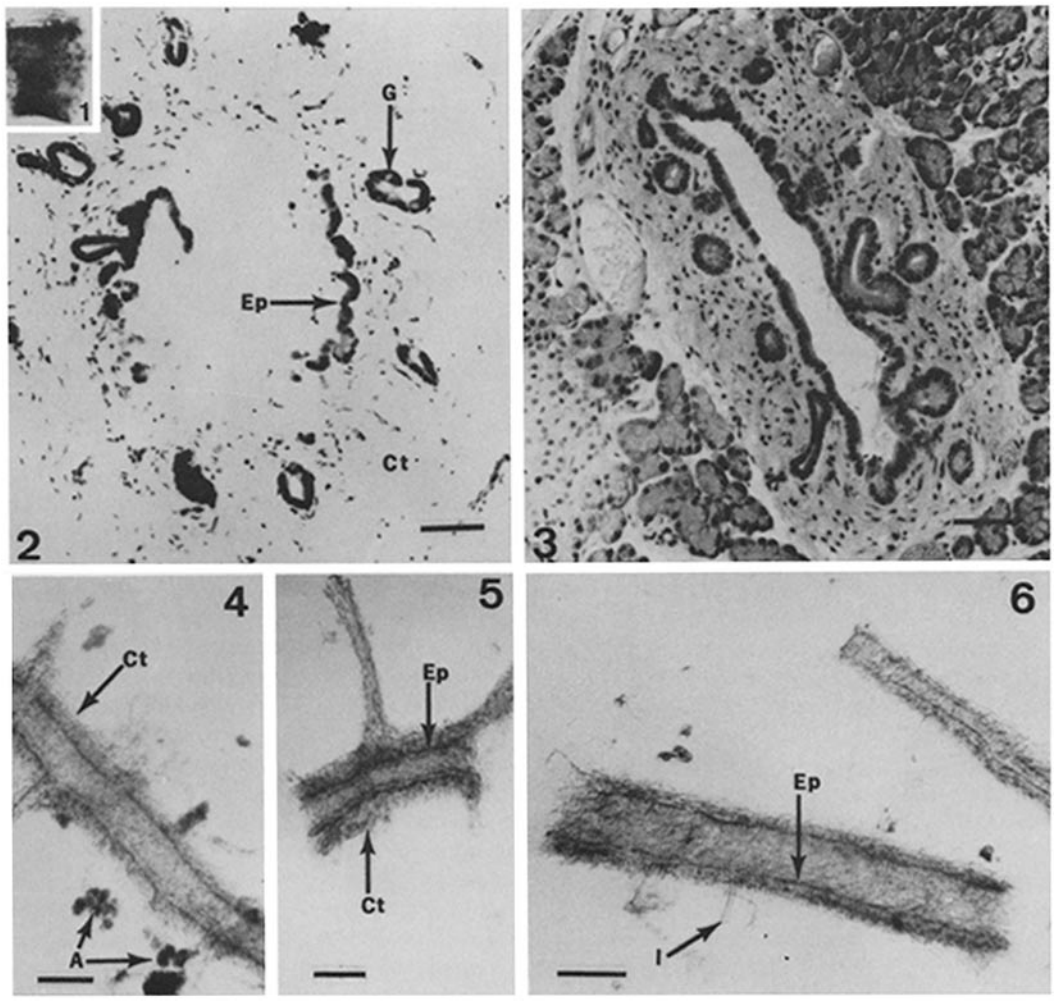

FIGURE 1 Dissecting microscope photograph of a common bile/main pancreatic duct. × 10.

FIGURE 2 Light micrograph of an isolated common bile/main pancreatic duct fragment. Note the disrupted lining epithelium (*Ep*), thinned connective tissue (*Ct*), and the intact epithelial evaginations containing goblet cells (*G*). Hematoxylin and eosin (H & E). × 165. Bar, 50 μm.

FIGURE 3 Light micrograph of an intrapancreatic region of the common bile duct *in situ*. Compare with Fig. 2. H & E. × 165. Bar, 50 μm.

FIGURES 4, 5, and 6 Dissecting microscope photographs of interlobular ducts. Note the epithelium (*Ep*) and connective tissue halo (*Ct*) of the larger ducts and the absence of an apparent lumen in the smaller ducts. Intercalated ducts (*I*) can be seen in Fig. 6, and acinar tissue (*A*) in Fig. 4. × 35, × 35, × 45. Bars, 200 μm.

sional goblet cells were noted within the epithelium of the evaginations. Some of these evaginations led directly to associated acini and were similar in size and structure to intralobular ducts (Fig. 15). Such small branches have been described previously (39). The distinguishing histological characteristics of the various pancreatic duct categories are summarized in Table I.

