INTRODUCTION

Pancreatic ducts comprise a very small percentage of the volume of the pancreas, 3.9% in the case of the guinea pig (1), although they perform the important function of adding a bicarbonate-rich fluid to the exocrine pancreatic secretion (2). Our understanding of pancreatic duct function, and its biochemical basis, has been limited until recently by the absence of methods for the isolation and culture of pancreatic ducts.

Pancreatic ducts of the rat (3) and the cat (4) have been isolated for biochemical studies, but efforts to culture those ducts have not been reported. Jones et al. (5) have cultured bovine and human pancreatic ducts for up to 12 weeks. The ducts were cut open and cultured as planar explants consisting of epithelium and underlying connective tissue with the latter in contact with the culture dish. These pioneering studies were restricted, however, to the main and largest interlobular ducts. In this paper, we describe the isolation and culture of interlobular and associated intralobular ducts of the pancreas of the rat.

MATERIALS AND METHODS

Isolation of ducts. The procedure for the isolation of ducts was a modification of that used for obtaining pancreatic islets (6). Pancreases were removed from 125- to 350-g Sprague-Dawley rats from our own colony, weighed, and minced with sharp scissors for 2 to 4 min in a vial containing, for each gram wet weight of pancreas, 6.5 ml of Hanks’ balanced salt solution (HBSS), 3 to 6 mg of collagenase/ml (Type IV, Worthington Biochemical Corp., Freehold, NJ), and 0.2 mg of a-chymotrypsin/ml (code CDI, Worthington). The HBSS was routinely supplemented with 0.2 mg bovine serum albumin/ml (Fraction V, Sigma, St. Louis, MO) and 0.1 mg soybean trypsin inhibitor/ml (code SIG, Worthington). In later 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 1600 rpm in an HN-S centrifuge (Damon/IEC Division, Damon Corp., Needham Heights, MA) in a swinging bucket rotor. The supernatant fluids were discarded and the pellets washed at least three times in HBSS as above, using a Vortex mixer to resuspend the pellets.

The fragments produced by digesting about 1 g of pancreas were resuspended in 6 ml HBSS and were pipetted onto a prewetted No. 60 mesh stainless steel sieve (pore size 200 × 300 μm, Belco Glass, Vineland, NJ). After the bulk of the fluid and small fragments had passed through the filter, the residue was washed with 20 ml of HBSS delivered from a syringe with moderate force. The large fragments remaining on the sieve, which included the ducts, were harvested by rinsing the inverted sieve with another 20 ml of HBSS.

Culture in soft agarose. The following methodology was suggested by the successful culture of pancreatic islets in an agarose matrix (7). Following the procedure of Macpherson as described for the culture of single cells (8), washed tissue fragments were suspended in 0.33% agarose (Sigma, Type II), 10% trypose phosphate broth (Difco Laboratories, Detroit, MI), 10% heat-inactivated fetal bovine serum (GIBCO, Grand Island, NY), and 80% defined medium. The defined medium adapted from Barrett et al. (9) was CMRL 1066 (GIBCO) containing 1 μM dexamethasone (Sigma), 1 μg bovine insulin/ml (Sigma), 0.7 mM L-glutamine (GIBCO), 100 μg soybean trypsin inhibitor/ml (Worthington, Code SIC), 100 U penicillin/ml (GIBCO), 100 μg streptomycin/ml (GIBCO), and 0.25 μg amphotericin B/ml (GIBCO). Gentamycin at 50 μg/ml replaced the penicillin and streptomycin in recent experiments. Aliquots of 1.5 ml were transferred to plastic culture dishes (160 × 15 mm, Falcon Plastics, Los Angeles, CA). The agar underlayer used by Macpherson (8) was omitted. After the agarose had solidified at room temperature, 1.5 ml of 90% defined medium;10% heat-inactivated fetal bovine serum was added to the dishes, which were placed in an incubator containing a humidified atmosphere of 5% CO₂:95% air at 37°C. In recent experiments 2.0 ml of tissue suspended in 0.67% agarose and 2.0 ml of liquid medium were added to culture flasks (25 cm², Corning Glass Works, Corning, NY), and the flasks were gassed with 5% CO₂:95% air. The higher concentration of agarose facilitated feeding and had no effect on the behavior of the ducts in culture. The medium was changed twice a week. The cultures were observed periodically with a dissecting microscope and photographed.

Each culture dish contained approximately 1.2 mg of tissue protein and individual structures were generally isolated from one another by at least a centimeter. Each dish contained a maximum of 40 interlobular duct fragments resulting from a maximal yield of 180 duct fragments/gram wet wt of pancreas.

Preparation of tissues for light and electron microscopy. Ducts were removed from the culture dishes with a small amount of adherent agarose and were fixed in cold Carnoy’s solution. After transferring the ducts to absolute ethanol, they were stained with neutral red to render them visible for processing. They were embedded in paraffin, sectioned at 5 to 7 μm and stained with hematoxylin-eosin-phloxin as were freshly excised Carnoy-fixed pancreases for comparison.

For electron microscopy, the ducts were fixed in a mixture of cold 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.2) (10). The tissue was fixed for 2 hr or held overnight in the refrigerator. The longer time of fixation did not produce any noticeable differences in morphology. The tissue was rinsed 10 to 12 hr with several changes in cold 0.1 M cacodylate buffer (pH 7.2) containing 0.2 M sucrose. Following the wash, the tissue was placed for 1½ to 2 hr in cold 1% osmium tetroxide buffered to pH 7.2 with 0.1 M cacodylate. The ducts were stained in block overnight in cold, aqueous 0.5% uranyl acetate followed by dehydration in ethanol. All material was flat embedded in a mixture of Epon-Araldite (11). Thin sections were cut on a Reichert OM-U2 ultramicrotome, stained with lead citrate (12), and viewed in a Philips EM 300 electron microscope.

**EXPERIMENTAL RESULTS**

**Ducts in the Intact Pancreas**

**Definition of duct size categories.** Once the pancreas has been minced and partially digested with collagenase, ducts can no longer be categorized according to their position in the gland (main, interlobular, etcetera) but solely on the basis of diameter, cells in circumference, and appearance. The identification of isolated and cultured pancreatic ducts is based on the following histological description of the various duct categories of the intact pancreas of the rat.