MAIN PANCREATIC DUCT: The main pancreatic ducts were lined by simple columnar epithelium with evaginations that were much less numerous than in the common bile duct and that

TABLE I

Pancreatic Duct Categories

Duct type	Epithelial cells in circumference	Type of simple epithelium	Amount of connective tissue
Intrapancreatic common bile	>100	Folded columnar with blind evaginations	Large
Main	75–100	Columnar	Moderate to large
Interlobular	15–60	Cuboidal to low columnar	Moderate
Small interlobular and intralobular	5–10	Cuboidal	Small
Intercalated (unbranched intralobular terminating in centroacinar cell)	2–3	Thick squamous	Sparse

more frequently led into small inter- or intralobular ducts. The main pancreatic ducts also gradually decreased in diameter and ultimately branched into large interlobular ducts.

INTERLOBULAR DUCTS: Interlobular duct fragments were distinguishable by their obvious lumina whose epithelial boundaries formed a cylinder, around which the adhering connective tissue appeared as a loosely organized halo (Figs. 4–6). Smaller ducts were often seen to branch from larger ducts. The identity of these isolated ducts was confirmed by histological examination, which revealed a simple cuboidal to low columnar lining epithelium ranging from 15 to 60 cells per cross section (Figs. 7, 8, and 15, isolated; 9 and 10, *in situ*). Small interlobular (Fig. 7) and intercalated ducts (Fig. 15) were occasionally observed to branch from this duct type. Interlobular duct cells exhibited a relatively large nuclear to cytoplasmic ratio and a densely granular nucleus surrounded by a fairly homogeneous eosinophilic cytoplasm. Occasional goblet cells were noted. The existence of loose connective tissue surrounding many of these ducts was confirmed histologically (Figs. 7 and 8); however, some ducts consisted only of an intact lining epithelium. Occasional clusters of acinar cells were seen (Figs. 4 and 15).

Small interlobular ducts were not easily identified with the dissecting microscope, except when they remained attached to larger ducts (Figs. 4 and 5). These ducts, in which a lumen was not obvious, were best characterized by their straightness and smooth outer margin. Histological examination (Fig. 7, isolated; Fig. 10, *in situ*) demonstrated that such ducts were lined by a low cuboidal epithelium. A cross section consisted of 5–10 cells with a relatively large nuclear to cytoplasmic ratio and a faintly stained homogeneous cytoplasm. These small isolated ducts were usually free of surrounding connective tissue, which accounts for their smooth outer margin under the dissecting microscope. *In situ*, ducts of this size are seen to extend into the lobule and are then termed "intralobular."

Ultrastructural examination of interlobular ducts revealed that many survived the isolation virtually intact (Figs. 11 and 12), although an occasional cell was noted as being displaced from the epithelium (Fig. 12). The nuclei of the epithelial cells were usually irregular in shape and displayed clumping of the chromatin in the region of the nuclear membrane. In most cases, extensive blebbing was noted on both the apical and basal surfaces of the cells (Fig. 13). Many of the mitochondria were slightly swollen (Fig. 14). Successful culture of these ducts (41), however, demonstrates that the blebbing and swelling of mitochondria are reversible and present no danger to the viability of the cells. In general, the cells were in close contact and revealed intact junctional complexes (Fig. 14), and many desmosomes were observed along the lateral borders of the cells (Figs. 13 and 14). Numerous microvilli (Fig. 13) and an occasional cilium (Fig. 14) were observed on the apical surfaces of these cells. None of the ducts examined possessed a basal lamina.

INTERCALATED DUCTS AND ACINI: Intercalated ducts were occasionally seen to remain attached to larger ducts (Fig. 6). This kind of arrangement has been observed histologically (Fig. 15). Intercalated ducts are also associated with isolated acinar cell clusters. These very small ducts, as illustrated by an *in situ* section (Fig. 16), consisted of one layer of thick squamous epithelial cells with large, pale, ovoid nuclei and pale, ho-

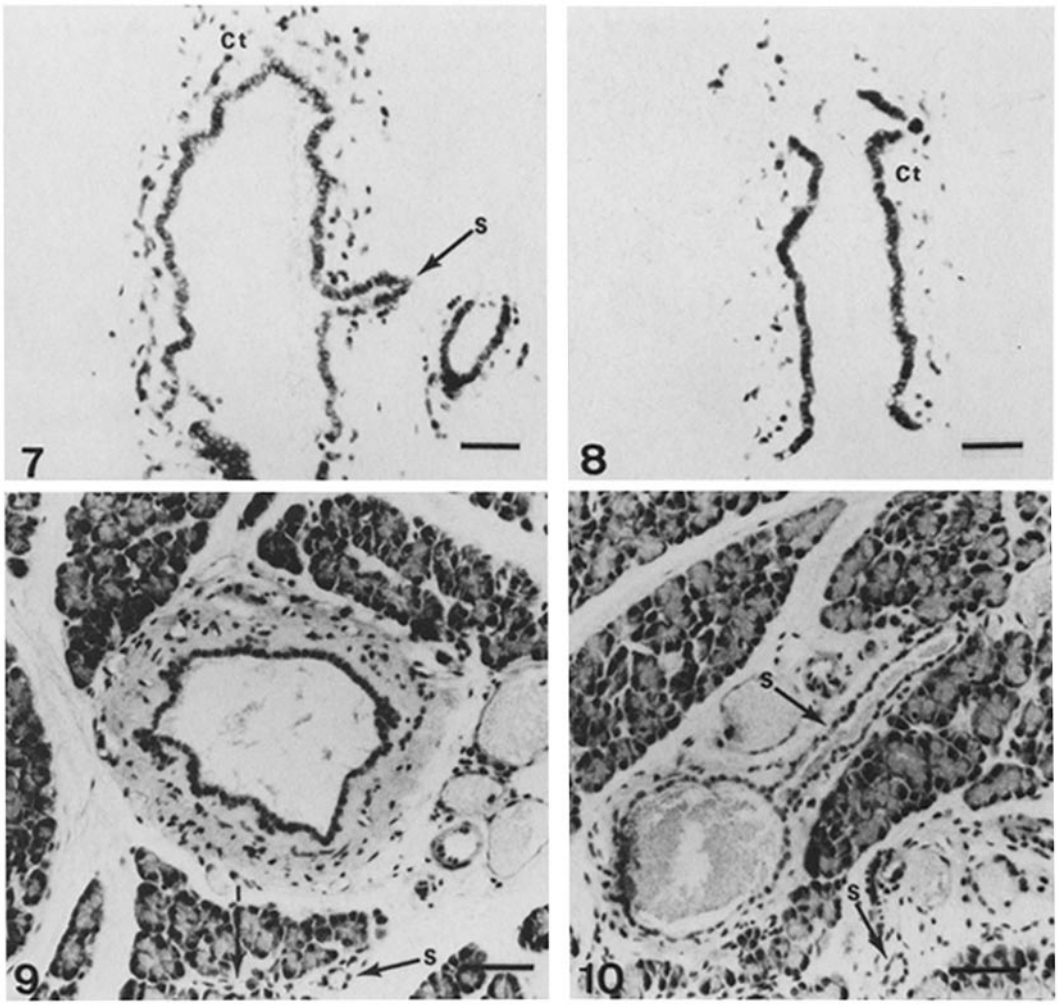

FIGURE 7 Fragment of a large, interlobular duct with a wide lumen. Remnants of the connective tissue (*Ct*) wall remain. Note a small interlobular duct (*S*) branching from the large duct and a second small duct in the lower right corner. H & E. × 260. Bar, 30 μm.

FIGURE 8 Fragment of an interlobular duct seen in longitudinal section. H & E. × 260. Bar, 30 μm.

FIGURE 9 Cross section of a large interlobular duct *in situ*. Compare with Fig. 7. Note small (*S*) and intercalated (*I*) ducts. H & E. × 185. Bar, 50 μm.

FIGURE 10 Smaller interlobular duct *in situ*. Compare with Fig. 8. Note small interlobular ducts (*S*). Compare with Fig. 7. H & E. × 185. Bar, 50 μm.

mogeneous cytoplasm. The intercalated duct is the last unbranched segment leading directly into the acinus and terminating in a centroacinar cell.

Acini were easily distinguished with the dissecting microscope from islets because of the latter's reddish-brown tint. There was considerable variation in the number of acinar cells in a given cluster, from fragments of lobules to clusters of a few acinar cells. Histological examination verified the presence of acinar and centroacinar cells usually free of, but sometimes (Fig. 15) still associated with, ducts. The ultrastructure of these cell clusters was similar to that described in previous reports (11), except that the basal lamina was missing.

OTHER STRUCTURES: Small veins were distinguished by the irregularity of their walls (Fig.

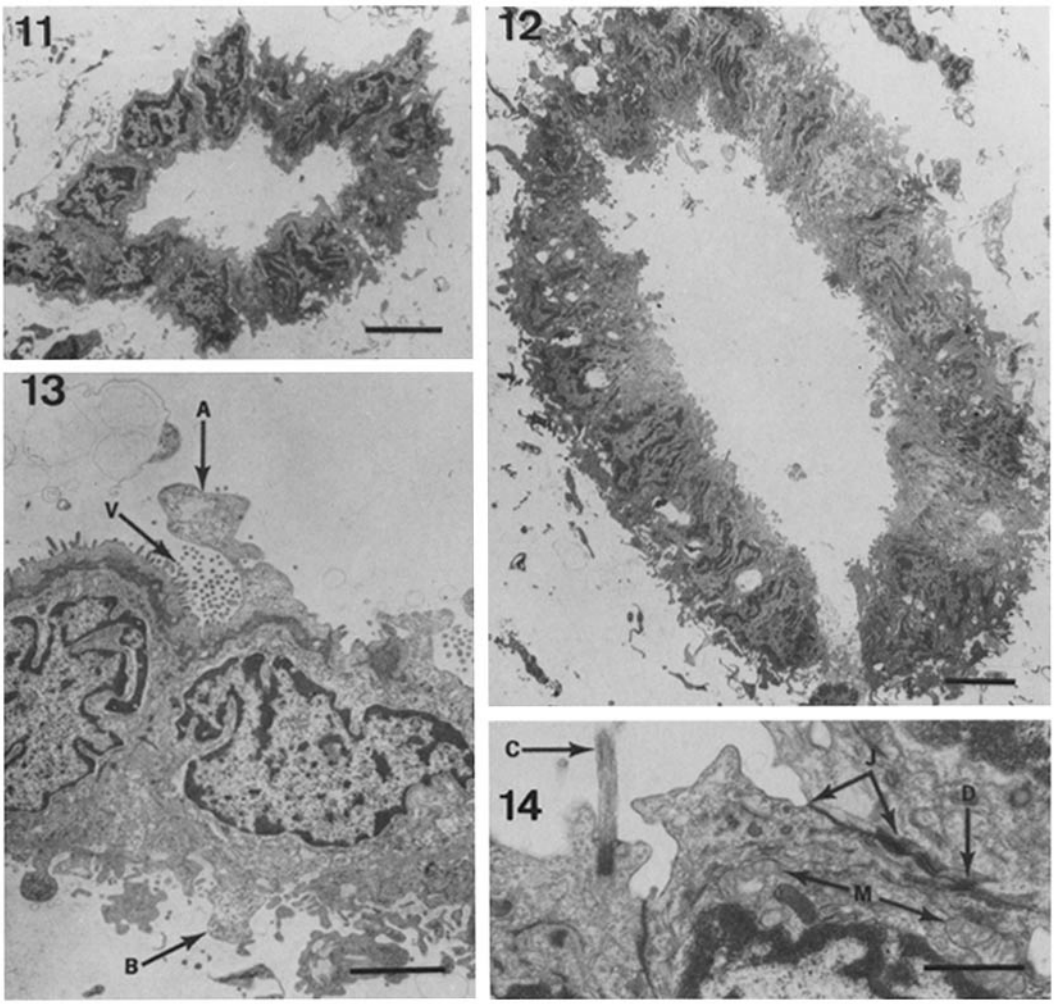

FIGURE 11 Electron micrograph of an isolated, small interlobular duct. × 2,500. Bar, 4 μm.

FIGURE 12 Electron micrograph of an isolated, large interlobular duct. At the bottom of the picture, note that a cell has been displaced from the duct. × 2,350. Bar, 4 μm.

FIGURE 13 Higher magnification of cells from an isolated, large interlobular duct. These cells exhibit apical (*A*) and basal (*B*) blebbing and numerous microvilli (*V*). Note the absence of a basal lamina. × 6,200. Bar, 2 μm.

FIGURE 14 Apical surface of duct cells of an isolated, large interlobular duct. These cells are held in close contact by junctional complexes (*J*) and numerous desmosomes (*D*). The mitochondria (*M*) are slightly swollen. Cilium (*C*). × 13,700. Bar, 1 μm.

17). Histologically, their venous character could be verified. Small arteries were distinguishable under the dissecting microscope by a very distinct outer margin and a dark central core of variable diameter, without an obvious lumen unless it was distended by erythrocytes (Fig. 18). Histologically, the dark central core proved to be the tunica intima, which had become detached from the smooth muscle. The basement membrane of the endothelium and the intercellular connective tissue of the tunica media of muscular vessels had been destroyed, presumably by the collagenase and protease. As a result, these vessels exhibited a very disorganized appearance in both their muscular and endothelial components. In contrast, the connective tissue of the tunica adventitia remained

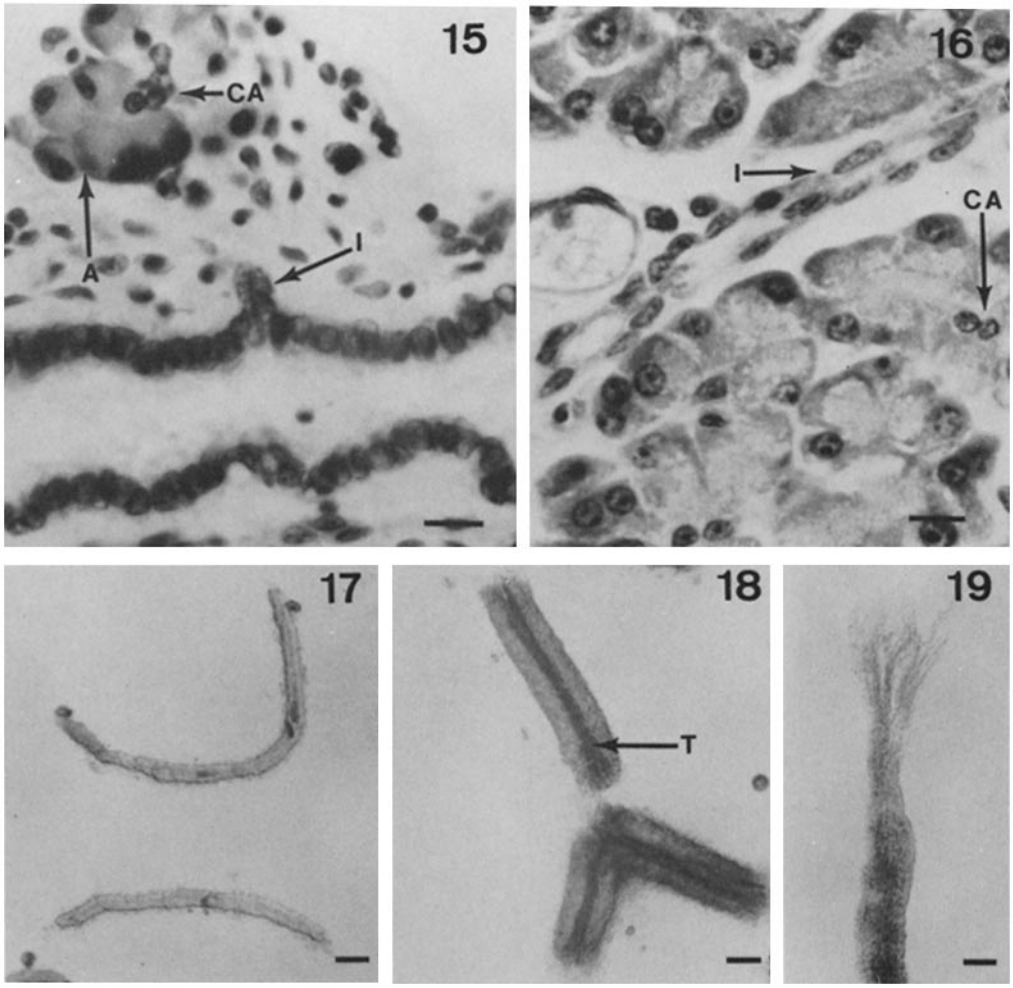

FIGURE 15 Isolated, small interlobular duct with an intercalated duct (*I*) branching from its wall. The intercalated duct could be followed in serial sections to its termination with the centroacinar cells (*CA*) in the acinus (*A*) above. H & E. × 750. Bar, 10 μm.

FIGURE 16 Intralobular (intercalated) duct (*I*) *in situ*. *CA*, centroacinar cells. Compare with Fig. 15. H & E. × 750. Bar, 10 μm.

FIGURE 17 Dissecting microscope photograph of small veins. × 45. Bar, 100 μm.

FIGURE 18 Dissecting microscope photograph of small arteries. Note the disrupted tunica intima (*T*). × 45. Bar, 100 μm.

FIGURE 19 Dissecting microscope photograph of nerve fragment. × 45. Bar, 100 μm.

relatively intact. Nerve fragments were identified by their frayed-rope-like appearance (Fig. 19).

Distribution of Enzyme Activities among Ficoll Fractions

In several experiments the Ficoll fractions were assayed for protein, amylase, and a group of enzymes suggested by functional and histochemical studies to be present in ducts. In keeping with the morphological results, no significant differences in specific activities were noted between P and I-1, or among I-2, I-3, and I-4. Therefore these fractions were grouped accordingly. As shown in Table II, larger specific activities of the putative duct-marker enzymes were obtained at I-2 + I-3 + I-4,

TABLE II

Biochemical Analysis of Ficoll Fractions

Enzyme	Specific activity*			Estimated yield§	Recovered activity in I-2 + I-3 + I-4
	Whole pancreas	P + I-1‡	I-2 + I-3 + I-4‡		
					%
Alkaline phosphatase	4.7 ± 2.6 (16)‖	14.3 ± 2.7	76.0 ± 9.9	62 ± 10	16.6 ± 6.3
Carbonic anhydrase	9.3 ± 5.7 (16)	1.2 ± 0.3	2.8 ± 0.4	2.4 ± 0.3	7.8 ± 4.3
Mg-ATPase	56.5 ± 18.8 (8)	85.3 ± 45.2	361 ± 250	49 ± 16	12.4 ± 9.4
HCO_3-ATPase	55.5 ± 21.5 (9)	31.5 ± 10.3	57.9 ± 16.4	11 ± 4	6.1 ± 2.6
Amylase	33.1 ± 7.6 (16)	27.6 ± 5.0	8.4 ± 1.4	14 ± 2	1.2 ± 0.6
Protein	—	—	—	—	3.5 ± 1.1

* Units per milligram of protein.

‡ P, pellet from Ficoll gradient; I-1, lowest interface in Ficoll gradient, etc.

§ Total recovered activity in Ficoll fractions divided by (pancreas wet weight × 180 mg protein/g wet weight × whole pancreas specific activity), or total recovered protein divided by (pancreas wet weight × 180 mg protein/g wet weight of whole pancreas).

‖ Mean ± SD. Number of experiments in parentheses. The fractionation data are based on four experiments for alkaline phosphatase, amylase, and protein, and on three experiments for the other enzymes.

as compared with P + I-1, which contained a larger amylase activity. The yield of each enzyme and of protein was estimated as indicated in Table II. The yield of alkaline phosphatase and Mg-ATPase was significantly greater than that of the other enzymes and total protein. Despite the higher specific activities of the duct marker enzymes in I-2 + I-3 + I-4, only 6–17% of the total recovered activities of these enzymes were found in those fractions, along with 3 and 1% of the total protein and amylase recovered, respectively.

Distribution of Enzyme Activities between Sieved Fractions

Sieving of the pancreatic digest produced a filtrate equivalent to I-2 + I-3 + I-4, except that the islets passed through the sieve and were found in the filtrand. The biochemical characteristics of these fractions are given in Table III.

The sieved fractions closely resembled the corresponding Ficoll fractions. Several significant differences were noted, including (*a*) a greater estimated yield of alkaline phosphatase and protein in the sieved fractions and (*b*) lower specific activities of alkaline phosphatase, carbonic anhydrase, and amylase in the sieved filtrates, as opposed to P + I-1. In addition, the percentage of the recovered activity in the nonacinar structures in the filtrand or I-2 + I-3 + I-4 was greater after sieving in the case of alkaline phosphatase, carbonic anhydrase, Mg-ATPase, and protein. γ-Glutamyltranspeptidase and DNA were also assayed in the sieved fractions. γ-Glutamyltranspeptidase specific activity was somewhat larger in the filtrate than in the filtrand, and most (97%) of the activity was recovered in that fraction. The DNA to protein ratio was significantly larger in the filtrand and 21% of the recovered DNA was found in that fraction.

All of the washes produced during the sieving procedure were pooled and assayed. This allowed the computation of overall recoveries, as indicated in Table III. These ranged from a low of 12% for carbonic anhydrase to 190% for alkaline phosphatase. Comparison with the estimated yields in filtrate plus filtrand revealed that considerable amounts of material were lost in the washes. The 190% recovery of alkaline phosphatase is attributable either to activation during the isolation procedure or to latency in whole-pancreas homogenates, although the detergent Triton X-100 was a component of the assay mix. The collagenase enzyme mixture contained no alkaline phosphatase.

Enzyme Activities of Isolated Tissues

Analysis of the isolated tissues (Table IV) showed that the common bile/main pancreatic ducts and the interlobular ducts contained very similar specific activities of each enzyme measured, except for carbonic anhydrase. Carbonic anhydrase could not be detected in the interlobular ducts because of the small number that could be isolated and the insensitivity of the assay. The consistently smaller specific activities of all of the enzymes in the larger ducts could be attributed to

TABLE III

Biochemical Analysis of Sieved Fractions

Enzyme	Specific activity*		Estimated‡ yield	Recovered activity in filtrand§	Estimated overall recovery‖
	Filtrate	Filtrand		%	
Alkaline phosphatase	7.9 ± 1.3¶	88.2 ± 33.5	95 ± 4	37.3 ± 11.4	190 ± 7
Carbonic anhydrase	0.5 ± 0.1	2.3 ± 0.3	2.6 ± 0.6	19.7 ± 7.2	12 ± 3
Mg-ATPase	81.2 ± 12.4	436 ± 68	58 ± 7	24 ± 1.4	86 ± 1
Amylase	17.8 ± 9.8	6.9 ± 5.5	21 ± 17	1.8 ± 0.4	39 ± 33
γ-Glutamyltranspeptidase**	331 ± 20	182 ± 88	51 ± 23	2.8 ± 0.9	144 ± 53
DNA	27.3 ± 0.3‡‡	135 ± 40	—	21.3 ± 6.7	—
Protein	—	—	38 ± 9	5.2 ± 0.8	66 ± 16

* Units per milligram of protein.

‡ See footnote § in Table II.

§ Total filtrand activity divided by (filtrate plus filtrand activity).

‖ Calculated in same manner as "estimated yield," except that protein, DNA, or activity recovered in the washes was added to the filtrate + filtrand values.

¶ Mean ± SD. Number of experiments in parentheses. Based on three experiments except for Mg-ATPase, which was based on two.

** The specific activity of this enzyme in whole pancreas homogenates is 240 ± 41 (10 determinations) when assayed in the presence of glycylglycine.

‡‡ Milligram of DNA per gram of protein.

TABLE IV

Enzyme-Specific Activities in Isolated Tissues‡*

	Common bile and main pancreatic ducts	Interlobular ducts	Acinar tissue	Small arteries	Small veins
Alkaline phosphatase	141 ± 56 (4)§	171 ± 51 (6)	10.5 ± 8.7 (5)	183 ± 34 (4)	66.3 ± 14.0 (4)
Carbonic anhydrase	20.2 ± 9.1 (3)	ND‖ (4)	2.0 ± 0.8 (4)	—	—
Mg-ATPase	612 ± 249 (5)	834 ± 316 (7)	117 ± 16 (4)	893 ± 354 (5)	696 ± 255 (4)
HCO_3-ATPase	144 ± 78 (4)	177 ± 67 (7)	74.8 ± 30.8 (3)	169 ± 91 (4)	122 ± 97 (4)
Amylase	6.3 ± 5.3 (5)	7.7 ± 8.9 (5)	23.1 ± 7.9 (5)	1.0 ± 0.6 (6)	2.5 ± 2.6 (4)
γ-Glutamyltranspeptidase¶	20.7 ± 9.2 (4)	30.2 ± 6.8 (6)	106 ± 19 (5)	8.5 ± 4.6 (4)	16.4 ± 10.5 (3)

* Units per milligram of protein.

‡ Isolated either from Ficoll gradients or sieved material except for acini, which were isolated by sieving.

§ Mean ± SD. Number of experiments in parentheses.

‖ Not detectable.

¶ Assayed in the absence of glycylglycine.

the greater amount of connective tissue associated with the larger ducts (cf. Fig. 2 with Figs. 7 and 8).

The ducts, when compared with the acinar tissue, contained larger specific activities of alkaline phosphatase, carbonic anhydrase, Mg-ATPase, and HCO_3-ATPase, whereas the acinar tissue contained larger amounts of amylase and γ-glutamyltranspeptidase. The small vessels resembled the ducts in their content of alkaline phosphatase, Mg-ATPase, and HCO_3-ATPase, but had less amylase and γ-glutamyltranspeptidase.

(Na+K)-ATPase was extremely difficult to measure with precision because it represented <10% of the total ATPase activity in whole-pancreas homogenates, although the average specific activity detected (6.2 U/mg protein) agreed well with the findings of Ridderstap and Bonting (40). Although detectable in the Ficoll fractions, this activity was not detectable in the specific nonaci-

nar structures that were assayed, suggesting that it was present in structures other than ducts and vessels.

The protein content of the ducts isolated by either method usually amounted to ≤0.1% of the protein content of the intact pancreas, although Bolender (5) states that duct cells make up 3.9% of the total pancreatic volume of the guinea pig. This low yield was probably in part a result of destruction during the isolation procedure, and also of the difficulty of discerning the smaller ducts with the dissecting microscope. These smaller ducts have manifested themselves in significant numbers during culture, however, by forming obvious cyst-like structures.[2]

DISCUSSION

The procedure for isolating rat pancreatic islets was found to be suitable for isolating pancreatic ducts, as predicted by the reported presence of small ducts and vessels at the same Ficoll interface where islets were found (28, 36). Small ducts were also reported to survive the extensive digestion required for the isolation of single acinar cells (2). Variation among different batches of collagenase gave the same difficulties experienced by others (1). The best results were obtained with collagenase with relatively high protease activity (as assayed by Worthington Biochemical Corp.). The addition of α-chymotrypsin allowed the use of collagenase with lower protease activity.

The small percentage of the total recovered protein found in Ficoll fractions I-2 + I-3 + I-4 (3.5%) and the sieved filtrand (5.2%), where the nonacinar structures congregated, was expected, because Bolender (5) had previously shown by morphometric analysis that only ~10% of the total volume of the guinea pig pancreas is taken up by nerve, islets, ducts, and blood vessels. Because acinar cells comprise 82% of the total pancreatic volume (5) and contain more protein per unit volume than the remaining pancreatic tissue (they remain in the densest part of the Ficoll gradient), <10% of the total protein would be expected in the nonacinar fractions.

The relatively higher specific activities of possible duct marker enzymes in I-2 + I-3 + I-4 and in the filtrand were consistent with the presence of recognizable ducts almost exclusively in those fractions, although blood vessels probably contributed equally. Indeed, isolated ducts and vessels exhibited significantly higher specific activities than in the crude nonacinar fractions, showing that the other structures present in those fractions contained smaller amounts of the activities.

Nevertheless, the majority of the total activities recovered were found in the acinar fractions (P + I-1 and sieved filtrate) where very few ducts and vessels were observed. Histological examination of the P and I-1 fractions revealed a predominance of acinar cells but also a small number of centroacinar and intercalated duct cells still associated with the relatively dense acinar cells. Either or both of these cell types may contain these enzymes, although previous studies (see the beginning of this article) would support a ductal localization.

Analyses of DNA, protein, and enzyme activities of the filtrate, filtrand, and pooled washes generated by the sieving procedure revealed the extensive destruction involved in the isolation procedure. Significant amounts of the recovered materials were found in the washes, which contained no intact cells. Extensive loss of carbonic anhydrase activity was noted in particular.

The principal disadvantage of both the modified islet-isolation technique and sieving of the collagenase digest was that both procedures produced a collection of ducts still contaminated with other tissues. Because the use of stainless-steel sieves proved to be more rapid and as effective as the Ficoll technique for removing the acinar tissue and islets from the ducts and other nonacinar structures, it has become the standard procedure. The sieve technique had the added advantage of improving the morphological appearance of the ducts and the yield of alkaline phosphatase, amylase, and protein in the recovered tissue fragments.

The collection of nonacinar structures isolated either way was suitable for the manual removal of ducts and blood vessels. The histological and ultrastructural appearances of these isolated structures showed that the isolation procedure had caused various amounts of damage such as the disruption of the basement membrane (basal lamina) and the apical and basal blebbing of the duct epithelial cells. Successful culture of the ducts has shown that these changes are reversible.[2] The enzyme treatment appeared to have been most effective in digesting the fibers of the delicate connective tissue associated with the basement membrane (basal lamina) of duct epithelium, endothelium, sarcolemma, and the intralobular stroma. The

[2] Githens, S., D. R. G. Holmquist, J. F. Whelan, and J. R. Ruby. Manuscript submitted for publication.

bulk of the interlobular loose connective tissue was freed by digestion and removed mechanically by the isolation procedure. The dense connective tissue of the main and interlobular duct walls was thinned by the digestion but remained intact.

The significant activities of amylase in the ducts and vessels can be attributed to contamination with small numbers of acinar cells or free amylase secreted during the isolation procedure. γ-Glutamyltranspeptidase, a membrane-bound enzyme, has been demonstrated histochemically primarily in acinar cells and to a lesser extent in duct cells in the rat (18), in agreement with our biochemical localization.

Because the acinar tissue isolated by filtration contained islets, which represent ~2% of pancreatic volume (5), it is possible that some of the enzyme activities associated with the acinar tissue were from islet cells. However, because similar specific activities were found in the Ficoll fractions P + I-1, which were essentially devoid of islets, the islet contribution could not have been very large.

Previous studies of the enzymatic profiles of pancreatic ducts and acinar tissue have revealed larger specific activities of the following enzymes in the ducts: alkaline phosphatase (52), carbonic anhydrase (27), Mg-ATPase (52), HCO_3-ATPase (27, 52), and 5′-nucleotidase (27, 52). Our results are in agreement with respect to the first four enzymes. However, as shown by Lakshmanan et al. (27) for bovine tissue, the lower specific activities of these enzymes in acinar tissue can be attributed in great part to the larger protein content of the acinar cells.

The protein to DNA ratios of rat acinar cells (25) and the sieved filtrate (primarily acinar cells) are 31 and 37, whereas the ratios for isolated major rat pancreatic ducts (46) and the sieved filtrand (ducts and other nonacinar structures) are 8.3 and 7.4. Thus, if the specific activity of a given enzyme were the same on a DNA basis, the acinar cell would have a fourfold lower specific activity of that enzyme when calculated on a protein basis. Specific activities of isolated ducts and vessels were not reported on a DNA basis in this study because the DNA assays would have consumed a prohibitively large amount of material. The difference in the DNA to protein ratio accounts for most of the differences in enzyme-specific activities in the filtrate as opposed to the filtrand (Table III). If the isolated pancreatic ducts (Table IV) have the same DNA to protein ratio as the filtrand, then only carbonic anhydrase and alkaline phosphatase can be regarded as preferentially localized in ducts as opposed to acinar tissue.

However, Lakshmanan et al. (27) eliminated alkaline phosphatase as a marker because they assayed predominantly epithelial cells obtained from scrapings of the luminal surface of the bovine duct. Our histochemical studies of the rat pancreas have confirmed the results of others (7) that this enzyme is in fact localized in the connective tissue of the ducts. None of the enzymes examined in the present study, with the possible exception of carbonic anhydrase, appears to be useful in differentiating ducts from small blood vessels, which closely resemble the ducts in size and density.

Recent studies of HCO_3-ATPase in several rodent organs, not including the pancreas, have indicated that this enzyme is localized in the mitochondria, not the plasma membrane (50, 51). Therefore it appears that HCO_3-ATPase is not involved in electrolyte secretion in the tissues studied. The presence of this enzyme in comparable levels in ductal, acinar, and vascular tissues in the present study is consistent with that conclusion.

Our results and those of others (27, 52) eliminate all of the enzymes studied as unique markers for ducts as opposed to other pancreatic components. An enzyme unique to the duct epithelium should have a specific activity in isolated ducts ~50 × that in pancreatic homogenates, if one assumes that the duct epithelium contains 1% of the total pancreatic protein and that half of the isolated duct protein is found in the associated connective tissue. None of the enzymes examined even approaches that figure. Carbonic anhydrase remains promising as a marker if a more sensitive assay can be developed. Other possible biochemical markers are secretin-stimulated adenylate cyclase (13) and specific lectin binding (33).

The methodology for isolating pancreatic ducts that is described in this paper is superior to dissection in that it permits the isolation of interlobular ducts in addition to common bile/main pancreatic ducts. The enzymatic treatment also has the potential for removing much of the associated connective tissue. The technique is apparently adaptable to the isolation of pancreatic "ductuli" (20) that we interpret to be small interlobular/intralobular ducts. We occasionally encounter such ducts as isolated entities in our collagenase digests, but they are more readily identified after a few weeks in culture when they form small cystlike structures.[2] The purification of significant quantities of small interlobular/intralobular and intercalated ducts is

a difficult problem, however, because they typically remain attached to larger ducts and/or acini, and their small size precludes manual selection even when unattached. The purification of these small ducts depends both on modification of the digestion protocol to free more of the ducts from other tissues, and on the development of separation techniques that exploit physical and biochemical properties of the ducts to separate them from other tissue fragments.

We thank Beth Underwood, Patti Breeden, Nan Tartt, and Joy Taylor for excellent technical assistance.

This study was supported by National Cancer Institute grant CA 19177 through the National Pancreatic Cancer Project and by BRSG grant RR 07196-01 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.

Received for publication 11 October 1979.

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